SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS OF A SERIES OF SIGMA RECEPTOR LIGANDS

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

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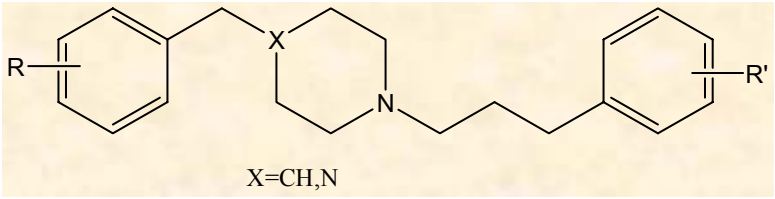
ABSTRACT

Sigma receptors are involved in several biological processes, and sigma ligands might be promising as cancer treatment agents, cocaine abuse medicines, and valuable psychiatric drugs. In an attempt to elucidate effectual structure-activity relationships, a series of *N*-phenylpropyl-*N'*-benzylpiperazine and *N*-phenylpropyl-4-benzylpiperidine analogs systematically substituted on both phenyl rings was synthesized. These ligands were specifically designed to have certain substituents and substitution pattern representing three physico-chemical parameters denoting size, hydrophobicity, and electronic characteristics, in order to study the effect of those properties on the biological activity.



Structure-activity relationships (SAR) were evaluated qualitatively to describe the effect of the systematic benzyl substitution in comparison to the phenylpropyl substitution. High quality mathematical equations were derived to quantitatively express the statistical correlation between the biological activity and the physico-chemical parameters associated with the benzyl ring substitution. Finally, the effect of the piperidine moiety was compared to the piperazine in a systematic and coherent fashion.

This SAR study will result in a better comprehension of the interaction between the sigma protein receptors and the ligands, a better understanding of the pharmacophore profile, and a more effective design for future potent sigma receptor ligands.

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CHAPTER I:

LITERATURE REVIEW

I.1 Introduction

I.1.1-*Protein receptors*:

Receptors are proteins that exist on the cell membrane, inside the cytoplasm or in the nucleus. Once they bind to a specific molecule, they can become activated resulting in several physiological functions which constitute the biological activity of the ligand. The biological significance of drug-receptor interaction caused by molecular recognition was clearly acknowledged when Donald Cram, Jean-Marie Lehn and Charles Pederson won the Nobel Prize in Chemistry in 1987.[1]

Multicellular eukaryotic organisms rely on small chemical molecules to coordinate the activities and communication between cells.[2] Unlike enzymes, proteins do not modify the structures of the ligands; they simply read the encoded information via an amazing process which is not fully elucidated yet.[3]

Complementarity in shape and properties between the protein receptor and the ligand results in a protein-receptor complex. Structurally dissimilar ligands belonging to different chemical classes can bind to the same protein receptor site, which suggests that the binding is associated with the physical and chemical properties of the ligand, and not necessarily the chemical structure itself.[4] The main intermolecular forces between the

receptor and the drug (ligand) are: hydrogen bonding, electrostatic interactions, hydrophobic interactions, and others.[4,5]

I.1.2-*Protein receptors pharmacology*:

A bioassay is an experiment that is conducted to measure and quantify the effect or the potency of a substance (drug) in terms of biological response. The interaction of a drug with a protein receptor can be represented by the following equation:

$$D + R \underset{k^{-1}}{\overset{k^{+1}}{\rightleftharpoons}} DR$$

where D represents the concentration of the drug, R is the concentration of unoccupied receptors, k^{+1} is the association rate constant, and k^{-1} is the dissociation rate constant. Drugs are usually characterized by their equilibrium dissociation constant K_d (k^{-1}/k^{+1}). According to an equation established by the physiologist A.V. Hill,[6] K_d is the concentration of the drug that can produce 50% occupancy and can be determined from radioligand-binding studies by using the "Scatchard Plot" of the following equation:

$$\frac{\beta}{D} = \frac{\beta_{max}}{K_d} - \frac{\beta}{K_d}$$

where β is the number of bound drug molecules, D is a radiolabeled drug (usually with ^{3}H or ^{125}I), and β_{max} is the total number of binding sites. Plotting β/D versus β has $1/K_d$ as a slope, and β_{max}/K_d as the x-axis intercept.[2]

Drugs can also be characterized by their binding affinity K_i (which is the dissociation constant also known as the inhibition constant of a non-radiolabeled inhibitor). The binding affinity can be determined by radioligand inhibition competitive binding assays, where the dissociation constant of an unlabeled compound is calculated from the concentration required to displace radiolabel binding by 50%. The concept is based on the fact that binding to a protein receptor depends on the concentration and the dissociation constant of the radioligand (of known K_d) as well as the binding affinity of the drug that is competing with the radiolabeled probe. An inhibition curve will result, where the dose-response sigmoidal curve (the dose being concentration of the drug; and the response being the inhibition of the radioligand binding of a concentration D^*) allows the determination of the IC_{50} (50% inhibition) which can be converted to a K_i number (the binding affinity of the drug) by using the Cheng-Prusoff equation:[7]

$$K_i = IC_{50} / \left(\frac{D^*}{K_d} + 1\right)$$

I.1.3-*Rational drug design and SAR*:

Studying the interactions of small molecules with protein receptors using crude receptors or tissue preparations *via* quantification of a response or competition with a radiolabeled probe is an integrated part of drug design.

Drug design includes lead finding and lead optimization. Approaches to finding a lead include but are not limited to searching for new compounds either of natural or artificial origin[1] identified by a receptor-based screening effort. Lead finding is followed by improving the affinity and selectivity in subsequent analogues (lead optimization).

The methodology called structure-activity relationships (SAR) consists of using a certain number of structurally modified compounds, testing their biological activity, and then identifying whether a pattern exist with the structural feature modification that may explain the changes in biological activity. The compounds are usually chosen based on availability, synthetic feasibility, and diversity of physico-chemical properties. The role of computers in the process of drug discovery consists of providing a tool for graphic modeling and computational chemistry to study models of drug-receptor interactions. Computers also allow collecting and viewing data of estimated or experimental data of molecular properties (also known as descriptors), as well as creating or simulating models of molecules and biological sites.

Quantitative structure-activity relationships (QSAR) is a specific type of SAR where computers can quantify hypotheses based on the fact that physico-chemical constants or descriptors of compounds are correlated with biological activities using computer software. The correlation is manifested in mathematical equations derived from statistical correlation.[2]

Advantages of QSAR include extending data collected from small organic systems (physico-chemical descriptors) to more complex systems, as well as quantification of predictions, with statistical confidence limits. Results and conclusions can be generalized and consequently applied beyond the particular analysis. However, applying a QSAR approach for SAR analysis assumes that conformational changes in receptors can be ignored and metabolism does not play a role in altering the activity.[8]

I.2 Sigma receptors

I.2.1-*History*:

Sigma receptors were first discovered in 1976 as a subtype of opioid receptors.[9] Nowadays, these receptors are a totally unique intracellular receptor family found in several tissues and organs[10,11] and are believed to be implicated in a multitude of biological processes, cellular functions, and medicinal applications.[12] Two subclasses are currently known: sigma-1 and sigma-2. The subclass differentiation is mostly determined by protein molecular size and the difference in binding affinity towards certain ligands, but it is also due to pharmacological studies based on anatomical distribution, biochemical characters, and function.[10,11,13,14]

There are more recognized facts concerning the sigma-1 receptor as opposed to the sigma-2 receptor. For instance, the sigma-1 receptor protein was coded from guinea pig and human sources[15,16] and found to consist of 223 amino acids (25 kDa), while the sigma-2 receptor protein is not yet coded.[17] The sigma-2 receptor protein is estimated to be about 18-21 kDa[13] but it is not as well known as sigma-1 due to the deficit of high affinity selective ligands for this subtype.[18] The presence of a sigma-3 subtype was never confirmed, although its existence was proposed in a few papers.[19,20] The major classification differences between sigma-1 and sigma-2 subtypes are summarized in the table below referred to in a study by Quirion and co-workers.[21]

Table 1. *Quirion and co-workers table summarizing the major pharmacological and functional differences between σ_1 and σ_2.*[21]

Ligand or assay	σ_1	σ_2
Discriminant ligands		
(+)-Pentazocine	High affinity	Low affinity
N-allylnormetazocine	Moderate to high affinity	Very low affinity
Dextromethorphan	Moderate to high affinity	Very low affinity
Nondiscriminant ligands		
Haloperidol	High affinity	High affinity
Ditolylguanidine (DTG)	High affinity	High affinity
Other characteristics		
Phenytoin sensitivity	Yes	No
Functional assays	Various gastrointestinal effects, inhibition of contraction of guinea pig ileum, inhibition of acetylcholine-induced phosphoinositide response	Dystonia upon injection into the rat red nucleus, modulation of K^+ channels
Radioligands	[^{3}H](+)-pentazocine	[^{3}H]DTG (with σ1 blockers)

I.2.2-*Sigma receptors: biology and pharmacology*:

Sigma receptors are present in the brain, as well as in many vital peripheral and internal organs and tissues.[10,11,20] It is believed that these receptors are related to several CNS (central nervous system) psychiatric and motor disorders such as depression,[22] schizophrenia,[14] movement disorders,[23] Alzheimer's disease,[24] epilepsy,[25] pain,[26] analgesia, amnesia,[11] memory deficit[27] and possibly involved in Parkinson's disease.[28]

Sigma receptors exist on the order of hundreds of thousands to millions per cancer cell, from a variety of cell lines. This fact suggests that sigma receptors are more than neurotransmitters. Today it is commonly recognized that both subtypes are widely expressed in a multitude of tumors from various organs,[29] and especially in human breast, while they are totally absent in normal mammary tissues.[30] Specifically, the sigma-2

subtype receptors are about 10-fold higher in proliferating tumor cells compared to dormant cells.[31] Sigma receptors might play a role associated with cancer growth and other functions through their involvement in ion channel regulation[32-34] and Ca^{2+} release.[35,36] This action subsequently affects cell growth, cell propagation and can stimulate a unique form of apoptosis.[30,37]

As time passes, sigma receptors are shown to be involved in the regulation of several unrelated body functions such as controlling retinal and gastrointestinal functioning,[38] inhibition of cell proliferation in human eye lens,[39] brain myelination regulation,[40] and treatment of endocrine, cardiovascular and immune systems.[12]

I.2.3-*Pharmacological potentials and clinical uses of sigma receptor ligands*:

Recent literature illustrates that both types of sigma receptors accommodate a wide array of structurally dissimilar compounds from different chemical classes.[41] It is commonly thought that some neurosteroids might be sigma endogenous ligands,[42] with progesterone being the most potent one.[26]

The specific participation and character of sigma receptors in the processes of the psychiatric and neurological disorders is still unclear,[43] but since sigma receptors are able to interact with many psychoactive ligands such as cocaine,[44] sigma receptor ligands have drawn attention first as potentially useful antipsychotics,[14] antidepressants,[45,46] anxyolitics,[47] antiamnesics, for mental improvement,[48] analgesics,[49] antiepileptics, anticonvulsants, for seizure reducing[50] and neuroprotective agents.[15,43] Moreover, some sigma-2 antagonists can suppress some side effects accompanying antipsychotic agents.[11]

Aside from their involvement in psychiatric disorders and nervous system diseases, it seems that sigma receptor ligands might be promising in dealing with several cancer cell types through a variety of strategies. It is already documented that many sigma receptor ligands (belonging to both subtypes) exert remarkable cytotoxicity and sustain cell viability,[51-53] which classifies them as potential anti-tumor agents. While cocaine and other agonists might promote *in vivo* lung cancer growth, the administration of sigma-1 antagonists could potentially reverse cancer growth.[44] Moreover, sigma-2 ligands could be used to efficiently induce apoptosis in tumor cells.[54] Sigma receptor ligands can also increase the effectiveness of cytotoxicity through reversing the drug resistance by tumor cells, they can be used as anti-neoplastic agents, and they can have chemosensitizing effects.[11]

Sigma receptors and their ligands offer a plethora of means available to treat cancer.[11] By their use *in vivo* as imaging agents,[29,55] the visualization of tumor cell proliferation *via* radiochemical analysis techniques such as PET (Positron Emission Tomography)[56] and SPECT (Single Emission Computed Tomography) scintigraphy[57] allow for non-invasive procedures for the tumor stage determination. This strategy is based on the over-expression of sigma receptors in cancer cell.[24] Radiotracers containing ^{123}I, ^{124}I, ^{125}I,[55,57-59] ^{18}F,[60] ^{99m}Tc,[61] and ^{11}C[62] sigma ligands were studied as tumor imaging agents. This subsequently contributes in providing a cost-effective means of diagnosis and early detection with widespread availability. It has also been suggested recently that sigma-1 receptor ligands might be potentially useful as PET analysis agents for imaging the brain of patients suffering from psychiatric disorders.[63]

The third useful application of sigma receptor ligands is the focus on cocaine abuse medication.[64,65] Sigma receptors are present in the brain and heart; therefore sigma receptor ligands (antagonists) can prevent the convulsions, locomotor activity, vasospastic disorders, lethality,[26,66,67] and toxic effects that are induced by cocaine, by competitively binding to the protein receptor domains.[68] Both sigma-1 and sigma-2 subtypes seem to be involved in this anti-cocaine activity. However, there is more solid evidence regarding the involvement of the sigma-1 subtypes, and (-)-cocaine itself bind to the sigma-1 subtype with a 10-fold higher affinity.[65,69,70] Derivatizing the phenyl ring of cocaine analogs is being studied recently in order to obtain more information on the pharmacophore profile of sigma-1 binding, and the discovery of derivatized ligands that might be useful for radioimaging.[71]

Because the pharmacological profile of the sigma ligands is crucial for the medicinal applications, it is of great importance and necessity to determine whether a ligand is an agonist or an antagonist. Cobos and co-workers[72,73] studied the effect of phenytoin (DPH) on modulating the binding affinity of sigma-1 receptors. They determined that DPH increases the binding affinity of sigma-1 agonists 10 fold (dextromethorphan, (+)-SKF-10,047, (+)-3-PPP and PRE-084). However, no notable effect was observed with sigma-1 antagonists (haloperidol, BD 1063, NE-100, progesterone, and BD 1047). This assay can potentially serve as a quick and preliminary test to screen the pharmacological profile (agonist / antagonist) of sigma-1 ligands.

I.2.4-*Sigma ligand selectivity*:

Sigma receptors recognize and interact with a wide array of compounds and drugs;[74] however, there have been only a few that bind with both a high affinity towards sigma receptors[75] and potency towards the subtypes. This fact was attributed to the lipophilic sterol-binding domain on the protein capable of binding a large array of lipophilic ligands.[24] Yet another explanation relied on the existence of multiple binding sites on the same protein.[76] Nowadays, modeling studies assume that the binding takes place on the same protein site.[77,78] In 1994, Glennon and co-workers[79] established one of the first pharmacophore models for sigma-1 binding consisting of an amine site flanked between two hydrophobic regions (Figure 1).

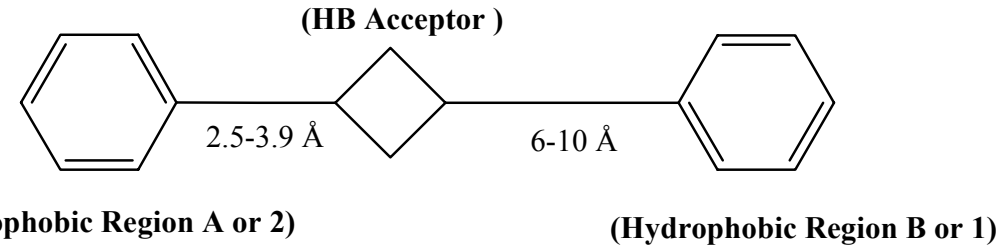


Figure 1. *Glennon and co-workers σ_1 binding pharmacophore model*.

In 2004, Cratteri and co-workers[78] described qualitatively a sigma-2 receptor binding pharmacophore similar to the widely accepted Glennon and co-workers sigma-1 pharmacophore. The difference in this model was the distances between the hydrogen bond acceptor site to each hydrophobic phenyl ring and the different distance between the two hydrophobic regions. They also proposed another binding site other than the nitrogen center that is an electron rich site (O, CO,...), (see Figure 2) able to behave as a strong H-

bond acceptor. A similar modeling study for building another sigma-1 receptor binding pharmacophore reported in 2004 by Gund and co-workers[77] revealed the presence of a similar electron rich site, behaving as a strong-H-bond acceptor (O in PD144181, CO in Haloperidol, and S in Spipethiane) (see Table 2). For high potency, the secondary electronegative binding groups should be in complementary positions.

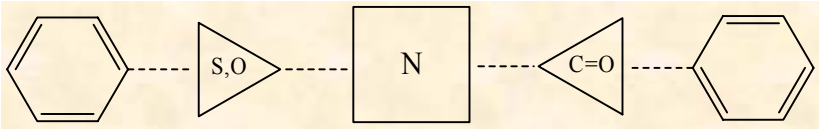


Figure 2. *The proposed electron-rich secondary binding site of the binding pharmacophore.*

The lack of selective sigma ligands (for the receptor type and each of the subtypes) has led to uncertainty in understanding the contribution of sigma receptors in many biological phenomena.[80] Although there have been many attempts to obtain selective sigma receptor ligands,[41] no specific sigma ligand has yet to make it to the pharmaceutical market.[81] This is most likely due to insufficient data from clinical trials, although it is recognized that pharmacological information is showing much potential.[82]

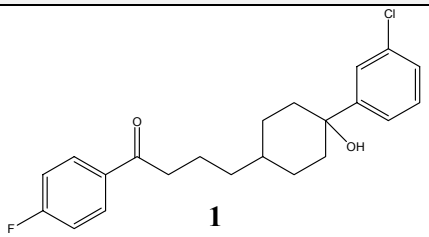
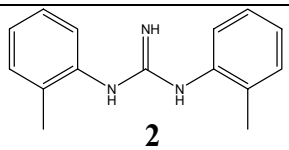
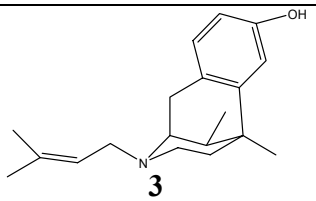
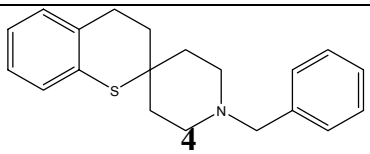
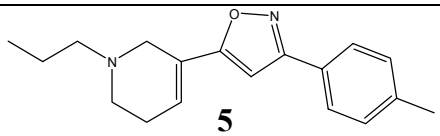
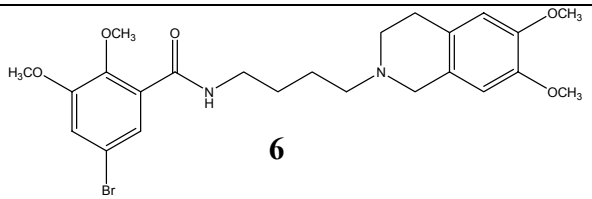
While some structures had high selectivity to sigma-1 receptors,[83] the sigma-2 receptor ligands showed low selectivity, especially over the sigma-1 subtype.[18] The lack of selective sigma-2 ligands is preventing the resolving of a pharmacophore model for sigma-2 ligand binding.[78] While several sigma-1 specific radioligands (functioning as probes) exist, there is none for the sigma-2 subtype.[29] The short list of sigma-2 agonists includes some low affinity, selective ligands and high affinity low selectivity ligands.[18]

Therefore, sigma-2 ligands with both high affinity and selectivity would greatly benefit the domain of sigma receptor research.

Many alkylamines, especially piperidine and piperazine derivatives,[74] have shown high affinity for sigma-1[77] as well as sigma-2 receptors and a quite strong selectivity for sigma receptors against dopamine D-2 and serotonin 5-HT_{1A} receptors.[84] Examples of such compounds belonging to this structural family appeared in many studies.[75,85-93] Moreover, (diphenylalkyl)piperidine and (diphenylalkyl)piperazine derivatives fit quite well into the proposed pharmacophore binding models. Therefore, we can conclude that (diphenylalkyl)piperidines and (diphenylalkyl)piperazines are very suitable compounds to study sigma-receptor binding (for both subtypes) due to their structural simplicity and compatibility with suggested pharmacophore models, which explains their manifestation in many sigma receptor studies.

Because of their binding affinity for neuroleptics in general, both sigma receptor subtypes exhibit high affinity for haloperidol (**1**) and ditolylguanidine (**2**), and the sigma-1 subtype exhibit high affinity for (+) pentazocine (**3**). Piperazine and piperidine derivatives are common sigma receptors ligands, and spipethiane (**4**) is a very potent and selective sigma-1 ligand. 1-Propyl-5-(3-*p*-tolyl-isoxazol-5-yl)-1,2,3,6-tetrahydropyridine (PD144418) (**5**) might be the most potent and selective sigma-1 ligand known hitherto, and finally, 5-bromo-N-(4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-2,3-dimethoxybenzamide (**6**) is the most selective sigma-2 known so far.

Table 2. *Structures, binding affinities and selectivity of some of the most potent and used sigma receptor ligands.*

Compound	***σ_1 Ki (nM)***	***σ_2 Ki (nM)***	***Selectivity (σ_2 Ki / σ_1 Ki)***	***Ref***
 **1**	2.2	16	7.27	[84]
 **2**	27.7	12.8	0.46	[94]
 **3**	5.8	1253	216	[95]
 **4**	0.5	416	832	[96]
 **5**	0.08	1377	17212	[95]
 **6**	12,900	8.2	0.00062	[97]

Investigating the phenyl ring substituted compounds can be very useful. For example the substitution at the aromatic ring revealed some potentially useful halogenated derivatives for PET or SPECT as well as ligands labeled with ^{11}C, ^{3}H, and other radiolabeled substituents.[58,98-101] Moreover, there are a large number of studies where the phenyl ring substitution resulted in a notable change in the binding affinity.[18,41,65,66,70,101-106] Although the effects of phenyl ring substitution are very important and effective for sigma receptor ligand binding affinity, amid the studies dealing with that effect, a relatively low number of papers have dealt with the phenyl ring substitution in a systematic way (permuting different substituents on different positions). Therefore, only few, if any, hypothetical explanations were given on the cause and effect. Among those above described studies, only a very limited number dealt with quantitative structure-activity relationships; Fujimura and co-workers[107] suggested that the sigma affinities are quantitatively dependent on the electronic natures of R_1 and R_2 (see Figure 3)..

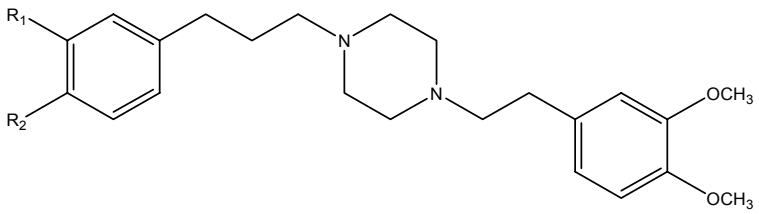


Figure 3. *General structure of substituted derivatives of 1-[2-(3,4-dimethoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazine.*[107]

Mascarella and co-workers[107] performed another QSAR study on a series of substituted benzyl-N-normetazocines (see Figure 4). Although they did not find any significant relationship between the electron-donating, electron-withdrawing or neutral substituent

effect on the binding affinity, they were able to relate the binding potency to the substituent volume, substituent position, hydrophobicity (π), and molar refractivity.

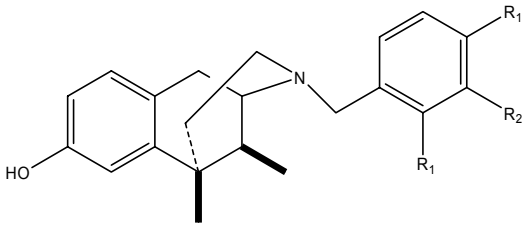


Figure 4. *General structure of substituted derivatives of benzyl-N-normetazocines.*

A third QSAR study was performed by Huang and co-workers[87] on a series of N-(1-benzyl-piperidin-yl)phenylacetamides (see Figure 5). They were able to relate sigma-1 binding affinity to substituent parameters such as electronic, hydrophobic and steric bulk effect, but their compounds were not suitable for sigma-2 binding affinity QSAR due to low affinity in general towards the latter sigma subtype.

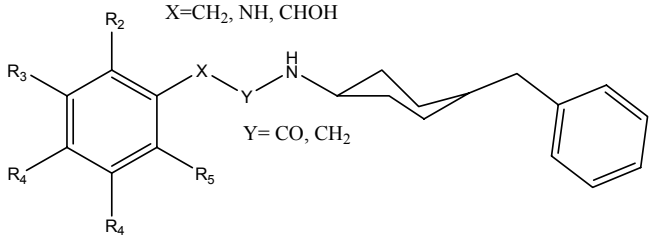


Figure 5. *Huang and co-workers series of substituted compounds.*

Finally Liu and co-workers,[108] related sigma ligand affinity to the lowest virtual orbital eigenvalues, steric parameters (molar refractivity) and substituent hydrophobicity.

CHAPTER II:

SPECIFIC AIMS AND HYPOTHESES

II.1 Objectives

1-Designing a series of structurally different compounds, each with a specifically selected and unique set of physico-chemical properties, and testing their binding affinity towards both subtypes of sigma receptors.

2- Elucidating the effects of the systematic structural variation on the binding, resulting in qualitative structure-activity relationships as well quantitative structure-activity relationships correlation equation (QSAR) attributed to the numerical quantification of the biological activity (K_i values) from one side, and the quantification of the physico-chemical properties associated with the structure from another side.

3-Understanding better the pharmacophore profile, and comprehending the interactions between the sigma proteins and their ligands.

4-Trying to uncover the major differences between the sigma-1 and the sigma-2 subtypes in terms of binding to ligands and what affects the selectivity for one of the subtypes versus the other.

5-Possibility of considering the QSAR analyses as a methodology or at least an option to consider when it comes to designing new potent ligands for either of the subtypes.

6-Achieving those goals involves selecting lead compounds, and a structural skeleton that is best suitable for such a study, synthesizing the various compounds in a time and cost efficient manner, as well as testing their biological assays by common standardized binding assays in order for the results to be comparable to other similar studies

II.2 Hypotheses

This "prospective approach" is based on carefully choosing a relatively small set of structures with precise and different physico-chemical properties that will enable one to obtain a certain analysis and conclusions regarding a certain biological activity.

Such a method is not often applied, and in fact it does not seem that it has been applied at all in studying the sigma receptors, where all the structure-activity relationships studies have been done in a retrospective fashion, where various structures are synthesized, biologically assessed, then the structural properties are looked back upon and conclusions are drawn.

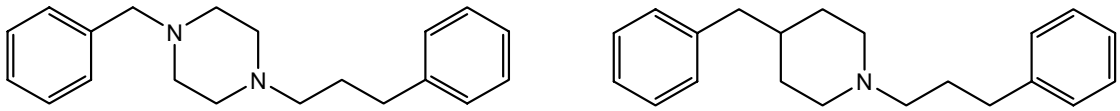
Perhaps such an approach is not very common because of the difficulty in designing compounds that can precisely and accurately represent a certain values of physico-chemical properties, especially in complex structures. Another obstacle might be the synthetic feasibility variation between different elements of the set of compounds (especially if the basic skeleton is not simple).

Hence, applying a prospective approach for designing a set of compounds for SAR should include finding a lead compound and a certain skeleton of compounds that offer foundations for structural simplicity (in order to solely study the effect of the desired

systematic structural modification), practicality in finding actual compounds that represent the desired values of the physico-chemical parameters, as well as synthetic feasibility in a similar fashion for most compounds.

II.3 Experimental steps

A- Choosing the lead compounds (***N*-phenylpropyl-*N'*-benzylpiperazine (Lead 1) and *N*-phenylpropyl-4-benzylpiperidine (Lead 2))**.



Lead-1 **Lead-2**

Figure 6. *Structures of lead compounds*.

B- Designing statistically the substituents, substitution pattern, and number of compounds in each series.

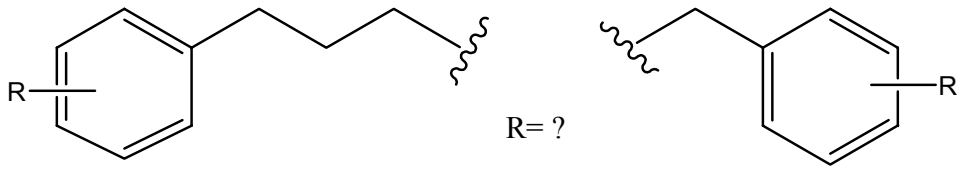


Figure 7. *Highlighted skeleton of substitution pattern*.

C- Synthesizing the selected phenyl ring substituted derivatives of lead compounds.

D- Testing their binding affinity for the sigma-1 and sigma-2 protein receptors.

E- Determining their Pharmacological profile (agonists / antagonists).

F- Establishing quantitative SAR by correlating the binding constants to the structure.

G- Establishing qualitative SAR by studying the effect of the substitution on each phenyl ring compared to the other, and in the piperidine model compared to the piperazine.

H- Elucidating conclusions on the pharmacophore profile, and the effective design of new potent ligand.

The next sections of this dissertation will describe explicitly each of these execution steps and the reason behind these choices.

Chapter III:

CHEMOMETRIC DESIGN

III. 1 Leads and assumptions

III.1.1-*Designation of leads and* choice *of structural skeleton*:

After citing in page 12 the reasons behind choosing (diphenylalkyl)piperidines and (diphenylalkyl)piperazines as a structural class for our study, the structural diversity of these compounds was narrowed down. It was specifically decided to investigate the structure-activity relationships in a series of *N*-phenylpropyl-*N'*-piperazine and *N*-phenylpropyl-4-benzylpiperadine substituted derivatives.

According to Costantino and co-workers,[84] a large number of benzyl piperazine and benzyl piperidine derivatives have remarkable affinity for sigma receptors that can be found in several studies.[52,70,77,87,98,109] On the other hand, another structural moiety resulted in several high potency sigma ligands, and that moiety is (phenylpropyl)piperidine or (phenylpropyl)piperazine.[18,68,99-101,110] (see Figure 8).

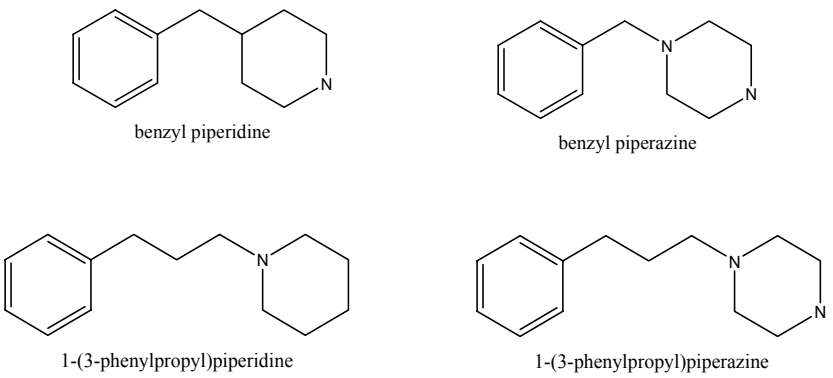


Figure 8. *The four different promising moieties found in search of lead compounds*.

By searching the literature in order to find some lead compounds having both of the above mentioned requirements (a benzyl group on one side and a phenylpropyl on the other side flanking a piperazine or a piperidine moiety in the middle) we have found the desired leads: **Lead 1** and **Lead 2**.

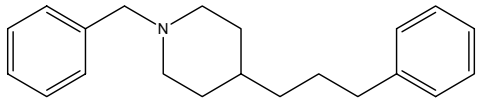
The lead compounds would be the unsubstituted [piperazine, 1-(phenylmethyl)-4-(3-phenylpropyl)] and [piperidine, 4-(phenylmethyl)-1-(3-phenylpropyl)]; designated consecutively as **Lead 1** and **Lead 2**.

Lead 1 appeared in a study in 2000 by Younes and co-workers[111] of structure activity relationships of aralkyl-(4-benzyl)piperazine derivatives. Although this compound was very selective for sigma-receptors (K_i = 20 nM) against serotonin $5HT_{1A}$ and dopamine D_2 receptors ($K_i > 10^5$), the affinity towards sigma subtypes (binding affinity

specification towards sigma-1 or sigma-2) was not indicated. Moreover, no phenyl substituted derivatives of this lead were studied.

Lead 2 seemed to appear first in 2002 in a study by Ablordeppey and co-workers following their development of the first sigma-1 binding pharmacophore.[112] In that study the carbon chain length was varied between the nitrogen atom of the piperidine moiety and the hydrophobic-B region phenyl ring, while the carbon chain between the nitrogen atom and the second phenyl group (hydrophobic-A) was left unchanged. **Lead 2** showed a sigma-1 site K_i of 0.4 nM and a sigma-2 site K_i of 3.3 nM. **Lead 2** also appeared and was used as a lead in a study performed by Costantino and co-workers in 2005[84] in an attempt to define structure-activity relationships of 1-arylalkyl-(4-benzyl)piperidine and 1-arylalkyl-(4-benzyl)piperazine derivatives. According to this study, **Lead 2** showed a sigma-1 site K_i of 1.4 nM and a sigma-2 site K_i of 0.49 nM. No phenyl substituted derivatives were reported in the sigma receptor binding studies from either paper. Although both studies showed different binding affinity numbers for sigma-1 and sigma-2 sites, the interesting fact is that both cases showed this compound to have high potency towards sigma-1 and sigma-2 subtypes.

A third lead compound, *N*-benzyl-4-phenylpropylpiperidine, was initially taken into consideration. This is a (1-benzyl)piperidine derivative instead of the previously mentioned (4-benzyl)piperidine derivative. This lead was disregarded, since a study by Ablordeppy and co-workers[113] proved that in the case of moieties with one nitrogen atom, the nitrogen attached to the longer carbon chain (1-phenylpropylpiperidine derivatives in this case) is more effective than when it is attached to the shorter carbon chain.



[Piperidine, 1-(phenylmethyl)-4-(3-phenylpropyl)]

Figure 9. *Structure of the lead that was not included in the study*.

Table 3. *Comparison of Lead 1 and Lead 2 binding affinities with some of the most used, and the most potent sigma receptor ligands.*

Compound	***σ_1 K_i (nM)***	***σ_2 K_i (nM)***	***Selectivity (σ_2 K_i/ σ_1 K_i)***	***Ref***
Haloperidol	2.2	16	7.27	[84]
(+)-Pentazocine	5.8 ±1.0	1253 ±519	216	[95]
Ditolylguanidine (DTG)	27.7 ±4.3	12.8 ±2.1	0.46	[94]
PD144418	0.08	1377	1721	[95]
Spipethiane	0.5 ±0.02	416 ±43	832	[96]
3-(1-piperidinoethyl)-6-propylbenzothiazolin-2-one	0.6 ±0.3	18.1 ±0.2	29	[114]
N-(N-Benzylpiperidin-4-yl)-2-fluorobenzamide	3.4	406	120	[115]
1-(2-Fluoroethyl)-4-[(iodophenoxy)methyl]piperdine	0.84	102	121.42	[116]
Spiro[2]benzopyran-1,4'-piperidine	IC_{50} =53	IC_{50}=0.9	0.017	[117]
5-Bromo-*N*-[4-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-butyl]-2,3-dimethoxy-benzamide	12,900	8.2	0.00062	[97]
Lead 1	**20**	ND	ND	[111]
Lead 2	**0.4**	**3.3**	**8.25**	[112]
Lead 2	**1.4**	**0.49**	**0.35**	[84]

II.1.2-*Phenyl ring substitution as structural modification for the SAR*:

Synthesizing and evaluating the sigma receptor binding affinities of phenyl ring substituted **Lead 1** and **Lead 2** derivatives is useful for the following purposes:

-Discovery of compounds with higher binding affinities than the lead compounds.

-Evaluation of qualitative and quantitative structure activity relationships by using different substituents at various positions, to yield a different binding affinity than the original compound.

-Identification of compounds valuable for radio-imaging, especially when the substituent is a radioisotope (^{18}F, ^{123}I), or when the substituent can incorporate a chelate to bear a radiometal (such as ^{99m}Tc).

Following the same systematic substitution pattern, the qualitative part of the structure-activity study will shed light on the substitution effect on the Hydrophobic-A region of the binding pharmacophore as opposed to the Hydrophobic-B region (see Figure 1 page 10), as well as the effect of the substitution on each phenyl ring of piperidine derivatives, as opposed to piperazine derivatives (see Figure 10 below).

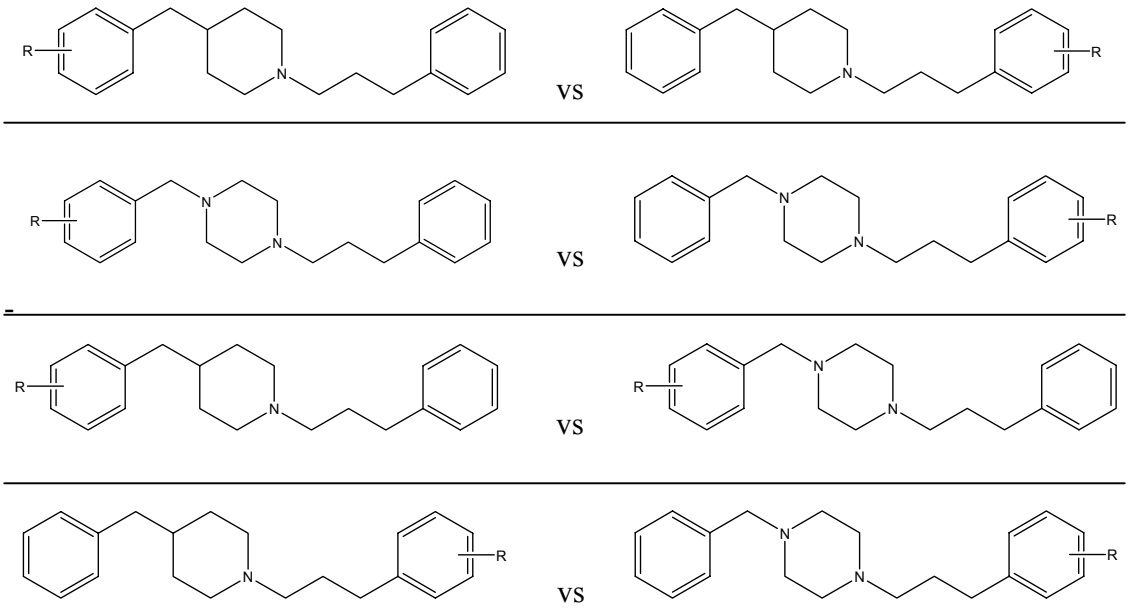


Figure 10. *Structural representation of the compounds for the qualitative SAR.*

III.2 Design of substituents, substitution pattern, and number of compounds

III.2.1-*Quantitative Structure Activity Relationships* (*QSAR*):

Physical organic chemistry will be heavily relied upon in order to illuminate the chemico-biological interactions.[118] Nowadays, QSAR is considered as an effective tool for drug design. Biological activity can be predicted prior to synthesis, subsequent to the establishment of a mathematical equation relating biological activity to physico-chemical parameters, or other parameters such as quantum-mechanics parameters, that can describe the ligand structure from a quantitative point of view. After validation, the equation can be used to predict the activity of molecules structurally similar to the ones used in building the equation.

The series of compounds being proposed has advantages over the before-mentioned studies. First, **Lead 1** and **Lead 2** contain structural simplicity, obvious discrimination between hydrophobic-A and hydrophobic-B regions, and exhibit high sigma receptor binding affinities. This affinity is a very crucial and necessary starting point for QSAR studies. Another advantage is the absence of the alternative electron-rich hydrogen-bond acceptor site on the pharmacophore model. The effect of this site is not fully understood. Lastly, the phenyl ring substitution will not be detrimental to the structural similarity between the compounds used in the study. This will enable us to exclusively study the effect of the substitution on the binding affinity.

Therefore, by synthesizing a number of *N*-phenylpropyl-4-benzylpiperidine and *N*-phenylpropyl-*N'*-benzylpiperazine phenyl substituted derivatives, we establish a multi-

variate regression equation relating multiple physico-chemical parameters to the compounds' sigma binding affinity. A different equation will describe the binding affinity on each of the two hydrophobic regions.

The choice of the physico-chemical descriptors was based on the biological relevance from the literature. The biological activity (binding affinity to sigma receptors) was previously shown to be potentially dependant on general descriptors such as steric effect, hydrophobicity and electronic parameters. Continuous physico-chemical parameters, such as Hammett σ values, the substituent hydrophobic contribution constant π, and molar refractivity MR are suitable for QSAR studies where a mathematical correlation equation is derived, as opposed to indicator variables (i.e., presence or absence of hydrogen bonding) or classifying descriptors (i.e., large, medium, small). In the case of descriptors not leading to a satisfactory correlation, modification of the descriptors will be considered (e.g. using σ^-, σ^+, σ^*, σ_I, $\sigma^{\cdot}$, or *F*). It might also be possible to use another descriptor for the same physical-property (i.e., the Taft's Steric Parameter E_s instead of the Molar Refractivity MR), to add a new descriptor (i.e., including a descriptor for the hydrogen-bonding capability of the aromatic substituent), or to delete a descriptor.

A QSAR equation of the following form is determined by multiple linear regression (MLR), principle component analysis (PC), partial least squares method (PLS), or by non-linear methods if necessary:[119]

$$\mathbf{Log\ (1/}\boldsymbol{K}_\mathbf{i}\mathbf{) = f}\ (k_1\boldsymbol{\sigma}^u, k_2\boldsymbol{\pi}_x^v, k_3\,\mathbf{MR}^y)$$

This can be done by correlating the biological activity (binding affinity K_i), to the following descriptors:

-**"Hammett σ"** values denote the electronic substituent parameter, and are determined from databases. σ accounts for: electron donating groups (OCH_3, CH_3), electron withdrawing groups (I, Br, Cl, F, NO_2, CH_3) , neutral (H), on different positions (ortho, meta, para).

-**"π_x"** values denote the hydrophobic contribution of each substituent: $\pi_x = \log P_X/P_H$, where P_X and P_H are the partition coefficients of substituted and unsubstituted compounds respectively.

-**"MR"** $[(n^2-1/ n^2+1)(MW/d)]$ values denote the substituent molar refractivity. The molar refractivity accounts for both the polarizibility and the substituent volume since "n" is a polarizibility dependent parameter, and MW / d is the actual substituent volume.

The squared correlation coefficient (r^2), cross-validation coefficient (q^2) and other parameters will be established to show the accuracy of the model.

III.2.2-*Factorial Design method*:

Benzyl ring substituents consisted of the so called "well behaved" substituents that have proven to give the sharpest correlations in SAR studies. [64] Specifically, a chosen set of 9 congeners was designed according to the factorial design method in a way to investigate a significantly large portion of three physicochemical parameters or structure descriptors space representing the electronic characteristics (σ Hammett constant values), the

substituent hydrophobic contribution (π_x), and the molar refractivity (MR). Each single compound represents a distinctive area of the descriptors numerical space.

Each parameter space is divided into high field, low field and intermediate field. Hammett sigma values are between -1.0, and 1.6; π_x values are between -1.0 and -0.02 and Molar Refractivity values are between 0.1 and 4.[119,120] Since a large portion of the substituents used for sigma-receptor binding affinity purposes have their MR values between 0.1 and 2.0, the molar refractivity high limit was set to 2.5. Since Yamashita and co-workers[121] proved that the hydrophobic region of the pharmacophore is not necessarily an aromatic site (i.e., phenyl ring), and since a statistically small proportion of substituents have a MR value higher than 2.5, our current study excludes such compounds (see Figure 11 below) because they might not behave differently than the original hydrophobic region (A or B) (see Figure 1, page 10) during binding instead of behaving as a substituent.

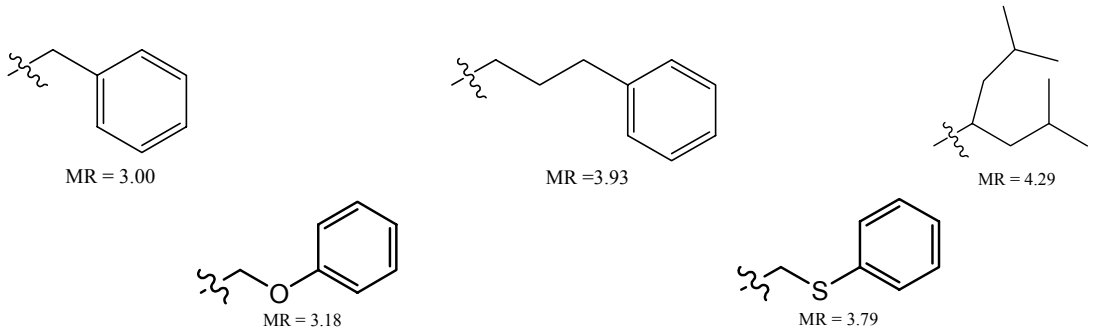


Figure 11. *Structures of some substituents capable of behaving as pharmacophore hydrophobic sites* (*values in ref*[119]).

Aside from the fact that the specific choice of the calibration set compounds turned out to be statistically valid, the choice of compounds was determined by the synthetic accessibility and availability of starting materials, their potential use where high potency can be obtained from the substituent at a particular position, or from the use of radioimaging agents (i.e., ^{123}I or ^{18}F), and finally by the fact that the same set of compounds will be used in the qualitative part of the structure-activity relationships study. Therefore, the nature of the substituents and the fact that they are commonly used would result in making the qualitative SAR simple to interpret.

Table 4. *The physicochemical properties that are investigated, the parameters that represent them, and the lower and upper limit of their numerical values.*

Property	Parameter	Low Limit (-)	Intermediate (0)	High Limit (+)
Lipophilicity	π_x	-1.0	-0.02	1.6
Electronics	$\sigma_{m,p}$	-0.4	0.12	0.8
Size	MR	0.1	0.79	2.5

According to the Factorial Design method,[120] the number of compounds needed to build the correlation equation is 2^n, n = the number of descriptors. In this case three descriptors (π, σ and MR) are used, so the number of necessary compounds = 8. One compound from the intermediate limit will be also used, bringing the total number of compounds to nine; each representing a distinctive physico-chemical value range. For instance the -*meta* Iodo substituted analog is a representation of a large, lipophylic, electron-withdrawing group, with a positive sign for each of these properties.

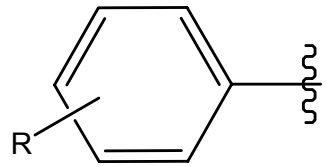
Table 5 below shows some possible substituents, their physico-chemical values, as well as their levels (whether each descriptor value belongs to a low, intermediate or high level). The numbers in bold correspond to the substituents that were picked, and represented in Table 6.

Table 5. *Physico-chemical values of some possible substituents.*

R	σ	π_x	MR	Levels	R	σ	π_x	MR	Levels
3-I	**0.35**	**1.12**	**1.39**	+ + +	**4-OCH_3**	**-0.27**	**-0.02**	**0.79**	**- 0 0**
4-I	0.18	1.12	1.39	+ + +	**3-OCH_3**	**0.12**	**-0.02**	**0.79**	**0 0 0**
3-F	**0.34**	**0.14**	**0.1**	**+ + -**	**3-SCF_3**	0.37	1.44	1.38	+ + +
4-F	0.06	0.14	0.1	- + -	**4-SCF_3**	0.42	1.44	1.38	+ + +
3-OH	0.12	-1.12	0.28	0 - -	**2-NO_2**	**-**	**-0.28**	**0.74**	**- - -**
4-OH	-0.37	-1.12	0.28	- - -	**3-NO_2**	**0.71**	**-0.28**	**0.74**	**+ - -**
3-SH	0.25	0.39	0.92	+ + +	**4-NO_2**	**0.78**	**-0.28**	**0.74**	**+ - -**
4-SH	0.15	0.39	0.92	+ + +	**3-CH_2CH_3**	-0.07	1.02	1.03	- + +
3-NH_2	-0.16	-1.23	0.54	- - -	**4-CH_2CH_3**	-0.15	1.02	1.03	- + +
4-NH_2	-0.66	-1.23	0.54	- - -	**3-CN**	0.56	-0.57	0.66	+ - -
2-Br	**-**	**0.86**	**0.89**	**- + +**	**4-CN**	0.66	-0.57	0.66	+ - -
3-Br	0.39	0.86	0.89	+ + +	**3-SCN**	0.41	0.51	1.34	+ + +
4-Br	0.23	0.86	0.89	+ + +	**4-SCN**	0.41	0.52	1.34	+ + +
2-Cl	-	0.71	0.60	- + -	**3-$CHCl_2$**	0.31	1.10	1.53	+ + +
3-Cl	0.37	0.71	0.60	+ + -	**4-$CHCl_2$**	0.32	1.10	1.53	+ + +
4-Cl	0.23	0.71	0.60	+ + -	**4-CBr_3**	0.54	0.88	0.50	+ + -
3-CBr_3	0.28	1.51	2.88	+ + +	**3-CO_2H**	0.37	-0.32	0.69	+ - -
4-CBr_3	0.29	1.51	2.88	+ + +	**4-CO_2H**	0.45	-0.32	0.69	+ - -
3-CH_2OH	0.00	-1.03	0.72	- - -	**3-CH_3**	-0.07	0.56	0.56	- + -
4-CH_2OH	0.00	-1.03	0.72	- - -	**4-CH_3**	**-0.17**	**0.56**	**0.57**	**- + -**

Table 6. *Calibration set of compounds and the "level" associated with each compound.*[119]

R	σ	π_x	MR	Levels	Actual
3-I	0.35	1.12	1.39	+ + +	+ + +
3-F	0.34	0.14	0.1	+ + -	+ + -
3-NO_2	0.71	-0.28	0.74	+ - +	+ - -
2-Br	-	0.86	0.89	- + +	- + +
4-OCH_3	-0.27	-0.02	0.79	- - +	**- 0 0**
4-CH_3	-0.17	0.56	0.57	- + -	- + -
4-NO_2	0.78	-0.28	0.74	+ - -	+ - -
3-OCH_3	0.12	-0.02	0.79	**0 0 0**	**0 0 0**
2-NO_2	-	-0.28	0.74	- - -	- - -
H	0	0	0.1	*Lead*	



R= *o*-Br, *o*-NO_2, *m*-I, *m*-NO_2, *m*-OCH_3
m-F,*p*-OCH_3, *p*-CH_3, *p*-NO_2, H

Figure 12.
"Calibration set" of compounds.

III.2.3-*Statistical validation of preliminary data*:

We tested the preliminary data in Table 6 above (numerical values from *ref* [119]), according to classical statistics notions in order to ensure that the final results will be statistically sound. First, each descriptor series of values was treated solely, and the mean (average), variance and standard deviation were all calculated. The results showed that the training set is sufficiently well dispersed in each parameter space.

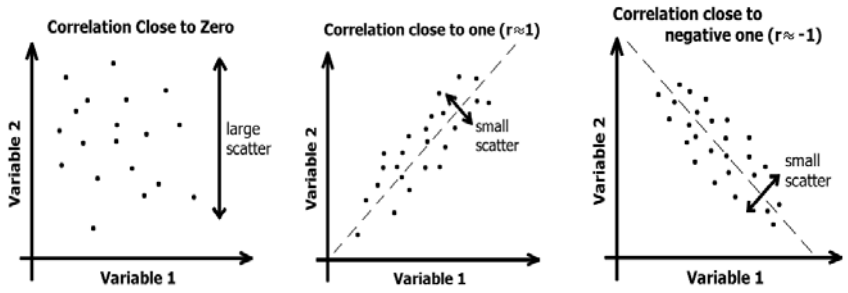
We then studied the distribution to discern whether it is “normal” or “abnormal”. Subsequently, we calculated the “skewness” (asymmetry of data) and the “kurtosis” (excess of data). The **skewness** is an indication of where the weight of the data is concentrated: a distribution skewed to the left is considered negatively skewed (left tail longest) and a distribution skewed to the right is positively skewed (right tail longest). The criteria on the skewness of a data distribution is usually determined by the Pearson’s coefficients.

$$\text{Skewness}= \frac{\sum_{i=1}^{N}(Y_i - \bar{Y})^8}{(N-1)s^8}$$

The **kurtosis** is a measure of the "peakedness" of the distribution. Frequent modestly sized deviations means low kurtosis, while higher kurtosis means that the variance is due to infrequent extreme deviations. Kurtosis of a normal distribution is ideally equal to zero. The higher the kurtosis, the sharper the peak, and wider the tails.

$$\text{Kurtosis}= \frac{\sum_{i=1}^{N}(Y_i - \bar{Y})^4}{(N-1)s^4} - 3$$

After treating each descriptor value series solely, a two dimensional analysis of data was carried out. The linear pair correlation coefficient between every two descriptors ($R = \frac{\text{Covariance}}{\text{Variance}}$) is an indicator of linear correlation of every two descriptors. If ”R” is significant (i.e. $r > 0.9$), then it is ineffective to use both of these descriptors in the same multiple regression analysis.

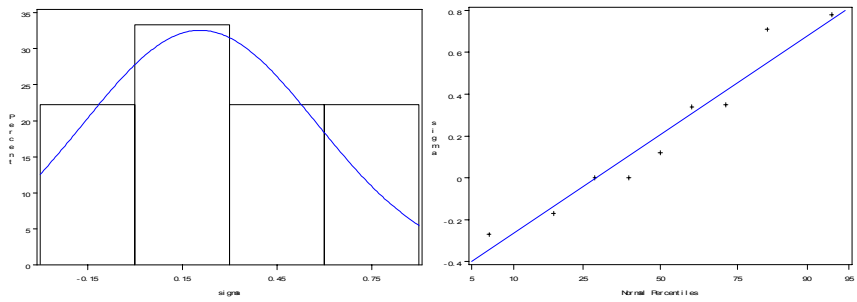


Graph 1. *The general graph of the linear correlation between two descriptors*.

To further investigate the parameter space of the descriptors, we have constructed Craig Plots. These plots symbolize the dispersion of every two descriptors in their dual numerical space (Graphs 5, 6, and 7).

Table 7. *Data statistical parameters and results of validation tests ran on SAS system, "The Univariate Procedure" (Linear Pair Correlation was run on Microsoft Excel).*

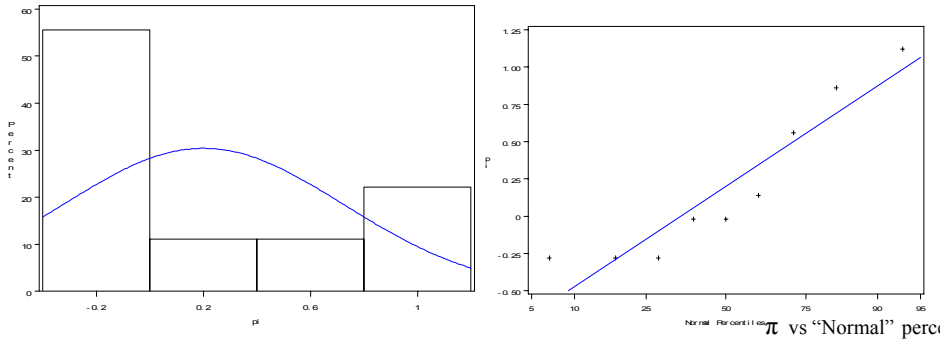
	Hammett σ	**π_x**	**MR**
Mean	0.2066	0.2	0.75
STDV	0.3678	0.5247	0.333
Variance	0.1353	0.2754	0.1109
Skewness (Asymmetry)	0.4561	0.8446	-0.0671
Kurtosis (Excess)	-0.9363	-0.7329	2.9947
Linear Pair Correlation	-0.25 (σ vs π)	0.41 (π_x vs MR)	0.01(σ vs MR)



σ vs "Normal" percentile

Graph 2. *Test for Normal Distribution of the σ descriptor values* (*SAS System*)

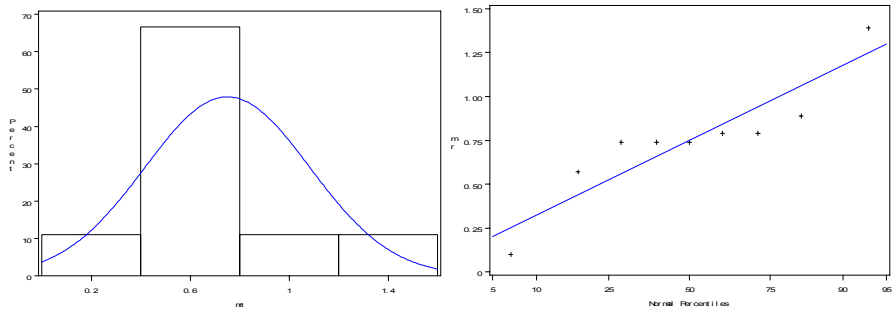
(*see appendix*).



π vs "Normal" percentile

Graph 3. *Test for Normal Distribution of the π descriptor values* (*SAS system*)

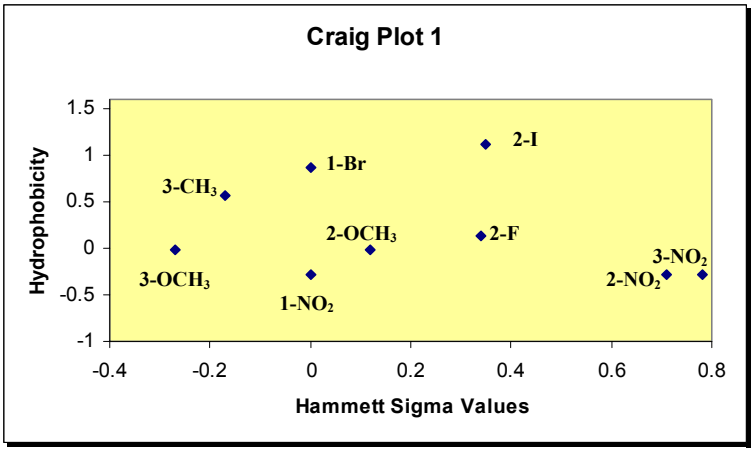
(*see appendix*).



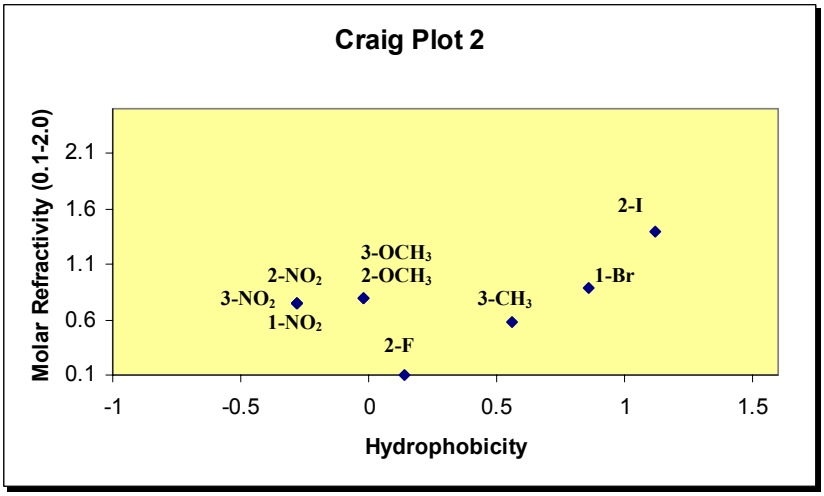
MR vs "Normal" percentile

Graph 4. *Test for Normal Distribution of the MR descriptor values* (*SAS system*)

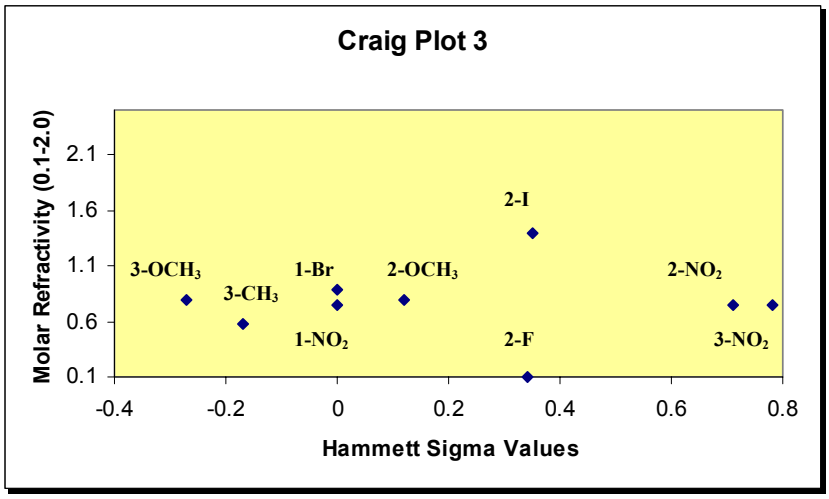
(*see appendix*).



Graph 5. *The "calibration set" dispersion in the hydrophobicity and Hammett electronic substituent constant dual parameter space.*



Graph 6. T*he "calibration set" dispersion in the molar refractivity and Hammett electronic substituent constant dual parameter space.*



Graph 7. *The "calibration set" dispersion in the molar refractivity and hydrophobicity dual parameter space.*

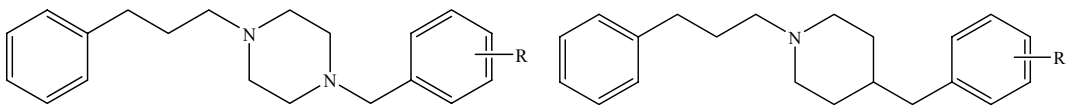
The results of all the previous tests showed that the compounds selected to build the correlation equation are statistically sound, and therefore, proceeding with this study is feasible. If the results were not statistically sound, additional compounds would have been added to the data, or descriptors may have been discarded, and subsequently replaced. The correlation equation will be established by first carrying out Multiple Linear Regression analysis. A principle component regression, partial least squares method, and non-linear methods (see experimental section) are to be considered if the MLR does not work.

Now that the specific features of the required compounds are known, synthesis of these compounds will be carried out first, followed by the determination of their binding affinity. Consequently, qualitative and quantitative structure-affinity relationships will be elucidated.

III.3 Synthesis schemes

The common feature in the design of the synthetic schemes for all four series is trying to use, as much as possible, the same schemes and reactions to synthesize all elements of a series as well as common intermediates.

III.3.1-*Series-1 and series-2*:

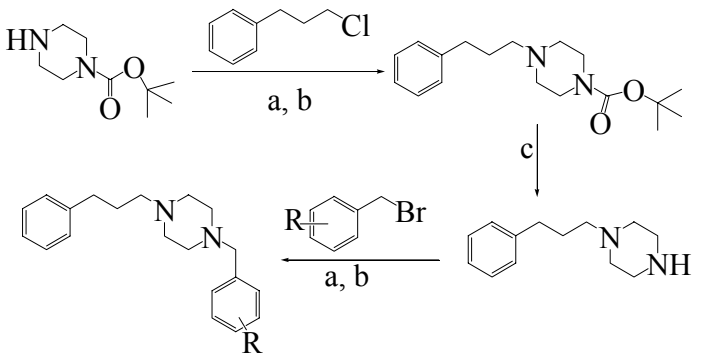


R= H, 2-Br, 2-NO_2, 3-I, 3-F, 3-OCH_3, 3-NO_3, 4-OCH_3, 4-NO_2, 4-CH_3

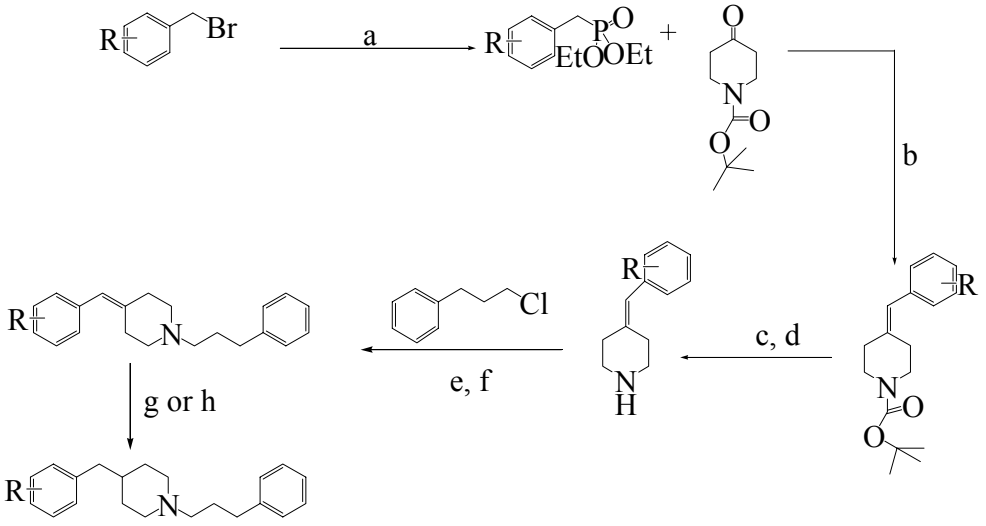
Figure 13. *Series-1 and series-2 compounds*.

Series-1 compounds are the benzyl substituted *N*-phenylpropyl-*N'*-benzylpiperazine derivatives, with substituents and substitution patterns based on the factorial design method. The compounds of that series are synthesized by a simple synthetic scheme (Figure 14) including the synthesis of a phenylpropyl piperazine as the final precursor yielding all analogs of the series when alkylated with the appropriate benzyl bromide substituted derivatives. Series-2 compounds are the benzyl substituted *N*-phenylpropyl-4-benzylpiperidine derivatives, with substituents and substitution patterns based on the factorial design method. The corresponding synthetic scheme in Figure 14 was assigned to make all the analogs of the series. All the steps are similar, including the Wittig-Horner reaction to alkylate the piperidine to the substituted benzyl bromide derivatives, followed by a straight forward alkylation of the corresponding benzyl piperidines with a

phenylpropyl chloride. The last step consists of reducing the double bond of the final precursor by catalytic hydrogenation under 1 atm with Pd on carbon (5%) as the catalyst, for all the analogs except the three nitro substituted ones, where a more selective catalytic hydrogenation was planned with a rhodium based catalyst.



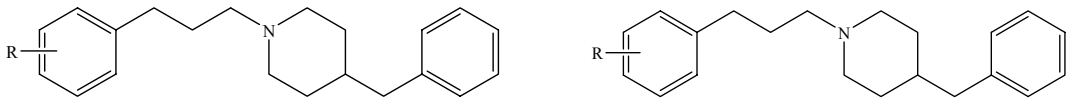
Reagents: (a) K_2CO_3; (b) NaI; (c) HCl



Reagents: (a) $(OEt)_3P$; (b) NaH; (c) TFA; (d) CH_2Cl_2; (e)NaI; (f) K_2CO_3;
(g) H_2/Pd-5%; (h) Wilkinson's catalyst

Figure 14. *Combined figure for synthetic schemes of series-1 and series-2 compounds*.

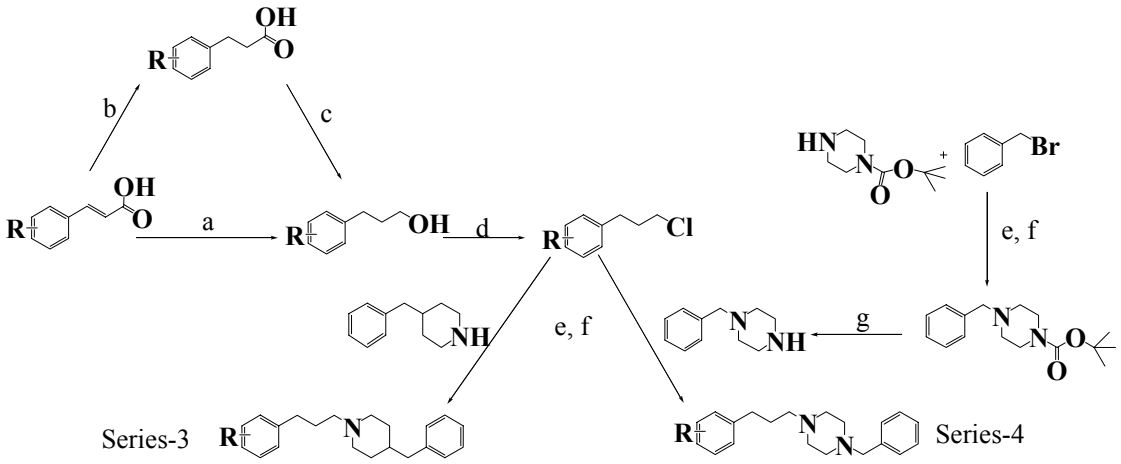
III.3.2-*Series-3 and series-4*:



R= H, 2-Br, 2-NO_2, 3-I, 3-F, 3-OCH_3, 3-NO_3, 4-OCH_3, 4-NO_2, 4-CH_3

Figure 15. *Series-3 and series-4 compounds*.

Series-3 and series-4 are respectively the phenylpropyl substituted *N*-phenylpropyl-4-benzylpiperidine and *N*-phenylpropyl-*N'*-piperazine derivatives with the same substituents and substituent pattern as series-1 and series-2. Synthesizing those compounds includes making the common precursor for both series: the substituted phenylpropyl chloride derivatives. The latter are synthesized from the corresponding substituted phenylpropyl alcohols, which result from the reduction of the corresponding cinnamic acid derivatives; with lithium aluminum hydride for the non-nitro substituted compounds (saving one step by reducing both the double bond and the carbonyl at once). A slightly different reduction was planned for the nitro substituted compounds; including reducing the double bond with the same rhodium based selective catalyst from series-3, followed by the reduction of the carbonyl with a mild reducing agent such as BH_3·THF (Figure 16).



Reagents: (a)LAH; (b) Wilkinson's catalyst; (c) BH_3·THF; (d) $SOCl_2$;(e) NaI; (f) K_2CO_3; (g) HCl

Figure 16. *Combined figure for synthetic schemes of series-3 and series-4*.

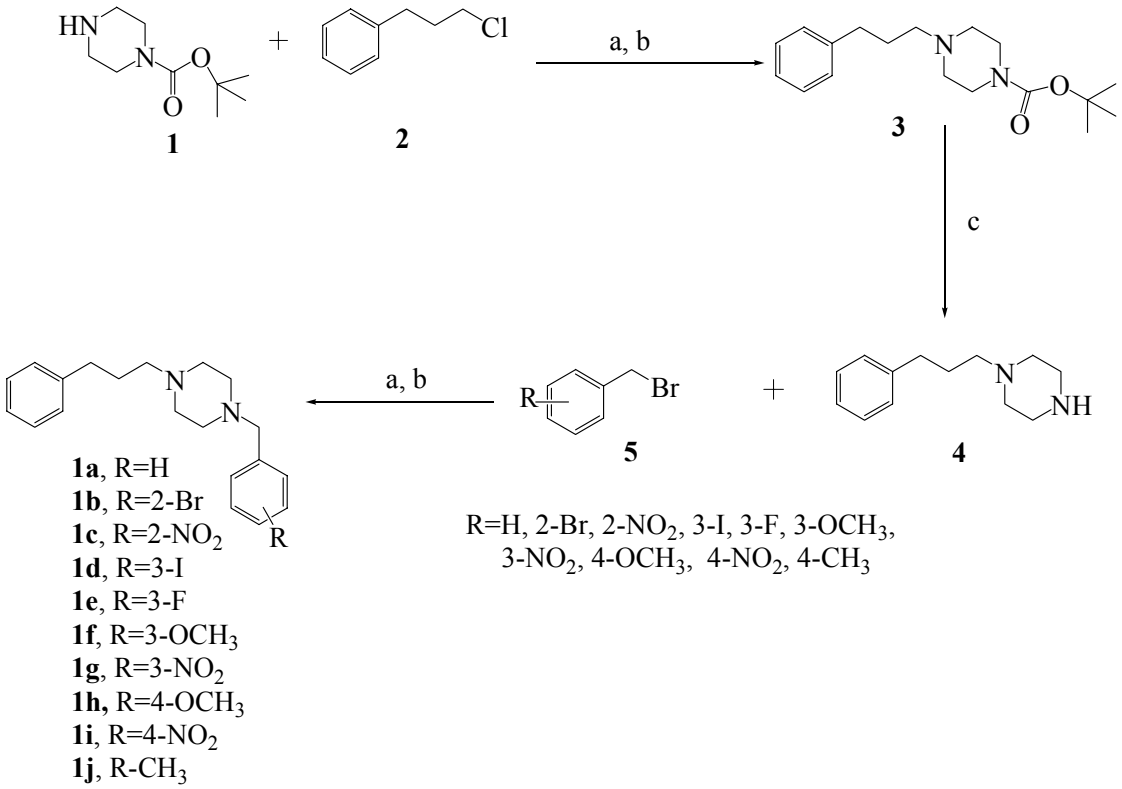
CHAPTER IV:

EXPERIMENTAL PROCEDURES

IV.1 Synthesis

Procedure: ^{1}H NMR spectra were determined on Bruker 250 or 300 MHz spectrometers. Chemical shifts are reported as parts per million (δ) relative to internal Me_4Si in $CDCl_3$, with coupling constants (*J*) given in Hertz (Hz). Elemental analyses were determined by Atlantic Microlab, Inc. (Norcross, GA), and were in agreement with calculated values (C, H, N: ± 0.4%) (see appendix). Short-path silica gel (Merck 7729, < 230 mesh) chromatography was conducted under N_2 pressure. Analytical TLC was performed with Macherey-Nagel silica gel 60 UV-254 plates (250 μm). Analytical reversed-phase HPLC was performed using a Symmetry C18 column (4.6 x 150 mm, 5 μm; Waters Corp., Milford, MA) and a ternary mobile phase of MeOH (25%), CH_3CN (25%), and water (50%) containing Et_3N (1.5%) and HOAc (2%) at a flow rate of 1 mL/min with detection at 254 nm. Other chemicals and solvents were the best grades available, and were used as received from commercial sources.

IV.1.1-*Series-1*:



Reagents: (a) K_2CO_3; (b) NaI; (c) HCl.

Figure 17. *Detailed synthetic scheme for series-1 compounds*.

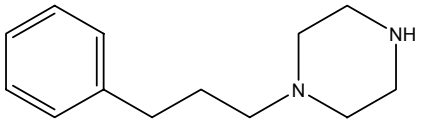
***N*-Phenylpropylpiperazine** was prepared as previously described.[99] *t*-Butyl-piperazine-1-carboxylate (compound **1**) (25 mmol, 4.64g), 3-phenyl-1-chloropropane (compound **2**) (25 mmol, 3.85g), potassium carbonate (75 mmol, 10.3g), and sodium iodide (25 mmol, 3.75g) were mixed in *N*,*N*-dimethylformamide (DMF) (100 mL) at 60 ^{o}C for 15 hours. The reaction mixture was filtered and evaporated to dryness under reduced pressure at 60 ^{o}C then diluted by water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated sodium chloride solution then dried over $MgSO_4$. Subsequently, it was filtered and the solvent evaporated to dryness. The residue was

purified by column chromatography using *n*-hexane/ethyl acetate (5/1, 4/1, 1/1, 1/2, 1/4, v/v) the fractions of *tert*-butyl 2-(4-(3-phenylpropyl)piperazin-1-yl)acetate (**3**) (judged by TLC) were combined and evaporated to dryness to give an oil (in yield of 92%) that had an R_f value on TLC of 0.4 in *n*-hexane/ethyl acetate (1/1, v/v).

4N HCl (40 mL) and 1,4-dioxane (200 mmol, 48 mL) were added to the oil of *tert*-butyl 2-(4-(3-phenylpropyl)piperazin-1-yl)acetate (compound **3**) (20 mmol, 6.08 g) at 0 ^{o}C. The mixture was diluted with methanol (40 mL), reacted at rt over night with stirring, and then evaporated to dryness under reduced pressure at 40 ^{o}C. The residue was diluted with 2 N NaOH, then extracted with ethyl acetate, dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography using chloroform/methanol (100/2, v/v), the fraction of *N*-phenylpropylpiperazine (compound **4**) was evaporated to dryness to give the oil in yield of 97% and an R_f value of 0.7 on TLC in chloroform/methanol (100/2, v/v).

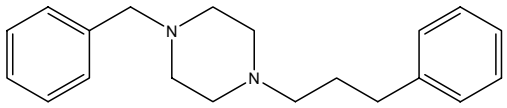
General Method for the Preparation of Compounds 1a – 1j (Figure 17). The appropriate benzyl bromide (1.0 mmol), N-phenylpropylpiperazine (1.0 mmol), NaI (150 mg, 1.0 mmol) and K_2CO_3 (0.41 g, 3.0 mmol) were heated in DMF (10 mL) for 2 h at 60 °C. The mixture was filtered, and then concentrated under reduced pressure at 60 °C. The residue was partitioned between water and EtOAc, and the organic layer washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography using a gradient of n-hexane:EtOAc (5:1 to 1:4) gave the target compounds in 70-88% yields as colorless to pale yellow oils that were stored in their free base forms. Analytical TLC R_f values ranged from 0.2 to 0.4 (1:1 *n*-hexane:EtOAc). ^{1}H NMR and elemental

analysis data agreed with the assigned structures. Analytical reversed-phase HPLC showed 98% purity for each compound and provided retention times (t_R) and capacity factors (k'). All compounds displayed appropriate ^{1}H NMR (300 MHz and 250 MHz) spectral data.



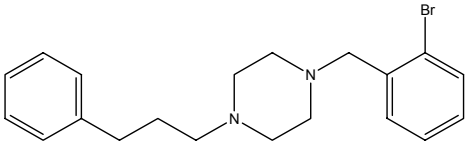
N-phenylpropyl piperazine

N-phenylpropylpiperazine (4): ^{1}H NMR $CDCl_3$ δ 1.72 (2H, dt, J= 7.5 Hz, 7.6 Hz, -CH_2-C$\underline{H_2}$-CH_2-), 2.15 (1H, s, -NH-), 2.22-2.28 (6H, m, -CH_2-C$\underline{H_2}$-(N-C$\underline{H_2}$-)-C$\underline{H_2}$-CH_2-), 2.53 (2H, t, J= 7.5 Hz, -CH_2-C$\underline{H_2}$-Ph), 2.77 (4H, t, J= 7.6 Hz, -CH_2-C$\underline{H_2}$-NH-C$\underline{H_2}$-CH_2), 6.90-7.27 (5H, m, Ph), in yield of 97%, kept in the free base form.



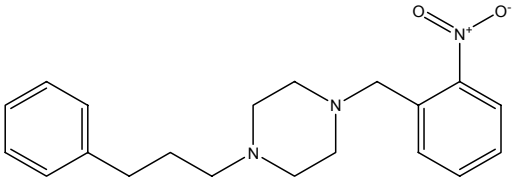
N-Phenylpropyl-N'-piperazine

***N*-phenylpropyl-*N'*-piperazine (1a)**: ^{1}H NMR $CDCl_3$ δ 1.85 (2H, dt, J= 7.5 Hz, 7.6 Hz, -CH_2-C$\underline{H_2}$-CH_2-), 2.42 (2H, t, J= 7.6 Hz, -CH_2-C$\underline{H_2}$-Ph), 2.53 (8H, m, Pip), 2.67 (2H, t, J= 7.5 Hz, -N-C$\underline{H_2}$-CH_2-), 3.56 (2H, s, Ph-C$\underline{H_2}$-N-), 7.19-7.38 (10H, m, Ph), and an HPLC retention time of 5.1 min and a capacity factor of 2, in yield of 78%, kept in the free base form.



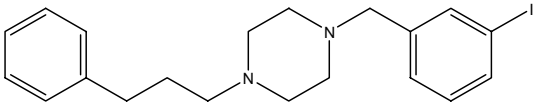
1-(2-Bromobenzyl)-4-(3-phenylpropyl)piperazine

1-(2-Bromobenzyl)-4-(3-phenylpropyl)piperazine (1**b**): ^{1}H NMR $CDCl_3$ δ 1.85 (2H, dt, J= 7.2 Hz, 7.5 Hz, -CH_2-C$\underline{H}_2$-CH_2-),2.4 (2H, t, J= 7.2 Hz, -CH_2-C$\underline{H}_2$-Ph), 2.52 (4H, m, Pip), 2.6 (4H, m, Pip), 2.67 (2H, t, J= 7.5 Hz, -N-C$\underline{H}_2$-CH_2-), 3.64 (2H, s, Ph-C$\underline{H}_2$-N-), 7.09-7.55 (8H, m, Ph), 7.57 (1H, d, CHar, J= 9 Hz) and an HPLC retention time of 9.3 min and a capacity factor of 4.8, in yield of 70%, kept in the free base form.



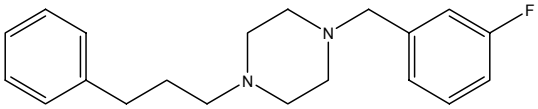
1-(2-Nitrobenzyl)-4-(3-phenylpropyl)piperazine

1-(2-Nitrobenzyl)-4-(3-phenylpropyl)piperazine (1c): ^{1}H NMR $CDCl_3$ δ 1.75 (2H, dt, J= 7.5 Hz, 7.6 Hz-CH_2-C$\underline{H}_2$-CH_2-), 2.35 (2H, t, J= 7.5 Hz, -CH_2-C$\underline{H}_2$-Ph), 2.47 (8H, m, Pip), 2.65 (2H, t, J= 7.6 Hz, -N-C$\underline{H}_2$-CH_2-), 3.8 (2H, s, Ph-C$\underline{H}_2$-N-), 7.15-7.79 (8H, m, Ph), 7.82 (1H, d, CHar, J= 12.5 Hz) and an HPLC retention time of 6.1 min and a capacity factor of 2.6, in yield of 83%, kept in the free base form.



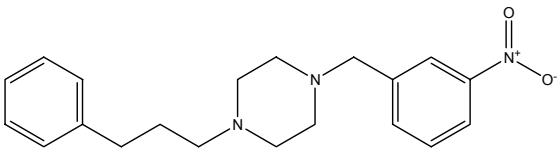
1-(3-Iodobenzyl)-4-(3-phenylpropyl)piperazine

1-(3-Iodobenzyl)-4-(3-phenylpropyl)piperazine (1d): ^{1}H NMR $CDCl_3$ δ 1.85 (2H, dt, J= 7.5 Hz, 7.6 Hz, -CH_2-C$\underline{H}_2$-CH_2-), 2.4 (2H, t, J= 7.5Hz, -CH_2-C$\underline{H}_2$-Ph), 2.49 (8H, m, Pip), 2.65 (2H, t, J=7.6 Hz, -N-C$\underline{H}_2$-CH_2-), 3.46 (2H, s, Ph-C$\underline{H}_2$-N-), 7.02-7.31 (7H, m, Ph), 7.60 (1H, d, J= 7.8 Hz, CHar), 7.71 (1H, s, CHar) and an HPLC retention time of 13.1 min and a capacity factor of 6.7, in yield of 76%, kept in the free base form.



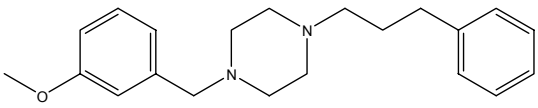
1-(3-Fluorobenzyl)-4-(3-phenylpropyl)piperazine

1-(3-Fluorobenzyl)-4-(3-phenylpropyl)piperazine (1e): ^{1}H NMR $CDCl_3$ δ 1.86 (2H, dt, J= 7.5 Hz, 7.7 Hz, -CH_2-C<u>*H*</u>$_2$-CH_2-), 2.42 (2H, t, J= 7.5 Hz, -CH_2-C<u>*H*</u>$_2$-Ph), 2.52 (8H, m, Pip), 2.69 (2H, t, J= 7.7 Hz, -N-C<u>*H*</u>$_2$-CH_2-), 3.59 (2H, s, Ph-C<u>*H*</u>$_2$-N-), 6.95-7.36 (9H, m, Ph), and an HPLC retention time of 5.9 min and a capacity factor of 2.5, in yield of 88%, kept in the free base form.



1-(3-nitrobenzyl)-4-(3-phenylpropyl)piperazine

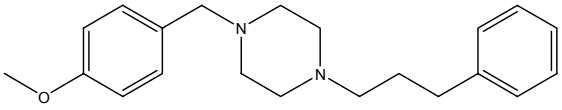
1-(3-Nitrobenzyl)-4-(3-phenylpropyl)piperazine (1f) : ^{1}H NMR $CDCl_3$ δ 1.81 (2H, dt, J= 7.5 Hz, 7.8 Hz, -CH_2-C<u>*H*</u>$_2$-CH_2-), 2.39 (2H, t, J= 7.5 Hz, -CH_2-C<u>*H*</u>$_2$-Ph), 2.50 (8H, m, Pip), 2.64 (2H, t, J= 7.8 Hz, -N-C<u>*H*</u>$_2$-CH_2-), 3.6 (2H, s, Ph-C<u>*H*</u>$_2$-N-), 7.16-7.68 (7H, m, Ph), 8.12 (1H, d, J= 9.6 Hz, CHar), 8.21 (1H, s, CHar) and an HPLC retention time of 5.6 min and a capacity factor of 2.3, in yield of 70%, kept in the free base form.



1-(3-Methoxybenzyl)-4-(3-phenylpropyl)piperazine

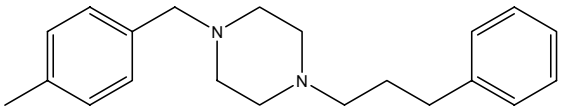
1-(3-Methoxybenzyl)-4-(3-phenylpropyl)piperazine (1g): ^{1}H NMR $CDCl_3$ δ 1.85 (2H, dt, J= 7.25 Hz, 7.5 Hz-CH_2-C<u>*H*</u>$_2$-CH_2-), 2.4 (2H, t, J= 7.25 Hz, -CH_2-C<u>*H*</u>$_2$-Ph), 2.51 (8H, m, Pip), 2.63 (2H, t, J= 7.5 Hz, -N-C<u>*H*</u>$_2$-CH_2-), 3.51 (2H, s, Ph-C<u>*H*</u>$_2$-N-), 3.83 (3H, s, Ph-

OC<u>H</u>$_3$), 6.78-7.34 (9H, m, Ph), and an HPLC retention time of 5.2 min and a capacity factor of 2.05, in yield of 84%, kept in the free base form.



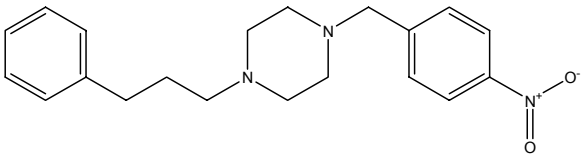
1-(4-Methoxybenzyl)-4-(3-phenylpropyl)piperazine

1-(4-Methoxybenzyl)-4-(3-phenylpropyl)piperazine (1h): ^{1}H NMR $CDCl_3$ δ 1.85 (2H, dt, J= 7.5 Hz, 7.8 Hz, -CH_2-C<u>H</u>$_2$-CH_2-), 2.39 (2H, t, J= 7.8 Hz, -CH_2-C<u>H</u>$_2$-Ph), 2.48 (8H, m, Pip), 2.64 (2H, t, J= 7.5 Hz, -N-C<u>H</u>$_2$-CH_2-), 3.46 (2H, s, Ph-C<u>H</u>$_2$-N-), 3.81 (3H, s, Ph-OC<u>H</u>$_3$), 6.78-7.31 (9H, m, Ph), and an HPLC retention time of 5.0 min and a capacity factor of 1.9, in yield of 84%, kept in the free base form.



1-(4-Methylbenzyl)-4-(3-phenylpropyl)piperazine

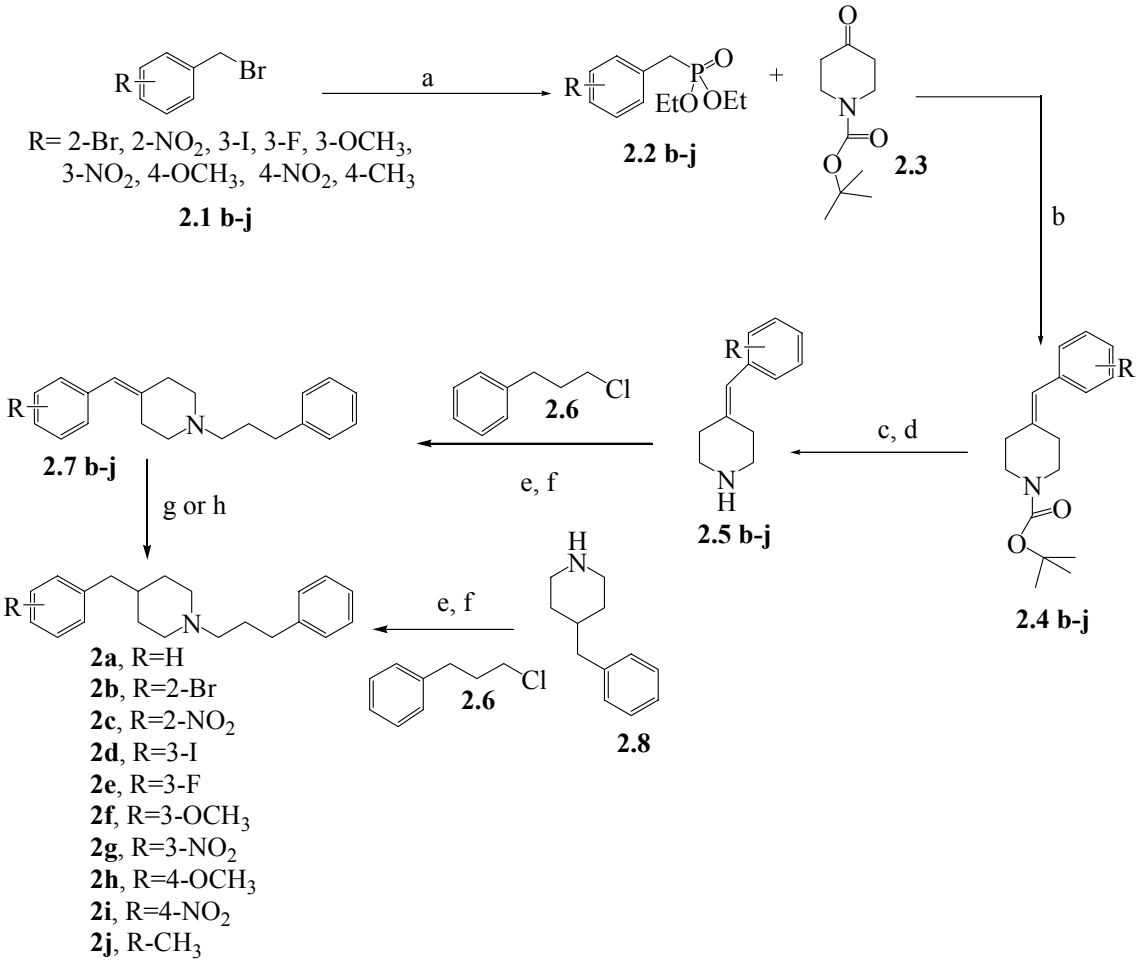
1-(4-Methylbenzyl)-4-(3-phenylpropyl)piperazine (1i): ^{1}H NMR $CDCl_3$ δ 1.85 (2H, dt, J= 7.2 Hz, 7.5 Hz, -CH_2-C<u>H</u>$_2$-CH_2-), 2.35 (2H, s, Ph-C<u>H</u>$_3$), 2.39 (2H, t, J= 7.2 Hz, -CH_2-C<u>H</u>$_2$-Ph), 2.65 (2H, t, J= 7.5Hz, N-C<u>H</u>$_2$-CH_2-), 3.49 (2H, s, Ph-C<u>H</u>$_2$-N), 7.12-7.29 (9H, m, Ph), and an HPLC retention time of 5.6 min and a capacity factor of 2.3, in yield of 82%, kept in the free base form.



1-(4-Nitrobenzyl)-4-(3-phenylpropyl)piperazine

1-(4-Nitrobenzyl)-4-(3-phenylpropyl)piperazine (1j): ^{1}H NMR $CDCl_3$ δ 1.84 (2H, dt, J= 7.2 Hz, 7.5 Hz, -CH_2-C*H_2*-CH_2-), 2.37 (2H, t, J= 7.2 Hz -CH_2-C*H_2*-Ph), 2.5 (8H, m, Pip), 2.67 (2H, t, J= 7.5 Hz, -N-C*H_2*-CH_2-), 3.6 (2H, s, Ph-C*H_2*-N-) 7.2-7.31 (5H, m, Ph), 7.52 (2H, d, J= 8.7 Hz, CHar), 8.18 (2H, d, J= 8.7 Hz, CHar) and an HPLC retention time of 6.5 min and a capacity factor of 2.8, in yield of 70%, kept in the free base form.

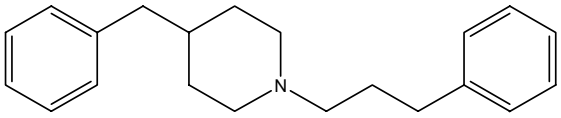
IV.1.2-*Series-2*:



Reagents: (a) $(OEt)_3P$; (b) NaH; (c) TFA; (d) CH_2Cl_2; (e)NaI; (f) K_2CO_3; (g) H_2/Pd-5%; (h) Wilkinson's catalyst

Figure 18. *Detailed synthetic scheme for series-2 compounds*.

Compound 2a. The commercially available 4-benzyl piperidine **2.8** (10 mmol, 1.75 g), 1-phenylpropyl chloride (10 mmol, 3.39 g), NaI (1.5 g, 10 mmol) and K_2CO_3 (4.1 g, 30 mmol) were heated in DMF (100 mL) overnight at 65 °C. The mixture was filtered, and then concentrated under reduced pressure at 60 °C. The residue was partitioned between water and EtOAc, and the organic layer washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography using a gradient of n-hexane:EtOAc (5:1 to 1:4) gave the target compounds in a 94% yield as colorless to pale yellow oil that was converted to the hydrochloric acid salt by dissolving it in ether and saturating the solution with HCl gas, followed by filtration of the precipitate and washing several times with ether. ^{1}H NMR and elemental analysis data agreed with the assigned structures. Analytical reversed-phase HPLC showed 99% purity. It also displayed appropriate ^{1}H NMR (300 MHz) spectral data.

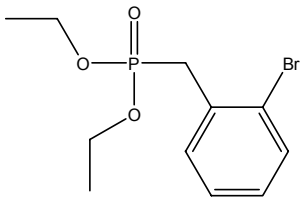


Benzyl-4-(3-phenylpropyl)piperidine

***N*-Phenylpropyl-4-benzylpiperidine (2a)**: ^{1}H NMR $CDCl_3$ δ 1.37-1.99 (7H, m, -C*H*-(C*H*$_2$-)$_2$-(CH$_2$-)$_2$-N-CH$_2$-C*H*$_2$-CH$_2$-), 2.42-3.02 (10H, m, Ph-C*H*$_2$-CH-(CH$_2$-)$_2$-(C*H*$_2$-)$_2$-N-C*H*$_2$-CH$_2$-C*H*$_2$-), 7.23-7.40 (10H, m, Ph), and an HPLC retention time of 6.5 min and a capacity factor of 2.8, in yield of 99.7%, kept in its acidic salt form.

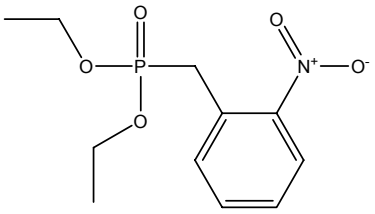
General method for the preparation of compounds 2.2 b-j (Figure 18). The appropriate benzyl bromide derivatives **2.1 b-j** (10 mmol) were heated with triethyl phosphite (1.66 g, 10 mmol) at 130-150 °C for 2 h under nitrogen according to slightly

modified, previously reported procedures.[122-124] The solution was extracted with chloroform, washed with water, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was purified on a column of silica gel with 1:1 *n*-hexane:EtOAc which gave the target compounds in 70%-91% yields as light brown oils. Analytical TLC R_f values ranged from 0.2-0.4 (1:1 *n*-hexane:EtOAc). Data agreed with the assigned structures.



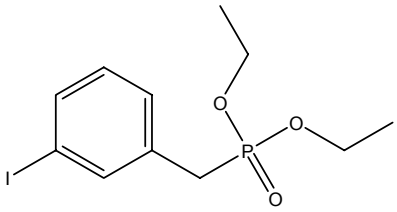
Diethyl 2-bromobenzylphosphonate

Diethyl 2-bromobenzylphosphonate (2.2 b): ^{1}H NMR $CDCl_3$ δ 1.16-1.22 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 3.19 (1H, s, Ph-(CH)$\underline{H}$-), 3.27 (1H, s, Ph-(CH)$\underline{H}$-), 3.93-4.04 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 7.03-7.50 (4H, m, Ph), in yield of 83%.



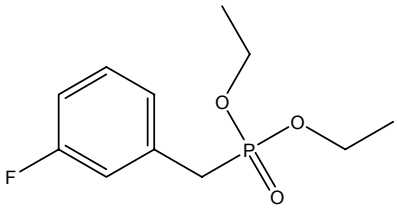
Diethyl 2-nitrobenzylphosphonate

Diethyl 2-nitrobenzylphosphonate (2.2 c): ^{1}H NMR $CDCl_3$ δ 1.04-1.08 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 3.50 (1H, s, Ph-(CH)$\underline{H}$-), 3.58 (1H, s, Ph-(CH)$\underline{H}$-), 3.81-3.95 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 7.23-7.79 (4H, m, Ph), in yield of 85%.



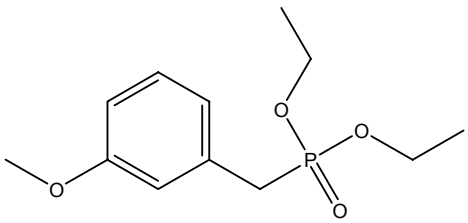
Diethyl 3-iodobenzylphosphonate

Diethyl 3-iodobenzylphosphonate (2.2 d): ^{1}H NMR $CDCl_3$ δ 0.95-1.00 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 2.77 (1H, s, Ph-(CH)$\underline{H}$-), 2.84 (1H, s, Ph-(CH)$\underline{H}$-), 3.70-3.80 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 6.73-7.38 (4H, m, Ph), in yield of 86%.



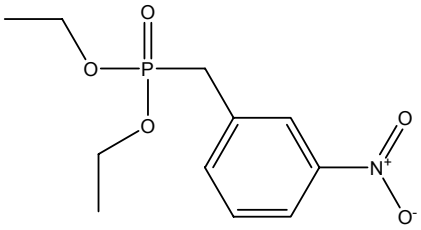
Diethyl 3-fluorobenzylphosphonate

Diethyl 3-fluorobenzylphosphonate (2.2 e): ^{1}H NMR $CDCl_3$ δ 1.14-1.21 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 3.04 (1H, s, Ph-(CH)$\underline{H}$-), 3.11 (1H, s, Ph-(CH)$\underline{H}$-), 3.92-4.01 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 6.84-7.24 (4H, m, Ph), in yield of 89%.



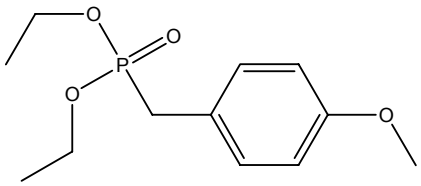
Diethyl 3-methoxybenzylphosphonate

Diethyl 3-methoxybenzylphosphonate (2.2 f): ^{1}H NMR $CDCl_3$ δ 1.16-1.24 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 3.06 (1H, s, Ph-(CH)$\underline{H}$-), 3.13 (1H, s, Ph-(CH)$\underline{H}$-), 3.73 (3H, s, -OC$\underline{H}_3$), 3.93-4.04 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 6.74-7.21 (4H, m, Ph), in yield of 70%.



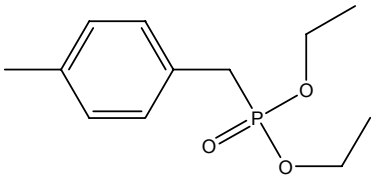
Diethyl 3-nitrobenzylphosphonate

Diethyl 3-nitrobenzylphosphonate (2.2 g): ^{1}H NMR $CDCl_3$ δ 1.20-1.25 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 3.17 (1H, s, Ph-(CH)$\underline{H}$-), 3.24 (1H, s, Ph-(CH)$\underline{H}$-), 3.97-4.07 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 7.27-8.12 (4H, m, Ph), in yield of 80%.



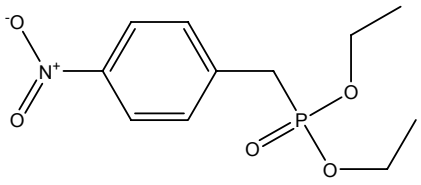
Diethyl 4-methoxybenzylphosphonate

Diethyl 4-methoxybenzylphosphonate (2.2 h): ^{1}H NMR $CDCl_3$ δ 0.74-0.82 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 2.62 (1H, s, Ph-(CH)$\underline{H}$-), 2.69 (1H, s, Ph-(CH)$\underline{H}$-), 3.32 (3H, s, -OC$\underline{H}_3$), 3.48-3.62 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 6.37-6.81 (4H, m, Ph), in yield of 86%.



Diethyl 4-methylbenzylphosphonate

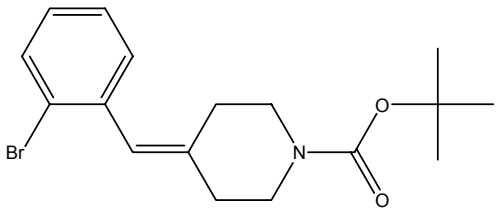
Diethyl 4-methylbenzylphosphonate (2.2 i): ^{1}H NMR $CDCl_3$ δ 1.12-1.19 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 2.23 (3H, s, -C$\underline{H}_3$), 2.99 (1H, s, Ph-(CH)$\underline{H}$-), 3.06 (1H, s, Ph-(CH)$\underline{H}$-), 3.98-3.38 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 7.02-7.13 (4H, m, Ph), in yield of 88%.



Diethyl 4-nitrobenzylphosphonate

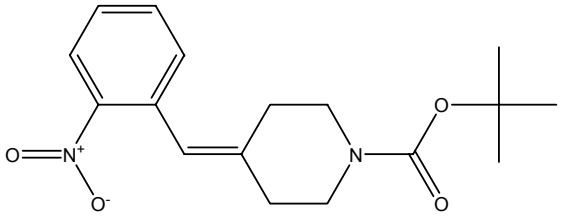
Diethyl 4-nitrobenzylphosphonate (2.2 j): ^{1}H NMR $CDCl_3$ δ 1.13-1.17 (6H, m,-(O-CH_2-C$\underline{H}_3$)$_2$), 3.12 (1H, s, Ph-(CH)$\underline{H}$-), 3.19 (1H, s, Ph-(CH)$\underline{H}$-), 3.90-4.00 (4H, m,-(O-C$\underline{H}_2$-CH_3)$_2$), 7.36-8.06 (4H, m, Ph), in yield of 91%.

General method for the preparation of compounds 2.4 b-j (Figure 18). Wittig-Horner-Emmons reaction was used to prepare compounds **2.4 b-j** according to Mavunkel and co-workers.[125] The substituted arylphosphonate derivatives **2.2 b-j** (3 mmol) and 60% dispersion of NaH in mineral oil were placed in a dry RBF and dissolved in 10 mL of dry THF. To the solution was added *N*-4-BOC-piperidone **2.3** (0.59g, 3 mmol) and refluxed for 3 h under nitrogen. The mixture was concentrated under reduced pressure at 60 °C. The residue was partitioned between water and DCM, and the organic layer washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography using a gradient of n-hexane:EtOAc (1:0 to 4:1) gave the target compounds in 15-41% yields as light brown oils. Analytical TLC R_f values ranged from 0.1 to 0.3 (1:1 *n*-hexane:EtOAc). ^{1}H NMR data agreed with the assigned structures.



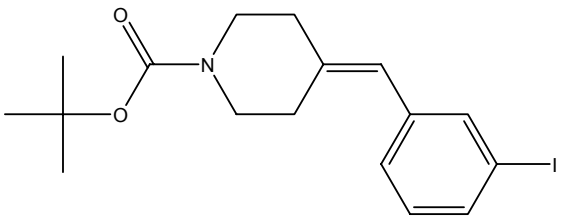
t-Butyl 4-(2-bromobenzylidene)piperidine-1-carboxylate

***t*-Butyl 4-(2-bromobenzylidene)piperidine-1-carboxylate (2.4 b)**: ^{1}H NMR $CDCl_3$ δ 1.46 (9H, s, -C(C<u>*H*</u>$_3$)$_3$), 2.27 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.35 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 3.39 (2H, t, J= 6 Hz, -CH$_2$-N-C<u>*H*</u>$_2$), 3.52 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH$_2$), 6.29 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 7.07-7.57 (4H, m, Ph), in yield of 36%.



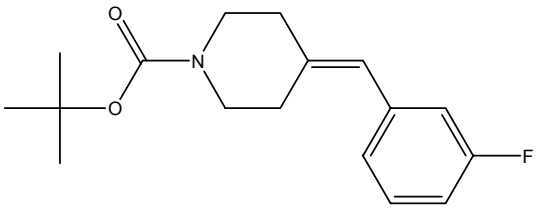
t-Butyl 4-(2-nitrobenzylidene)piperidine-1-carboxylate

***t*-Butyl 4-(2-nitrobenzylidene)piperidine-1-carboxylate (2.4 c)**: ^{1}H NMR $CDCl_3$ δ 1.46 (9H, s, -C(C<u>*H*</u>$_3$)$_3$), 2.18 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.34 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 3.35 (2H, t, J= 6 Hz, -CH$_2$-N-C<u>*H*</u>$_2$), 3.51 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH$_2$), 6.56 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 7.24-7.97 (4H, m, Ph), in yield of 15%.



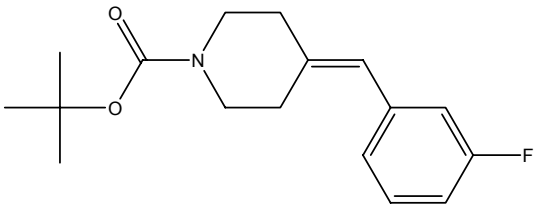
t-Butyl 4-(3-iodobenzylidene)piperidine-1-carboxylate

***t*-Butyl 4-(3-iodobenzylidene)piperidine-1-carboxylate (2.4 d)**: ^{1}H NMR $CDCl_3$ δ 1.48 (9H, s, -C(C<u>*H*</u>$_3$)$_3$), 2.32 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.42 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 3.40 (2H, t, J= 6 Hz, -CH$_2$-N-C<u>*H*</u>$_2$), 3.50 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH$_2$), 6.26 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 7.04-7.55 (4H, m, Ph), in yield of 41%.



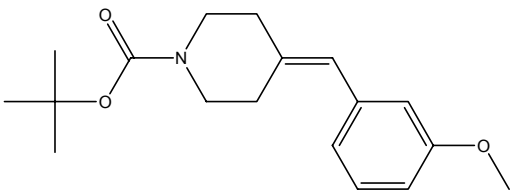
t-Butyl 4-(3-fluorobenzylidene)piperidine-1-carboxylate

***t*-Butyl 4-(3-fluorobenzylidene)piperidine-1-carboxylate (2.4 e)**: ^{1}H NMR $CDCl_3$ δ 1.48 (9H, s, -C(C<u>*H*</u>$_3$)$_3$), 2.34 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.45 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 3.42 (2H, t, J= 6 Hz, -CH$_2$-N-C<u>*H*</u>$_2$), 3.52 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH$_2$), 6.32 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 6.88-7.27 (4H, m, Ph), in yield of 34%.



t-Butyl 4-(3-fluorobenzylidene)piperidine-1-carboxylate

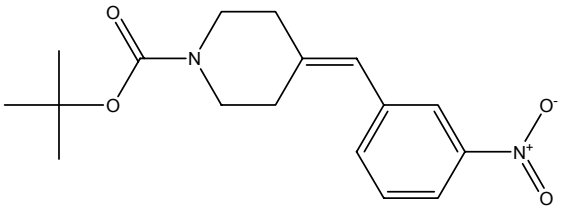
***t*-Butyl 4-(3-fluorobenzylidene)piperidine-1-carboxylate (2.4 e)**: ^{1}H NMR $CDCl_3$ δ 1.48 (9H, s, -C(C<u>*H*</u>$_3$)$_3$), 2.34 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.45 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 3.42 (2H, t, J= 6 Hz, -CH$_2$-N-C<u>*H*</u>$_2$), 3.52 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH$_2$), 6.32 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 6.88-7.27 (4H, m, Ph), in yield of 34%.



t-Butyl 4-(3-methoxybenzylidene)piperidine-1-carboxylate

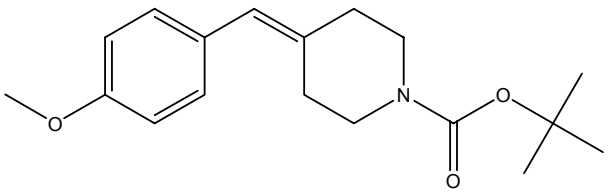
***t*-Butyl 4-(3-methoxybenzylidene)piperidine-1-carboxylate (2.4 f)**: ^{1}H NMR $CDCl_3$ δ 1.48 (9H, s, -C(C<u>*H*</u>$_3$)$_3$), 2.32 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.47 (2H, t, J= 6 Hz, -

CH=C(C$\underline{H}_2$)$_2$-), 3.40 (2H, t, J= 6 Hz, -CH$_2$-N-C$\underline{H}_2$), 3.51 (2H, t, J= 6Hz, -C$\underline{H}_2$-N-CH$_2$), 3.79 (3H, s, -OC$\underline{H}_3$), 6.33 (1H, s,-(Ph-C$\underline{H}$=C(CH$_2$)2-), 6.74-7.25 (4H, m, Ph), in yield of 22%.



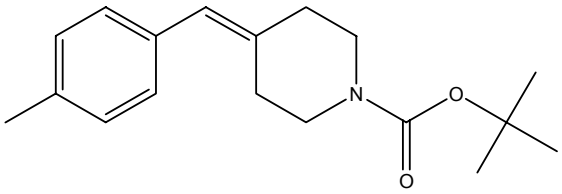
t-Butyl 4-(3-nitrobenzylidene)piperidine-1-carboxylate

***t*-Butyl 4-(3-nitrobenzylidene)piperidine-1-carboxylate (2.4 g)**: ^{1}H NMR CDCl$_3$ δ 1.47 (9H, s, -C(C$\underline{H}_3$)$_3$), 2.37 (2H, t, J= 6 Hz, -CH=C(C$\underline{H}_2$)$_2$-), 2.44 (2H, t, J= 6 Hz, -CH=C(C$\underline{H}_2$)$_2$-), 3.42 (2H, t, J= 6 Hz, -CH$_2$-N-C$\underline{H}_2$), 3.51 (2H, t, J= 6Hz, -C$\underline{H}_2$-N-CH$_2$), 6.38 (1H, s,-(Ph-C$\underline{H}$=C(CH$_2$)2-), 7.47-8.05 (4H, m, Ph), in yield of 16%.



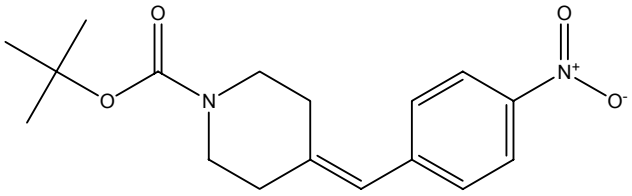
t-Butyl 4-(4-methoxybenzylidene)piperidine-1-carboxylate

***t*-Butyl 4-(4-methoxybenzylidene)piperidine-1-carboxylate (2.4 h)**: ^{1}H NMR CDCl$_3$ δ 1.48 (9H, s, -C(C$\underline{H}_3$)$_3$), 2.32 (2H, t, J= 6 Hz, -CH=C(C$\underline{H}_2$)$_2$-), 2.32 (2H, t, J= 6 Hz, -CH=C(C$\underline{H}_2$)$_2$-), 2.46 (2H, t, J= 6 Hz, -CH$_2$-N-C$\underline{H}_2$), 3.41 (2H, t, J= 6Hz, -C$\underline{H}_2$-N-CH$_2$), 3.50 (3H, s, -OC$\underline{H}_3$), 3.80 (3H, s, -OC$\underline{H}_3$), 6.30 (1H, s,-(Ph-C$\underline{H}$=C(CH$_2$)2-), 6.85-7.15 (4H, m, Ph), in yield of 18%.



t-Butyl 4-(4-methylbenzylidene)piperidine-1-carboxylate

***t*-Butyl 4-(4-methylbenzylidene)piperidine-1-carboxylate (2.4 i)**: ^{1}H NMR $CDCl_3$ δ 1.48 (9H, s, -C(C<u>*H*</u>$_3$)$_3$), 2.33 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.35 (3H, s, -C<u>*H*</u>$_3$), 2.47 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 3.41 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH$_2$), 3.51 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH$_2$), 6.34 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 7.08-7.52 (4H, m, Ph), in yield of 18%.

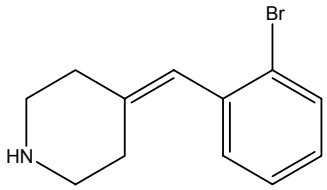


t-Butyl 4-(4-nitrobenzylidene)piperidine-1-carboxylate

***t*-Butyl 4-(4-nitrobenzylidene)piperidine-1-carboxylate (2.4 i)**: ^{1}H NMR $CDCl_3$ δ 1.49 (9H, s, -C(C<u>*H*</u>$_3$)$_3$), 2.38 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.47 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 3.44 (2H, t, J= 6 Hz, -CH$_2$-N-C<u>*H*</u>$_2$), 3.54 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH$_2$), 6.41 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 7.32-8.20 (4H, m, Ph), in yield of 23%.

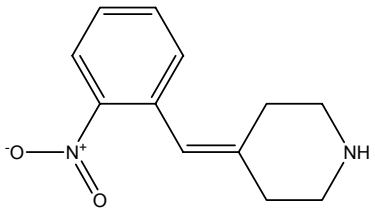
General method for the preparation of compounds 2.5 b-j (Figure 18). Deprotection of compounds **2.4 b-j** (0.5 mmol) was carried out by stirring in 20 mL 1:1 dichloromethane-trifluoroacetic acid for 1 h. It was evaporated and dried under reduced pressure to remove all traces of TFA. It was partitioned between 2.5 M NaOH and DCM, and the organic layer washed with brine, dried ($MgSO_4$) and concentrated under reduced

pressure and gave the compounds in yield of 73-89% as pale oils. ^{1}H NMR data agreed with the assigned structures.



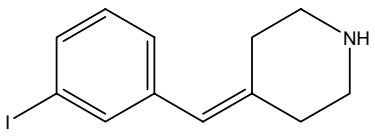
4-(2-Bromobenzylidene)piperidine

4-(2-Bromobenzylidene)piperidine (2.5 b): ^{1}H NMR $CDCl_3$ δ 1.26 (1H, s, -N*H*), 2.37 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.45 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.93 (2H, t, J= 6 Hz, -CH_2-N-C*H*$_2$), 3.07 (2H, t, J= 6Hz, -C*H*$_2$-N-CH_2), 6.29 (1H, s,-(Ph-C*H*=C(CH_2)2-), 7.07-7.59 (4H, m, Ph), in yield of 89%.



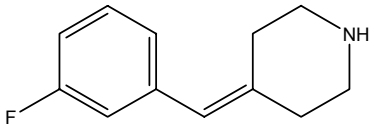
4-(2-Nitrobenzylidene)piperidine

4-(2-Nitrobenzylidene)piperidine (2.5 c): ^{1}H NMR $CDCl_3$ δ 1.88 (1H, s, -N*H*), 2.20 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.36 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.82 (2H, t, J= 6 Hz, -CH_2-N-C*H*$_2$), 2.97 (2H, t, J= 6Hz, -C*H*$_2$-N-CH_2), 6.50 (1H, s,-(Ph-C*H*=C(CH_2)2-), 7.30-7.97 (4H, m, Ph), in yield of 76% as brown oils.



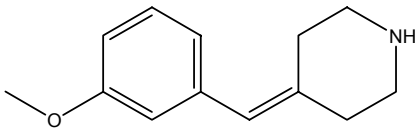
4-(3-Iodobenzylidene)piperidine

4-(3-Iodobenzylidene)piperidine (2.5 d): ^{1}H NMR $CDCl_3$ δ 1.69 (1H, s, -N*H*), 2.32 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.42 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 3.41 (2H, t, J= 6 Hz, -CH_2-N-C*H*$_2$), 3.51 (2H, t, J= 6Hz, -C*H*$_2$-N-CH_2), 6.27 (1H, s,-(Ph-C*H*=C(CH_2)2-), 7.05-7.55 (4H, m, Ph), in yield of 77%.



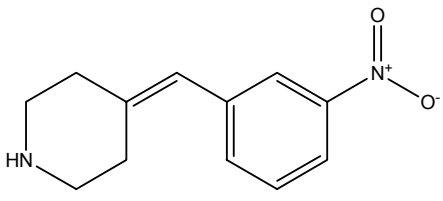
4-(3-Fluorobenzylidene)piperidine

4-(3-Fluorobenzylidene)piperidine (2.5 e): ^{1}H NMR $CDCl_3$ δ 1.67 (1H, s, -N*H*), 2.33 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.45 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.86 (2H, t, J= 6 Hz, -CH_2-N-C*H*$_2$), 2.97 (2H, t, J= 6Hz, -C*H*$_2$-N-CH_2), 6.25 (1H, s,-(Ph-C*H*=C(CH_2)2-), 6.87-7.30 (4H, m, Ph), in yield of 87%.



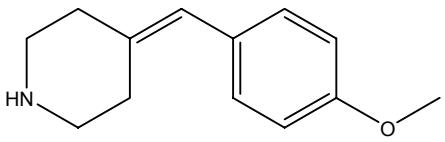
4-(3-Methoxybenzylidene)piperidine

4-(3-Methoxybenzylidene)piperidine (2.5 f): ^{1}H NMR $CDCl_3$ δ 1.72 (1H, s, -N*H*), 2.32 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.47 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.85 (2H, t, J= 6 Hz, -CH_2-N-C*H*$_2$), 2.96 (2H, t, J= 6Hz, -C*H*$_2$-N-CH_2), 3.80 (3H, s, -OC*H*$_3$), 6.26 (1H, s,-(Ph-C*H*=C(CH_2)2-), 6.74-7.27 (4H, m, Ph), in yield of 80%.



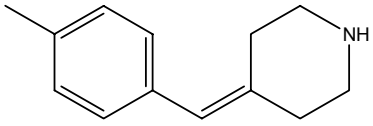
4-(3-Nitrobenzylidene)piperidine

4-(3-Nitrobenzylidene)piperidine (2.5 g): ^{1}H NMR $CDCl_3$ δ 1.71 (1H, s, -N*H*), 2.36 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.43 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.87 (2H, t, J= 6 Hz, -CH_2-N-C*H*$_2$), 3.13 (2H, t, J= 6Hz, -C*H*$_2$-N-CH_2), 6.30 (1H, s,-(Ph-C*H*=C(CH_2)2-), 8.05-7.44 (4H, m, Ph), in yield of 73%.



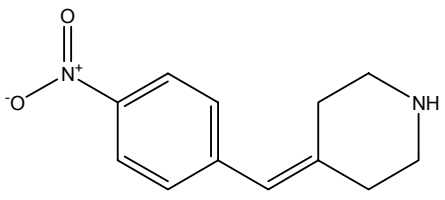
4-(4-Methoxybenzylidene)piperidine

4-(4-Methoxybenzylidene)piperidine (2.5 h): ^{1}H NMR $CDCl_3$ δ 1.72 (1H, s, -N*H*), 2.15 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.20 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.87 (2H, t, J= 6 Hz, -CH_2-N-C*H*$_2$), 2.95 (2H, t, J= 6Hz, -C*H*$_2$-N-CH_2), 3.70 (3H, s, -OC*H*$_3$), 6.25 (1H, s,-(Ph-C*H*=C(CH_2)2-), 7.01-7.60 (4H, m, Ph), in yield of 76%.



4-(4-Methylbenzylidene)piperidine

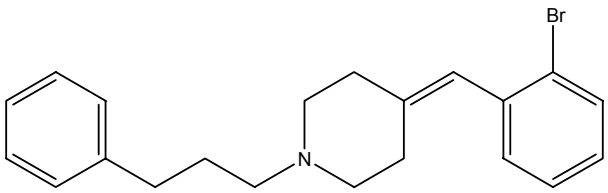
4-(4-Methylbenzylidene)piperidine (2.5 i): ^{1}H NMR $CDCl_3$ δ 1.57 (1H, s, -N*H*), 2.32 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.34 (3H, s, -C*H*$_3$), 2.46 (2H, t, J= 6 Hz, -CH=C(C*H*$_2$)$_2$-), 2.85 (2H, t, J= 6 Hz, -CH_2-N-C*H*$_2$), 2.96 (2H, t, J= 6Hz, -C*H*$_2$-N-CH_2), 6.25 (1H, s,-(Ph-C*H*=C(CH_2)2-), 7.12-7.27 (4H, m, Ph), in yield of 76%.



4-(4-Nitrobenzylidene)piperidine

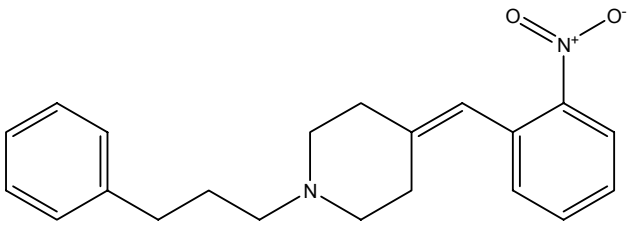
4-(4-Nitrobenzylidene)piperidine (2.5 j): ^{1}H NMR $CDCl_3$ δ 1.64 (1H, s, -N<u>*H*</u>), 2.34 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.43 (2H, t, J= 6 Hz, -CH=C(C<u>*H*</u>$_2$)$_2$-), 2.87 (2H, t, J= 6 Hz, -CH_2-N-C<u>*H*</u>$_2$), 2.96 (2H, t, J= 6Hz, -C<u>*H*</u>$_2$-N-CH_2), 6.22 (1H, s,-(Ph-C<u>*H*</u>=C(CH_2)2-), 7.22-8.16 (4H, m, Ph), in yield of 88%.

General method for the preparation of compounds 2.7 b-j (Figure 18). Each of compounds **2.5 b-j** (0.35 mmol), 1-phenylpropyl chloride (0.35 mmol, 118 mg), NaI (53 mg, 0.35 mmol) and K_2CO_3 (0.41 g, 3.0 mmol) were heated in acetonitrile (8 mL) for 18h at 60 °C. The mixture was filtered, and then concentrated under reduced pressure at 60 °C. The residue was partitioned between water and EtOAc, and the organic layer washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography using a gradient of *n*-hexane:EtOAc (5:1 to 1:4) gave the target compounds in 58-75% yields as pale yellow oils that were stored in free base form. Analytical TLC R_f values ranged from 0.2 to 0.4 (1:1 *n*-hexane:EtOAc). ^{1}H NMR data agreed with the assigned structures.



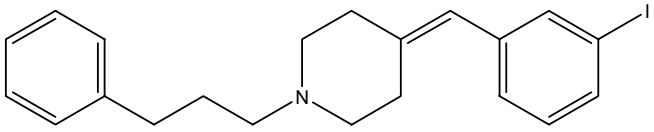
4-(2-Bromobenzylidene)-1-(3-phenylpropyl)piperidine

4-(2-Bromobenzylidene)-1-(3-phenylpropyl)piperidine (2.7 b): ^{1}H NMR $CDCl_3$ δ 1.88 (2H, app. p, J= 7.8 Hz, CH_2-C<u>*H*</u>$_2$-CH2-Ph), 2.42-2.69 (12H, m, -CH=C-[(C<u>*H*</u>$_2$)$_2$]$_2$-N-C<u>*H*</u>$_2$-CH_2-C<u>*H*</u>$_2$-Ph), 6.24 (1H, s,-(Ph-C<u>*H*</u>=C(CH_2)2-), 7.06-7.59 (9H, m, Ph), in yield of 65%.



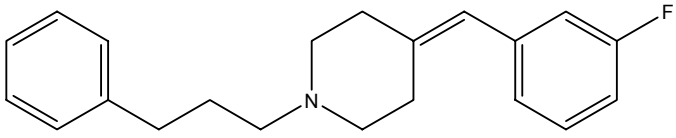
4-(2-Nitrobenzylidene)-1-(3-phenylpropyl)piperidine

4-(2-Nitrobenzylidene)-1-(3-phenylpropyl)piperidine (2.7 c): ^{1}H NMR $CDCl_3$ δ 1.85 (2H, app. p, J= 7.8 Hz, CH_2-C<u>*H*</u>$_2$-CH2-Ph), 2.27-2.67 (12H, m, -CH=C-[(C<u>*H*</u>$_2$)$_2$]$_2$-N-C<u>*H*</u>$_2$-CH_2-C<u>*H*</u>$_2$-Ph), 6.52 (1H, s,-(Ph-C<u>*H*</u>=C(CH_2)2-), 7.06-7.59 (9H, m, Ph), in yield of 70%.



4-(3-Iodobenzylidene)-1-(3-phenylpropyl)piperidine

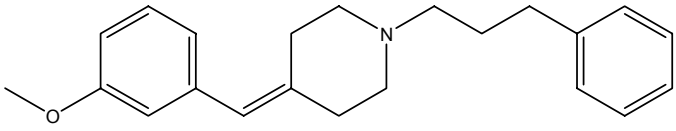
4-(3-Iodobenzylidene)-1-(3-phenylpropyl)piperidine (2.7 d): ^{1}H NMR $CDCl_3$ δ 1.85 (2H, app. p, J= 7.5 Hz, CH_2-C<u>*H*</u>$_2$-CH2-Ph), 2.36-2.69 (12H, m, -CH=C-[(C<u>*H*</u>$_2$)$_2$]$_2$-N-C<u>*H*</u>$_2$-CH_2-C<u>*H*</u>$_2$-Ph), 6.38 (1H, s,-(Ph-C<u>*H*</u>=C(CH_2)2-), 7.01-7.60 (9H, m, Ph), in yield of 66%.



4-(3-Fluorobenzylidene)-1-(3-phenylpropyl)piperidine

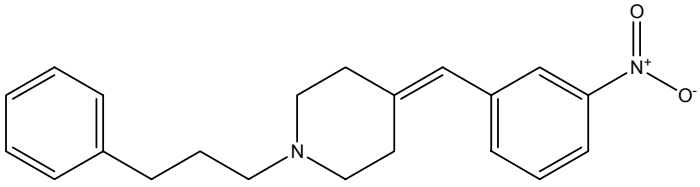
4-(3-Fluorobenzylidene)-1-(3-phenylpropyl)piperidine (2.7 e): ^{1}H NMR $CDCl_3$ δ 1.86 (2H, app. p, J= 7.5 Hz, CH_2-C<u>*H*</u>$_2$-CH2-Ph), 2.36-2.79 (12H, m, -CH=C-[(C<u>*H*</u>$_2$)$_2$]$_2$-N-

C<u>*H*</u>$_2$-CH$_2$-C<u>*H*</u>$_2$-Ph), 6.35 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 6.87-7.40 (9H, m, Ph), in yield of 58%.



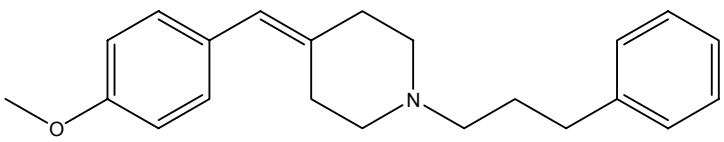
4-(3-Methoxybenzylidene)-1-(3-phenylpropyl)piperidine

4-(3-Methoxybenzylidene)-1-(3-phenylpropyl)piperidine (2.7 f): ^{1}H NMR $CDCl_3$ δ 1.87 (2H, app. p, J= 7.8 Hz, CH$_2$-C<u>*H*</u>$_2$-CH2-Ph), 2.38-2.69 (12H, m, -CH=C-[(C<u>*H*</u>$_2$)$_2$]$_2$-N-C<u>*H*</u>$_2$-CH$_2$-C<u>*H*</u>$_2$-Ph), 3.82 (3H, s, -OC<u>*H*</u>$_3$), 6.27 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 6.76-7.33 (9H, m, Ph), in yield of 71%.



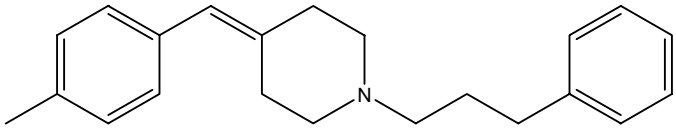
4-(3-Nitrobenzylidene)-1-(3-phenylpropyl)piperidine

4-(3-Nitrobenzylidene)-1-(3-phenylpropyl)piperidine (2.7 g): ^{1}H NMR $CDCl_3$ δ 1.86 (2H, tt, J= 7.5 Hz, J= 7.5 Hz, CH$_2$-C<u>*H*</u>$_2$-CH2-Ph), 2.39-2.69 (12H, m, -CH=C-[(C<u>*H*</u>$_2$)$_2$]$_2$-N-C<u>*H*</u>$_2$-CH$_2$-C<u>*H*</u>$_2$-Ph), 6.31 (1H, s,-(Ph-C<u>*H*</u>=C(CH$_2$)2-), 7.17-8.08 (9H, m, Ph), in yield of 63%.



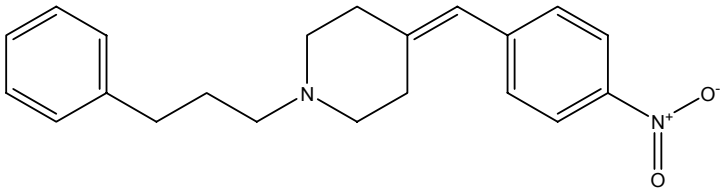
4-(4-Methoxybenzylidene)-1-(3-phenylpropyl)piperidine

4-(4-Methoxybenzylidene)-1-(3-phenylpropyl)piperidine (2.7 h): ^{1}H NMR $CDCl_3$ δ 1.88 (2H, app. p, J= 7.8 Hz, CH_2-C<u>H_2</u>-CH2-Ph), 2.23-2.73 (12H, m, -CH=C-[(C<u>H_2</u>)$_2$]$_2$-N-C<u>H_2</u>-CH_2-C<u>H_2</u>-Ph), 6.19 (1H, s,-(Ph-C<u>H</u>=C(CH_2)2-), 7.02-7.56 (9H, m, Ph), in yield of 70%. (Absence of 3H, s, -OC<u>H_3</u>).



4-(4-Methylbenzylidene)-1-(3-phenylpropyl)piperidine

4-(4-Methylbenzylidene)-1-(3-phenylpropyl)piperidine (2.7 i): ^{1}H NMR $CDCl_3$ δ 1.86, 2.34 (3H, s, -C<u>H_3</u>), (2H, app. p, J= 7.5 Hz), 2.34-2.68 (12H, m, -CH=C-[(C<u>H_2</u>)$_2$]$_2$-N-C<u>H_2</u>-CH_2-C<u>H_2</u>-Ph), 6.19 (1H, s,-(Ph-C<u>H</u>=C(CH_2)2-), 7.08-7.31 (9H, m, Ph), in yield of 66%.

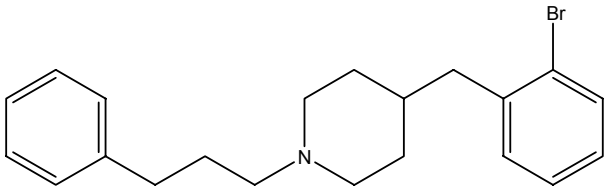


4-(4-Nitrobenzylidene)-1-(3-phenylpropyl)piperidine

4-(4-Nitrobenzylidene)-1-(3-phenylpropyl)piperidine (2.7 j): ^{1}H NMR $CDCl_3$ δ 1.86 (2H, app. p, J= 7.8 Hz, CH_2-C<u>H_2</u>-CH2-Ph), 2.38-2.69 (12H, m, -CH=C-[(C<u>H_2</u>)$_2$]$_2$-N-C<u>H_2</u>-CH_2-C<u>H_2</u>-Ph), 6.34 (1H, s,-(Ph-C<u>H</u>=C(CH_2)2-), 7.12-8.20 (9H, m, Ph), in yield of 75**%.**

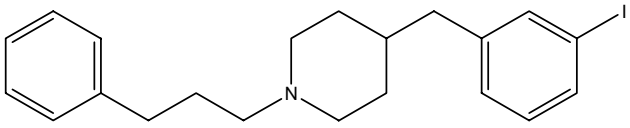
General method for the preparation of compounds 2b, 2d, 2e, 2f. These compounds were prepared by catalytic hydrogenation of the precursors (**2.7 b, 2.7 d, 2.7 e, 2.7 f**) with 5% Pd on carbon under 1 atmosphere. (0.2 mmol) of each precursor was dissolved in 4

mL of MeOH (THF for precursor **2.7 d**). Catalyst (10 mg) was added and the mixture was hydrogenated (1 atm) for at least 18 h (48 h for precursor **2.7 b**) with rapid stirring. The catalyst was removed by filtration through celite and the organic solution was evaporated under reduced pressure. ^{1}H NMR data were as follows:



4-(2-Bromobenzyl)-1-(3-phenylpropyl)piperidine

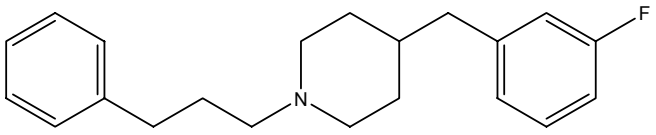
4-(2-Bromobenzyl)-1-(3-phenylpropyl)piperidine (2b): Spectral analysis showed a mixture of unreacted compound and product. ^{1}H NMR $CDCl_3$ δ 1.23-3.46 (~17H, m, *precursor 2.7 b and product 2b*), 6.40 (0.4H, s,-(Ph-C*H*=C(CH_2)2- *precursor*), 7.09-7.55 (~9H, m, Ph *precursor 2.7 b and product 2b*).



4-(3-Iodobenzyl)-1-(3-phenylpropyl)piperidine

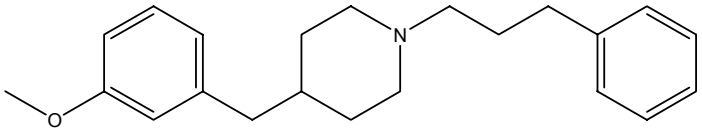
4-(3-Iodobenzyl)-1-(3-phenylpropyl)piperidine (2d): Spectral analysis showed the reduction of the iodine occurring before the reduction of the double bond (after 12 h) ^{1}H NMR $CDCl_3$ δ 2.26-3.04 (~16H, m, -CH=*Pip*-(CH_2)$_3$-Ph *unsubstituted precursor that has lost the iodine*), 6.45 (0.9H, s,-(Ph-C*H*=C(CH_2)2- *unsubstituted precursor that has lost the iodine*), 7.12-7.35 (~9H, m, Ph, *the unsubstituted precursor has lost the iodine*). (After 48h): ^{1}H NMR $CDCl_3$ δ 1.44-3.44 (~17H, m, Ph-CH_2-*Pip*-(CH_2)$_3$-Ph *unsubstituted*

product that has lost the iodine), 7.12-7.35 (~9H, m, Ph, *the unsubstituted product has lost the iodine*).



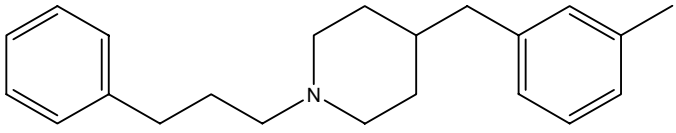
4-(3-Fluorobenzyl)-1-(3-phenylpropyl)piperidine

4-(3-Fluorobenzyl)-1-(3-phenylpropyl)piperidine (2e): ^{1}H NMR $CDCl_3$ δ 1.22-1.91 (7H, m, -C*H*[(C*H*$_2$)$_2$]$_2$-N-), 2.33-2.94 (8H, m, Ph-C*H*$_2$-Pip-C*H*$_2$-C*H*$_2$-C*H*$_2$-Ph), 6.83-7.30 (9H, m, Ph). Quantitative yield, kept in the free base form.



4-(3-Methoxybenzyl)-1-(3-phenylpropyl)piperidine

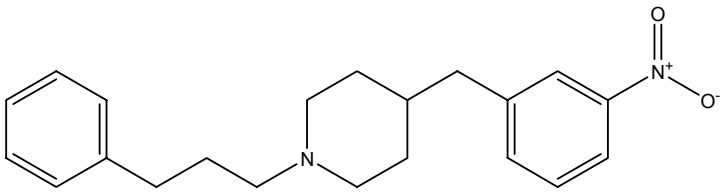
4-(3-Methoxybenzyl)-1-(3-phenylpropyl)piperidine (2f): ^{1}H NMR $CDCl_3$ δ 1.28-1.94 (7H, m, -C*H*[(C*H*$_2$)$_2$]$_2$-N-), 2.36-2.97 (8H, m, Ph-C*H*$_2$-Pip-C*H*$_2$-C*H*$_2$-C*H*$_2$-Ph), 3.82 (3H, s, -OC*H*$_3$), 6.71-7.31 (9H, m, Ph). Quantitative yield, kept in the HCl form.



4-(3-Methylbenzyl)-1-(3-phenylpropyl)piperidine

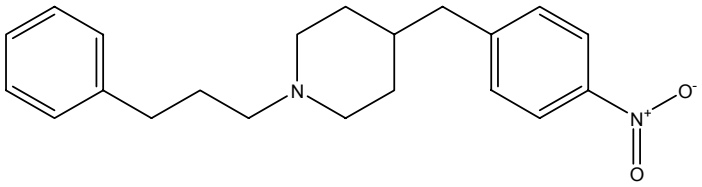
4-(3-Methylbenzyl)-1-(3-phenylpropyl)piperidine (2i): ^{1}H NMR $CDCl_3$ δ 1.25-2.01 (7H, m, -C*H*[(C*H*$_2$)$_2$]$_2$-N-), 2.32 (3H, s, -C*H*$_3$), 2.55-3.18 (8H, m, Ph-C*H*$_2$-Pip-C*H*$_2$-C*H*$_2$-C*H*$_2$-Ph), 3.82 (3H, s, -OC*H*$_3$), 7.08-7.37 (9H, m, Ph). Quantitative yield, kept in the free base form form.

General method for the preparation of compounds 2c and 2j (Figure 18). These compounds were prepared by catalytic hydrogenation of 0.2 mmol of each precursors (**2.7 c and 2.7 j**) with 4% chlorotris(triphenylphosphine)rhodium(I) (Wilkinson's catalyst) under 1 atmosphere according to Jourdant and co-workers.[126] The solution was stirred under a hydrogen atmosphere at room temperature for 48 h with rapid stirring. The reaction mixture was filtered through a thin pad of Celite and the organic solution was evaporated under reduced pressure. Purification of the saturated compounds was attempted by silica gel column chromatography with *n*-hexane:EtOAc (5:1 to 2:1) but did not give the target compounds. ^{1}H NMR data were as following:



4-(3-Nitrobenzyl)-1-(3-phenylpropyl)piperidine

4-(3-Nitrobenzyl)-1-(3-phenylpropyl)piperidine (2c): ^{1}H NMR $CDCl_3$ *Same features of precursor 2.7 c, complete absence of product*.



4-(4-Nitrobenzyl)-1-(3-phenylpropyl)piperidine

4-(4-Nitrobenzyl)-1-(3-phenylpropyl)piperidine (2j): ^{1}H NMR $CDCl_3$ δ 1.69-2.25 (7H, m, -C<u>*H*</u>[(C<u>*H*</u>$_2$)$_2$]$_2$-N-), 2.43-3.43 (8H, m, Ph-C<u>*H*</u>$_2$-Pip-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-Ph), 7.13-7.62 (9H, m, Ph). It was kept in the free base form as a brown oil.

It also showed the presence of Wilkinson's Catalyst (δ 6.5-8.2, Rh-(P*Ph*$_3$)$_3$). At that point, it was decided to purify and isolate compound **2j** by semipreparative RP-HPLC and characterized by LC-MS:

A Waters (Milford, MA) NovaPak C18 column (3.9 x 300 mm) was used for the LC-MS analysis. Analytical and semipreparative RP-HPLC were performed on a Beckman Coulter System Gold chromatography equipped with a 168 diode array detector, a 507e auto-injector, and the 32 KARAT software package (Beckman Coulter, Fullerton, CA). A keystone Scientific, Inc. (San Jose, CA) C-18 Kromosil column (4.6 x 150 mm, 5 μm, 100 Å) was used for analytical HPLC. For semipreparative HPLC, a Waters Prep NovaPak, HR-C18 column (7.8 x 300 mm, 6 μm, 60 Å) was used. The flow rate was maintained at 1.0 mL for analytical runs and at 4.0 mL/min for semipreparative purification. The wavelengths used for UV detection were 214 and 280 nm for analytical RP-HPLC and 225 and 280 for semipreparative RP-HPLC, respectively. Eluents used in all runs consisted solvent A (0.1% TFA/H_2O) and solvent B (0.1% TFA/CH_3CN). A linear gradient was used for analytical RP-HPLC from 15% to 80% in 80 min. For the semipreparative run the gradient was from 15% to 25% in 5 min, isocratic (25%) for 5 min, 25% to 40% in 30 min, 40% to 80% in 5 min, isocratic conditions (80%) were applied for the final 5 minutes.

During the semipreparative run, every fraction with a significant UV absorbance was collected, and analyzed by LC-MS. The fraction containing the desired product exhibited the appropriate ESI-MS *m*/z signal 339 $(M + H)^{1+}$ (analytical HPLC t_R= 40.2 min), and was lyophilized and stored in the free base form.

IV.1.3-*Series-3 and series-4*:

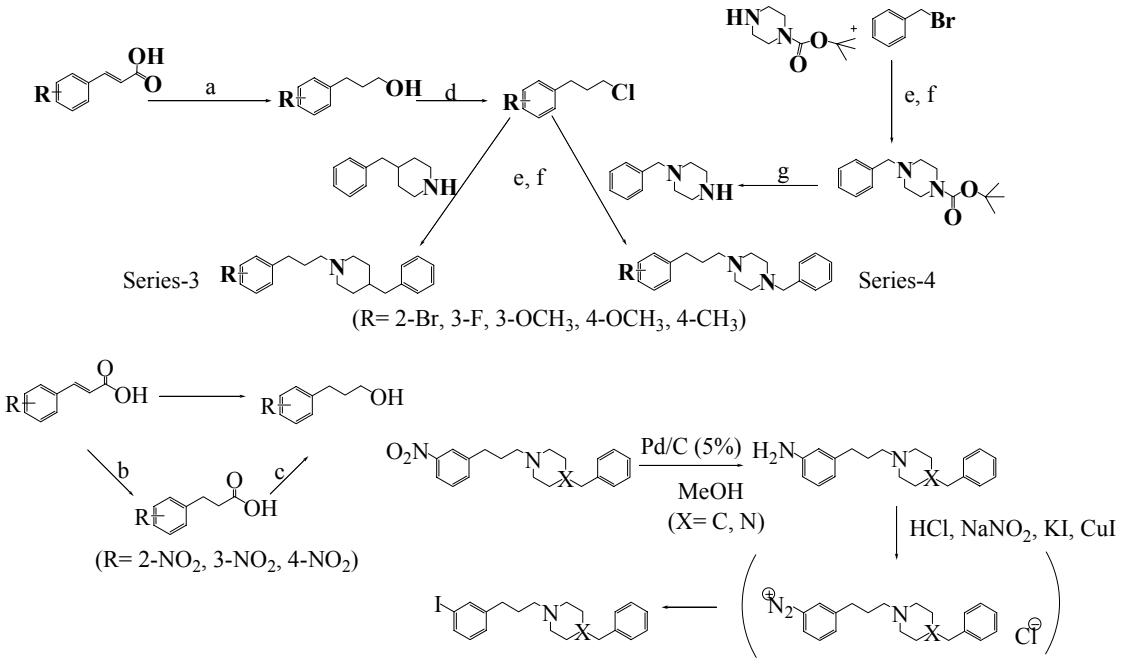


Figure 19. *Detailed synthetic scheme for series-3 and series-4 compounds*.

Experimental details on the synthesis of these compounds in Figure 19 above are present in the master thesis of Mr. Yu Lu (*Ligands for the Sigma receptors and the μ-opioid receptor*).

IV.2 Binding assays

Sigma-1 and sigma-2 receptor binding assays were carried out as previously described[127] using membranes from fresh-frozen, male English Hartley guinea pig brains (Rockland Immunochemicals, Inc.; Gilbertsville, PA), [^{3}H](+)-pentazocine as a sigma-1 radiotracer and [^{3}H]DTG in the presence of (+)-pentazocine as sigma-2 radiotracer.

Opioid receptor binding assays were conducted in membranes from guinea pig (μ, κ) or mouse brains (δ) using [^{3}H]NTI (δ), [^{3}H]DAMGO (μ), [^{3}H]U69,593 (κ) as previously reported.[128]

Phenytoin modulation of ligand binding to sigma-1 was investigated using minor modifications of reported procedures.[72,73]

Data from binding assays were analyzed with the non-linear curve-fitting computer programs Prism 4.0b (Graph-Pad Software, San Diego, CA) and Radlig 6.0 (KELL, Suite, Biosoft, Inc., Ferguson, MO). Statistical analyses were carried out with Prism program. Each experiment was repeated three to six times, and data points repeated in duplicates, yielding means and standard errors. The goodness-of-fit between one- and two-site models was compared with the *F*-ratio test. The *F*-ratio test was also applied for testing of pseudo-Hill slopes (n_H) against a hypothetical value of 1.0 by comparison of the variable-slope, four-parameter fit against the null hypothesis of a three-parameter fit against the null hypothesis of a three-parameter model with n_H fixed. Apparent binding affinities (K_i) were calculated by the Cheng and Prussof equation[7] using IC_{50} values, the radioligand concentration, and the radioligand experimentally determined K_d of the radioligand.

IV.2.1-*In Vitro Inhibition of [^{3}H](+)-Pentazocine (sigma-1)*:

Guinea pig brain membrane aliquots were thawed, and then diluted to 1 mg/mL by adding an appropriate volume of sigma-1 assay buffer (50 mM Tris-HCl; pH 7.4, 25 ^{o}C). Each glass assay tube contained ~0.25 mg protein in 1 mL total volume, and was

incubated for 150 minutes at 37 °C with [^{3}H(+)-pentazocine (1.0 nM), along with haloperidol (1.0 μM) for nonspecific binding, and the competing ligands, used at 10 increasing concentrations equally spaced on the log scale, centered around the suspected IC_{50}. Assays were terminated by addition of the sigma-1 buffer (5 mL), followed by filtration using a cell harvester (Brandel, Gaithersburg, MD), through glass filters (GF/B) that had been pre-treated with polyethyleneimine (0.5%) for 60 minutes. Subsequently, filter discs and tubes were washed three times with ice-cold sigma-1 buffer, and the filter discs dried under vacuum. The resulting discs were incubated for at least 18 hours with scintillation cocktail, and then counted for 5 minutes/sample.

IV.2.2-*In Vitro Inhibition of [3H] DTG (sigma-2)*:

Guinea pig brain membrane aliquots were thawed, and then diluted to 1 mg/mL by adding an appropriate volume of sigma-2 assay buffer (50 mM Tris-HCl; pH 8, 25 °C). Each glass assay tube contained ~0.25 mg protein in 0.5 mL total volume, and was incubated for 120 minutes at 25 ^{o}C with [^{3}H]DTG (3.0 nM) in the presence of (+)-pentazocine (200 nM), along with DTG (100 μM) for nonspecific binding, and the competing ligands, used 10 increasing concentrations equally spaced on the log scale, centered around the assumed IC_{50}. Assays were terminated by addition of sigma-1 buffer (5 mL), followed by filtration using a cell harvester (Brandel, Gaithersburg, MD), through glass filters (GF/B) that had been pre-treated with polyethyleneimine (0.5%) for 60 minutes. Subsequently, filter discs and tubes were washed three times with ice-cold sigma-2 buffer, and the filter discs dried under vacuum. Extraction and counting was similar to the sigma-1 binding assay procedure.

IV.2.3-*Phenytoin modulation of ligand binding to sigma-1 receptors*:

Assays were conducted as above for [^{3}H]PTZ, except DPH (50 μL, 20 mM) in NaOH vehicle (0.15 M) was added to every tube. Control experiments, where only NaOH (50 μL, 0.15 M) was added, were also conducted. The incubation medium for these assays was TRIS-HCl buffer and the pH was 7.44 at 37 °C, while the medium had pH 7.06 at 37 °C for [^{3}H]PTZ assays done in the absence of NaOH or DPH / NaOH.

IV.2.4-*In Vitro Inhibition of [^{3}H]NTI (δ), [^{3}H]DAMGO (μ), and [^{3}H]U69,593 (κ)*:

The competing ligands were introduced at a concentration suspected to be higher than a certain IC_{50} (1-2 μM) (when no specific binding was observed at that concentration, we assumed that the IC_{50} of the studied compound is higher than that concentration).

Delta: membranes from fresh, whole CD-1 mouse brain homogenates in a buffer comprised of TRIS-HCl (pH 7.4, 50 mM), 0.1% protease-free BSA, 50 mg/mL bacitracin, 30 mg/mL bestatin, 10 mM captopril and 0.1 mM phenylmethylsulfonyl fluoride were diluted to 2.5 mg/mL by adding an appropriate volume of buffer. Each glass assay tube contained ~0.6 mg protein in 1 mL total volume, and was incubated for 90 minutes at 37 °C with [^{3}H](+)-NTI (0.113 nM), with NTI (1.0 μM) for nonspecific binding, and the competing ligands. Assays were terminated by addition of TRIS-HCl buffer (5 mL), followed by filtration using a cell harvester (Brandel, Gaithersburg, MD), through glass filters (GF/B) that had been pre-treated with polyethyleneimine (0.5%) for 60 minutes. Subsequently, filter discs and tubes were washed three times with ice-cold sigma-1 buffer, and the filter discs dried under vacuum.

The resulting discs were incubated for at least 72 hours with scintillation cocktail, and then counted for 5 minutes/sample.

Mu: Guinea pig brain membrane aliquots were thawed, and then diluted to 2 mg/mL by adding an appropriate volume of buffer (50 mM Tris-HCl; pH 7.4, 25 ^{o}C). Each glass assay tube contained ~0.4 mg protein in 0.25 mL total volume, and was incubated for 75 minutes at 25 °C with [^{3}H](+)-DAMGO (0.6 nM), with DAMGO (5.0 μM) for nonspecific binding, and the competing ligands. Assays were terminated upon addition of the Tris buffer (5 mL), followed by filtration using a cell harvester (Brandel, Gaithersburg, MD), through glass filters (GF / B) that had been pre-treated with polyethyleneimine (0.5%) for 60 minutes. Subsequently, filter discs and tubes were washed three times with the ice-cold sigma-1 buffer, and the filter discs dried under vacuum. The resulting discs were incubated for at least 72 hours with scintillation cocktail, and then counted for 5 minutes / sample.

Kappa: Guinea pig brain membrane aliquots were thawed, and then diluted to ~ 2000 mg/ mL by adding an appropriate volume of buffer (50 mM Tris-HCl; pH 7.4, 25 ^{o}C). Each glass assay tube contained ~0.4 mg protein in 0.25 mL total volume, and was incubated for 90 minutes at 25 °C with [^{3}H]U69,593 (0.6 nM), with U69,593 (10 μM) for nonspecific binding, and the competing ligands. Assays were terminated by addition of TRIS-HCl buffer (5 mL), followed by filtration using a cell harvester (Brandel, Gaithersburg, MD), through glass filters (GF/B) that had been pre-treated with polyethyleneimine (0.5%) for 60 minutes. Subsequently, filter discs and tubes were washed three times with the ice-cold sigma-1 buffer, and the filter discs dried under

vacuum. The resulting discs were incubated for at least 72 hours with scintillation cocktail, and then counted for 5 minutes / sample.

IV.3 QSAR

The QSAR of the compounds were analyzed by the Hansch-Fujita method,[129] with several reported physico-chemical descriptors that represent lipophilic, electronic and steric effects. All physico-chemical parameters were taken from Hansch, Leo and Hoekman.[130]

IV.3.1-*Descriptors used*:

π_x values denote the hydrophobic contribution of each substituent: $\pi_x = \log P_X/P_H$, where P_X and P_H are the partition coefficients of substituted and unsubstituted compounds, respectively.

MR values, which are equal to $[(n^2-1/\ n^2+1)(MW/d)]$ (scaled by 0.1), denote the substituent molar refractivity. The molar refractivity accounts for both the polarizability and the substituent volume. On the other hand, the Taft steric effect E_s values reflect only the steric effect of a substituent.

Finally, Hammett substituent constants $\sigma_{m,p}$ (based upon the acid dissociation of the unsubstituted benzoic acid and *meta* and *para* substituted benzoic acid in water at 25 ^oC) denote the electronic characteristics of the substituents at the meta and para positions. σ^- is employed when an augmented electronic withdrawing effect is observed between the substituent and the receptor counter-part of the interaction. σ^- is basically defined as $\sigma^- =$

log K_X – log $K_{H,}$ where K refers to the ionization of phenols or anilines (K_X for the substituted moiety, and K_H for the unsubstituted moiety). There are no uniform sigma constants for the *ortho* substituents as the Hammett sigma constants are restricted to the *para* and *meta* substituents and that is because unlike the cases of *meta* and *para* substitutions, the electronic effect in the *ortho* position is difficult to separate from other contributors such as the steric and proximity effects. Fujita and Nishioka[131] proposed their own extended Hammett equation to represent the *ortho* electronic characteristic as $\log k_{ortho} = \rho\sigma_p + \delta E_s + fF + c$ (as opposed to $\log k_{m,p} = \rho\sigma_{m,p} + c$) where k refers to the ionization of benzoic acid, E_s and F are the terms respectively for the steric effect and the polar proximity effect. "C" is a constant, and ρ, δ, and f are the equation coefficients and their numerical values depend on the reaction system and the molecular structure. Charton[132] discussed the quantitative treatment of the *ortho* effect, and he proposed the use of an extended Hammett equation: $\sigma_{ortho} = \alpha\sigma_I + \beta\sigma_R + h$, where σ_I and σ_R are respectively, the localized contribution (inductive) and delocalized (resonance). α and β are coefficients dependent on the type of reaction and molecular structure and varying between 0 and ∞, h is a constant. Charton[132] came up with the conclusion that it is impossible to define a "*single pure characteristic ortho electrical effect*". Consequently, he recommended the use of the constants giving the best correlation with the data, and thus calculating the extended Hammett equation coefficients from that. And while in some QSAR studies σ_p of the corresponding *para* substituent was used to denote the Hammett constant in the *ortho* position (clearly disregarding the steric and proximity effects in the ortho positions), in some other studies, the Fujita and Nishioka[131] F constant is used with σ_p to delineate the proximity effect (clearly disregarding the steric effect, and

considering that *f* which is the *F* equation coefficient is equal to +1). Hansch and Leo[130] reported several series of σ_o constant values from various sources, and we employed σ_o values for 2-Br and 2-NO_2 in equation-3 from those sources.[133,134]

IV.3.2-*Regression type*:

According to Hadjipavlou-Litina and co-workers,[135] the Multiple Linear Regression (MLR) using the ordinary least squares methods is the best method for analyzing small size data, especially when the number of physico-chemical descriptors (or variables) is smaller than the number of compounds (or observations), therefore, the correlation was analyzed by MLR on a PC with the 9.0 version SAS statistical software. While a multitude of regression equations were judged significant, the regression equations shown below represent the ones that have best described the data variance, based primarily on the correlation coefficient of the regression (r^2) of each equation, as well as the *F* statistic. This permits comparison to the statistical significance of multiple regression models, as the maximum value of the *F* criterion corresponds to the multiple regression equation with the maximum description of the variance of dependent variable (which is the property that is being studied). The *t* parameter gave decisive information regarding the importance of a single independent variable in a model involving all other independent variable (the higher the *t* value of a certain variable, the more significant its presence is in the model). The standard error of the multiple linear regression *s* (also known as standard error of the estimate) was also taken into consideration (usually a model with a smaller standard error is more likely to be selected from among other models with higher standard errors). "*Q*" is the cross-validated coefficient is the square

root of the cross-validated coefficient of determination q^2 defined as $1-\frac{PRESS}{\sum_{i=1}^{n}(Pi-\overline{P})^2}$

where PRESS is the prediction sum of squares. "*Q*" denotes the predictive effectiveness of the model. *n* is the number of compounds used to establish the equation and the figures in parentheses are the 95% confidence of the regression coefficients.

CHAPTER V:

RESULTS AND DISCUSSION

V.1 Series-1

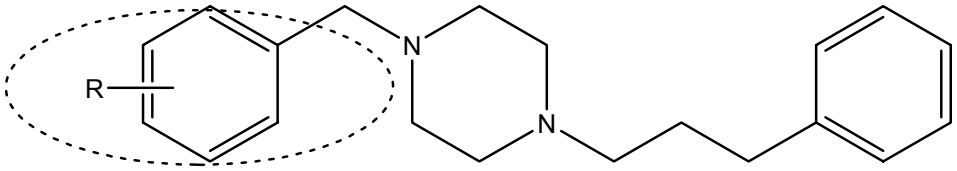


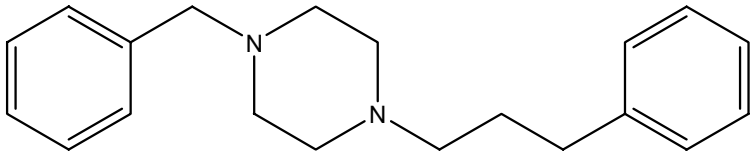
Figure 20. *Series-1 is the benzyl substituted piperazine containing series of compounds*.

V.1.1-*Results*:

Compounds of this series were synthesized according to the planned synthetic scheme, all the steps were straight forward, and final yields varied from 70-88%. All 10 compounds were kept in the free base form, then each was submitted to sigma-1 and sigma-2 binding assays, opioid receptor affinity screening, and three of them were tested according to the agonist /antagonist phenytoin assay.

Each binding assay experiment was repeated 3-6 times (values were duplicate) yielding 6 data sets of specific binding percentages, each specific binding data set (10 values) was plotted against the corresponding concentrations (10 values). Consequently, 3-6 sigmoidal curves resulted, each giving rise to a K_i, IC_{50} and n_H (Hill Slope). Final K_i, IC_{50} and n_H values are means of 3-6 values (from 3-6 data sets). The standard error, standard deviation, and 95% confidence intervals were generated, as well as a normality test. The

following is an example of the data output for compound **1a** (Lead 1) (the rest of the data for the other compounds is in the appendix section):

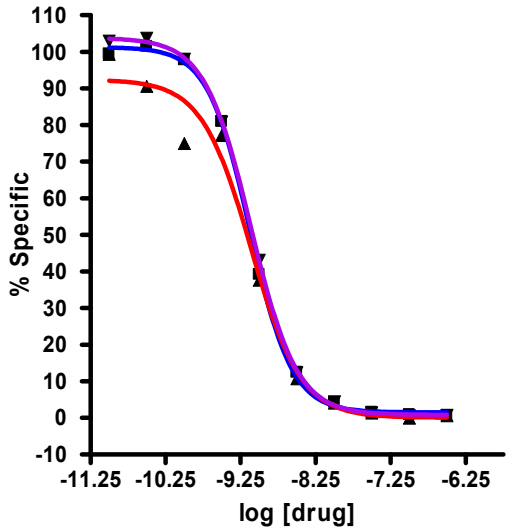


Compound 1a (lead 1)

Figure 21. *Compound **1a** (Lead 1).*

Table 8. *Ten different concentrations of compound **1a** and the corresponding specific binding % values performed 3 times (3 data sets) for σ_1.*

log [drug] (M) (σ_1)	(1a) (Lead1)(1) (%)	(1a) (Lead1)(2) (%)	(1a) (Lead1)(3) (%)
3.16E-07	0.645	0.560	0.702
1.00E-07	0.896	-0.022	0.576
3.16E-08	1.548	1.300	1.409
1.00E-08	4.243	4.023	4.415
3.16E-09	12.472	10.711	12.332
1.00E-09	39.227	37.621	43.104
3.16E-10	80.978	77.116	80.822
1.00E-10	97.785	74.938	97.987
3.16E-11	101.500	90.579	103.742
1.00E-11	99.423	99.308	102.889



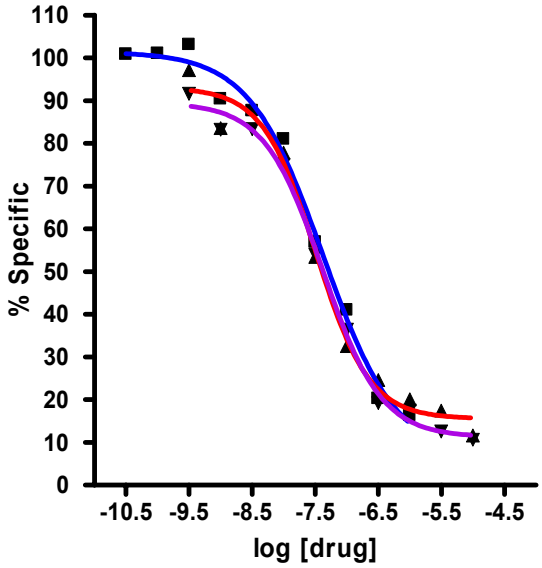
Graph 8. *Three sigmoidal curves (3 data sets) representing the specific binding % of the radioligand vs. the logarithmic concentration of compound* ***2a*** *for* σ_1.

Table 9. *Mean values for* σ_1, IC_{50}, K_i *and* n_H, *the corresponding standard deviation, standard error, and 95 % confidence interval as well as the normality test results for* σ_1.

(σ_1)	***IC_{50} (M)***	***K_i (M)***	***Hill***
Number of values	3	3	3
Mean	7.641e-010	5.342e-010	-1.427
Std. Deviation	1.605e-011	1.119e-011	0.119
Std. Error	9.265e-012	6.460e-012	0.069
Lower 95% CI of mean	7.242e-010	5.064e-010	-1.723
Upper 95% CI of mean	8.040e-010	5.620e-010	-1.132
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

Table 10. *Ten different concentrations of compound **1a** and the corresponding specific binding % values performed 3 times (3 data sets) for σ_2.*

log [drug] (M) (σ_2)	(1a) (Lead1)(1) (%)	(1a) (Lead1)(2) (%)	(1a) (Lead1)(3) (%)
1.00E-06		11.616	10.623
3.16E-07		17.448	12.538
1.00E-07	16.334	20.073	17.442
3.16E-08	20.285	24.625	19.198
1.00E-08	41.034	32.586	36.364
3.16E-09	56.911	53.426	53.925
1.00E-09	81.039	77.830	74.989
3.16E-10	87.683	88.147	83.354
1.00E-10	90.391	83.567	83.230
3.16E-11	103.135	97.146	91.609
1.00E-11	101.071		
3.16e-012	100.867		



Graph 9. *Three sigmoidal curves (3 data sets) representing the specific binding % of the radioligand vs. the logarithmic concentration of compound* ***1a*** *for* σ_2.

Table 11. *Mean values for* σ_1, IC_{50}, K_i *and* n_H, *the corresponding standard deviation, standard error, 95 % confidence interval, and the normality test results for* σ_2.

(σ_2)	***IC_{50} (M)***	***K_i (M)***	***Hill***
Number of values	3	3	3
Mean	3.864e-008	3.433e-008	-0.905
Std. Deviation	5.256e-009	4.670e-009	0.143
Std. Error	3.035e-009	2.696e-009	0.083
Lower 95% CI of mean	2.558e-008	2.273e-008	-1.261
Upper 95% CI of mean	5.169e-008	4.593e-008	-0.549
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

Table 12. *Series-1 affinity and subtype selectivity. Numbers are means (n= 3-6) ± SEM.*

Compound	IC_{50} (nM) σ_1	K_i (nM) σ_1	IC_{50} (nM) σ_2	K_i (nM) σ_2	Selectivity = $K_i \sigma_2 / K_i \sigma_1$
1a (R= H)	0.95 ± 0.09	0.66 ± 0.06	38.64 ± 3.03	34.33 ± 2.69	52.01
1b (R= 2-Br)	0.86 ± 0.02	0.6 ± 0.01	4.64 ± 0.22	4.12 ± 0.19	6.86
1c (R= 2-NO_2)	4.01 ± 0.06	2.8 ± 0.04	4.26 ± 0.26	3.79 ± 0.23	1.35
1d (R= 3-I)	0.56 ± 0.07	0.39 ± 0.05	1.15 ± 0.09	1.03 ± 0.08	2.64
1e (R= 3-F)	1.94 ± 0.1	1.36 ± 0.08	15.59 ± 1.1	13.85 ± 0.98	10.18
1f (R= 3-OCH_3)	1.25 ± 0.02	0.87 ± 0.01	15.97 ± 0.29	14.19 ± 0.26	16.31
1g (R= 3-NO_2)	1.33 ± 0.04	0.93 ± 0.02	1.79 ± 0.09	1.59 ± 0.08	1.7
1h (R= 4-OCH_3)	1.09 ± 0.01	0.76 ± 0.07	36.93 ± 3.3	32.81 ± 2.93	43.17
1i (R= 4-CH_3)	1.68 ± 0.02	1.17 ± 0.01	19.71 ± 2.82	17.51 ± 2.51	14.96
1j (R= 4-NO_2)	0.52 ± 0.01	0.37 ± 0.01	3.70 ± 0.38	3.29 ± 0.34	8.89
Haloperidol	1.19 ± 0.06	0.83 ± 0.03	34.33 ± 2.69	9.57 ± 0.97	11.53
SA4503	6.21 ± 0.44	4.34 ± 0.31	101.3 ± 9.02	89.51 ± 7.97	20.62
Dextromethorphan	232.3 ± 8.75	162.9 ± 6.12			
Dextro. + DPH	15.18 ± 0.56	10.65 ± 0.33			
Lead1 + NaOH	1.86 ± 0.21	1.31 ± 0.15			
Lead1 + DPH	2.30 ± 0.22	1.62 ± 0.16			
1d + DPH	0.71 ± 0.01	0.49 ± 0.00			
1d + NaOH	0.57 ± 0.06	0.39 ± 0.04			
1f + DPH	1.04 ± 0.04	0.72 ± 0.02			
1f + NaOH	0.60 ± 0.02	0.42 ± 0.01			

V.1.2-*Discussion*:

Qualitative SAR. All the *N*-phenylpropyl-*N'*-benzylpiperazines displayed very high sigma-1 binding affinities (K_i) varying from 0.37 nM to 2.8 nM, seven of them exhibited subnanomolar affinities, with 3-iodo (**1d**) (0.39 nM) and 4-nitro (**1i**) (0.39 nM) exhibiting particularly strong effects. On the other hand, a wider range of affinities was observed for sigma-2 receptor binding ranging from 1.03 nM to 34.33 nM with 3-iodo (**1d**) being the most potent (1.0 nM), and the parent compound (R= H) (**1a**) and the 4-methoxy analog (**1h**) being the least potent with, respectively, 32.81 nM and 34.33 nM binding potencies. Consequently, the higher sigma-1 affinities resulted in a σ_1/σ_2 selectivities ranging from 1.35 to 52.01 toward the sigma-1 subtype, with 40-50 fold sigma-1 selectivities noted for the parent compound (**1a**) and the 4-methoxy (**1h**) analog. Haloperidol and SA4503 (*N*-phenylpropyl-*N*'-3,4-dimethoxyphenethylpiperazine) were included and gave K_i values near those previously reported.[127] Haloperidol purchased from Sigma-Aldrich, and SA4503 prepared by Dr. Rong Xu in Dr. Susan Lever's research laboratory according to published procedures.[127]

The compounds were also tested for the three opioid receptors (μ, δ and κ), and uniformly exhibited poor affinities (<5% displacement of radioligand at 1-2 μM)

The sigma-1 receptor binding proved to be sensitive in regard to the nitro substitution showing almost an eight-fold decrease in affinity observed over the 2-nitro, 3-nitro and 4-nitro benzyl substituted *N*-phenylpropyl-*N'*-benzylpiperazines (**1i** > **1g** > **1c**). On the other hand, notable changes were not observed regarding the sigma-2 binding affinity (less than three-fold decrease in affinity over **1i** > **1g** > **1c**).

The analog with the electron-withdrawing 4-nitro substituent (**1i**) showed an increased affinity for both sigma-1 and sigma-2 receptor subtypes (0.37 nM for sigma-1 and 3.29 nM for sigma-2) in contrast to the electron-donating methoxy (0.76 nM for sigma-1 and 32.81 for sigma-2) and methyl groups (1.17 nM for sigma-1 and 17.51 nM for sigma-2) in the *para*-position, with a greater detrimental effect on sigma-2 binding than sigma-1 binding (2-3 fold decrease for sigma-1 and 4-6 decrease for sigma-2).

The halogen series showed a trend between size, hydrophobicity and polarizability on one side, and affinity on another as higher sigma-1 and sigma-2 affinities were observed for 3-iodo **1d** (0.39 nM for sigma-1 and 1.03 nM for sigma-2) > 3-bromo **1b** (0.60 nM for sigma-1 and 4.10 nM for sigma-2) > 3-fluoro **1e** (1.36 nM for sigma-01 and 13.85 nM for sigma-2).

Quantitative SAR. Equation 1 was derived for sigma-1 binding data, and it shows that the binding affinity follows a parabolic dependence on the hydrophobicity of the substituent, with a negative sign for the $(\pi_x)^2$ term of the equation. It is also dependent on the Molar Refractivity, and the sigma Hammett constant values for the corresponding *meta* and *para* positions $\sigma_{m,p}$. Using σ^- for compound 1j (R= 4-NO_2) instead of the normal σ_p improved all the equation parameters. The inclusion of compound **1a** (R= H) gave a non-satisfactory equation in term of statistical significance, hence it was decided to exclude this compound while building the correlation equation, however its predicted value was calculated from the equation.

Log (1 / K_i) = -0.63 (±0.469) -1.535 (±1.45) $(\pi_x)^2$ +1.255 (±1.01) (π_x) +

0.965 (±0.788) MR + 0.617 (±0.371) $\sigma_{m,p}$ **(1)**

***n* = 9;** **r^2 = 0.888;** **$F_{4,8}$ = 7.93;** ***s* = 0.129;** **q^2 = 0.434;**

0.01 < *P* < 0.05

Equation 2 was derived for sigma-2 binding data:

Log (1 / K_i) = -1.498 (±0.342) +1.497 (±1.14) $(\pi_x)^2$ -0.958 (±0.878) (π_x)

-0.408 (±0.483) E_s + 0.505 (±0.526) $\sigma_{m,p}$ **(2)**

***n* = 10;** **r^2 = 0.947;** **$F_{4,9}$= 22.22;** ***s* = 0.167;** **q^2 = 0.789;**

***P* < 0.01**

Introducing Hammett sigma values for the two *ortho*-substituted compounds among the 10 studied compounds gave a poorer performance in the equation derived for sigma-1 binding, but a better performance for sigma-2 binding, shown in equation (3):

Log (1 / K_i) = -1.499(±0.297) +1.485 (±0.974) $(\pi_x)^2$ -0.989 (±0.724) (π_x) -0.375 (±0.421) E_s + 0.547 (±0.450) $\sigma_{m,p,o}$ **(3)**

***n* = 10;** **r^2 = 0.960;** **$F_{4,9}$= 29.95;** ***s* = 0.145;** **q^2 = 0.862;** ***P* < 0.001**

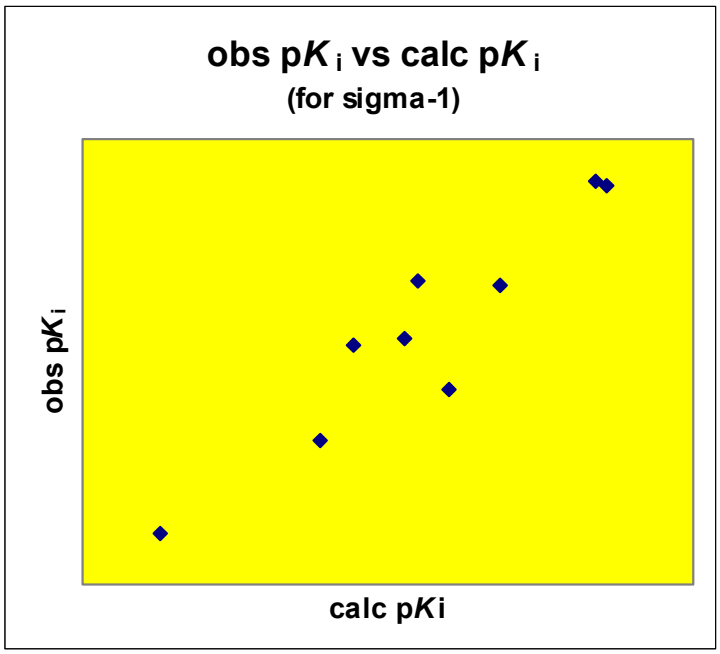
"n" Is the number of compounds used to build the equation (9 for the equation-1 and 10 for equations-2 and 3). The regression coefficient "r^2" is considered of best statistical quality, especially for equations-2 and 3. The statistics *F* ($F_{4,8}$ *and* $F_{4,9}$) is a sign of how well the overall model correlates in consideration with the number of parameters (the subscript 4 is the number of independent terms in the equation, 8 and 9 are, respectively, the number of compounds n – 1), *s* is the standard deviation (the lower, the less error), q^2 is the predictive ability of the model (q^2= 0.862 in equation-3 means 86% predictive ability), and *P* is the probability to which the equation is the result of coincidence as

opposed to real correlation ($P < 0.01$ in equation-2 denotes a probability smaller than 1% that the correlation occurred by coincidence).

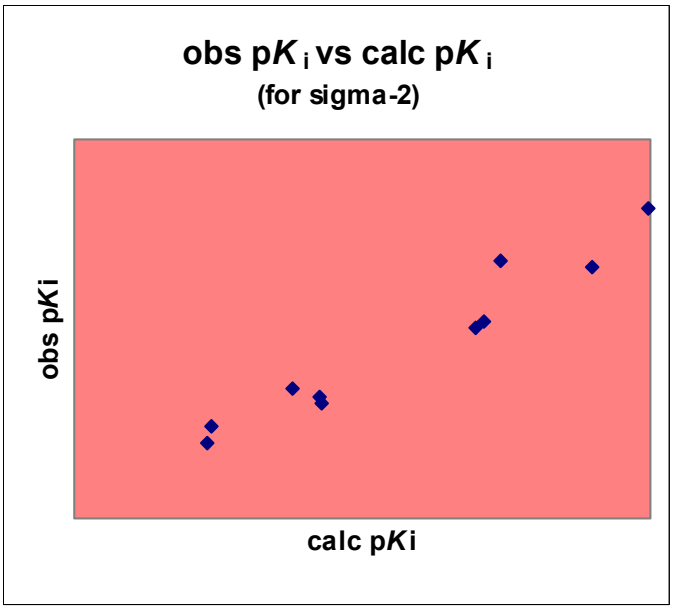
Table 13. *Actual log ($1/K_i$) (observed) values and the predicted log ($1/K_i$) (calculated) values of the same compounds from equations 1and 3 for σ_1 and σ_2 binding, respectively.*

Compound	Obsd pK_i (σ_1)	Calc pK_i (σ_1)	Obsd pK_i (σ_2)	Calc pK_i (σ_2)
1a (R= H)	0.02	-0.57	-1.54	-1.50
1b (R= 2-Br)	0.17	0.22	-0.61	-0.74
1c (R= 2-NO_2)	-0.39	-0.45	-0.58	-0.70
1d (R= 3-I)	0.41	0.41	-0.01	0.05
1e (R= 3-F)	-0.18	-0.13	-1.14	-1.24
1f (R= 3-OCH_3)	0.18	0.06	-1.15	-1.19
1g (R= 3-NO_2)	0.05	0.03	-0.20	-0.34
1h (R= 4-OCH_3)	-0.06	0.12	-1.52	-1.39
1i (R= 4-CH_3)	0.04	-0.07	-1.24	-1.15
1j (R= 4-NO_2)	0.40	0.43	-0.52	-0.30

pK_i (σ_1) and pK_i (σ_2) experimental values were plotted respectively against pK_i (σ_1) and pK_i (σ_2) equation predicted values. Graphs 10 and 11 are characterized by a relatively pseudo-straight line indicating a good correlation between the predicted activity and the actual activity; and consequently indicating visually the good quality of the QSAR equations.

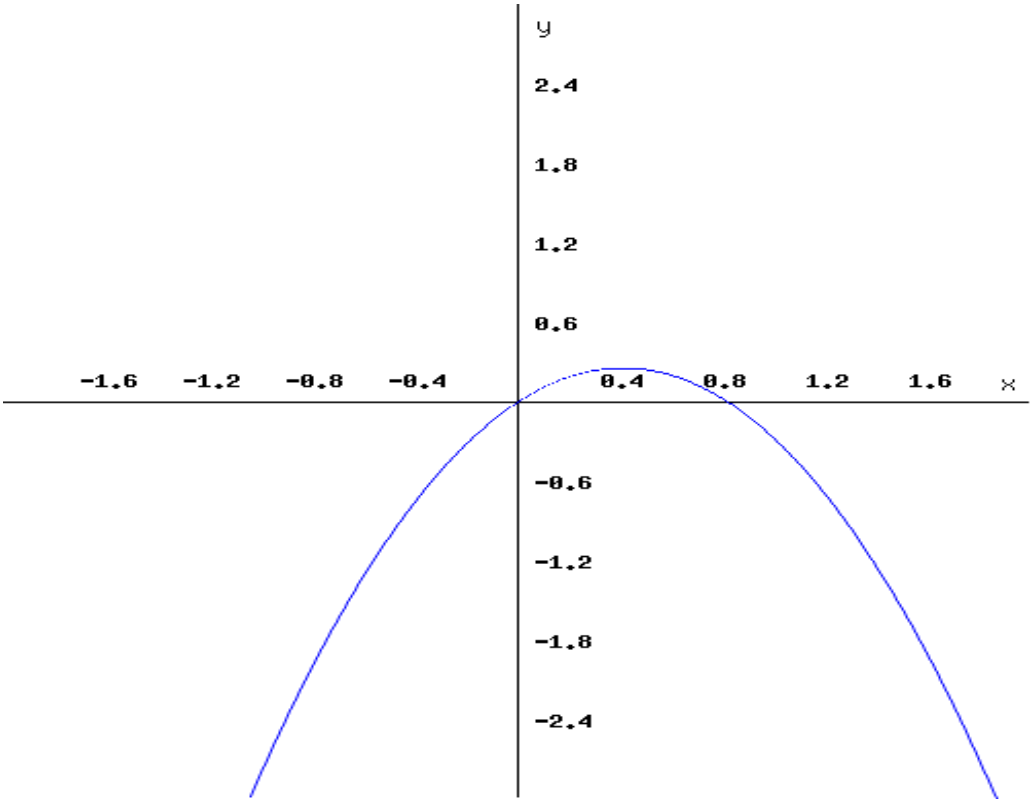


Graph 10. *Plot of actual or observed affinity against calculated or predicted affinity for σ_1 binding data based on equation 1.*

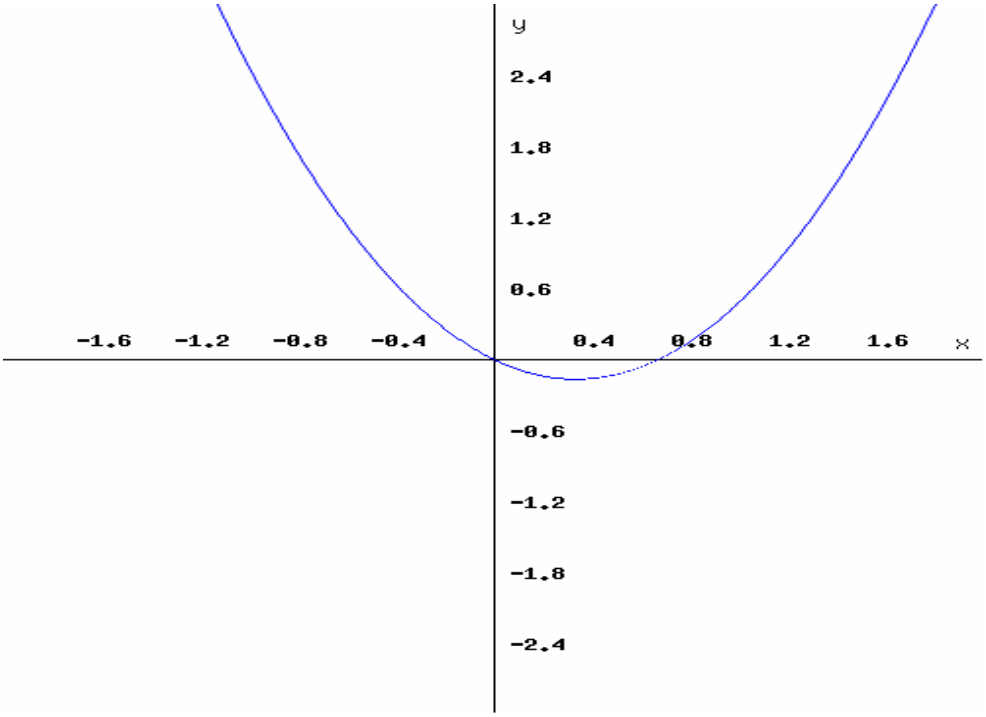


Graph 11. *Plot of actual or observed affinity against calculated or predicted affinity for σ_2 binding data based on equation 3.*

A major difference between the sigma-1 and sigma-2 QSAR correlation regression multivariate equations is the positive parabolic form of the hydrophobicity term of equations 2 and 3 as opposed to a negative sign in the case of equation-1. This constitutes a major binding pharmacophore difference between the two receptor subtypes, and how the hydrophobicity affects the binding of each. While the hydrophobicity might seem to affect the affinity in a similar manner for both subtypes within a limited range, the extrapolation of binding affinity versus hydrophobicity clearly underlines the difference.



Graph 12. *The binding affinity (pK_i) (y-axis) versus the hydrophobicity contribution (x-axis) in equation 1 for σ_1 binding.*

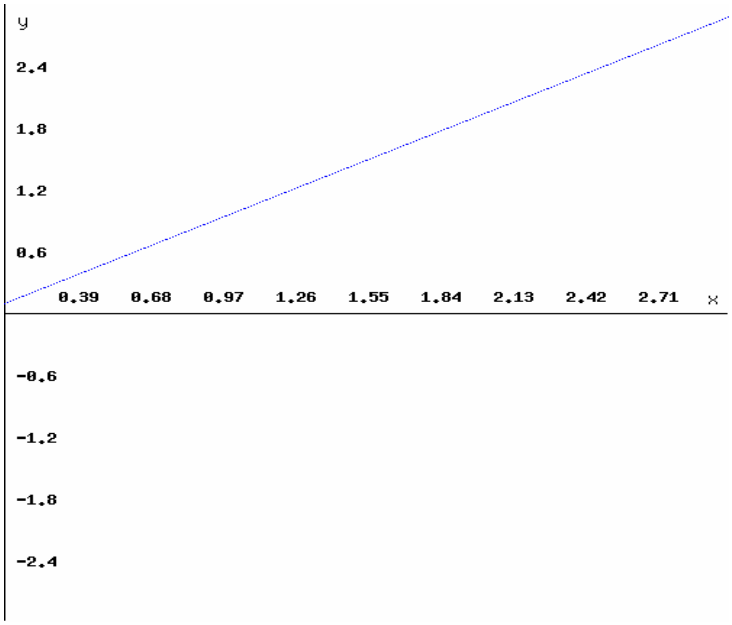


Graph 13. *The binding affinity (pK_i) (y-axis) versus the hydrophobicity contribution (x-axis) in equation 3 for σ_2 binding.*

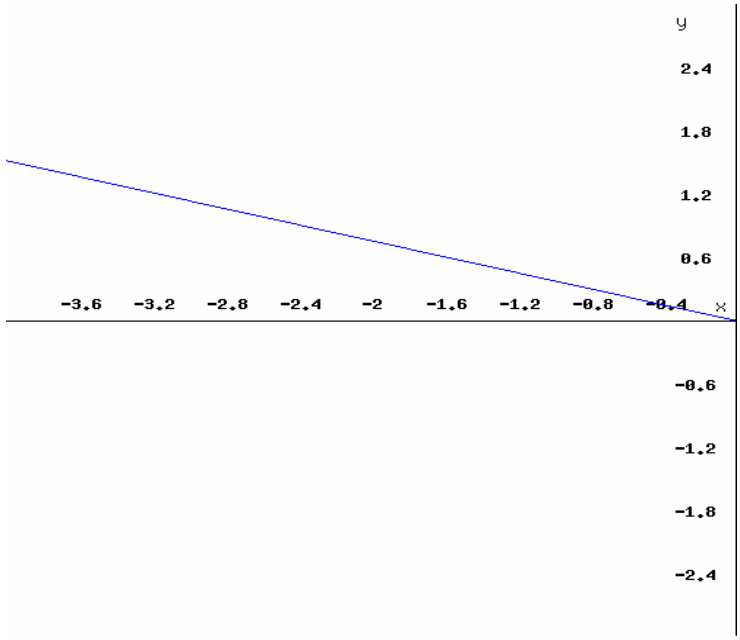
Graphs 12 and 13 represent, respectively, two parabolic equations of opposite signs (same magnitudes and weights as the ones in equations 1 and 3) for equations 1 and 3. The y axis has a range varying between -3 and +3 on the log scale (binding affinity ranging between 0.001 nM and 1000 nM). The x axis has a range varying between -2 and +2 on the hydrophobicity scale (π_x) (a very hydrophilic group such as $SO_2(NH_2)$ has a π_x of -1.82 and a highly lipophilic group such as C_6H_5 has a π_x of 2.0).

A second difference between the two subtypes appears from utilizing E_s in equations 2 and 3 instead of MR. While both MR and E_s account for the size of the substituent; E_s accounts also for the polarizability. This fact suggests that while size has a similar effect regarding both subtypes (as size increases, the affinity increases), the polarizability has a

more pronounced effect on sigma-2 than on sigma-1. The equation terms for MR (equation 1) and E_s (equation 3) suggest that the dependence of the binding affinity on the size is apparently linear for both subtypes.



Graph 14. *The binding affinity (pK_i) (y-axis) versus the size contribution (MR) (x-axis) in equation 1 for σ_1 binding.*

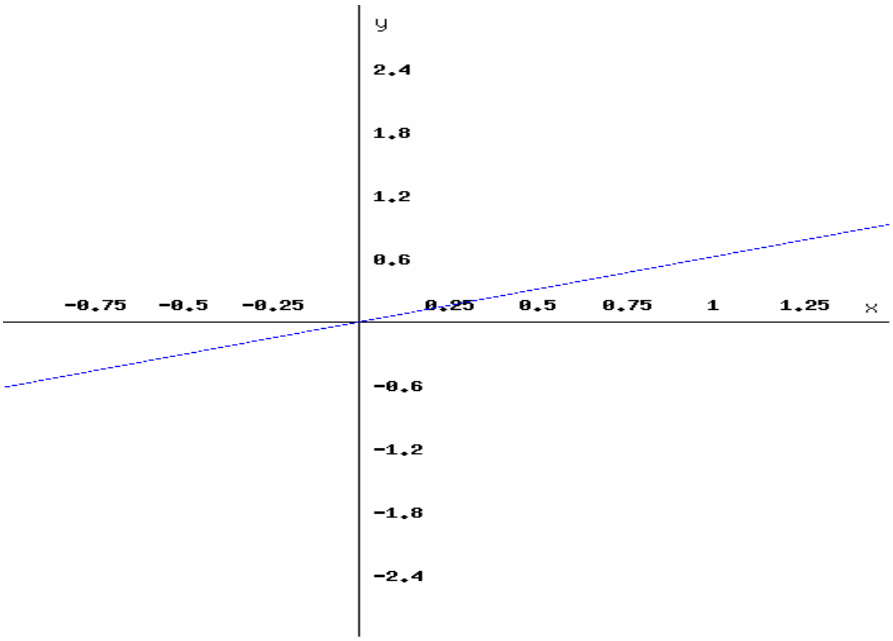


Graph 15. *The binding affinity (pK_i) (y-axis) vs. the size contribution (E_s) (x-axis) in equation 1 for σ_1 binding.*

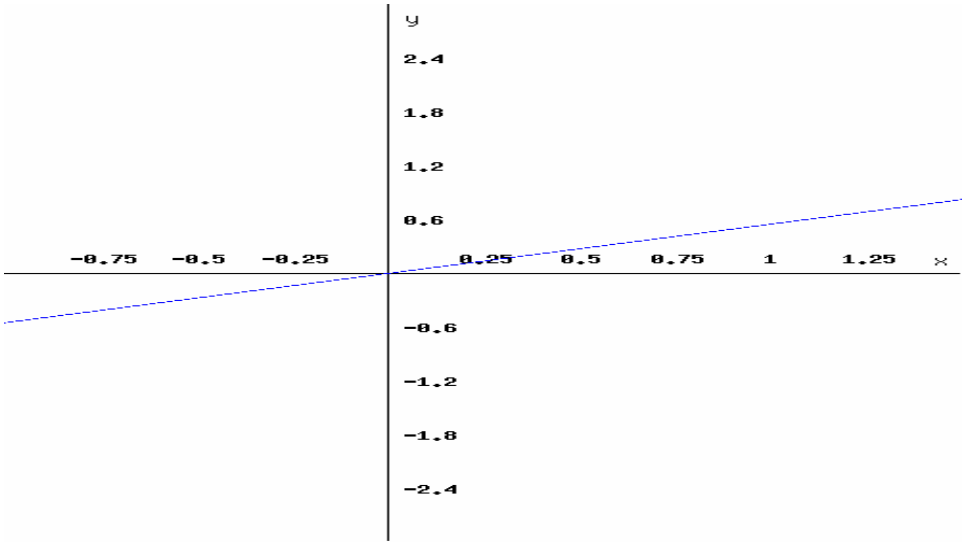
Graphs 14 and 15 represent, respectively, two linear equations of the same sign (same magnitudes and weights as the ones in equation-1 and equation-3). The y-axis has a range varying between -3 and +3 on the log scale (binding affinity ranging between 0.001 nM and 1000 nM). The x-axis in graph 14 has a range varying between 0 and 3 on the MR scale (a relatively large group such as CBr_3 has a MR of 2.88 and the smallest group is H and has a MR of 0.1). The x-axis in Graph 15 has a range varying between 0 and -4.0 on the E_s scale (a relatively large group such as CBr_3 has an E_s of -3.67, and the smallest is H as well, with an E_s equal to 0).

A third apparent difference stems from the comparison of the electronic characteristics parameter (σ Hammett parameter) in both equations (1 and 3). Both equations describe a binding affinity increase with electron withdrawing groups in the *meta* and *para* positions

(as $\sigma_{m,p}$ increases, the pK_i increases for both subtypes). Nevertheless, including σ_o values for the substituents in the *ortho* position (2-Br and 2-NO_2, respectively, **1.b** and **1.c**) improves only the quality of the correlation equation for sigma-2 binding, which signify the existence of a favorable interaction with the sigma-2 receptor, leading to slightly enhanced ligand binding.



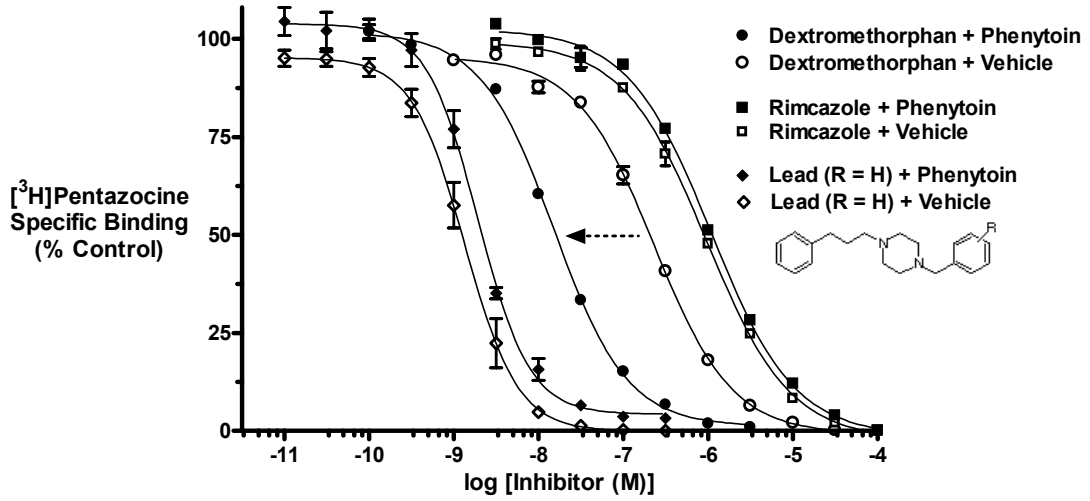
Graph 16. *The binding affinity (pK_i) (y-axis) vs. the electronic characteristics ($\sigma_{m,p}$) contribution (x-axis) in equation 1 for σ_1 binding.*



Graph 17. *The binding affinity (pK_i) (y-axis) vs. the electronic characteristics ($\sigma_{m,o,p}$) contribution (x-axis) in equation 3 for σ_2 binding.*

Graphs 16 and 17 represent respectively two linear equations of the same sign (same magnitudes and weights as the ones in equation-1 and 3). The y-axis has a range varying between -3 and +3 on the log scale (binding affinity ranging between 0.001 nM and 1000 nM). The x-axis has a range varying between -1 and 1.5 on the Hammett σ scale (a strong electron withdrawing group such as NH_2 has a σ_p of 0.94, while a strong electron withdrawing group such as $SO_2(Cl)$ has a σ_m of 1.20).

Agonist / Antagonist profiling.



Graph 18. *Specific binding % against the drug concentration (dextromethorphan (σ_1 agonist), rimcazole (σ_1 antagonist), and the lead compound in series-1 (compound **1a**, R= H)) in presence of DPH and in its absence (NaOH).*

As shown in Graph 18, we have validated the previously reported[72,73] 15-fold shift to higher affinity for the sigma-1 agonist dextromethorphan as its IC_{50} changed from 232 nM in absence of phenytoin to 15.2 nM in presence of phenytoin. Rimcazole (a low affinity sigma-1 receptor antagonist that can attenuate (-)-cocaine effects *in vivo*),[136] was also tested by Ms. Sarah Violand in Dr. John Lever's research laboratory. The binding affinity difference in the presence or absence of phenytoin was not significant; the IC_{50} ratio was less that unity as predicted.

Binding parameters for the parent compound (R=H, compound **1a**) in the presence and absence of phenytoin were not significantly different, but the ratio IC_{50} (with

vehicle)/IC_{50} (with phenytoin) was less than unity (0.8), as previously found for the sigma-1 antagonists haloperidol, NE100, BD1063, BD1047 and progesterone.[72,73]

The results of the 3-iodo (**1d**) and 4-methoxy (**1h**) compounds were consistent with the behavior of an antagonist in this agonist/antagonist assay.

This assay served as a quick and practical preliminary screening method to obtain information on the possible pharmacological profile of this family of structures, although agonist and antagonist properties are best determined in functional assays. These results are useful in determining the potential pharmacological application of these *N*-phenyl-*N'*-benzylpiperazines as possible blockers of some (-)-cocaine actions *in vivo*.

V.2 Series-3

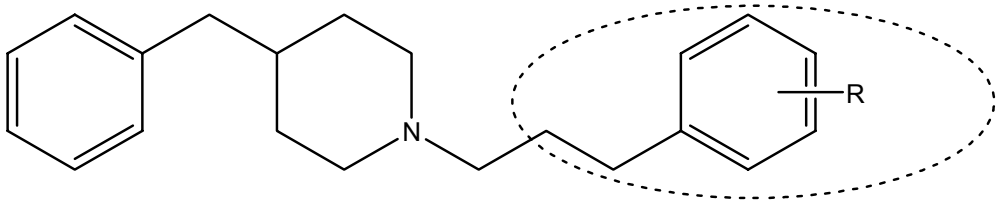


Figure 22. *Series-3 is the phenylpropyl substituted piperidine containing series of compounds*.

V.2.1-*Results*:

Table 14. *Series-3 affinity and subtype selectivity. Numbers are means (n= 3-6) ± SEM.*

Compound	IC_{50} (nM) σ_1	K_i (nM) σ_1	IC_{50} (nM) σ_2	K_i (nM) σ_2	Selectivity = $K_i \sigma_2 / K_i \sigma_1$
3b (R= 2-Br)	0.46 ± 0.02	0.32 ± 0.01	2.03 ± 0.13	1.81 ± 0.12	5.6
3c (R= 2-NO_2)	0.86 ± 0.03	0.60 ± 0.02	2.95 ± 0.27	2.62 ± 0.24	4.37
3d (R= 3-I)	0.50 ± 0.00	0.34 ± 0.00	1.07 ± 0.09	0.95 ± 0.08	2.79
3e (R= 3-F)	0.79 ± 0.11	0.56 ± 0.08	2.18 ± 0.33	1.94 ± 0.27	3.50
3f (R= 3-OCH_3)	0.94 ± 0.06	0.64 ± 0.04	4.10 ± 0.15	3.65 ± 0.14	5.70
3g (R= 3-NO_2)	0.95 ± 0.02	0.66 ± 0.01	0.81 ± 0.05	0.72 ± 0.04	1.09
3h (R= 4-OCH_3)	0.70 ± 0.07	0.49 ± 0.05	7.40 ± 0.39	6.59 ± 0.34	13.45
3i (R= 4-CH_3)	0.47 ± 0.01	0.33 ± 0.01	3.97 ± 0.60	3.53 ± 0.53	10.70
3j (R= 4-NO_2)	0.16 ± 0.00	0.11 ± 0.00	1.13 ± 0.12	1.00 ± 0.10	9.09

V.2.2-*Discussion*:

Qualitative SAR. All the *N*-phenylpropyl-benzylpiperidines displayed remarkably high sigma-1 binding affinities (K_i) varying from around 100 pM to 0.66 nM with 4-nitro (3**d**) (0.11 nM) exhibiting a particularly strong effect. On the other hand, a relatively wider range of affinities was observed for sigma-2 receptor binding ranging from 0.72 nM to 6.59 nM with the 3-nitro analog (**3g**) being the most potent (0.72 nM), and the 4-methoxy analog (**1i**) being the least potent with 6.59 nM. The relatively high affinities for the sigma-2 resulted in σ_1/σ_2 selectivities ranging from 2.79 to 13.45 toward the sigma-1 subtype.

The sigma-1 receptor binding was somehow sensitive in regard to the nitro substitution, with a 6-fold decrease in affinity observed over the 4-nitro (**3j**) on one side, and the 3-nitro (**3f**) and 2-nitro (**3c**) on the other (there was no notable change between the 2-nitro and the 3-nitro isomers). The sigma-2 binding affinity of the nitro isomers showed less than a 4-fold decrease in affinity observed over the 4-nitro (**3j**) and the 2-nitro (**3c**) (4-NO_2 (**3j**) > 3-NO_2 (**3f**) > 2-NO_2(**3c**)).

The analog with the electron-withdrawing 4-nitro substituent (**2i**) exhibited an increased affinity for both sigma-1 and sigma-2 receptor subtypes (0.11 nM for sigma-1 and 1.00 nM for sigma-2) in contrast to the electron-donating methoxy (0.49 nM for sigma-1 and 6.59 for sigma-2) and methyl groups (0.33 nM for sigma-1 and 3.88 nM for sigma-2) in the *para*-position, with a slightly greater detrimental effect on sigma-2 binding than sigma-1 binding (3-5 fold decrease for sigma-1 and 4-7 decrease for sigma-2).

The halogen series did not exhibit a trend between size, hydrophobicity and polarizability on one side, and sigma-1 affinity (2-Br (**2b**) > 3-I (**2d**) > 3-F (**2e**)). However, a trend was observed for sigma-2 (3-I (**2d**) > 2-Br (**2b**) > 3-F (**2e**)).

Quantitative SAR. Several equations were derived for sigma-1 and sigma-2 data. None was proven to be statistically significant based on the statistical parameters discussed in the QSAR section in Chapter 4 (Experimental Procedure) (r^2, F, t, P). Different approaches were tested: univariate (where the p$K_i(\sigma_1)$ and p$K_i(\sigma_2)$ were correlated with one parameter at a time (π_x, $\sigma_{m,p}$, MR). $(\pi_x)^2$ was also introduced to explore possible non-linear possible correlation. Subsequently, a bi-variate, tri- and multi-variate approaches were tested (two, three, and four parameters at a time, with all possible combinations). Finally, MR was replaced with E_s, and the same tests were performed again.

Table 15. *The QSAR analysis matrix for series-3 representing the different approaches applied.*

	Sigma-1				***Sigma-2***		
	R	**S**	***F***		***R***	**s**	***F***
$(\pi_x)^2$	0.23	0.24	0.44		0.44	0.41	1.95
π_x	0.146	0.24	0.17		0.46	0.42	1.58
MR	0.145	0.24	0.17		0.186	0.45	0.28
E_s	0.28	0.24	0.70		0.23	0.45	0.44
$\sigma_{m,p}$	0.33	0.23	0.9		0.03	0.46	0.00
	(univariate approach correlation matrix)						

	Sigma-1				***Sigma-2***		
	r	**S**	***F***		***R***	**S**	***F***
$(\pi_x)^2$, π_x	0.27	0.26	0.26		0.44	0.44	0.86
$(\pi_x)^2$, **MR**	0.23	0.26	0.19		0.48	0.43	1.05
$(\pi_x)^2$, E_s	0.29	0.25	0.31		0.45	0.44	0.90
$(\pi_x)^2$, $\sigma_{m,p}$	0.39	0.24	0.65		0.44	0.44	0.86
π_x, **MR**	0.17	0.26	0.10		0.41	0.45	0.70
π_x, E_s	0.28	0.25	0.30		0.41	0.45	0.70
π_x, $\sigma_{m,p}$	0.40	0.24	0.69		0.43	0.79	0.79
MR, $\sigma_{m,p}$	0.35	0.25	0.47		0.44	0.43	0.85
E_s, $\sigma_{m,p}$	0.39	0.24	0.64		0.23	0.48	0.20
	(bivariate approach correlation matrix)						

	Sigma-1				***Sigma-2***		
	r	**s**	***F***		***R***	**S**	***F***
$(\pi_x)^2$, π_x, **MR**	0.23	0.24	0.44		0.44	0.41	1.95
$(\pi_x)^2$, π_x, $\sigma_{m,p}$	0.146	0.24	0.17		0.46	0.42	1.58
$(\pi_x)^2$, **MR**, $\sigma_{m,p}$	0.40	0.26	0.39		0.48	0.46	0.60
π_x, **MR**, $\sigma_{m,p}$	0.145	0.24	0.17		0.43	0.48	0.46
$(\pi_x)^2$, π_x, E_s	0.30	0.27	0.19		0.45	0.47	0.51
$(\pi_x)^2$, E_s, $\sigma_{m,p}$	0.41	0.26	0.39		0.45	0.47	0.52
π_x, E_s, $\sigma_{m,p}$	0.42	0.26	0.42		0.43	0.48	0.72
	(tri-variate approach correlation matrix)						

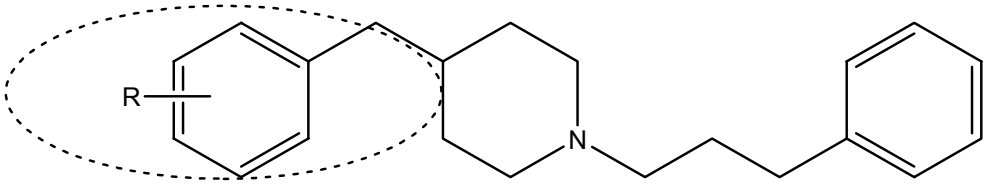
	Sigma-1				***Sigma-2***		
	R	**s**	***F***		***R***	**S**	***F***
$(\pi_x)^2$, π_x, **MR**, $\sigma_{m,p}$	0.40	0.29	0.25		0.49	0.50	0.40
$(\pi_x)^2$, π_x, E_s, $\sigma_{m,p}$	0.42	0.28	0.27		0.45	0.51	0.32
	(multivariate approach correlation matrix)						

None of the generated equations above met the acceptable criteria for a true predictive correlation equation. However, using r instead of r^2 gives a better sense of comparison among the various generated equations because of the wider range provided by r as opposed to r^2. Several observations can be made regarding those results:

The *r* and *F* values were higher for all the equations generated for sigma-2 binding, which means that there is a more obvious correlation between the studied physico-chemical parameters and the sigma-2 binding affinity, and a less obvious one between the parameters and the sigma-1 binding affinity. This can be attributed to the very narrow range of binding affinities for sigma-1 (0.11 nM-0.66 nM), which resulted in slightly better correlation coefficients for the equations describing the sigma-2 binding (although those were narrow as well being between 0.72 nM-6.69 nM).

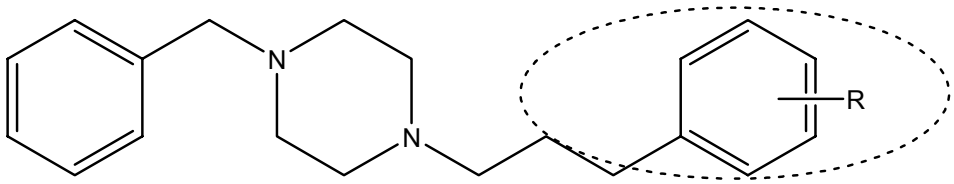
Another noteworthy observation is that the correlation coefficients of the equations with only the molar refractivity (MR)/Taft steric effect (E_s) and/or the Hammett sigma parameter ($\sigma_{m,p}$) showed the lowest *r* and *F* values (*r* = 0.03-0.39 and *F*= 0.00-0.44), which suggests that the hydrophobicity (both its terms $(\pi_x)^2$ and (π_x) correlate more closely with the binding affinity (towards both subtypes) than the size and the electronic characteristics parameters. However, this hydrophobicity contribution is not lucid enough to the extent of resulting in any equation with a predictive potential.

V.3 Series-2 and 4 selected compounds and piperazine versus piperidine SAR



Series-2

Figure 23. *Series-2 is the benzyl substituted piperidine containing series of compounds*.

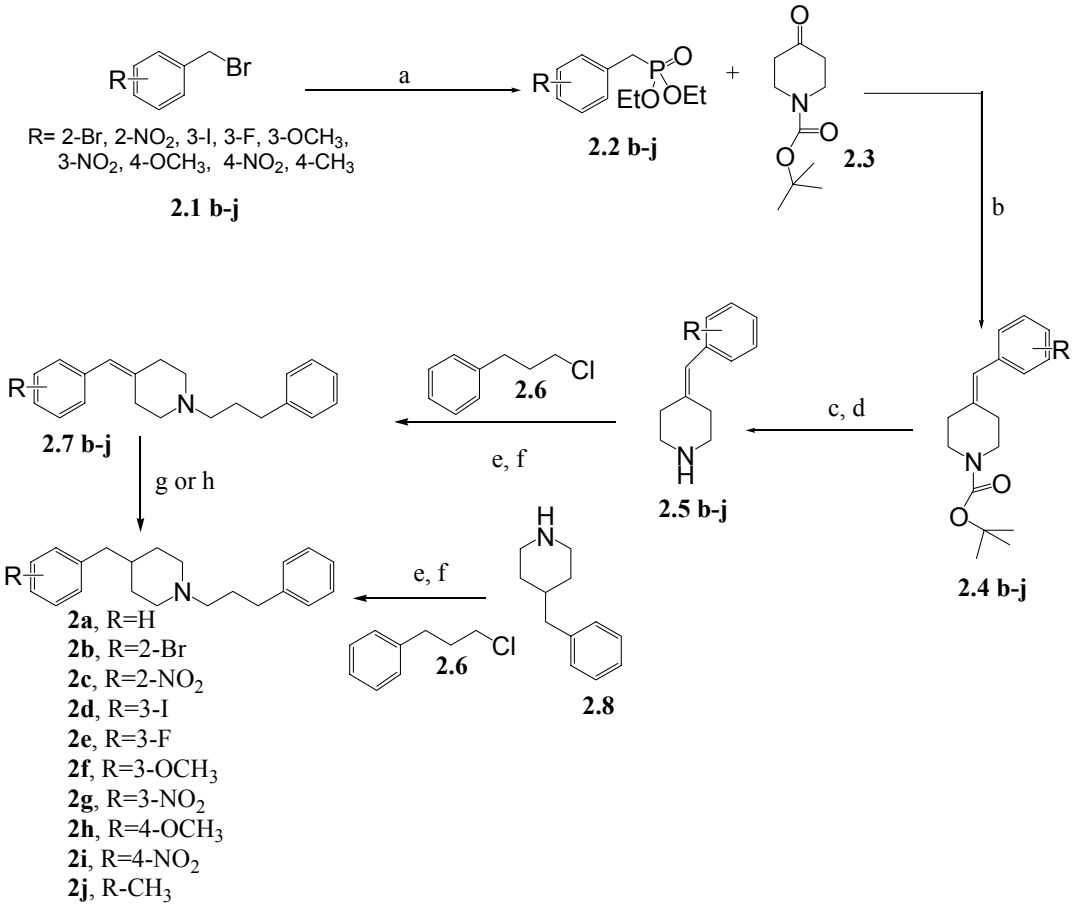


Series-4

Figure 24. *Series-4 is the phenylpropyl substituted piperazine containing series of compounds*.

V.3.1-*Results of selected compounds from series-2 and series-4*:

Synthesis of series-2 was successful for the 3-F, 3-OCH_3, 4-NO_2 and 4-CH_3 benzyl substituted piperidine compounds (respectively, **2d**, **2f**, **2i** and **2j**), was not successful for the 2-Br, 2-NO_2, 3-I and compounds (respectively, **2b**, **2c** and **2e**), and was not attempted for the 3-NO_2 and 4-OCH_3 compounds (respectively, **2g** and **2h**), although the corresponding precursors are present (respectively, **2.7g** and **2.7h**).



Reagents: (a) $(OEt)t_3P$; (b) NaH; (c) TFA; (d) CH_2Cl_2; (e)NaI; (f) K_2CO_3; (g) H_2/Pd-5%; (h) Wilkinson's catalyst

Figure 25. *Detailed synthetic scheme for series-2 compounds*.

The first step which included the preparation of the benzyl substituted phosphonate derivatives from the appropriate benzyl bromides and triethyl phosphite worked out well for all compounds, with yields ranging from 70-91%. The second step consisted of the Wittig-Horner reaction to attach the substituted benzyl bromides to the 4-end of the piperidine moiety. Although precautions were taken in order to dry and remove any residual humidity or solvents from the flasks or starting materials, and slightly different concentrations of sodium hydride were assayed, as well as different order of adding the reagents and reactants, the yields for that step were the lowest (15-41%) compared to the

other steps of the synthetic route, which affected the overall consequent masses for all subsequent steps. The third step consisted of deprotecting the BOC protecting group under acidic conditions. The original plan was to utilize hydrochloric acid with 1,4-dioxane in MeOH, because this combination seems to work for BOC piperazines. However, it did not seem to work well for this set of piperidine derivatives, especially for the lipophilic analogs like the 3-iodo substituted derivative, which might be due to the fact that piperidines do not dissolve as well in MeOH as piperazines. Alternatively, a combination of DCM and THF was used, and deprotection was successful for all analogs, in yields between 73-89%. The subsequent step was the alkylation of the substituted benzyl piperidine with a phenylpropyl bromide, and occurred for all compounds in yields varying between 58-75%. Perhaps the yields were slightly low with respect to the same alkylation steps of the piperazine analogs from series-1, but that is probably because the masses of the starting materials were small (in the order of 100 mg) which contributed in decreasing the yield.

The last step, which consisted of reducing the double bond of the final precursor, was not the same for all compounds. Catalytic hydrogenation with Pd on carbon was supposed to reduce the olefinic double bond rather than reducing a halogen (including I and Br). The original intention was to reduce all precursors with Pd on carbon, with the exception of the nitro substituted precursors, where a more selective catalytic hydrogenation is to be used (specifically with Wilkinson's catalyst, which reduces the double bond selectively in the presence of nitro groups according to literature[126]). The ^{1}H NMR spectra of the 2-bromo compound (**2b**) showed a mixture of the precursor and the product, which were difficult to isolate efficiently. The spectroscopic data of the 3-iodo (**2d**) showed the

reduction of the iodine and its replacement by a hydrogen occurring first, followed by the reduction of the double bond. On the other hand, it seems that the 3-fluoro, and 3-methoxy (respectively, **2e** and **2h**) analogs resisted the reduction, which occurred exclusively at the olefinic bond.

Regarding the nitro compounds, Wilkinson's catalyst seemed to work for the 4-NO_2 (**2j**) compound, but not for the 2-NO_2 (**2c**) as the ^{1}H NMR spectra showed the starting material in a dominant ratio compared to the product).

At that stage, it was decided to stop working on the synthesis of these compounds, in view of the results of the binding assays from series-1 and series-3. Series-1 compounds (benzyl substituted piperazines) gave us a good idea on how the benzyl moiety substitution affects the binding, and so in order to predict the behavior of the benzyl substituted piperidines, it is needed to understand the behavior of corresponding piperazine analogs in comparison. Hence, it was chosen to pursue this study with the 3-OCH_3 analogs, in all four series, as well as the comparison of the unsubstituted piperidine compound with the unsubstituted piperazine compound.

All series-4 compounds (substituted phenylpropyl piperazines) were synthesized as planned by Mr. Yu Lu (*Ligands for the Sigma receptors and the μ-opioid receptor*), among which, the 3-OCH_3 analog (**4g**) was tested. However, the other compounds are available for testing if needed.

In case compounds of series-2 will be required to conduct further studies on them, an alternative reduction of the precursor might be possible. This alternative step includes

reducing the olefin (in the bromo, nitro, and iodo phenyl substituted derivatives) with potassium diazocarboxylate (PADA),[137] which can be prepared from azodicarbonamide in aqueous KOH solution according to modified procedures.[138]

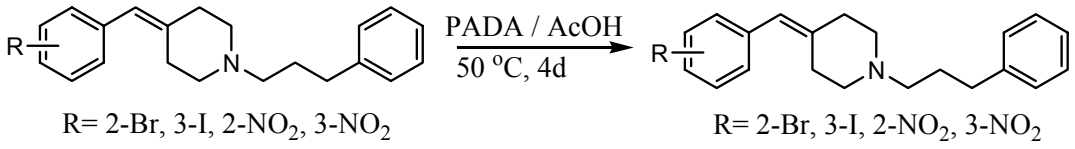


Figure 26. *Alternative method for the selective reduction of olefin in presence of sensitive groups on the phenyl ring.*

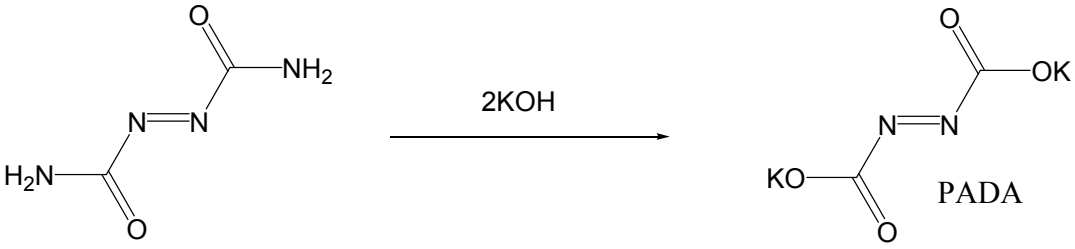


Figure 27. *Preparation of PADA.*

If the 3-iodo substituent does not survive this selective reduction, an alternative method to synthesize the 3-iodo compound (**2d**) would be through the Sandmeyer reaction, by first synthesizing the 3-NO_2 analog (**compound 2f**) by PADA selective reduction from its olefinic precursor, then reducing the nitro substituted compound by catalytic hydrogenation with Pd on carbon into its corresponding amine. Subsequently, the amino substituted intermediate is converted into the diazonium salt by sodium nitrite, and finally the diazonium salt will decompose under copper catalysis and in presence of sodium iodide to give the corresponding iodo substituted compound (**2d**).

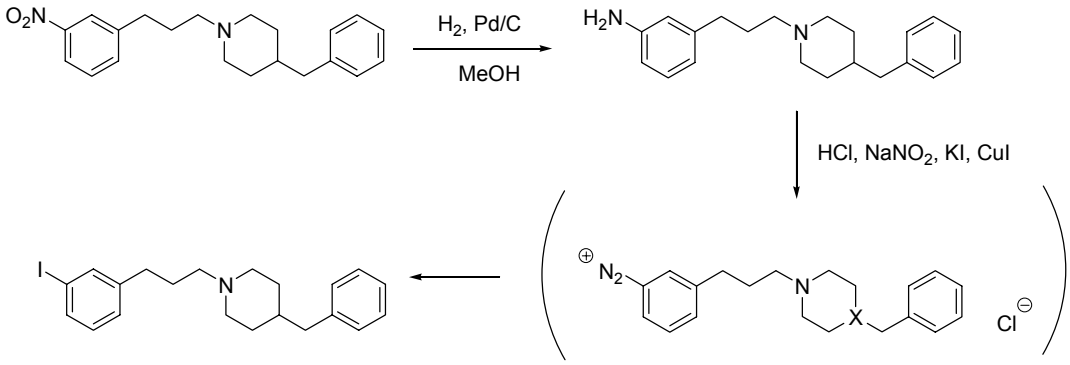


Figure 28. *Sandmeyer reaction as an alternative method for the preparation of the iodo substituted compound.*

Table 16. *Affinity and subtype selectivity of selected compounds. Numbers are means (n= 3-6) ± SEM.*

Compound	**IC_{50} (nM)** **σ_1**	**K_i (nM)** **σ_1**	**IC_{50} (nM)** **σ_2**	**K_i (nM)** **σ_2**	**Selectivity = $K_i \sigma_2 / K_i \sigma_1$**
2a (R= H)	0.6 ± 0.03	0.38 ± 0.02	3.88 ± 0.02	3.50 ± 0.02	9.21
2f (R= 3-OCH_3)	0.48 ± 0.07	0.33 ± 0.05	3.40 ± 0.47	3.03 ± 0.42	9.18
4f (R= 3-OCH_3)	0.99 ± 0.02	0.68 ± 0.02	29.70 ± 2.89	26.43 ± 2.57	5.70
Lead 2 + NaOH	0.59 ± 0.1	0.41 ± 0.07			
Lead 2 + DPH	0.74 ± 0.06	0.52 ± 0.04			

V.3.2-*Qualitative SAR and discussion*:

Assessing the binding affinity of series-1 and series-3 compounds, and consequently establishing structure-activity relationships provided information regarding the effect of the benzyl and phenylpropyl moieties on both sigma-1 and sigma-2 binding. In order to establish a relationship between the effect of one or two nitrogen atoms in the central

moiety (piperidine or piperazine) and link the four series all together, it was decided to proceed only with selected compounds from series-2 and series-4 and study their binding affinity. We specifically chose the 3-OCH_3 analogs to compare because of the physico-chemical properties of this substituent, which represent the central values in term of size, electronic characteristics and hydrophobicity (see table 16 below).

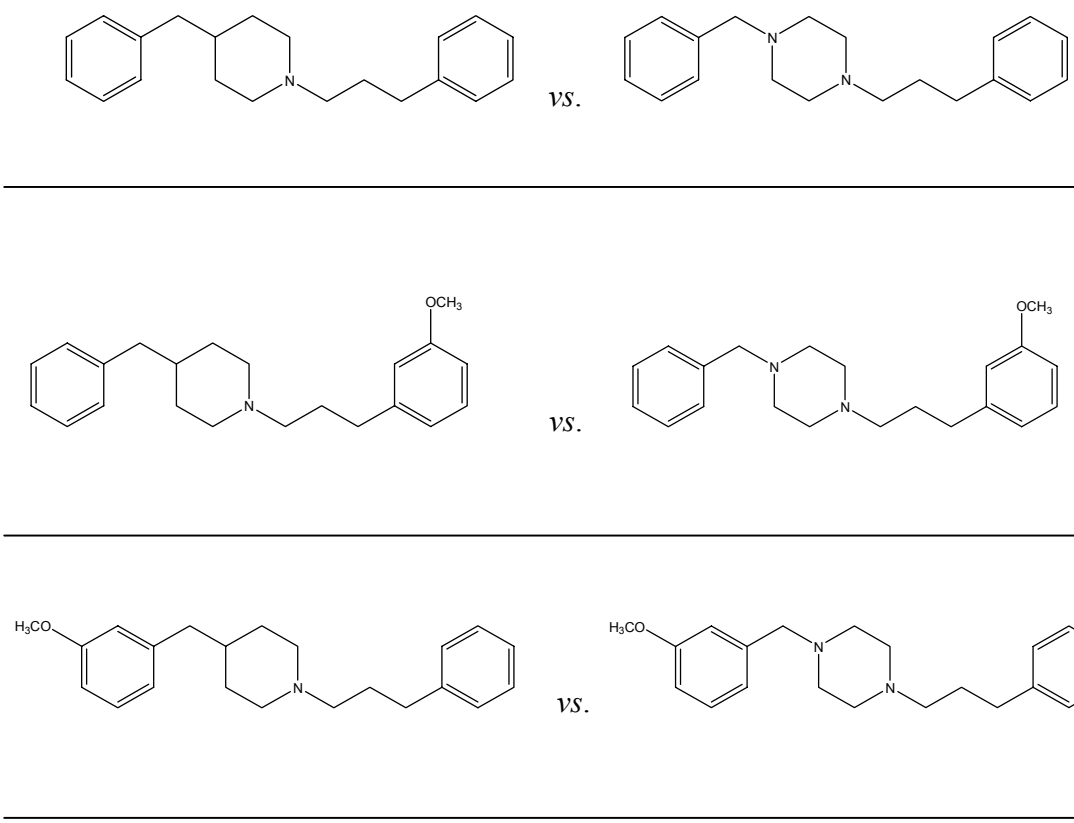


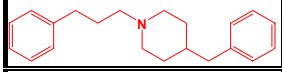
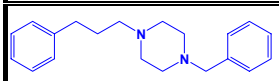
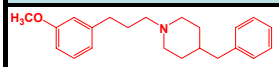
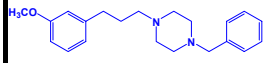
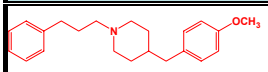
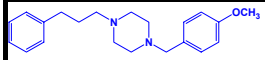
Figure 29. *Structural representation of the compounds for the qualitative SAR.*

Table 17. *Physico-chemical properties of the 3-OCH_3 substituent.*

R	σ	Πx	MR	Levels
3-OCH_3	0.12	-0.02	0.79	**0 0 0**

The compounds that we selected for the comparison as well as their binding affinities for sigma-1 and sigma-2 are represented in the table below:

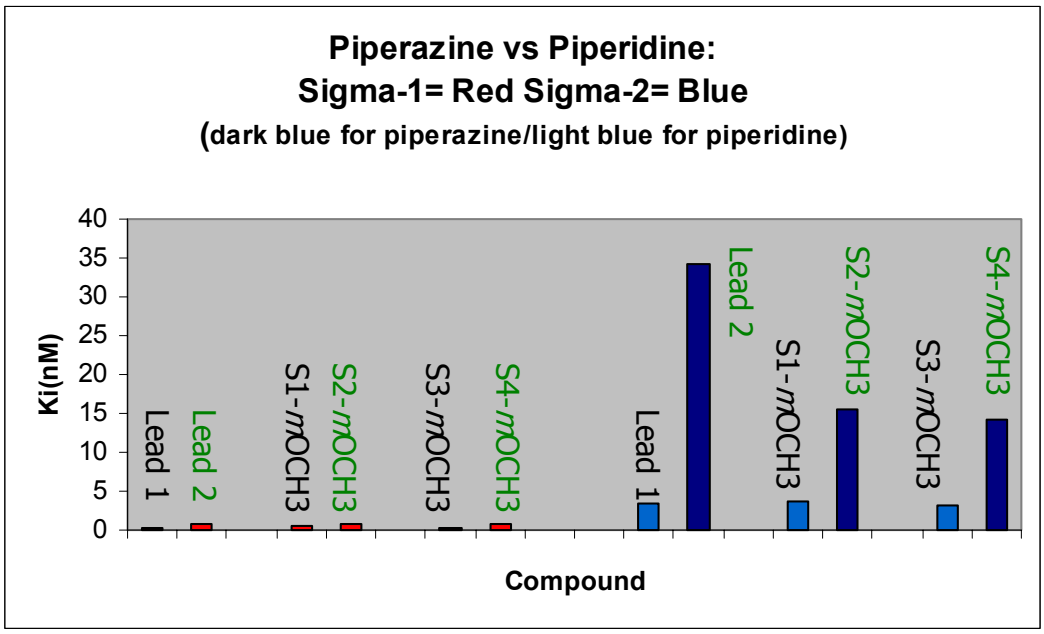
Table 18. *Affinity and subtype selectivity of compounds selected for qualitative SAR.*

Compound	*Ki σ_1 (nM)*	*Ki σ_2 (nM)*	*Ki σ_2 / Ki σ_1*
	**0.38**	**0.8**	**9.21**
	**0.8**	**34.33**	**42.91**
	**0.64**	**3.65**	**5.70**
	**0.68**	**26.43**	**38.87**
	**0.33**	**3.03**	**9.18**
	**0.87**	**14.19**	**16.31**

As all the sigma-1 binding affinities varied between 0.33 and 0.87 nM, there did not seem to be a fundamental difference in the sigma-1 binding affinity of compounds with the piperidine moiety in comparison to the compounds with a piperidine moiety.

In contrast, there has been a big difference in sigma-2 binding affinity of the compounds with a piperidine moiety, and the ones with a piperazine moiety. The three compounds with a piperidine moiety showed five to 43-fold higher sigma-2 affinities than the ones with a piperazine moiety. This observation consisted of the lead unsubstituted compounds (43-fold) as well as the phenylpropyl 3-methoxy substituted compounds (7-fold), and the benzyl substituted 3-methoxy compounds (5-fold).

In conclusion, it seems that the one nitrogen in the central moiety (piperidine) results in a high affinity for both subtypes. However, two nitrogen atoms (piperazine) exhibit more or less the same sigma-1 affinity, but significantly lower sigma-2 affinity.



Graph 19. *Representation of the binding affinity of all compounds used for comparison.*

This graph shows the significant variation within the sigma-2 affinities between the compounds with piperazine moiety (dark blue) and piperidine moiety (light blue), and the

non-significant variation within the sigma-1 affinities between compounds with piperidine and piperazine moiety (both in red).

CHAPTER VI:

EVALUATION OF RESULTS, CONCLUSIONS AND FUTURE GOALS

VI.1 Prediction power of the QSAR equations, limitations, and future goals for series-1 compounds

The sigma-1 affinity prediction equation form is $pK_i = -b(\pi_x)^2 + c\pi_x + dMR + e\sigma + f$ as opposed to $pK_i = b'(\pi_x)^2 - c'\pi_x - d'E_s + e'\sigma + f'$ for sigma-2. Besides the fact of how different those equations are in terms of explaining the pharmacophore profile and the interactions of the ligands with the proteins, which was discussed in the previous section; the only algebraic difference between the two equations resides in the sign of the hydrophobicity terms ($-b(\pi_x)^2 + c\pi_x$ as opposed to $b'(\pi_x)^2 - c'\pi_x$). The other apparent difference is +dMR as opposed to $-d'E_s$ but that is not a real difference because the MR and E_s values for the same substituents are almost of the same magnitude but of different sign, which will make the overall sign identical in both cases. This algebraic hydrophobicity difference can be used to design sigma-1 or sigma-2 selective ligands.

Substituents with π_x values < 0 will have a high sigma-2 affinity and a low sigma-1 affinity (the lower the π_x, the higher the selectivity for sigma-2).

Substituents with π_x values > 0.66 will have high affinity for sigma-2, and they will have a low affinity for sigma-1 when $\pi_x > 0.817$ (the higher the π_x, the higher selectivity for sigma-2).

The sigma-1 affinity increases with π_x values in the range of 0-0.817 (highest affinity with π_x= 0.4). In contrast, the sigma-2 affinity decreases with π_x values in the range of 0-0.66 (lowest affinity with π_x= 0.66).

According to the hydrophobic contribution to the binding affinity, a very hydrophilic or very hydrophobic substituent would result in a sigma-2 selective ligand (the more hydrophilic or hydrophobic, the more sigma-2 selective). A substituent with a π_x in the range of 0.33-0.40 would result in an optimum sigma-1 selective ligand. Nevertheless, the contribution of the hydrophobicity within such an interval where π_x is between 0 and +1 is small because $(- b(\pi_x)^2 + c\pi_x$ and $b'(\pi_x)^2 - c'\pi_x)$ will yield a small number as the second degree term and the first degree term will cancel out each other.

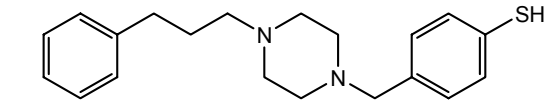
However, and regardless of the cases described above, the other descriptors also play a role: the bigger the substituent (within limits defined in Chapter III), and the more electron withdrawing it is, the higher the affinity (for both subtypes).

In conclusion, as shown by the correlation equations, it is easier to design a predicted sigma-2 selective compounds, when using a highly lipophilic or hydrophilic substituent. For instance, 3-SH, which has a π_x of 0.39 (within the optimal hydrophobicity range for sigma-1 selective ligands), MR of 0.92, E_s of -1.07, and a σ_m of 0.25[130] has a projected K_i of 0.6-0.7 nM for sigma-1 and 2-3 nM for sigma-2 according to equations 1 and 3, resulting in a sigma-1 selectivity of only 3-5 fold.

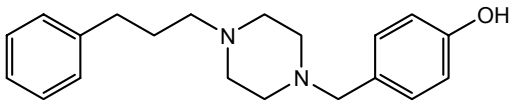
On the other hand, a substituent such as 4-OH with a π_x of -0.67, MR of 0.28, E_s of -0.55, and a σ_p of -0.37[130] would have a sigma-1 affinity of 125-135 nM and a sigma-2 affinity of 1-1.5 nM, resulting in almost 100-fold sigma-2 selectivity.

A substituent such as 4-CH_2OH with a π_x of -1.03, MR of 0.72, E_s of -1.21, and a σ_p of -0.05[130] would have a sigma-1 affinity close to 700 nM and a sigma-2 affinity of about 0.05 nM, resulting in highly selective sigma-2 ligand.

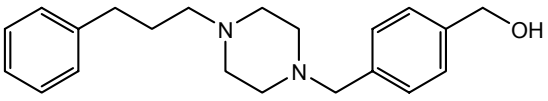
Finally, a highly hydrophilic substituent such as 4-NH_2 (π_x= -1.23, MR= 0.54, E_s= -0.61, and σ_p= -0.66[130]) would have a sigma-1 affinity > 1000 nM and sigma-2 affinity < 1 nM, which theoretically results in an extremely selective sigma-2 ligand.



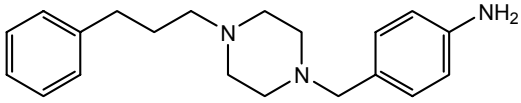
predicted K_i= 0.67 nM (sigma-1) and K_i= 2.4 nM (sigma-2)



predicted K_i= 130 nM (sigma-1) and K_i= 1.44 nM (sigma-2)



predicted K_i= 700 nM (sigma-1) and K_i= 0.03 nM (sigma-2)



predicted K_i > 700 nM (sigma-1) and K_i < 0.03 nM (sigma-2)

Figure 30. *Structures and predicted binding affinities for possible ligands with promising σ_1 and σ_2 affinities and selectivities*.

Like other empirical relationships, extrapolations can frequently lead to false predictions. In spite of that, a future goal for this part of the project is synthesis of those aforementioned sigma-2 ligands. Series-1 synthesis scheme in Figure 17 (p. 44) can be used as a straight forward synthetic route. However, hydroxyl and amino substituents in

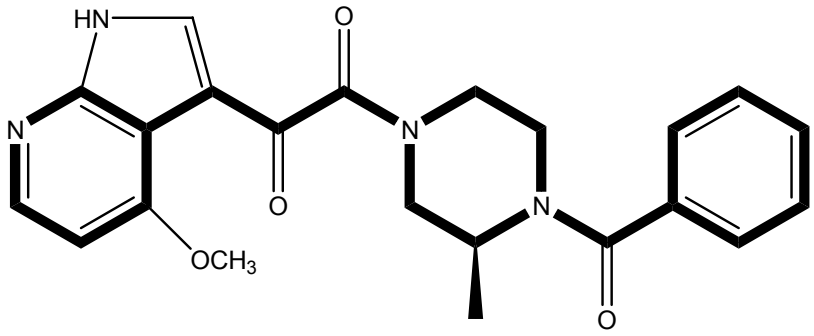
general are not known to behave well in a series of congeners and several times they do not exhibit the predicted activities, and hence become outliers. This fact is often attributed to the strong hydrogen-bonding ability of these substituents, which can overshadow the studied physico-chemical parameters, and consequently mask the predicted behavior. Another potential obstacle lies in the fact that all used physico-chemical constants are determined at acidic or basic pHs, where all the structures are fully protonated or fully deprotonated. The assays are done at a pH varying between 7 and 8, which is close to the pK_a values of some of the studied analogs. This results in a medium which is different that the one used to determine experimentally the physico-chemical parameters.

Another possible future project related to series-1 compounds is synthesizing few anti-HIV analogs similar in structure to the compounds occurring in our study, and testing their sigma receptor potency.

Currently, there are four FDA approved classes of drugs to combat HIV infection: nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, and one fusion inhibitor.[139] Researchers at Bristol-Meyers Squibb designed a potent small molecule viral-entry inhibitor, BMS-378806. That was one of the few successes known so far in designing a small molecule that inhibits HIV entry.[140] This compound does not interact with any of the receptors known to inhibit viral entry. The mechanism of the mode of action of this drug was not clear, and a unique mode of action was proposed for a series of similar, yet smaller, compounds.[141]

HIV infection of CD4+ lymphocytes and release of virions occurs in lipid rafts; cholesterol- and sphingolipid-rich microdomains of the plasma membrane. It has been shown that reducing membrane cholesterol content also reduces HIV infectivity of lymphocytes and diminishes virulence of the virions released. Reducing membrane sphingomyelin may produce similar effects. It has been shown that sigma-2 receptor-activation reduces levels of membrane sphingomyelin in breast tumor cells. It has also been found that the sigma-2 receptors are localized in lipid rafts. In addition, sigma-2 receptor activation may inhibit P-I-3' kinase signaling, an effect that should inhibit HIV infection of lymphocytes and macrophages.[142,143]

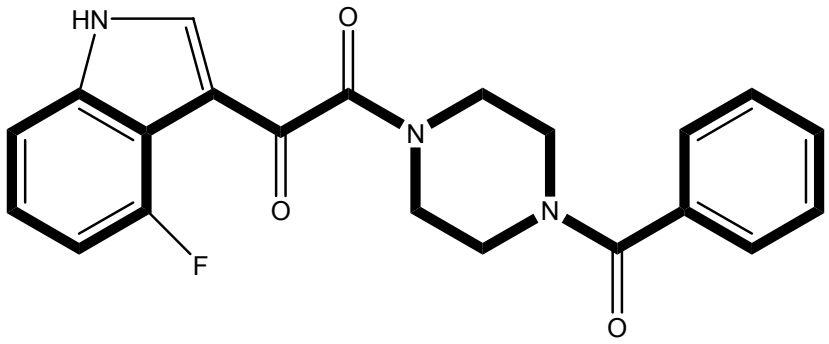
Due to the structural similarity between BMS-378806 (1-(4-benzoylpiperazin-1-yl)-2-(4-methoxy-1H-pyrrolo[2,3-b]pyridin-3-yl)ethane-1,2-dione) and Lead 1, BMS-378806 might show potency for sigma receptors, which can explain and add more information towards the mode of action of this drug, and towards the rising theory that sigma-receptor agonists might have anti-HIV activity. The structural differences between BMS-378806 and Lead 1 reside only in the non-pharmacophoric region, where it has proven that bulk is tolerated (sigma-1 and sigma-2 pharmacophores).



1-(4-benzoylpiperazin-1-yl)-2-(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)ethane-1,2-dione

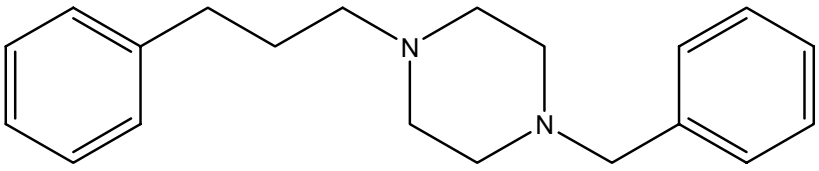
BMS-378806

Moreover, another analogue of BMS-378806 (the 4-fluoro derivative that lacks the methyl substitution on the piperazine moiety) was more potent than the latter, but had poor pharmaceutical properties. This derivative is even more structurally related to Lead 1 than BMS-378806.



1-(4-benzoylpiperazin-1-yl)-2-(4-fluoro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)ethane-1,2-dione

2-F Derivative



Lead 1

VI.2 Conclusions on the SAR of series-3, and future plans for series-4

All the compounds of series-3 exhibited subnanomolar affinities for sigma-1 (< 0.66 nM), and high affinities for sigma-2 as well (< 6.5 nM). The variation in binding affinity between various analogs did not exceed six-fold (for both subtypes) (the variation was 7 to 33-fold for series-1 compounds). While some qualitative SAR were drawn in regard to the sensitivity of the nitro substitution, and the increased affinity of the analog with the electron withdrawing 4-nitro substituent in contrast to the electron-donating methoxy and methyl groups, no high quality quantitative SAR were established in terms of statistics parameters. However, two observations were realized: it appeared that hydrophobicity had more impact on the binding affinity than the size and electronic characteristics. And the statistics parameters for the sigma-2 binding were relatively better than the ones for sigma-1. Nevertheless, these observations cannot be used to predict the activity of new compounds.

Three different reasons might be behind not getting QSAR equations for series-3 compounds equally statistically valid as series-1 equations:

A) The relationships between the parameters are neither linear nor parabolic (i.e., they can be logarithmic, exponential, 3rd degree, etc.). Such relationships are highly unexpected while studying interactions of this type. They would not be potentially exploitable either in terms of understanding the pharmacophore and trying to translate the mathematical terms into pharmacological interactions.
B) The descriptors used were not representative of the type of interactions between the ligands and the receptors. This is not likely either, as those same descriptors

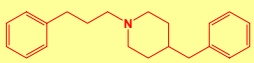
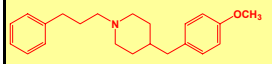
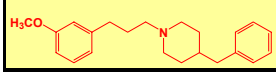
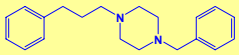
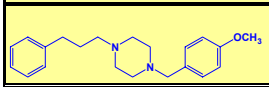
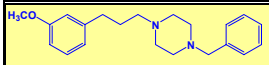
worked well for series-1 (structurally similar). Besides, the descriptors used were general, and usually substituting a descriptor with another can improve the quality of an equation, but the equations of series-3 compounds were not even close to being statistically valid.

C) The variation interval was too narrow to understand how the physico-chemical parameters controlled the binding affinity. The compounds were designed such that each covers a distinctive area of the numerical scale of the three physico-chemical parameters. However, the binding affinity values that resulted for series-1 had a very tight range (less than 0.5 nM for sigma-1 and less than 6 nM for sigma-2). This lack of binding affinity variation can have two different explanations:

    1) The phenylpropyl moiety is not very sensitive towards the substitution on the phenyl ring due to the pharmacophore requirements (i.e., lack of a pocket in the hydrophobic region of the protein, which prevents such interactions with a substituent).
    2) There is a substitution effect; however, it is masked by the very strong binding affinity of the skeleton of the lead compound (Lead 1). In other terms, the phenylpropyl is sensitive for substitution, but that effect is less than the one of the original skeleton.

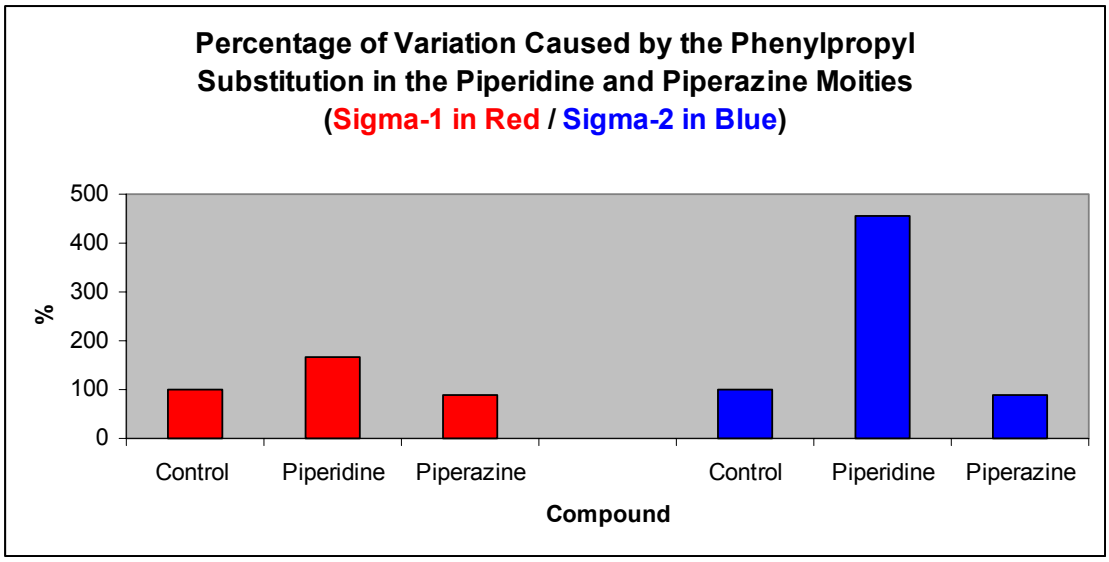
In order to determine which of these two explanations is the most probable, the effect of the benzyl and phenylpropyl 4-methoxy substitution on the binding affinity was analyzed in comparison to the unsubstituted compound. This illustrates how sensitive is the substitution on each phenyl ring in each moiety (piperidine and piperazine).

Table 19. *Binding affinity variation caused by the substitution at each phenyl ring, in each model (piperidine or piperazine) percentage wise and nM wise.*

Compound	***Ki σ_1 (nM)*** (variation caused)	***Ki σ_2 (nM)*** (variation caused)
	0.38 (*control*)	0.8 (*control*)
	0.33 **(*-13%*) (*-0.05 nM*)**	3.03 **(*+312%*) (*+2.5 nM*)**
	0.64 **(*+68%*) (*+0.26 nM*)**	3.65 **(*356%*) (*+2.85 nM*)**
	0.8 (control)	34.33 (control)
	0.87 **(*+9%*) (*+0.07 nM*)**	14.19 **(*-59%*) (*-20.14 nM*)**
	0.68 **(*-18%*) (*-0.12 nM*)**	26.43 **(*-22%*) (*-7.60 nM*)**

Percentage wise, the phenylpropyl moiety substitution with 4-methoxy causes a 68% (sigma-1) and a 356% (sigma-2) variation (piperidine moiety), and 18% (sigma-1) and 22% (sigma-2) (piperazine moiety). However, and regardless of the percentage variation, the actual variation in nM is very small for the piperidine moiety compared to the piperazine moiety (specifically for sigma-2).

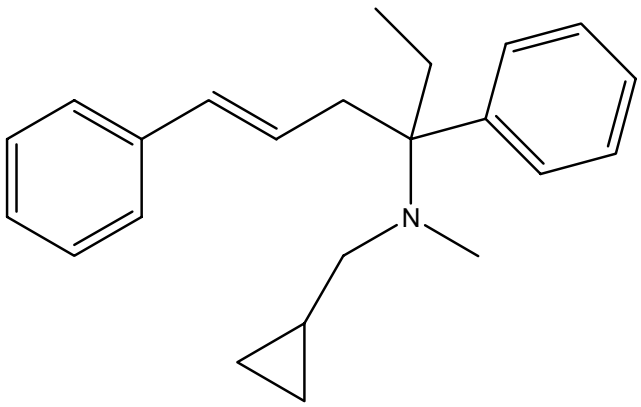
All this might suggest that there is a substitution effect on the phenylpropyl and it might be more obvious on the phenylpropyl moiety attached to a piperazine moiety (series-4). Assessing the binding affinity constants for all the compounds of that series can answer that question.



Graph 20. *Percentage of the variation caused on the K_i (σ_1 and σ_2) by the phenylpropyl substitution in the piperidine and piperazine moieties.*

A future project for the compounds of series-2 that have not been assessed stems from the following idea:

Igmesine



(*E*)-*N*-(cyclopropylmethyl)-*N*-methyl-3,6-diphenylhex-5-en-3-amine

Figure 31. *Structure of igmesine.*

Igmesine is a well know selective sigma-1 agonist, proven to possess anti-depressant effects and delays memory deficit.[63,144-146]

There is a structural resemblance between igmesine and the final precursors of series-2 compounds:

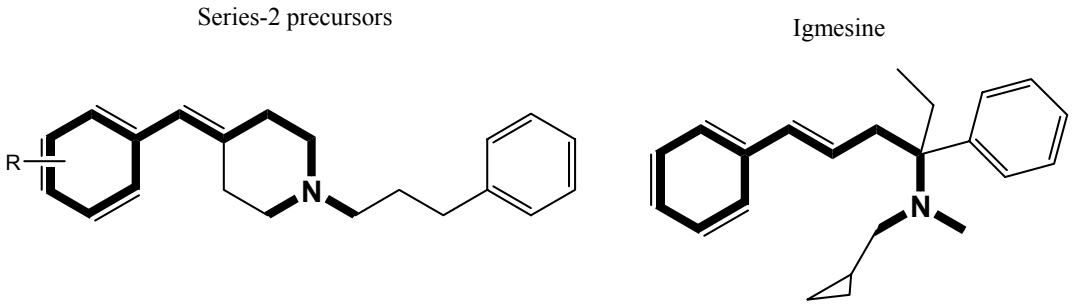


Figure 32. *Structural similarity in the skeleton of series-2 compounds with igmesine*.

Assessing the binding affinity of few of the series-2 precursors (including the unsubstituted analog), as well as determining their pharmacological profile (agonist/antagonist) according to the quick preliminary sigma-1 determination assay will be interesting for the following purposes:

1- Possibility of identification of sigma-1 selective agonists, which can have pharmacological applications totally different than the final products (series-2 compounds).
2- It would be noteworthy in that case to elucidate that the difference in pharmacological action (antagonist/agonist) resides in the unsaturated double bond/saturated bond.

VI.3 Qualitative SAR (piperidine versus piperazine)

It appears that a piperidine moiety results in a high affinity for both subtypes. The piperazine moiety exhibits about the same sigma-1 affinity, but significantly lower sigma-2 affinity. This leads to the following conclusions:

1- The nitrogen attached to the longer carbon chain (which exists in both moieties) is an important element for binding in both sigma-1 and sigma-2 pharmacophore models. Therefore, a favorable hydrogen bonding might occur between the nitrogen attached to the longer carbon chain and the corresponding sigma-1 protein receptor region where it binds.

2- The second nitrogen atom (the one attached to the shorter carbon chain) does not affect the sigma-1 binding but decreases the sigma-2 binding affinity. Therefore, this nitrogen atom does not favorably interact through hydrogen bonding with the corresponding part of the protein receptor, resulting in a lower sigma-2 affinity (in fact there might be a disfavorable repulsion).

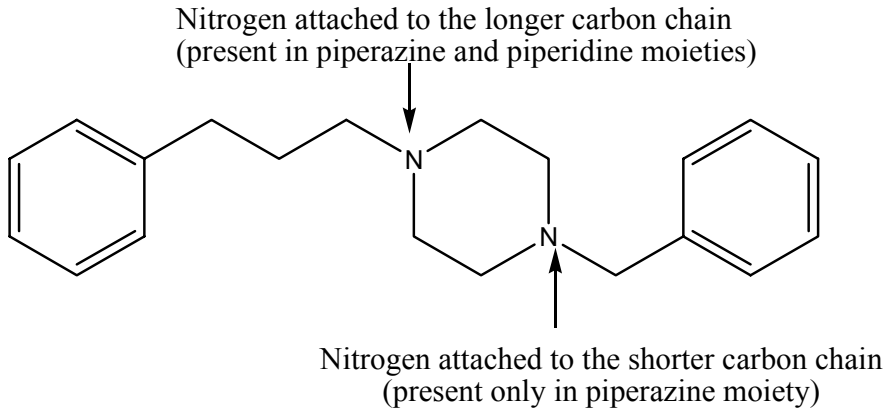
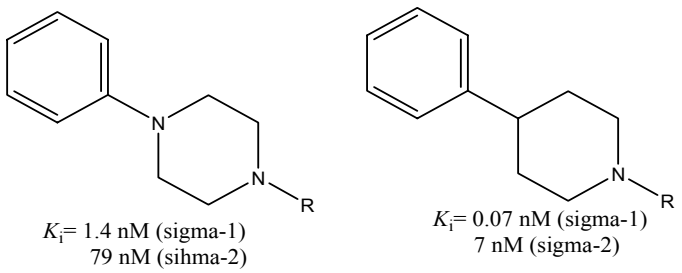


Figure 33. *Differentiation of both nitrogen atoms in skeleton*.

Ablordeppey and co-workers[113] synthesized a series of phenylalkylpiperidines and phenylalkylpiperazines and demonstrated that the nitrogen attached to the longer carbon chain is important for the binding of these two classes of compounds to the signa-1 pharmacophore. The second nitrogen atom (if it exists) was deemed as non-effective toward the sigma-1 binding. The effect of two nitrogen atoms versus one on sigma-2 binding was not discussed thoroughly in that study and the sigma-2 binding affinity was assessed only for some selected compounds. However, it was noted that the nitrogen attached to the longer chain, might be an important element for the sigma-1 binding as well as the sigma-2. They also suggested that the sigma-2 subtypes are not tolerant of the phenyl ring substitution as compared to sigma-1. Among the multitude of compounds reported in that study they had only three pairs of compounds where each member of a pair is identical to the other and the only difference residing in a piperidine moiety as opposed to a piperazine. The sigma-2 affinity was only assessed for compounds of pair **1**.



1

Figure 34. *K_i values (σ_1 and σ_2) of pair 1 compounds*.

The major subsequent studies discussing the binding pharmacophore models for sigma receptors used the conclusions of Ablordeppey and co-workers on which to base their work.[77,78,112,147]

In their effort to better understand the role of sigma-1 receptors, Corbera and co-workers[148] synthesized three series including numerous cycloalkyl-annelated pyrazoles, and established SAR. Among all the reported compounds, only two pairs (**2** and **3**) displayed the same structural features with only the exception of a piperidine moiety in one and a piperazine in the other with reported K_i values for sigma-1 and sigma-2 (although they were not discussed).

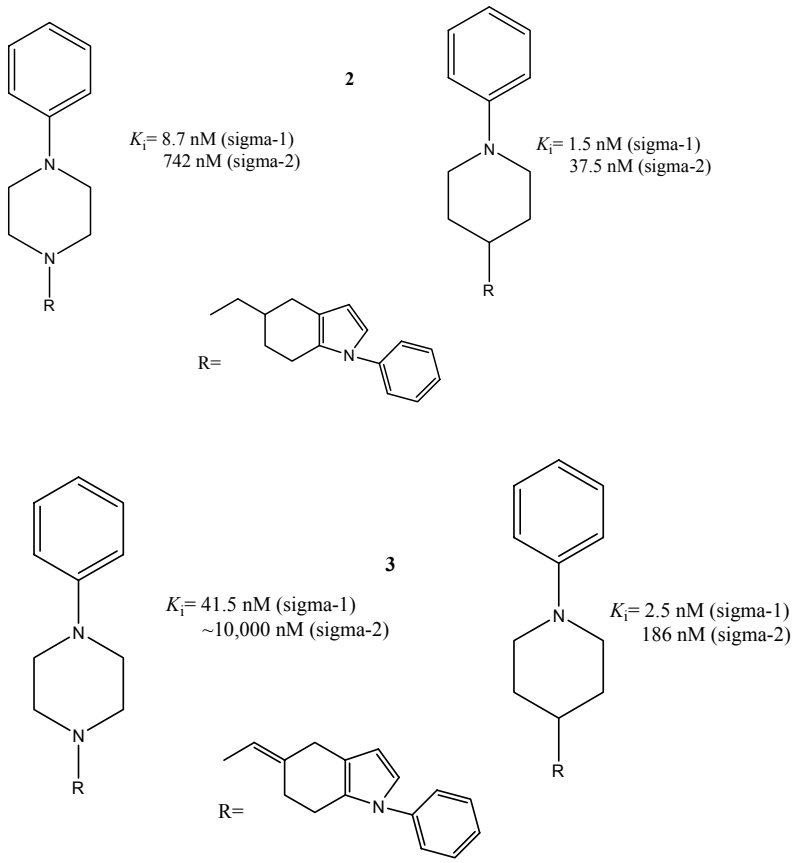


Figure 35. *K_i values (σ_1 and σ_2) of pairs 2 and 3 compounds*.

First, similar to Ablordeppey and co-workers' conclusion that the phenyl ring substitution is tolerant for sigma-1 but not as tolerant for sigma-2, our results also show that the sigma-2 receptor binding is more sensitive to the phenyl ring substitution as compared to sigma-1 binding. While Ablordeppey and co-workers look at it as "tolerance" (because

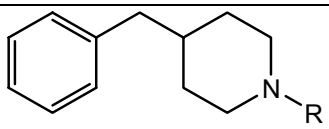
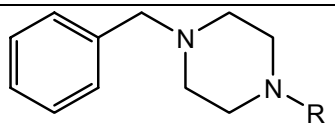
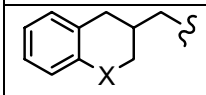
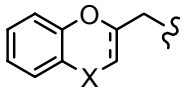
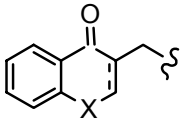
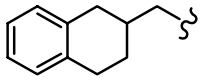
the substituted compounds they studied showed a decrease in binding affinity), we look at it as “sensitivity”, which can cause a variation in both directions (increasing or decreasing the binding affinity), hence, potentially leading to more potent or selective ligands.

Secondly, the three aforementioned reported compounds[114,138] with a piperidine moiety showed 11- to 24-fold higher sigma-2 affinities than their analogs with a piperazine moiety. This observation is consistent with the three compound pairs from our current study. Therefore, compounds with a piperidine display a higher sigma-1 and sigma-2 affinity. Besides, their affinity for sigma-2 exceeds significantly that of their piperidine containing analogs.

All these observations solidify the suggested fact that the nitrogen attached to the shorter atom (in presence of a nitrogen attached to the longer carbon chain) does not favorably interact through hydrogen bonding with the corresponding part of the protein receptor, and results in a lower sigma-2 affinity.

However, in a 2005 study by Constantino and co-workers[84] of the bulk tolerance of the longer carbon chain attached to the piperidine or piperazine moiety in a series of 1-aralkyl-4-benzylpiperidines and 1-aralkyl-4-benzylpiperazines, 12 pairs of analogs (piperazine versus piperidine) occurred, and comparison of different moieties was available.

Table 20. *Affinities and selectivities of selected compounds from Constantino and co-workers[84] study.*

						
R	**σ_1 K_i (nM)**	**σ_2 K_i (nM)**	**σ_2 / σ_1**	**σ_1 K_i (nM)**	**σ_2 K_i (nM)**	**σ_2 / σ_1**
						
X: O	2.50	5.98	2	0.30	1.48	5
X: C=O	1.40	4.63	3	0.30	1.49	5
						
X: CH2	11.6	4.80	0.4	0.30	3.02	10
X: O	16.2	28.4	2	15.4	25.6	2
X: C=O	1.40	7.90	6	1.20	4.75	4
X: C=O; double bond	115	285	2	2.66	5.35	2
						
X: CH2	24.0	3.38	0.1	0.40	1.40	3
X: O	700	370	0.5	18.0	32.8	2
X: C=O; double bond	100	21.5	0.2	3.80	14.1	4
	1.40	0.49	0.4	0.80	1.70	2

The findings of the aforementioned group showed that the piperazine compounds bind with a stronger affinity than their piperidine analogs. They commented on this by suggesting that piperazine binds to receptors differently than piperidines. Although the structural skeleton of compounds described in the paper[84] is similar to the skeleton of compounds reported in our study, the former are overcomplicated by the presence of various bulky groups along with heteroatoms in between the piperidine or piperazine moiety and the phenyl ring. In order to study the effect on one nitrogen *vs*. two, we

decided to choose our lead compounds as being simple in structure and have no groups belonging to the secondary binding pharmacophore, like the ones present in the series of compounds by Constantino and co-workers[84]. The latter probably had a different effect on piperidines than the one exerted on the piperazines, leading to confusion in assessing the bare effect of one nitrogen *vs*. two.

VI.4 Significance of results and contribution towards sigma receptor research

Sigma receptors are being studied currently for their involvement in several biological functions. Sigma receptor ligands are potentially useful for several pharmacological applications especially in cancer treatment and cocaine abuse medication. Hence, there is a great importance in understanding the pharmacophore profile of the binding and comprehend the factors that lead to the design of potent and selective ligands with a defined pharmacological profile (agonist/antagonist). Phenylalkylpiperidines and phenylalkylpiperazines seem to constitute the largest structural group of sigma ligands, and they are studied and used by many research groups.

Glennon and co-workers[79] established the sigma-1 binding pharmacophore in 1994, which consisted of a hydrogen bond accepting area, flanked in between two hydrophobic regions (one attached to the central site through a longer carbon chain). From then onwards, Ablordeppey and co-workers[113] studied the effect of one nitrogen atom in the central moiety *vs*. two nitrogens in regard to the sigma-1 binding, followed with a second study by the same group in 2002,[112] where they assessed the effect of varying the length of both carbon chains on the sigma-1 and sigma-2 binding. Younes and co-workers[111] and Constantino and co-workers[84] studied the effect of the bulk tolerance of the longer carbon

chaine on the binding. In 2004, Cratteri and co-workers[78] established a pharmacophore model for sigma-2 binding, very similar to the one for sigma-1, but with slightly different distances between each hydrophobic region, and the hydrogen bond acceptor moiety in the center. Finally, Gund and co-workers[77] proposed the existence of secondary binding pharmacophore sites, in between the hydrophobic regions and the central moiety, consisting of a heteroatomic entity such as a carbonyl or an oxygen atom.

It did not seem that those abovementioned studies assessed the effect of the systematic phenyl ring substitution on the binding, nor the comparison of the same compounds with piperazine with their analogs with piperidine. The QSAR equations we established for sigma-1 and sigma-2 for the benzyl substitution gave a good idea qualitatively and quantitatively on how the ring substitution can affect the binding; the statistical parameters of the equations were of very high quality, and good predictive ability. Moreover, the selected compounds for the piperidine *vs*. piperazine comparison, gave solid results regarding that effect, supported by the structural simplicity of the structures, which enabled us to study solely the effect of one nitrogen *vs*. two in the central moiety.

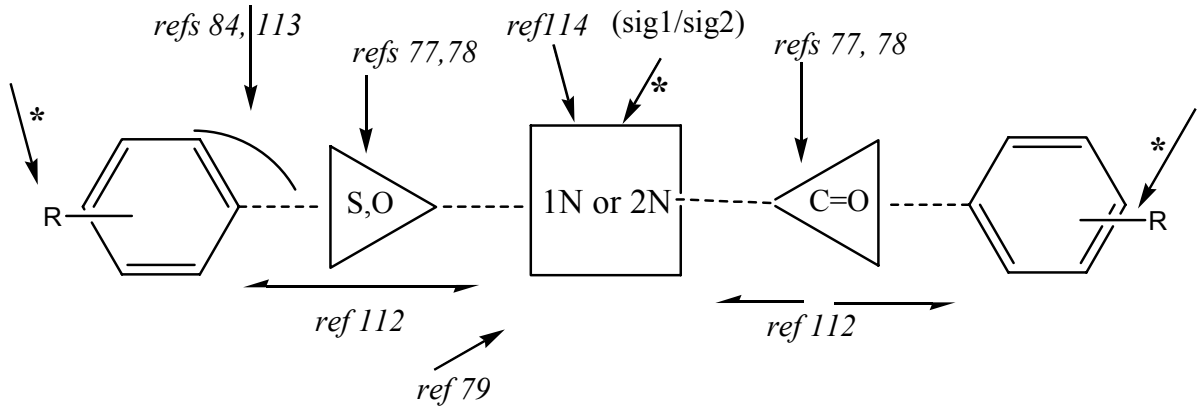


Figure 36. *Representation of major contribution performed on various locations of sigma receptor binding pharmacophore model. (Contribution of this study is symbolized by the asterisk sign *).*

Concerning the "prospective design" that led to those results, it seems that this method presents several advantages; it minimizes the number of compounds involved in the study and hence it is time and effort efficient. In most of the other SAR studies of sigma receptors, the number of compounds was larger. The qualitative results we came up with, as well as the quantitative SAR were solid and well characterized. Its limitations however, reside in the fact that this methodology serves only as lead optimization technique and not a lead discovery technique due to the limited number of compounds and restricted structural modifications.

APPENDIX

STATISTICAL EVALUATION OF DESIGNED COMPOUNDS

The SAS System

Obs	sigma	pi	mr
1	0.35	1.12	1.39
2	0.34	0.14	0.10
3	0.71	-0.28	0.74
4	0.00	0.86	0.89
5	-0.27	-0.02	0.79
6	-0.17	0.56	0.57
7	0.78	-0.28	0.74
8	0.12	-0.02	0.79
9	0.00	-0.28	0.74

The SAS System

The UNIVARIATE Procedure
Variable: sigma

Moments

N	9	Sum Weights	9
Mean	0.20666667	Sum Observations	1.86
Std Deviation	0.36783148	Variance	0.1353
Skewness	0.45611646	Kurtosis	-0.9363442
Uncorrected SS	1.4668	Corrected SS	1.0824
Coeff Variation	177.982976	Std Error Mean	0.12261049

Basic Statistical Measures

Location		Variability	
Mean	0.206667	Std Deviation	0.36783
Median	0.120000	Variance	0.13530

Mode	0.000000	Range	1.05000
		Interquartile Range	0.35000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------
Student's t	t	1.685554	Pr > |t| 0.1304
Sign	M	1.5	Pr >= |M| 0.4531
Signed Rank	S	9	Pr >= |S| 0.1563

Tests for Normality

Test	--Statistic---		-----p Value------
Shapiro-Wilk	W	0.933439	Pr < W 0.5148
Kolmogorov-Smirnov	D	0.157336	Pr > D >0.1500
Cramer-von Mises	W-Sq	0.042827	Pr > W-Sq >0.2500
Anderson-Darling	A-Sq	0.285771	Pr > A-Sq >0.2500

Quantiles (Definition 5)

Quantile	Estimate
100% Max	0.78
99%	0.78
95%	0.78
90%	0.78
75% Q3	0.35
50% Median	0.12
25% Q1	0.00

The UNIVARIATE Procedure
Variable: sigma

Quantiles (Definition 5)

Quantile	Estimate
10%	-0.27
5%	-0.27
1%	-0.27
0% Min	-0.27

Extreme Observations

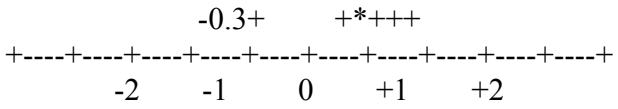
----Lowest----		----Highest---	
Value	Obs	Value	Obs
-0.27	5	0.12	8
-0.17	6	0.34	2
0.00	9	0.35	1
0.00	4	0.71	3
0.12	8	0.78	7

```
Stem Leaf              #          Boxplot
   6 18                2            |
    4                                |
  2 45                 2          +--+--+
  0 002                3          *-----*
   -0 7                1            |
   -2 7                1            |
       ----+----+----+----+
```

Multiply Stem.Leaf by 10**-1

Normal Probability Plot

```
 0.7+                          * ++++*+
    |                        +++++
    |                     +++*++*
    |                  *++*++*
    |               ++*+++
```



The UNIVARIATE Procedure
Fitted Distribution for sigma

Parameters for Normal Distribution

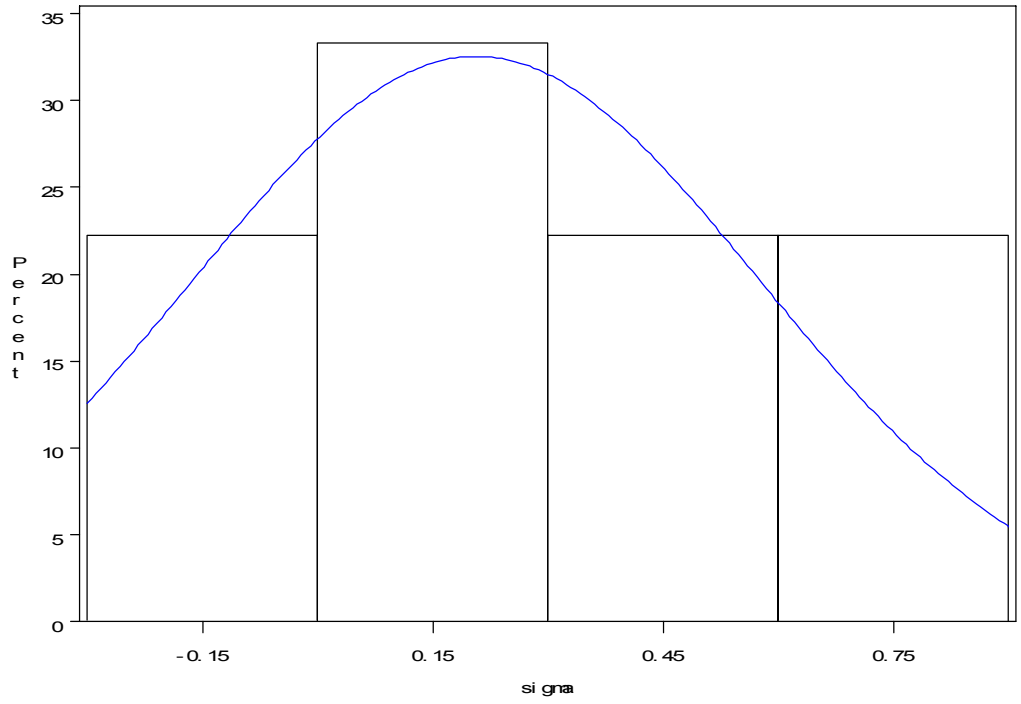
Parameter	Symbol	Estimate
Mean	Mu	0.206667
Std Dev	Sigma	0.367831

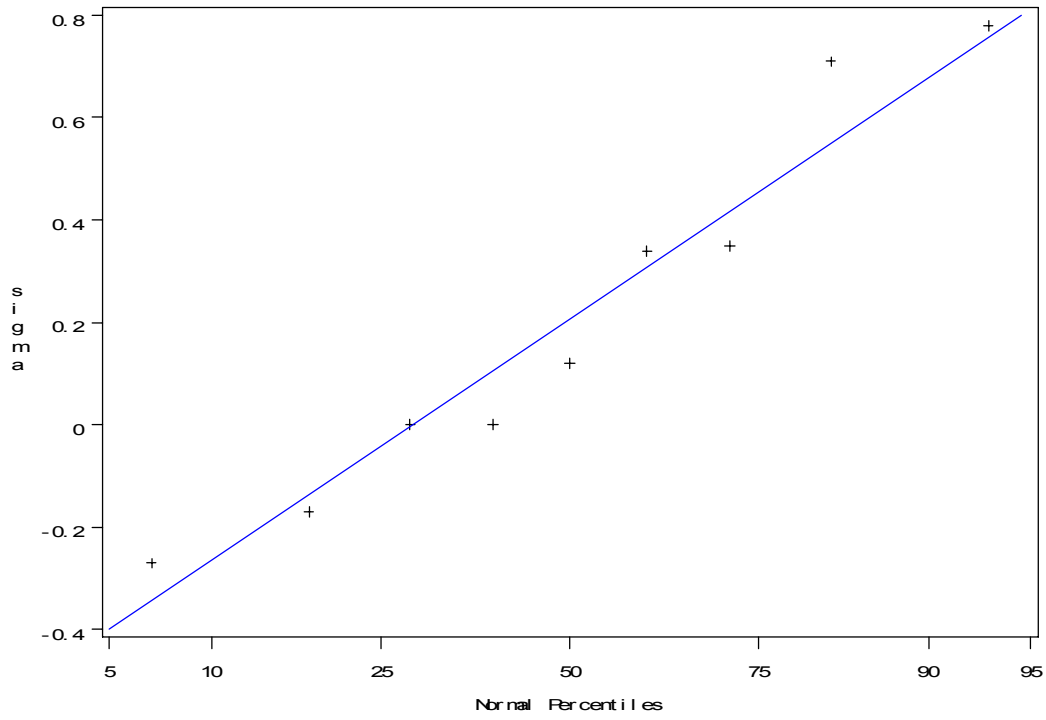
Goodness-of-Fit Tests for Normal Distribution

Test	---Statistic----		-----p Value-----	
Kolmogorov-Smirnov	D	0.15733584	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.04282678	Pr > W-Sq	>0.250
Anderson-Darling	A-Sq	0.28577081	Pr > A-Sq	>0.250

Quantiles for Normal Distribution

	------Quantile------	
Percent	Observed	Estimated
1.0	-0.27000	-0.64904
5.0	-0.27000	-0.39836
10.0	-0.27000	-0.26473
25.0	0.00000	-0.04143
50.0	0.12000	0.20667
75.0	0.35000	0.45477
90.0	0.78000	0.67806
95.0	0.78000	0.81170
99.0	0.78000	1.06237


35
30
25
20
15
10
5
0
Percent
-0.15
0.15
0.45
0.75
sigma


0.8
0.6
0.4
0.2
0
-0.2
-0.4
sigma
5
10
25
50
75
90
95
Normal Percentiles

The UNIVARIATE Procedure
Variable: pi

Moments

N	9	Sum Weights	9
Mean	0.2	Sum Observations	1.8
Std Deviation	0.52478567	Variance	0.2754
Skewness	0.8446292	Kurtosis	-0.7329443
Uncorrected SS	2.5632	Corrected SS	2.2032
Coeff Variation	262.392835	Std Error Mean	0.17492856

Basic Statistical Measures

Location		Variability	
Mean	0.20000	Std Deviation	0.52479
Median	-0.02000	Variance	0.27540
Mode	-0.28000	Range	1.40000
		Interquartile Range	0.84000

Tests for Location: Mu0=0

Test		-Statistic-		-----p Value------
Student's t	t	1.143324	Pr > |t|	0.2860
Sign	M	-0.5	Pr >= |M|	1.0000
Signed Rank	S	4.5	Pr >= |S|	0.6250

Tests for Normality

Test		--Statistic---		-----p Value------
Shapiro-Wilk	W	0.858733	Pr < W	0.0929
Kolmogorov-Smirnov	D	0.218027	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.093195	Pr > W-Sq	0.1216
Anderson-Darling	A-Sq	0.547177	Pr > A-Sq	0.1164

Quantiles (Definition 5)

Quantile	Estimate
100% Max	1.12
99%	1.12
95%	1.12
90%	1.12
75% Q3	0.56
50% Median	-0.02
25% Q1	-0.28

The UNIVARIATE Procedure
Variable: pi

Quantiles (Definition 5)

Quantile	Estimate
10%	-0.28
5%	-0.28
1%	-0.28
0% Min	-0.28

Extreme Observations

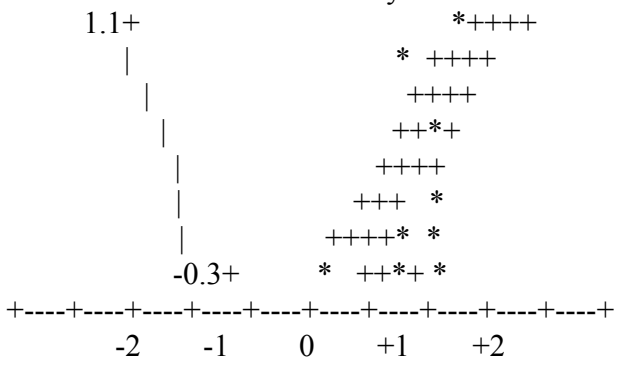
----Lowest----		----Highest---	
Value	Obs	Value	Obs
-0.28	9	-0.02	8
-0.28	7	0.14	2
-0.28	3	0.56	6
-0.02	8	0.86	4
-0.02	5	1.12	1

Stem	Leaf	#	Boxplot
10	2	1	|
8	6	1	|
6			|
4	6	1	+-----+
2			| + |
0	4	1	| |

```
-0 22              2          *-----*
-2 888             3          +-----+
          ----+----+----+----+
```

Multiply Stem.Leaf by 10**-1

Normal Probability Plot



The UNIVARIATE Procedure
Fitted Distribution for pi

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	0.2
Std Dev	Sigma	0.524786

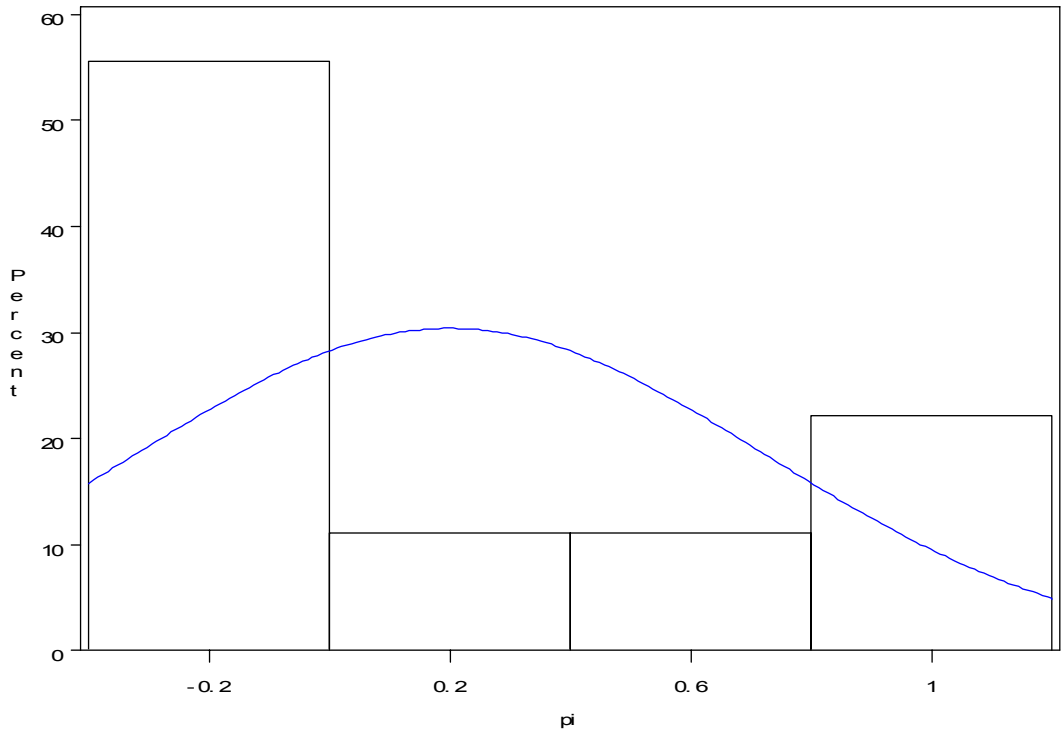
Goodness-of-Fit Tests for Normal Distribution

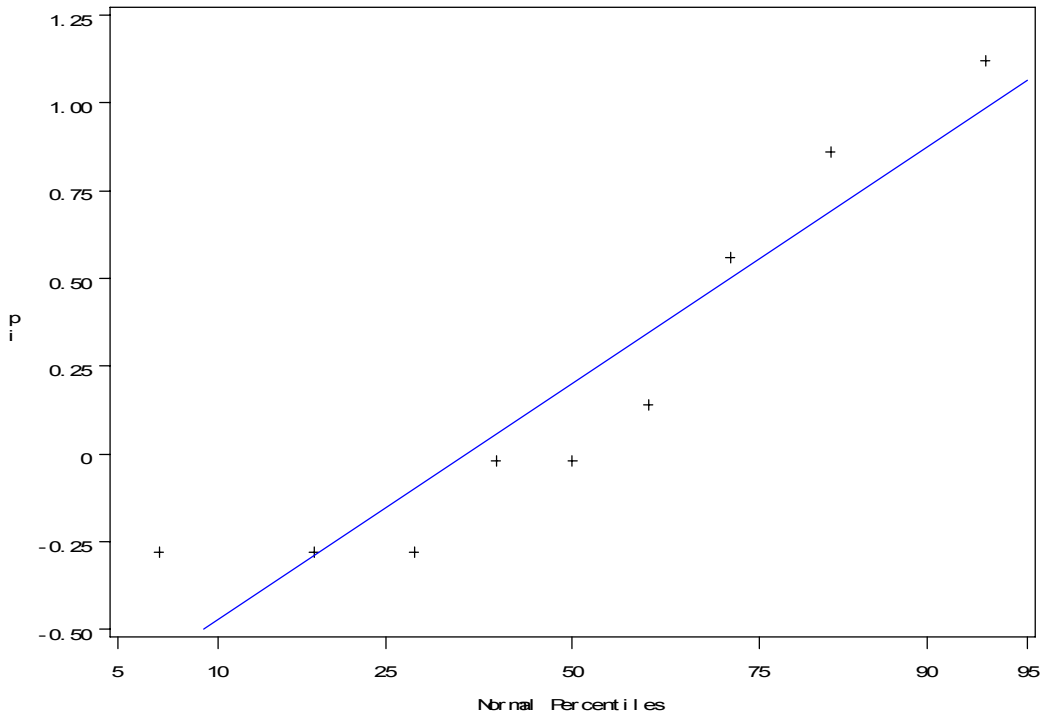
Test	---Statistic----		-----p Value-----	
Kolmogorov-Smirnov	D	0.21802743	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.09319546	Pr > W-Sq	0.122
Anderson-Darling	A-Sq	0.54717716	Pr > A-Sq	0.116

Quantiles for Normal Distribution

------Quantile------		
Percent	Observed	Estimated

1.0	-0.28000	-1.02083
5.0	-0.28000	-0.66320
10.0	-0.28000	-0.47254
25.0	-0.28000	-0.15396
50.0	-0.02000	0.20000
75.0	0.56000	0.55396
90.0	1.12000	0.87254
95.0	1.12000	1.06320
99.0	1.12000	1.42083





The UNIVARIATE Procedure
Variable: mr

Moments

N	9	Sum Weights	9
Mean	0.75	Sum Observations	6.75
Std Deviation	0.33309158	Variance	0.11095
Skewness	-0.067162	Kurtosis	2.99472483
Uncorrected SS	5.9501	Corrected SS	0.8876
Coeff Variation	44.4122105	Std Error Mean	0.11103053

Basic Statistical Measures

Location		Variability	
Mean	0.750000	Std Deviation	0.33309
Median	0.740000	Variance	0.11095
Mode	0.740000	Range	1.29000

Interquartile Range 0.05000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	6.754899	Pr > |t|	0.0001
Sign	M	4.5	Pr >= |M|	0.0039
Signed Rank	S	22.5	Pr >= |S|	0.0039

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.868133	Pr < W	0.1174
Kolmogorov-Smirnov	D	0.265803	Pr > D	0.0664
Cramer-von Mises	W-Sq	0.144959	Pr > W-Sq	0.0225
Anderson-Darling	A-Sq	0.737109	Pr > A-Sq	0.0362

Quantiles (Definition 5)

Quantile	Estimate
100% Max	1.39
99%	1.39
95%	1.39
90%	1.39
75% Q3	0.79
50% Median	0.74
25% Q1	0.74

The SAS System
The UNIVARIATE Procedure
Variable: mr

Quantiles (Definition 5)

Quantile	Estimate
10%	0.10
5%	0.10
1%	0.10
0% Min	0.10

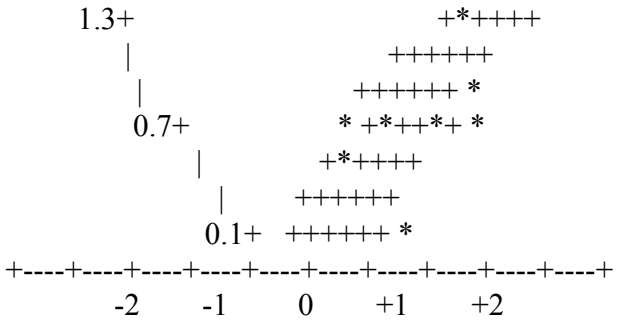
Extreme Observations

----Lowest----		----Highest---	
Value	Obs	Value	Obs
0.10	2	0.74	9
0.57	6	0.79	5
0.74	9	0.79	8
0.74	7	0.89	4
0.74	3	1.39	1

```
Stem Leaf              #          Boxplot
  12 9                 1             *
                      10
   8 9                 1             0
  6 44499              5            +--+--+
   4 7                 1             *
                       2
   0 0                 1             *
          ----+----+----+----+
```

Multiply Stem.Leaf by 10**-1

Normal Probability Plot



The UNIVARIATE Procedure
Fitted Distribution for mr

Parameters for Normal Distribution

Parameter	Symbol	Estimate
Mean	Mu	0.75
Std Dev	Sigma	0.333092

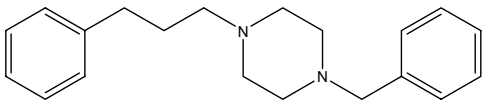
Goodness-of-Fit Tests for Normal Distribution

Test		---Statistic----		-----p Value-----
Kolmogorov-Smirnov	D	0.26580262	Pr > D	0.066
Cramer-von Mises	W-Sq	0.14495859	Pr > W-Sq	0.022
Anderson-Darling	A-Sq	0.73710898	Pr > A-Sq	0.036

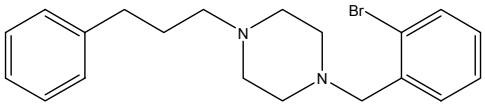
Quantiles for Normal Distribution

	------Quantile------	
Percent	Observed	Estimated
1.0	0.10000	-0.02489
5.0	0.10000	0.20211
10.0	0.10000	0.32313
25.0	0.74000	0.52533
50.0	0.74000	0.75000
75.0	0.79000	0.97467
90.0	1.39000	1.17687
95.0	1.39000	1.29789
99.0	1.39000	1.52489

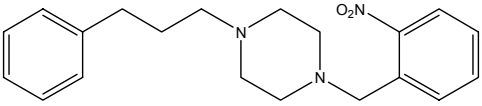
SERIES-1



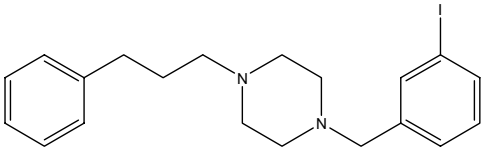
Compound 1a



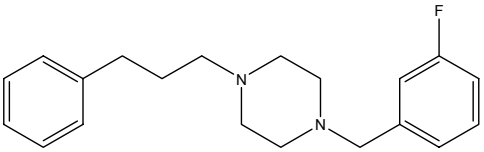
Compound 1b



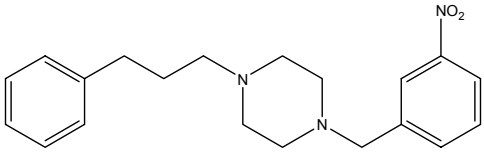
Compound 1c



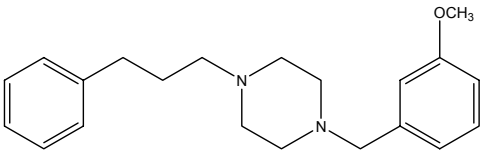
Compound 1d



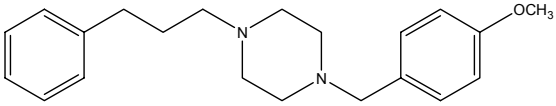
Compound 1e



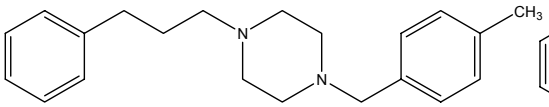
Compound 1f



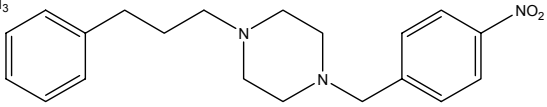
Compound 1g



Compound 1h

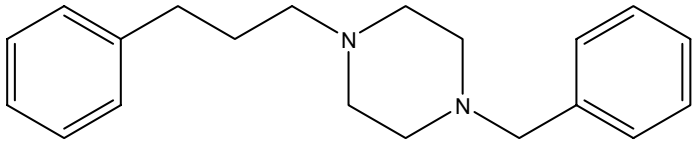


Compound 1i



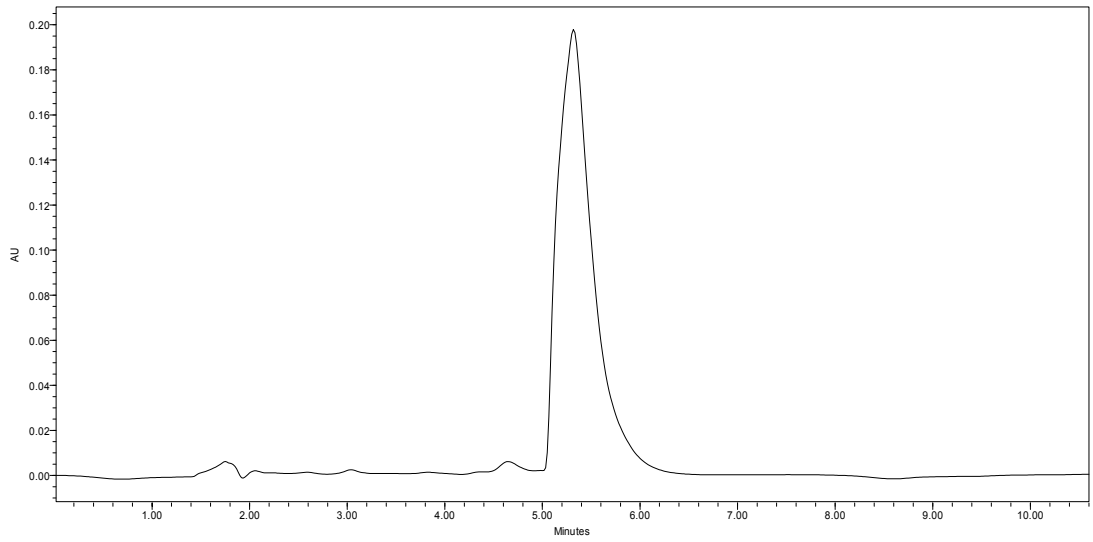
Compound 1j

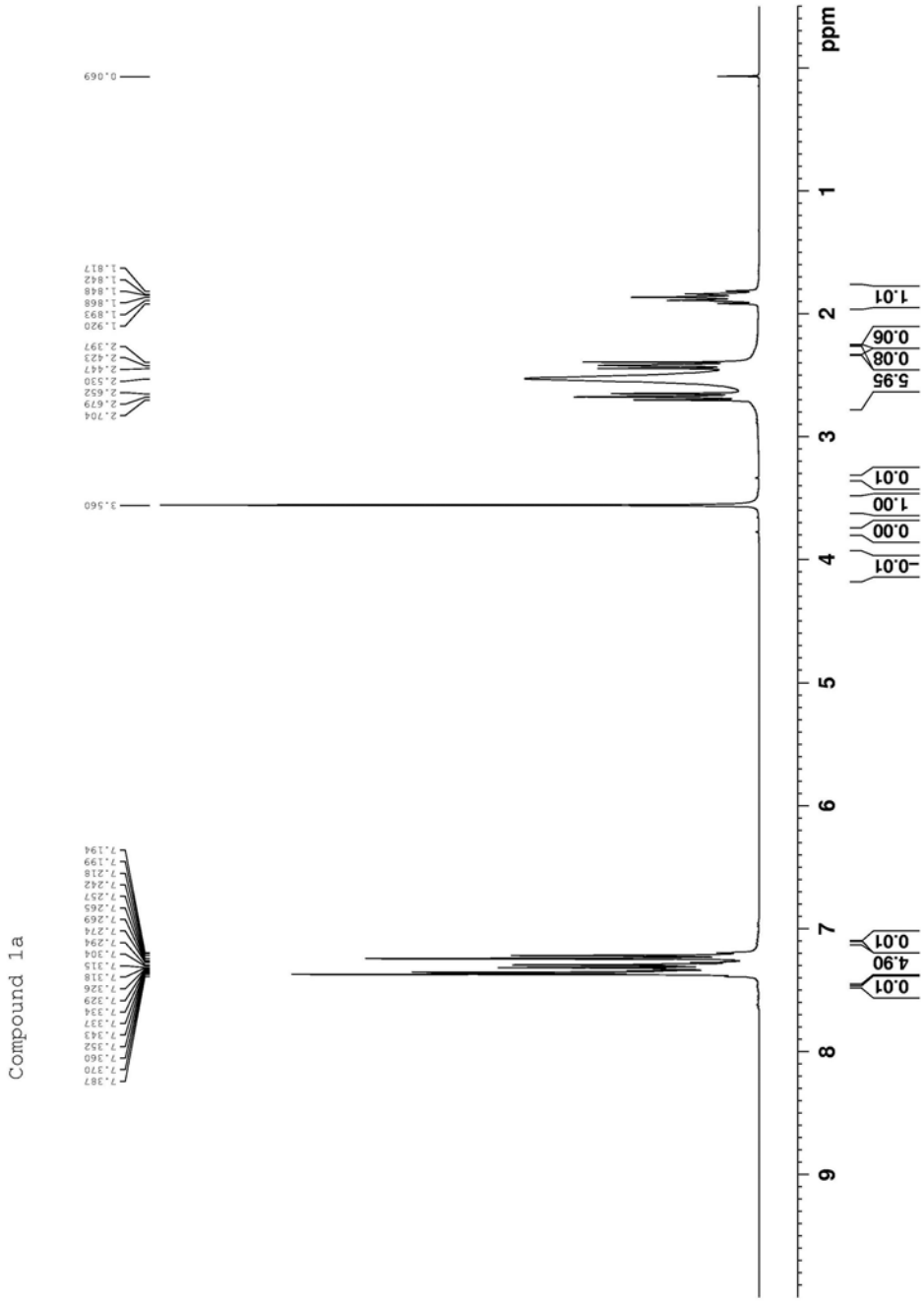
Compound 1a:



Compound 1a

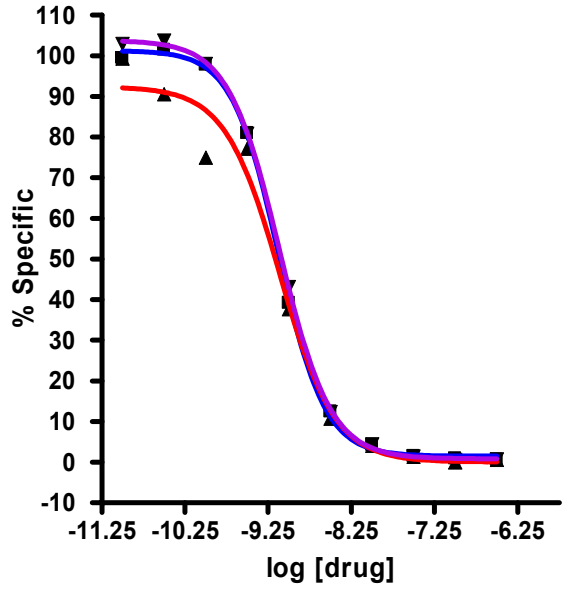
Elemental analysis $C_{20}H_{26}N_2$	**C %**	**H %**	**N %**
Calculated	81.59	8.90	9.51
Found	81.22	8.95	9.59




Compound 1a
ppm
0.01
4.90
0.01
0.01
1.00
0.00
0.01
5.95
0.08
0.06
1.01

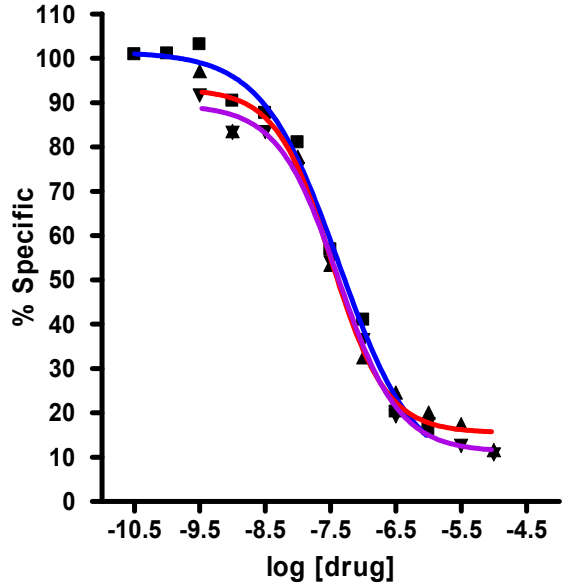
log [drug] (M) (σ_1)	(1a) (R= H)(1) (%)	(1a) (R= H)(2) (%)	(1a) (R= H)(3) (%)
3.16E-07	0.645	0.5601	0.702
1.00E-07	0.896	-0.022	0.576
3.16E-08	1.548	1.300	1.409
1.00E-08	4.243	4.023	4.415
3.16E-09	12.472	10.711	12.332
1.00E-09	39.227	37.621	43.104
3.16E-10	80.978	77.116	80.822
1.00E-10	97.785	74.938	97.987
3.16E-11	101.500	90.579	103.742
1.00E-11	99.423	99.308	102.889

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	7.641e-010	5.342e-010	-1.427
Std. Deviation	1.605e-011	1.119e-011	0.119
Std. Error	9.265e-012	6.460e-012	0.069
Lower 95% CI of mean	7.242e-010	5.064e-010	-1.723
Upper 95% CI of mean	8.040e-010	5.620e-010	-1.132
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

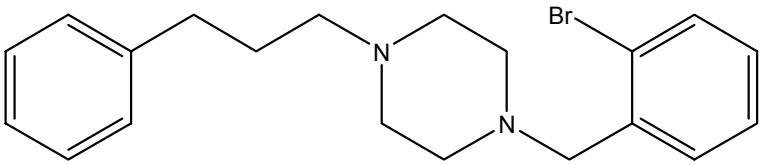


log [drug] (M) (σ_2)	(1a) (R= H)(1) (%)	(1a) (R= H)(2) (%)	(1a) (R= H)(3) (%)
1.00E-06		11.616	10.623
3.16E-07		17.448	12.538
1.00E-07	16.334	20.073	17.442
3.16E-08	20.285	24.625	19.198
1.00E-08	41.034	32.586	36.364
3.16E-09	56.911	53.426	53.925
1.00E-09	81.039	77.830	74.981
3.16E-10	87.683	88.147	83.353
1.00E-10	90.391	83.567	83.230
3.16E-11	103.135	97.146	91.609
1.00E-11	101.071		
3.16e-012	100.867		

(σ_2)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	3.864e-008	3.433e-008	-0.905
Std. Deviation	5.256e-009	4.670e-009	0.143
Std. Error	3.035e-009	2.696e-009	0.083
Lower 95% CI of mean	2.558e-008	2.273e-008	-1.261
Upper 95% CI of mean	5.169e-008	4.593e-008	-0.549
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

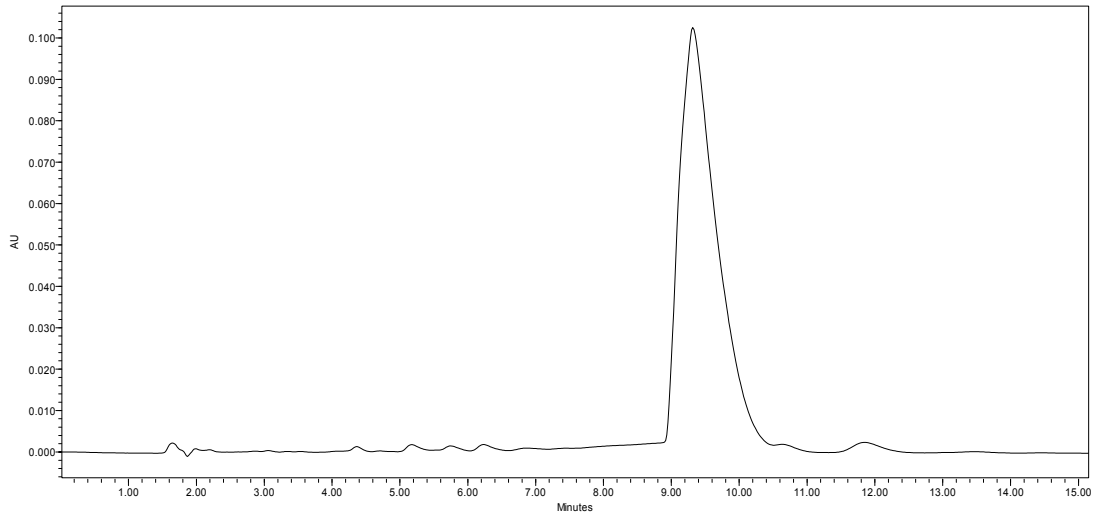


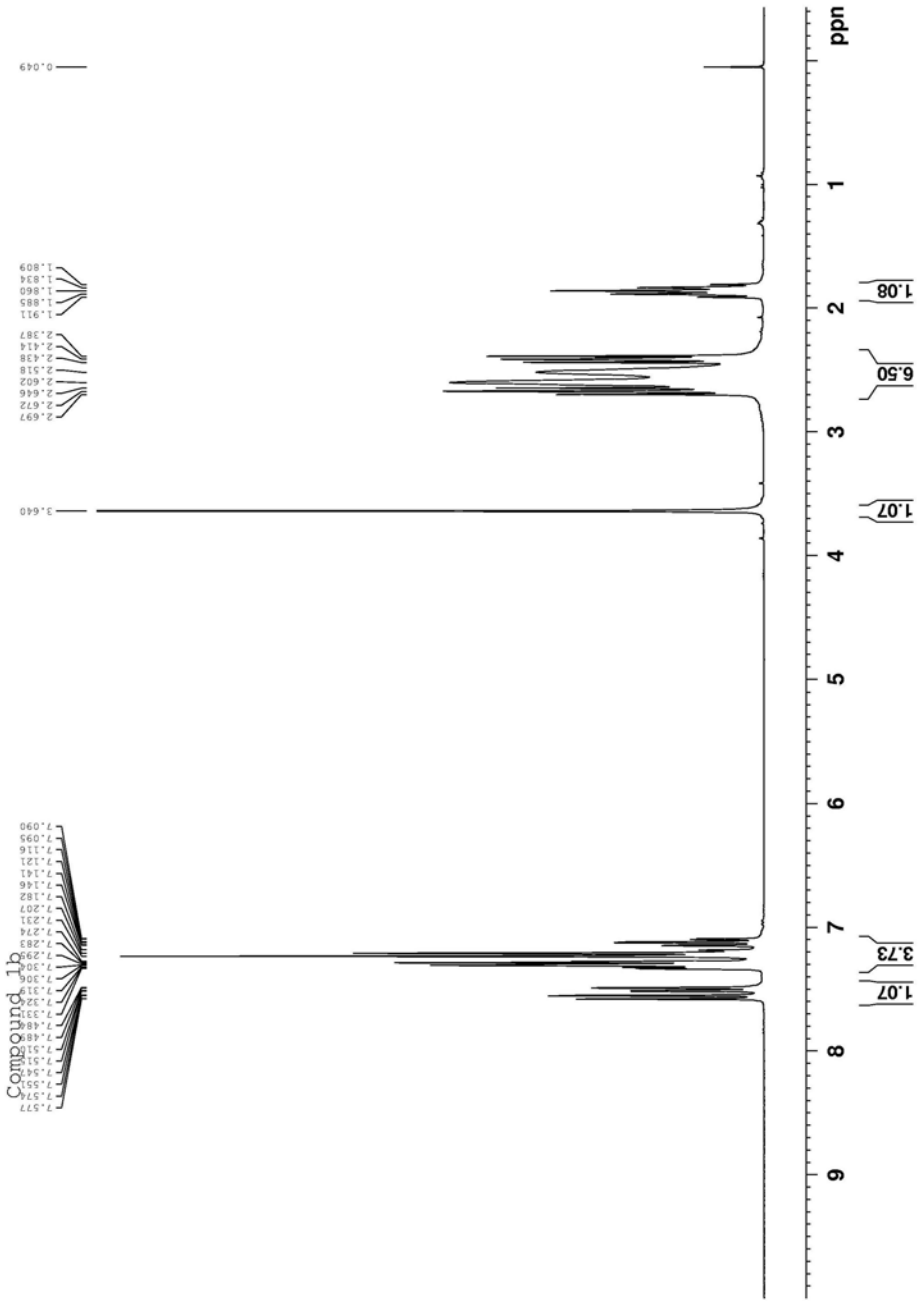
Compound 1b:



Compound 1b

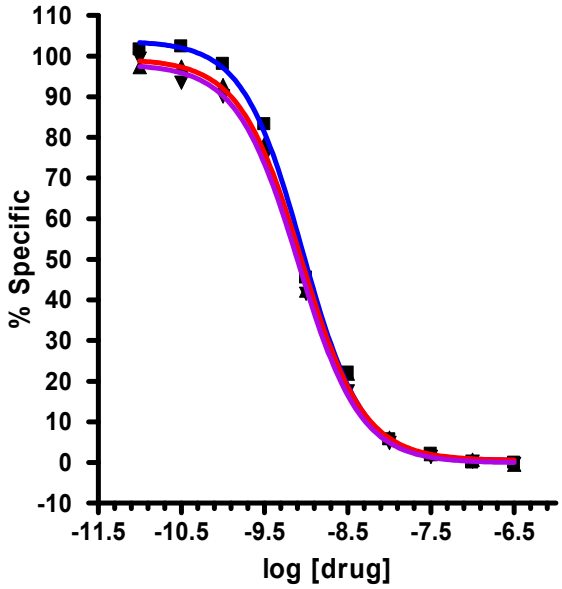
Elemental analysis $C_{20}H_{25}N_2Br$	**C %**	**H %**	**N %**
Calculated	64.34 %	6.75 %	7.50 %
Found	64.05 %	6.77 %	7.49 %




Compound 1b
ppm
1.07
3.73
1.07
6.50
1.08

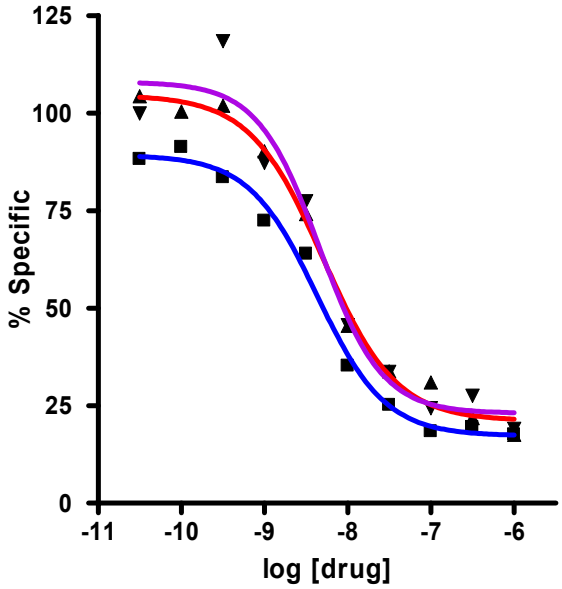
log [drug] (M) (σ_1)	(1b) (R= 2-Br)(1) (%)	(1b) (R= 2-Br)(2) (%)	(1b) (2-Br)(3) (%)
3.16E-07	-0.026	-0.522	-0.315
1.00E-07	-0.026	0.716	0.245
3.16E-08	2.140	2.064	1.413
1.00E-08	5.682	6.089	4.824
3.16E-09	22.070	21.916	17.555
1.00E-09	45.477	42.348	41.536
3.16E-10	83.247	78.443	74.953
1.00E-10	98.048	92.858	90.092
3.16E-11	102.359	97.463	93.454
1.00E-11	101.591	97.303	99.348

(σ_1)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	8.610e-010	6.019e-010	-1.185
Std. Deviation	4.540e-011	3.175e-011	0.034
Std. Error	2.621e-011	1.833e-011	0.020
Lower 95% CI of mean	7.482e-010	5.231e-010	-1.271
Upper 95% CI of mean	9.738e-010	6.808e-010	-1.100
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

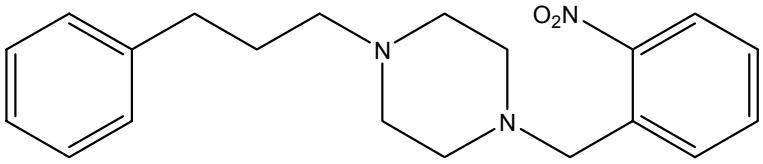


log [drug] (M) (σ_2)	(1b) (R= 2-Br)(1) (%)	(1b) (R= 2-Br)(2) (%)	(1b) (R= 2-Br)(3) (%)
1.00E-06	17.648	17.534	10.622
3.16E-07	19.500	21.801	12.538
1.00E-07	18.440	30.957	17.442
3.16E-08	25.208	33.779	19.198
1.00E-08	35.231	45.499	36.364
3.16E-09	63.921	74.076	53.925
1.00E-09	72.420	90.208	74.981
3.16E-10	83.544	101.899	83.354
1.00E-10	91.292	100.360	83.230
3.16E-11	88.233	104.322	91.609

(σ_2)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	4.637e-009	4.120e-009	-1.070
Std. Deviation	3.797e-010	3.373e-010	0.086
Std. Error	2.192e-010	1.948e-010	0.050
Lower 95% CI of mean	3.693e-009	3.282e-009	-1.284
Upper 95% CI of mean	5.580e-009	4.957e-009	-0.856
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

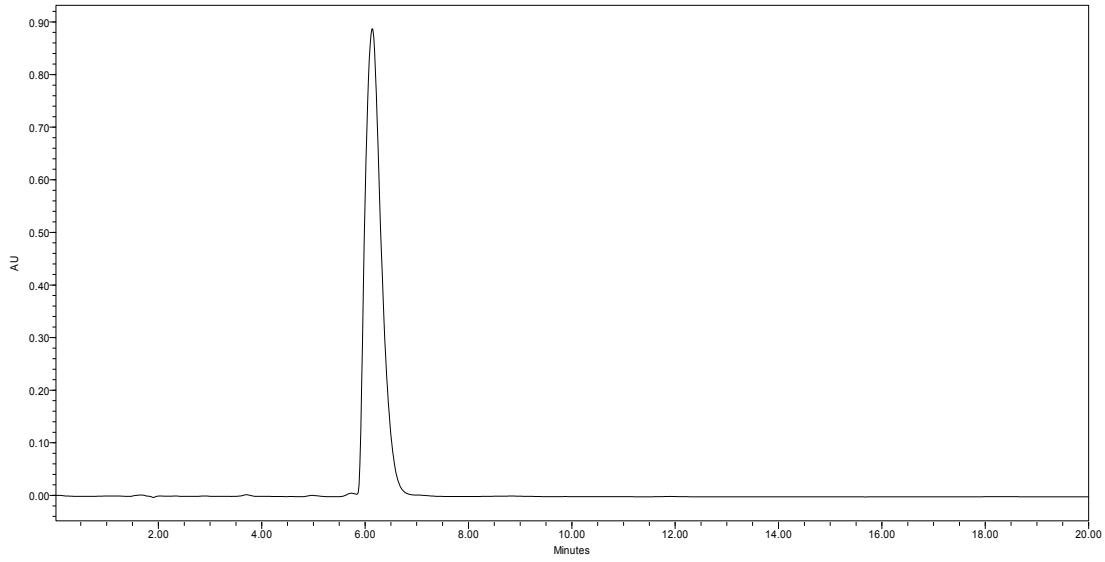


Compound 1c:



Compound 1c

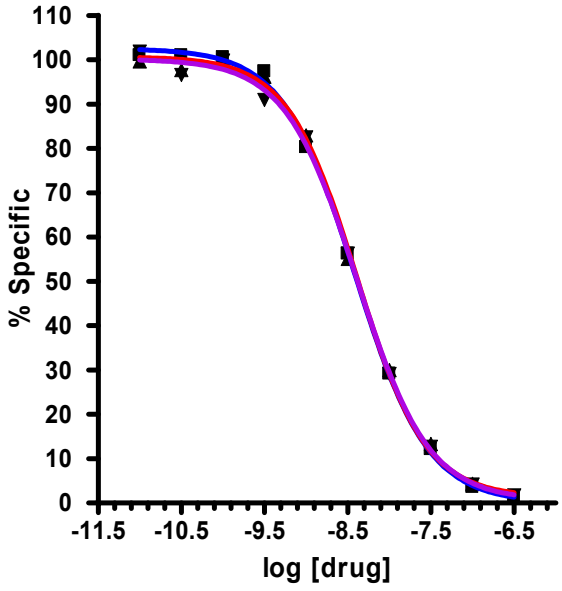
Elemental analysis $C_{20}H_{25}N_3O_2$	**C %**	**H %**	**N %**
Calculated	70.77	7.42	12.38
Found	70.26	7.48	12.26



Compound 1f
7.825
7.820
7.793
7.788
7.619
7.613
7.588
7.583
7.557
7.552
7.527
7.522
7.497
7.492
7.416
7.409
7.384
7.379
7.355
7.349
7.314
7.309
7.303
7.275
7.271
7.252
7.200
7.187
7.177
7.171
7.156
7.150
7.144
3.798
2.665
2.634
2.603
2.464
2.397
2.367
2.336
1.874
1.843
1.813
1.789
1.781
1.752
0.019
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
4.46
1.00
0.02
1.08
4.96
0.98

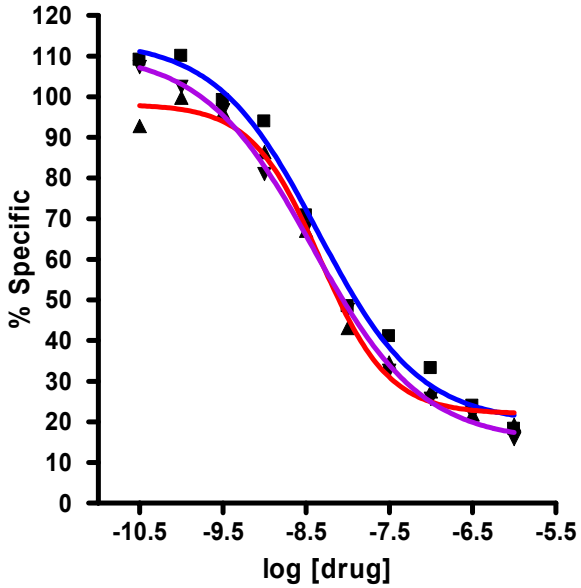
log [drug] (M) (σ_1)	(1c) (R= 2-NO_2)(1) (%)	(1c) (R= 2-NO_2)(2) (%)	(1c) (R= 2-NO_2)(3) (%)
3.16E-07	0.971	1.203	1.740
1.00E-07	3.626	4.386	4.164
3.16E-08	12.145	13.290	12.694
1.00E-08	29.161	29.967	29.098
3.16E-09	56.266	55.050	56.266
1.00E-09	80.234	82.958	82.450
3.16E-10	97.353	96.207	90.918
1.00E-10	100.594	100.248	99.801
3.16E-11	101.007	97.706	96.576
1.00E-11	100.978	99.622	101.839

(σ_1)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	4.017e-009	2.809e-009	-1.017
Std. Deviation	1.040e-010	7.262e-011	0.027
Std. Error	6.005e-011	4.193e-011	0.015
Lower 95% CI of mean	3.759e-009	2.628e-009	-1.083
Upper 95% CI of mean	4.275e-009	2.989e-009	-0.950
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

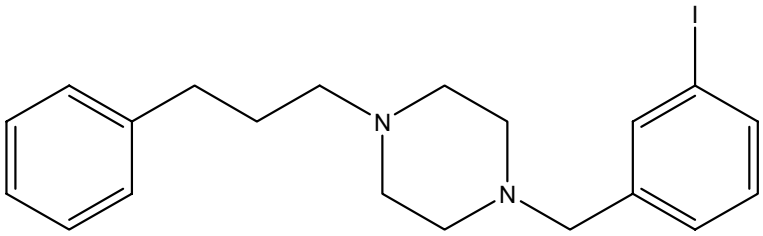


log [drug] (M) (σ_2)	(1c) (R= 2-NO_2)(1) (%)	(1c) (R= 2-NO_2)(2) (%)	(1c) (R= 2-NO_2)(3) (%)
1.00E-06	18.270	19.336	15.799
3.16E-07	23.941	21.836	21.842
1.00E-07	33.205	27.494	25.643
3.16E-08	41.069	34.600	32.611
1.00E-08	48.475	43.095	47.548
3.16E-09	70.839	67.045	67.712
1.00E-09	93.927	86.383	81.007
3.16E-10	99.120	96.165	96.658
1.00E-10	110.071	99.790	102.460
3.16E-11	109.061	92.799	107.348

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	4.637e-009	4.120e-009	-1.070
Std. Deviation	3.797e-010	3.373e-010	0.086
Std. Error	2.192e-010	1.948e-010	0.050
Lower 95% CI of mean	3.693e-009	3.282e-009	-1.284
Upper 95% CI of mean	5.580e-009	4.957e-009	-0.856
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

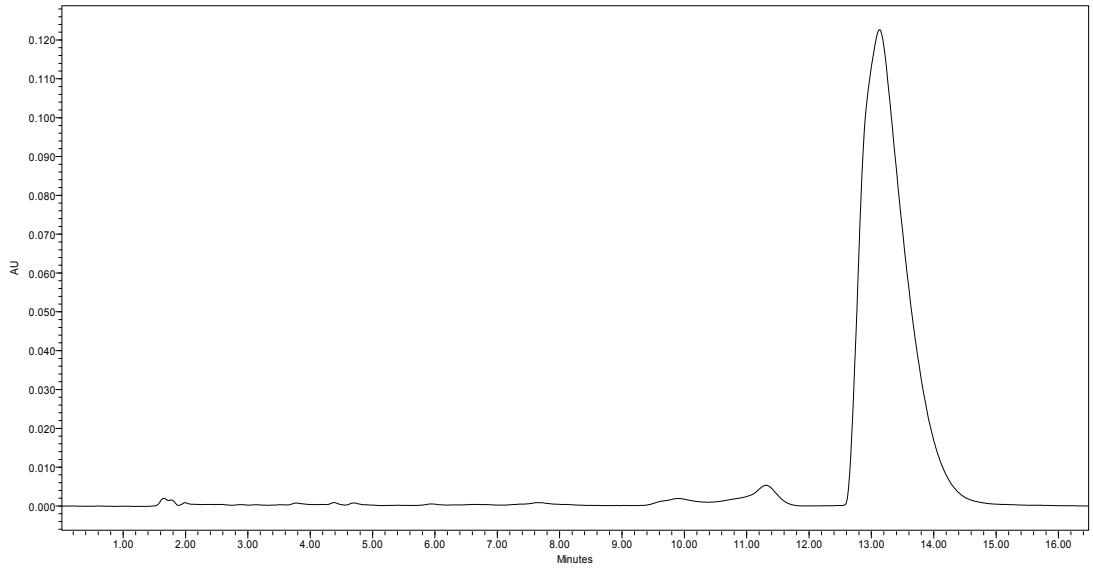


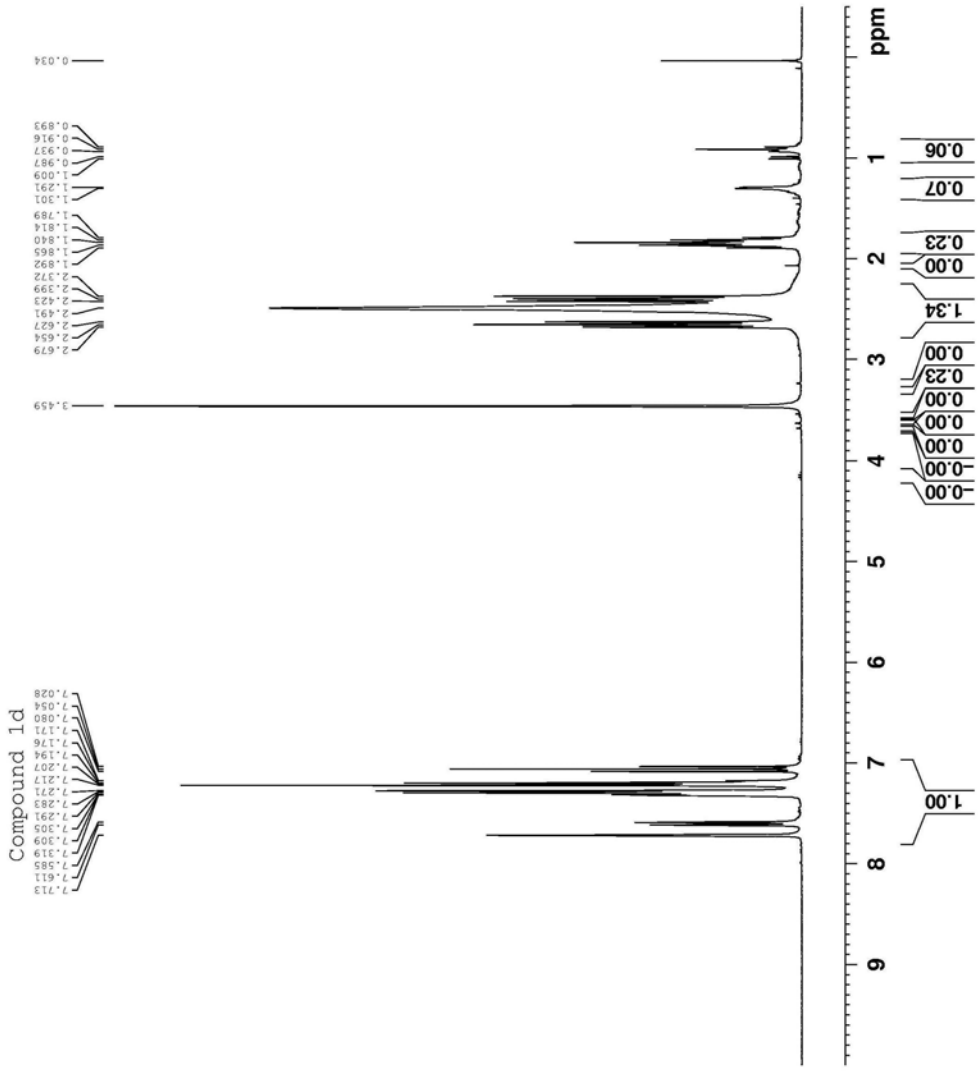
Compound 1d:



Compound 1d

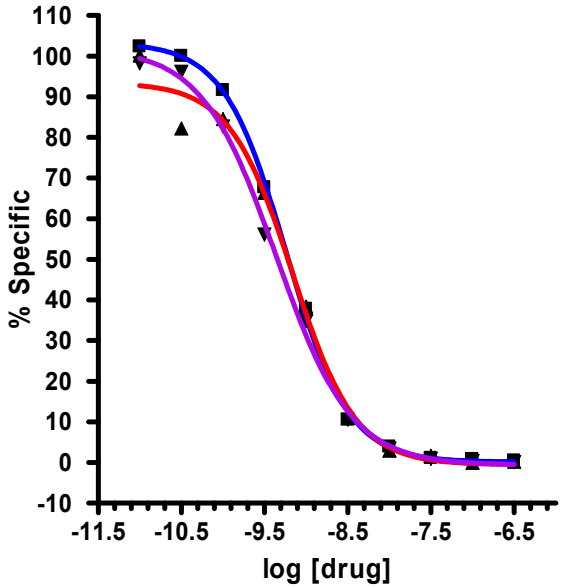
Elemental analysis $C_{20}H_{25}N_2I$	**C %**	**H %**	**N %**
Calculated	57.12	5.99	6.66
Found	57.03	5.95	6.61




Compound 1d
7.713
7.611
7.585
7.319
7.309
7.305
7.291
7.283
7.271
7.217
7.207
7.194
7.176
7.171
7.080
7.054
7.028
3.459
2.679
2.654
2.627
2.491
2.423
2.399
2.372
1.892
1.865
1.840
1.814
1.789
1.301
1.291
1.009
0.987
0.937
0.916
0.893
0.034
ppm
1.00
1.34
0.06
0.07
0.23
0.00
0.00
0.23
0.00
0.00
0.00
0.00
0.00
0.00

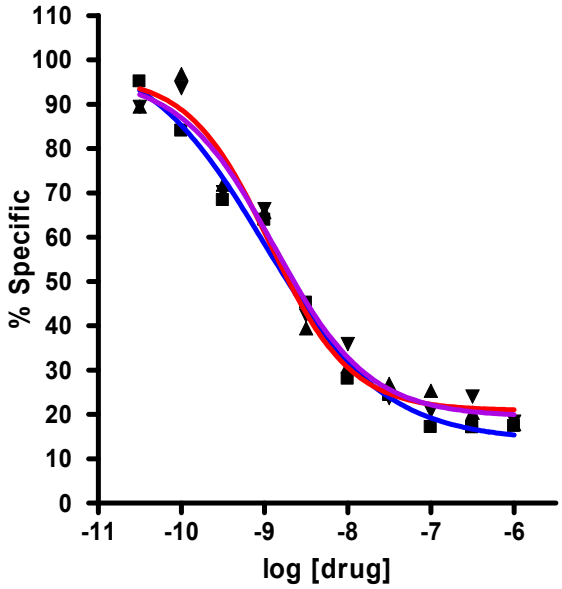
log [drug] (M) (σ_1)	(1d) (R= 3-I)(1) (%)	(1d) (R= 3-I)(2) (%)	(1d) (R= 3-I)(3) (%)
3.16E-07	0.471	0.242	0.110
1.00E-07	0.836	-0.061	0.355
3.16E-08	1.147	1.648	0.928
1.00E-08	3.882	2.969	3.424
3.16E-09	10.550	10.879	10.548
1.00E-09	37.844	38.407	34.769
3.16E-10	67.695	66.387	56.079
1.00E-10	91.647	84.555	82.672
3.16E-11	100.046	82.218	96.137
1.00E-11	102.384	100.203	98.221

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	5.677e-010	3.969e-010	-1.090
Std. Deviation	1.252e-010	8.758e-011	0.107
Std. Error	7.231e-011	5.057e-011	0.062
Lower 95% CI of mean	2.566e-010	1.794e-010	-1.357
Upper 95% CI of mean	8.788e-010	6.145e-010	-0.823
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

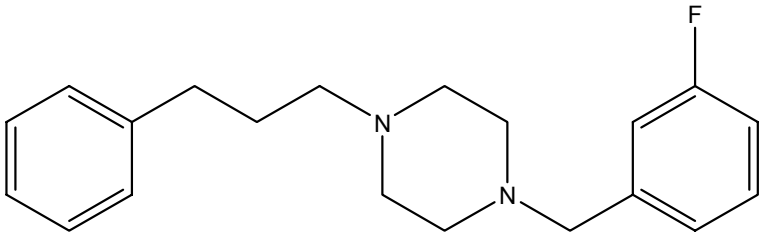


log [drug] (M) (σ_2)	(1d) (R= 3-I)(1) (%)	(1d) (R= 3-I)(2) (%)	(1d) (R= 3-I)(3) (%)
1.00E-06	17.407	17.854	18.401
3.16E-07	17.0971	20.429	24.011
1.00E-07	17.172	25.379	20.767
3.16E-08	24.361	26.925	23.754
1.00E-08	28.060	30.889	35.854
3.16E-09	45.260	39.471	42.308
1.00E-09	63.930	65.663	66.359
3.16E-10	68.344	71.842	70.164
1.00E-10	84.026	96.803	93.593
3.16E-11	95.125	89.463	89.419

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	1.155e-009	1.026e-009	-0.752
Std. Deviation	1.520e-010	1.348e-010	0.147
Std. Error	8.778e-011	7.782e-011	0.082
Lower 95% CI of mean	7.773e-010	6.913e-010	-1.107
Upper 95% CI of mean	1.533e-009	1.361e-009	-0.398
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

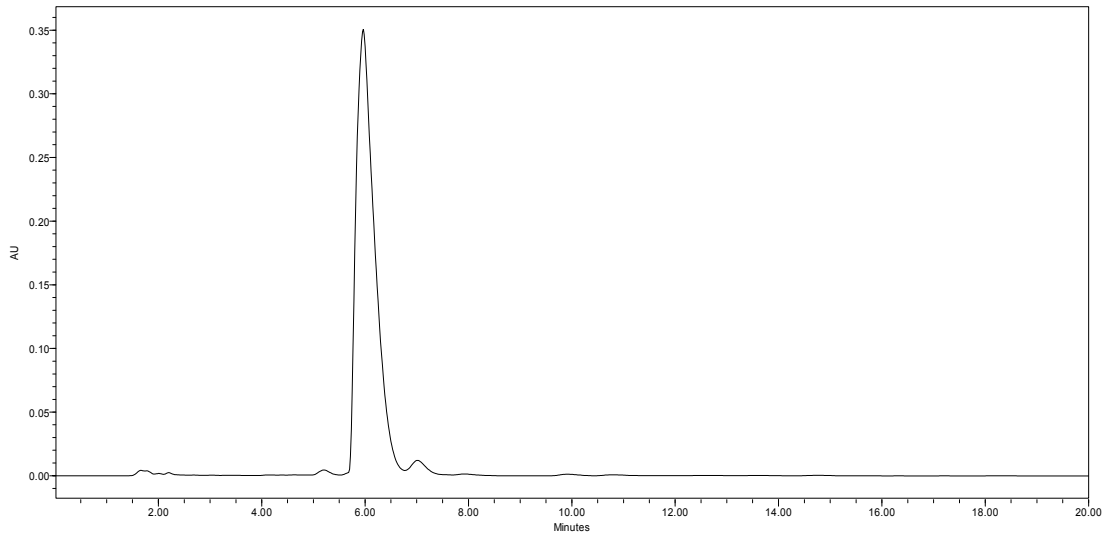


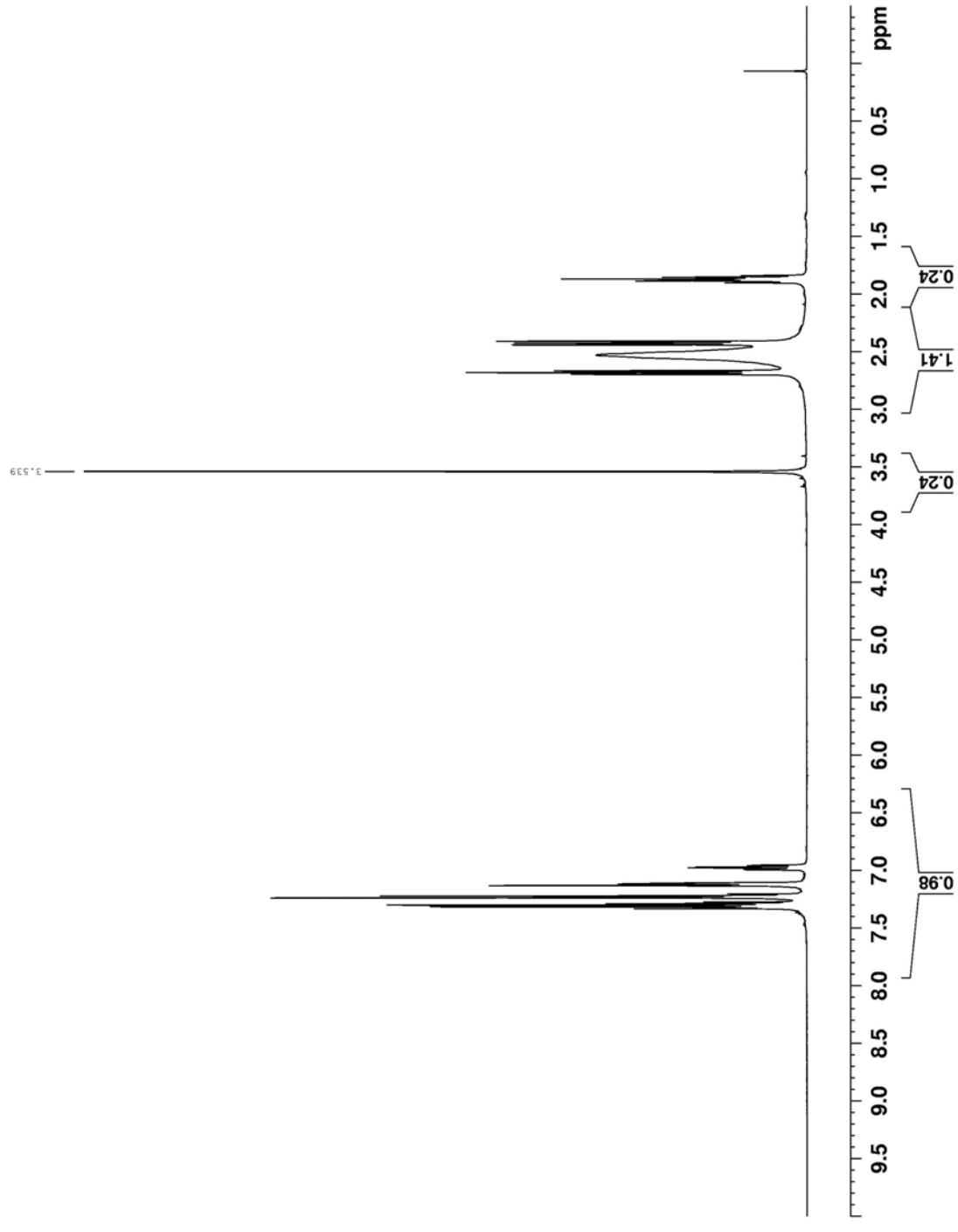
Compound 1e:



Compound 1e

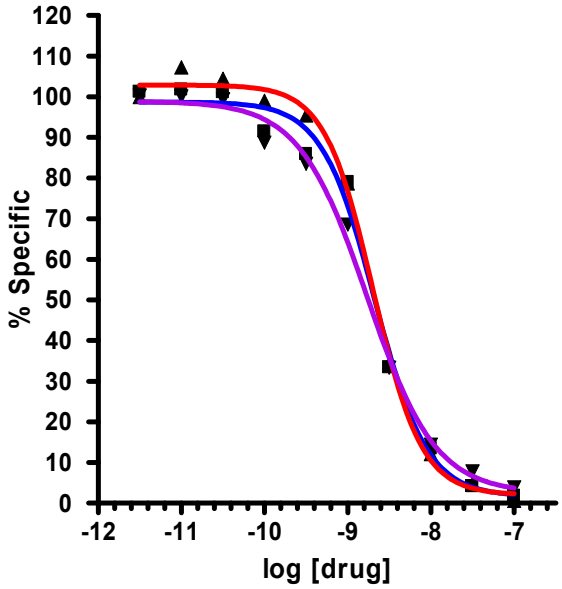
Elemental analysis $C_{20}H_{25}N_2F$	**C %**	**H %**	**N %**
Calculated	76.89	8.07	8.97
Found	76.91	8.08	9.10




3.539
0.98
0.24
1.41
0.24
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

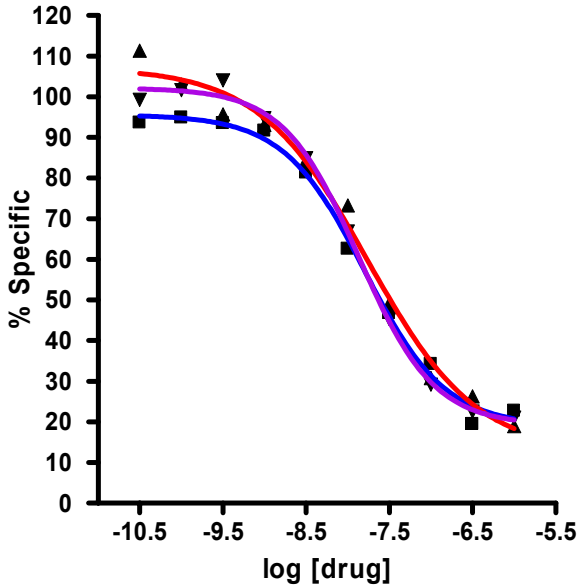
log [drug] (M) (σ_1)	(1e) (R= 3-F)(1) (%)	(1e) (R= 3-F)(2) (%)	(1e) (R= 3-F)(3) (%)
3.16E-07	1.794	0.559	3.877
1.00E-07	4.153	4.853	7.853
3.16E-08	13.427	12.035	14.388
1.00E-08	33.375	34.438	33.311
3.16E-09	79.027	78.677	68.552
1.00E-09	85.882	95.408	83.494
3.16E-10	91.475	99.028	88.714
1.00E-10	101.078	104.473	99.414
3.16E-11	101.775	107.227	100.076
1.00E-11	101.253	99.938	100.321

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	1.947e-009	1.361e-009	-1.309
Std. Deviation	2.023e-010	1.418e-010	0.223
Std. Error	1.168e-010	8.185e-011	0.129
Lower 95% CI of mean	1.445e-009	1.009e-009	-1.863
Upper 95% CI of mean	2.450e-009	1.713e-009	-0.755
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

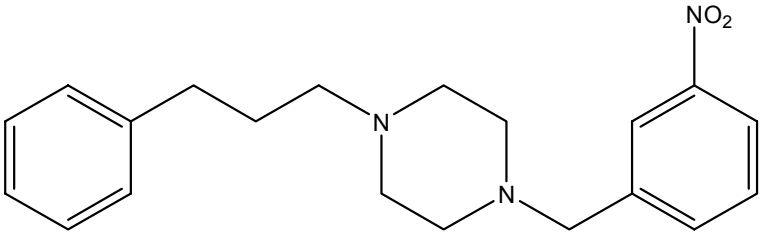


log [drug] (M) (σ_2)	(1e) (R= 3-F)(1) (%)	(1e) (R= 3-F)(2) (%)	(1e) (R= 3-F)(3) (%)
1.00E-06	22.723	19.005	21.022
3.16E-07	19.474	26.319	22.527
1.00E-07	34.253	30.774	29.177
3.16E-08	46.757	49.122	45.288
1.00E-08	62.616	73.283	66.857
3.16E-09	81.327	84.412	84.868
1.00E-09	91.660	93.072	94.706
3.16E-10	93.490	95.685	104.064
1.00E-10	94.843	103.042	101.622
3.16E-11	93.669	111.340	99.256

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	1.559e-008	1.385e-008	-0.839
Std. Deviation	1.920e-009	1.707e-009	0.155
Std. Error	1.109e-009	9.857e-010	0.090
Lower 95% CI of mean	1.082e-008	9.609e-009	-1.224
Upper 95% CI of mean	2.036e-008	1.809e-008	-0.452
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

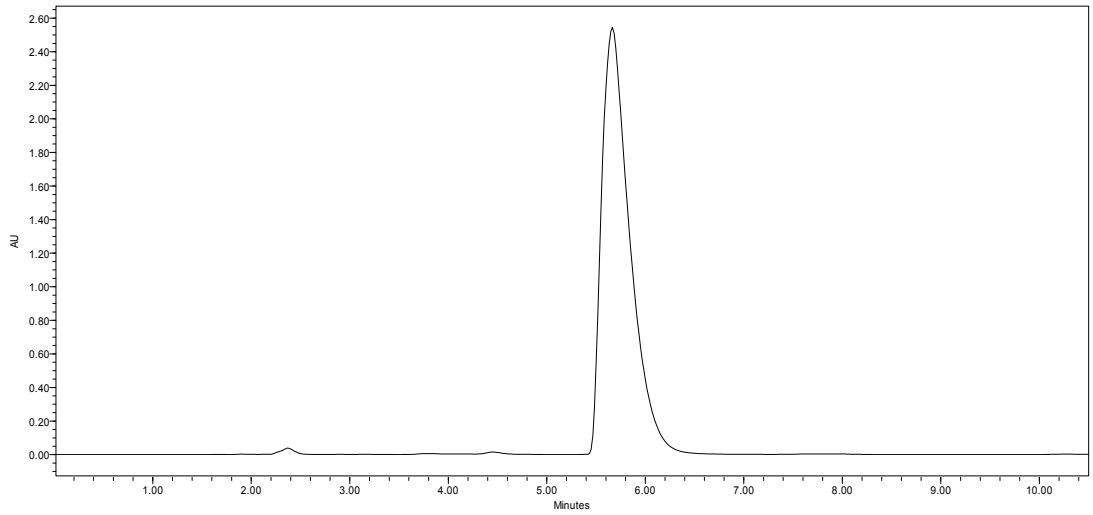


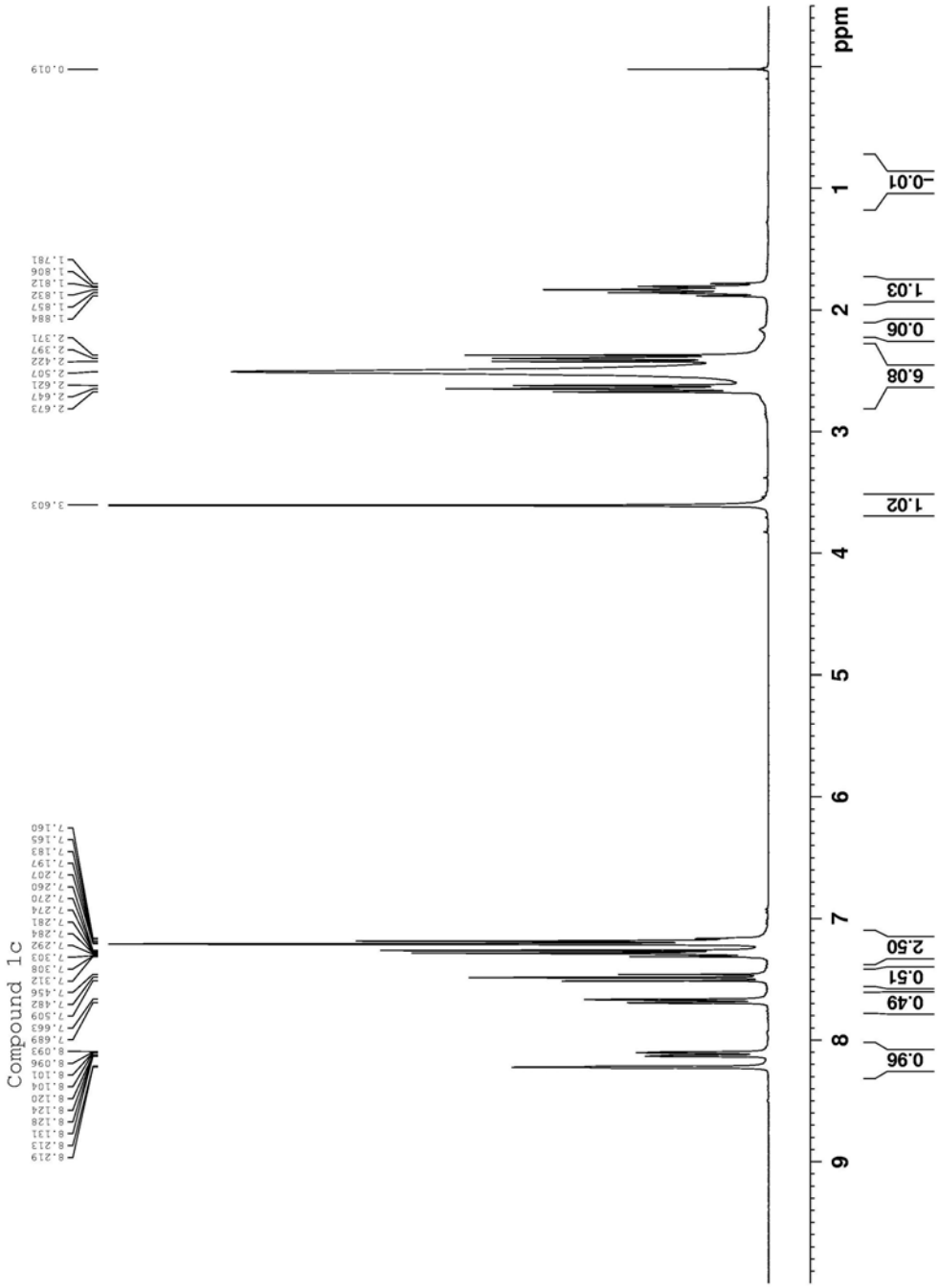
Compound 1f:



Compound 1f

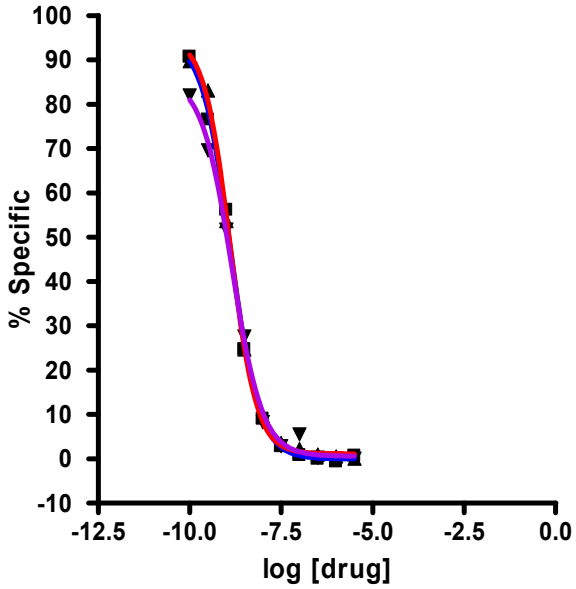
Elemental analysis $C_{20}H_{25}N_3O_2.025H_2O$	**C %**	**H %**	**N %**
Calculated	70.67	7.44	12.37
Found	70.31	7.50	12.12




Compound 1c
ppm
0.01
1.03
0.06
6.08
1.02
2.50
0.51
0.49
0.96

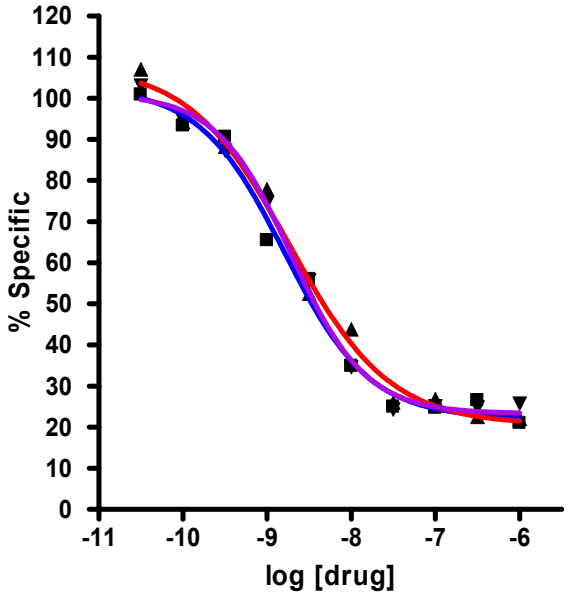
log [drug] (M) (σ_1)	(1f) (R= 3-NO_2)(1) (%)	(1f) (R= 3-NO_2)(2) (%)	(1f) (R= 3-NO_2)(3) (%)
3.16E-06	0.694	0.052	0.038
1.00E-07	-0.606	0.563	-0.005
3.16E-07	0.017	0.995	0.274
1.00E-07	0.812	2.247	5.541
3.16E-08	2.847	3.557	2.877
1.00E-08	8.998	9.628	8.252
3.16E-09	24.677	24.691	27.571
1.00E-09	56.171	53.661	51.724
3.16E-10	76.516	83.134	69.542
1.00E-10	90.706	89.665	81.960

(σ_1)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	1.334e-009	9.328e-010	-1.122
Std. Deviation	7.586e-011	5.296e-011	0.084
Std. Error	4.380e-011	3.057e-011	0.0483
Lower 95% CI of mean	1.146e-009	8.012e-010	-1.330
Upper 95% CI of mean	1.523e-009	1.064e-009	-0.914
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

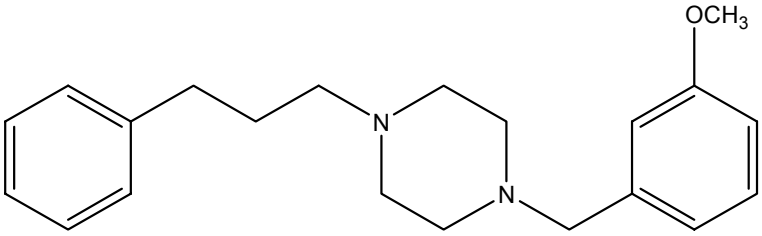


log [drug] (M) (σ_2)	(1f) (R= 3-NO_2)(1) (%)	(1f) (R= 3-NO_2)(2) (%)	(1f) (R= 3-NO_2)(3) (%)
1.00E-06	21.055	22.080	25.750
3.16E-07	26.596	22.550	24.777
1.00E-07	24.714	26.837	25.125
3.16E-08	25.013	25.999	24.317
1.00E-08	34.881	43.798	34.540
3.16E-09	56.046	52.488	55.783
1.00E-09	65.441	77.838	74.589
3.16E-10	90.615	88.244	87.281
1.00E-10	93.394	94.1740	94.572
3.16E-11	100.918	107.038	103.090

(σ_2)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	1.789e-009	1.589e-009	-0.854
Std. Deviation	1.567e-010	1.392e-010	0.123
Std. Error	9.048e-011	8.039e-011	0.071
Lower 95% CI of mean	1.399e-009	1.243e-009	-1.159
Upper 95% CI of mean	2.178e-009	1.935e-009	-0.548
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

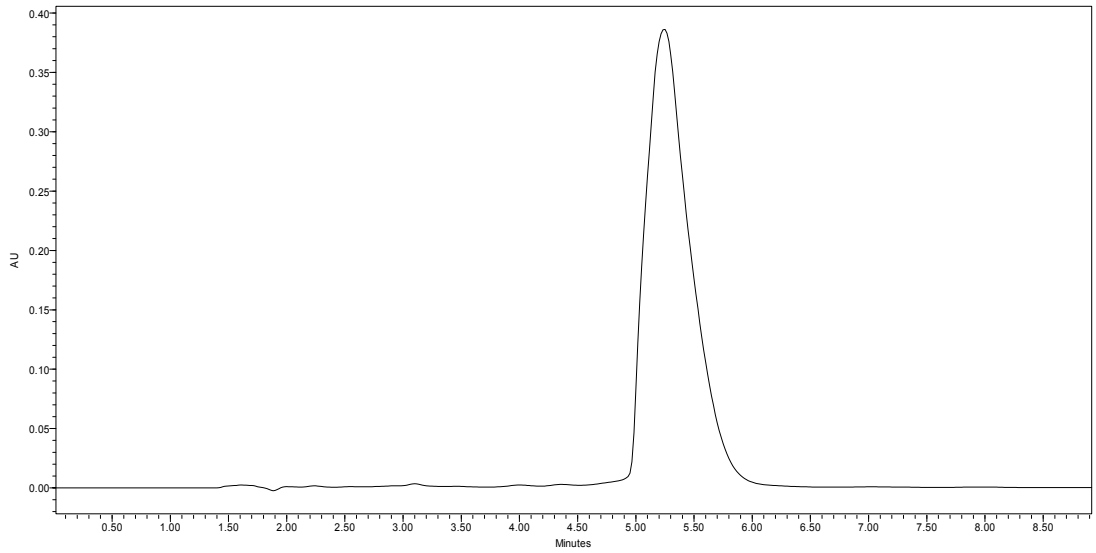


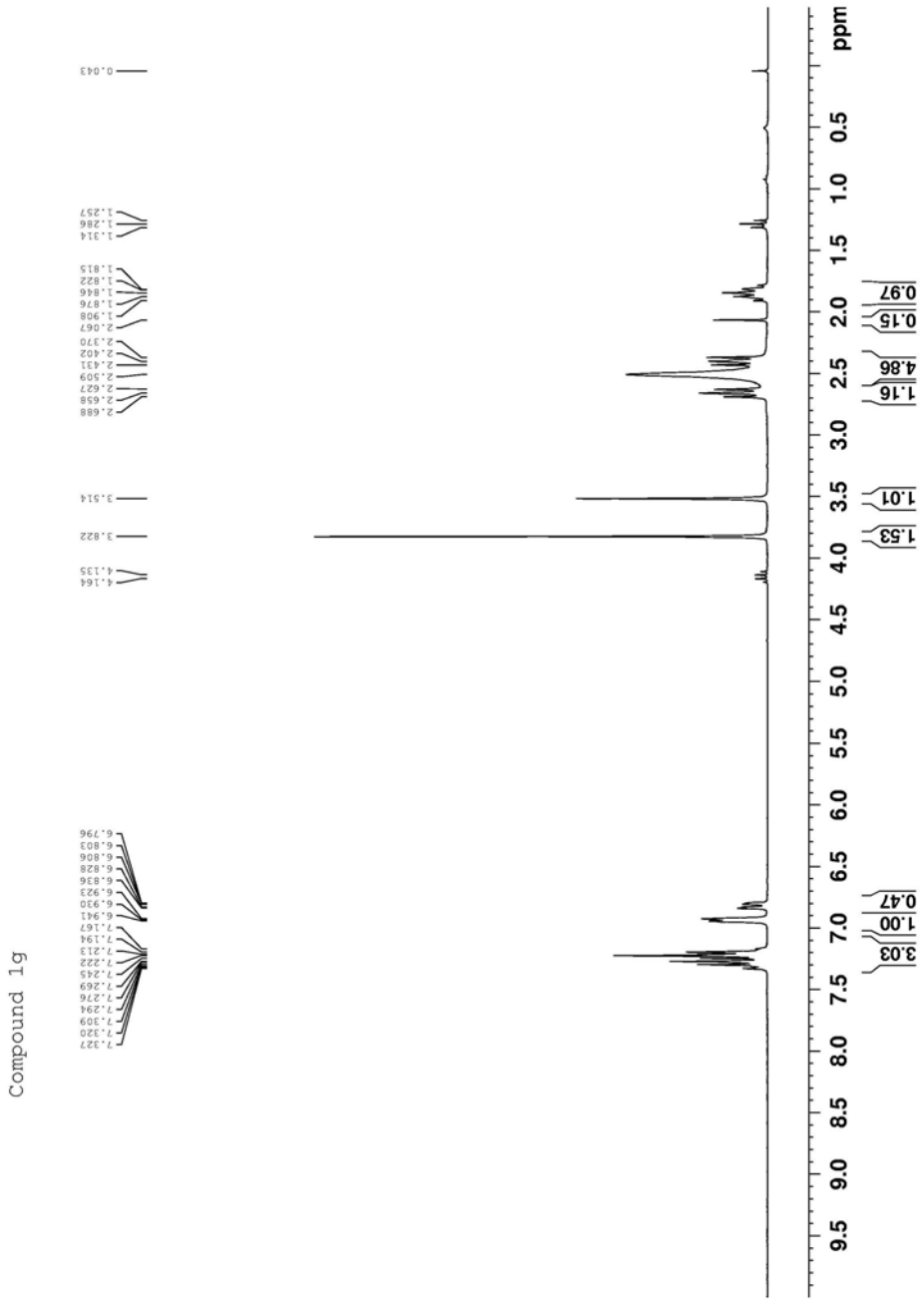
Compound 1g:



Compound 1g

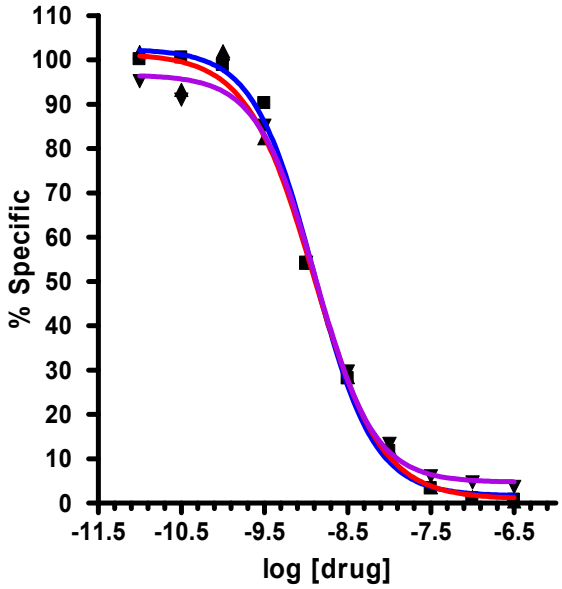
Elemental analysis $C_{21}H_{28}N_2O.25H_2O$	**C %**	**H %**	**N %**
Calculated	76.67	8.73	8.52
Found	76.82	8.73	8.53




Compound 1g
7.327
7.320
7.309
7.294
7.276
7.269
7.245
7.222
7.213
7.194
7.167
6.941
6.930
6.923
6.836
6.828
6.806
6.803
6.796
4.164
4.135
3.822
3.514
2.688
2.658
2.627
2.509
2.431
2.402
2.370
2.067
1.908
1.876
1.846
1.822
1.815
1.314
1.286
1.257
0.043
3.03
1.00
0.47
1.53
1.01
1.16
4.86
0.15
0.97
ppm

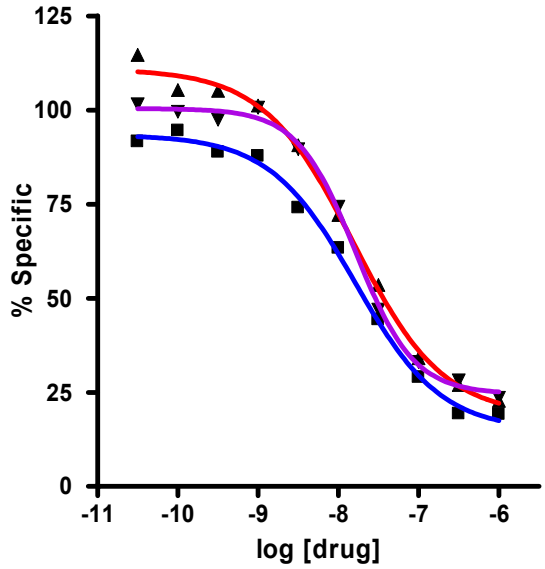
log [drug] (M) (σ_1)	(1g) (R= 3-OCH_3)(1) (%)	(1g) (R= 3-OCH_3)(2) (%)	(1g) (R= 3-OCH_3)(3) (%)
3.16E-06	0.791	0.434	3.686
1.00E-07	1.102	1.321	4.665
3.16E-07	3.338	3.588	6.043
1.00E-07	11.733	12.281	13.372
3.16E-08	28.193	28.430	29.659
1.00E-08	53.923	54.764	54.278
3.16E-09	90.214	82.339	85.120
1.00E-09	98.785	101.967	98.662
3.16E-10	100.497	93.264	91.065
1.00E-10	100.132	101.777	95.170

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	1.258e-009	8.793e-010	-1.171
Std. Deviation	3.754e-011	2.628e-011	0.0808
Std. Error	2.167e-011	1.517e-011	0.0466
Lower 95% CI of mean	1.164e-009	8.140e-010	-1.371
Upper 95% CI of mean	1.351e-009	9.445e-010	-0.970
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

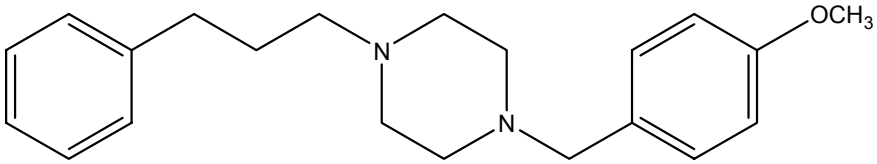


log [drug] (M) (σ_2)	(1g) (R= 3-OCH_3)(1) (%)	(1g) (R= 3-OCH_3)(2) (%)	(1g) (R= 3-OCH_3)(3) (%)
1.00E-06	19.192	22.776	23.509
3.16E-07	19.360	26.953	28.160
1.00E-07	29.001	34.091	33.119
3.16E-08	44.306	53.558	46.962
1.00E-08	63.355	72.137	74.319
3.16E-09	74.010	90.654	89.538
1.00E-09	87.807	101.146	100.605
3.16E-10	88.854	105.082	97.372
1.00E-10	94.518	105.336	99.522
3.16E-11	91.665	114.642	101.511

(σ_2)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	1.597e-008	1.419e-008	-0.928
Std. Deviation	5.025e-010	4.459e-010	0.234
Std. Error	2.901e-010	2.574e-010	0.135
Lower 95% CI of mean	1.473e-008	1.308e-008	-1.508
Upper 95% CI of mean	1.722e-008	1.530e-008	-0.347
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

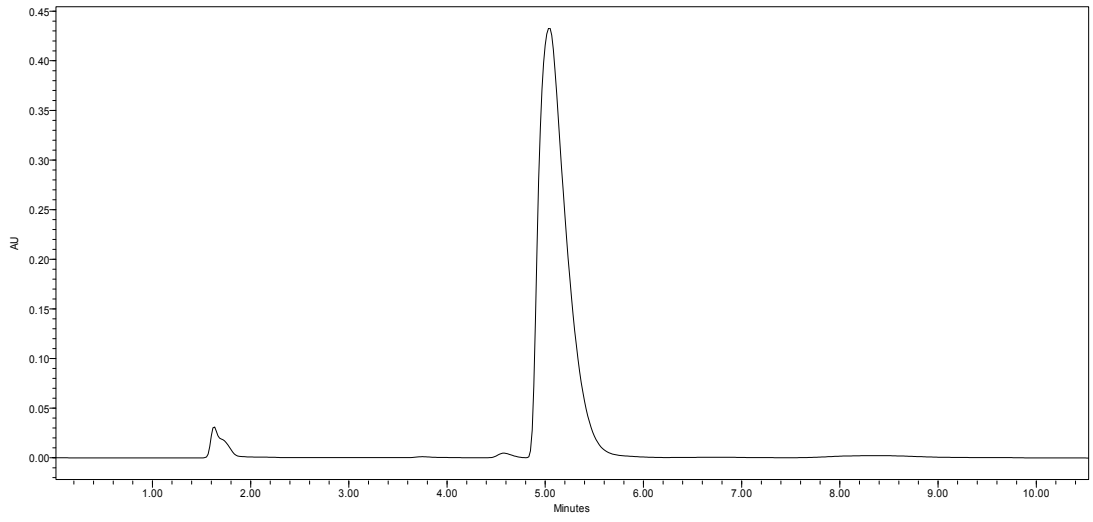


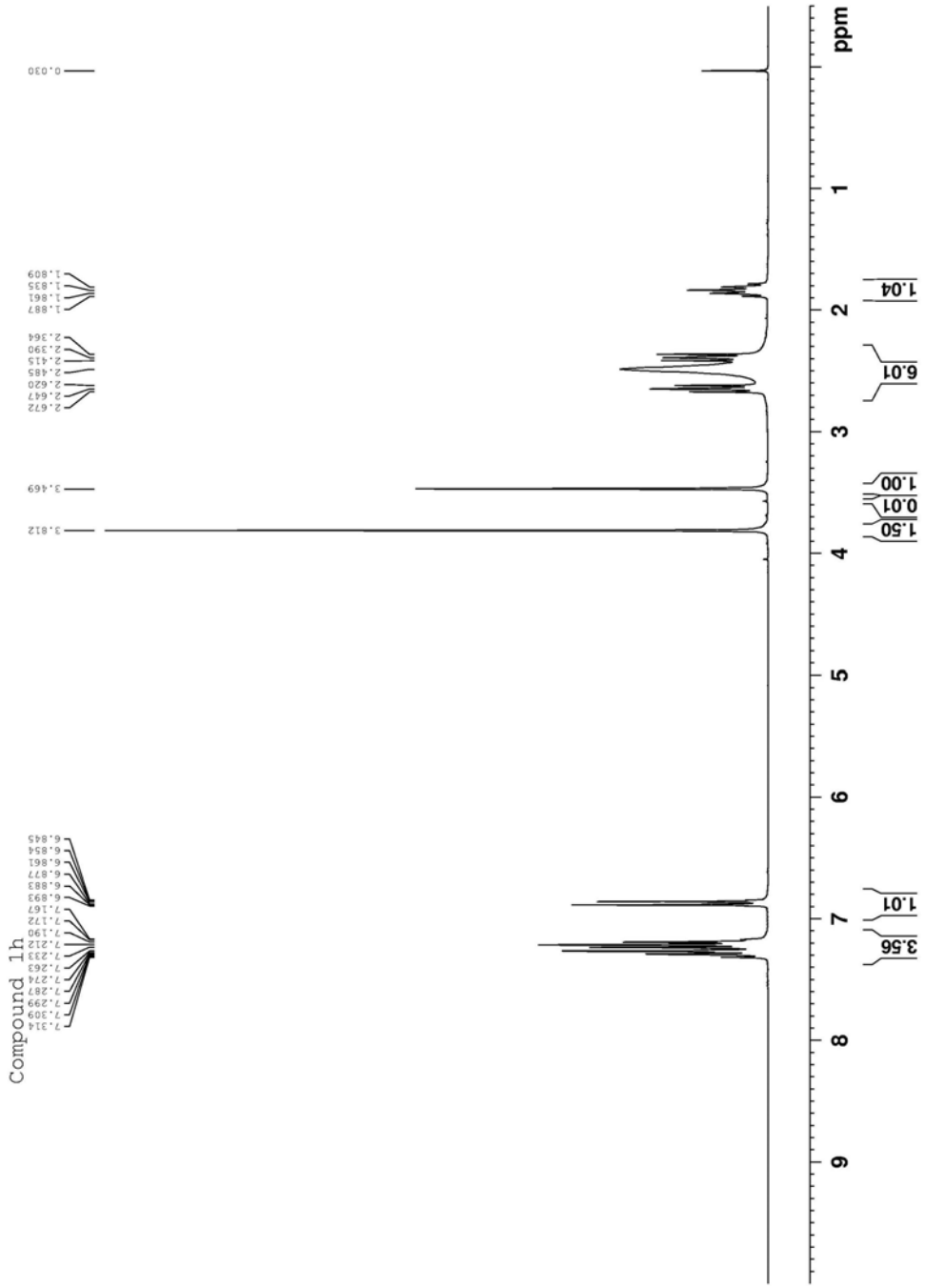
Compound 1h:



Compound 1h

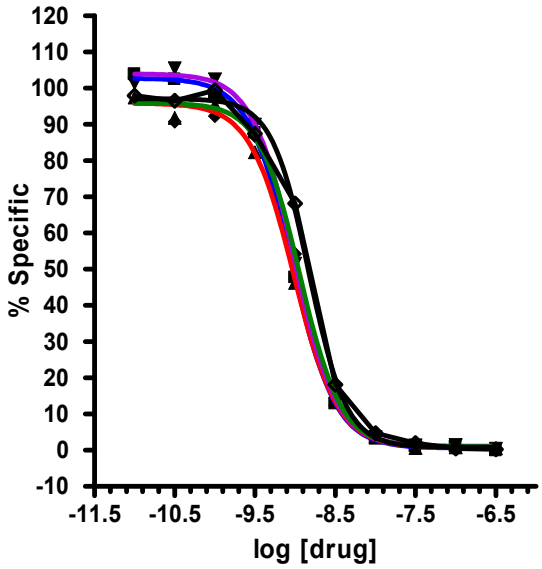
Elemental analysis $C_{21}H_{28}N_2O.5H_2O$	**C %**	**H %**	**N %**
Calculated	75.68	8.55	8.40
Found	76.06	8.60	8.25




Compound 1h
7.314
7.309
7.299
7.287
7.274
7.263
7.233
7.212
7.190
7.172
7.167
6.893
6.883
6.877
6.861
6.854
6.845
3.812
3.469
2.672
2.647
2.620
2.485
2.415
2.390
2.364
1.887
1.861
1.835
1.809
0.030
ppm
1
2
3
4
5
6
7
8
9
1.04
6.01
1.00
0.01
1.50
1.01
3.56

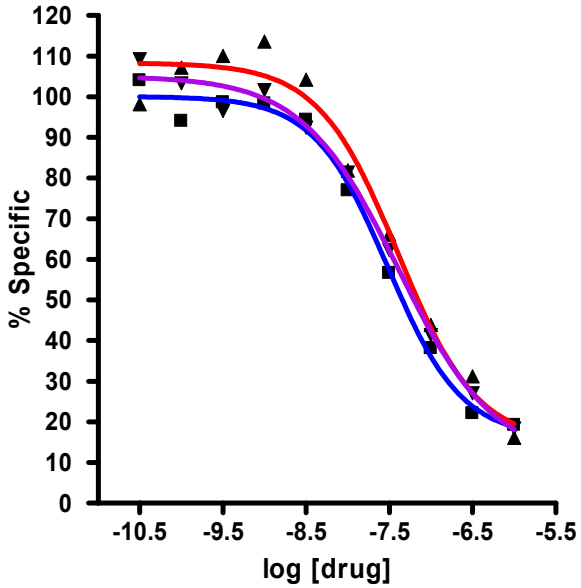
log [drug] (M) (σ_1)	(1h) (R= 4-OCH_3)(1) (%)	(1h) (R= 4-OCH_3)(2) (%)	(1h) (R= 4-OCH_3)(3) (%)	(1h) (R= 4-OCH_3)(4) (%)
3.16E-06	0.545	0.420	0.403	0.545
1.00E-07	0.812	1.472	0.717	0.812
3.16E-07	0.631	1.365	1.734	0.631
1.00E-07	3.496	3.684	4.430	3.496
3.16E-08	14.091	14.571	14.510	14.091
1.00E-08	46.287	51.499	54.249	46.287
3.16E-09	82.323	89.958	86.799	82.323
1.00E-09	95.377	102.477	92.365	95.377
3.16E-10	91.794	105.552	90.749	91.794
1.00E-10	97.429	101.335	102.522	97.429

(σ_1)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	4	4	4
Mean	1.098e-009	7.678e-010	-1.678
Std. Deviation	2.358e-010	1.650e-010	0.119
Std. Error	1.054e-010	7.378e-011	0.0530
Lower 95% CI of mean	8.054e-010	5.630e-010	-1.825
Upper 95% CI of mean	1.391e-009	9.726e-010	-1.531
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

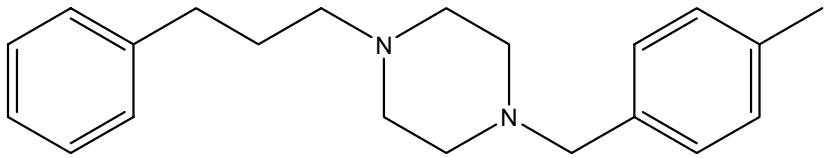


log [drug] (M) (σ_2)	(1h) (R= 4-OCH_3)(1) (%)	(1h) (R= 4-OCH_3)(2) (%)	(1h) (R= 4-OCH_3)(3) (%)
1.00E-06	19.263	16.095	18.389
3.16E-07	22.148	31.239	27.031
1.00E-07	38.143	43.848	41.300
3.16E-08	56.661	66.078	62.357
1.00E-08	76.988	81.863	81.317
3.16E-09	94.342	104.247	92.386
1.00E-09	98.343	113.638	101.666
3.16E-10	98.611	110.031	96.483
1.00E-10	94.044	107.151	103.309
3.16E-11	104.038	98.128	109.263

(σ_2)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	3.693e-008	3.281e-008	-0.866
Std. Deviation	5.722e-009	5.080e-009	0.176
Std. Error	3.303e-009	2.933e-009	0.101
Lower 95% CI of mean	2.272e-008	2.019e-008	-1.303
Upper 95% CI of mean	5.115e-008	4.543e-008	-0.4297
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

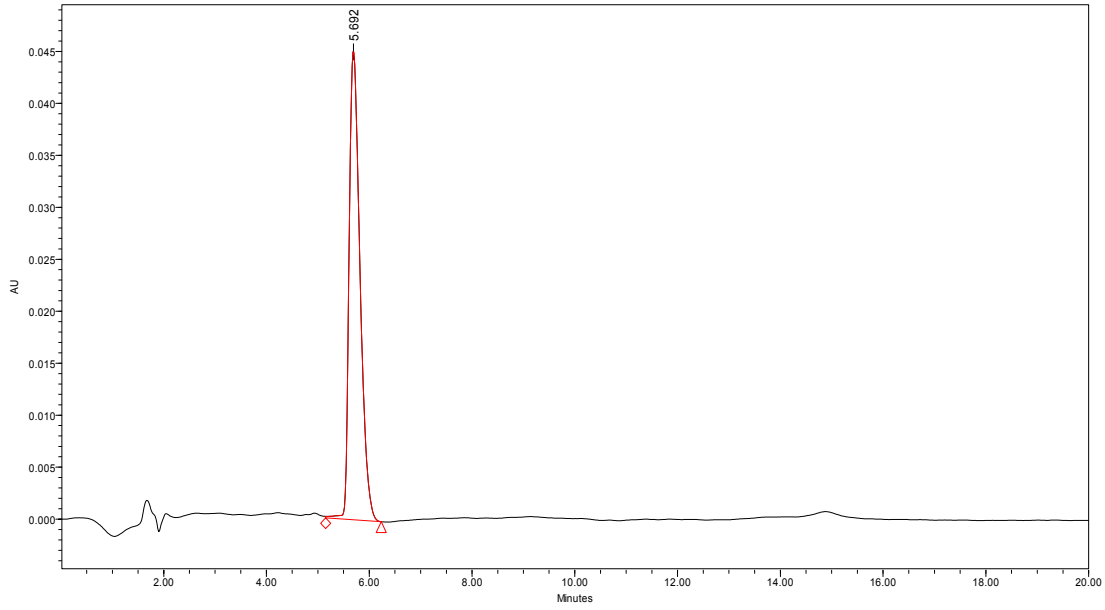


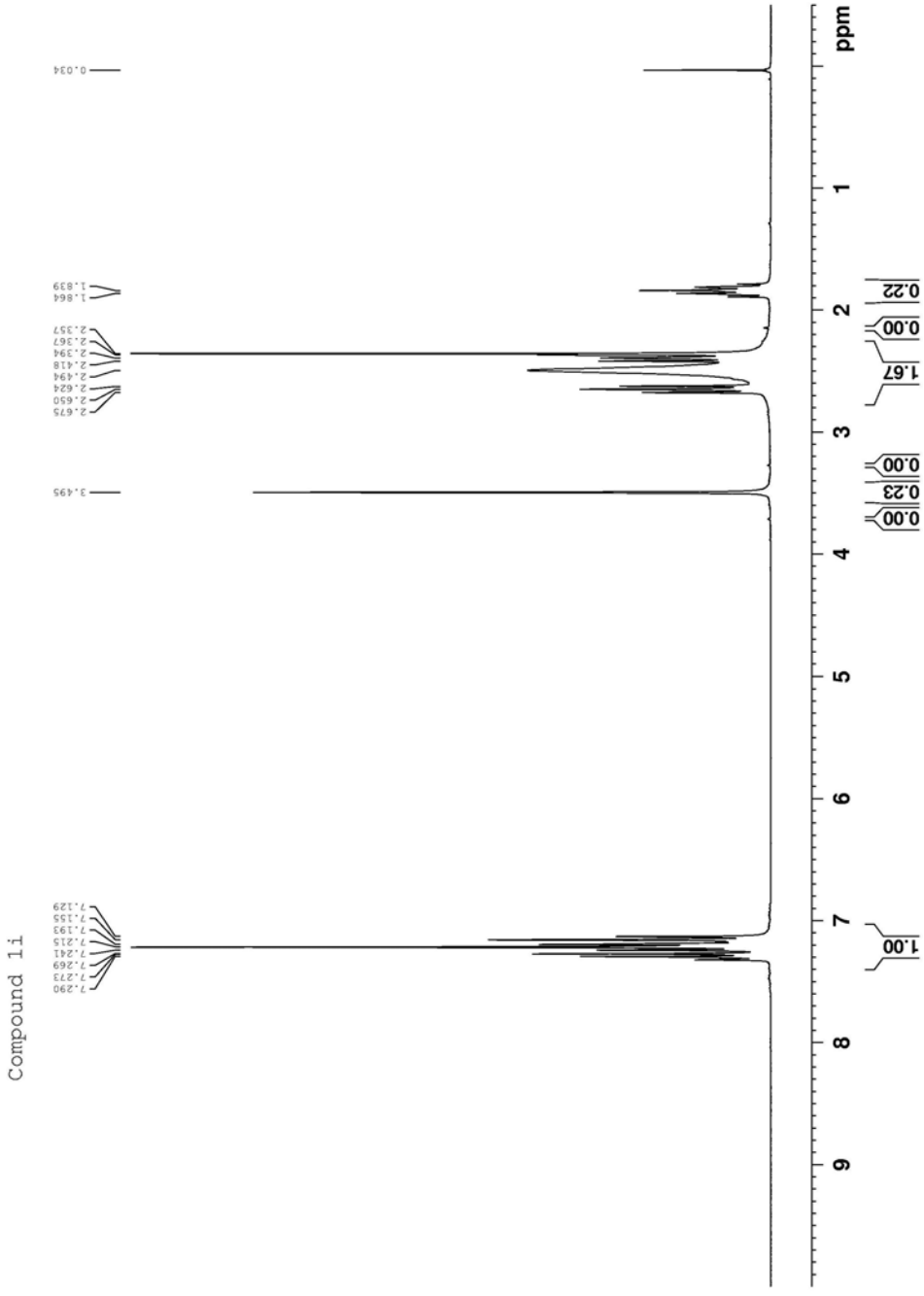
Compound 1i:



Compound 1i

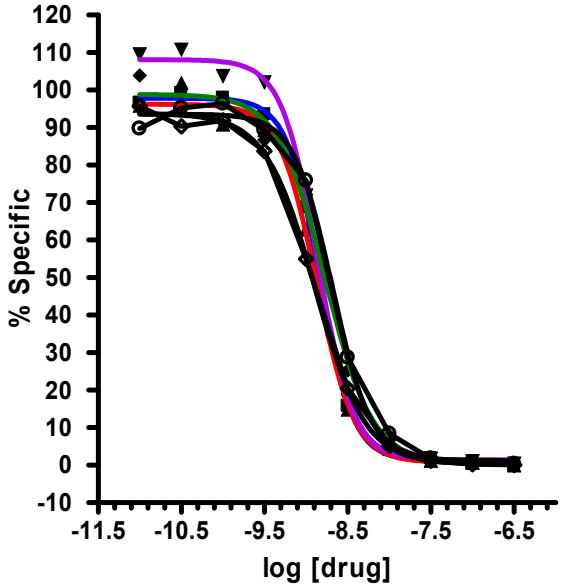
Elemental analysis $C_{21}H_{28}N_2$	**C %**	**H %**	**N %**
Calculated	81.77	9.15	9.08
Found	81.91	9.30	9.19




Compound 1i
7.290
7.273
7.269
7.241
7.215
7.193
7.155
7.129
3.495
2.675
2.650
2.624
2.494
2.418
2.394
2.367
2.357
1.864
1.839
0.034
1.00
1.67
0.00
0.22
0.00
0.23
0.00
ppm

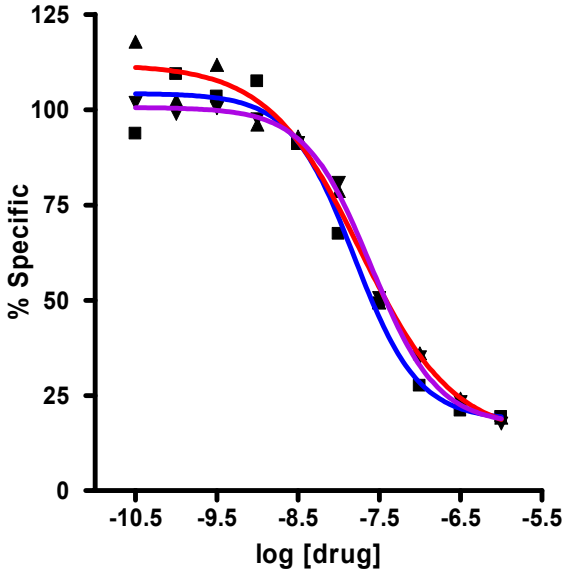
log [drug] (M) (σ_1)	(1i) (R= 4-CH_3)(1) (%)	(1i) (R= 4-CH_3)(2) (%)	(1i) (R= 4-CH_3)(3) (%)	(1i) (R= 4-CH_3)(4) (%)	(1i) (R= 4-CH_3)(5) (%)	(1i) (R= 4-CH_3)(6) (%)
3.16E-06	0.003	-0.005	0.384	-0.236	0.139	0.316
1.00E-07	0.472	0.633	1.110	0.887	0.263	0.473
3.16E-07	1.793	1.214	1.772	2.023	1.191	1.734
1.00E-07	4.733	4.399	5.407	7.830	5.130	8.397
3.16E-08	15.837	14.846	17.283	25.267	20.480	28.704
1.00E-08	68.789	62.823	71.854	72.078	54.988	75.859
3.16E-09	93.494	89.350	102.061	86.701	83.717	89.378
1.00E-09	97.961	90.874	103.729	97.988	91.878	96.419
3.16E-10	99.203	101.916	110.743	96.034	90.353	95.244
1.00E-10	96.629	95.920	109.536	103.885	95.764	89.738

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	6	6	6
Mean	1.538e-009	1.075e-009	-1.826
Std. Deviation	2.954e-010	2.066e-010	0.278
Std. Error	1.206e-010	8.434e-011	0.113
Lower 95% CI of mean	1.228e-009	8.586e-010	-2.117
Upper 95% CI of mean	1.848e-009	1.292e-009	-1.534
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

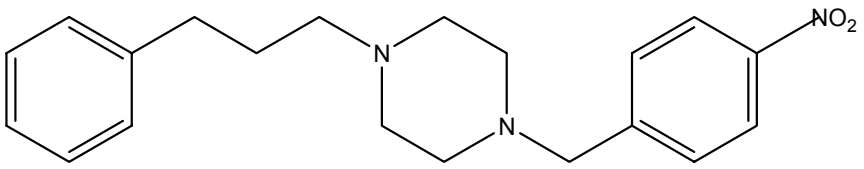


log [drug] (M) (σ_2)	(1i) (R= 4-CH_3)(1) (%)	(1i) (R= 4-CH_3)(2) (%)	(1i) (R= 4-CH_3)(3) (%)
1.00E-06	19.371	19.175	17.595
3.16E-07	20.9791	24.198	23.273
1.00E-07	27.563	36.071	34.976
3.16E-08	49.206	49.429	50.523
1.00E-08	67.443	78.697	80.784
3.16E-09	90.888	92.956	91.264
1.00E-09	107.471	96.107	97.454
3.16E-10	103.420	111.768	100.405
1.00E-10	109.266	102.427	98.899
3.16E-11	93.663	117.842	101.821

(σ_2)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	1.971e-008	1.751e-008	-0.975
Std. Deviation	4.895e-009	4.349e-009	0.187
Std. Error	2.826e-009	2.511e-009	0.108
Lower 95% CI of mean	7.546e-009	6.704e-009	-1.440
Upper 95% CI of mean	3.187e-008	2.831e-008	-0.510
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

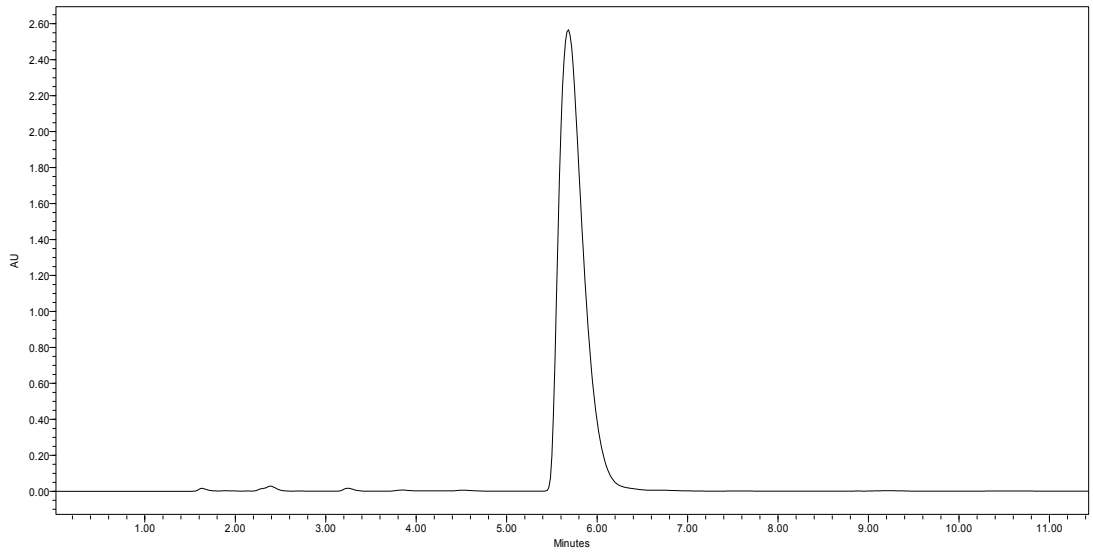


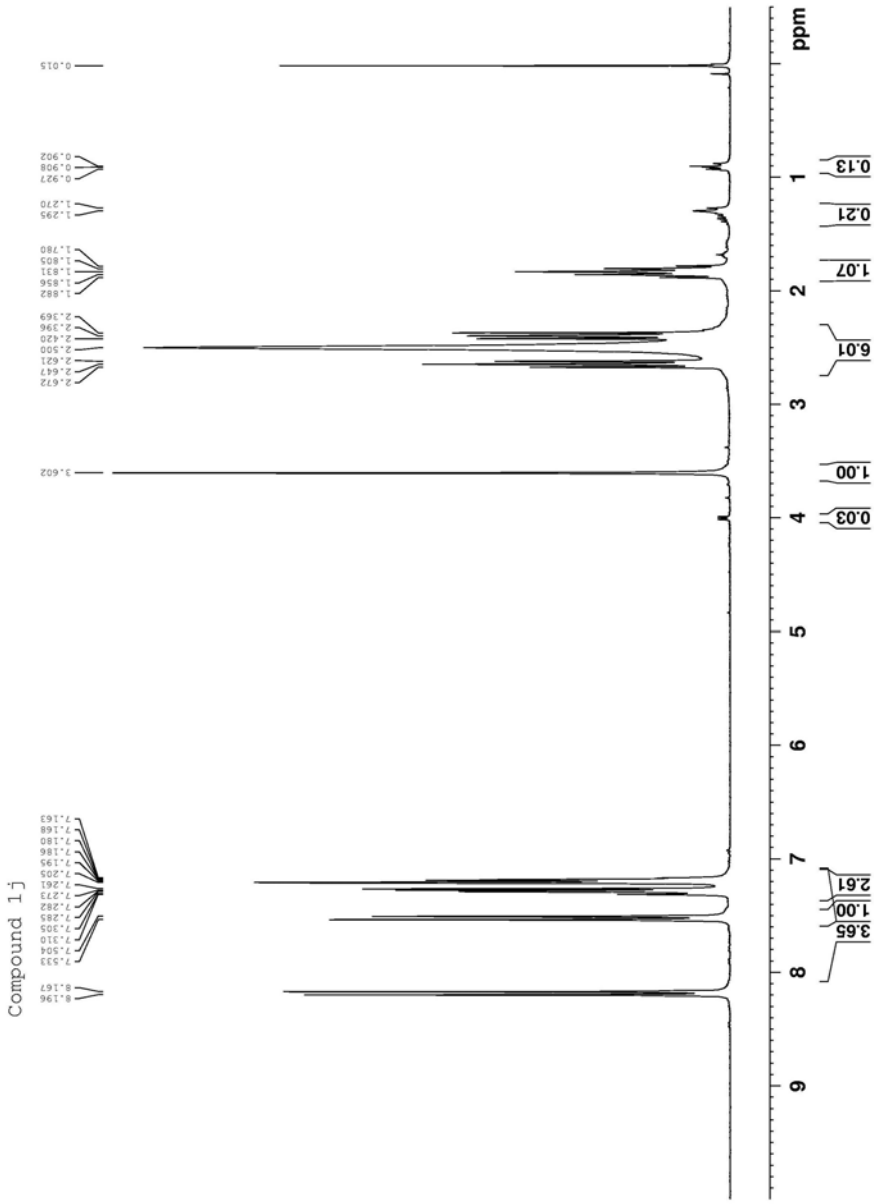
Compound 1j:



Compound 1j

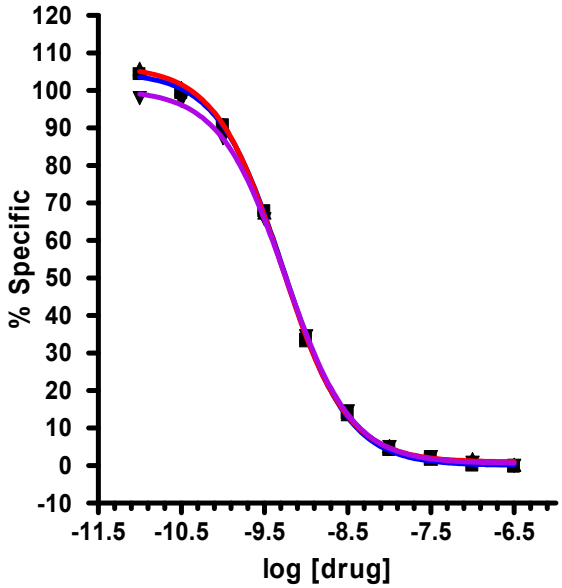
Elemental analysis $C_{20}H_{25}N_3O_2$	**C %**	**H %**	**N %**
Calculated	70.77	7.42	12.38
Found	70.65	7.51	12.20




Compound 1j
8.196
8.167
7.533
7.504
7.316
7.305
7.285
7.282
7.273
7.261
7.205
7.195
7.186
7.180
7.168
7.163
3.602
2.672
2.647
2.621
2.508
2.420
2.396
2.369
1.882
1.856
1.831
1.805
1.780
1.295
1.270
0.927
0.908
0.902
0.015
ppm
1
2
3
4
5
6
7
8
9
0.13
0.21
1.07
6.01
1.00
0.03
2.61
1.00
3.65

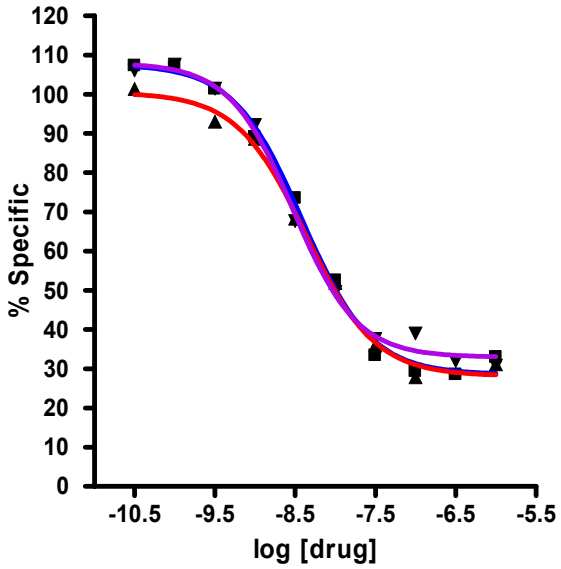
log [drug] (M) (σ_1)	(1j) (R= 4-NO_2)(1) (%)	(1i) (R= 4-NO_2)(2) (%)	(1i) (R= 4-NO_2)(3) (%)
3.16E-06	-0.355	0.252	-0.014
1.00E-07	-0.005	1.736	0.764
3.16E-07	1.764	1.897	2.253
1.00E-07	4.078	5.239	4.911
3.16E-08	13.861	13.742	14.504
1.00E-08	33.493	33.445	34.518
3.16E-09	67.836	67.484	65.663
1.00E-09	90.680	90.817	87.264
3.16E-10	99.293	100.610	97.280
1.00E-10	104.353	105.843	97.980

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	5.293e-010	3.701e-010	-1.098
Std. Deviation	3.190e-011	2.230e-011	0.005
Std. Error	1.842e-011	1.288e-011	0.003
Lower 95% CI of mean	4.501e-010	3.147e-010	-1.111
Upper 95% CI of mean	6.085e-010	4.255e-010	-1.085
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

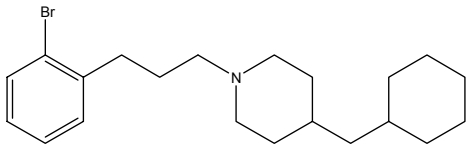


log [drug] (σ_2)	(1j) (R= 4-NO_2)(1) (%)	(1i) (R= 4-NO_2)(2) (%)	(1i) (R= 4-NO_2)(3) (%)
1.00E-06	33.009	31.156	30.803
3.16E-07	28.565		31.936
1.00E-07	29.384	27.851	39.033
3.16E-08	33.377	35.947	37.630
1.00E-08	52.573	51.834	49.593
3.16E-09	73.569	68.266	67.633
1.00E-09	89.096	88.682	92.189
3.16E-10	101.341	92.993	101.422
1.00E-10	107.055		107.568
3.16E-11	107.342	101.395	106.077

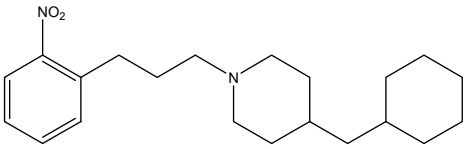
(σ_2)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	3.709e-009	3.296e-009	-1.039
Std. Deviation	6.580e-010	5.846e-010	0.045
Std. Error	3.799e-010	3.375e-010	0.026
Lower 95% CI of mean	2.075e-009	1.844e-009	-1.152
Upper 95% CI of mean	5.344e-009	4.748e-009	-0.926
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



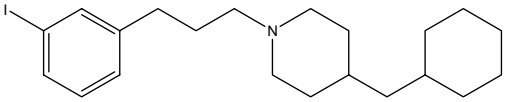
SERIES-3



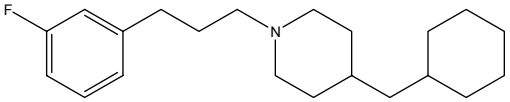
Compound 3b



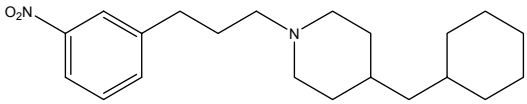
Compound 3c



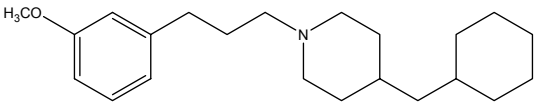
Compound 3d



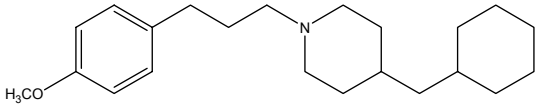
Compound 3e



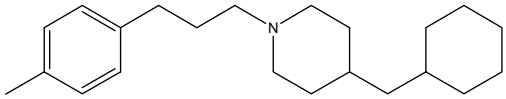
Compound 3f



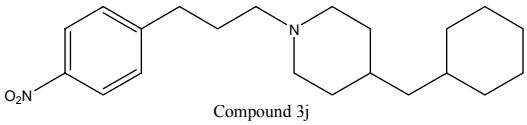
Compound 3g



Compound 3h

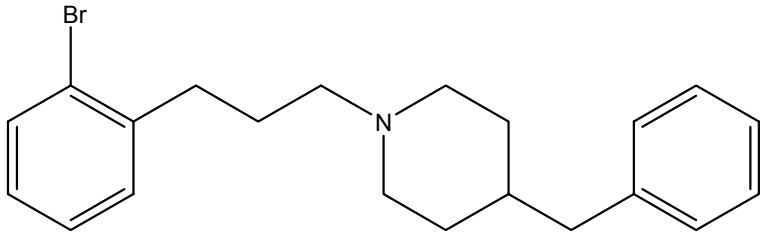


Compound 3i



Compound 3j

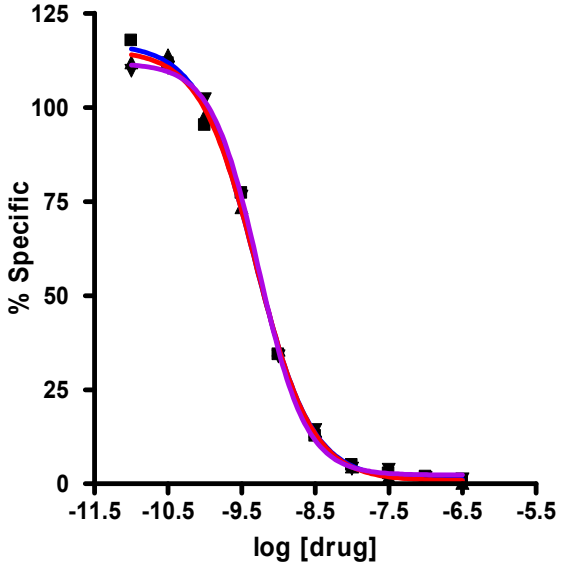
Compound 3b:



Compound 3b

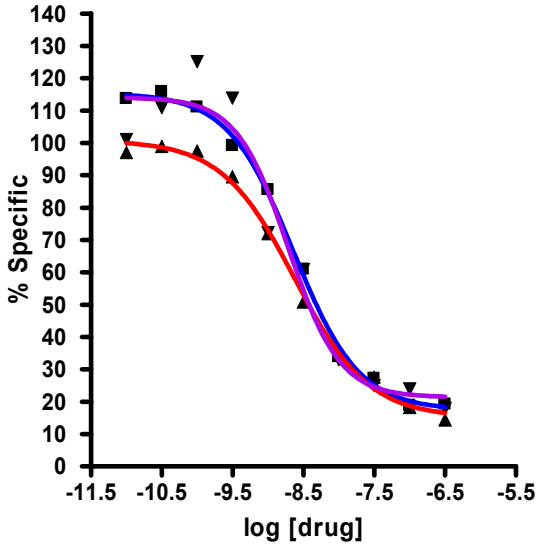
log [drug] (M) (σ_1)	(3b) (R= 2-Br)(1) (%)	(3b) (R= 2-Br)(2) (%)	(3b) (R= 2-Br)(3) (%)
3.16E-07	0.910	0.262	1.155
1.00E-07	1.920	1.313	1.500
3.16E-08	2.770	1.858	3.732
1.00E-08	4.990	4.617	3.999
3.16E-09	12.670	14.082	14.294
1.00E-09	34.351	35.648	33.877
3.16E-10	77.260	73.447	76.317
1.00E-10	95.249	97.829	102.189
3.16E-11	111.711	113.870	110.475
1.00E-11	117.785	111.904	109.733

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	5.070e-010	3.533e-010	-1.208
Std. Deviation	2.964e-011	2.066e-011	0.125
Std. Error	1.711e-011	1.193e-011	0.072
Lower 95% CI of mean	4.334e-010	3.020e-010	-1.518
Upper 95% CI of mean	5.806e-010	4.047e-010	-0.897
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

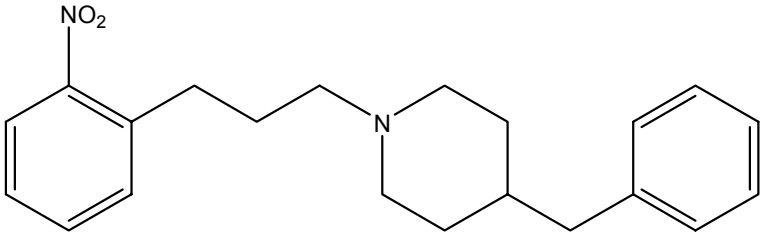


log [drug] (M) (σ_2)	(3b) (R= 2-Br)(1) (%)	(3b) (R= 2-Br)(2) (%)	(3b) (R= 2-Br)(3) (%)
3.16E-07	19.285	14.391	17.923
1.00E-07	18.831	18.236	23.987
3.16E-08	27.219	27.609	24.997
1.00E-08	33.964	35.703	32.963
3.16E-09	60.947	50.831	60.793
1.00E-09	85.557	71.985	72.291
3.16E-10	99.183	89.512	113.861
1.00E-10	111.043	97.561	125.094
3.16E-11	115.850	98.965	110.949
1.00E-11	113.688	97.011	100.996

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	2.205e-009	1.962e-009	-0.988
Std. Deviation	2.461e-010	2.195e-010	0.181
Std. Error	1.421e-010	1.267e-010	0.104
Lower 95% CI of mean	1.593e-009	1.416e-009	-1.437
Upper 95% CI of mean	2.816e-009	2.507e-009	-0.540
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



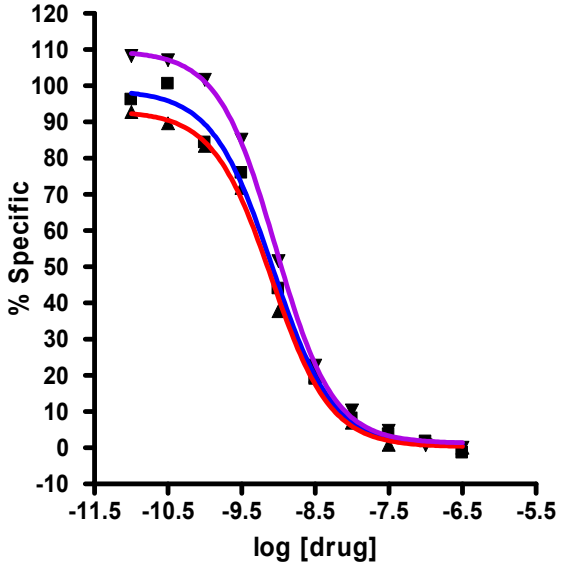
Compound 3c:



Compound 3c

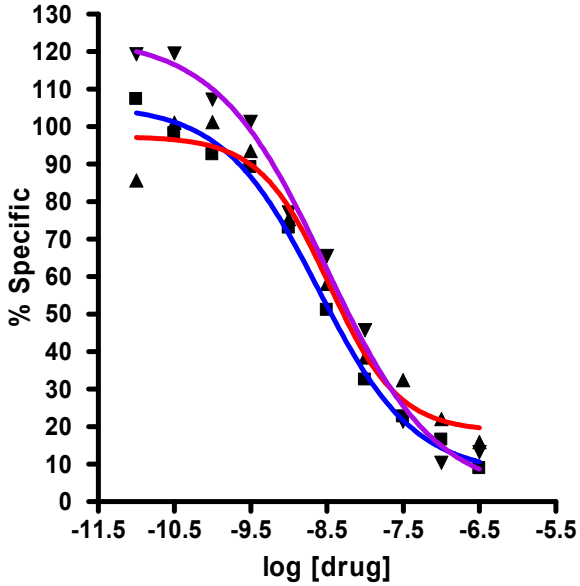
log [drug] (M) (σ_1)	(3c) (R= 2-NO_2)(1) (%)	(3c) (R= 3-NO_2)(2) (%)	(3c) (R= 3-NO_2)(3) (%)
1.00E-06	-1.360	0.018	0.062
3.16E-07	1.771		0.688
1.00E-07	3.742	0.836	4.667
3.16E-08	8.100	6.963	10.327
1.00E-08	19.021	19.873	22.671
3.16E-09	43.959	37.721	51.601
1.00E-09	75.972	71.849	85.146
3.16E-10	84.312	83.472	101.652
1.00E-10	100.543	89.682	107.089
3.16E-11	96.194	92.799	108.287

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	8.527e-010	5.943e-010	-1.086
Std. Deviation	5.331e-011	3.715e-011	0.0281
Std. Error	3.078e-011	2.145e-011	0.0162
Lower 95% CI of mean	7.202e-010	5.020e-010	-1.156
Upper 95% CI of mean	9.851e-010	6.866e-010	-1.017
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

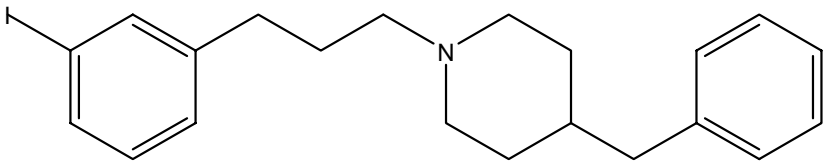


log [drug] (M) (σ_2)	(3c) (R= 2-NO_2)(1) (%)	(3c) (R= 3-NO_2)(2) (%)	(3c) (R= 3-NO_2)(3) (%)
3.16E-07	8.904	15.905	13.192
1.00E-07	16.566	22.032	10.429
3.16E-08	22.703	32.384	21.399
1.00E-08	32.567	38.508	45.767
3.16E-09	51.141	58.086	65.540
1.00E-09	73.074	75.387	77.158
3.16E-10	89.190	93.512	101.284
1.00E-10	92.649	101.246	107.211
3.16E-11	98.241	101.063	119.551
1.00E-11	107.364	85.642	119.326

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	2.946e-009	2.621e-009	-0.748
Std. Deviation	4.614e-010	4.108e-010	0.179
Std. Error	2.664e-010	2.372e-010	0.103
Lower 95% CI of mean	1.799e-009	1.601e-009	-1.194
Upper 95% CI of mean	4.092e-009	3.642e-009	-0.304
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



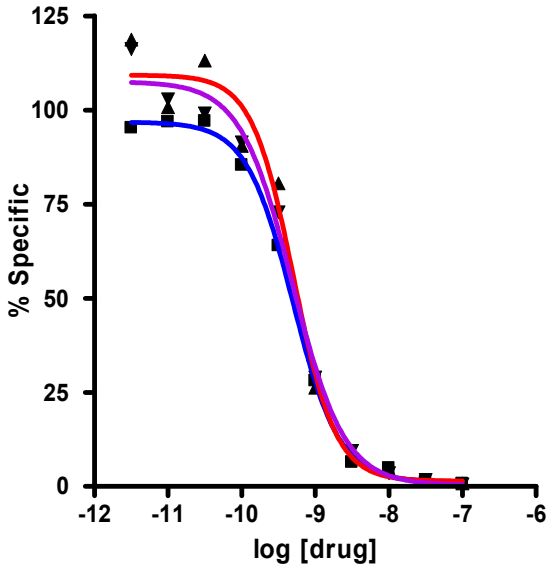
Compound 3d:



Compound 3d

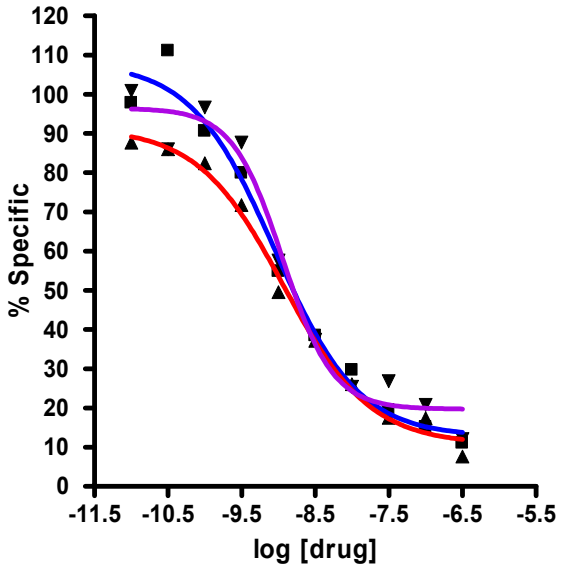
log [drug] (M) (σ_1)	(3d) (R= 3-I)(1) (%)	(3d) (R= 3-I)(2) (%)	(3d) (R= 3-I)(3) (%)
1.00E-07	0.778	0.836	0.546
3.16E-08	1.248	1.668	1.753
1.00E-08	4.861	4.547	3.500
3.16E-09	6.421	7.774	9.456
1.00E-09	28.086	26.251	28.944
3.16E-10	63.994	80.543	72.805
1.00E-10	85.348	90.489	91.429
3.16E-11	97.017	113.128	99.081
1.00E-11	96.873	100.714	102.787
3.16E-12	95.230	118.678	116.213

(σ_1)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	4.993e-010	3.480e-010	-1.377
Std. Deviation	1.054e-011	7.346e-012	0.147
Std. Error	6.087e-012	4.241e-012	0.0852
Lower 95% CI of mean	4.731e-010	3.297e-010	-1.744
Upper 95% CI of mean	5.255e-010	3.662e-010	-1.011
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

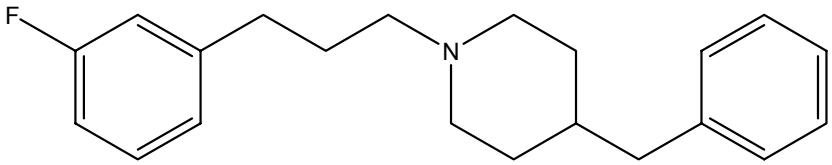


log [drug] (M) (σ_2)	(3d) (R= 3-I)(1) (%)	(3d) (R= 3-I)(2) (%)	(3d) (R= 3-I)(3) (%)
3.16E-07	11.103	7.635	12.036
1.00E-07	15.163	17.387	20.831
3.16E-08	19.528	17.550	26.842
1.00E-08	29.682	26.095	25.371
3.16E-09	38.436	37.182	37.411
1.00E-09	54.889	49.557	57.595
3.16E-10	79.938	71.767	87.681
1.00E-10	90.621	82.414	96.627
3.16E-11	111.136	85.949	85.962
1.00E-11	97.783	87.695	100.923

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	1.755e-008	1.561e-008	-0.933
Std. Deviation	4.427e-010	3.941e-010	0.109
Std. Error	2.556e-010	2.275e-010	0.0631
Lower 95% CI of mean	1.645e-008	1.463e-008	-1.204
Upper 95% CI of mean	1.865e-008	1.659e-008	-0.661
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



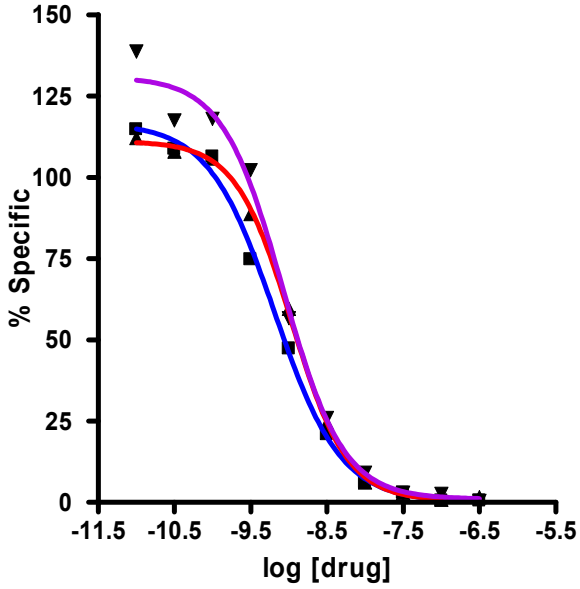
Compound 3e:



Compound 3e

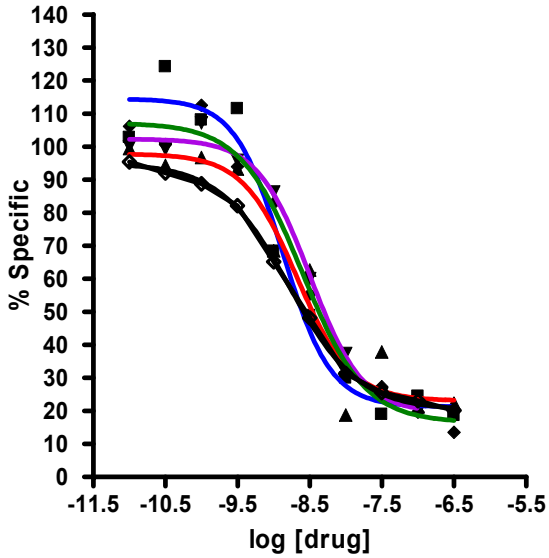
log [drug] (M) (σ_1)	(3e) (R= 3-F)(1) (%)	(3e) (R= 3-F)(2) (%)	(3e) (R= 3-F)(3) (%)
3.16E-07	0.588	1.581	0.458
1.00E-07	0.514	0.789	2.586
3.16E-08	2.962	2.874	2.972
1.00E-08	6.155	6.141	9.143
3.16E-09	20.937	24.432	25.935
1.00E-09	47.341	59.531	56.639
3.16E-10	74.784	88.666	102.151
1.00E-10	106.360	105.752	117.984
3.16E-11	108.781	107.931	117.503
1.00E-11	114.729	112.084	138.652

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	8.540e-010	5.952e-010	-1.064
Std. Deviation	2.136e-010	1.488e-010	0.0549
Std. Error	1.233e-010	8.592e-011	0.0317
Lower 95% CI of mean	3.233e-010	2.255e-010	-1.200
Upper 95% CI of mean	1.385e-009	9.649e-010	-0.927
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

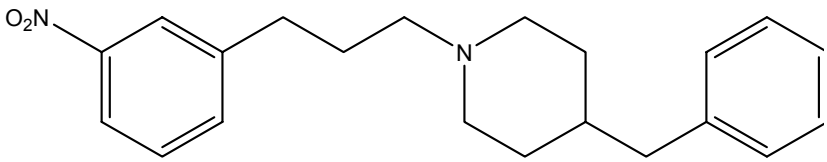


log [drug] (M) (σ_2)	(3e) (R= 3-F)(1) (%)	(3e) (R= 3-F)(2) (%)	(3e) (R= 3-F)(3) (%)	(3e) (R= 3-F)(4) (%)	(3e) (R= 3-F)(5) (%)
1.00E-07	18.664	22.406		13.516	20.145
3.16E-08	24.477	21.259	19.815	19.654	23.132
1.00E-08	18.958	37.887	25.183	27.328	25.560
3.16E-09	29.952	18.826	37.337	33.186	31.283
1.00E-09	48.879	62.670	59.993	55.288	48.142
3.16E-10	68.347	68.500	86.295	82.818	65.140
1.00E-10	111.470	93.199	95.884	93.915	82.127
3.16E-11	108.062	96.714	107.208	112.494	88.736
1.00E-11	124.145	94.352	99.299	100.639	92.033
3.16E-12	102.758	99.439	99.978	106.111	95.329

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	5	5	5
Mean	2.364e-009	2.103e-009	-1.06
Std. Deviation	6.546e-010	5.825e-010	0.136
Std. Error	3.273e-010	2.912e-010	0.068
Lower 95% CI of mean	1.322e-009	1.176e-009	-1.278
Upper 95% CI of mean	3.405e-009	3.030e-009	-0.846
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



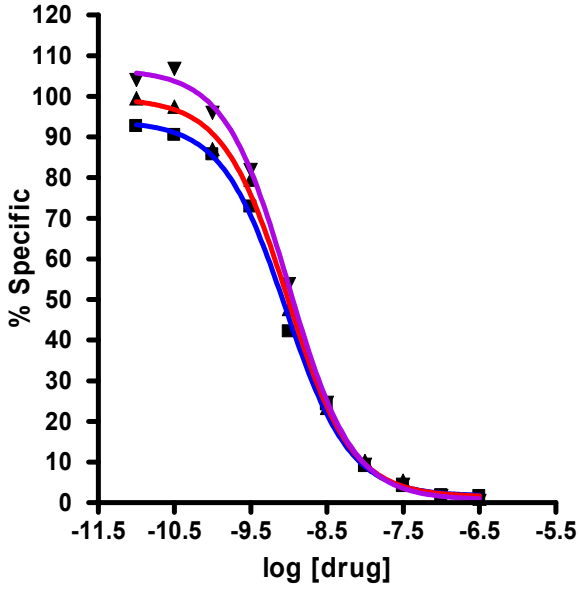
Compound 3f:



Compound 3f

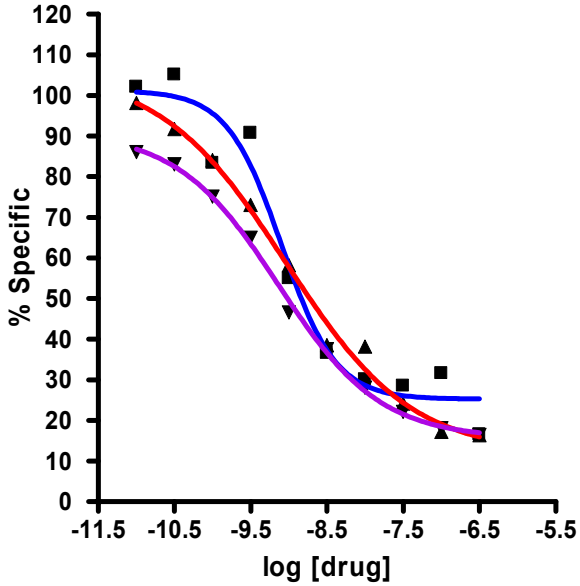
log [drug] (M) (σ_1)	(3f) (R= 3-NO_2)(1) (%)	(3f) (R= 3-NO_2)(2) (%)	(3f) (R= 3-NO_2)(3) (%)
3.16E-07	1.588	0.999	0.424
1.00E-07	1.803	1.268	1.706
3.16E-08	4.048	5.462	4.392
1.00E-08	8.960	10.316	9.324
3.16E-09	24.193	23.321	24.578
1.00E-09	42.185	47.695	53.812
3.16E-10	72.829	79.514	81.884
1.00E-10	85.703	86.984	95.984
3.16E-11	90.473	97.438	106.780
1.00E-11	92.684	99.518	104.047

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	9.519e-010	6.635e-010	-1.052
Std. Deviation	3.832e-011	2.670e-011	0.0325
Std. Error	2.212e-011	1.542e-011	0.0188
Lower 95% CI of mean	8.567e-010	5.971e-010	-1.133
Upper 95% CI of mean	1.047e-009	7.298e-010	-0.971
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

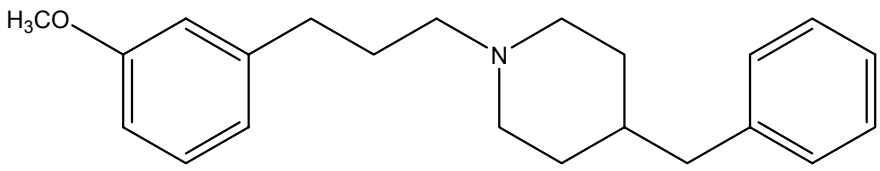


log [drug] (M) (σ_2)	(3f) (R= 3-NO_2)(1) (%)	(3f) (R= 3-NO_2)(2) (%)	(3f) (R= 3-NO_2)(3) (%)
3.16E-07	16.584	16.383	16.424
1.00E-07	31.620	17.196	18.069
3.16E-08	28.522	24.186	22.154
1.00E-08	30.103	38.205	28.036
3.16E-09	36.594	38.518	37.569
1.00E-09	55.036	58.237	46.621
3.16E-10	90.749	73.052	64.961
1.00E-10	83.416	84.060	75.099
3.16E-11	105.102	91.720	83.039
1.00E-11	102.123	98.178	86.061

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	5	5	5
Mean	8.133e-010	7.237e-010	-0.798
Std. Deviation	8.822e-011	7.851e-011	0.367
Std. Error	5.093e-011	4.532e-011	0.212
Lower 95% CI of mean	5.941e-010	5.287e-010	-1.708
Upper 95% CI of mean	1.032e-009	9.187e-010	0.113
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



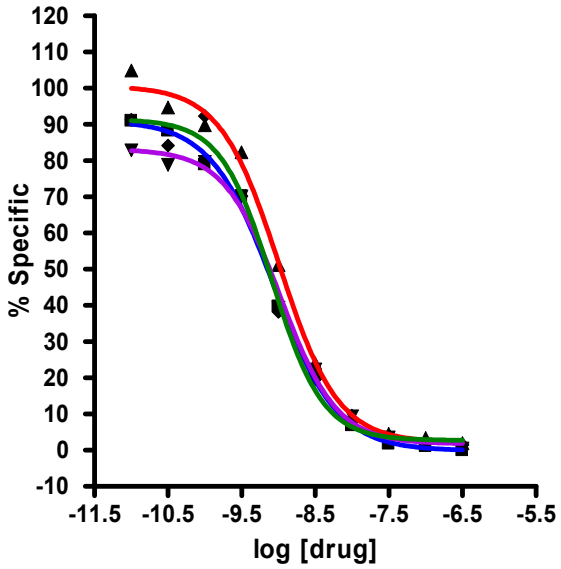
Compound 3g:



Compound 3g

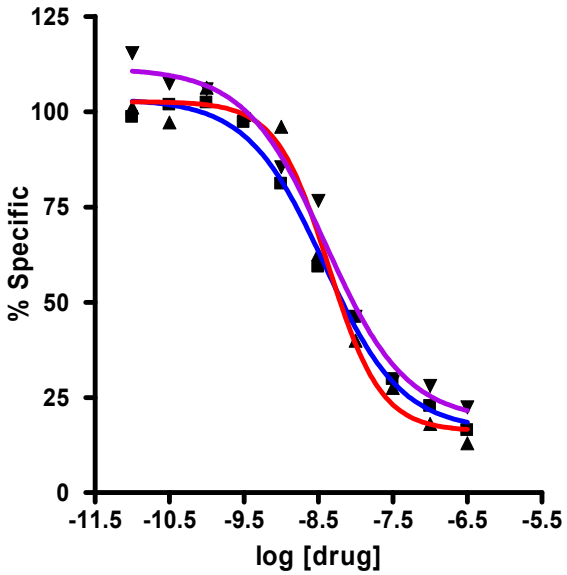
log [drug] (M) (σ_1)	(3g) (R= 3-OCH_3) (1) (%)	(3g) (R= 3-OCH_3) (2) (%)	(3g) (R= 3-OCH_3) (3) (%)	(3g) (R= 3-OCH_3) (4) (%)
3.16E-07	-0.033	1.956	0.470	1.534
1.00E-07	1.071	3.349	1.617	1.957
3.16E-08	1.728	4.532	3.514	2.741
1.00E-08	6.863	9.592	9.341	8.374
3.16E-09	20.657	24.074	22.341	19.646
1.00E-09	39.757	51.144	39.392	38.274
3.16E-10	70.207	82.360	70.122	70.189
1.00E-10	78.920	89.783	79.703	92.328
3.16E-11	88.244	94.671	78.868	84.160
1.00E-11	90.966	104.950	82.888	91.308

(σ_1)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	4	4	4
Mean	9.400e-010	6.415e-010	-1.120
Std. Deviation	1.197e-010	8.171e-011	0.100
Std. Error	5.987e-011	4.086e-011	0.050
Lower 95% CI of mean	7.494e-010	5.115e-010	-1.279
Upper 95% CI of mean	1.130e-009	7.715e-010	-0.960
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

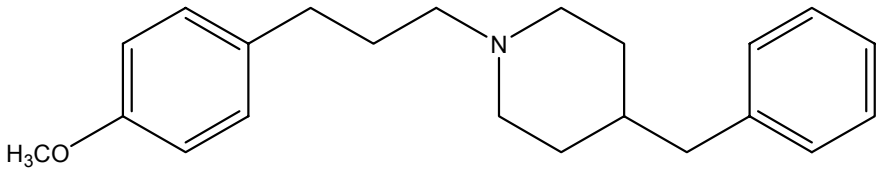


log [drug] (M) (σ_2)	(3g) (R= 3-OCH_3) (1) (%)	(3g) (R= 3-OCH_3) (2) (%)	(3g) (R= 3-OCH_3) (3) (%)
3.16E-07	16.426	13.003	22.408
1.00E-07	22.757	18.040	28.046
3.16E-08	29.792	27.503	29.033
1.00E-08	46.087	39.949	46.189
3.16E-09	59.290	62.609	76.611
1.00E-09	81.065	96.060	85.451
3.16E-10	97.274	99.089	98.009
1.00E-10	102.365	106.281	105.868
3.16E-11	101.792	97.181	107.377
1.00E-11	98.607	101.084	115.376

(σ_2)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	4.035e-009	3.590e-009	-0.9531
Std. Deviation	2.678e-010	2.383e-010	0.2353
Std. Error	1.546e-010	1.376e-010	0.1359
Lower 95% CI of mean	3.369e-009	2.998e-009	-1.538
Upper 95% CI of mean	4.700e-009	4.182e-009	-0.3685
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



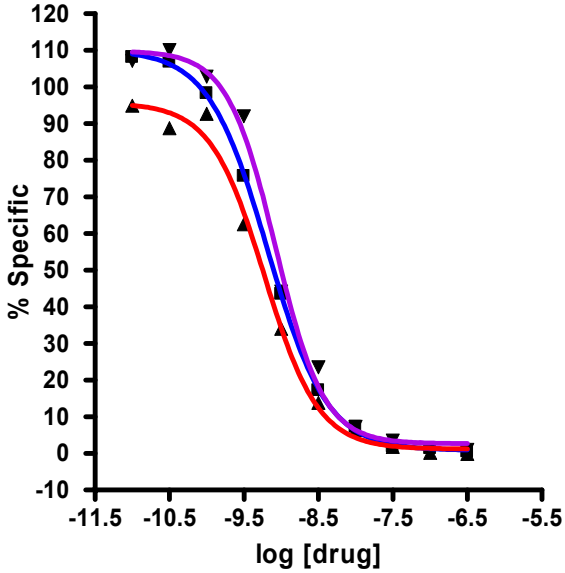
Compound 3h:



Compound 3h

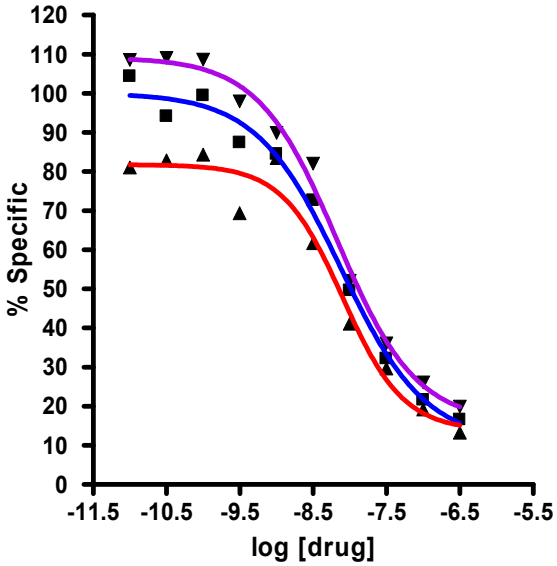
log [drug] (M) (σ_1)	(3h) (R= 4-OCH_3) (1) (%)	(3h) (R= 4-OCH_3) (2) (%)	(3h) (R= 4-OCH_3) (3) (%)
3.16E-07	0.586	-0.098	1.012
1.00E-07	1.442	0.221	1.340
3.16E-08	1.959	1.851	3.486
1.00E-08	7.271	6.415	7.329
3.16E-09	17.242	13.782	23.555
1.00E-09	43.548	34.048	44.220
3.16E-10	75.732	62.534	92.015
1.00E-10	98.343	92.709	102.873
3.16E-11	106.824	88.741	110.148
1.00E-11	108.212	94.927	107.171

(σ_1)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	6.997e-010	4.876e-010	-1.211
Std. Deviation	1.269e-010	8.842e-011	0.126
Std. Error	7.325e-011	5.105e-011	0.072
Lower 95% CI of mean	3.845e-010	2.680e-010	-1.525
Upper 95% CI of mean	1.015e-009	7.073e-010	-0.898
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

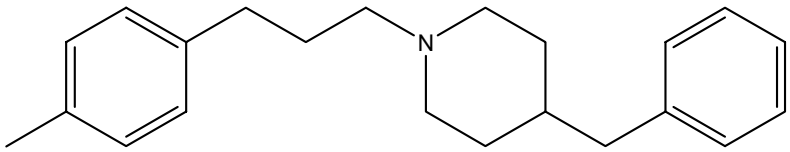


log [drug] (M) (σ_2)	(3h) (R= 4-OCH_3) (1) (%)	(3h) (R= 4-OCH_3) (2) (%)	(3h) (R= 4-OCH_3) (3) (%)
3.16E-07	16.584	13.240	19.930
1.00E-07	21.623	19.195	26.058
3.16E-08	32.160	29.654	36.079
1.00E-08	49.625	41.116	52.055
3.16E-09	72.638	61.713	82.028
1.00E-09	84.451	83.450	89.881
3.16E-10	87.392	69.391	97.918
1.00E-10	99.430	84.314	108.643
3.16E-11	94.134	82.914	109.115
1.00E-11	104.362	81.075	108.548

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	7.403e-009	6.587e-009	-0.870
Std. Deviation	6.688e-010	5.951e-010	0.158
Std. Error	3.861e-010	3.436e-010	0.091
Lower 95% CI of mean	5.742e-009	5.109e-009	-1.263
Upper 95% CI of mean	9.064e-009	8.065e-009	-0.478
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



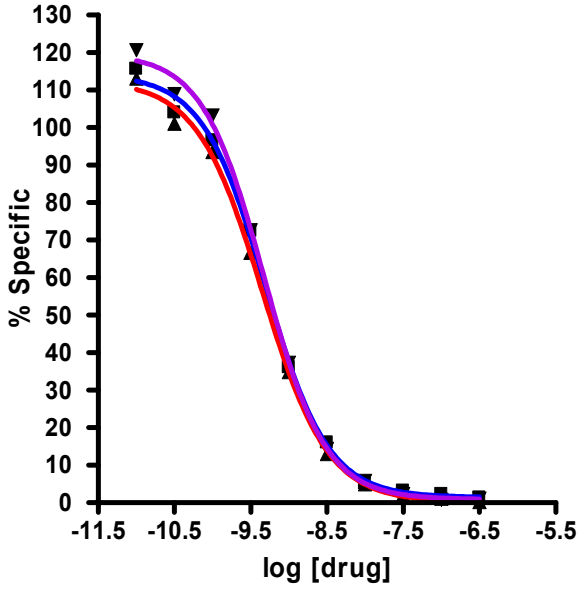
Compound 3i:



Compound 3i

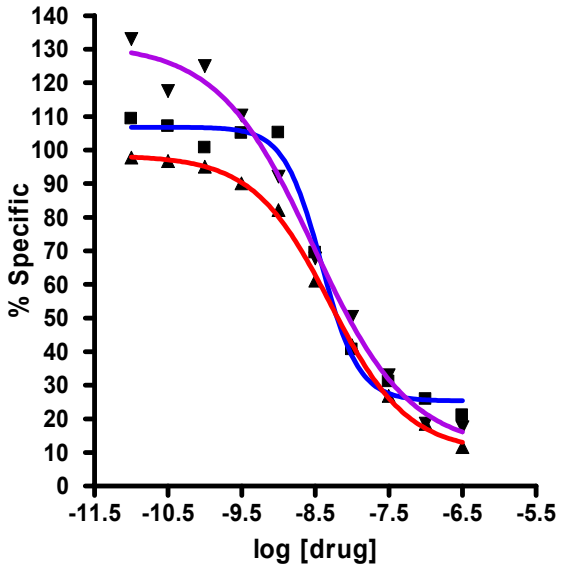
log [drug] (M) (σ_1)	(3i) (R= 4-CH_3) (1) (%)	(3i) (R= 4-CH_3) (2) (%)	(3i) (R= 4-CH_3) (3) (%)
3.16E-07	1.372	0.161	1.061
1.00E-07	2.304	1.099	0.750
3.16E-08	3.188	2.069	2.320
1.00E-08	5.158	4.897	5.829
3.16E-09	16.059	13.012	14.338
1.00E-09	36.119	34.816	37.283
3.16E-10	72.510	66.638	72.620
1.00E-10	96.629	93.503	103.205
3.16E-11	104.047	101.105	108.902
1.00E-11	115.684	113.054	120.624

(σ_1)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	4.709e-010	3.282e-010	-1.048
Std. Deviation	2.148e-011	1.497e-011	0.033
Std. Error	1.240e-011	8.643e-012	0.019
Lower 95% CI of mean	4.175e-010	2.910e-010	-1.130
Upper 95% CI of mean	5.242e-010	3.654e-010	-0.966
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

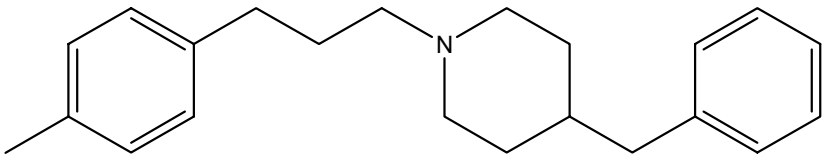


log [drug] (M) (σ_2)	(3i) (R= 4-CH_3) (1) (%)	(3i) (R= 4-CH_3) (2) (%)	(3i) (R= 4-CH_3) (3) (%)
3.16E-07	21.062	11.631	17.479
1.00E-07	25.863	18.566	18.633
3.16E-08	31.157	26.856	32.976
1.00E-08	40.619	41.960	50.387
3.16E-09	69.435	61.183	67.764
1.00E-09	105.204	82.102	92.076
3.16E-10	105.002	90.071	110.290
1.00E-10	100.706	95.069	124.938
3.16E-11	107.095	96.737	117.521
1.00E-11	109.307	97.792	132.984

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	3.967e-009	3.530e-009	-1.064
Std. Deviation	1.038e-009	9.243e-010	0.556
Std. Error	5.995e-010	5.337e-010	0.321
Lower 95% CI of mean	1.387e-009	1.234e-009	-2.447
Upper 95% CI of mean	6.546e-009	5.826e-009	0.318
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



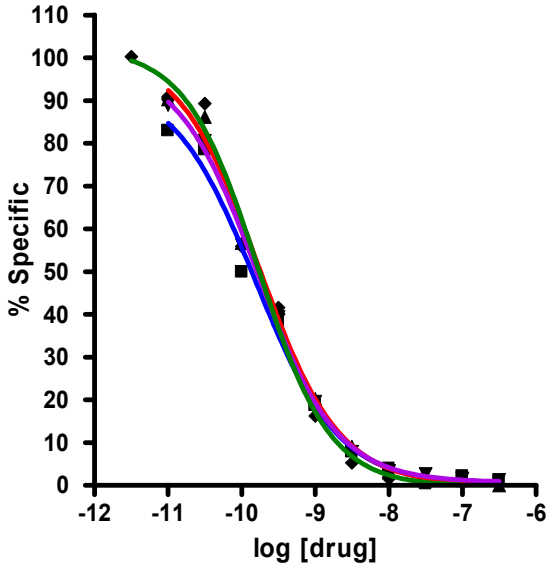
Compound 3j:



Compound 3j

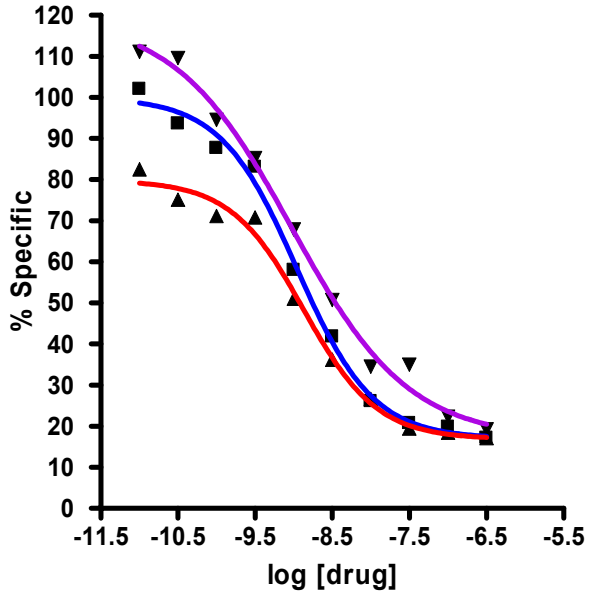
log [drug] (M) (σ_1)	(3j) (R= 4-NO_2) (1) (%)	(3j) (R= 4-NO_2) (2) (%)	(3j) (R= 4-NO_2) (3) (%)	(3j) (R= 4-NO_2) 4) (%)
3.16E-07	0.945	0.035	1.286	
1.00E-07	2.162	1.221	1.588	0.908
3.16E-08	0.411	2.057	2.691	1.521
1.00E-08	3.998	3.374	3.348	1.616
3.16E-09	7.841	9.075	7.835	5.281
1.00E-09	18.735	20.243	19.567	16.268
3.16E-10	38.535	41.408	39.369	41.586
1.00E-10	49.906	56.673	56.258	55.847
3.16E-11	78.512	86.176	80.593	89.306
1.00E-11	82.968	90.299	88.958	90.838
3.16E-11				100.246

(σ_1)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	4	4	4
Mean	1.642e-010	1.144e-010	-0.813
Std. Deviation	6.173e-012	4.299e-012	0.049
Std. Error	3.086e-012	2.149e-012	0.025
Lower 95% CI of mean	1.543e-010	1.076e-010	-0.891
Upper 95% CI of mean	1.740e-010	1.212e-010	-0.734
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



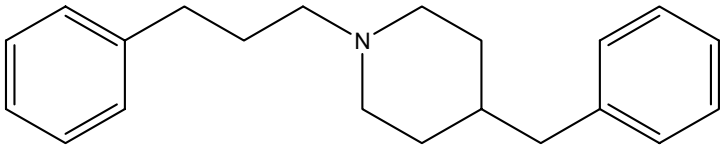
log [drug] (M) (σ_2)	(3j) (R= 4-NO_2) (1) (%)	(3j) (R= 4-NO_2) (2) (%)	(3j) (R= 4-NO_2) (3) (%)
3.16E-07	17.191400	17.102200	19.233600
1.00E-07	19.844700	18.444300	22.343000
3.16E-08	20.735200	19.454100	34.979600
1.00E-08	26.057500	26.598500	34.462700
3.16E-09	41.786100	36.228800	50.672700
1.00E-09	58.028000	51.036700	67.853700
3.16E-10	83.118700	70.793800	85.261400
1.00E-10	87.695400	71.160000	94.546600
3.16E-11	93.623500	75.108800	109.584000
1.00E-11	102.026000	82.489100	111.084000
3.16E-11	17.191400	17.102200	19.233600

(σ_2)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	1.130e-009	1.003e-009	-0.788
Std. Deviation	2.032e-010	1.804e-010	0.189
Std. Error	1.173e-010	1.042e-010	0.109
Lower 95% CI of mean	6.256e-010	5.548e-010	-1.256
Upper 95% CI of mean	1.635e-009	1.451e-009	-0.319
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

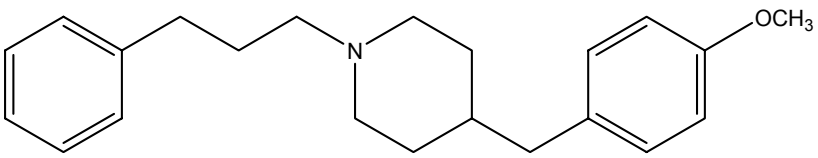


SELECTED COMPOUNDS FROM SERIES-2

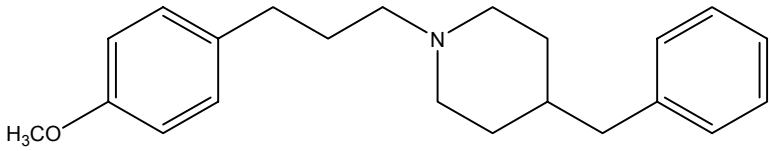
AND SERIES-4



Compound 2a

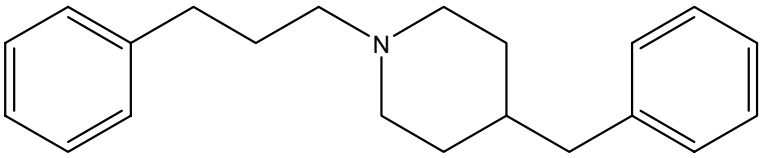


Compound 2g



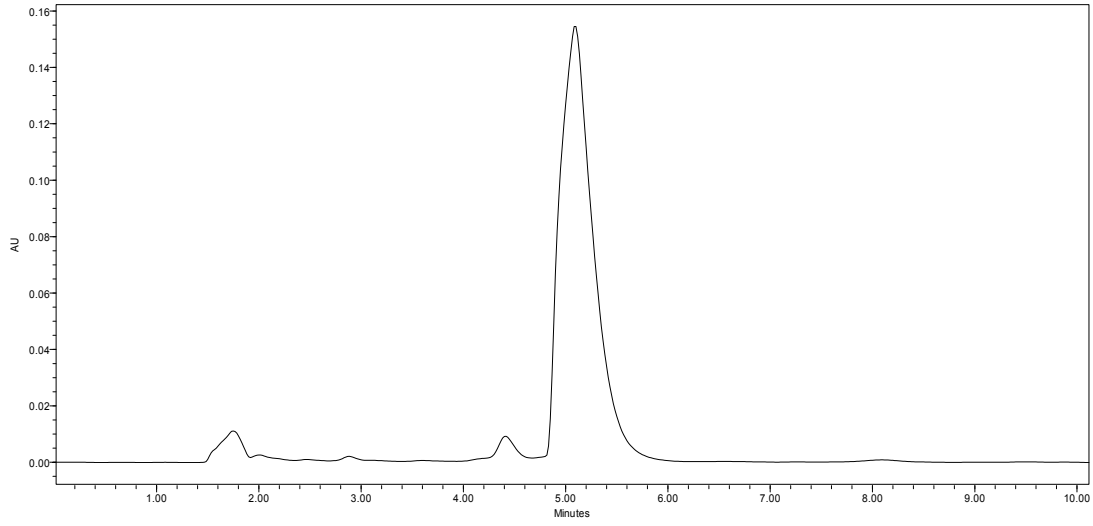
Compound 4g

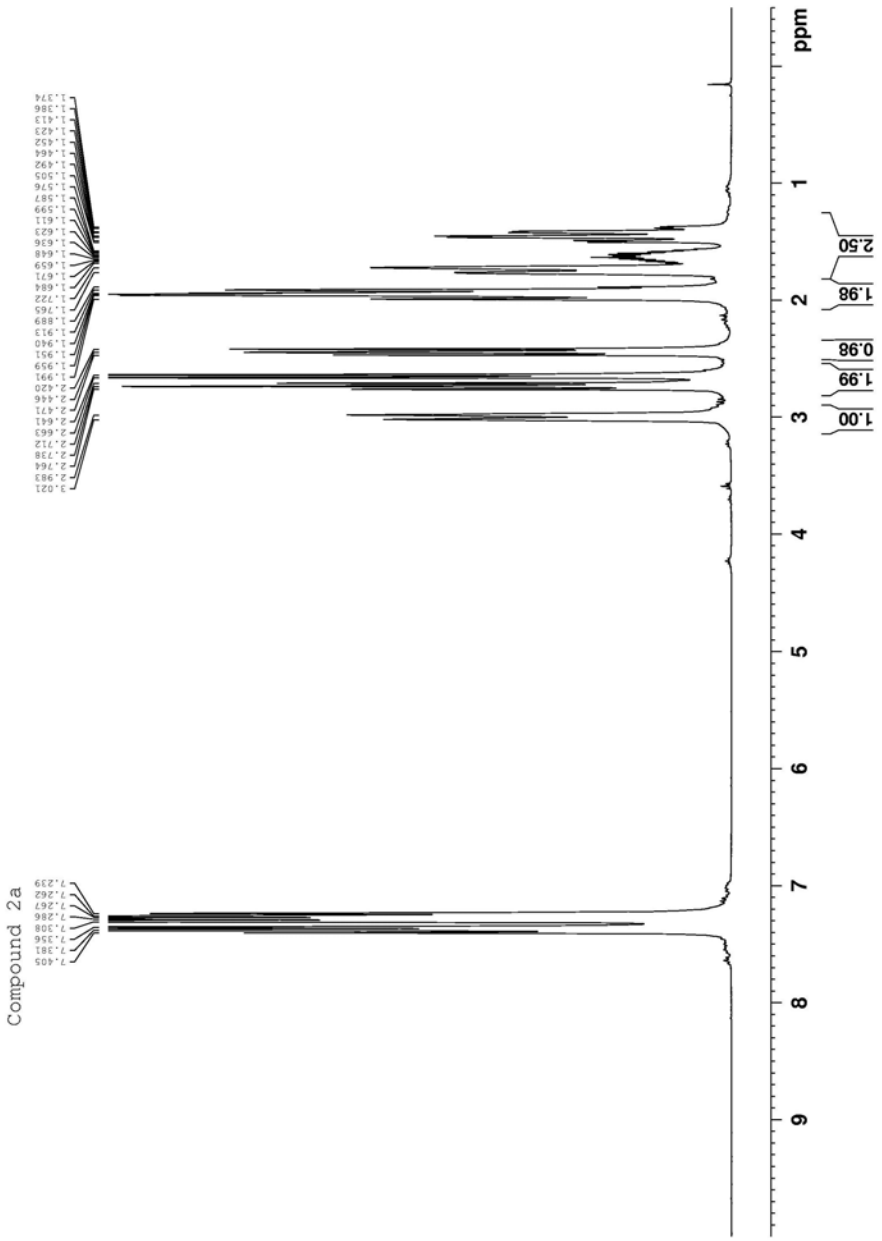
Compound 2a:



Compound 2a

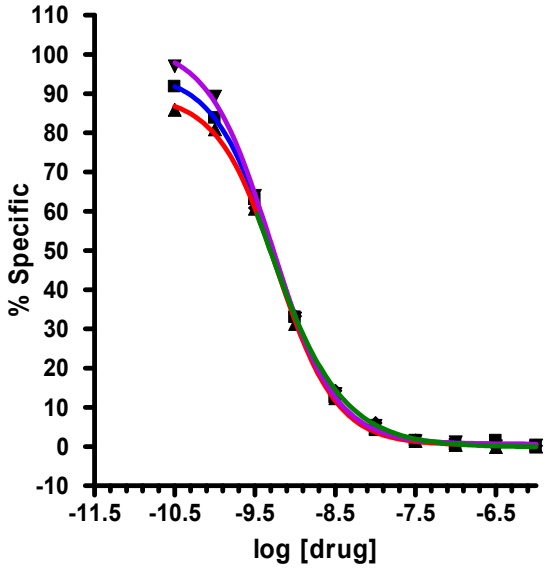
Elemental analysis $C_{21}H_{27}N$	**C %**	**H %**	**N %**
Calculated	85.95	9.27	4.77
Found	85.68	9.26	4.92




Compound 2a
ppm
1.00
1.99
0.98
1.98
2.50

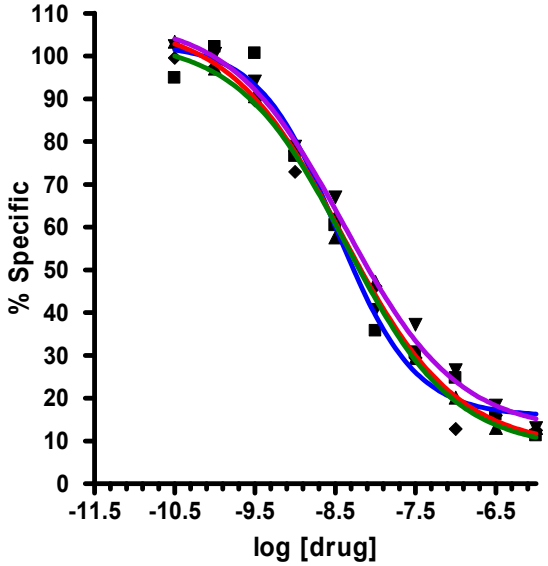
log [drug] (M) (σ_1)	(2a) (R= H)(1) %	(2a) (R= H)(2) %	(2a) (R= H)(3) %	(2a) (R= H)(4) %
3.16E-06	0.014	-0.014	0.309	0.236
1.00E-07	1.551	-0.132	0.055	-0.024
3.16E-07	0.488	0.429	1.125	0.634
1.00E-07	1.536	1.448	1.376	1.603
3.16E-08	4.119	4.718	5.262	6.065
1.00E-08	12.563	12.311	13.443	14.265
3.16E-09	32.951	31.275	32.600	33.274
1.00E-09	63.021	60.691	63.980	60.842
3.16E-10	83.755	80.880	89.267	
1.00E-10	91.758	85.865	97.005	
3.16E-11	0.014	-0.014	0.309	

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	4	4	4
Mean	5.554e-010	3.848e-010	-1.131
Std. Deviation	4.705e-011	3.260e-011	0.0311
Std. Error	2.716e-011	1.882e-011	0.018
Lower 95% CI of mean	4.385e-010	3.038e-010	-1.208
Upper 95% CI of mean	6.723e-010	4.657e-010	-1.053
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

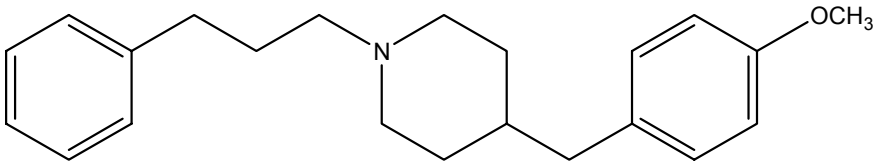


log [drug] (M) (σ_2)	(2a) (R= H)(1) %	(2a) (R= H)(2) %	(2a) (R= H)(3) %	(2a) (R= H)(4) %
3.16E-06	11.245	13.066	13.031	12.500
1.00E-07	16.118	13.090	18.245	15.986
3.16E-07	24.725	20.176	26.532	12.830
1.00E-07	30.725	29.360	37.194	29.424
3.16E-08	35.763	47.177	40.640	46.187
1.00E-08	60.368	57.557	66.979	62.062
3.16E-09	76.577	79.146	78.828	72.922
1.00E-09	100.612	90.617	94.067	89.619
3.16E-10	102.112	97.054	100.485	97.510
1.00E-10	94.887	103.335	102.351	99.575
3.16E-11	11.245	13.066	13.031	12.500

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	4	4	4
Mean	3.879e-009	3.459e-009	-0.690
Std. Deviation	4.283e-010	3.821e-010	0.134
Std. Error	2.141e-010	1.910e-010	0.0672
Lower 95% CI of mean	3.198e-009	2.851e-009	-0.904
Upper 95% CI of mean	4.560e-009	4.067e-009	-0.476
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

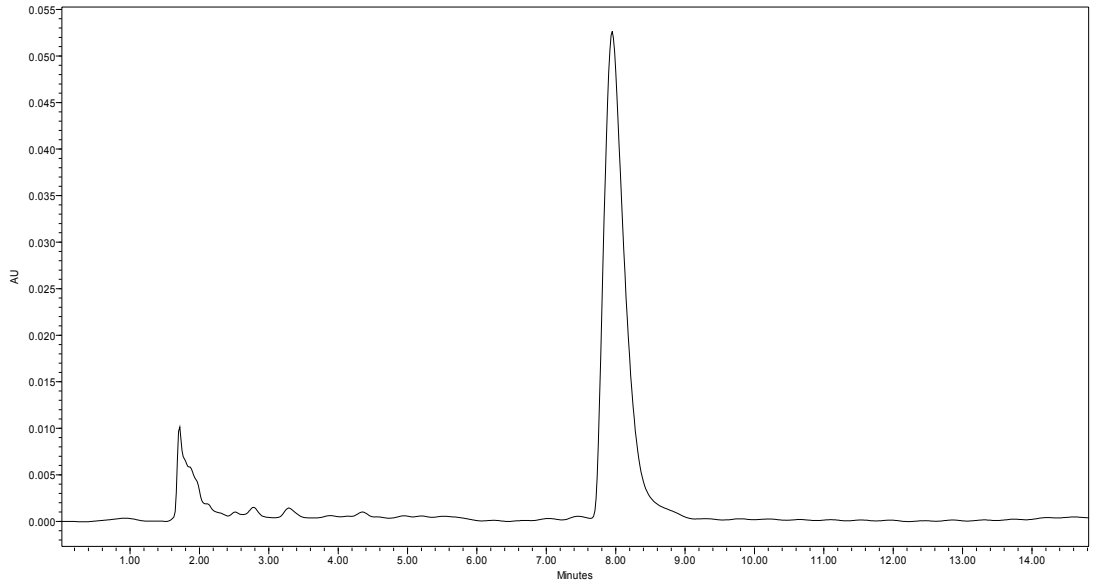


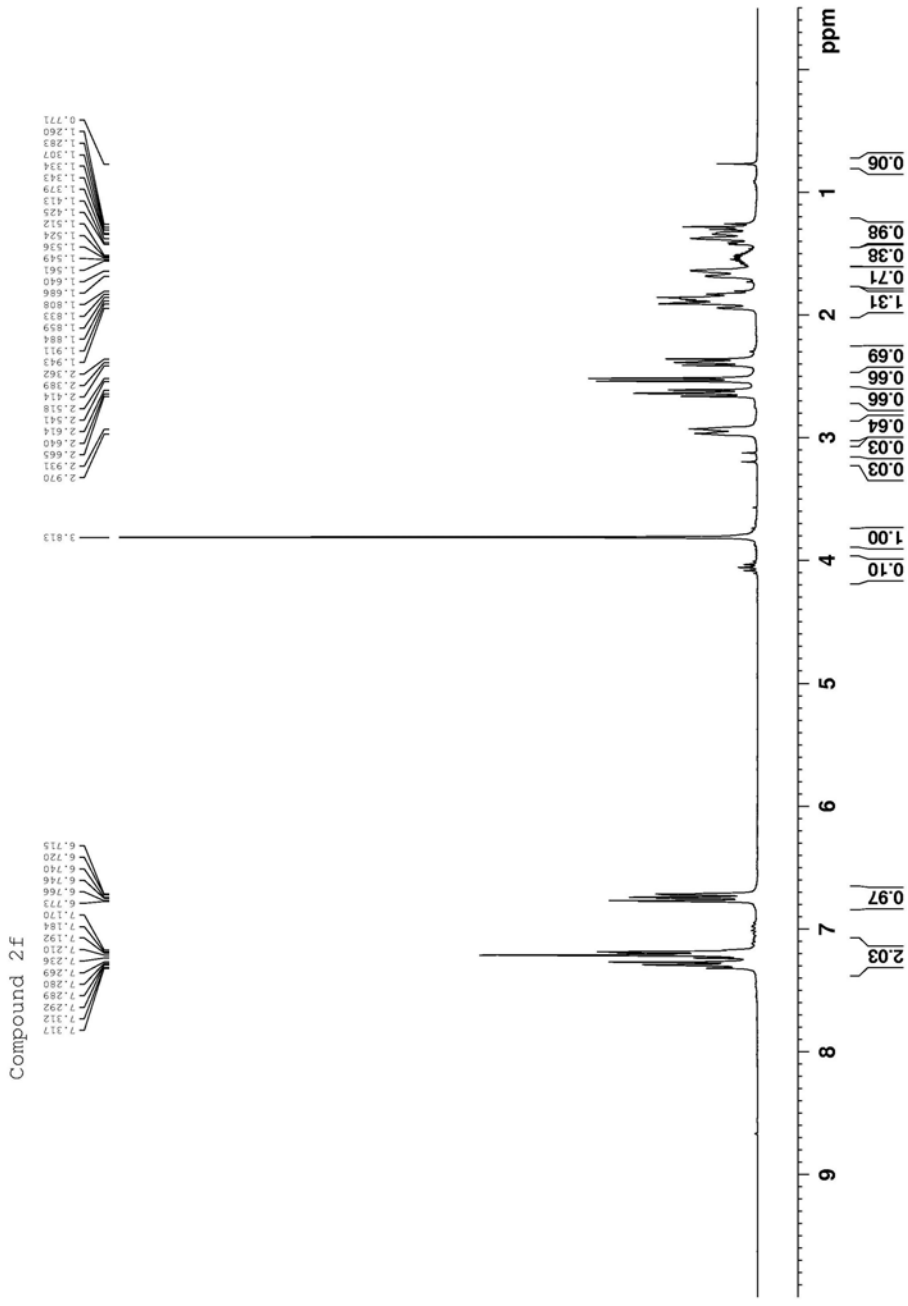
Compound 2g:



Compound 2g

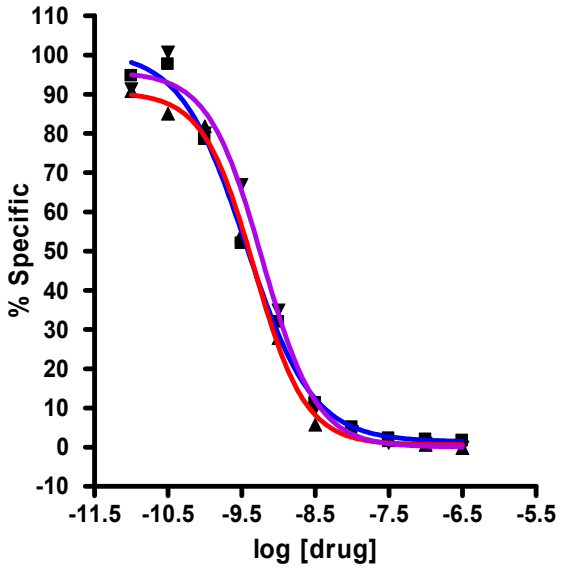
Elemental analysis $C_{22}H_{29}N.HCl.0.25H_2O$	**C %**	**H %**	**N %**
Calculated	72.51	8.44	3.84
Found	72.80	8.38	3.84


AU
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
Minutes


Compound 2f
7.317
7.312
7.292
7.289
7.280
7.269
7.236
7.210
7.192
7.184
7.170
6.773
6.766
6.746
6.740
6.720
6.715
3.813
2.970
2.931
2.665
2.660
2.614
2.541
2.518
2.414
2.389
2.362
1.943
1.911
1.884
1.859
1.833
1.808
1.686
1.660
1.561
1.549
1.536
1.524
1.512
1.425
1.413
1.379
1.343
1.334
1.307
1.283
1.260
0.771
ppm
0.06
0.98
0.38
0.71
1.31
0.69
0.66
0.66
0.64
0.03
0.03
0.10
1.00
0.97
2.03

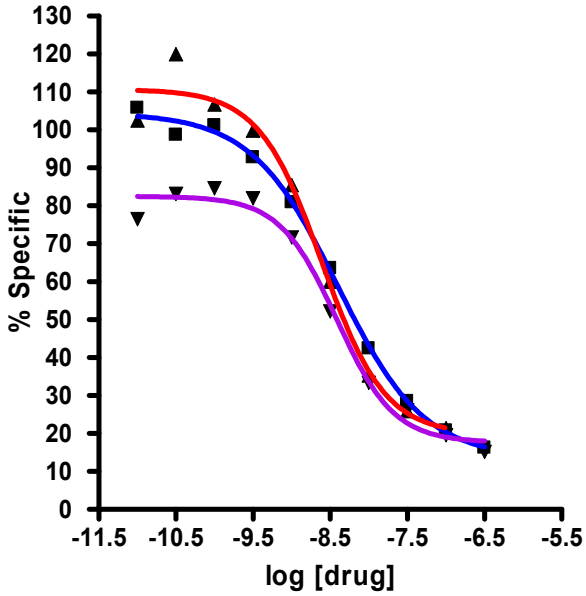
log [drug] (M) (σ_1)	(2g) (R= 3-OCH_3) (1) (%)	(2g) (R= 4-OCH_3) (2) (%)	(2g) (R= 4-OCH_3) (3) (%)
3.16E-07	1.754	-0.177	-0.038
1.00E-07	1.997	0.641	0.902
3.16E-08	2.295	1.769	1.086
1.00E-08	5.052	4.137	3.872
3.16E-09	11.269	5.753	9.802
1.00E-09	31.953	27.866	34.858
3.16E-10	51.995	54.303	66.868
1.00E-10	78.539	81.838	79.989
3.16E-11	97.672	85.048	100.674
1.00E-11	94.689	90.826	91.311

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	4.792e-010	3.340e-010	-1.135
Std. Deviation	1.180e-010	8.223e-011	0.146
Std. Error	6.815e-011	4.747e-011	0.084
Lower 95% CI of mean	1.859e-010	1.297e-010	-1.497
Upper 95% CI of mean	7.724e-010	5.382e-010	-0.773
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

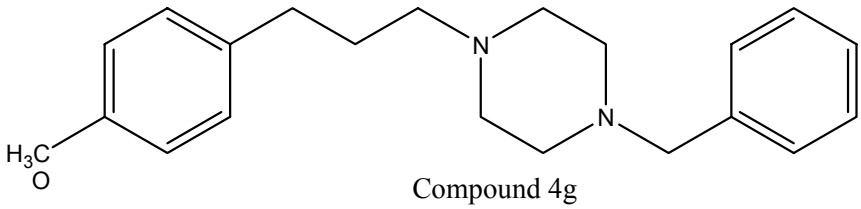


log [drug] (σ_2)	(2f) (3-OCH_3) (1) (%)	(2f) (4-OCH_3) (2) (%)	(2f) (4-OCH_3) (3) (%)
3.16E-07	16.267		15.140
1.00E-07	20.759	21.467	19.509
3.16E-08	28.557	25.892	26.075
1.00E-08	42.434	35.327	33.391
3.16E-09	63.576	60.066	52.149
1.00E-09	80.889	85.443	71.645
3.16E-10	92.753	99.790	81.928
1.00E-10	101.247	106.692	84.652
3.16E-11	98.719	119.965	83.176
1.00E-11	105.830	102.497	76.456

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	3.404e-009	3.029e-009	-1.004
Std. Deviation	8.239e-010	7.329e-010	0.203
Std. Error	4.757e-010	4.231e-010	0.117
Lower 95% CI of mean	1.358e-009	1.209e-009	-1.509
Upper 95% CI of mean	5.451e-009	4.850e-009	-0.499
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



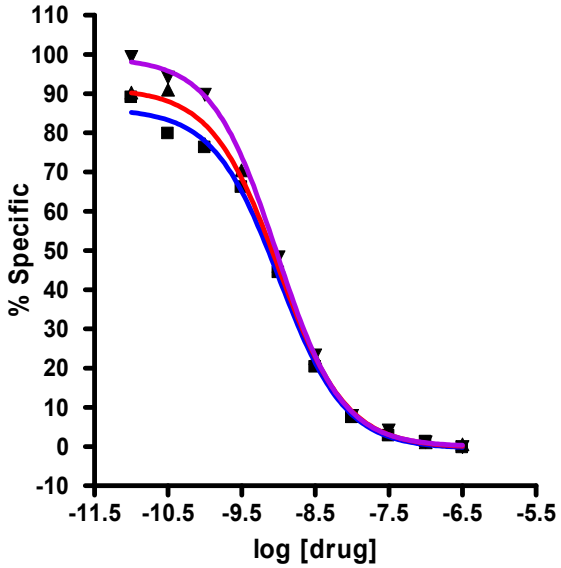
Compound 4g:



Compound 4g

log [drug] (M) (σ_1)	(4f) (R= 3-OCH_3) (1) (%)	(4f) (R= 4-OCH_3) (2) (%)	(4f) (R= 4-OCH_3) (3) (%)
3.16E-07	-0.276	0.583	-0.094
1.00E-07	1.266	1.091	1.123
3.16E-08	2.765	3.285	4.107
1.00E-08	7.424	7.811	7.826
3.16E-09	20.348	22.597	23.232
1.00E-09	44.399	46.073	48.311
3.16E-10	66.130	70.440	
1.00E-10	76.227	77.141	89.743
3.16E-11	79.837	91.013	94.070
1.00E-11	89.065	90.276	99.357

(σ_1)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	9.912e-010	6.765e-010	-0.967
Std. Deviation	4.265e-011	2.910e-011	0.018
Std. Error	2.462e-011	1.680e-011	0.010
Lower 95% CI of mean	8.853e-010	6.042e-010	-1.012
Upper 95% CI of mean	1.097e-009	7.488e-010	-0.922
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

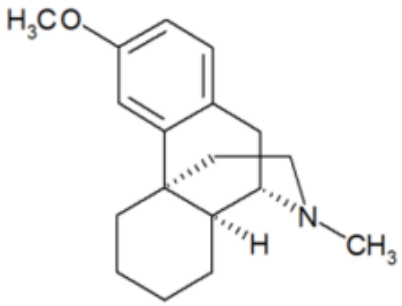


log [drug] (M) (σ_2)	(4f) (R= 3-OCH_3) (1) (%)	(4f) (R= 4-OCH_3) (2) (%)	(3f) (R= 4-OCH_3) (3) (%)
3.16E-07	11.811	10.318	14.029
1.00E-07	21.287	15.562	23.421
3.16E-08	29.669	27.746	36.328
1.00E-08	44.355	46.353	51.450
3.16E-09	81.722	59.465	62.831
1.00E-09	95.569	72.054	87.235
3.16E-10	81.723	80.083	96.469
1.00E-10	95.668	85.980	90.237
3.16E-11	112.916	85.930	81.007
1.00E-11	111.019	103.185	82.826

(σ_2)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	2.970e-008	2.643e-008	-0.715
Std. Deviation	4.998e-009	4.449e-009	0.277
Std. Error	2.886e-009	2.569e-009	0.160
Lower 95% CI of mean	1.728e-008	1.537e-008	-1.403
Upper 95% CI of mean	4.212e-008	3.748e-008	-0.026
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

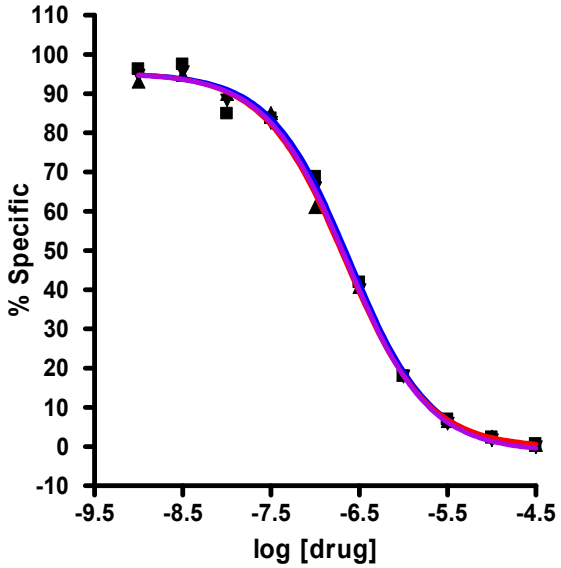
PHENYTOIN MODULATION ASSAY DATA

Dextromethorphan:



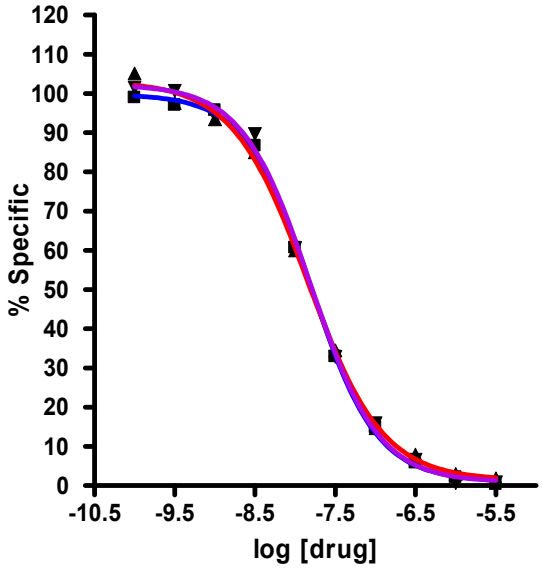
log [drug] (σ_1)	Dextromethorphan(1) (+NaOH) (%)	(Dextromethorphan(2) (+NaOH) (%)	Dextromethorphan(3) (+NaOH) (%)
0.0000316	0.613	0.444	-0.222
0.000010	2.344	2.536	1.563
0.00000316	6.926	6.530	5.842
0.000001	17.942	18.398	17.850
3.160000e-007	41.855	40.673	39.853
1.000000e-007	68.769	61.103	65.831
3.160000e-008	83.701	85.063	82.560
1.000000e-008	84.865	89.964	88.262
3.160000e-009	97.421	94.770	95.465
1.000000e-009	96.193	93.054	94.608

(σ_1) (+NaOH)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	2.323e-007	1.629e-007	-0.951
Std. Deviation	1.516e-008	1.061e-008	0.012
Std. Error	8.750e-009	6.123e-009	0.007
Lower 95% CI of mean	1.726e-007	1.206e-007	-1.088
Upper 95% CI of mean	3.414e-007	2.386e-007	-0.621
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

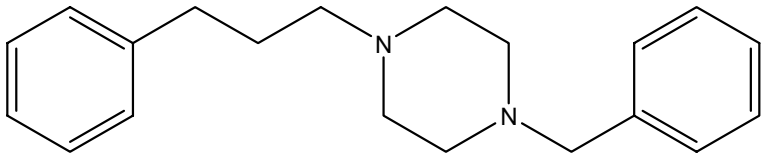


log [drug] (σ_1)	Dextromethorphan(1) + DPH (%)	(Dextromethorphan(2) + DPH (%)	Dextromethorphan(2) + DPH(%)
0.00000316	0.404	1.911	0.832
0.000001	2.173	2.984	0.618
3.160000e-007	5.880	7.882	6.570
1.000000e-007	14.854	14.682	15.911
3.160000e-008	32.905	34.481	32.866
1.000000e-008	60.705	59.989	60.558
3.160000e-009	86.669	85.050	89.642
1.000000e-009	95.738	93.458	94.217
3.160000e-010	97.065	97.521	100.674
1.000000e-010	98.943	105.099	101.480

(σ_1) (+DPH)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	1.518e-008	1.065e-008	-1.005
Std. Deviation	8.128e-010	5.701e-010	0.06721
Std. Error	4.693e-010	3.292e-010	0.03881
Lower 95% CI of mean	1.316e-008	9.230e-009	-1.172
Upper 95% CI of mean	1.720e-008	1.206e-008	-0.8381
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



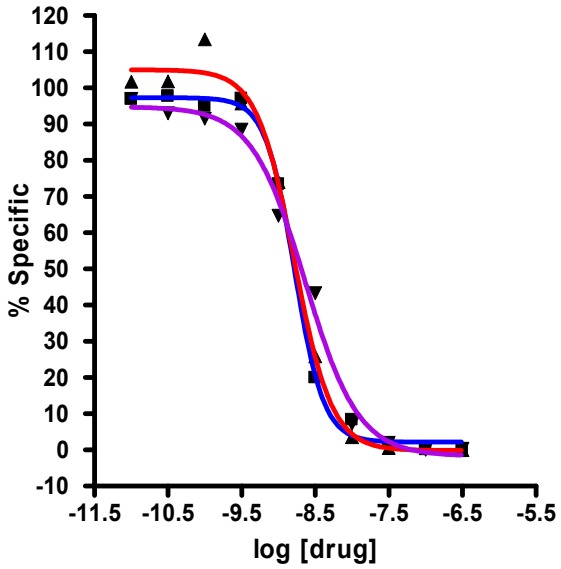
Compound 1a:



Compound 1a

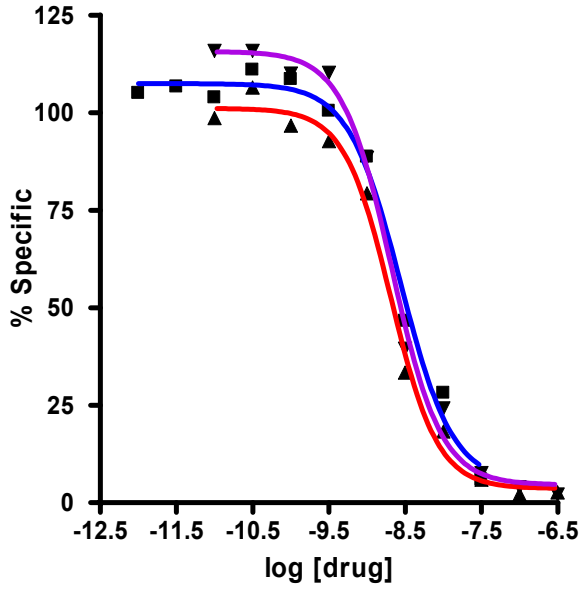
log [drug] (M) (σ_1)	(1a) (R= H)(1) (+NaOH)(%)	(1a) (R= H)(2) (+NaOH)(%)	(1a) (R= H)(3) (+NaOH)(%)
3.16E-07	0.279	-0.008	0.315
1.00E-07	0.803	0.479	0.053
3.16E-08	1.856	0.529	1.997
1.00E-08	8.319	3.537	7.171
3.16E-09	19.946	25.885	43.260
1.00E-09	73.421	74.010	64.689
3.16E-10	97.067	95.716	88.464
1.00E-10	95.006	113.415	91.507
3.16E-11	97.747	101.779	92.983
1.00E-11	96.957	101.751	96.969

(σ_1) (+NaOH)	*IC50 (M)*	*Ki (M)*	*Hill*
Number of values	3	3	3
Mean	1.865e-009	1.312e-009	-1.701
Std. Deviation	3.799e-010	2.676e-010	0.4951
Std. Error	2.194e-010	1.545e-010	0.2858
Lower 95% CI of mean	9.215e-010	6.472e-010	-2.930
Upper 95% CI of mean	2.809e-009	1.977e-009	-0.4709
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

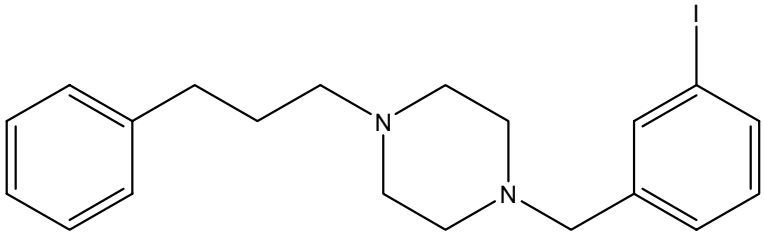


log [drug] (M) (σ_1)	(1a) (R= H)(1) (+ DPH)(%)	(1a) (R= H)(2) (+ DPH)(%)	(1a) (R= H)(3) (+ DPH)(%)
3.160000e-007		2.667	2.088
1.000000e-007		2.196	3.979
3.160000e-008	5.925	5.933	7.581
1.000000e-008	28.139	18.299	24.152
3.160000e-009	46.593	33.349	39.484
1.000000e-009	88.717	79.310	88.437
3.160000e-010	100.441	92.686	110.228
1.000000e-010	108.570	96.716	110.001
3.160000e-011	110.983	106.417	115.841
1.000000e-011	103.915	98.607	115.836
3.160000e-012	106.630		
1.000000e-012	105.045		

(σ_1) (+DPH)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	2.303e-009	1.620e-009	-1.349
Std. Deviation	3.840e-010	2.700e-010	0.06716
Std. Error	2.217e-010	1.559e-010	0.03877
Lower 95% CI of mean	1.349e-009	9.491e-010	-1.516
Upper 95% CI of mean	3.257e-009	2.291e-009	-1.183
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



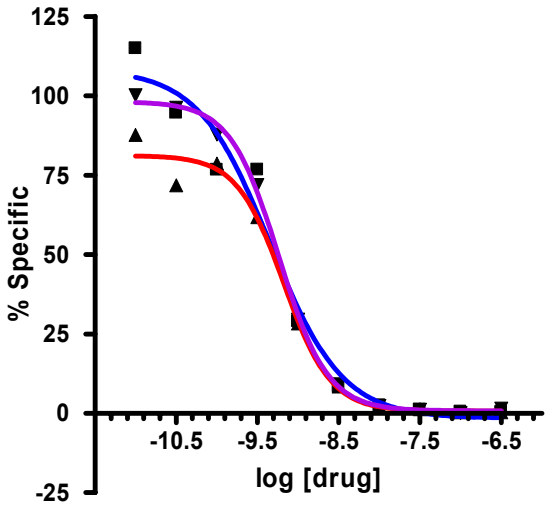
Compound 1d:



Compound 1d

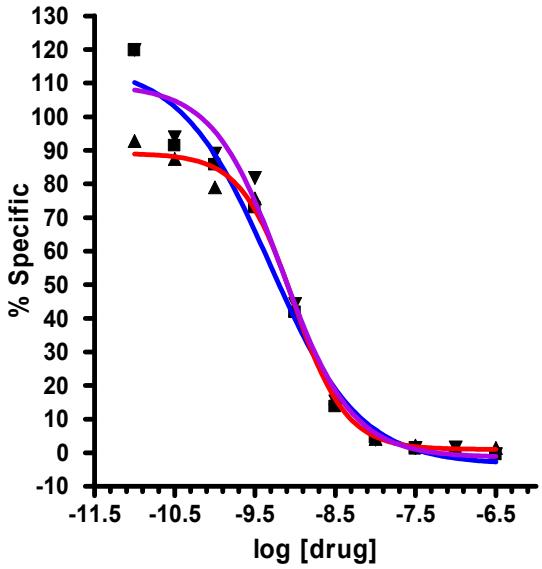
log [drug] (M) (σ_1)	(1d) (R= 3-I)(1) +NaOH (%)	(R= 1d) (3-I)(2) +NaOH (%)	(1d) (R= 3-I)(3) +NaOH (%)
3.16E-07	0.375	0.328	1.411
1.00E-07	0.550	0.369	0.054
3.16E-08	1.071	1.312	1.118
1.00E-08	2.445	2.126	2.302
3.16E-09	8.457	8.467	9.144
1.00E-09	28.937	28.310	29.501
3.16E-10	76.762	61.828	71.906
1.00E-10	76.667	78.635	87.744
3.16E-11	94.647	71.810	96.264
1.00E-11	114.962	87.654	100.193

(σ_1) (+NaOH)	*IC_{50} (M)*	*K_i (M)*	*Hill*
Number of values	3	3	3
Mean	5.657e-010	3.919e-010	-1.312
Std. Deviation	1.064e-010	7.368e-011	0.288
Std. Error	6.142e-011	4.254e-011	0.166
Lower 95% CI of mean	3.014e-010	2.089e-010	-2.027
Upper 95% CI of mean	8.300e-010	5.749e-010	-0.596
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

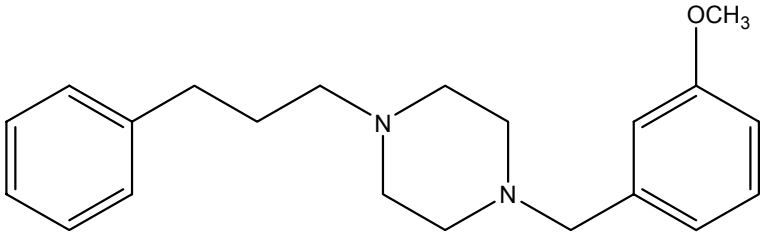


log [drug] (M) (σ_1)	(1d) (R= 3-I)(1) +DPH (%)	(1d) (R= 3-I)(2) +DPH (%)	(1d) (R= 3-I)(3) +DPH (%)
3.16E-07	-0.554	1.421	-0.353
1.00E-07	-0.188	1.073	1.621
3.16E-08	1.149	2.133	1.433
1.00E-08	4.478	4.194	3.947
3.16E-09	13.734	16.285	15.315
1.00E-09	41.803	42.504	44.278
3.16E-10	72.901	75.720	81.816
1.00E-10	85.653	78.961	89.064
3.16E-11	91.345	87.442	93.906
1.00E-11	119.689	92.789	119.933

(σ_1) (+DPH)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	7.093e-010	4.914e-010	-1.033
Std. Deviation	2.335e-010	1.618e-010	0.270
Std. Error	1.348e-010	9.340e-011	0.156
Lower 95% CI of mean	1.294e-010	8.951e-011	-1.705
Upper 95% CI of mean	1.289e-009	8.932e-010	-0.361
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



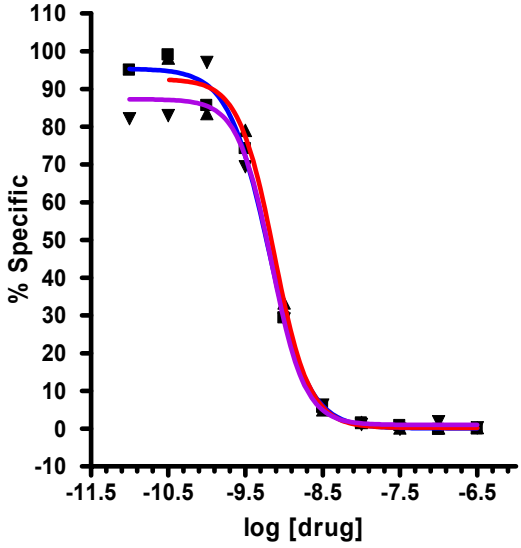
Compound 1g:



Compound 1g

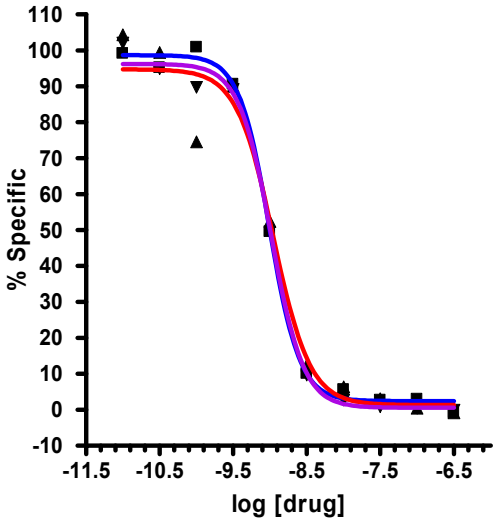
log [drug] (M) (σ_1)	(1d) (R= 3-OCH_3)(1) +NaOH (%)	(1d) (R= 3-OCH_3)(2) +NaOH (%)	(1d) (R= 3-OCH_3)(3) +NaOH (%)
3.16E-07	0.094	-0.281	0.924
1.00E-07	0.201	0.008	-0.121
3.16E-08	0.811	0.331	0.689
1.00E-08	1.662	1.028	1.937
3.16E-09	6.534	6.338	7.574
1.00E-09	29.603	29.323	31.577
3.16E-10	69.211	71.869	72.976
1.00E-10	100.886	94.824	91.862
3.16E-11	98.049	93.153	97.752
1.00E-11	101.901	95.106	103.231

(σ_1) (+NaOH)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	6.047e-010	4.220e-010	-1.615
Std. Deviation	4.984e-011	3.306e-011	0.2479
Std. Error	2.035e-011	1.350e-011	0.1012
Lower 95% CI of mean	5.524e-010	3.873e-010	-1.875
Upper 95% CI of mean	6.570e-010	4.567e-010	-1.355
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

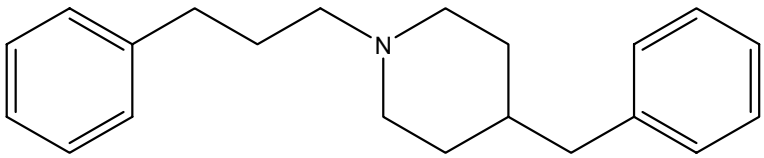


log [drug] (M) (σ_1)	(1d) (R= 3-OCH_3)(1) +DPH (%)	(1d) (R= 3-OCH_3)(2) +DPH (%)	(1d) (R= 3-OCH_3)(3) +DPH (%)
3.16E-07	-0.877	-0.897	-0.094
1.00E-07	2.973	0.406	0.403
3.16E-08	2.710	3.103	0.845
1.00E-08	5.652	6.378	2.706
3.16E-09	10.094	12.367	9.642
1.00E-09	49.503	52.367	
3.16E-10	90.632	89.989	89.101
1.00E-10	100.842	74.566	89.733
3.16E-11	95.208	99.465	94.950
1.00E-11	99.150	104.352	102.055

(σ_1) (+DPH)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	1.046e-009	7.244e-010	-1.738
Std. Deviation	6.432e-011	4.458e-011	0.1827
Std. Error	3.714e-011	2.574e-011	0.1055
Lower 95% CI of mean	8.858e-010	6.137e-010	-2.396
Upper 95% CI of mean	1.205e-009	8.351e-010	-1.488
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



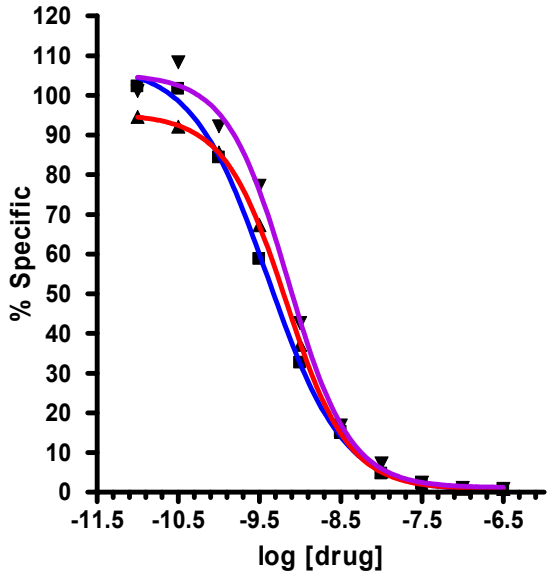
Compound 2a:



Compound 2a

log [drug] (M) (σ_1)	(2a) (R= H)(1) (+NaOH)(%)	(2a) (R= H)(2) (+NaOH)(%)	(2a) (R= H)(3) (+NaOH)(%)
3.16E-07	0.575	0.861	0.840
1.00E-07	0.551	0.990	1.117
3.16E-08	2.196	1.924	2.436
1.00E-08	4.704	5.260	7.259
3.16E-09	15.240	15.193	16.862
1.00E-09	32.661	37.158	42.635
3.16E-10	58.784	67.317	77.242
1.00E-10	84.343	85.611	92.221
3.16E-11	101.657	92.113	108.308
1.00E-11	102.272	94.606	101.061

(σ_1) (+NaOH)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	5.947e-010	4.120e-010	-1.070
Std. Deviation	1.699e-010	1.177e-010	0.120
Std. Error	9.812e-011	6.796e-011	0.069
Lower 95% CI of mean	1.725e-010	1.196e-010	-1.368
Upper 95% CI of mean	1.017e-009	7.044e-010	-0.771
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

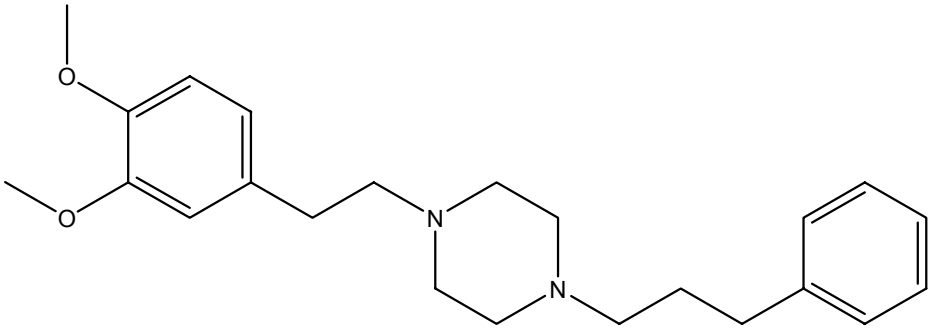


log [drug] (M) (σ_1)	(2a) (R= H)(1) (+DPH)(%)	(2a) (R= H)(2) (+DPH)(%)	(2a) (R= H)(3) (+DPH)(%)
3.16E-07	0.713	0.096	1.938
1.00E-07	1.718	0.882	1.065
3.16E-08	3.056	3.726	5.450
1.00E-08	7.685	8.229	8.313
3.16E-09	20.308	21.022	26.037
1.00E-09	41.365	47.099	46.453
3.16E-10	64.367	63.383	64.268
1.00E-10	83.448	85.550	85.167
3.16E-11	100.000	92.865	89.568
1.00E-11	93.874	98.263	94.357

(σ_1) (+DPH)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	7.450e-010	5.162e-010	-0.852
Std. Deviation	1.063e-010	7.366e-011	0.045
Std. Error	6.138e-011	4.253e-011	0.026
Lower 95% CI of mean	4.810e-010	3.332e-010	-0.963
Upper 95% CI of mean	1.009e-009	6.991e-010	-0.740
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

VALIDATION OF BINDING ASSAYS

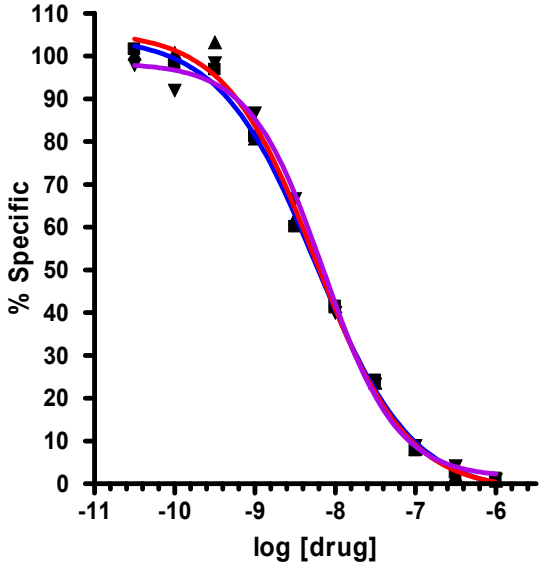
SA4503:



1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine dihydrochloride

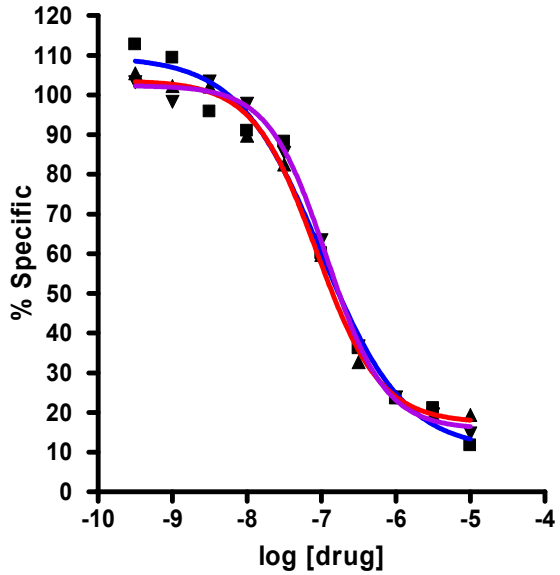
log [drug] (σ_1)	SA4503 (1) (%)	SA4503 (2) (%)	SA4503 (3) (%)
1.00E-06	1.205	0.747	0.963
3.16E-07	2.538	2.049	4.101
1.00E-07	7.735	8.142	8.860
3.16E-08	24.193	23.606	23.237
1.00E-08	41.463	41.469	39.986
3.16E-09	60.096	62.069	66.553
1.00E-09	81.142	80.715	86.566
3.16E-10	96.783	103.192	98.288
1.00E-10	97.899	100.763	91.919
3.16E-11	101.584	100.515	97.899

(σ_1)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	6.210e-009	4.341e-009	-0.815
Std. Deviation	7.771e-010	5.432e-010	0.116
Std. Error	4.487e-010	3.136e-010	0.067
Lower 95% CI of mean	4.280e-009	2.992e-009	-1.102
Upper 95% CI of mean	8.140e-009	5.691e-009	-0.527
Passed normality test (alpha=0.05)?	Yes	Yes	Yes

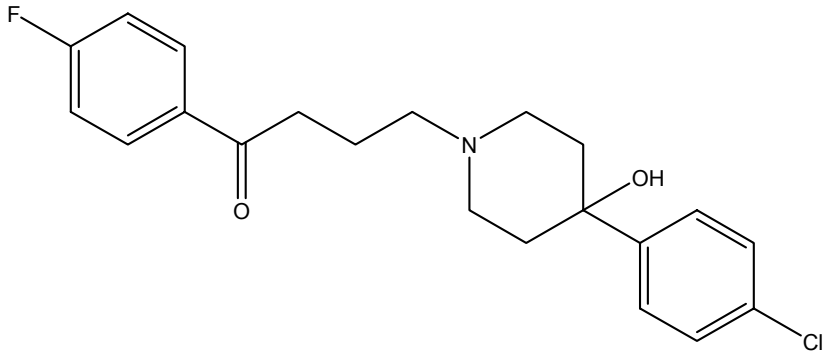


log [drug] (M) (σ_2)	SA4503 (1) (%)	SA4503 (2) (%)	SA4503 (3) (%)
1.00E-05	11.734	19.399	14.754
3.16E-06	21.127	19.399	19.502
1.00E-06	23.457	23.959	23.853
3.16E-07	36.113	32.691	36.696
1.00E-07	60.113	59.693	63.417
3.16E-08	88.256	82.487	85.403
1.00E-08	90.952	89.722	97.843
3.16E-09	95.811	102.114	103.415
1.00E-09	109.383	102.283	98.305
3.16E-10	112.729	105.442	103.201

(σ_2)	IC_{50} (M)	K_i (M)	Hill
Number of values	3	3	3
Mean	1.013e-007	8.951e-008	-0.968
Std. Deviation	1.563e-008	1.381e-008	0.174
Std. Error	9.025e-009	7.972e-009	0.101
Lower 95% CI of mean	6.248e-008	5.521e-008	-1.401
Upper 95% CI of mean	1.401e-007	1.238e-007	-0.535
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



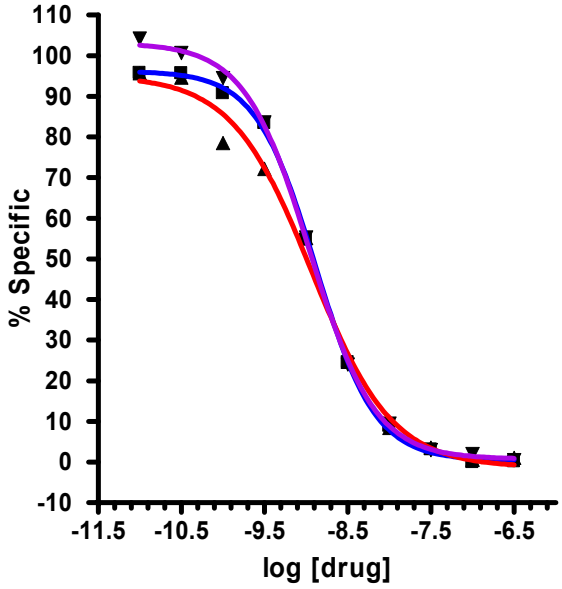
Haloperidol:



4-(4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)-1-(4-fluorophenyl)butan-1-one

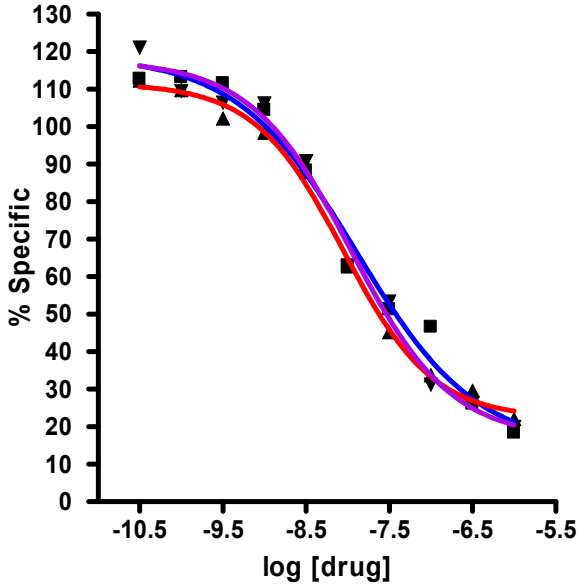
log [drug] (M) (σ_1)	Haloperidol (1) (%)	Haloperidol (2) (%)	Haloperidol (3) (%)
3.16E-07	0.152	1.070	0.530
1.00E-07	-0.040	0.551	1.968
3.16E-08	3.064	3.544	2.925
1.00E-08	8.900	8.457	9.473
3.16E-09	24.593	24.729	24.007
1.00E-09	54.700	55.099	55.275
3.16E-10	83.421	72.167	83.590
1.00E-10	90.754	78.481	94.488
3.16E-11	95.643	94.661	100.645
1.00E-11	95.718	95.656	104.194

(σ_1)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	1.190e-009	8.319e-010	-1.075
Std. Deviation	9.635e-011	6.735e-011	0.159
Std. Error	5.563e-011	3.889e-011	0.091
Lower 95% CI of mean	9.507e-010	6.646e-010	-1.468
Upper 95% CI of mean	1.429e-009	9.993e-010	-0.681
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



log [drug] (M) (σ_2)	Haloperidol (1) (%)	Haloperidol (2) (%)	Haloperidol (3) (%)
1.00E-05	18.430	22.074	19.935
3.16E-06	26.129	29.581	26.298
1.00E-06	46.638	33.708	31.220
3.16E-07	51.282	45.194	53.354
1.00E-07	62.795	62.760	62.986
3.16E-08	88.330	87.867	90.780
1.00E-08	104.494	98.436	106.220
3.16E-09	111.576	102.177	106.403
1.00E-09	113.192	109.739	109.490
3.16E-10	112.772	112.339	121.071

(σ_2)	IC_{50} (*M*)	K_i (*M*)	*Hill*
Number of values	3	3	3
Mean	1.084e-008	9.577e-009	-0.708
Std. Deviation	1.921e-009	1.694e-009	0.104
Std. Error	1.109e-009	9.783e-010	0.060
Lower 95% CI of mean	6.070e-009	5.368e-009	-0.966
Upper 95% CI of mean	1.562e-008	1.379e-008	-0.451
Passed normality test (alpha=0.05)?	Yes	Yes	Yes



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VITA

Roger I. Nahas was born in Beirut, Lebanon, on April 24th, 1978. He studied Chemistry at the Lebanese University (UL) where he worked on a thesis entitled "*Towards Asymmetric Syntheses*" under the guidance of Professor Dunia Chahine, and received a Bachelor of Science degree in 2001. After graduating, he was awarded a full support scholarship from CIHEAM organization and joined the Chemistry of Natural Products & Biotechnology program at the Mediterranean Agronomic Institute of Chania (MAICH) in Chania, Crete, Greece. There, he was awarded a post-graduation diploma (D.S.P.U.) subsequent to the first year of the program, and a Master of Science after accomplishing a thesis entitled "*Evaluation of the Antioxidant Activity of 15 Different Algae from the Island of Crete, and Isolation of Bioactive Compounds from the Brown Alga Taonia Atomaria*" under the supervision of Professor Vassilios Roussis (University of Athens). In August 2003, he was accepted by the Department of Chemistry at the University of Missouri-Columbia. Since then, he has been working in the area of medicinal chemistry under the guidance of Professor Susan Z. Lever, and received a PhD in organic chemistry in August 2007, entitled "*Synthesis and Structure-Activity Relationships of a Series of Sigma Receptor Ligands*." Besides his published research, he was actively involved in teaching at the University of Missouri-Columbia, and has won a number of awards among which are the "*The Breckenridge/Lyons Award for Outstanding Graduate Teaching*" and the "*The European Union Special Prize at the RCAF*".