Abstract

Title of Dissertation: Phenylpropyloxyethylamines: Opioids lacking a tyrosine mimetic

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The μ opioid agonist morphine is the standard for severe pain management. Despite the ability of morphine to treat severe pain, there are significant side effects which often cause undermedication in clinical settings. Such effects are respiratory depression, tolerance, constipation, and dependence. Accordingly, investigation of novel classes of opioid analgesics would provide great therapeutic benefits. 14-Phenylpropyloxymorphinans are agonists that exhibit extreme potency at μ receptors, suggesting that the 14-phenylpropyloxy group has a major effect on receptor binding and is responsible for the dramatic increase in potency. Our hypothesis is that both a basic amine and a phenylpropyloxy group alone are required for opioid activity, and the aromatic A-ring, that was historically considered essential, is not required. By removing the A-ring, this allows the skeleton to adopt an alternate binding mode with the receptor, thereby potentially causing alternate receptor trafficking events and post-receptor mechanisms, all of which are involved in the development of tolerance. During initial studies, a conformationally sampled pharmacophore approach was utilized to confirm that the aromatic moiety in the novel series does not mimic the A-ring. In order to further substantiate our hypothesis, a series of phenylpropyloxyethylamines and

cinnamyloxyethylamines were synthesized, and analyzed for opioid receptor binding affinity. Opioid binding studies showed that the optimal *N*-substituent is the *N*-phenethyl, specifically analog 2-(cinnamyloxy)-N-methyl-N-phenethylethanamine which has an affinity of 1680 nM for μ opioid receptors. Subsequently, rings B, C, and D from the morphine skeleton were systematically re-introduced as ring-constrained analogs. Binding studies showed that the B-ring analog containing a *N*,*N*-dimethyl substituent produced the highest affinity of 2340 nM, while the C- and D-ring analogs were fully inactive. Furthermore, by combining the B-ring with the optimal *N*-substituent, phenethyl, we were able to achieve 1640 nM affinity at μ. Moreover, upon introduction of an indole group into the C-ring analog, *N*,*N*-dimethyl-1-(3-(3-phenylpropoxy)-2,3,4,9-tetrahydro-1H-carbazol-3-yl)methanamine, the affinity was increased to 1110 nM, which represents a viable lead compound for optimization studies.

Phenylpropyloxyethylamines: Opioids lacking a tyrosine mimetic

By
Lidiya Stavitskaya

Dissertation submitted to the faculty of the Graduate School
of the University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2011

Dedication

This thesis is dedicated to my parents, Luba and Boris Stavitsky for their undying love and endless support

Acknowledgements

I would first and foremost like to thank my advisor Dr. Andrew Coop for giving me the opportunity to pursue my passion for research. Dr. Coop has always been committed to the role of mentoring and despite his busy schedule, he was always available for discussion. His guidance and critique has helped me grow as a scientist.

I would like to thank Dr. Alexander MacKerell, Jr., Dr. Sarah Michel, Dr. James Polli, and Dr. Rae Matsumoto for serving on my committee and providing constructive criticism throughout my career as a graduate student. In addition, I would like to thank my collaborators Dr. MacKerell for performing computational chemistry, Dr. Matsumoto for performing opioid and sigma binding analyses, and Dr. Wayne Bowen for performing sigma calcium assays. I am especially grateful for the opportunity I was given to participate in the on-going experiments in Dr. Matsumoto's laboratory.

I would also like to thank Dr. Kellie Hom for her technical assistance with the NMR. Special thanks to my collaborators Jason Healy and Michael Seminerio for their great efforts in performing *in vivo* and *in vitro* pharmacology studies and Jihyun Shim for performing the computational chemistry studies. Many thanks to my present and former labmates, Dr. Matthew Metcalf, Dr. Susan Mercer, Dr. Christopher Cunningham, Dr. Trudy Smith, Dr. Marilyn Tsourounis, Dr. Sucheta Kudrimoti, and Dr. Padmavani Bezawada. Additionally, I would like to thank my "other" labmate, Dr. Rana Rais. They truly made my experiences as a graduate student more enjoyable with their sense of

humor and their enthusiasm. I am especially grateful for their support and constructive criticism and I look forward to our continued friendships.

I would like to take this opportunity to thank my parents, Luba and Boris Stavitsky, for their love and support. I would not be where I am if not for their hard work and sacrifices. They may not have agreed with every decision that I have made, but they have always supported me in my decisions. I would also like to thank my brother, Andrey Stavitskiy for always being there for me. I am truly grateful for having such a loving family.

Lastly, I would like to thank my fiancé, Sgt. Matthew Linkenhoker for being my strength and support. His military service to our country inspires me to be a better person in my daily life. I am so grateful to have him in my life. I would also like to thank his family for their support throughout my career as a graduate student.

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List of Abbreviations

ABNR	Adopted Basis Newton-Raphson
AD_{50}	Antagonist dose 50%
C,H,N	Combustion analysis
C-CAM	Clocinnamox
CCD	Charge-coupled device
CHARMM	Chemistry at Harvard Molecular Mechanics
CNS	Central nervous system
CONJ	Conjugate gradient
CPM	Cyclopropylmethyl
CSP	Conformationally sampled pharmacophore
DEC	Drug Evaluation Committee
DOC-CAM	Deoxyclocinnamox
E.A.S.E.	Entereg Access Support and Education
GBSW	Generalized born continuum solvent model
GIT	Gastrointestinal tract
HMBC	Heteronuclear multiple bond correlation spectroscopy
HMQC	Heteronuclear multiple quantum correlation spectoscopy
HP	Hot plate assay
i.c.	Intracerebral
i.p.	Intraperitoneal
IC_{50}	Inhibitory concentration 50

IL-2	Interleukin-2
LCQ MS	Liquid chromatography mass spectrometry
m/z	Mass-to-charge-ratio
MC-CAM	Methocinnamox
MD	Molecular dynamics
MLR	Mixed lymphocyte reaction
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
NTX	Naltrexone
s.c.	Subcutaneous
SAR	Structure-activity relationship
SD	Steepest descents
TF	Tail flick assay
TLC	Thin layer chromatography
TREX-MD	Temperature replica exchange-molecular dynamic
TW	Tail withdrawal

Chapter 1. Most recent developments and modifications of 14-alkylamino and 14-alkoxy-4,5-epoxymorphinan derivatives

Reproduced in part with permission from Stavitskaya, L; Coop, A. Most recent developments and modifications of 14-alkylamino and 14-alkoxy-4,5-epoxymorphinan derivatives. *Mini-Rev Med Chem.* **2011**, accepted.

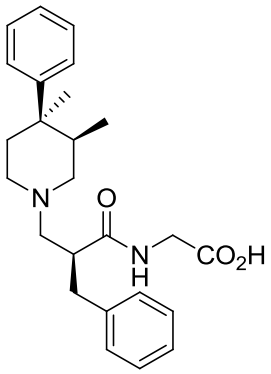
1.1 INTRODUCTION

The provision of effective pain management is essential in a clinical setting where pain is common in individuals treated for cancer, post-operative patients, or in cases of severe trauma. There are two major classes of drugs that are commonly used in treating moderate to severe clinical pain; opioids and nonsteroidal anti-inflammatory agents (Block and Beale, 2004). Even though opioids are known to be most problematic, (Fries, 1995) they are the mainstay of treatment of severe clinical pain(Zieglgansberger et al., 1995; Stein et al., 2003). Undesirable side effects such as tolerance, dependence,(Kieffer and Evans, 2002) respiratory depression, constipation and nausea(McNicol et al., 2003) have been the leading cause of under-medication and inadequate pain management (Hill, 1993; Cherny et al., 2001). Patients that receive opioid treatment often receive additional medications to treat or prevent some of the undesirable side effects. For example, constipation can be managed with stool softeners and laxatives, but not chronically (Klaschik et al., 2003). More recently, alvimopan and methylnaltrexone have been approved as selective antagonists of gastrointestinal opioid receptors to treat constipation (Hipkin et al.). While additional medication may lessen or even prevent some of the adverse effects, in some cases it may dramatically decrease the effectiveness of the opioid itself due to drug-drug interaction (Armstrong and Cozza, 2003). Another problem associated with taking additional medication is that it adds to the regimen of drugs already taken by the patients.

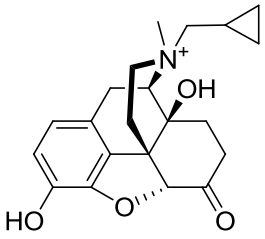
Opioid receptors are G-protein coupled receptors that contain seven transmembrane domains and are primarily located in the brain and the spinal cord as well as the gastrointestinal tract (GIT) (Ossipov et al., 2004). The three types of opioid receptors that have been cloned and pharmacologically characterized are κ (Mansson et al., 1994), δ (Evans et al., 1992; Kieffer et al., 1992), and μ (Wang et al., 1994), and each exhibits unique pharmacological response upon stimulation. μ Agonists produce analgesia, euphoria, respiratory depression, tolerance, and constipation (Kieffer, 1999). Agonists of the κ receptor have been shown to produce dysphoria, by interacting though central nervous system (CNS) mechanisms, tremendously limiting the use of κ agonists in a clinical setting (Hasebe et al., 2004). δ Agonists are not effective against severe pain and are known to produce convulsions (Comer et al., 1993; Broom et al., 2002). The growing body of evidence concerning the physiological relevance of homo- and heterodimers of opioid receptors (Bouvier, 2001; George et al., 2002), leads to the potential of designing ligands that target the dimers and give rise to different effects. However, at present, μ opioid receptors remain the preferred target for more severe pain therapeutics.

Tremendous effort has been put towards the development of novel opioids lacking side effects that are commonly seen in opioid treatment (Casy and Parfitt, 1986). For example, analgesics such as orvinols, bupronorphine, developed by Bentley, exhibit extreme potency but are unsuccessful in elimination of the frequently seen side effects (Lewis et al., 1971). Ziconotide, an N-type calcium channel blocker has been recently approved for clinical use, but has the disadvantage of intrathecal administration (Klotz, 2006). More recently, several μ-receptor antagonists have been approved for treatment of opioid

induced constipation: alvimopan (Lavine, 2008), and methylnaltrexone bromide (Yuan et al., 2005) (Figure 1.1). Alvimopan's large molecular weight, zwitterionic form, and polarity reduce its CNS penetration, thereby allowing the agent to selectively antagonize the effect of opioids on μ receptors in the GIT (Lavine, 2008). Another significant limitation to prolonged use is the risk of a heart attack. Consequently, alvimopan is only available as a short-term treatment, in hospitals approved by the Entereg Access Support and Education (E.A.S.E.) program, and cannot be dispensed to patients after discharge (Chappelle, 2008; Lavine, 2008). Methylnaltrexone bromide is a derivative of naltrexone which has a high peripheral selectivity resulting from the low lipid solubility due to its quaternary salt form (Yuan et al., 2005). Moreover, methylnaltrexone must be administered subcutaneously as it exhibits poor oral bioavailability (Yuan et al., 2005).



Alvimopan



Methylnaltrexone

Figure 1.1 Stuctures of alvimopan and methylmaltrexone

In the last decade, modifications at position 14 have opened a new realm of possibilities. Though natural opiates are unsubstituted at position 14, introduction of 14-OH and $14NH_2$ has been achieved starting from thebaine (Bentley et al., 1969; Greiner et al., 2003). Substituents in position 14 have shown to not only improve potency but also selectivity for certain receptor types. For example, Schmidhammer et. al., showed that

extremely high potency can be achieved at all three opioid receptors with 14-alkoxymorphinan derivatives (Schmidhammer and Spetea, 2010). While, Husbands' group presented modest selectivity with 14-aminodihydromorphinones and 14-aminodihydrocodeinones, clocinnamox analogs (Lewis and Husbands, 2010). Most recently, studies by Zhang et. al., showed that high binding affinity for the μ opioid receptor with high selectivity over the δ and the κ receptors can be achieved with 14-O-heterocyclic substituted naltrexone (Li et al., 2009). This review will present the most recent developments and modifications in the 14 position of the morphine analogs as potential therapeutic opportunities.

1.2 4,5-EPOXYMORPHINAN DERIVATIVES

1.2.1 14-Alkoxymorphinans

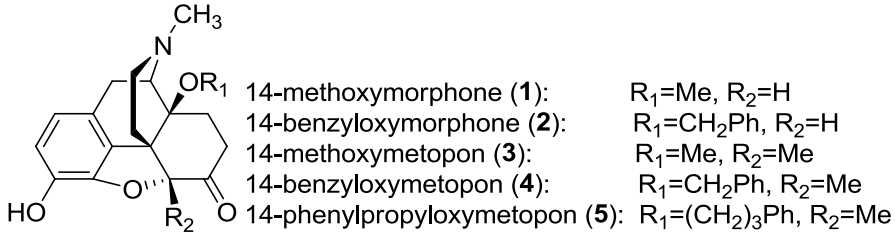


Figure 1.2 Structures of alkoxymorphinans

One of the most promising subclass of opioids with the potential for reduced undesired effects is the 14-alkoxymorphinans, which were developed by Schmidhammer et. al. (Schmidhammer and Spetea, 2010). During the initial structure-activity relationship (SAR) studies, Schmidhammer's group showed that introduction of a 14-methoxy in oxymorphone (**1**, Figure 1.2) result in increased binding affinities at all three opioid receptors (0.10 nM at μ receptor; 4.80 nM at δ receptor; and 10.2 nM at κ receptor). (Lattanzi et al., 2005) The 14-O-methoxymorphone was reported to possess agonist

properties with 400-fold greater potency than morphine and 800-fold greater potency than the parent compound oxymorphone by hot-plate test in mice (Schmidhammer et al., 1984). Like the parent compound, 14-O-oxymorphone induced respiratory depression, physical dependence, and constipation (Schmidhammer et al., 1984).

Further studies revealed that introduction of a 14-benzyloxy group (**2,** Figure 1.2) compared to 14-methoxy group produced similar μ binding affinities (0.12 nM and 0.10 nM respectively), but lower selectivity over δ opioid receptors (2.14 nM and 4.80 nM, respectively) and κ opioid receptors (1.18 nM and 10.2 nM, respectively) (Lattanzi et al., 2005). Moreover, 14-O-benzyloxymorphone was reported to have 4-fold greater potency than the 14-methoxy analog and 700-fold greater potency than morphine. (Lattanzi et al., 2005) Most interestingly, 14-O-benzyloxymorphone (ED_{50} CBE vs AD_{50} HP = 2.8) displayed 2.5-fold less constipative activity as compared to morphine and 7.0-fold less constipation effects than 14-O- methoxymorphone in mice after s.c. administration (Lattanzi et al., 2005).

Subsequently, the same group showed that introduction of a 14-methoxy in an N-methylmorphinan-6-one series (**3,** Figure 1.2), produced similar μ binding affinity as 14-O- methoxymorphone (0.15 nM and 0.10 nM, respectively) with a slightly better selectivity over δ opioid receptors (13.3 nM and 4.80 nM, respectively) and κ opioid receptors (25.2 nM and 10.2 nM, respectively) (Spetea et al., 2003). Remarkably high antinociceptive activity was reported for 14-methoxymetopon, which exhibited approximately 20,000-fold greater potency than morphine and 1500-fold greater potency

than oxymorphone by the acetylcholine-writhing test in rats and mice (Furst et al., 1993a). Upon supraspinal administration, 14-methoxymetopon can elicit potency of up to one million-fold greater than morphine (King et al., 2003). Perhaps the most exciting finding was that 14-methoxymetopon lacked tolerance and physical dependence after repeated treatment(Furst et al., 1993b). Studies also showed that 14-methoxymetopon had reduced constipation (King et al., 2003) and respiratory depression (Furst et al., 1993b) commonly associated with highly potent opioids. These results indicate that a more favorable interaction is possible with the receptor via position 14 in the *N*-methylmorphinan-6-one series.

Furthermore, the 14-alkoxymorphinan series shows that potency can be further magnified by C_{14} arylalkyl substituents as seen with 14-benzyloxy (**4**, Figure 1.2) (Lattanzi et al., 2005) and 14-phenylpropyloxymetopon (**5**, Figure 1.2) (Schutz et al., 2003) derivatives. These 14-arylalkyloxymetapon derivatives displayed enhanced δ and κ affinities while maintaining high μ affinities (Schutz et al., 2003). Though the 14-phenylpropyloxymetopon derivative exhibited complete loss in μ-selectivity with 0.20 nM at μ receptors, 0.14 nM at δ receptors, and 0.40 nM at κ receptors, it was reported to have extreme potency (24,000-fold higher in the tail flick assay and 8,500-fold higher in the hot plate assay as compared to morphine) (Schutz et al., 2003). This analog is even more potent than etorphine which makes 14-phenylpropyloxymetapon unsuitable for clinical use due to its extreme potency (Schutz et al., 2003).

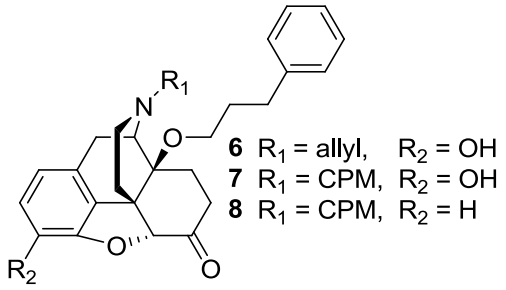


Figure 1.3 Stuctures of 14-O-phenylpropyl derivatives

While developing novel μ agonists for the treatment of pain is beneficial, their reinforcing properties make for strong abuse potential (Compton and Volkow, 2006). Thus, there has been a growing interest in the development of μ antagonists to block the actions of the abused μ agonists (Husbands and Lewis, 2003). For many years, it has been general knowledge that the introduction of either cyclopropylmethyl or allyl groups on the nitrogen position 17 typically results in complete loss of agonist activity (Casy and Parfitt, 1986). However, in contrast to the generally accepted antagonist SAR models, 14-O-phenylpropyl derivatives containing *N*-cyclopropylmethyl and *N*-allyl groups (**6-8**, Figure 1.3) displayed very potent agonist activity (Greiner et al., 2003). Both analogs **6** and **7** displayed enhanced potency, about 100-400-fold more potent in the HP than morphine.(Greiner et al., 2003) Moreover, 14-alkoxymorphinans such as 14-O-phenylpropyloxy-3-desoxy NTX (**8**) was capable of maintaining subnanomolar affinity for μ (0.84 nM) even when there is no C_3 oxygen function.(Spetea et al., 2004) These results indicate that the *N*-substituent itself does not determine the efficacy, but rather the position of the *N*-substituent can be used to dictate the efficacy. In addition, it is evident that the substituents in position 3 that were previously considered essential for μ activity are not required in the 14-alkoxymorphinone subclass.

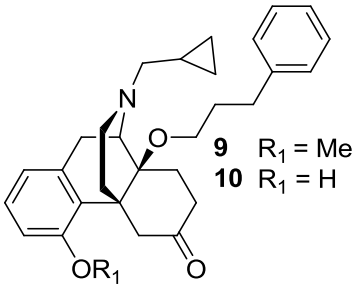


Figure 1.4 Stuctures of cyprodime derivatives

Further SAR studies revealed that partial agonism at μ and δ can be attained by introducing a 14-phenylpropyl group into cyprodime (Spetea et al., 2004), a selective μ-antagonist. Although antagonism was observed at κ opioid receptors by GTPγS functional assays, the cyprodime derivatives, **9** and **10** (Figure 1.4) showed no antagonist activity against morphine in the mouse tail flick assay (Spetea et al., 2004). The presence of 14-alkoxy showed an increase in binding affinity at all three opioid receptors and acted as a potent antinociceptive agent *in vivo* with potency similar to that of 14-metoxymetopon (Spetea et al., 2004). These results further imply that the overall conformation of the *N*-substituent in relation to its skeleton, rather than the substituent itself, dictates the efficacy.

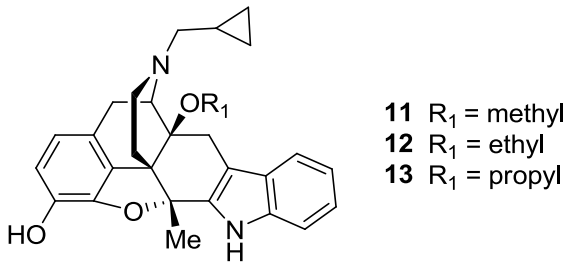


Figure 1.5 Stuctures of naltincole derivatives

Schmidhammer's group also showed that conversion of a hydroxyl to alkoxy in naltrindole with a methyl moiety located at position 5 produced lower affinity for δ while increasing δ selectivity when compared to naltrindole (Biyashev et al., 2001). Further

studies showed that the nature of the substituent in position 14 determines the binding strength (Biyashev et al., 2001). The 14-ethoxy substituent (**12**, Figure 1.5) showed increased interaction with the δ receptor (K_i = 0.78 nM) when compared to the 14-methoxy (**11**: K_i = 1.15 nM) and 14-propoxy (**13**: K_i = 5.3 nM) naltrindole derivatives. (Biyashev et al., 2001) All 14-alkoxy derivatives possessed antagonist activity in the GTPγS functional assay. Some loss in δ affinity and selectivity was seen with the 14-arylalkoxy naltrindole derivatives (8-30 nM) (Biyashev et al., 2001).

Evidence that δ antagonists such as naltrindole and 7-benzylspiroindanylnaltrexone may be involved in allograft survival (Linner et al., 1998) persuaded Schmidhammer's group to investigate such a phenomena with analog **12**, which was previously shown to be superior to naltrindole (Biyashev et al., 2001). The results showed that **12** inhibited rat lymphocyte proliferation *in vitro* (IC_{50} = 0.54 μM) (Spetea et al., 2001b). Additionally, compound **11** showed immunosuppressive activity *in vitro* and reduced interleukin-2 (IL-2) production in mouse and human lymphocytes (D'Ambrosio et al., 2004). In contrast to the previous finding, these naltrindole derivatives did not exhibit immunosupression via δ opioid receptors as seen in the MLR assay that uses μ/δ/κ receptor knock-out mice (Gaveriaux-Ruff et al., 2001). Furthermore, it has been suggested that the indolo moiety is involved in immunosuppressive activity (Gaveriaux-Ruff et al., 2001).

1.2.2 14-Aminomorphinones and codeinones

Another important subclass of opioids contains 14-aminomorphinones and codeinones.

Compounds **14** (C-CAM) and **15** (MC-CAM) (Figure 1.6) were the first analogs developed in their structural class by Lewis et al. (Lewis et al., 1988). MC-CAM and its parent compound C-CAM had very similar affinities (μ = 0.46 nM and 7.2 nM; δ = 29 nM and 7.2 nM; and κ = 4.5 nM and 1.6 nM respectively) (Zernig et al., 1996). While C-CAM displayed μ antagonism with no agonist activity (Comer et al., 1992), MC-CAM was reported to have higher efficacy, displaying partial agonism at the μ receptor after peripheral administration *in vivo* (Woods et al., 1995). Potentially, the most exciting finding was that MC-CAM had pseudo-irreversible effects with its extremely long duration of antagonist action similar to that of buprenorphine (Aceto et al., 1989). Initially, MC-CAM was believed to exhibit its delayed long-term antagonist effect via its de-methylated metabolite C-CAM (Lewis and Husbands, 2010). However, it was later shown that MC-CAM was capable of producing μ-antagonist effects after i.c.v. administration (Lewis and Husbands, 2010). Although long duration of action μ-antagonists can be used to treat drug abuse by blocking the effects of the drug upon subsequent administration, MC-CAM does not possess a profile superior to buprenorphine (Cowan and Lewis, 1995).

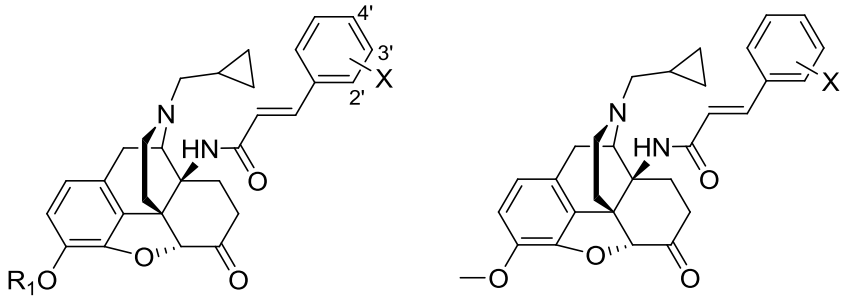


Figure 1.6 Stuctures of 14-aminomorphinones and codeinones

Other studies presented by the groups of Husbands and Lewis looked at the effect of the aryl ring substituent orientation (**16**).(Nieland et al., 2006) In these studies, the μ efficacy decreases in the order: ortho- > meta- > para- for the methyl and chloro substituents while no effect was seen with the fluoro substituent.(Nieland et al., 2006) In contrast, a reduction in μ agonist efficacy and potency was seen when the nitro orientation was changed from the para- to the ortho- position, possibly due to the lipophilicity rather than steric or electronic effects.(Nieland et al., 2006) Conclusions drawn from these studies showed that 2'-chloro, 2'-methyl, 4'-fluoro and 4'-nitro substituted cinnamylaminomorphinone analogs possessed potent agonist effects, with ED_{50} of 0.003 mg/kg to 0.014 mg/kg compared to morphine's 0.66 mg/kg in the rat tail pressure *in vivo* assay.(Lewis and Husbands, 2010) Interestingly, the 4'-nitro analog acted as a short-term agonist in the TW assay (McLaughlin et al., 1999). However, when pretreated for 24 hours, the 4'-nitro analog had morphine antagonist activity with a long duration of action (McLaughlin et al., 1999; Nieland et al., 2006).

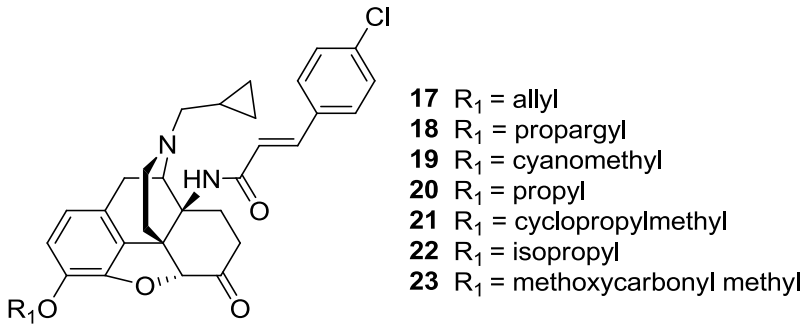
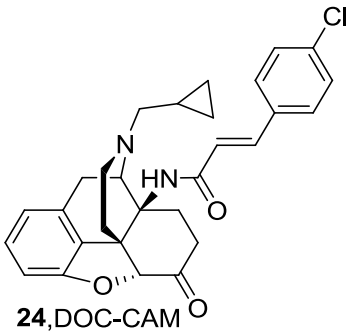


Figure 1.7 Structures of 3-alkyl ether derivatives

Subsequently, the groups of Lewis and Husbands studied the effect of a variety of 3-alkyl ethers (Figure 1.7) to further investigate the possibility of the MC-CAM's delayed long duration of action antagonism to be a result of the C-CAM metabolite. Interestingly,

higher efficacy was achieved with 3-alkyl ether C-CAM analogs (Husbands et al., 1998; Husbands and Lewis, 2003). Specifically, 3-allyl (**17**), 3-propargyl (**18**), cyanomethyl (**19**), and propyl (**20**) ethers displayed higher efficacy than MC-CAM, with 3-propargyl ether analog having the greatest activity by TW assay (Husbands et al., 1998). The 3-propargyl ether analog was reported to have similar potency to morphine with higher efficacy than buprenorphine in mice, meanwhile a lack of change in efficacy was seen in rhesus monkeys (Husbands et al., 1998). Other substituents like cyclopropylmethyl, isopropyl and methoxycarbonyl methyl ether were reported to have antagonist activity by warm water TW assay in mice (Husbands et al., 1998). All the ether analogs were reported to have long-term antagonism effects in the TW assay when administered 24 hours prior to morphine administration (Husbands et al., 1998). In this series, the propagyl ether analog had the preferred long-lived μ-antagonist effects in mice and rhesus monkeys in addition to the increased efficacy when compared to buprenorphine (Husbands et al., 1998). These results further indicate that the delayed antagonist activity of MC-CAM is not related to its metabolism (Husbands et al., 1998).



24,DOC-CAM

Figure 1.8 Structure of DOC-CAM

Similar to Schmidhammer's compounds (Spetea et al., 2004), the removal of the 3-hydroxy group from C-CAM to give DOC-CAM, **24** (Figure 1.8) resulted in similar μ

affinity as its parent compounds MC-CAM and C-CAM (K_i= 0.54 nM, 0.46 nM, and 0.25 nM, respectively) (Derrick et al., 2000; Lewis and Husbands, 2010). Although DOC-CAM was reported to be an antagonist, it did not exhibit irreversible effects as its parent compound *in vivo* (Derrick et al., 2000; Lewis and Husbands, 2010). Therefore, even though it is evident that the 3-hydroxyl substituent is not required for μ-opioid activity, it is essential for the irreversible μ antagonist activity in the 14-cinnamoylamino series (Derrick et al., 2000; Lewis and Husbands, 2010).

1.2.3 14-O-heterocyclic naltrexones

Antagonists such as naloxone and naltrexone are the approved drugs used for treatment of opiate overdose (Ling and Wesson, 1990). Since there is no crystal structure of the μ receptor in existence to date, these μ antagonists play an important role in the study of opioid receptors (Li et al., 2009). Recently, studies showed that μ antagonists can be used to treat obesity, psychosis, and Parkinson's disease (Goodman et al., 2007), making the development of novel μ antagonists a valuable tool not only for studying the structure of opioid receptors, but also for the development of much needed therapeutics. 14-O-heterocyclic substituted naltrexone derivatives were most recently developed by Guo et. al. (Li et al., 2009), using a constructed homology model based on bovine rhodopsin. This model contained transmembrane helical domains with extracellular and intracellular loops, and was further optimized in a membrane-aqueous system using molecular dynamic simulations. The model revealed that the non-conserved residues, Tyr212 and Trp320, may interact with the receptor via hydrogen bonding interactions with the ligand (Li et al., 2009). Thus, a new series of compounds were developed to incorporate a

hetero-aromatic moiety on position 14 of naltrexone enabling hydrogen bonding and/or aromatic stacking interactions with Tyr212 and Trp320 (Li et al., 2009).

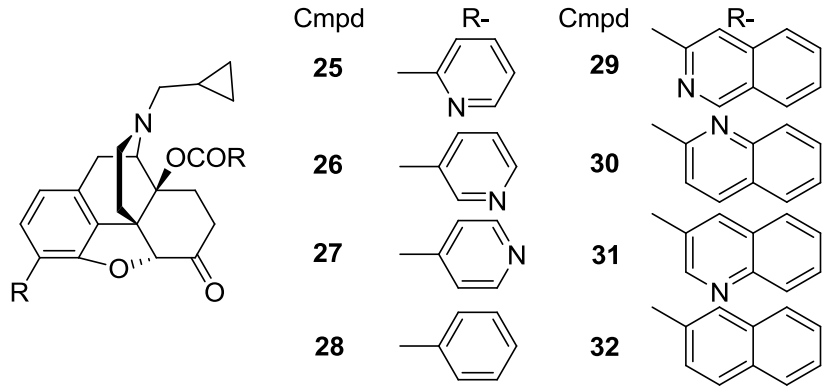


Figure 1.9 Structures of 14-O-heterocyclic naltrexones

Zhang's group further investigated the effect of the pyridyl nitrogen position and bulkiness via additional aromatic moieties on the 14-O-heterocyclic naltrexone derivatives (Figure 1.9). Almost all compounds were reported to have antagonist activity in GTPγS assays except for compound **31** (Li et al., 2009). When compared to previously reported compounds by Schmidhammer[31] and Husbands (Lewis and Husbands, 2010; Schmidhammer and Spetea, 2010) this series had similar binding affinities; however, compound **25** had higher selectivity, approximately 800-fold selectivity for the μ over δ and 200-fold selectivity for the μ over κ (Li et al., 2009). Introduction of an additional aromatic moiety (compounds **29**-**32**) did not improve the interaction with the μ receptor, but rather lowered their selectivity (Li et al., 2009).

1.3 CONCLUSION

Advances in the development of highly potent and selective opioid agonists and antagonists via position 14 in 14-alkoxymorphinan, 14-aminomorphinone, and 14-O-

heterocyclic naltrexone series provide valuable insights into opioid ligand-receptor interactions. It is evident that the nature of the substituent on position 14 and its orientation has a strong influence on receptor binding and post-receptor mechanisms. The advances in SAR illustrated in this review serve as a valuable tool for designing novel molecules with optimal configuration that may aid in identification of ideal opioid medications.

1.4 METHODS

1.4.1 Chemical Methods

Compounds discussed in this thesis were prepared using standard methods or following novel synthetic routes. These compounds were purified using standard chemical techniques (column chromatography, crystallization, etc.) and characterized using standard spectroscopic methods such as NMR (^{1}H, ^{13}C, HMBC, HMQC, NOESY) and LCQ MS. The purity of compounds was confirmed by combustion analysis, TLC, and melting point. Once characterized, the final products were converted to water soluble salts. All optically active compounds were prepared and evaluated as racemates.

1.4.2 Pharmacological Methods for Opioid Analogs

Binding affinity, potency, and efficacy of compounds were determined at all three opioid receptors (μ, δ, κ) using standard *in vitro* methods (Spetea et al., 2001a) provided by the laboratory of R. Matsumoto (West Virginia University, Morgantown, WV) and DEC.

This thesis dissertation is mainly focused on activity at μ, but analysis of κ and δ were performed for full evaluation of the opioid activity of these compounds.

Competition Binding Assay. Binding affinity (K_i) was assessed by radiolabled ligand displacement from cloned human receptors. Briefly, hMOR membrane protein were labeled with 1.3 nM [^{3}H]DAMGO (53.4 Ci/mmol). hDOR membrane protein were labeled with 1.2 nM [^{3}H]DPDPE (45 Ci/mmol). hKOR membrane protein were labeled with 1.7 nM [^{3}H]U69,593 (42.7 Ci/mmol). Non-specific binding was determined in the presence of 1 μM unlabelled DAMGO, DPDPE and U69,593 for the respective subtypes. Competition binding studies were performed using 12 concentrations of each test compound and were incubated for 1 h at 25^{o}C. Reactions were terminated by rapid vacuum filtration through GF/B glass fiber filters previously soaked in 0.5% polyethyleneimine . Bound radioactivity was quantified by liquid scintillation counting. Affinities (K_i) were calculated using the Cheng-Prusoff equation.

GTPγS assay. The efficacy (% stimulation) and potency (EC_{50}) were determined using the GTPγS assay by described procedures.(Aceto et al., 2007) The [^{35}S]GTPγS binding assay measures the amount of G protein activated (Figure 1.10). Activation of the receptor results in the exchange of GTP for GDP on the G_α subunit. Next, the G_αGTP exhibits dissociation from the Gβγ subunit followed by the hydrolysis of GTP to GDP by the GTPase activity of the G_α subunit. The Gα and Gβγ subunits reform and the cycle repeats. However, in this assay, GTPγS contains a γ-thiophosphate bond, which is resistant to hydrolysis by the GTPase. As a result, the [^{35}S]GTPγS labeled Gα remains

uncoupled following activation and its accumulation is measured by counting the radioactivity on the glass-fiber filter (Harrison and Traynor, 2003). The efficacy is determined as the % maximal effect with respect to the defined full agonists (DAMGO for μ, U69,593 for κ, and DPDPE for δ). The potency is measured as the amount of ligand required to reach 50% of the maximal response.

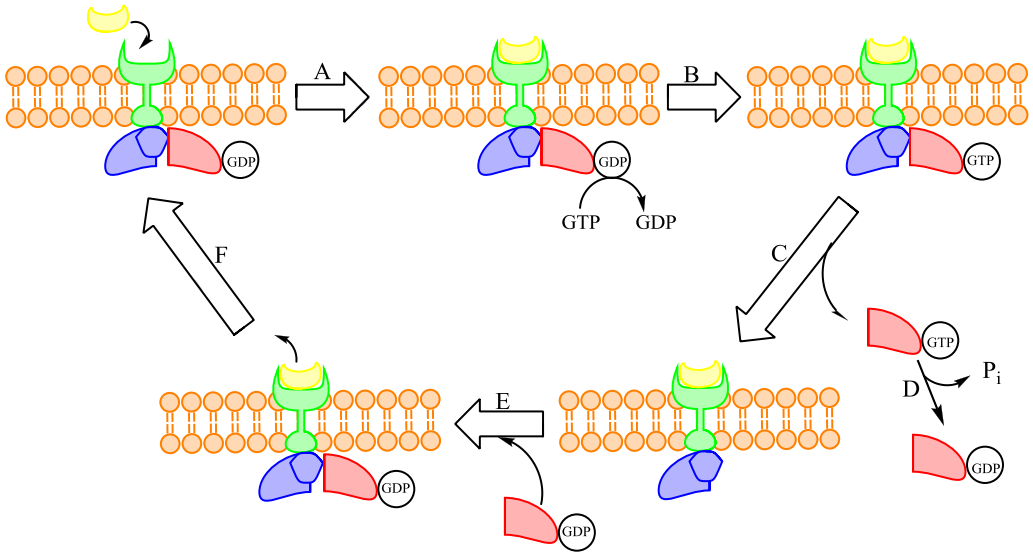


Figure 1.10 The GDP stimulation cycle A). Ligand binds to the receptor producing conformational change in the $G\alpha(GDP)_{\beta\gamma}$ heterotrimer. B). Once activated, GDP dissociates from the G_α subunit and GTP binds to G_α. C). The G_α-GTP dissociates from the $G_{\beta\gamma}$ dimer subunit. D). The GTPase activity hydrolyzes the GTP to GDP forming $G_\alpha GDP$. E). The $G_\alpha GDP$ $G_{\beta\gamma}$ recombines to form the complex. F). The ligand is displaced and the cycle repeats.(Harrison and Traynor, 2003)

1.4.3 Pharmacological Methods for Sigma Analogs

Competition Binding Assay. *In vitro* competition binding assays were performed as follows. Preparation of rat brain membrane and binding assays for the σ_1 and σ_2 receptor were performed as previously described in detail.(Matsumoto et al., 1995; Matsumoto et al., 2008) In brief, σ_1 receptors were labeled with 5 nM [^{3}H](+)-pentazocine. The σ_2 receptors were labeled with 3 nM [^{3}H]di-o-tolylguanidine (DTG) in the presence of 300

nM (+)-pentazocine to block σ_1 receptors. Nonspecific binding was determined in the presence of 10 μM haloperidol. Ten concentrations of each sigma compound (0.1–10,000 nM) were used in the assays. The compounds were incubated for 120 min at 25°C to measure their ability to displace the radioligands from their binding sites. Termination of the reactions was achieved through rapid vacuum filtration over glass fiber filters which were previously soaked in 1% polyethyleneimine for at least 45 min. K_i values were calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

Cocaine- Induced Convulsions. To probe for anticonvulsant actions against cocaine, male, Swiss Webster mice were pretreated (i.p.) with compounds **46** (0, 1, 10, and 30 mg/kg i.p.) and **49** (0, 0.1, 1, 10 mg/kg i.p.) 15 min prior to administration of a convulsive dose of cocaine (70 mg/kg i.p.). The mice were observed for the occurrence of convulsions for 30 min following the injection and results were recorded. Convulsions were operationally defined as clonic or tonic limb movements, which were accompanied by the loss of righting reflexes for at least 5 s, and/or popcorn jumping. Fisher's exact test was utilized to determine significant differences between the effects produced by pretreatment with the test compounds and the effects produced by the pretreatment with saline.

$[Ca^{2+}]_i$ Measurement. Cytosolic Ca^{2+} was monitored with the ratiometric indicator Fura-2 (InCyt Im2 Dual-wavelength Fluorescence Imaging System; Intracellular Imaging, Cincinnati, OH). The SK-N-SH neuroblastoma cells (human neuroblastoma, HTB-11; American Type Culture Collection, Manassas, VA) were grown on glass coverslips and

then washed twice in Dulbecco's phosphate-buffer saline (DPBS) before incubation in DPBS containing 2.0 to 3.0 μM Fura-2 AM and 0.066% Pluronic F-127 (Invitrogen). After incubating for 60 to 75 min at 37°C in darkness, cultures were washed twice in DPBS to remove extracellular dye and kept at room temperature in the dark for more than 30 min before use in the experiments. All measurements were performed in DPBS or, where specified, in Ca^{2+}-free DPBS. Compounds **46** and **49** were added to cells in the presence of DPBS in the Petri dishes. The dishes with dye-loaded cells were mounted on the stage of a Nikon TS-100 fluorescence inverted microscope with a Cohu model 4915 charge-coupled device (CCD) camera (Nikon, Melville, NY). Fluorescent images were captured alternately at the excitation wavelengths of 340 and 380 nm with an emission wavelength of 520 nm, which were analyzed with InCyt Im2 version 4.62 imaging software (Intracellular Imaging, Cincinnati, OH).

A standard curve was used to derive experimental $[Ca^{2+}]_i$ values. The standard curve was generated by using various concentrations of Ca^{2+} (Calcium Calibration Buffer Kit) in the presence of indicator dye Fura-2 free acid (Invitrogen). During each experiment, background fluorescence was estimated for a region without cells, and this value was automatically subtracted from the measured emission of each channel. The ratios of cell emissions were compared with the standard curve stored in the computer, and both the ratio and $[Ca^{2+}]_i$ were displayed on screen. Preliminary measurement of $[Ca^{2+}]_i$ was taken on various cells in the field before any tested compounds were applied. Only cells with basal $[Ca^{2+}]_i$ in the range of 90 to 120 nM were chosen for the experiments described here.

1.5 SPECIFIC AIMS

Opioid analgesics are a class of agents used clinically to treat moderate to severe pain. Due to the effectiveness in treating severe pain, morphine is typically the drug of choice, though its use is most problematic. Serious side effects, such as respiratory depression, tolerance, constipation, dependence, and nausea, limit the effectiveness of opioids. Thus, there is a continuing need to investigate novel structural opioid classes in an effort to develop opioids that exhibit more favorable interactions with the receptor. Previous studies show that 14-phenylpropyloxymorphinans are agonists that exhibit extreme potency at μ receptors when compared to morphine. However, such compounds are unsuitable for clinical use due to their high potency. This suggests that the 14-phenylpropyloxy group has a major effect on receptor binding and is responsible for the dramatic increase in potency. As a result, this class can act as a lead skeleton for analgesic development. **Our hypothesis is that both a basic amine and a phenylpropyloxy group alone are required for opioid activity, and the aromatic A-ring, that is considered essential, is not required.** Accordingly, a series of phenylpropyloxyethylamines will be synthesized and will be analyzed for opioid receptor binding affinity, and efficacy. Differing N-substituents will be evaluated in order to develop a SAR. The optimal spatial orientation of the basic amine and the phenylpropyloxy group will be determined via syntheses of conformationally constrained analogs of phenylpropyloxyethylamine using single ring systems that mimic rings of morphine. Subsequently, a multiple ring system will be synthesized by combining the

previously determined optimal single ring orientations to produce optimal μ opioid activity. The ultimate goal will be to introduce the optimal *N*-substituent into the optimal skeleton. The following specific aims will help achieve our goals for this proposal:

Specific Aim 1. Optimize the *N*-substituents and length of the carbon linker for high affinity at μ receptors. Preliminary results have shown that 2-(cinnamyloxy)-*N*,*N*-dimethylethanamine exhibits codeine-like affinity for μ receptors *in vitro*. Using this scaffold, lead compound optimization will be explored through synthesis of phenylpropyloxyethylamine analogs with flexible and ring-constrained *N*-substituents. Specifically, phenylpropyloxyethylamines containing *N*,*N*-dimethyl, diethyl, dipropyl, dibutyl, pyridine and pyrrolidine (azetidine, aziridine) substituents will be synthesized. In addition, a cinnamyloxyethylamine series containing identical *N*-substituents will be generated in an effort to understand the effect of saturation in this group. This process will aid in the development of structure-activity relationships for this series, and the *N*-substituents that produced the desired profile of high binding affinity and agonist efficacy will be selected for further optimization.

Specific Aim 2. Incorporate constraining rings into the phenylpropyloxyethylamines which mimic rings B, C, and D in opioids. It is hypothesized that the compounds synthesized in Specific Aim 1 will optimize interactions with the μ receptor to give greater affinity. To determine the bioactive conformation, and aid in future modeling studies, constrained rings B, C, and D (see background section) will be re-introduced back into the system iteratively. This will determine which conformations and 3D spatial

relationships are required for specific opioid binding affinity and agonist activity. To date, every pharmacophore describing binding affinity of opioids to μ receptors includes the A-ring. In this Specific Aim, the conformationally sampled pharmacophore (CSP) approach will be used to examine all accessible conformations of the single ring system analogs. The predictions obtained from the pharmacophore will guide the subsequent synthesis of poly ring system analogs in Specific Aim 3.

Specific Aim 3. Design and synthesize analogs of the phenylpropyloxyethylamines containing multiple rings from the opioid skeleton. Continuing the approach from Specific Aim 2, a multiple B/D ring system will be synthesized subsequently in order to investigate specific opioid activity. The rings, which are determined to have the greatest effect on opioid activity from Specific Aim 2, will be combined to produce a more potent opioid ligand. The optimal *N*-substituents determined in Specific Aim 1 will be combined with the rings selected from Specific Aim 2 to optimize this lead as a novel μ opioid agonist.

The goal of this research is to determine the minimal structural requirements for high affinity and efficacy at μ opioid receptors in ligands that lack the A-ring, traditionally considered to be essential for opioid activity. Compounds synthesized in Specific Aims 1, 2, and 3 will be analyzed for opioid receptor binding affinity and efficacy, and the results will be used in the design of further generations of compounds. Compounds with high affinity and efficacy at μ opioid receptors will be assayed for antinociceptive activity in

mice, with the top candidates further considered for development into novel analgesic agents.

Specific Aim 4. Determine the structural requirements for σ_1 and σ_2 receptor recognition. Since the proposed compounds closely resemble AC927 (*N*-phenethylpiperidine oxalate), they will be further investigated as partial opioid structures, lacking the A-ring, at the two established σ receptors subtypes (σ_1, σ_2). Compounds which show the highest affinity will be tested in functional assays.

Additionally, in an effort to design a pharmacophore for selective σ_2 antagonism, we have investigated the effect of pyridyl nitrogen position and chain length in the phenylalkylpiperazinepyridine series. A series of pyridylpiperazines will be synthesized and analyzed for sigma receptor binding affinity to determine the optimal pyridyl nitrogen position and chain length for σ_1 and σ_2 receptor recognition.

Chapter 2. Opioids lacking a tyrosine mimetic. Part 1: Phenylpropyloxyethylamines

2.1 INTRODUCTION

The μ opioid agonist morphine (**I**) is the standard for severe pain management (Zieglgansberger et al., 1995; Stein et al., 2003). Despite the ability of **I** to treat severe pain, there are significant side effects which often cause undermedication in clinical settings. Such effects are tolerance, dependence (Kieffer and Evans, 2002), constipation, nausea, and respiratory depression (McNicol et al., 2003; Benyamin et al., 2008).

Opioid therapy is often accompanied by additional medications to treat or prevent some of the undesirable side effects (Klaschik et al., 2003). For example, constipation can be managed with stool softners and laxatives, but not chronically (Klaschik et al., 2003). While additional medication may lessen or even prevent some of the adverse effects, in some cases it may dramatically decrease the effectiveness of the opioid itself due to drug-drug interaction (Armstrong and Cozza, 2003). Another problem associated with taking additional medication is that it adds to the regimen of drugs already taken by the patients.

Recently, peripherally- restricted μ opioid receptor antagonists have been approved for treatment of opioid induced constipation: alvimopan (Lavine, 2008), and methylnaltrexone bromide (Yuan et al., 2005). Alvimopan is a zwitterionic phenylpiperidine, which is unable to penetrate the BBB due to its hydrophobicity and therefore it selectively antagonize the effect of opioids on μ receptors in the GIT (Lavine, 2008). A significant limitation to prolonged use of Alvimopan is the risk of a heart attack (Chappelle, 2008; Lavine, 2008). Consequently, alvimopan is only available

as a short-term treatment, in hospitals approved by the Entereg Access Support and Education (E.A.S.E.) program, and cannot be dispensed to patients after discharge (Chappelle, 2008; Lavine, 2008). Methylnaltrexone bromide is a derivative of naltrexone which has a high peripheral selectivity that comes from the low lipid solubility due to its quaternary salt form (Yuan et al., 2005). Moreover, methylnaltrexone must be administered subcutaneously as it exhibits poor oral bioavailability (Yuan et al., 2005).

Lack of tolerance and physical dependence has been observed after repeated treatment with 14-methoxymetopon (**II**, Figure 2.1), a member of the alkoxymorphinan opioid series.(Furst et al., 1993b) Studies also showed that **II** has reduced constipation(King et al., 2003) and respiratory depression (Furst et al., 1993b) as compared to **I** and has been characterized as a μ selective opioid with 500-fold greater systemic antinociceptive potency than **I** (Furst et al., 1993a). Upon superaspinal administration, **II** can elicit potency of up to one million-fold greater than morphine (King et al., 2003).

The 14-phenylpropyloxymorphinan (**III**, Figure 2.1), a derivative that belongs to the 14-alkoxymorpinan family, is an agonist which is even more potent than **II** (24000-fold higher in the tail flick assay and 8500-fold higher in the hot plate assay as compared to **I**) (Schutz et al., 2003). Although **III** is unsuitable for clinical use due to its extreme potency, it can serve as a lead compound for structural development of a novel opioid skeleton.

The structure of **I** is comprised of 5 rings: aromatic A, cyclohexyls B and C, piperidine D, and epoxy E (Figure 2.1) (Casy and Parfitt, 1986). Opioids lacking rings B-E were developed in an effort to eliminate undesirable effects, but all continue to produce these side effects (Casy and Parfitt, 1986). Common among all structural classes of opioids (phenylpiperidines, benzomorphans, morphinans) is an aromatic ring - the A-ring (Casy and Parfitt, 1986). The phenolic A-ring of morphine is thought to mimic the tyrosine residue of enkephalin, strongly suggesting its requirement for opioid receptor binding (Andersson et al., 1995). Point mutation studies supported this, as the histidine located in TM VI (His VI:17) hydrogen-bonds to the C_3 oxygen substituent on the A-ring (Kane et al., 2006). The C_3 oxygen substituents are generally associated with high affinity and potency (Aldrich, 1993). Furthermore, the 14-alkoxymorphinan series shows that potency can be magnified by C_{14} alkyl substituents (Schmidhammer and Spetea, 2010). Moreover, 14-alkoxymorphinans are capable of maintaining high affinity for μ even when there is no C_3 oxygen function (Spetea et al., 2004). Our hypothesis is that opioid activity can be achieved in presence of both a basic amine and a phenylpropyloxy group, and that the A-ring, that is considered essentiall (Casy and Parfitt, 1986), is not required. By removing the A-ring, this allows the skeleton to adopt an alternate binding mode with the receptor, thereby potentially causing alternate receptor trafficking events(Ignatova et al., 1999) and post-receptor mechanisms,[37] all of which are involved in the development of tolerance. (Kieffer and Evans, 2002). Further evidence that the A-ring can be removed is seen in the case of ozonolysis of 6, 14-endo-ethenotetrahydrothebaines (Casy and Parfitt, 1986). Although the cleavage of the aromatic ring, to give lactonic esters, diminished activity, morphine-like potency was achieved (Casy and Parfitt, 1986).

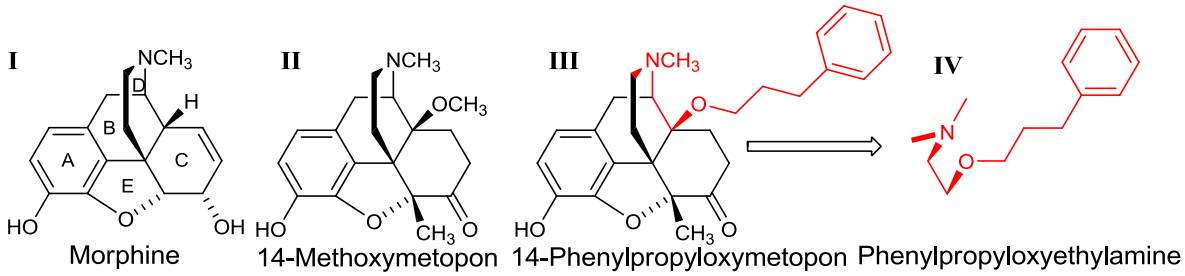
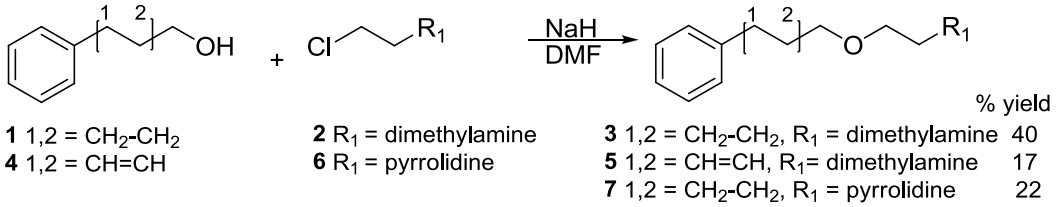


Figure 2.1 Opioids used for hypothesis and the proposed analog

The work described in this chapter aimed to develop a novel opioid class that exhibits high affinity and efficacy at μ opioid receptors. According to our hypothesis, phenylpropyloxyethylamine (**IV**, Figure 2.1) analogs with flexible and ring-constrained *N*-substituents were synthesized, characterized, and (through our collaborators) pharmacologically evaluated. Specifically, the synthesis of phenylpropyloxyethylamines containing *N*,*N*-dimethyl, diethyl, dipropyl, and dibutyl, as well as pyrrolidine, pyridine, and azepane substituents will be discussed. In addition, a cinnamyloxyethylamine series containing identical *N*-substituents were generated in an effort to understand the effect of saturation in this group. In order to investigate differences and similarities between the morphinans and this series, *N*-benzyl and *N*-allyl analogs, which traditionally tend to antagonism, and *N*-phenethyl and *N*-phenylpropyl analogs, which tend to confer μ agonism, have been synthesized. This series of compounds allowed for the development of structure-activity relationships, and the *N*-substituents that produced the desired profile of highest binding affinity were selected for further optimization.

2.2 CHEMISTRY

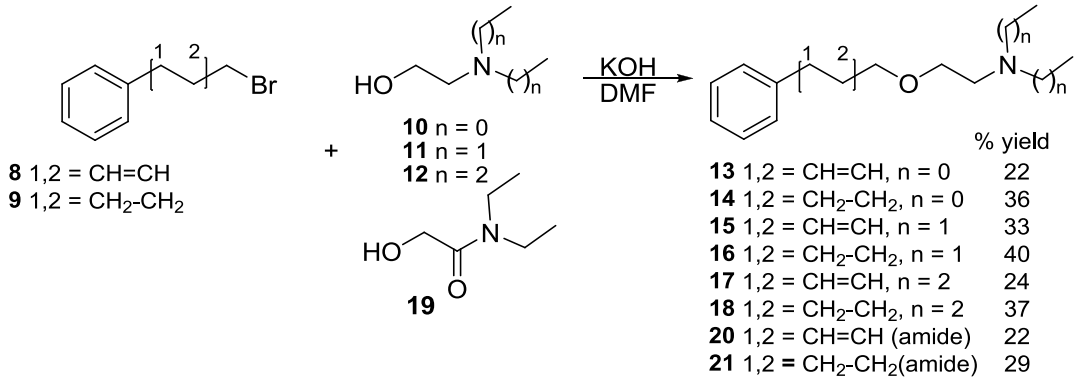
In our initial studies, *N*,*N*-dimethyl-2-(3-phenylpropoxy)ethanamine (**3**), 2-(cinnamyloxy)-*N*,*N*-dimethylethanamine (**5**), and 1-(2-(3-phenylpropoxy)ethyl)pyrrolidine (**7**) were synthesized and characterized following the literature procedure (Rist et al., 2001) (Scheme 2.1). The appropriate chloroethylamines **2** and **6** were treated with the alcohols **1** and **4** in the presence of NaH and heated to 50°C for 3 hours. Compounds **3, 5** and **7** were successfully synthesized in moderate yields (**3,** 40%; **5,** 17%; **7,** 22%). Due to the hygroscopic nature of the salts, the final products remained in oil form.



Scheme 2.1 Synthesis of analogs **3**, **5** and **7** and their yields

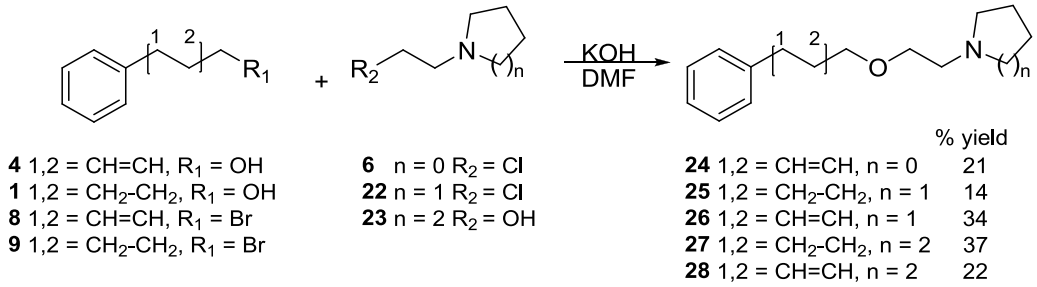
To improve the yield of amino ethers and use a less hazardous compound than NaH, alternate conditions were considered. However, the improvement of yield met with limited success. Ultimately, a less hazardous approach was developed and utilized for subsequent reactions. Potassium hydroxide (KOH), a weaker base, proved to be a good substitute for hazardous NaH and reactions were found to proceed well, with less side-products, at room temperature. Targets **13-18**, **20**, and **21** (Scheme 2.2) were prepared following the new procedure. Compound **13** was synthesized from starting materials **8** and **10**; **14** from **9** and **10**; **15** from **8** and **11**; **16** from **9** and **11**; **17** from **8** and **12**; **18**

from **9** and **12**; **20** from **8** and **19**; and **21** from **9** and **19**. The final products were converted into oxalic salts using diethyl ether and oxalic acid. All of the compounds were synthesized in moderate 22-37% yields.



Scheme 2.2 Synthesis of *N,N*-dialkyl analogs and their yields

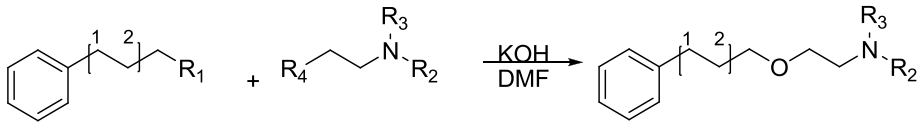
To investigate the effect of constrained *N*-substituents, targets **24-28** were synthesized (Scheme 2.3) following the same procedure as developed for the alkyl analogs. Compound **24** was prepared from **4** and **6**; **25** from **1** and **22**; **26** from **4** and **22; 27** from **9** and **23**; and **28** from **8** and **23**.



Scheme 2.3 Synthesis of pyrrolidine, piperdine and azepane containing analogs and their yields

The *N*-arylalkyl and *N*-allyl series (Scheme 2.4) was selected based on the established SAR of agonism and antagonism in the opioids. Specifically, *N*-allyl tended to yield high affinity antagonists and *N*-benzyl groups tended to yield low affinity antagonists, whereas

longer chains confer μ agonism (Casy and Parfitt, 1986). Compounds **35** (**8**, **30**), **36** (**9**, **30**), **37** (**8**, **31**), and **38** (**9**, **31**) were synthesized and the *N*-phenethyl and *N*-phenylpropyl substituents are anticipated to possess agonist activity. The *N*-benzyl and *N*-allyl substituents are understood to impart antagonism (Casy and Parfitt, 1986) and therefore we have synthesized compounds **33** (**8**, **29**), **34** (**9**, **29**), **39** (**8**, **32**), and **40** (**9**, **32**) in order to investigate the differences and similarities that compounds without the traditional A-ring will have in this series.



4 1,2 = CH=CH, R_1 = OH
1 1,2 = CH_2-CH_2, R_1 = OH
8 1,2 = CH=CH, R_1 = Br
9 1,2 = CH_2-CH_2, R_1 = Br

29 R_2= CH_2Ph, R_3=CH_3, R_4=OH
30 R_2= $(CH_2)_2Ph$, R_3=CH_3, R_4=Cl
31 R_2= $(CH_2)_3Ph$, R_3=CH_3, R_4=Cl
32 R_2, R_3= $CH_2CH=CH_2$, R_4=Cl

	% yield
33 1,2 = CH=CH, R_2 = CH_2Ph, R_3 = CH_3	20
34 1,2 = CH_2-CH_2, R_2 = CH_2Ph, R_3 = CH_3	32
35 1,2 = CH=CH, R_2 = $(CH_2)_2Ph$, R_3 = CH_3	19
36 1,2 = CH_2-CH_2, R_2 = $(CH_2)_2Ph$, R_3=CH_3	34
37 1,2 = CH=CH, R_2 = $(CH_2)_3Ph$, R_3= CH_3	19
38 1,2 = CH_2-CH_2, R_2 = $(CH_2)_3Ph$, R_3=CH_3	27
39 1,2 = CH=CH, R_2,R_3 = $CH_2CH=CH_2$	23
40 1,2 = CH_2-CH_2, R_2,R_3 = $CH_2CH=CH_2$	22

Scheme 2.4 Synthesis of *N*-arylalkyl and *N*-diallyl analogs and their yields

2.3 RESULTS AND DISCUSSION

The goal of this research was to develop novel opioid skeletons that possess high affinity and efficacy for μ receptors. Whereas the compounds **3**, **5**, and **7** were exclusively pharmacologically evaluated by the Drug Evaluation Committee (DEC), all other compounds were biologically evaluated by Jason Healy, a graduate student in the laboratory of Dr. Rae Matsumoto (WVU, Morgantown, WV).

2.3.1 Opioid Receptor Binding

Opioid binding studies for compounds **3**, **5**, and **7** (Table 2.1) were performed against all three opioid receptors (μ, δ, and κ) by the Drug Evaluation Committee (DEC) via a displacement assay, following standard procedures (Spetea et al., 2001a). Compound **7** showed weak affinity (3100 nM) for μ-opioid receptors, and negligible (>10,000 nM) affinity for κ and δ receptors. The interaction with the μ-opioid receptor significantly improved when phenylpropyl group, **3** was modified to a cinnamyl group **5.** As a result, compound **5** had codeine-like affinity for μ-receptors (338 nM). Hence, the nature of the *N*-substituent was modified to include functional groups which were anticipated to confer agonism (e.g. methyl, ethyl, butyl, phenylpropyl) and possibly antagonism (e.g. benzyl and allyl) (Casy and Parfitt, 1986).

Table 2.1 Opioid Receptor Binding Affinities for Compounds **3**, **5**, and **7**

	K_i± SEM (nM)		
Compound	[^{3}H] DAMGO (μ)	[^{3}H] DPDPE (δ)	[^{3}H] U69,593 (κ)
3	>10,000	>10,000	>10,000
5	338*	6100*	6500*
7	3210 ± 430	>10,000	>10,000
Codeine[1]	727 ± 128	52207 ± 25421	25411 ± 10015
Morphine[2]	6.55 ± 0.74	217 ± 19	113 ± 9

*Statistical data not available
[1] ref. (Peckham and Traynor, 2006)
[2] ref. (King et al., 2003)

In the current series, the *N*-diethyl analog was previously studied along with related compounds as potential antiarrythmic agents but were shown to have undesired effects (Molimard, 1970). This, coupled with the fact that *N*-ethyl tends to lead to low analgesic activity with opioids (Casy and Parfitt, 1986), prompted our decision to eliminate the

diethyl analogs from our design as potential therapeutics. Although the *N*-diethyl analogs did not undergo any *in vivo* analysis, *in vitro* studies were performed to assist in the modeling studies.

In order to be able to compare data between laboratories, compounds **3**, **5** and **7** were biologically re-evaluated by Jason Healy, a graduate student in the laboratory of Dr. Rae Matsumoto at WVU (Morgantown, WV). DAMGO (μ opioid peptide), DPDPE (δ opioid peptide), and U69,593 (κ opioid peptide) were utilized as the positive controls in order to validate the method (Table 2.3). Unexpectedly, and in contrast to the previous affinity data, compounds **3**, **5** (Table 2.2) and **7** (Table 2.3) did not have significant affinity. However, the *N*-dibutyl analogs (**17**, **18**) in the *N*-dialkyl series both displayed similar weak binding affinity (2494 nM) for the μ binding receptor and negligible (>10,000 nM) affinity for δ receptors (Table 2.2). Despite the previously described preference for the unsaturated chain in the DEC tested compounds **3** and **5**, compounds **18** and its unsaturated analog **17** displayed identical binding affinities independent of the level of the unsaturation.

Table 2.2 Opioid Receptor Binding Affinities for *N*-dialkyl Analogs

	K_i± SEM (nM)		
Compound	[^{3}H] DAMGO (μ)	[^{3}H] DPDPE (δ)	[^{3}H] U69,593 (κ)
3	>10,000	ND*	ND*
5	>10,000	ND*	ND*
13	>10,000	ND*	ND*
14	>10,000	ND*	ND*
15	>10,000	>10,000	ND*
16	>10,000	>10,000	ND*
17	2490 ± 206	>10,000	ND*
18	2490 ± 165	>10,000	ND*
20	>10,000	>10,000	ND*
21	>10,000	>10,000	ND*
DAMGO	1.47 ± 0.37	>10,000	> 10,000
DPDPE	618 ± 64	2.44 ± 0.33	>10,000
U69,593	> 10,000	>10,000	1.13 ± 0.49

ND* = not determined

N-heterocycles were examined to delineate the effect of conformational freedom of these substituents on opioid activity. From our results, it appears that constraining the flexible chains in the *N*-dialkyl analogs to give ring constrained *N*-heterocyclic analogs (**7**, **24**-**27**) is not favorable for interactions with the opioid receptors (Table 2.3).

Table 2.3 Opioid Receptor Binding Affinities for *N*-heterocyclic Analogs

	$K_i \pm$ SEM (nM)		
Compound	[^{3}H] DAMGO (μ)	[^{3}H] DPDPE (δ)	[^{3}H] U69,593 (κ)
7	>10,000	ND*	ND*
24	>10,000	ND*	ND*
25	>10,000	ND*	ND*
26	>10,000	>10,000	ND*
27	>10,000	ND*	ND*
28	>10,000	ND*	ND*

ND* = not determined

The *N*-arylalkyl and *N*-diallyl analogs were selectively synthesized based on the established SAR of agonism and antagonism in the opioids. From the results in Table 2.4, it appears that the second aromatic ring is important for binding. Among the *N*-arylalkyl analogs (Table 2.4), compounds **35** and **38** displayed the highest affinity for the μ opioid receptor binding site (1680 nM and 1520 nM, respectively), weak affinity for δ (6850 nM and 6650 nM, respectively), and negligible (>10,000 nM) affinity for κ. The *N*-benzyl analogs (**33**, **34**), which tend to yield low antagonism displayed low binding affinity for the μ opioid receptor (2760 nM - 3040 nM) and negligible binding affinity (>10,000 nM) for δ while the *N*-diallyl analogs showed no activity at either receptor type.

Table 2.4 Opioid Receptor Binding Affinities for *N*-arylalkyl and *N*-diallyl Analogs

	K_i± SEM (nM)		
Compound	[^{3}H] DAMGO (μ)	[^{3}H] DPDPE (δ)	[^{3}H] U69,593 (κ)
33	3040 ± 250	>10,000	ND*
34	2760 ± 146	>10,000	ND*
35	1680 ± 155	6850 ± 453	>10,000
36	2310 ± 193	8530 ± 669	>10,000
37	6450 ± 315	>10,000	ND*
38	1520 ± 175	6650 ± 405	>10,000
39	>10,000	ND*	ND*
40	>10,000	ND*	ND*

ND* = not determined

The affinity results showed that the optimal *N*-substituents include *N*-phenethyl and *N*-phenylpropyl. Overall, there was no noticeable trend between the saturated and unsaturated derivatives in the phenylpropyloxyethylamine series.

2.3.2 [^{35}S]GTPγS Binding Assays

The efficacy (% stimulation) and potency (EC_{50}) were determined using the GTPγS assay by described procedures.(Aceto et al., 2007) The efficacy is determined as the % maximal effect with respect to the defined full agonists (DAMGO for μ, U69,593 for κ, and DPDPE for δ). The potency is measured as the amount of ligand required to reach 50% of the maximal response. GTPγS studies were performed by DEC with compound **5**, which appeared to have the highest affinity for the μ receptor prior to being retested in Dr. Matsumoto's lab. Results showed that compound **5** produced high efficacy (78% vs. DAMGO) and low potency (EC_{50}=9200 nM) at μ receptors. Despite the low potency, it was evident that the new series of compounds were suitable for further optimization into a clinically acceptable μ opioid analog.

GTPγS studies are currently underway for the rest of the synthesized compounds. These compounds will aid in the understanding of the SAR of the series and the optimal *N*-substituent will be utilized in Specific Aims 2 and 3.

2.3.3 Molecular Modeling Studies

The novel series of compounds consist of an aromatic moiety, similar to that of morphine and its analogs. In order to verify that the aromatic moiety on compound **5** mimics the aromatic moiety coming off position 14 on 14-cinnamyloxymetopon, and not the A-ring on morphine, the conformationally sampled pharmacophore (CSP) (Rais et al.; Bernard et al., 2003; Bernard et al., 2005; Bernard et al., 2007) modeling approach was applied and pharmacophore models were designed. The CSP method, developed by Dr. MacKerell and coworkers at the University of Maryland, is a novel approach for ligand-based drug design (Rais et al.; Bernard et al., 2003; Bernard et al., 2005; Bernard et al., 2007). This method maximizes the probability of inclusion of the bioactive conformation for model development by considering all the energetically accessible conformations of each ligand in the set rather than individual lowest energy conformers traditionally used. The CSP method has been previously used to predict the affinity and efficacy of the peptidic and nonpeptidic delta opioids (Bernard et al., 2003; Bernard et al., 2005; Bernard et al., 2007). This method was also applied to highly flexible ligands, bile acids (Rais et al.; Rais et al.) and relationship between affinity and various substituents has been proposed. Considering that compound **5** also has a high degree of conformational freedom, the CSP

method was performed by Jihyun Shim, a member of Dr. MacKerell's laboratory in order to secure the conformational diversity.

Compound **5** and cinnamyloxymetopon were modeled using the program CHARMM (Brooks et al., 2009) with the CHARMM General Force Field (Vanommeslaeghe et al.) (CGenFF) parameters and they were energy-minimized using a combination of minimization algorithms in CHARMM such as steepest descent and adopted basis Newton-Raphson (ABNR) to a RMS gradient of 10^{-6} kcal/mol Å. For conformational sampling, the molecules were subjected to Temperature Replica Exchange-Molecular Dynamic (TREX-MD) simulations (Sugita and Okamoto, 1999). TREX-MD is an efficient methods currently used to overcome local minima and to sample diverse conformational space (Sugita and Okamoto, 1999). TREX-MD performs a range of independent MD simulations (replicas) in which each replica is under different temperatures, representing system of different degrees of kinetic energy to overcome energy barriers (Sugita and Okamoto, 1999). Exchanges of configurations occur between the adjacent replicas when the energy differences are small, such that the lower energy is always accepted and the higher energy is conditionally accepted according to Metropolis criterion (Metropolis and Ulam, 1949). Such exchanges are utilized to sample different conformations, which can overcome the energy barriers while satisfying Boltzmann distribution of conformations. In this study, 8 replicas with exponential scaling of temperatures between 300K to 400K (300K, 313K, 326K, 339K, 354K, 368K, 384K, 400K) were used. MD simulations on each replica were carried out for 5 ns using Langevin dynamics (Allen and Tildesley, 1989) in implicit solvent using the GBMV

(Generalized Born using molecular volume) method in CHARMM (Lee et al., 2003). Exchange was attempted every 0.5 ps. To confirm that the simulation was sampling distinctive conformations, the probability of geometric distributions was compared with the increment of simulation time. For example, the probability distribution of distances between basic nitrogen and aromatic ring of compound 5 was calculated over 0.5 ns intervals. Overlap between probability distributions at 4.5ns and 5ns reached 99% and a significant shift in the population was not observed. Therefore 5ns sampling was deemed converged enough to perform further analysis.

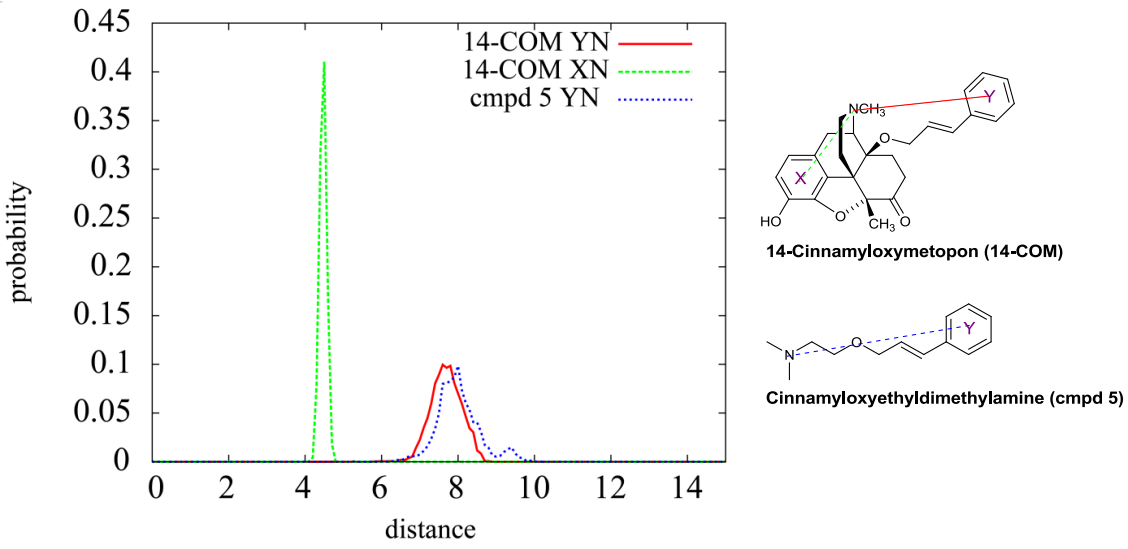


Figure 2.2 CSP-generated data showing 1D probability distribution of distances between the basic nitrogen (N) and the aromatic moieties (X, Y) of compound **5** and 14-cinnamyloxymetopon. Green represents the probability distribution of the XN distance on the 14-cinnamyloxymetopon; red the YN on the 14-cinnamyloxymetopon; blue the YN on compound **5**.

For the analysis, only the first replica corresponding to room temperature was used from which 2500 conformations were obtained. The 1D probability distribution of distances between the basic nitrogen (N) and the centroid of the aromatic moieties (X and Y) of both molecules, with bin size of 0.1 Å, are displayed in Figure 2.2. As expected, there is a

significant overlap for the YN distances in both of the molecules (indicated in red and blue) and no overlap is observed with the A-ring (XN: indicated in green), further suggesting that the aromatic moiety on phenylpropyloxyethylamines does not mimic the A-ring.

Additionally, the same approach was utilized to determine the 1D probability distribution of distances between the centroid of the aromatic moieties on the *N*-arylalkyl series (compounds **34**-**38**) and the basic nitrogen and compared to the 1D probability distribution of distances between the aromatic A-ring and the basic nitrogen on morphine. In earlier studies, it was found that the aromatic moiety coming off the oxygen on compound **5** did not sample the same space as the A-ring. Unexpectedly, results displayed in Figure 2.3 illustrate that the distance between the aromatic ring coming off the oxygen position and the nitrogen on the phenylpropyloxyethylamines had some overlap with the conformations that are sampled by the aromatic A-ring and the basic nitrogen on morphine. However, is evident that the cinnamyl analogs are least likely to mimic the A-ring as they are less flexible and therefore sample a relatively narrow range of conformations

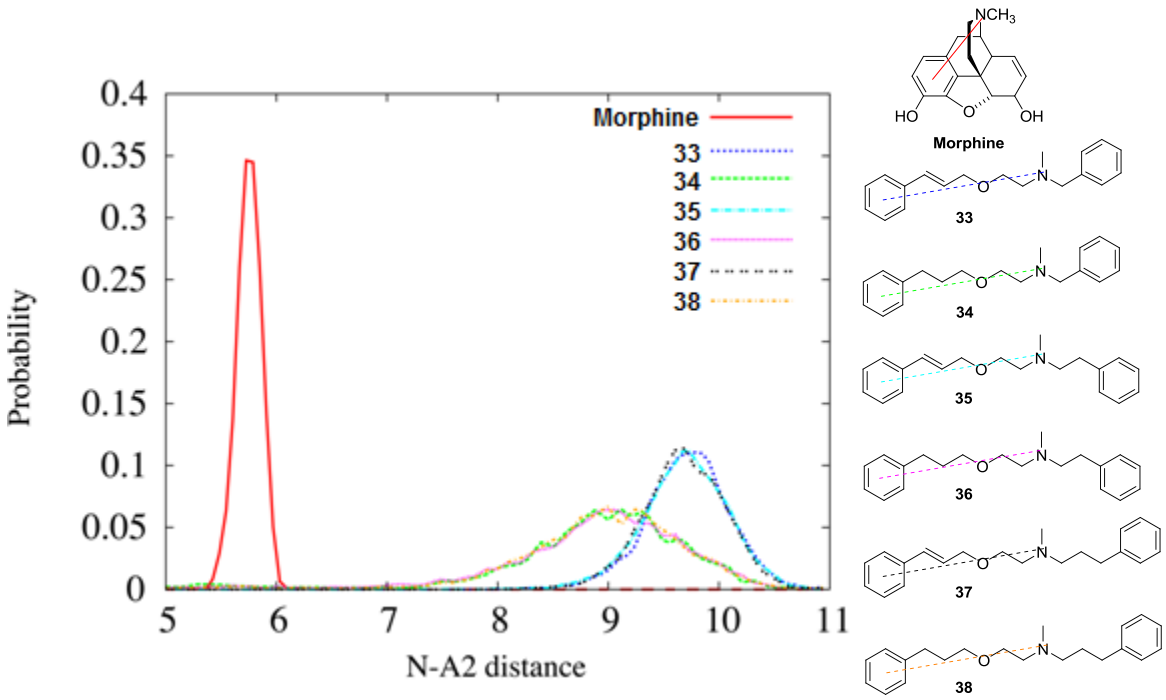


Figure 2.3 Figure showing 1D probability distribution of distances between the basic nitrogen and the aromatic moiety coming off the oxygen on compounds **33-38** and the aromatic A-ring on morphine.

Similar to the previous results, the *N*-benzyl derivatives do not appear to mimic the A-ring (Figure 2.3). In contrast, some overlap in the conformational space between the basic nitrogen and the aromatic ring on the *N*-phenethyl and *N*-phenylpropyl is observed with the A-ring on morphine. These results indicate that the aromatic moiety on compounds **35-38** may be mimicking the A-ring. Though the affinity of the *N*-phenethyl derivative was slightly lower compared to *N*-phenylpropyl, the *N*-phenethyl has been identified as the optimal *N*-substituent because the molecular modeling data indicated that the *N*-phenylpropyl derivatives had a higher overlap coefficient than the *N*-phenethyl derivatives.

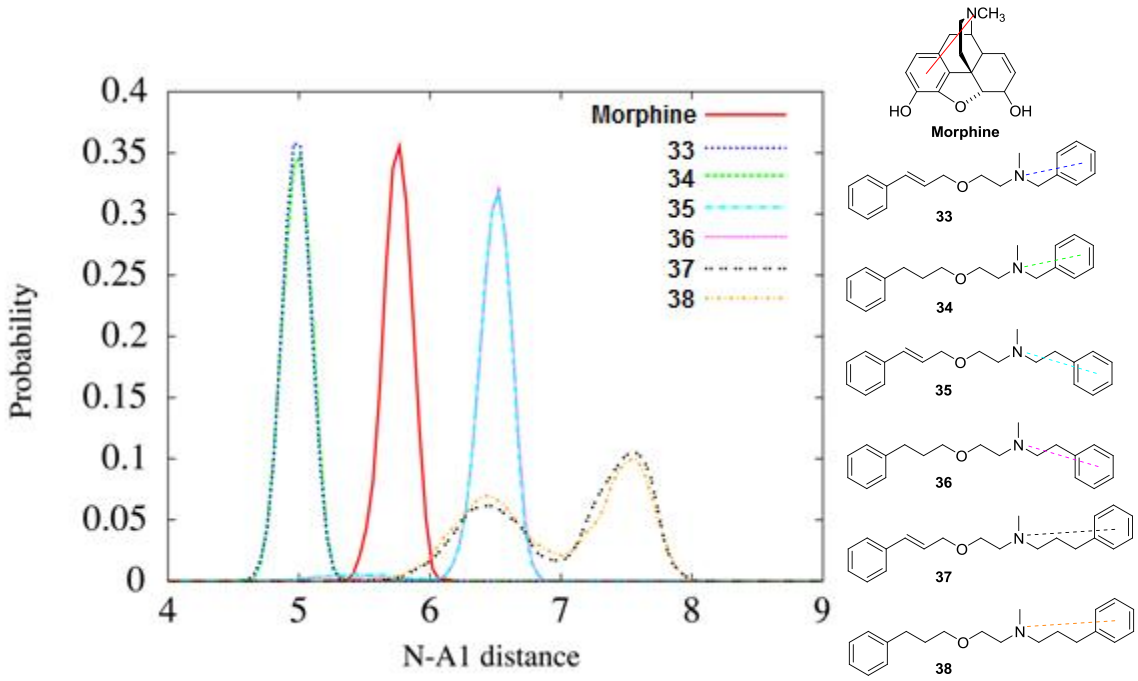


Figure 2.3 Figure showing 1D probability distribution of distances between the basic nitrogen and the aromatic moiety coming off the nitrogen on compounds **33-38** and the aromatic A-ring on morphine.

2.3 CONCLUSIONS

In this chapter, a series of phenylpropyloxyethylamines with differing *N*-substituents were synthesized to test the hypothesis that opioid activity can be achieved in the presence of a basic amine and a phenylpropyloxy group, and that the A-ring is not necessarily required. Using the CSP approach, we predicted that the aromatic moiety coming off the oxygen does not mimic the A-ring on the cinnamyl analogs. However, slight overlap in the conformational space between the basic nitrogen and the aromatic ring on the *N*-phenethyl and *N*-phenylpropyl derivatives is observed with the A-ring on morphine, indicating that it may be mimicking the A-ring. Nonetheless, the

phenylpropyloxyethylamines are capable of binding to the μ opioid receptor possessing a fairly weak affinity while maintaining negligible affinity for κ and δ receptors. Based on the molecular modeling and opioid binding studies, we have identified the optimal *N*-substituent as the *N*-phenethyl contained in analog **35**, with 1680 nM affinity for the μ opioid receptor. Furthermore, compound **35** will serve as the novel lead compound for further optimization. In chapter 3, we will discuss the subsequent re-introduction of rings B, C, and D from the morphine skeleton as ring-constrained analogs containing the optimal *N*-substituent, *N*-phenethyl. The ultimate goal of this research is to develop ring-constrained phenylpropyloxyethylamine analogs that will enhance future modeling studies and aid in the design of improved opioid analgesics.

2.4 EXPERIMENTAL

All reagents and solvents were purchased from Sigma Aldrich, Inc. unless stated otherwise and used without further purification. All reactions were carried out under an atmosphere of nitrogen. Thin layer chromatography was performed on silica 60 F_{254} plated (Analtech, Inc., Newark, DE). All compounds were purified using standard techniques (crystallization, etc) and characterized using standard spectroscopic methods such as ^{1}H NMR (Varian Inova 500 MHz) and MS (ThermoFinnigan LCQ Classic, Waltham, MA). Melting points were determined using Mel-Temp (Laboratory Devises, city, state) apparatus. Elemental analysis was performed by Atlantic Microlabs (Norcross, GA).

***N,N*-Dimethyl-2-(3-phenylpropoxy)ethanamine (3, UMB205)**

Method 1: A solution of 3-phenyl1-propanol, **1** (5.99 mL, 44.6 mmol) in dry DMF was added to a stirring solution of 2.39 g (104 mmol) of NaH at room temperature. After 30 min, 1.60 g (14.9 mmol) of 2-(dimethylamino)ethylchloride hydrochloride, **2** was added in small portions over a 30 min period. The resulting mixture was allowed to stir for another 3 hours at 50°C and 30 min at room temperature. After completion by TLC, the reaction mixture was quenched with ethanol and the solvent was removed under reduced pressure. The crude product was dissolved in H_2O and extracted with Et_2O. The product was then extracted into 6M HCl from Et_2O. The solution was made basic (pH 12-13) with 5M NaOH (aq) and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, 5% $CHCl_3$/MeOH/1% NH_4OH) followed by formation of the oxalate salt from ether. Yield 40% (1.23 g); mp 120-121°C

Method 2: To obtain target **3,** alcohol **1** (1.25 ml, 9.30 mmol) was reacted with **2** (1 g, 9.30 mmol) in the presence of KOH (2.5 eq., 1.30 g) in DMF (20 mL/g). The reaction mixture was allowed to stir overnight at room temperature. After completion by TLC, the crude reaction mixture was dissolved in H_2O and extracted with Et_2O. The product was then extracted into 6M HCl from Et_2O. The solution was made basic (pH 12-13) with 5M NaOH (aq) and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, 5%

$CHCl_3$/MeOH/1% NH_4OH) followed by formation of the oxalate salt from ether. Yield 57%, (1.10 g); mp 120-121°C

^1H NMR (D_2O) δ 7.36-7.42 (m, 2H), δ 7.27-7.35 (m, 3H), δ 3.77-3.81 (m, 2H), δ 3.56-3.61 (m, 2H), δ 3.33-3.38 (m, 2H), δ 2.90-2.93 (m, 6H), δ 2.69-2.75 (m, 2H), δ 1.91-1.98 (m, 2H); MS ESI m/z = 208 ($M+ H^+$); Anal. ($C_{13}H_{21}NO$ $(C_2H_2O_4)_1$) C, H, N.

2-(Cinnamyloxy)-*N*,*N*-dimethylethanamine (5, UMB207) was prepared through alkylation of cinnamyl alcohol, **4** (3.74 g 27.9 mmol) with 2-(dimethylamino)ethylchloride hydrochloride, **2** (1 g, 9.30 mmol) following both method 1 and 2 described above. Yield 17% (0.32 g); ^1H NMR (D_2O) δ 7.54 (d, 3.50 Hz, 2H), δ 7.42-7.49 (m, 3H), δ 6.75-6.80 (m, 1H), δ 6.39-6.46 (m, 1H), δ 4.29 (d, 3.33 Hz, 2H), δ 3.88 (t, 5.25 Hz, 2H), δ 3.41 (t, 4.90 Hz, 2H), δ 2.91-2.96 (m, 6H); MS ESI m/z = 206 ($M+ H^+$); Anal. ($C_{13}H_{19}NO$ $(C_2H_2O_4)_1$) C, H, N.

1-(2-(3-Phenylpropoxy)ethyl)pyrrolidine (7, UMB206) was prepared through alkylation of 3-phenyl1-propanol, **1** (2.01 mL, 15.0 mmol) with 1-(2-chloroethyl)pyrrolidine hydrochloride (1 g, 7.48 mmol) following both method 1 and 2 described above. Yield 22% (0.38 g); ^1H NMR (D_2O) δ 7.41 (t, 7.67 Hz, 2H), δ 7.27-7.35 (m, 3H), δ 3.76-3.80 (m, 2H), δ 3.64-3.70 (m, 2H), δ 3.58 (t, 6.61 Hz, 2H), δ 3.39 (t, 4.84 Hz, 2H), δ 3.09-3.18 (m, 2H), δ 2.71 (t, 7.44 Hz, 2H), δ 2.11-2.21 (m, 2H), δ 1.91-2.07 (m, 4H); MS ESI m/z = 234 ($M+ H^+$); Anal. ($C_{15}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

2-(Cinnamyloxy)-*N,N*-diethylethanamine (13, UMB365) was prepared through alkylation of 2-(diethylamino)ethanol, **10** (1.14 mL, 8.53 mmol) with cinnamyl bromide, **8** (1.85 g, 9.39 mmol) in presence of KOH (1.19 g, 21.3 mmol) following method 2 described previously. Yield 22% (0.44 g); ^{1}H NMR (D_2O) δ 7.54 (d, 3.94 Hz, 2H), δ 7.44 (t, 7.42 Hz, 2H), δ 7.35-7.41 (m, 1H), δ 6.74-6.80 (m, 1H), δ 6.38-6.46 (m, 1H), δ 4.27 (d, 3.48 Hz, 2H), δ 3.87 (t, 4.87 Hz, 2H), δ 3.40 (t, 4,87 Hz, 2H), δ 3.22-3.36 (m, 4H), δ 1.31 (t, 7.19 Hz, 6H); MS ESI m/z = 234 (M+ H^+); Anal. ($C_{15}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

***N,N*-Diethyl-2-(3-phenylpropoxy)ethanamine (14, UMB364)** was prepared through alkylation of 2-(diethylamino)ethanol, **10** (1.14 mL, 8.53 mmol) with 1-bromo-3-phenylpropane, **9** (1.43 mL, 9.39 mmol) in presence of KOH (1.19 g, 21.3 mmol) following method 2 described previously. Yield 36% (0.72 g); ^{1}H NMR (D_2O) δ 7.39 (t, 7.37 Hz, 2H), δ 7.26-7.35 (m, 3H), δ 3.76-3.81 (m, 2H), δ 3.58 (t, 6.47Hz, 2H), δ 3.19-3.37 (m, 6H), δ 2.71 (t, 7.37 Hz, 2H), δ 1.90-1.98 (m, 2H), δ 1.30 (t, 7.19 Hz, 6H); MS ESI m/z = 236 (M+ H^+); Anal. ($C_{15}H_{25}NO$ $(C_2H_2O_4)_1$) C, H, N.

2-(Dipropylamino)ethanol (11) A mixture of dipropylamine (1.35 mL, 9.88 mmol), 2-chloroethanol (0.86 mL, 9.88 mmol) and K_2CO_3 (13.7 g, 99 mmol) in DMF (20 mL/g) was vigorously stirred at room temperature under N_2. After completion by TLC, H_2O was added and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, 1-3% $CHCl_3$/MeOH/1% NH_4OH). Yield 78% (1.12 g); MS ESI m/z = 146 (M + H^+).

2-(Cinnamyloxy)-*N,N*-diethylethanamine (15, UMB398) was prepared through alkylation of 2-(dipropylamino)ethanol, **11** (0.80 g, 5.51 mmol) with cinnamyl bromide, **8** (1.09 g, 5.51 mmol) in the presence of KOH (0.46 g, 8.26 mmol), following method 2 described previously. Yield 33% (0.48 g); mp °C; ^{1}H NMR (D_2O) δ 7.49-7.54 (m, 2H), δ 7.43 (t, 7.57 Hz, 2H), δ 7.31 (m, 1H), δ 6.71-6.77 (m, 1H), δ 6.35-6.44 (m, 1H), δ 4.25 (d, 3.53 Hz, 2H), δ 3.87 (t, 4.44 Hz, 2H), δ 3.41 (t, 4.70 Hz, 2H), δ 3.08-3.21 (m, 4H), δ 1.66-1.77 (m, 4H), δ 0.94 (t, 7.31 Hz, 6H); MS ESI m/z = 262 (M+ H^+); Anal. ($C_{17}H_{27}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.75}$) C, H, N.

***N,N*-Diethyl-2-(3-phenylpropoxy)ethanamine (16, UMB397)** was prepared through alkylation of 2-(dipropylamino)ethanol, **11** (0.8 g, 5.51 mmol) with 1-bromo-3-phenylpropane, **9** (0.84 mL, 5.51 mmol) in the presence of KOH (0.46 g, 8.26 mmol), following method 2 described previously. Yield 40% (0.58 g); mp °C; ^{1}H NMR (D_2O) δ 7.37 (t, 6.98 Hz, 2H), δ 7.25-7.33 (m, 3H), δ 3.75-3.81 (m, 2H), δ 3.53-3.59 (m, 2H), δ 3.33-3.38 (m, 2H), δ 3.07-3.20 (m, 4H), δ 2.70 (t, 7.46 Hz, 2H), δ 1.88-1.97 (m, 2H), δ 1.66-1.77 (m, 4H), δ 0.96 (t, 6.98 Hz, 6H); MS ESI m/z = 264 (M+ H^+); Anal. ($C_{17}H_{29}NO$ $(C_2H_2O_4)_1$) C, H, N.

2-(Cinnamyloxy)-*N,N*-dibutylethanamine (17, UMB366) was prepared through alkylation of 2-(dibutylamino)ethanol, **12** (1.16 g, 5.77 mmol) with cinnamyl bromide, **8** (1.25 g, 6.35 mmol) in the presence of KOH (0.81 g, 14.4 mmol), following method 2 described previously. Yield 24% (0.40 g); ^{1}H NMR (D_2O) δ 7.53 (d, 3.65 Hz, 2H), δ 7.43

(t, 7.57 Hz, 2H), δ 7.33-7.38 (m, 1H), δ 6.72-6.78 (m, 1H), δ 6.37-6.44 (m, 1H), δ 4.24 (d, 3.13 Hz, 2H), δ 3.84-3.88 (m, 2H), δ 3.41 (t, 4.83 Hz, 2H), δ 3.12-3.25 (m, 4H), δ 1.64-1.73 (m, 4H), δ 0.92 (t, 7.31 Hz, 6H); MS ESI m/z = 290 (M+ H^+); Anal. ($C_{19}H_{31}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$) C, H, N.

***N,N*-Dibutyl-2-(3-phenylpropoxy)ethanamine (18, UMB384)** was prepared through alkylation of 2-(dibutylamino)ethanol, **12** (1.16 mL, 5.77 mmol) with 1-bromo-3-phenylpropane, **9** (0.96 mL, 6.35 mmol) in the presence of KOH (0.81 g, 14.4 mmol), following method 2 described previously Yield 37% (0.62 g); mp 94-96°C; ^{1}H NMR (D_2O) δ7.39 (t, 7.50 Hz, 2H), δ 7.26-7.35 (m, 3H), δ 3.76-3.82 (m, 2H), δ 3.58 (t, 6.52 Hz, 2H), δ 3.34-3.40 (m, 2H), δ 3.12-3.26 (m, 4H), δ 2.72 (t, 7.50 Hz, 2H), δ 1.90-1.97 (m, 2H), δ 1.63-1.74 (m, 4H), δ 1.33-1.43 (m, 4H), δ 0.90-0.98 (m, 6H); MS ESI m/z = 292 (M+ H^+); Anal. ($C_{19}H_{33}NO$ $(C_2H_2O_4)_1$) C, H, N.

2-(Cinnamyloxy)-*N,N*-diethylacetamide (20, UMB383) was prepared through alkylation of *N,N*-diethyl-2-hydroxyacetamide, **19** (1.00 mL, 7.62 mmol) with cinnamyl bromide, **8** (1.65 g, 8.39 mmol) in the presence of KOH (1.07 g, 19.1 mmol), following method 2 described previously. Yield 22% (0.42 g); mp °C; ^{1}H NMR (D_2O) δ 7.39 (d, 3.74 Hz, 2H), δ 7.32 (t, 7.65 Hz, 2H), δ 7.22-7.28, (m, 1H), δ 6.60-6.66 (m, 1H), δ 6.27-6.35 (m, 1H), δ 4.24-4.28 (m, 2H), δ 4.19 (s, 2H), δ 3.39 (q, 7.12 Hz, 2H), δ 3.31 (q, 7.12 Hz, 2H), δ 1.11-1.22 (m, 6H); MS ESI m/z = 248 (M+ H^+); Anal. ($C_{15}H_{21}NO$) C, H, N.

***N,N*-Diethyl-2-(3-phenylpropoxy)acetamide (21, UMB382)** was prepared through alkylation of *N,N*-diethyl-2-hydroxyacetamide, **19** (1.00 mL, 7.62 mmol) with 1-bromo-3-phenylpropane, **9** (1.27 mL, 8.39 mmol) in the presence of KOH (1.07 g, 19.1 mmol), following method 2 described previously. Yield 29% (0.55 g); ^{1}H NMR (D_2O) δ 7.91-7.98 (m, 2H), δ 7.81-7.89 (m, 3H), δ 4.20 (t, 6.49 Hz, 2H), δ 3.96-4.08 (m, 4H), δ 3.38 (t, 7.79 Hz, 2H), δ 2.52-2.66 (m, 2H), δ 2.29 (s, 2H), δ 1.76-1.89 (m, 6H); MS ESI m/z = 250 (M+ H^+); Anal. ($C_{15}H_{23}NO$) C, H, N.

1-(2-(Cinnamyloxy)ethyl)pyrrolidine (24, UMB361) was prepared through alkylation of cinnamyl alcohol, **4** (3.01 g, 22.5 mmol) with 1-(2-chloroethyl)pyrrolidine hydrochloride, **6** (1.00 g, 7.48 mmol) in the presence of KOH (1.05 g, 18.7 mmol), following method 2 described previously. Yield 21%; ^{1}H NMR (D_2O) δ 7.55 (d, 10.79 Hz, 2H), δ 7.46 (t, 7.73 Hz, 2H), δ 7.39 (t, 7.33 Hz, 1H), δ 6.76-6.82 (m, 1H), δ 6.40-6.48 (m, 1H), δ 4.29 (d, 3.05 Hz, 2H), δ 3.88 (t, 16.90 Hz, 2H), δ 3.67-3.76 (m, 2H), δ 3.44-3.49 (m, 2H), δ 3.11-3.21 (m, 2H), δ 2.11-2.23 (m, 2H), δ 1.98-2.09 (m, 2H); MS ESI m/z = 232 (M+ H^+); Anal. ($C_{13}H_{21}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$) C, H, N.

1-(2-(3-Phenylpropoxy)ethyl)piperidine (25, UMB362) was prepared through alkylation of 3-phenyl1-propanol, **1** (0.92 mL, 6.77 mmol) with 1-(2-chloroethyl)piperidine hydrochloride, **22** (1 g, 6.77 mmol) in the presence of KOH (0.95 g, 16.9 mmol), following method 2 described previously. Yield 14 % (0.24 g); ^{1}H NMR (D_2O) δ 7.41 (t, 7.43 Hz, 2H), δ 7.29-7.36 (m, 3H), δ 3.82 (t, 5.04 Hz, 2H), δ 3.59 (t, 6.44 Hz, 2H), δ 3.56 (d, 3.56 Hz, 2H), δ 3.32 (t, 5.04 Hz, 2H), δ 3.00 (t, 12.87 Hz, 2H), δ 2.76

(t, 7.57 Hz, 2H), δ 1.92-2.00 (m, 4H), δ 1.71-1.88 (m, 3H), δ 1.46-1.55 (m, 1H); MS ESI m/z = 248 (M+ H^+); Anal. ($C_{16}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

1-(2-(Cinnamyloxy)ethyl)piperidine (26, UMB363) was prepared through alkylation of cinnamyl alcohol, **4 (**1.82 g, 13.6 mmol) with 1-(2-chloroethyl)piperidine hydrochloride, **22** (1.00 g, 6.77 mmol) in the presence of KOH (0.95 g, 16.9 mmol), following method 2 described previously. Yield 34% (0.57 g); ^{1}H NMR (D_2O) δ 7.53 (d, 3.86 Hz, 1H), δ 7.43 (t, 7.61 Hz, 2H), δ 7.37 (t, 7.17 Hz, 1H), δ 6.73-6.79 (m, 1H), δ 6.38-6.45 (m, 1H), δ 4.26 (d, 3.20 Hz, 2H), δ 3.88 (t, 4.97 Hz, 2H), δ 3.57 (d, 6.40 Hz, 2H), δ 3.35 (t, 4.85 Hz, 2H), δ 2.99 (t, 12.35 Hz, 2H), δ 1.94 (d, 7.50 Hz, 2H), δ 1.69-1.85 (m, 3H), δ 1.43-1.54 (m, 1H); MS ESI m/z = 246 (M+ H^+); Anal. ($C_{16}H_{25}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$) C, H, N.

1-(2-(3-Phenylpropoxy)ethyl)azepane (27, UMB381). was prepared through alkylation of 2-(1-azepanyl)ethanol, **23** (1 g, 6.98 mmol) with 1-bromo-3-phenylpropane, **9** (1.17 mL, 7.68 mmol) in the presence of KOH (0.98 g, 17.5 mmol), following method 2 described previously. Yield 37% (0.68 g); mp °C; ^{1}H NMR (D_2O) δ 7.36-7.43 (m, 2H), δ 7.26-7.36 (m, 3H), δ 3.77-3.83 (m, 2H), δ 3.58 (t, 6.10Hz, 2H), δ 3.45-3.53 (m, 2H), δ 3.33-3.39 (m, 2H), δ 3.19-3.27 (m, 2H), δ 2.72 (t, 7.09 Hz, 2H), δ 1.64-2.00 (m, 10H); MS ESI m/z = 262 (M+ H^+); Anal. ($C_{17}H_{27}NO$ $(C_2H_2O_4)_1$) C, H, N.

1-(2-(Cinnamyloxy)ethyl)azepane (28, UMB389) was prepared through alkylation of 2-(1-azepanyl)ethanol, **23** (1.00 g, 6.98 mmol) with cinnamyl bromide, **8** (1.51 g, 7.68 mmol) in the presence of KOH (0.98 g, 17.45 mmol), following method 2 described

previously. Yield 22% (0.39 g); ^{1}H NMR (D_2O) δ 7.49-7.54 (m, 2H), δ 7.43 (t, 7.40 Hz, 2H), δ 7.37 (t, 7.40 Hz, 1H), δ 6.71-6.78 (m, 1H), δ 6.36-6.44 (m, 1H), δ 4.22-4.26 (m, 2H), δ 3.87 (t, 4.81 Hz, 2H), δ 3.46-3.53 (m, 2H), δ 3.39 (t, 4.81 Hz, 2H), δ 3.18-3.27 (m, 2H), δ 1.77-1.95 (m, 4H), δ 1.61-1.74 (m, 4H); MS ESI m/z = 260 (M+ H^+); Anal. ($C_{17}H_{25}NO$ $(C_2H_2O_4)_1$) C, H, N.

***N*-Benzyl-2-(cinnamyloxy)-*N*-methylethanamine (33, UMB367)** was prepared through alkylation of *N*-benzyl-*N*-methylethanolamine, **29** (0.99 mL, 6.05 mmol) with cinnamyl bromide, **8** (1.31 g, 6.66 mmol) in the presence of KOH (0.85 g, 15.1 mmol), following method 2 described previously. Yield 20% (0.34 g); mp 94-96°C; ^{1}H NMR (D_2O) δ 7.47-7.55 (m, 7H), δ 7.43 (t, 7.48 Hz, 2H), δ 7.32-7.38 (m, 1H), δ 6.67-6.73 (m, 1H), δ 6.32-6.40 (m, 1H), δ 4.26-4.50 (m, 2H), δ 4.15-4.24 (m, 2H), δ 3.80-3.92 (m, 2H), δ 3.26-3.54 (m, 2H), δ 2.88 (s, 3H); MS ESI m/z = 282 (M+ H^+); Anal. ($C_{19}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

***N*-Benzyl-*N*-methyl-2-(3-phenylpropoxy)ethanamine (34, UMB385)** was prepared through alkylation of *N*-benzyl-*N*-methylethanolamine, **29** (0.99 mL, 6.05 mmol) with 1-bromo-3-phenylpropane, **9** (1.01 mL, 6.05 mmol) in the presence of KOH (0.85 g, 15.13 mmol), following method 2 described previously. Yield 32% (0.55 g); ^{1}H NMR (D_2O) δ 7.22-7.58 (m, 10H), δ 4.37-4.47 (m, 1H), δ 4.23-4.35 (m, 1H), δ 3.69-3.87 (m, 2H), δ 3.38-3.59 (m, 3H), δ 3.21-3.33 (m, 1H), δ 2.80-2.90 (m, 3H), δ 2.66 (t, 7.09 Hz, 2H), δ 1.84-1.93 (m, 2H)); MS ESI m/z = 284 (M+ H^+); Anal. ($C_{19}H_{25}NO$ $(C_2H_2O_4)_1$) C, H, N.

2-(Methyl(phenethyl)amino)ethanol (**30)** A mixture of (2-bromoethyl)benzene (5.48 mL, 39.9 mmol), 2-chloroethanol (1.08 mL, 13.3 mmol), and K_2CO_3 (18.4 g, 133 mmol) in DMF (20 mL/g) was vigorously stirred at room temperature under N_2. After completion by TLC, H_2O was added and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, 1-3% $CHCl_3$/MeOH/1% NH_4OH). Yield 64% (1.53g); MS ESI m/z = 180 ($M + H^+$).

2-(Cinnamyloxy)-*N*-methyl-*N*-phenethylethanamine (35, UMB391) was prepared through alkylation of 2-(methyl(phenethyl)amino)ethanol, **30** (0.75g, 4.18mmol) with cinnamyl bromide, **8** (2.47 g, 12.6 mmol) in the presence of KOH (0.59 g, 10.5 mmol), following method 2 described above. Yield 19%; ^{1}H NMR (D_2O) δ 7.16-7.4o (m, 10H), δ 6.57-6.63 (m, 1H), δ 6.26-6.33 (m, 1H), δ 4.18 (d, 2.97 Hz, 2H), δ 3.62-3.69 (m, 2H), δ 2.82-2.88 (m, 2H), δ 2.73-2.80 (m, 4H), δ 2.44 (s, 3H); MS ESI m/z = 296 ($M+ H^+$); Anal. ($C_{20}H_{25}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.5}$) C, H, N.

***N*-Methyl-*N*-phenethyl-2-(3-phenylpropoxy)ethanamine (36, UMB390)** was prepared through alkylation of 2-(methyl(phenethyl)amino)ethanol, **30** (0.75 g, 4.18 mmol) with 1-bromo-3-phenylpropane, **9** (1.91 mL, 12.6 mmol) in the presence of KOH (0.59 g, 10.46 mmol), following method 2 described previously. Yield 34% (0.42 g); ^{1}H NMR (D_2O) δ 7.31-7.43 (m, 6H), δ 7.23-7.30 (m, 4H), δ 3.78 (t, 4.47 Hz, 2H), δ 3.44-3.60 (m, 4H), δ

3.28-3.43 (m, 2H), δ 3.02-3.16 (m, 2H), δ 2.94 (s, 3H), δ 2.66 (t, 7.45 Hz, 2H), δ 1.84-1.93 (m, 2H); MS ESI m/z = 298 (M+ H^+); Anal. ($C_{20}H_{27}NO$ ($C_2H_2O_4$)$_1$) C, H, N.

2-(Methyl(3-phenylpropyl)amino)ethanol (31) A mixture of 1-bromo-3-phenylpropane, **9** (6.07 mL, 39.9 mmol), 2-chloroethanol (1.08 mL, 13.3 mmol), and K_2CO_3 (18.4 g, 133 mmol) in DMF (20 mL/g) was vigorously stirred at room temperature under N_2. After completion by TLC, H_2O was added and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, 1-3% $CHCl_3$/MeOH/1% NH_4OH). Yield 71% (1.83 g); MS ESI m/z = 194 (M + H^+).

2-(Cinnamyloxy)-*N*-methyl-*N*-phenylpropylethanamine (37, UMB413) was prepared through alkylation of 2-(methyl(3-phenylpropyl)amino)ethanol, **31** (1.0 g, 5.17 mmol) with cinnamyl bromide, **8** (3.06 g, 15.5 mmol) in the presence of KOH (7.26 g, 12.9 mmol), following method 2 described previously. Yield 19% (0.30 g); mp °C; ^{1}H NMR (D_2O) δ 7.15-7.40 (m, 10H), δ 6.57-6.63 (m, 1H), δ 6.25-6.34 (m, 1H), δ 4.14-4.18 (m, 2H), δ 3.57 (t, 6.23 Hz, 2H), δ 2.60-2.66 (m, 4H), δ 2.42-2.48 (m, 2H), δ 2.30 (s, 3H), δ 1.79-1.87 (m, 2H); MS ESI m/z = 310 (M+ H^+); Anal. ($C_{21}H_{27}NO$ ($C_2H_2O_4$)$_1$) C, H, N.

***N*-Methyl-*N*-phenylpropyl-2-(3-phenylpropoxy)ethanamine (38, UMB403)** was prepared through alkylation of 2-(methyl(3-phenylpropyl)amino)ethanol, **31** (1 g, 5.17 mmol) with 1-bromo-3-phenylpropane, **9** (2.36 mL, 15.5 mmol) in the presence of KOH

(0.73 g, 12.9 mmol), following method 2 described previously. Yield 27% (0.44 g); ^{1}H NMR (D_2O) δ 7.26-7.32 (m, 4H), δ 7.15-7.25 (m, 6H), δ 3.66 (t, 4.88 Hz, 2H), δ 3.42 (t, 6.21, 2H), δ 3.28 (s, 2H), δ 3.09 (s, 2H), δ 2.79 (s, 3H), δ 2.59-2.65 (m, 4H), δ 1.96 (q, 7.84 Hz, 2H), δ 1.76-1.84 (m, 2H); MS ESI m/z = 312 (M+ H^+); Anal. ($C_{21}H_{29}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$) C, H, N.

2-(Diallylamino)ethanol (32) A mixture of diallylamine (1.27 mL, 10.3 mmol), 2-chloroethanol (0.89 mL, 10.3 mmol), and K_2CO_3 (14.2 g, 103 mmol) in DMF (20 mL/g) was vigorously stirred at room temperature under N_2. After completion by TLC, H_2O was added and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, 1-3% $CHCl_3$/MeOH/1% NH_4OH). Yield 49% (0.71 g); MS ESI m/z = 142 (M + H^+).

2-(Cinnamyloxy)-*N,N*-diallylethanamine (39, UMB402) was prepared through alkylation of 2-(diallylamino)ethanol, **32** (2.00 g, 14.2 mmol) with cinnamyl bromide, **8** (2.79 g, 14.2 mmol) in the presence of KOH (1.19 g, 21.2 mmol), following method 2 described above. Yield 22% (0.84 g); mp °C; ^{1}H NMR (D_2O) δ 7.52 (d, 3.54 Hz, 2H), δ 7.42 (t, 7.58 Hz, 2H), δ 7.32-7.37 (m, 1H), δ 6.72-6.78 (m, 1H), δ 6.35-6.46 (m, 1H), δ 5.86-6.00 (m, 2H), δ 5.56-5.65 (m, 4H), δ 4.22-4.29 (m, 2H), δ 3.80-3.91 (m, 6H), δ 3.39-3.44 (m, 2H); MS ESI m/z = 258 (M+ H^+); Anal. ($C_{17}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

***N,N*-Diallyl-2-(3-phenylpropoxy)ethanamine (40, UMB399)** was prepared through alkylation of 2-(diallylamino)ethanol, **32** (0.71 g, 5.03 mmol) with 1-bromo-3-phenylpropane, **9** (0.76 mL, 5.03 mmol) in the presence of KOH (0.42 g, 7.54 mmol), following method 2 described previously. Yield 22% (0.29 g); ^{1}H NMR (D_2O) δ 7.38 (t, 7.36 Hz, 2H), δ 7.23-7.33 (m, 3H), δ 5.86-5.97 (m, 2H), δ 5.56-5.65 (m, 4H), δ 3.75-3.85 (m, 6H), δ 3.56 (t, 6.56 Hz, 2H), δ 3.32-3.37 (m, 2H), δ 2.70 (t, 7.52 Hz, 2H), δ 1.87-1.96 (m, 2H); MS ESI m/z = 260 (M+ H^+); Anal. ($C_{17}H_{25}NO$ $(C_2H_2O_4)_1$) C, H, N.

2-(3-(4-Hydroxyphenyl)propoxy)-*N,N*-dimethylethanamine (UMB388) was prepared through alkylation of cinnamyl alcohol, **4** with 1-(2-Chloroethyl)pyrrolidine hydrochloride, following method 2 described above. ^{1}H NMR (Cl_3CH) δ 7.25-7.30 (m, 2H), δ 7.15-7.22 (m, 2H), δ 3.50 (t, 5.91 Hz, 2H), δ 3.45 (t, 6.40 Hz, 2H), δ 2.66-2.71 (m, 2H), δ 2.52 (t, 5.84 Hz, 2H), δ 2.27 (s, 6H), δ 1.88-1.95 (m, 2H), δ 1.77 (s, 1H); MS ESI m/z = 238 (M+ H^+); Anal. ($C_{14}H_{23}NO_2$ $(C_2H_2O_4)_1$) C, H, N.

Chapter 3. Opioids lacking a tyrosine mimetic. Part 2: Ring-Constrained Phenylpropyloxyethylamines

3.1 INTRODUCTION

Opioid receptors are G-protein coupled receptors that contain seven transmembrane domains and are primarily located in the brain and the spinal cord as well as the gastrointestinal tract (Ossipov et al., 2004). There are three cloned opioid receptor types known as μ (Wang et al., 1994), κ (Mansson et al., 1994), and δ (Evans et al., 1992; Kieffer et al., 1992). Each type of opioid receptor produces unique pharmacological effects upon stimulation. For example, κ agonists have been shown to exhibit dysphoria, by interacting though central nervous system (CNS) mechanisms, thus tremendously limiting the use of κ agonists in a clinical setting (Hasebe et al., 2004). δ Agonists are not effective against severe pain and are known to produce convulsions (Comer et al., 1993; Broom et al., 2002). Most commonly used opioid analgesics such as morphine, fentanyl, and oxycodone act at μ receptors (Zieglgansberger et al., 1995; Stein et al., 2003). Though there are indisputable benefits to opioid treatment in a clinical setting, their use is often limited due to a number of adverse actions including development of tolerance, dependence (Kieffer and Evans, 2002), constipation, nausea, and respiratory depression (McNicol et al., 2003; Benyamin et al., 2008).

One of the most problematic side effects associated with the μ opioids is constipation (Hipkin et al.), which becomes more severe as the dosage increases due to analgesic tolerance (Kieffer and Evans, 2002). Alvimopan (Lavine, 2008) and methylnaltrexone (Yuan et al., 2005) are μ opioid receptor antagonists have been recently approved by the Food and Drug Administration as the peripherally acting agents. These agents do not

cross the BBB, thus avoiding the antagonist effect in the CNS while reversing the unwanted side effects in the GIT (Yuan et al., 2005; Lavine, 2008).

The structure of morphine is comprised of 5 rings: aromatic A, cyclohexyls B and C, piperidine D, and epoxy E. Modifications to the morphinan class included removal of rings B-E in an effort to eliminate undesirable effects, however, all continue to produce these side effects (Ling and Wesson, 1990). A common structural feature among phenylpiperidines, benzomorphans, and morphinans is the aromatic A-ring (Casy and Parfitt, 1986). The phenolic A-ring of morphine is thought to mimic the tyrosine residue of enkephalin and it is therefore suggested to a requirement for opioid receptor binding. Point mutation studies support this, as the histidine located in TM VI (His VI:17) is predicted to hydrogen-bonds to the C_3 oxygen substituent on the A-ring (Kane et al., 2006). Moreover, studies show that the C_3 hydroxyl substituent is generally associated with high affinity and potency (Aldrich, 1993). Furthermore, the 14-alkoxymorphinan series shows that potency can be magnified by C_{14} alkyl substituents (Furst et al., 1993a; Schutz et al., 2003). For example, the 14-phenylpropyloxymorphinan, a derivative which belongs to the 14-alkoxymorpinan family, is an agonist which elicits extreme potency (24,000-fold higher in the tail flick assay and 8,500-fold higher in the hot plate assay as compared to morphine) (Schutz et al., 2003). Moreover, 14-alkoxymorphinans are capable of maintaining high affinity for μ even when there is no C_3 oxygen function (Spetea et al., 2004). Perhaps the most interesting finding about a member of the 14-alkoxy morphinan series is that 14-methoxy metopon elicits minimal physical dependence and tolerance and has been shown to have reduced constipation (King et al.,

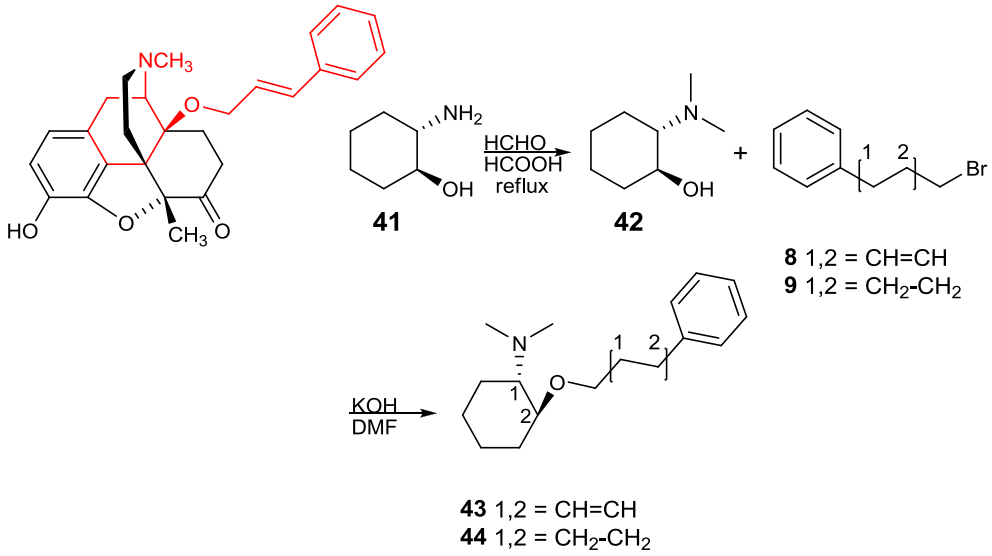
2003) and respiratory depression (Furst et al., 1993a) as compared to morphine. These results indicate that it is indeed possible to develop opioids that display functional selectivity and have reduced side effects (Paakkari et al., 1992; Paakkari et al., 1993; King et al., 2003).

On the basis of these findings, we theorize that both a basic amine and an alkoxy group such as phenylpropyloxy group alone are required for opioid activity, and the aromatic A-ring, that was historically considered essential, is not required (Casy and Parfitt, 1986). By removing the A-ring, this allows the skeleton to adopt an alternate binding mode with the receptor interacting with different residues, thereby potentially causing alternate receptor trafficking events (Ignatova et al., 1999) and post-receptor mechanisms, all of which are involved in the development of tolerance (Kieffer and Evans, 2002). In our previous studies (Chapter 2), we showed that phenylpropyloxyethylamines are capable of binding to μ opioid receptors and we identified the *N*-phenethyl analog, 2-(cinnamyloxy)-*N*-methyl-*N*-phenethylethanamine as the optimal *N*-substituent analog with an affinity of 1680 nM. In an effort to investigate the optimal configuration between the basic amine and the phenylpropyloxy group, constraining rings B, C, and D of 4,5-epoxymorphinans were incorporated. Investigations were initially focused on the synthesis of ring constrained phenylpropylethylamines containing the *N*–methyl substituent which were then further optimized by incorporating the optimal *N*-substituent, phenethyl, which confers increased affinity and potency.

3.2 CHEMISTRY

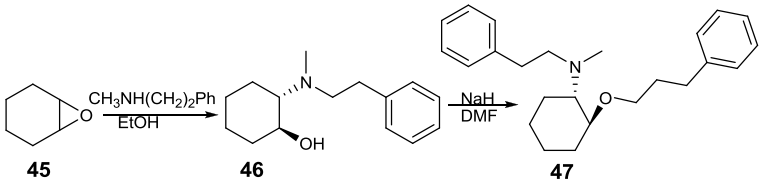
3.2.1 B-ring *Cis* and *Trans* Analogs

From Scheme 3.1, it is evident that *cis* configuration occurs at positions 9 and 14 of 14-phenylpropyloxymetopon. The *trans* conformer **43** was initially prepared, to confer the configuration and determine appropriate experimental procedures. Eschweiler–Clarke methylation reaction (Overman and Sugai, 1985) was utilized in the first step to obtain the dimethyl substituted amine **42** outlined below in Scheme 3.1. Compound **41** formed an imine with formaldehyde (HCHO), followed by reduction to a secondary amine using formic acid. In the presence of excess formic acid (HCOOH), this reaction repeats until a tertiary amine is produced. The last step of this synthesis was achieved according to a known method (Rist et al., 2001) to give compounds **43** and **44**. The resulting compounds were obtained as a crude product in 82-86% yield. The final products, **43** and **44,** were made into oxalate salts in 10 % and 7% yields, respectively.



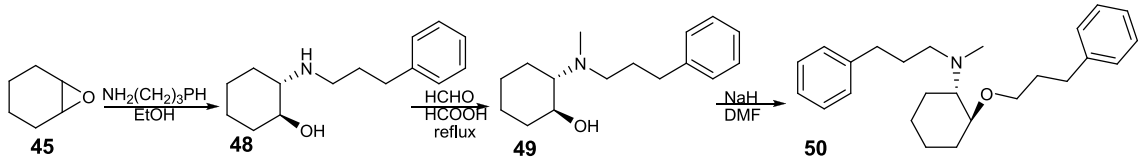
Scheme 3.1 Synthesis of *trans*-*N*,*N*-dimethyl-2-(sz-phenylpropoxy) cyclohexanamine and *trans*-2-(cinnamyloxy)-*N*,*N*-dimethylcyclohexanamine

The *N*-phenethyl analog **47** was synthesized as shown in Scheme 3.2. Compound **46** was synthesized in 70% yield by addition of *N*-methyl-phenethylamine to epoxide, **45** under the S_N2 conditions (Rogers et al., 1989). Alkylation with 1-bromo-3-phenylpropane in the presence of NaH gave the desired product **47**. The resulting compound was converted into an oxalate salt in 6% yield.



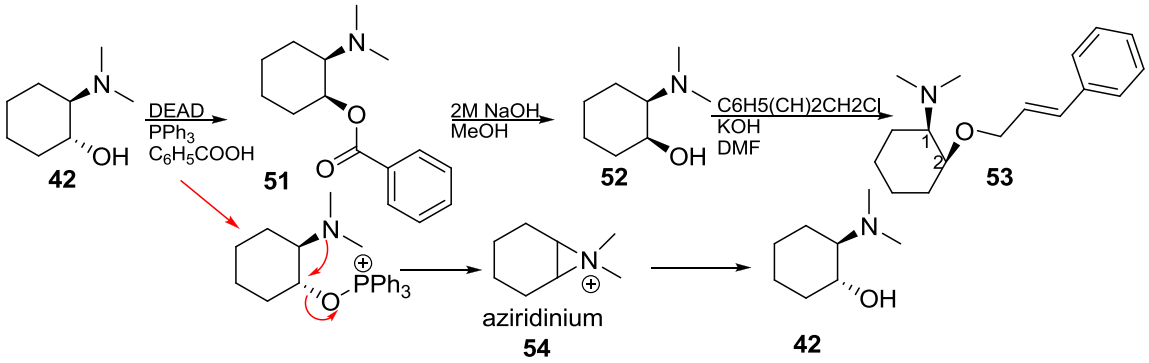
Scheme 3.2 Synthesis of *trans*-*N*-methyl-*N*-phenethyl-2-(3-phenylpropoxy) cyclohexanamine

As shown in Scheme 3.3, the *N*-phenylpropyl substituent was introduced from an epoxide ring-opening reaction(Rogers et al., 1989) with 3-phenylpropylamine under reflux conditions. The resulting compound **48**, was *N*-methylated using Eschweiler–Clarke methylation(Overman and Sugai, 1985) described previously to give compound **49** in 52% yield. The final step was achieved via a previously described alkylation method(Rist et al., 2001) resulting in target **50**. This compound was afforded in 33% yield as an oxalate salt.



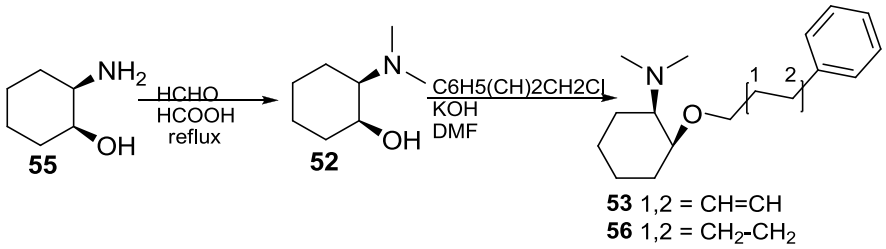
Scheme 3.3 Synthesis of *trans*-*N*-methyl-2-(3-phenylpropoxy)-*N*-(3-phenylpropyl) cyclohexanamine

To synthesize the *cis* conformer (Scheme 3.4), esterification and inversion of the hydroxyl group was performed via a Mitsunobu reaction (Hughes, 1992), to give cis-2-(dimethylamino)cyclohexyl benzoate (**51**). The benzoic ester was cleaved by NaOH to give compound **52**. The final step of this synthesis was achieved by a previously developed methodology (Rist et al., 2001) to obtain the final product **53**. The final product was afforded in 2% yield, which is too low for further characterization. Scale-up attempts resulted in lower yields. In addition, there is a possibility that the reaction may proceed with the retention of configuration via the proposed mechanism displayed in Scheme 3.4. (Poelert et al., 1994) If the aziridinium intermediate (Poelert et al., 1994) is generated, it can undergo *trans* addition of either a hydroxyl or a benzoic acid resulting in the *trans* product rather than the *cis*.



Scheme 3.4 Synthesis of cis-2-(cinnamyloxy)-*N*,*N*-dimethylcyclohexanamine

To improve the yield and to ensure that the *cis* analog is formed, Eschweiler–Clarke methylation reaction (Overman and Sugai, 1985) was utilized to obtain the dimethyl substituted amine **52** (Scheme 3.5). Compounds **53** and **56** were obtained utilizing the previously conceived method (Rist et al., 2001). The final products **53** and **56** were made into oxalate salts in 14% yield and 16% respectively.

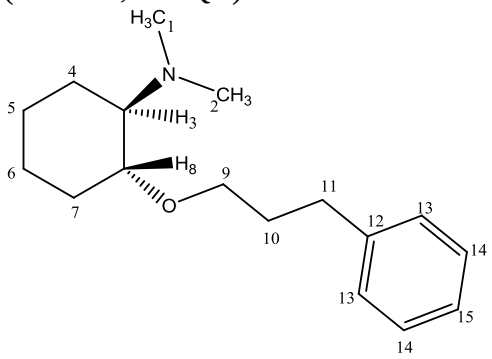


Scheme 3.5 Synthesis of cis-2-(cinnamyloxy)-*N*,*N*-dimethylcyclohexanamine and cis-*N*,*N*-dimethyl-2-(3-phenylpropoxy)cyclohexanamine

3.2.1.1 Structural assignments of 44 and 56

Formation of the *cis* and *trans* conformers was fully anticipated as the starting materials **41** and **55** were purchased in the appropriate conformations. Proton and carbon assignments of analogs **44** and **56** were determined using ^{13}C, ^{1}H, HMBC, and HMQC, which are shown in Table 5 and 6. Proton-proton correlations were determined using NOESY. While these experiments were useful tools for assignment of protons and carbons, the identification of the overall stereochemistry was not obtained.

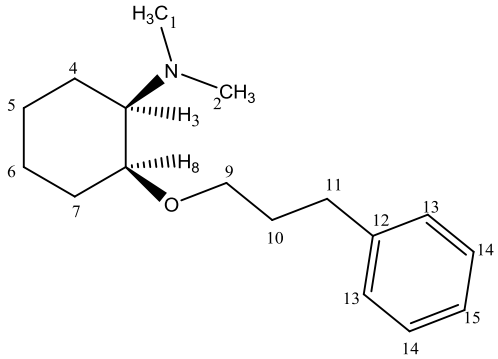
Table 3.1 Proton and carbon assignments for **44** as determined by 2D NMR experiments (NOESY, HMQC)



^{13}C Shift (δ, ppm)[a]	Carbon ID	^{1}H Correlation (δ, ppm)[b]	Proton ID	H/H Correlation (δ, ppm)[c]
44.32	C-1	2.83	H-1	2.07, 2.47, 1.47
39.32	C-2	2.80	H-2	
71.55	C-3	3.17	H-3	1.33, 1.25, 1.20
24.76	C-4	2.09	H-4α	2.82, 1.47, 1.87, 1.32
		1.51	H-4β	1.47, 2.07, 2.81
26.42	C-5	1.89	H-5α	2.07
		1.33	H-5β	3.15, 2.07, 2.31, 3.62
25.68	C-6	1.80	H-6α	2.32, 3.65
		1.24	H-6β	3.15, 2.31, 3.55
32.47	C-7	2.31	H-7α	1.32, 1.78, 1.25, 1.20, 3.72, 3.51
		1.22	H-7β	3.15, 3.55, 3.72
78.60	C-8	3.56	H-8	2.82, 1.47, 1.32, 1.77, 1.25, 2.32, 1.20
70.75	C-9	3.73	H-9α	2.32, 1.21,7.33
		3.52	H-9β	2.32, 2.71, 7.33
33.53	C-10	1.97	H-10	2.82
34.33	C-11	2.73	H-11	3.72, 3.51
145.0	C-12			
131.4	C-13	7.31	H-13	1.95, 2.70, 3.50, 3.72, 7.38, 7.28
131.5	C-14	7.38	H-14	1.95, 2.70, 3.50, 7.31, 7.28
129.0	C-15	7.28	H-15	7.31, 7.38

[a] ^{13}C NMR; [b] HMQC; [c] NOESY

Table 3.2 Proton and Carbon assignments for **56** as determined by 2D NMR experiments (NOESY, HMQC)

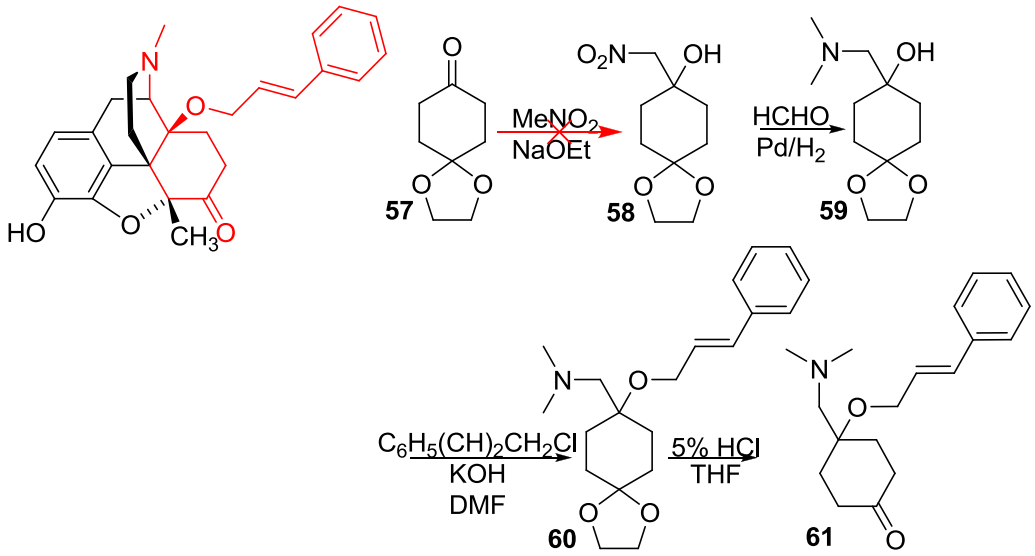


^{13}C Shift (δ, ppm)[a]	Carbon ID	^{1}H Correlation (δ, ppm)[b]	Proton ID	H/H Correlation (δ, ppm)[c]
43.77	C-1, C-2	2.88	H-1,H-2	3.17, 2.03, 1.67, 4.03, 3.38
69.99	C-3	3.15	H-3	1.33, 1.37, 1.29, 4.03
25.52	C-4	2.02	H-4α	2.88
		1.65	H-4β	2.88, 4.03
26.48	C-5	1.86	H-5α	2.04
		1.33	H-5β	3.16, 2.04
20.58	C-6	1.38	H-6	3.16, 4.03, 3.68
29.14	C-7	2.20	H-7α	1.87, 1.33, 4.03, 3.67, 3.39
		1.28	H-7β	4.02, 3.16, 4.03
74.06	C-8	4.03	H-8	1.67, 1.37, 2.20, 1.30, 1.96, 3.67, 3.39, 4.03, 2.88, 3.16
70.06	C-9	3.66	H-9α	1.42, 1.38, 2.20, 4.03,7.33
		3.37	H-9β	2.20, 2.88, 4.03, 1.96, 7.33
33.66	C-10	1.96	H-10	4.03, 7.33
34.57	C-11	2.75	H-11	7.33
145.4	C-12			
131.36	C-13	7.32	H-13	3.38, 3.67, 1.96, 1.96, 2.75, 7.41, 7.70
131.49	C-14	7.41	H-14	7.30, 732
128.86	C-15	7.30	H-15	7.32, 7.32

[a] ^{13}C NMR; [b] HMQC; [c] NOESY

3.2.2 C-ring Analogs

The synthesis of target **61** was proposed as illustrated in Scheme 3.6. Preparation of compound **58** was anticipated by a reaction of nitromethane with **57** in the presence of NaOEt according to literature method (Nightingale et al., 1952). Reduction and dimethylation of nitrogendioxide in order to obtain compound **53** was planned according to literature methods (Greenfield, 1994) using formaldehyde in the presence of H_2, Pd/C. Compound **54** was going to then be obtained using the previously devised alkylation method (Rist et al., 2001). Subsequently, acid hydrolysis was going to give the desired product **55** (Grieco et al., 1977).

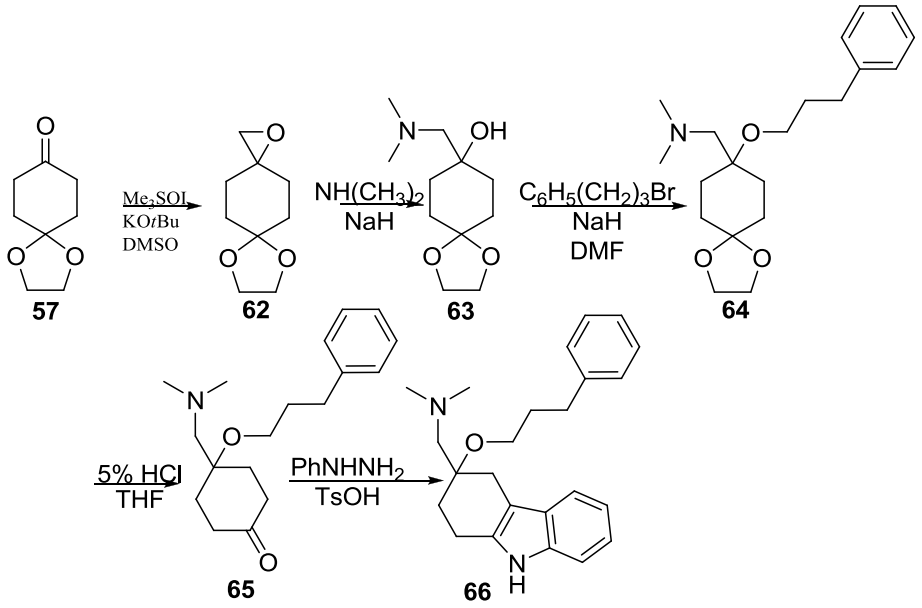


Scheme 3.6 *Method 1:* Synthesis of 4-(cinnamyloxy)-4-((dimethylamino)methyl) cyclohexanone

The conditions attempted in the first step of Scheme 3.6 did not result in the formation of the product, **58**. Previous reports showed that 3- and 4-methyl-1-cyclohexanone does not react with nitromethane in presence of NaOEt, however product was formed when piperidine was used as the catalyst (Nightingale et al., 1950). Thus, we investigated the

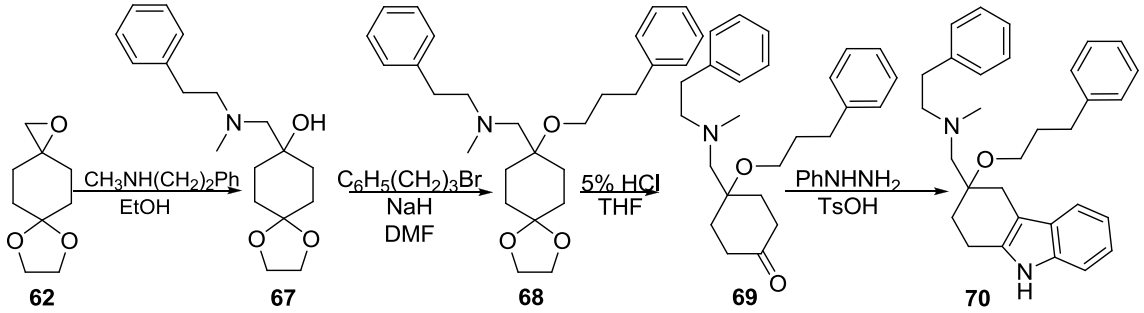
effect of piperidine as the catalyst as well as the base. In addition, we attempted to prepare compound **58** with NaH as our base; however, the desired product was not produced (Nightingale et al., 1950). It was hypothesized that steric interference could be occurring during the nitromethane addition stage due to the ketal moiety in the starting material. Therefore, 1,4-cyclohexanedione was utilized instead of 1,4-cyclohexanedione monoethylene acetal in the subsequent synthesis but was unsuccessful. Additionally, the proposed synthesis was attempted with cyclohexanone as the starting material, using a literature method (Nightingale et al., 1950; Nightingale et al., 1952), in order to validate that steric hinderance hypothesis. This reaction proceeded as anticipated, giving 1-(nitromethyl)cyclohexanol in 78% yield. The product was confirmed by LCQ MS. These results suggest that the proposed methodology suffers from steric hinderance.

Since the proposed method proved to be difficult, an alternative method for preparation of the C-ring analogs was devised (Scheme 3.7). Following a reported procedure for "Instant Methylide," a modified version of Corey-Cheykovsky epoxide synthesis (Ciaccio et al., 2003), compound **62** was successfully synthesized. Ring-opening of epoxide was achieved with dimethylamine salt in presence of NaH to produce **63** (Szakonyi et al., 2008). Compound **64** was obtained in 64% yield using 1-bromo-3-phenylpropane in the presence of NaH since the previously devised method of alkylation did not produce the desired product (Rist et al., 2001). Subsequently, acid hydrolysis was utilized to give **65**, 78% yield (Grieco et al., 1977). The final step was achieved following standard Fisher-Indole synthesis conditions (Kubota et al., 1998). The final product was converted into an oxalate salt in 43% yield.



Scheme 3.7 *Method 2:* Synthesis of 4-(cinnamyloxy)-4-((dimethylamino)methyl) cyclohexanone

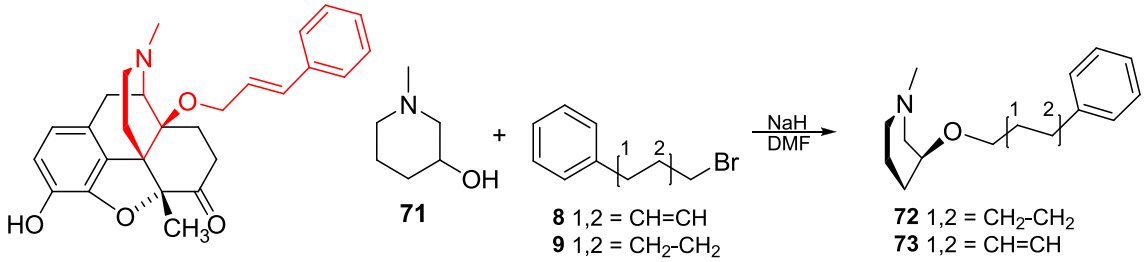
Synthesis of the *N*-phenethyl series (Scheme 3.8) was achieved following the previously proposed method. In order to obtain the *N*-phenethyl derivative **67**, the epoxide ring-opening was achieved with *N*-methyl-phenethylamine in the presence of NaH (Szakonyi et al., 2008). Following the conditions utilized in the synthesis of the *N*-methyl series, compounds **68**-**70** were produced in moderate to good yields (30%, 74%, and 21% respectively) (Grieco et al., 1977; Kubota et al., 1998; Rist et al., 2001).



Scheme 3.8 Synthesis of 4-(cinnamyloxy)-4-((dimethylamino)methyl)cyclohexanone

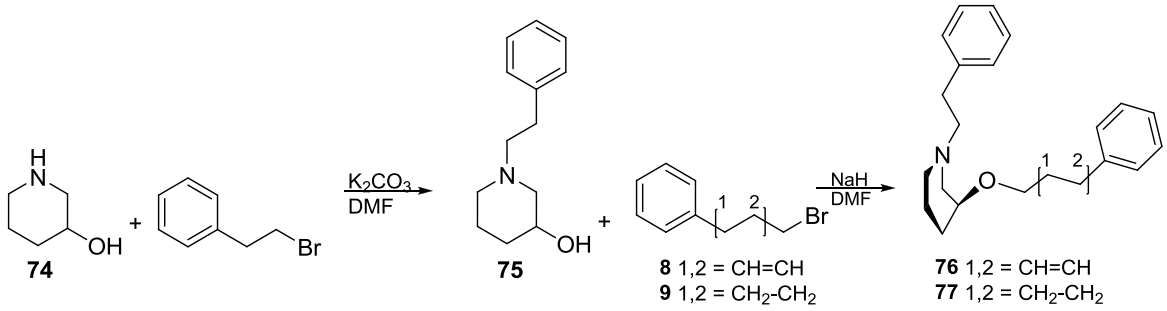
3.2.3 D-ring Analogs

The D-ring analogs **72** and **73** (Scheme 3.9) were prepared in moderate yields (40% and 31% respectively) as racemic mixtures through methods previously conceived, in the presence of NaH (Rist et al., 2001). Resolution using chiral preparative HPLC or crystallization as chiral salts to characterize the activity of each isomer of compounds **72** and **73** was not performed, as they were showing low μ affinity (K_i > 1000 nM) (Leusen, 2003).



Scheme 3.9 Synthesis of 1-methyl-3-(3-phenylpropoxy)piperidine and 3-(cinnamyloxy)-1-methylpiperidine

The *N*-phenethyl derivative **75** was synthesized by an alkylation reaction in the presence of K_2CO_3 (Scheme 3.10) (Maeda et al., 2002a). The final step of this synthesis was achieved by a previously developed methodology (Rist et al., 2001) to obtain the final products **76** and **77** in 22% and 46% yields respectably as an oxalate salt.



Scheme 3.10 Synthesis of 1-phenethyl-3-(3-phenylpropoxy)piperidine and 3-(cinnamyloxy)-1-phenethylpiperidine

3.3 RESULTS AND DISCUSSION

3.3.1 Molecular Modeling Studies: Conformer Prediction

The CSP method (Rais et al.; Bernard et al., 2003; Bernard et al., 2005; Bernard et al., 2007) was performed by Jihyun Shim, a member of Dr. MacKerell's laboratory, on the *cis* and *trans* compounds to determine the favored conformation in the new series (Figure 3.1). Compounds **44** (*trans* conformer), **56** (*cis* conformer), and 14-phenylpropyloxymetopon were modeled using the program CHARMM (Brooks et al., 2009) with the CHARMM CGenFF (Vanommeslaeghe et al.) and they were energy-minimized using a combination of minimization algorithms known as steepest descents and ABNR with a RMS gradient of 10^{-6} kcal/mol Å. After energy-minimization, TREX-MD(Sugita and Okamoto, 1999) was used to obtain conformations as described in section 2.3.3. Among conformations sampled the *cis* and *trans* molecules possessing the highest overlap with the 14-phenylpropyloxymetopon were displayed in Visual Molecular Dynamics (VMD) software (Humphrey et al., 1996). Molecular modeling studies

revealed that the *cis* conformation exhibits a better fit with the parent compound and therefore may be overall favored by the receptor.

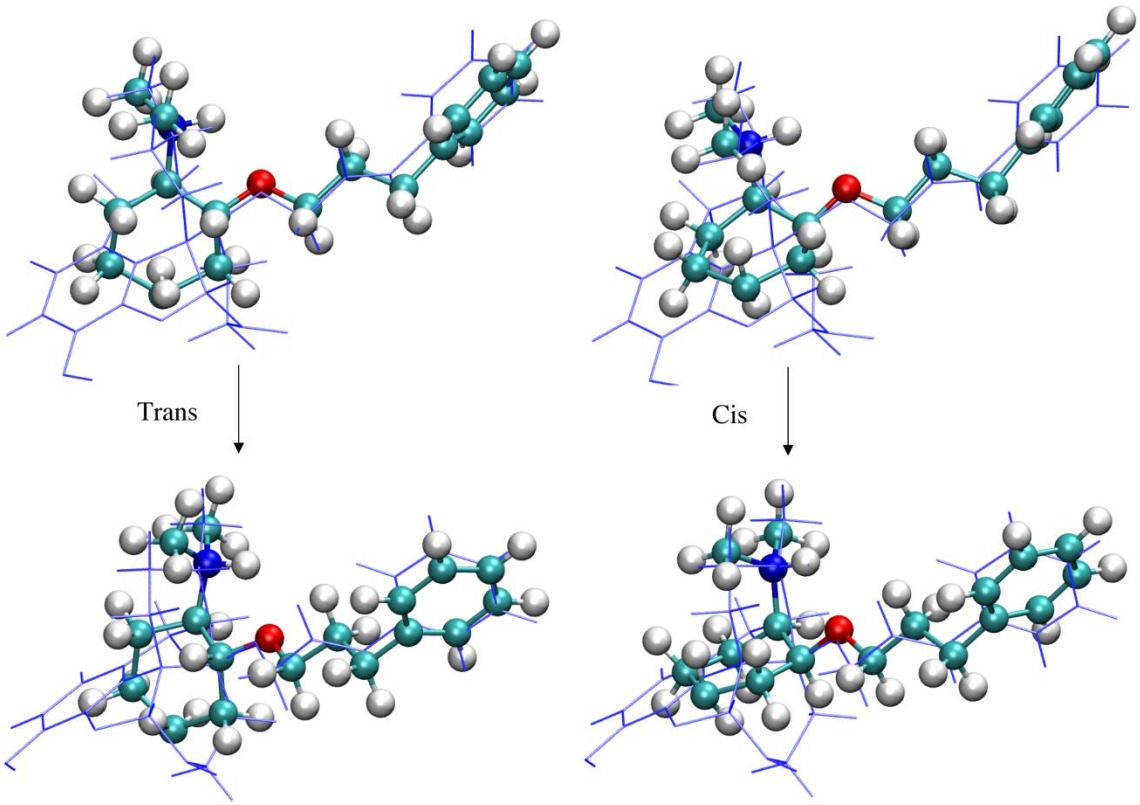


Figure 3.1 Superimposed images of the *cis* and *trans* conformer on 14-phenylpropyloxymetopon

3.3.2 Opioid Receptor Binding

Opioid binding affinities for all the newly synthesized compounds were performed against all three opioid receptors (μ, δ, and κ) by Jason Healy, a graduate student in the laboratory of Dr. Rae Matsumoto, WVU via a displacement assay using a previously described method (Spetea et al., 2001a). Constrained rings B, C, and D were re-introduced back into the system to determine the bioactive conformation. The binding

data for the B- ring analogs are expressed as inhibition constants (K_is) in Table 3.3. Surprisingly, the *trans* conformer appeared to exhibit better binding affinity than the *cis* conformer though it is evident that the *cis* conformation is occurring in the parent compound, 14-phenylpropyloxymetopon. The *trans* conformers (**43** and **44**) showed weak affinity (4200 nM and 2300 nM, respectively) for μ opioid receptor, and negligible (>10,000 nM) affinity for the κ receptor. Introduction of a phenylpropyl group at N_{17} resulted in a slightly lower binding affinity at μ receptors (3100 nM). The interaction with the μ opioid receptor improved when the optimal *N*-substituent (phenethyl) was introduced into the *trans* conformer **47** resulting in 1600 nM affinity for the μ opioid receptor. Perhaps the most interesting finding was that the compounds lacking a phenylpropyl group (**46** and **49**) were not capable of binding to the μ opioid receptors. This finding validates our hypothesis that the phenylpropyloxy group is essential for binding activity.

Table 3.3 Opioid Receptor Binding Affinities for B-ring Analogs

	K_i± SEM (nM)		
Compound	[^{3}H] DAMGO (μ)	[^{3}H] DPDPE (δ)	[^{3}H] U69,593 (κ)
43	4200 ± 135	>10,000	ND*
44	2340 ± 74	>10,000	ND*
53	>10,000	ND*	ND*
56	7080 ± 1790	ND*	ND*
46	>10,000	ND*	ND*
49	>10,000	ND*	ND*
47	1640 ± 40.7	>10,000	>10,000
50	3090 ± 61.9	6210 ± 774	ND*

ND* = not determined

The binding data for the C- ring analogs are expressed as inhibition constants (K_is) in Table 3.4. Among the C-ring analogs, compound **66** displayed the highest binding affinity (1100 nM) for μ opioid receptors. Surprisingly and in contrast to the published data on indole containing opioids, introduction of the indole moiety (**66** and **70**) produced weak (6400 nM) to negligible (>10,000 nM) affinities, respectively, for δ (Portoghese et al., 1988). As expected, the phenethyl substituent at N_{17} increased the binding affinity at the μ receptor (Casy and Parfitt, 1986) for compounds **68** and **69**. However, a reduction in the affinity for compound **70** was observed upon introduction of the N-phenethyl substituent. These results suggest that the current series does not follow the SAR of the morphinan class.

Table 3.4 Opioid Receptor Binding Affinities for C-ring Analogs

	K_i± SEM (nM)		
Compound	[^{3}H] DAMGO (μ)	[^{3}H] DPDPE (δ)	[^{3}H] U69,593 (κ)
64	>10,000	ND*	ND*
65	>10,000	ND*	ND*
66	1110 ± 90.9	6390 ± 206	ND*
68	5840 ± 533	ND*	ND*
69	3480 ± 57.5	ND*	ND*
70	2190 ± 73.9	>10,000	ND*

ND* = not determined

The binding data for the D- ring analogs are expressed as inhibition constants (K_is) in Table 3.5. Re-introduction of the D- ring as seen in the case of **72** and **73** was detrimental to the opioid receptor affinity, displaying negligible (>10,000 nM) affinity for the μ

opioid receptor. Binding studies of the synthesized compounds **76** and **77** are currently underway.

Table 3.5 Opioid Receptor Binding Affinities for D-ring Analogs

	K_i± SEM (nM)		
Compound	[^{3}H] DAMGO (μ)	[^{3}H] DPDPE (δ)	[^{3}H] U69,593 (κ)
72	>10,000	ND*	ND*
73	>10,000	ND*	ND*
76	ND*	ND*	ND*
77	ND*	ND*	ND*

ND* = not determined

3.4 CONCLUSION

To determine the bioactive conformation, and aid in future modeling studies, constrained rings B, C, and D were re-introduced back into the system iteratively. In agreement with our hypothesis, compounds lacking a phenylpropyl group (**46** and **49**) were not capable of binding to the opioid receptors indicating that the phenylpropyloxy group is essential for binding activity. Binding studies showed that the B-ring analog containing the *N*,*N*-dimethyl substituent, **44** produced the highest affinity of 2340 nM, while the C- and D-ring analogs were fully inactive. Further optimization was achieved by combining the B-ring with the optimal *N*-substituent, phenethyl, to give **47** which had 1640 nM affinity at μ. The interaction with the μ opioid receptor greatly improved when the C-ring analog was modified to contain an indole group, **66**. The resulting compound had an affinity of 1110 nM for the μ opioid receptor. Unexpectedly, and in contrast to the published data on

indole containing opioids, introduction of the indole moiety **66** produced weak affinity (6400 nM) for δ.(Portoghese et al., 1988) These results indicate that **66** is a viable lead compound for optimization studies at the μ opioid receptor.

3.5 EXPERIMENTAL

All reagents and solvents were purchased from Sigma Aldrich Inc. unless stated otherwise and used without further purification. All reactions were carried out under an atmosphere of nitrogen. Thin layer chromatography was performed on silica 60 F_{254} plated (Analtech, Inc. Newark, DE). All compounds were purified using standard techniques (crystallization, etc.) and characterized using standard spectroscopic methods such as ^{1}H NMR (Varian Inova 500 MHz) and MS (ThermoFinnigan LCQ Classic, Waltham, MA). Melting points were determined using Mel-Temp (Laboratory Devises, city, state) apparatus. Elemental analysis was performed by Atlantic Microlabs (Norcross, GA).

***trans*-2-(Dimethylamino)cyclohexanol** (**42**) A solution of *trans*-2-aminocyclohexanol, **41** (1 g, 8.68 mmol) 37% formaldehyde (HCHO) (14 mL, 471 mmol) and formic acid (HCOOH) (14 mL, 365 mmol) was refluxed over night. The resulting crude mixture was concentrated and dissolved with ether and washed with 5N NaOH. The organic extracts were combined, dried with K_2CO_3, and evaporated. Yield 83% (1.03 g);

***trans*-2-(Cinnamyloxy)-N,N-dimethylcyclohexanamine** (**43**, **UMB400**) To a solution of DMF and NaH (1.21 g, 50.3 mmol) was added trans-2-(dimethylamino)cyclohexanol, **42**

(1.03 g, 7.19 mmol) dropwise and allowed to stir at room temperature for 30 min prior to adding cinnamyl bromide (4.25 g, 21.6 mmol). The reaction was heated for 3 hours at 50°C. After the reaction reached completion by TLC, it was quenched with 20ml of ethanol and the solvent was reduced under pressure. The crude product was dissolved in H_2O and extracted with Et_2O. The reaction mixture was then extracted into 6M HCl and made basic (pH 12-13) with 5M NaOH (aq) and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, $CHCl_3$/ 5%MeOH/ 1% NH_4OH) followed by formation of the oxalate salt from ether. Yield 10% (0.18 g); ^{1}H NMR (D_2O) δ 7.52 (d, 3.56 Hz, 2H), δ 7.42 (t, 7.33 Hz, 2H), δ 7.33-7.38 (m, 1H), δ 6.73-6.79 (m, 1H), δ 6.36-6.44 (m, 1H), δ 4.36-4.41 (m, 1H), δ 4.20-4.26 (m, 1H), δ 3.65-3.72 (m, 1H), δ 3.15-3.22 (m, 1H), δ 2.80 (s, 6H), δ 2.38-2.44 (m, 1H), δ 2.07 (d, 5.86 Hz, 1H) δ 1.87 (d, 6.07 Hz, 1H), δ 1.76-1.81 (m, 1H), δ 1.41-1.51 (m, 1H), δ 1.19-1.38 (m, 4H); MS ESI m/z = 260 (M+ H^+); Anal. ($C_{19}H_{31}NO$ $(C_2H_2O_4)_1$) C, H, N.

***trans*-*N*,*N*-Dimethyl-2-(3-phenylpropoxy)cyclohexanamine** (**44**, **UMB401**) was prepared through alkylation of trans-2-(dimethylamino)cyclohexanol, **42** (0.72 g, 5.03 mmol) with 1-bromo-3-phenylpropane, (2.29 mL, 15.1 mmol) in the presence of NaH (0.84 g, 35.2 mmol), following the method described above (see compound 43). Yield 7% (0.092 g); mp °C; ^{1}H NMR (D_2O) δ 7.41 (t, 7.67 Hz, 2H), δ 7.26-7.33 (m, 3H), δ 3.69-3.76 (m, 1H), δ 3.47-3.59 (m, 2H), δ 3.11-3.19 (m, 1H), δ 2.68-2.94 (m, 8H), δ 2.28-

2.35 (m, 1H), δ 2.04-2.11 (m, 1H), δ 1.91-1.99 (m, 2H), δ 1.89 (d, 6.79 Hz, 1H), δ 1.75-1.81 (m, 1H); MS ESI m/z = 262 (M+ H^+); Anal. ($C_{19}H_{29}NO$ $(C_2H_2O_4)_2$) C, H, N.

***trans*-2-(Methyl(phenethyl)amino)cyclohexanol (46, UMB408)** *N*-methylphenethylamine (2.70 mL, 18.8 mmol) and cyclohexene oxide, **45** (3.70 mL, 36.6 mmol) were dissolved in 50mL of ethanol and refluxed overnight. The ethanol was evaporated under reduced pressure and purified by column chromatography (silica gel, $CHCl_3$/ 5%MeOH/ 1% NH_4OH). Yield 70% (3.04 g); ^{1}H NMR (D_2O) δ 7.44 (t, 7.23 Hz, 2H), δ 7.35-7.40 (m, 3H), δ 3.74-3.86 (m, 1H), δ 3.51-3.64 (m, 1H), δ 3.38-3.46 (m, 1H), δ 3.01-3.31 (m, 3H), δ 2.98 (s, 1H), δ 2.83 (s, 2H), δ 2.01-2.15 (m, 2H), δ 1.82-1.90 (m, 1H), δ 1.71-1.79 (m, 1H), δ 1.21-1.55 (m, 4H); MS ESI m/z = 234 (M+ H^+); Anal. ($C_{15}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

***N*-Methyl-*N*-phenethyl-2-(3-phenylpropoxy)cyclohexanamine (47, UMB404)** was prepared through alkylation of trans-2-(methyl(phenethyl)amino)cyclohexanol, **46** (1.5 g, 6.43 mmol) with 1-bromo-3-phenylpropane, (2.93 mL, 19.3 mmol) in the presence of NaH (1.08 g, 45.0 mmol), following the method described previously (see compound 43). Yield 6% (0.14 g); ^{1}H NMR (D_2O) δ 7.33-7.47 (m, 6H), δ 7.26-7.33 (m, 4H), δ 3.59-3.69 (m, 3H), δ 3.39-3.48 (m, 1H), δ 3.25-3.32 (m, 1H), δ 3.16-3.24 (m, 1H), δ 3.09-3.15 (m, 1H), δ 2.94-3.00 (m, 2H), δ 2.80-2.84 (m, 1H), δ 2.59-2.68 (m, 2H), δ 2.27-2.33 (m, 1H), δ 2.01-2.14 (m, 1H), δ 1.73-1.90 (m, 4H), δ 1.44-1.56 (m, 1H), δ 1.11-1.39 (m, 4H) ; MS ESI m/z = 352 (M+ H^+); Anal. ($C_{24}H_{33}NO$ $(C_2H_2O_4)_1$) C, H, N.

***trans*-2-(3-Phenylpropylamino)cyclohexanol (48)** Phenylpropylamine (1.58 mL, 11.1 mmol) and cyclohexene oxide, **45** (2.36 mL, 23.3 mmol) were dissolved in 50mL of ethanol and refluxed overnight. The ethanol was evaporated under reduced pressure and purified by column chromatography (silica gel, 94% $CHCl_3$/ 5%MeOH/ 1% NH_4OH). Yield 89% (2.30 g); MS ESI m/z = 234 (M+ H^+)

***trans*-2-(Methyl(3-phenylpropyl)amino)cyclohexanol (49, 414)** A solution of *trans*-2-(3-phenylpropylamino)cyclohexanol, **48** (2.27 g, 9.73 mmol) 37% formaldehyde (HCHO) (21 mL, 706 mmol) and formic acid (HCOOH) (21 mL, 548 mmol) was refluxed over night. The resulting crude mixture was concentrated and dissolved with ether and washed with 5N NaOH. The organic extracts were combined, dried with K_2CO_3, and evaporated. Yield 52.4% (1.26 g); ^{1}H NMR (D_2O) δ 7.41 (t, 7.41 Hz, 2H), δ 7.30-7.30 (m, 3H), δ 3.71-3,79 (m, 1H), δ 3.05-3.34 (m, 3H), δ 2.91-3.00 (m, 1H), δ 2.88 (s, 1H), δ 2.70-2.83 (m, 4H), δ 2.00-2.20 (m, 3H), δ 1.89-1.96 (m, 1H), δ 1.79-1.88 (m, 1H), δ 1.70-1.78 (m, 1H), δ 1.21-1.48 (m, 4H); MS ESI m/z = 248 (M+ H^+); Anal. ($C_{16}H_{25}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$) C, H, N.

***trans*-*N*-Methyl-2-(3-phenylpropoxy)-*N*-(3-phenylpropyl)cyclohexanamine (50, UMB415)** was prepared through alkylation of *trans*-2-(methyl(3-phenylpropyl)amino)cyclohexanol, **49** (0.6 g, 2.43 mmol) with 1-bromo-3-phenylpropane, (1.84 mL, 12.1 mmol) in the presence of NaH (0.41 g, 17.0 mmol), following the method described previously (see compound 43). Yield 33% (0.29 g); ^{1}H NMR (D_2O) δ 7.23-7.32 (m, 5H), δ 7.14-7.24 (m, 5H), δ 3.54-3.62 (m, 1H), δ 3.40-3.47

(m, 1H), δ 3.18-3.26 (m, 1H), δ 2.54-2.75 (m, 5H), δ 2.42-2.51 (m, 1H), δ 2.28-2.38 (m, 3H), δ 2.06-2.14 (m, 1H), δ 1.84-1.93 (m, 2H), δ 1.72-1.83 (m, 3H), δ 1.63-1.72 (m, 2H), δ 1.53-1.60 (m, 1H), δ 1.04-1.30 (m, 4H); MS ESI m/z = 366 (M+ H^+); Anal. ($C_{25}H_{35}NO$ $(C_2H_2O_4)_1$) C, H, N.

***cis*-2-(Cinnamyloxy)-*N,N*-dimethylcyclohexanamine (53, UMB420)** was prepared through alkylation of *cis*-2-(dimethylamino)cyclohexanol, **55** (0.77 g, 5.40 mmol) with cinnamyl bromide, (0.91 mL, 5.94 mmol) in the presence of NaH (0.91 g, 37.8 mmol), following the method described previously (see compound 43). The final product was converted into a HBr salt. Yield 14% (0.26 g); ^{1}H NMR (D_2O) δ 7.53 (d, 3.83 Hz, 2H), δ 7.44 (t, 7.46 Hz, 2H), δ 7.33-7.39 (m, 1H), δ 6.74-6.80 (m, 1H), δ 6.40-6.48 (m, 1H), δ 4.32-4.41 (m, 1H), δ 4.11-4.21 (m, 2H), δ 3.16-3.23 (m, 1H), δ 2.88 (s, 6H), δ 2.26-2.33 (m, 1H), δ 2.02-2.10 (m, 1H), δ 1.84-1.93 (m, 1H), δ 1.61-1.72 (m, 1H), δ 1.28-1.54 (m, 4H); MS ESI m/z = 260 (M+ H^+); Anal. $C_{17}H_{25}NO$ $(HBr)_1$ $(H_2O)_{0.75}$ C, H, N.

***cis*-2-(Dimethylamino)cyclohexanol** (**55**) A solution of *cis*-2-aminocyclohexanol, **52** (1.00 g, 8.68 mmol) 37% formaldehyde (HCHO) (14.0 mL, 471 mmol) and formic acid (HCOOH) (14.0 mL, 365 mmol) was refluxed over night. The resulting crude mixture was concentrated and dissolved with ether and washed with 5N NaOH. The organic extracts were combined, dried with K_2 CO_3, and evaporated. Yield 61.9% (0.77 g); MS ESI m/z = 144 (M+ H^+);

***cis*-*N*,*N*-Dimethyl-2-(3-phenylpropoxy)cyclohexanamine (56, UMB419)** was prepared through alkylation of *cis*-2-(dimethylamino)cyclohexanol, **55** (0.61 g, 4.26 mmol) with 1-bromo-3-phenylpropane (1.94 mL, 12.8 mmol) in the presence of NaH (0.72 g, 29.8 mmol), following the method described previously (see compound 43). The final product was converted into a HBr salt. Yield 16%; ^{1}H NMR (D_2O) δ 7.35 (t, 7.33 Hz, 2H), δ 7.24-7.33 (m, 3H), δ 4.01 (s, 1H), δ 3.62-3.69 (m, 1H), δ 3.33-3.40 (m, 1H), δ 3.11-3.18 (m, 1H), δ 2.83-2.90 (m, 6H), δ 2.74 (t, 7.60 Hz, 2H), δ 2.20 (d, 7.60 Hz, 1H), δ 1.82-2.05 (m, 4H), δ 1.59-1.70 (m, 1H), δ 1.21-1.46 (m, 4H); MS ESI m/z = 262 (M+ H^+); Anal. $C_{17}H_{27}NO\ (HBr)_1\ (H_2O)_{0.25}$ C, H, N.

1,7,10-Trioxadispiro[2.2.4.2]dodecane (62). The sulfoxonium salt and base mixture was prepared by combining trimethyl sulfoxonium iodide, Me3SOI (5.90 g, 26.8 mmol) and potassium t-butoxide, KOt-Bu (3.00 g, 26.7 mmol). A solution of anhyd. DMSO, 1,4-cyclohexanedione monoethylene acetal, **57** (4.20 g, 26.9 mmol), and the sulfoxonium salt and base mixture (17.9 g, 53.8 mmol) were added all at once and stirred in an oil bath heated to 55°C for 45 min. The reaction mixture was treated with H_2O and extracted with Et_2O. The organic layer was washed with brine solution, dried over Na_2SO_4, and evaporated under reduced pressure. Yield 70% (3.19 g); ^{1}H NMR ($CDCl_3$) δ 4.00 (s, 4H), δ 2.71 (s, 2H), δ 1.88-1.98 (m, 4H), δ 1.75-1.86 (m, 2H), δ 1.54-1.65 (m, 2H); MS ESI m/z = 171 (M+ H^+).

8-((Dimethylamino)methyl)-1,4-dioxaspiro[4.5]decan-8-ol (63). To a stirring solution of DMF, NaH (1.21 g, 50.6 mmol), and 1,7,10-trioxadispiro[2.2.4.2]dodecane, **62** (1.23

g, 7.23 mmol), *N*,*N*-dimethylamine (2.95 g, 36.1 mmol) was added slowly. The reaction mixture was heated to 50-60°C overnight. The reaction was treated with cold H_2O and extracted with Et_2O. The organic extract was then washed with brine, dried over $MgSO_4$, and filtered. Solvent was evaporated under reduced pressure to afford the crude product. Purification was performed via column chromatography (silica gel, 96%$CHCl_3$/ 3%MeOH/ 1% NH_4OH). Yield 64% (1.01 g) ^{1}H NMR ($CDCl_3$) δ 3.90-4.00 (m, 4H), δ 3.18 (s, 1H), δ 2.36 (s, 6H), δ 2.30 (s, 2H), δ 1.92-2.00 (m, 2H), δ 1.49-1.70 (m, 6H); MS ESI m/z = 216 (M+ H^+).

***N*,*N*-Dimethyl-1-(8-(3-phenylpropoxy)-1,4-dioxaspiro[4.5]decan-8-yl)methanamine (64, UMB410)** was prepared through alkylation of 8-(dimethylamino)methyl)-1,4-dioxaspiro[4.5]decan-8-ol, **63** (1.01 g, 4.64 mmol) with 1-bromo-3-phenylpropane, (3.53 mL, 23.2 mmol) in the presence of NaH (0.78 g, 32.5 mmol) according to the previously described method (see compound 43). Yield 65% (0.5 g); ^{1}H NMR (D_2O) δ 7.34-7.39 (m, 2H), δ 7.24-7.33 (m, 3H), δ 4.02 (s, 4H), δ 3.42 (t, 6.48 Hz, 2H), δ 3.32 (s, 2H), δ 2.92 (s, 6H), δ 2.75 (t, 7.34 Hz, 2H), δ 1.90-1.99 (m, 4H), δ 1.77 (t, 13.10 Hz, 2H), δ 1.65-1.72 (m, 2H), δ 1.57 (t, 13.10 Hz, 2H); MS ESI m/z = 334 (M+ H^+); Anal. ($C_{20}H_{31}NO_3$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$) C, H, N.

4-((Dimethylamino)methyl)-4-(3-phenylpropoxy)cyclohexanone (65, UMB411) A solution of **64** (0.5 g, 1.5 mmol) in THF was cooled to 0°C and treated with 5 mL of 1 M HCl (5 mmol). The reaction mixture was allowed to stir overnight at room temperature. The crude mixture was neutralized with 2 M NaOH and extracted with Et_2O. The organic

extract was then washed with brine, dried over $MgSO_4$, concentrated and purified by column chromatography (silica gel, 96%CH_2Cl_2/ 3%MeOH/ 1% NH_4OH) Yield 78% (0.34 g); ^1H NMR (D_2O) δ 7.23-7.41 (m, 5H), δ 3.49-3.55 (m, 2H), δ 3.40 (s, 2H), δ 2.95 (s, 6H), δ 2.72-2.80 (m, 2H), δ 2.50 (t, 12.68 Hz, 3H), δ 2.25-2.32 (m, 2H), δ 2.16-2.24 (m, 2H), δ 1.96-2.05 (m, 2H), δ 1.80-1.89 (m, 2H); MS ESI m/z = 290 (M+ H^+); Anal. ($C_{18}H_{27}NO_2$ $(C_2H_2O_4)_1$ $(H_2O)_{1.25}$) C, H, N.

***N,N*-Dimethyl-1-(3-(3-phenylpropoxy)-2,3,4,4a,9,9a-hexahydro-1H-carbazol-3-yl) methanamine (66, UMB412)** Phenylhydrozine HCl (0.12 g, 0.84 mmol) and tosic acid (0.29 g, 1.52 mmol) were added to a solution of **65** (0.22 g, 0.76 mmol) in EtOH and refluxed at 95°C for 2 hours. The reaction was monitored by TLC and upon completion, neutralized with ammonia and extracted with chloroform. The organic extract was then washed with brine, dried over $MgSO_4$, concentrated and purified by column chromatography (silica gel, 95.5%CH_2Cl_2/ 4%MeOH /0.5% NH_4OH). Yield 43% (0.12g); ^1H NMR (D_2O) δ 7.48 (t, 8.24 Hz, 2H), δ 7.10-7.24 (m, 5H), δ 6.98-7.04 (m, 2H), δ 3.52-3.58 (m, 1H), δ 3.50-3.52 (m, 1H), δ 3.38-3.45 (m, 1H), δ 3.07-3.14 (m, 1H), δ 2.90-3.02 (m, 7H), δ 2.74-2.86 (m, 3H), δ 2.50-2.64 (m, 3H), δ 2.28-2.35 (m, 1H), δ 1.93-2.01 (m, 1H), δ 1.70-1.88 (m, 1H); MS ESI m/z = 365 (M+ H^+); Anal. ($C_{24}H_{32}N_2O$ $(C_2H_2O_4)_1$) C, H, N.

8-((Methyl(phenethyl)amino)methyl)-1,4-dioxaspiro[4.5]decan-8-ol (67). To a stirring solution of EtOH and 1,7,10-trioxadispiro[2.2.4.2]dodecane, **62** (3.20 g, 18.8 mmol), *N*-methyl-phenethylamine (2.73 mL, 18.8 mmol) was added. The reaction mixture was

heated to a reflux and allowed to stir overnight. The reaction was treated with cold H_2O and extracted with Et_2O. The organic extract was then washed with brine, dried over $MgSO_4$, and filtered. Solvent was evaporated under reduced pressure to afford the crude product. Purification was performed via column chromatography (silica gel, 96%$CHCl_3$/ 3%MeOH/ 1% NH_4OH). Yield 73% (4.17 g); MS ESI m/z = 306 ($M+ H^+$)

***N*-Methyl-2-phenyl-*N*-((8-(3-phenylpropoxy)-1,4-dioxaspiro[4.5]decan-8-yl)methyl) ethanamine (68, UMB416)** was prepared through alkylation of **67** (2.0 g, 6.55 mmol) with 1-bromo-3-phenylpropane, (4.98 mL, 32.7 mmol) in the presence of NaH (1.1 g, 45.8 mmol), according to a previously described method (see compound 43). Yield 29.6% (0.82 g); ^1H NMR (CD_3OD) δ 7.05-7.30 (m, 10H), δ 3.84-3.94 (m, 4H), δ 3.28-3.42 (m, 6H), δ 2.94-3.06 (m, 5H), δ 2.65 (t, 7.39 Hz, 2H), δ 1.87-1.98 (m, 2H), δ 1.78-1.87 (m, 2H), δ 1.68-1.77 (m, 2H), δ 1.56 (t, 14.49 Hz, 4H) ESI m/z = 424 ($M+ H^+$); Anal. ($C_{25}H_{35}NO$ $(C_2H_2O_4)_1$) C, H, N.

4-((Methyl(phenethyl)amino)methyl)-4-(3-phenylpropoxy)cyclohexanone (69, UMB417) A solution of **68** (0.36 g, 0.85 mmol) in THF was cooled to 0°C and treated with 1.70 mL of 1M HCl (1.7 mmol). The reaction mixture was allowed to stir overnight at room temperature. The crude mixture was neutralized with 2M NaOH and extracted with Et_2O. The organic extract was then washed with brine, dried over $MgSO_4$, concentrated and purified by column chromatography (silica gel, 96%CH_2Cl_2/ 3%MeOH/ 1% NH_4OH) Yield 74.4% (0.24g); ^1H NMR (CD_3OD) δ 7.26 (t, 7.23 Hz, 2H), δ 7.14-7.24 (m, 5H), δ 7.05-7.11 (m, 3H), δ 3.47 (t, 6.20 Hz, 1H), δ 3.28-3.39 (m, 5H), δ 2.91-

3.03 (m, 5H), δ 2.60-2.69 (m, 2H), δ 2.40-2.50 (m, 1H), δ 2.19 (d, 5.37 Hz, 2H), δ 1.63-1.93 (m, 6H), δ 1.44-1.59 (m, 1H); MS ESI m/z = 380 (M+ H^+); Anal. ($C_{25}H_{35}NO$ $(C_2H_2O_4)_1$) C, H, N.

***N*-Methyl-2-phenyl-*N*-((3-(3-phenylpropoxy)-2,3,4,9-tetrahydro-1H-carbazol-3-yl)methyl)ethanamine (70, UMB418)** Phenylhydrozine HCl (0.08 g, 0.70 mmol) and tosic acid (0.24 g, 1.27 mmol) were added to a solution of **69** (0.24 g, 0.63 mmol) in EtOH and refluxed at 95°C for 2 hours. The reaction was monitored by TLC and upon completion, neutralized with ammonia and extracted with chloroform. The organic extract was then washed with brine, dried over $MgSO_4$, concentrated and purified by column chromatography (silica gel, 95.5%CH_2Cl_2/ 4%MeOH /0.5% NH_4OH). Yield 21% (0.60 g); ^{1}H NMR (CD_3OD) δ 6.88-7.33 (m, 14H), δ 3.40-3.50 (m, 2H), δ 3.30-3.39 (m, 4H), δ 3.07-3.14 (m, 2H), δ 2.87-3.02 (m, 6H), δ 2.68-2.82 (m, 2H), δ 2.48-2.62 (m, 2H), δ2.30-2.38 (m, 1H), δ 1.89-1.97 (m, 1H), δ 1.70-1.86 (m, 2H); MS ESI m/z = 453 (M+ H^+); Anal. $C_{31}H_{36}N_2O$ $(C_2H_2O_4)_1$ $(H_2O)_{0.50}$ C, H, N.

1-Methyl-3-(3-phenylpropoxy)piperidine (72, UMB386) To a solution of 1-methyl-3-piperidinol (1.00 g, 8.68 mmol) in DMF, potassium hydroxide (1.22 g, 21.7 mmol) was added and stirred for 30 min under nitrogen. Next, 1-bromo-3-phenylpropane (1.32 mL, 8.68 mmol) was added and allowed to stir at room temperature overnight. After completion by TLC, the crude reaction mixture was dissolved in H_2O and extracted with Et_2O. The reaction mixture was then extracted into 6M HCl and made basic (pH 12-13) with 5M NaOH (aq) and extracted with Et_2O. The combined organic layers were washed

with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, 94%$CHCl_3$/ 5%MeOH/ 1% NH_4OH), followed by formation of the oxalate salt from ether. Yield 40% (0.81 g); ^{1}H NMR (D_2O) δ 7.41 (t, 7.46 Hz, 2H), δ 7.27-7.35 (m, 3H), δ 3.89 (s, 1H), δ 3.59-3.68 (m, 1H), δ 3.52-3.58 (m, 2H), δ 3.46 (d, 6.22 Hz, 1H), δ 3.07-3.14 (m, 1H), δ 3.01 (t, 12.73 Hz, 1H), δ 2.90 (s, 1H), δ 2.84 (s, 2H), δ 2.68-2.77 (m, 3H), δ 1.88-2.06 (m, 4H), δ 1.53-1.63 (m, 2H); MS ESI m/z = 234 (M+ H^+); Anal. ($C_{15}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

3-(Cinnamyloxy)-1-methylpiperidine (73, UMB387) was prepared through alkylation of 1-methyl-3-piperidinol, **71** (1.00 g, 8.68 mmol) with cinnamyl bromide, **8** (1.71 g, 8.68 mmol) in the presence of KOH (1.22 g, 21.7 mmol), following the method described above (see compound 43). Yield 31% (0.62g); ^{1}H NMR (D_2O) δ 7.50 (d, 3.84 Hz, 2H), δ 7.32-7.47 (m, 3H), δ 6.71-6.77 (m, 1H), δ 6.34-6.46 (m, 1H), δ 4.22-4.34 (m, 2H), δ 4.05 (s, 1H), δ 3.59 (d, 6.53 Hz, 1H), δ 3.44 (d, 6.15 Hz, 1H), δ 3.12 (d, 6.53 Hz, 1H), δ 3.02 (t, 12.11 Hz, 1H), δ 2.83-2.92 (m, 3H), δ 1.97-2.12 (m, 2H), δ 1.78-1.88 (m, 1H), δ 1.58-1.68 (m, 1H); MS ESI m/z = 232 (M+ H^+); Anal. ($C_{18}H_{26}NO$ $(C_2H_2O_4)_1$ $(H_2O)_?$) C, H, N.

1-Phenethylpiperidin-3-ol (75) A mixture of 3-hydroxypiperidine (1.0g, 9.89mmol), *N*-methyl-phenethylamine, **74** (1.47 mL, 10.9mmol), and K_2CO_3 (13.7 g, 99.0 mmol) in DMF (20 mL/g) was vigorously stirred at room temperature under N_2. After completion by TLC, H_2O was added and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under

reduced pressure, the crude product was purified by column chromatography (silica gel, 94%$CHCl_3$/ 5%MeOH/ 1% NH_4OH). Yield 88% (1.79 g); MS ESI m/z = 206 ($M + H^+$).

3-(Cinnamyloxy)-1-phenethylpiperidine (76, UMB424) was prepared through alkylation of 1-phenethylpiperidin-3-ol, **75** (2.24 g, 10.9 mmol) with cinnamyl bromide (6.45 g, 32.7 mmol) in the presence of NaH (1.76 g, 76 mmol), following the method described above (see compound 43). Yield 22% (0.77 g); ^{1}H NMR (D_2O) δ 7.30-7.57 (m, 10H), δ 6.72-6.78 (m, 1H), δ 6.36-6.45 (m, 1H), δ 4.21-4.36 (m, 3H), δ 4.09 (s, 1H), δ 3.73-3.80 (m, 1H), δ 3.51-3.59 (m, 1H), δ 3.42 (t, 7.75 Hz, 2H), δ 3.04-3.18 (m, 5H), δ 2.13-2.24 (m, 2H), δ 1.82-1.89 (m, 1H), δ 1.65-1.76 (m, 1H); MS ESI m/z = 322 ($M+ H^+$); Anal. $C_{22}H_{27}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$ C, H, N.

1-Phenethyl-3-(3-phenylpropoxy)piperidine (77, UMB423) was prepared through alkylation of 1-phenethylpiperidin-3-ol, **75** (0.55 g, 2.68 mmol) with 1-bromo-3-phenylpropane (1.22 mL, 8.04 mmol) in the presence of NaH (0.43 g, 18.8 mmol), following a method described previously (see compound 43). Yield 46% (0.40 g); ^{1}H NMR (D_2O) δ 7.27-7.48 (m, 10H), δ 3.91 (s, 1H), δ 3.62-3.70 (m, 2H), δ 3.47-3.59 (m, 4H), δ 3.36-3.45 (m, 2H), δ 3.02-3.17 (m, 4H), δ 2.69-2.76 (m, 2H), δ 1.98-2.11 (m, 2H), δ 1.88-1.96 (m, 2H); MS ESI m/z = 324 ($M+ H^+$); Anal. $C_{22}H_{29}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$ C, H, N.

Chapter 4. Opioids lacking a tyrosine mimetic. Part 3: Phenylpropyloxyethylamines Containing Multiple Rings from the Opioid Skeleton

4.1. INTRODUCTION

Opioids such as morphine, fentanyl and oxycodone are typically used to treat moderate to severe clinical pain (Zieglgansberger et al., 1995; Stein et al., 2003). Despite the clinically beneficial properties of opioids (e.g. analgesia, euphoria), these opioids share a common side effect profile which includes the development of tolerance, dependence (Kieffer and Evans, 2002), constipation, nausea, and respiratory depression(McNicol et al., 2003; Benyamin et al., 2008). While additional medication may lessen or even prevent some of the adverse effects, there is a lack of effective treatment for opioid induced constipation (Bell et al., 2009). Subsequently, peripherally restricted μ opioid receptor antagonists, alvimopan (Lavine, 2008) and methylnaltrexone (Yuan et al., 2005) have been approved for treatment of opioid induced constipation. Significant limitations to alvimopan include heart attack and diarrhea (Chappelle, 2008; Lavine, 2008). Additionally, methylnaltrexone has poor bioavailability, thus it has to be administered s.c. on daily basis (Yuan et al., 2005).

Investigations of other biological systems have been conducted to avoid the existing problems associated with opioids. Among systems investigated were NMDA receptor agonists,(McCartney et al., 2004) GABA agonists (Kjaer and Nielsen, 1983), and nicotinic agonists (Decker et al., 2004). All have undesirable effects and thus have seen little promise as pain therapeutics (Kjaer and Nielsen, 1983; Decker et al., 2004; McCartney et al., 2004). In December of 2004, the Food and Drug Administration approved Ziconotide, a N-type calcium blocking agent for treatment of chronic pain (Staats et al., 2004). Although Ziconotide shows no evidence of tolerance or addiction

that is commonly seen in opioid therapy, it is administered intrathecally (directly into the spine) making it less attractive in the clinical setting (Staats et al., 2004). Therefore, mu-opioids remain the gold standard for the treatment of severe pain.

Tremendous efforts have been put towards the development of opioid analgesics displaying a more favorable pharmacological profile (Casy and Parfitt, 1986). Modifications at position 14 of 4,5-epoxymorphinans have opened a new realm of possibilities with a major impact on the receptor-ligand interaction (Li et al., 2009; Lewis and Husbands, 2010; Schmidhammer and Spetea, 2010). Specifically, 14-methoxy metopon, a member of a 14-alkoxy morphinan opioid series has been characterized as a μ-selective opioid with 500-fold greater systemic antinociceptive potency than morphine (Furst et al., 1993a). Minimal physical dependence and tolerance has been observed after repeated treatment in mice (Furst et al., 1993a). Moreover, reduced constipation (King et al., 2003) and respiratory depression (Furst et al., 1993a) were reported as compared to morphine.

Further investigations of the 14-alkoxy morphinans lead to the discovery of 14-phenylpropyloxy metopon, which is even more potent than 14-methymetopon (24,000-fold higher in tail flick as compared to morphine) (Schutz et al., 2003). Although 14-phenylpropyloxy metopon is unsuitable for clinical use, it can serve as a lead compound for the development of a novel opioid skeleton. Therefore, it was of interest to develop a novel opioid skeleton that contains a phenylpropyloxy group and a basic nitrogen, but

lacks the A-ring, historically required (Casy and Parfitt, 1986; Casy, 1993) for opioid activity.

In previous studies, we showed that phenylpropyloxyethylamines are able to bind to μ opioid receptors and we identified the *N*-phenethyl analog, 2-(cinnamyloxy)-*N*-methyl-*N*-phenethylethanamine as the optimal *N*-substituent with an affinity of 1680 nM. Binding studies showed that the B-ring analog containing the *N*,*N*-dimethyl substituent, *N*,*N*-dimethyl-2-(3-phenylpropoxy)cyclohexanamine produced the highest affinity (2340 nM) of the single ring containing phenylpropyloxyethylamines, while the C- and D-ring analogs were fully inactive. The affinity was regained by combining the B-ring with the optimal *N*-substituent, phenethyl, to give *trans*-*N*-methyl-*N*-phenethyl-2-(3-phenylpropoxy)cyclohexanamine achieving 1640 nM affinity at μ receptors. Furthermore, the μ opioid receptor interaction was improved when the C-ring analog was modified to contain an indole moiety, *N*,*N*-dimethyl-1-(3-(3-phenylpropoxy)-2,3,4,9-tetrahydro-1H-carbazol-3-yl)methanamine giving 1110 nM affinity for the μ opioid receptor.

This chapter focuses on the design and synthesis of phenylpropyloxyethylamines analogs containing multiple rings from the opioid skeleton. A polycyclic ring system will be introduced mimicking rings B and D in order to investigate the optimal configuration between the basic nitrogen and phenylpropyloxy group required to achieve opioid activity. Initial compounds will be synthesized containing the dimethyl *N*-substituent.

Subsequent optimization of the final product will be achieved by introducing the optimal *N*-substituent, *N*-phenethyl, determined in Chapter 2.

4.2. CHEMISTRY

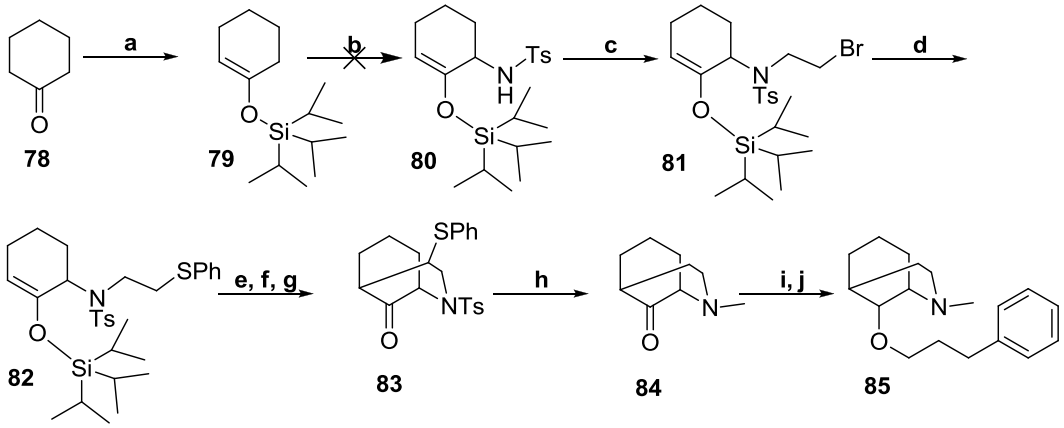
4.2.1. B/D-ring system analogs

Numerous attempts have been made towards the synthesis of the phenylpropyloxyethylamines analogs containing the B/D rings from the opioid skeleton. The initially proposed synthesis (Scheme 4.1) of this compound consisted of preparation of triisopropylsilyl enol ether (**79)** (Yu et al., 2005), introduction of –NHTs functional group into the axial position with $(TsN)_2Se$ to give **80** (Magnus et al., 1995), followed by *N*-alkylation in order to obtain **81** (Magnus et al., 1995). Conversion of bromide to sulfide (**82)** would be achieved with sodium thiophenate (Magnus et al., 1995), which would be further converted to the sulfoxide with *m*CPBA (Magnus et al., 1995). The resulting sulfoxide compound would be cyclized with trifluoroacetic acid anhdride/2,6-dibutyl-4-methylpyridine and then chlorobenzene to give **83** (Magnus et al., 1995). The removal of the –SPh functional group and the tosyl group and simultaneous *N*-methylation to give **84** would be accomplished by with treatment of sodium amide, followed by methyl iodide (Magnus et al., 1995). Further alkylation to give compound **85** would be achieved as discussed previously in Chapter 3 (Rist et al., 2001).

As an alternative method (Scheme 4.2), we considered starting with 2-chlorocyclohexanone, **86**, and preparing **87** through alkylation of the amine with 2-

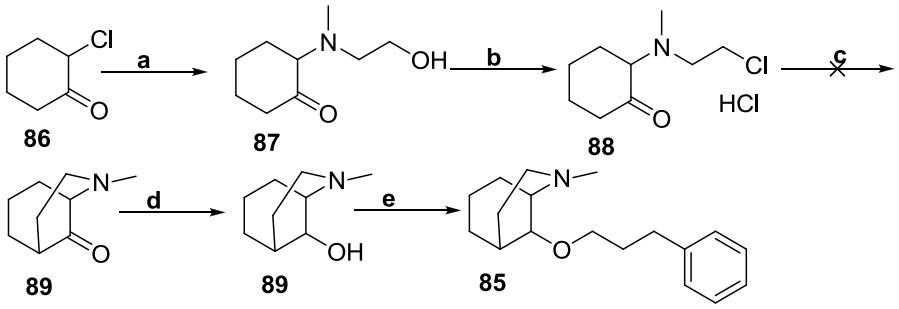
bromoethanol.(Maeda et al., 2002a) The hydroxyl group would then be converted to a chlorine to give compound **88** (Smith and March, 2007). In order to attain the kinetic enolate which would attack from the same face to give the desired bridged product **84**, we chose to use a strong, hindered base such as LDA (Coop et al., 1995). Reduction with LAH will yield compound **89** (Smith and March, 2007). A previously described method for alkylation would be utilized to give compound **85** (Rist et al., 2001).

Scheme 4.1 *Method 1:* Proposed synthesis of 2-methyl-9-(3-phenylpropoxy)-2-azabicyclo[3.3.1]nonane.



Reagent and Conditions. a) Triisopropylsilyl triflate, Et_3N, DCM, rt. b) Selenium powder, anhydrous chloramines-T, DCM, rt. c) NaH, 1,2-dibromoethane, THF, reflux. d) Thiophenol, NaH, THF, reflux for 1h. e) m-Chloroperoxybenzoic acid, DCM, -78°C for 20min. f) TFAA, 2,6-di-*tert*-butyl-4-methylpyridine, DCM, 0°C. g) Chlorobenzene, DCM, 130°C. h) sodium amide, MeI, THF. i) LAH, THF, reflux j) NaH, 1-bromo-3-phenylpropane, DMF.

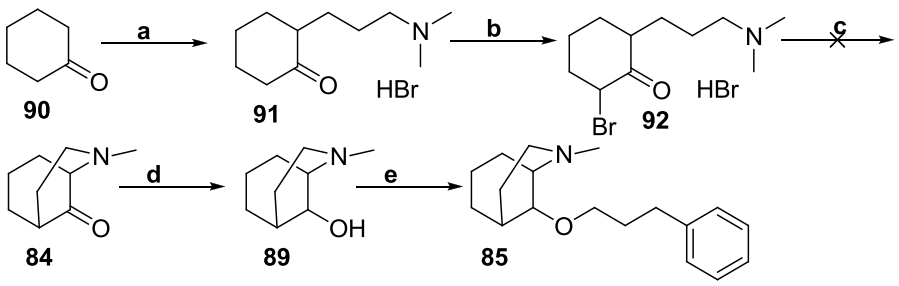
Scheme 4.2 *Method 2:* Proposed Synthesis of 2-methyl-9-(3-phenylpropoxy)-2-azabicyclo[3.3.1]nonane.



Reagent and Conditions. a) K_2CO_3, 2-(methylamino)ethanol, DMF, overnight. b) $SOCl_2$, DCM, 0°C c) LDA, THF -78°C. d) LAH, THF, 0°C e) NaH, 1-bromo-3-phenylpropane, DMF.

In an alternative pathway (Scheme 4.3) compound **91** would be prepared by a known alkylation procedure used to synthesize (-)-5-*m*-hydroxyphenyl-2-methylmorphan, (Rogers and May, 1974) followed by bromination of the Mannich ketone with HBr and Br_2 to give rise to compound **92**.(Rogers and May, 1974) Cyclization (**84**) of the bromo ketone would be achieved as a freebase at room temperature followed by dry distillation using a literature method (May and Murphy, 1954; Rogers and May, 1974). Reduction with LAH will yield compound **89** (Smith and March, 2007)**.** Target **85** will be attained by the developed alkylation reaction (Rist et al., 2001).

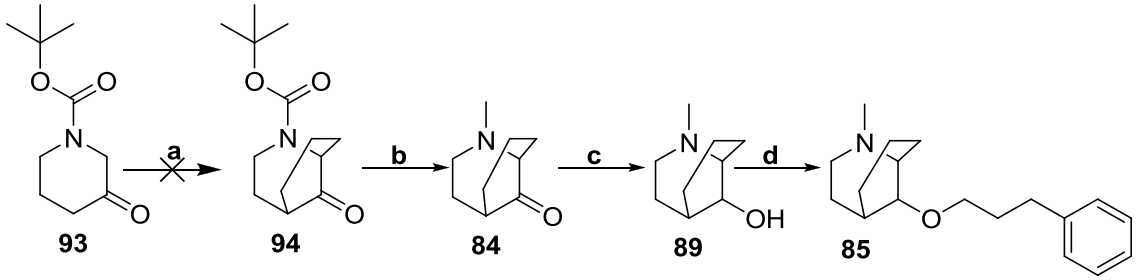
Scheme 4.3 *Method 3:* Proposed synthesis of 2-methyl-9-(3-phenylpropoxy)-2-azabicyclo[3.3.1]nonane.



Reagent and Conditions. a) NaH, 3-Dimethylamino-1-propyl chloride HCl, DMF, overnight. b) Br_2, AcOH c) NaOH, distillation. d) LAH, THF, reflux e) NaH, 1-bromo-3-phenylpropane, DMF.

Our fourth method entertained the idea of performing cyclization in one-step with 1,3-dibromopropane to produce the desired analog, **94** as shown in Scheme 4.4. Once the cyclization step is complete, the BOC group will be removed under acidic conditions (Smith and March, 2007) and *N*-methylated using Eschweiler–Clarke methylation (Overman and Sugai, 1985) to give **84**. Reduction with LAH will yield compound **89** (Smith and March, 2007). Target **85** will be attained by the developed alkylation reaction (Rist et al., 2001).

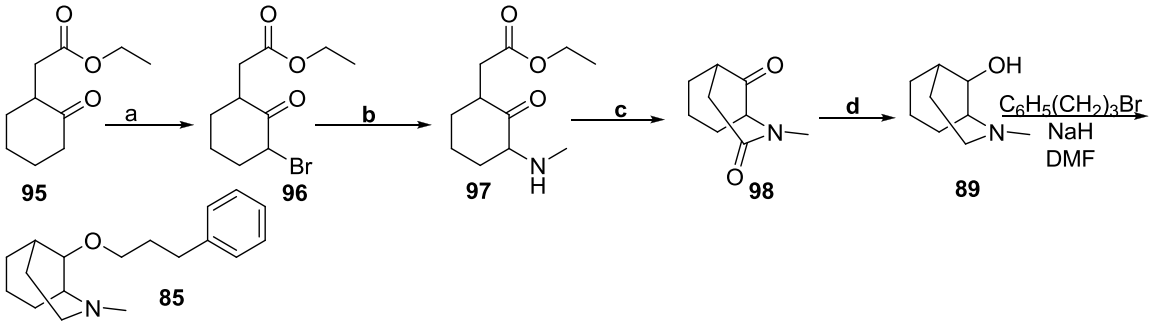
Scheme 4.4. *Method 4:* Proposed synthesis of 2-methyl-9-(3-phenylpropoxy)-2-azabicyclo[3.3.1]nonane.



Reagent and Conditions. a) LiHMDS, 1,3-Dibromopropane, THF. b) TFA, DCM, 0°C. c) LAH, THF, reflux d) NaH, 1-bromo-3-phenylpropane, DMF.

In our most recently designed synthesis of the B/D ring system (Scheme 4.5), ethyl 2-cyclohexanoneacetate (**95**) will be utilized as the starting material. Bromination of the Mannich ketone(Rogers and May, 1974) will yield compound **96** which will be aminated(Iddon and Yat, 1990) to give **97** followed by a cyclization step to give rise to **98**.(Iddon and Yat, 1990) Reduction of the amide with LAH in THF will yield compound **89**. (Smith and March, 2007) Target **85** will be attained by the developed alkylation reaction. (Rist et al., 2001)

Scheme 4.5 *Method 5:* Proposed synthesis of 2-methyl-9-(3-phenylpropoxy)-2-azabicyclo[3.3.1]nonane.



Reagent and Conditions. a) Br_2, HBr, AcOH. b) NH_2CH_3, K_2CO_3, DMF c) EtOH. d) LAH, THF, reflux e) NaH, 1-bromo-3-phenylpropane, DMF.

4.3 RESULTS AND DISCUSSION

Reactions with triisopropylsilyl groups have been widely explored due to their effective protective properties for enol ethers (Magnus et al., 1995). Preparation of the triisopropylsilyl enol ether (**79**) (Scheme 4.1) was accomplished in 80% yield according to literature methods (Yu et al., 2005). Preparation of anhydrous chloramines-T was

attempted by heating the dehydrate form of the reagent under vacuum conditions(Magnus et al., 1995). Inadequate dehydration resulted in formation of NaOH in solution and subsequent cleavage of the triisopropylsilyl group resulting in the formation of starting material. Reports have been made that the dehydration reaction is extremely explosive(Bishop and Jennings, 1958) and after numerous attempts it was in the best interest to devise an alternative method for preparation of the B/D ring analogs.

In our second attempt (Scheme 4.2), compound **86** was successfully obtained in 91% yield via the alkylation reaction described above (Maeda et al., 2002a). In our initial studies, we tried to activate the hydroxyl group using tosyl chloride (Smith and March, 2007). However, once tosylated the product proceeded to react with itself forming a complex reaction mixture. To overcome this problem, the hydroxyl was converted to chlorine, (Smith and March, 2007) **88** with thionyl chloride and made into a hydrochloride salt to avoid reactions at the nitrogen such as formation of aziridinium, which could reopen to give other products. Full conversion of hydroxyl to chlorine was assumed and no further purification was performed before the LDA reaction (Coop et al., 1995). Unfortunately, the cyclization step to give **89** proved to be problematic giving a complex reaction mixture. After several attempts, an alternative method was proposed in Scheme 4.3.

For the third method (Scheme 4.3), alkylation of **90** was achieved with 33% yield (Rogers and May, 1974). Compound **91** was successfully converted to the hydrobromide salt from ether and brominated to give impure **92** in 2% yield (May and Murphy, 1954;

Rogers and May, 1974). Since the yield was very low and the product was impure, we could not proceed with the cyclization step. Additionally, it was very difficult to reproduce the same results each time and therefore an alternative method was developed.

The one-step cyclization pathway was not successful. As anticipated, alkylation occurred on the on the oxygen instead of the α-carbon as confirmed by the LCQ MS. The reaction was repeated with LDA as the base; however, similar results were obtained.

In our most recent attempt, bromination of the ketone with Br_2 and HBr was afforded in 4.2% yield. However, the amination step (Iddon and Yat, 1990) was not as successful. It appears that more substituted product is forming, as seen on the LCQ MS, due to over alkylation of the starting material, **96** to the amine. In future studies, the reaction should to be repeated in excess of methylamine and under more diluted solvent conditions to avoid over alkylation. This reaction requires monitoring by MS to assure that the alkylation only occurs once. In the event that alkylation occurs twice, *N*-protected methylamine should be utilized in this step.

4.4. CONCLUSION

In this chapter, we attempted to re-introduced a combination of the B and D rings back into the systems to determine the bioactive conformation and aid in future modeling studies. Though the cyclization step proved to be difficult for all the attempted methods, we were able to produce compounds leading up to the cyclization step. In our last

attempt, the reaction proceeded to form the more substituted product. In the future studies, the reaction should to be repeated in excess of methylamine and under more diluted solvent conditions to avoid over alkylation. Additionally, this reaction should be monitored by MS to assure that the alkylation only occurs once and to avoid formation of a complex reaction mixture. In the event that alkylation occurs twice, *N*-protected methylamine should be utilized in this step. The methodology explored in this chapter will aid in the future synthesis of phenylpropyloxyethylamines.

4.5. EXPERIMENTAL

All reagents and solvents were purchased from Sigma Aldrich Inc. unless stated otherwise and used without further purification. All reactions were carried out under an atmosphere of nitrogen. Thin layer chromatography was performed on silica 60 F_{254} plated (Analtech, Inc., Newark, DE). All compounds were purified using standard techniques (crystallization, etc.) and characterized using standard spectroscopic methods such as ^{1}H NMR (Varian Inova 500 MHz) and MS (ThermoFinnigan LCQ Classic, Waltham, MA).

Cyclohexenyloxytriisopropylsilane (79). To a solution of ketone (1.58 mL, 15.3 mmol) and triethylamine (4.30 mL, 30.6 mmol) in DCM was added triisopropylsilyl triflate (4.19 mL, 15.60 mmol). The reaction was monitored by TLC and once it was complete, the crude reaction mixture was diluted with DCM and washed with sodium bicarbonate. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced

pressure. The crude product was purified by column chromatography (basic alumina, 100% hexane). Yield 80% (3.11 g); ^{1}H NMR ($CDCl_3$) δ 4.92 (s, 1H), δ 2.00-2.15 (m, 4H), δ 1.66-1.76 (m, 2H), δ 1.50-1.61 (m, 2H), δ 1.08-1.22 (m, 21H).

4-Methyl-*N*-(2-(triisopropylsilyloxy)cyclohex-2-enyl)benzenesulfonamide (80). Anhydrous chloramines-T was prepared in a two-neck flask under vacuum. A solution of chloramines-T (0.90 g, 3.93 mmol) and chloroform was heated for 5 hours until the moisture around the neck of the flask was no longer seen. The reaction mixture was allowed to come to room temperature and the vacuum line was removed. Next, selenium powder (0.14 g, 1.79 mmol) was added to the solution and the reaction mixture was stirred for 2 days. A solution of silyl enol ether, **79** (0.5 g, 1.97 mmol) in chloroform was added to the reaction mixture at 0°C and allowed to stir for an additional 2 days, followed by addition of sodium hydroxide (0.1 M). After 30 min, the reaction mixture was filtered through celite and washed with chloroform. The product was extracted with chloroform, dried over Na_2SO_4, and concentrated. Yield 0%;

2-((2-Hydroxyethyl)(methyl)amino)cyclohexanone (87). A mixture of 2-(methylamino)ethanol (0.61 mL, 7.54 mmol), 2-chlorocyclohexanone (0.86 mL, 7.54 mmol), and K_2CO_3 (10.42 g, 75 mmol) in DMF (20 mL/g) was vigorously stirred at room temperature under N_2. After completion by TLC, H_2O was added and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography (silica gel, $CHCl_3$/ 1-3%MeOH/ 1%NH_4OH). Yield

91% (1.18 g); MS ESI m/z = 172 ($M + H^+$); ^{1}H NMR ($CDCl_3$) δ 4.45 (t, 12.83 Hz, 1H), δ 3.80-3.85 (m, 1H), δ 3.41-3.47 (m, 1H), δ 3.33-3.39 (m, 1H), δ 3.14-3.20 (m, 1H), δ 2.83 (s, 3H), δ 2.11-2.20 (m, 1H), δ 1.84-1.91 (m, 2H), δ 1.46-1.71 (m, 5H), δ 1.32-1.43 (m, 1H).

2-((2-Chloroethyl)(methyl)amino)cyclohexanone hydrochloride (88). To a solution of **87** (0.1 g, 0.58 mmol) in DCM was added dropwise thionyl chloride (0.43 mL, 5.84 mmol) at 0°C. The reaction was then stirred for overnight at room temperature. The solvent was then removed with a rotary evaporator, re-dissolved in chloroform and concentrated to remove traces of thionyl chloride. Due to the instability of the product, full conversion was assumed without further purification (Yield 100%; 0.11 g).

2-Methyl-2-azabicyclo[3.3.1]nonan-9-one (89). Compound **88** (0.11 g, 0.59 mmol) was re-dissolved in THF and cooled to -78°C, and a 2M solution of LDA (0.73 mL, 1.46 mmol) was added drop wise. The reaction was monitored by TLC and upon completion, diluted with H_2O and extracted with ether. The organic extracts were then washed with brine, dried over $MgSO_4$, and concentrated. Yield 0%.

2-(3-(Dimethylamino)propyl)cyclohexanone hydrobromide (91). A solution of cyclohexanone, **90** (2.59 mL, 25 mmol) in dry DMF was added to a stirring solution of 0.69 g (30 mmol) of NaH at room temperature. After 30 min, 5.38 g (50 mmol) of 3-dimethylamino-1-propyl chloride HCl was added in small portions over a 15 min period. The resulting mixture was allowed to stir overnight at room temperature. After

completion by TLC, the reaction mixture was quenched with ice H_2O and extracted with Et_2O. The product was then extracted into 3M HCl from Et_2O. The acid extract was warmed to 90°C for 30 min. The solution was made basic (pH 12-13) with 2M NaOH (aq) and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, an HBr salt was formed from ether. Yield 33% (1.40 g); MS ESI m/z = 170 ($M + H^+$).

2-Bromo-6-(3-(dimethylamino)propyl)cyclohexanone hydrobromide (92). To a solution of acetic acid-water was added **91** (0.14 g, 0.85 mmol) at 0°C. Next, bromide (0.044 mL, 0.85 mmol) was added dropwise and the reaction was warmed to room temperature. After 1 hour, the reaction stopped and triturated several times with ether. Yield 2% (4.11 g); MS ESI m/z = 249 ($M + H^+$).

***tert*-Butyl 9-oxo-2-azabicyclo[3.3.1]nonane-2-carboxylate (94)**. To a solution of **93** (0.5 g 2.51 mmol) was added dropwise LiHMDS (0.84 g, 5.02 mmol) at -78°C and stirred for 30 min. 1,3-Dibromopropane (0.27 mL, 2.51 mmol) was then added to the reaction mixture and stirred for an hour. Next, the reaction mixture was allowed to warm to 0° and stirred for an additional 1 hour. The reaction was stopped and treated with H_2O followed by extractions with Et_2O. The organic layers were combined, washed with brine solution, dried over Na_2SO_4, and evaporated under reduced pressure. Yield 0%.

Ethyl 2-(3-bromo-2-oxocyclohexyl)acetate (96). To a solution of acetic acid and water was added **95** (2.0 mL, 11.07 mmol) at 0°C. Next, bromide (0.57 mL, 11.07 mmol) was

added dropwise and the reaction was warmed to room temperature. After 1 hour, the reaction was stopped, diluted with chloroform, and washed with water. The organic layer was washed with brine solution, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, CH_2Cl_2/ 1-3%MeOH/ 1%NH_4OH). Yield 4.2% (0.12 g).

Ethyl- 2-(3-(methylamino)-2-oxocyclohexyl)acetate (97). A mixture of **96** (0.12 g, 0.47 mmol), methylamine (0.10 mL, 0.82 mmol), and K_2CO_3 (0.13 g, 0.94 mmol) in DMF (20 mL/g) was vigorously stirred at room temperature under N_2. After completion by TLC, H_2O was added and extracted with Et_2O. The combined organic layers were washed with brine solution, dried over Na_2SO_4, and concentrated. Yield 0%

Chapter 5. The Effect of Ring-Constrained Phenylpropyloxyethylamines on Sigma Receptors

5.1 INTRODUCTION

Finding effective pharmacotherapies to treat cocaine abuse and addiction remains a major challenge (Carrera et al., 2004). Considerable efforts have been put forth towards the development of potential anti-cocaine therapeutics that attenuate the toxic and addictive effects, yet success has been limited (Matsumoto, 2009). Our approach utilized the fact that cocaine interacts with σ receptors (Matsumoto et al., 2002; Maurice et al., 2002; Shibayama et al., 2002) and σ antagonists attenuating acute (convulsions, lethality, locomotor activity) and subchronic (sensitization, place conditioning) effects of cocaine, makes σ a promising target for cocaine treatment (Romieu et al., 2000; Matsumoto and Mack, 2001; Matsumoto et al., 2001; Matsumoto et al., 2002; Romieu et al., 2004).

σ Receptors were initially proposed by Martin and coworkers as a subtype of opioid receptor to account for some benzomorphan activity (Martin et al., 1976). However, due to the inability of naloxone to antagonize σ effects, σ receptors were later considered to be a unique class of receptors (Largent et al., 1987). σ Receptors are comprised of two subtypes, σ_1 and σ_2 (Quirion et al., 1992). To date, σ_1 receptors are the only cloned σ receptors (Mei and Pasternak, 2001; Matsumoto et al., 2003; Guitart et al., 2004; Matsumoto, 2007). Studies have shown that σ_1 receptors are involved in intracellular signaling, synaptic transmission, modulation of inositol phosphates, protein kinases, and calcium (Morin-Surun et al., 1999; Hayashi et al., 2000; Hayashi and Su, 2001; Guitart et al., 2004; Bermack and Debonnel, 2005). Though not yet cloned, σ_2 receptors appear to exist as heterodimers and are smaller in size compared to σ_1 (Hellewell and Bowen, 1990;

Moebius et al., 1993; Hellewell et al., 1994). Studies have shown that σ_2 are associated with the inhibition of cell proliferation and induction of apoptosis, producing transient and sustained release of calcium ions (Vilner et al., 1995).

Prior to the discovery of the two subtypes, initial σ receptor-ligand structure-activity relationship (SAR) studies were performed on a range of opioid related compounds and it was determined that (a) phenylpiperidine containing analogs produce high potency at the σ receptor, (b) *N*-alkyl lipophilic substituents produce greater affinity, and (c) there is no predetermined set of rigid constraints for the intramolecular distances required for σ receptor binding (Largent et al., 1987). Though these initial σ ligands helped gain insight of the σ SAR, their interaction with other biological systems such as opioid receptors, dopamine transporters, or *N*-methyl-D-aspartate (NMDA) receptors,[14] impeded the understanding of their true biological function. Subsequent studies included partial opioid structure of the *N*-phenylpropyl derivative of a ring opened benzomorphinan (PPAP), which had high selectivity for the σ receptor versus the PCP sites and dopamine, D1 and D2 receptors (Glennon et al., 1990) and thus it served as the lead compound for years to come in detailed structure activity investigation (Prasad et al., 1998; Mei and Pasternak, 2001; Glennon, 2005). Specifically, the effect of longer-chain, aryl substituents, as well as conformational constraint on PPAP derivates was examined (Glennon, 2005). These studies resulted in agents which were selective for σ over other biological systems while displaying equivalent or higher affinity for σ_1 and σ_2 receptor subtypes (Glennon, 2005). Earlier studies from our laboratory had shown that AC927 (*N*-phenethylpiperidine), a mixed σ_1 and σ_2 antagonist, demonstrated high selectivity for the σ receptors (Maeda et

al., 2002b). Additional studies showed that AC927 attenuated the locomotor stimulant effects of cocaine in mice (Matsumoto et al., 2007a). However, AC927 has a relatively narrow therapeutic window (unpublished finding; R. R. Matsumoto, Morgantown, WV). Accordingly analogs of AC927 are required to determine the optimal substituents needed to improve selectivity for each σ subtype and to increase the therapeutic window for cocaine treament.

The compounds discussed in this chapter were initially synthesized as B-, C-, and D-ring constrained phenylpropyloxyethylamine analogs, opioid ligands (refer to Chapter 3). Their close resemblance to AC927 prompted our decision to further investigate the partial opioid structures, lacking the A-ring, at the two established σ receptors subtypes (σ_1, σ_2). The structural differences of the novel compounds will aid in the design of σ_1 and σ_2 antagonists for the treatment of cocaine abuse. Additionally, these compounds have low to negligible affinity for opioid receptors making them a desirable candidate for drug development.

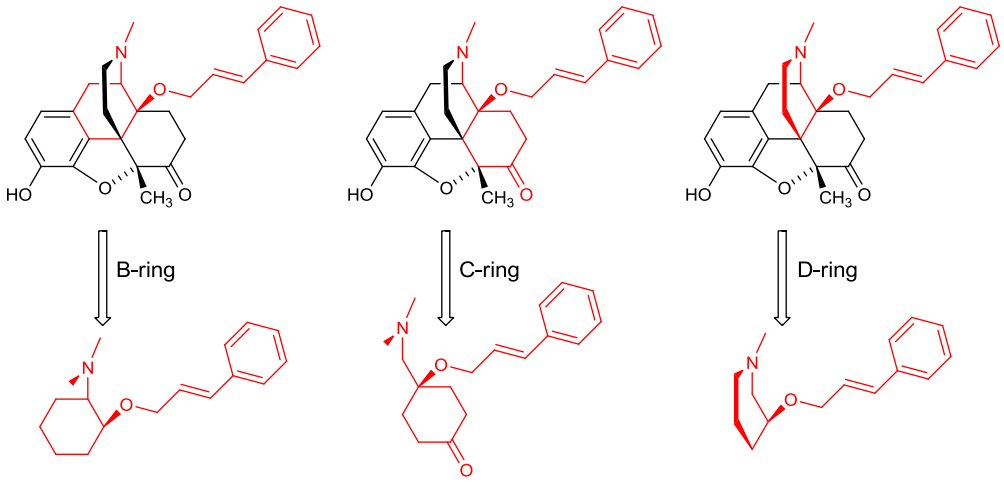


Figure 5.1 Structures of B-, C-, and D-ring analogs

5.2 CHEMISTRY

Preparation of the discussed ring constrained phenylpropyloxyethylamines (Figure 5.2) is described in detail in Chapter 3. In brief, analogs **43** and **44** have been synthesized via *N*-methylation of *trans*-2-aminocyclohexanol hydrochloride using the Eschweiler-Clark methylation(Overman and Sugai, 1985) followed by alkylation with the corresponding phenylalkyl bromide in the presence of NaH.(Rist et al., 2001) Compound **46** and the intermediate of **49** were synthesized by addition of the appropriate *N*-alkylamine to an epoxide.(Rogers et al., 1989) The Eschweiler-Clark methylation(Overman and Sugai, 1985) reaction was utilized again to obtain target **49**. Analogs **47**, **50**, **72**, and **73** were achieved by alkylation with the appropriate phenylalkyl bromide in the presence of NaH.(Rist et al., 2001) The preparation of target **64** required epoxide synthesis (Ciaccio et al., 2003), which was then opened with *N*,*N*-dimethylamine (Szakonyi et al., 2008) followed by O-alkylation with 1-bromo-3-phenylpropane to yield the desired product.

(Rist et al., 2001) To synthesize compound **66**, acid hydrolysis (Grieco et al., 1977) was utilized to first cleave off the ketal moiety on compound **64** which subsequently underwent standard Fisher indole synthesis conditions (Kubota et al., 1998) to give the final product. All final targets were purified by column chromatography and converted into oxalate salts from ether. All salt targets were characterized using NMR and MS and all elemental analyses of salts were within ±0.4%.

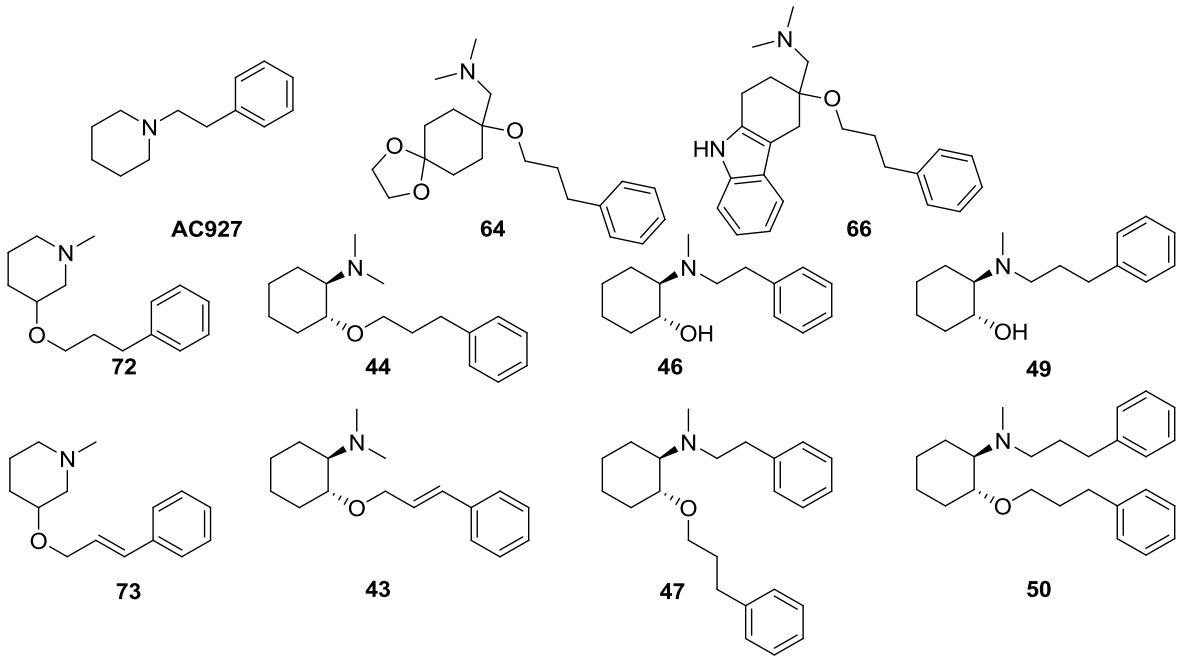


Figure 5.2 Structures of ring-contrained phenylpropyloxyethylamines and AC927

5.3 PHARMACOLOGY

5.3.1 Opioid Binding Assays

Binding affinity of compounds has been determined at all three opioid receptors (μ, δ, and κ expressed in CHO cells) using standard *in vitro* displacement methods(Dooley et

al., 1997) provided by Jason Healy, a graduate student in the laboratory of R. Matsumoto (WVU, Morgantown, WV). Briefly, hMOR membrane protein were labeled with 1.3 nM [^{3}H]DAMGO (53.4 Ci/mmol). hDOR membrane protein were labeled with 1.2 nM [^{3}H]DPDPE (45 Ci/mmol). hKOR membrane protein were labeled with 1.7 nM [^{3}H]U69,593 (42.7 Ci/mmol). Nonspecific binding was determined in the presence of 1 μM unlabelled DAMGO, DPDPE or U69,593 for the respective subtypes. Competition binding studies were performed using 12 concentrations of each test compound and were incubated for 1 h at 25^{o}C. Reactions were terminated by rapid vacuum filtration through GF/B glass fiber filters previously soaked in 0.5% polyethyleneimine. Bound radioactivity was quantified by liquid scintillation counting. Affinities (K_i) were calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

5.3.2 Sigma Binding Assays

In vitro competition binding assays were performed in the laboratory of our collaborator, R. Matsumoto (WVU, Morgantown, WV). Preparation of rat brain membrane and binding assays for the σ_1 and σ_2 receptor were performed as previously described in detail (Matsumoto et al., 1995; Matsumoto et al., 2008). In brief, σ_1 receptors were labeled in rat brain membrane with 5 nM [^{3}H](+)-pentazocine. The σ_2 receptors were labeled with 3 nM [^{3}H]di-o-tolylguanidine (DTG) in the presence of 300 nM (+)-pentazocine to block σ_1 receptors. Nonspecific binding was determined in the presence of 10 μM haloperidol. Ten concentrations of each test compound (0.1–10,000 nM) were used in the assays. The compounds were incubated for 120 min at 25°C to measure their ability to displace the radioligands from their binding sites. Termination of the reaction was achieved through rapid vacuum filtration over glass fiber filters which were previously soaked in 1%

polyethyleneimine for at least 45 min. K_i values were calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

5.3.3 Cocaine-Induced Convulsions

Determination of the anticonvulsant actions of compounds **46** and **49** was performed in the laboratory of our collaborator, R. Matsumoto (WVU, Morgantown, WV) using methods(Matsumoto et al., 2003) approved by the Institutional Animal Care and Use Committee. To probe for anticonvulsant actions against cocaine, male Swiss Webster mice (n = 80) were pretreated (i.p.) with compounds **46** (0, 1, 10, and 30 mg/kg) and **49** (0, 0.1, 1, 10 mg/kg) 15 min prior to administration of a convulsive dose of cocaine (70 mg/kg, i.p.). The mice were observed for the occurrence of convulsions for 30 min, which were operationally defined as clonic or tonic limb movements, accompanied by the loss of righting reflexes for at least 5 s, and/or popcorn jumping. Fisher's exact test was utilized to determine significant differences between the effects produced by pretreatment with the test compounds compared to the effects produced by pretreatment with saline (vehicle control).

5.3.4 Sigma Efficacy: $[Ca^{2+}]_i$ Measurements

The calcium assays were performed in the laboratory of our collaborator, W. Bowen (Brown University, Providence, RI) using standard methods(Vilner and Bowen, 2000) to determine the σ_2 efficacy. Cytosolic Ca^{2+} was monitored with the ratiometric indicator Fura-2 (InCyt Im2 Dual-wavelength Fluorescence Imaging System; Intracellular Imaging, Cincinnati, OH). The SK-N-SH neuroblastoma cells were grown on glass

coverslips and then washed twice in Dulbecco's Phosphate Buffered Saline (DPBS) before incubation in DPBS containing 2.0 to 3.0 μM Fura-2 AM and 0.066% Pluronic F-127 (Invitrogen, Carlsbad, CA). After incubating for 60 to 75 min at 37°C in darkness, cultures were washed twice in DPBS to remove extracellular dye and kept at room temperature in the dark for more than 30 min before use in the experiments. All measurements were performed in DPBS or, where specified, in Ca^{2+}-free DPBS. Compounds **46** and **49** were added to cells in the presence of DPBS in the Petri dishes. The dishes with dye-loaded cells were mounted on the stage of a Nikon TS-100 fluorescence inverted microscope with a Cohu model 4915 charge-coupled device (CCD) camera (Nikon, Melville, NY). Fluorescent images were captured alternately at the excitation wavelengths of 340 and 380 nm with an emission wavelength of 520 nm, which were analyzed with InCyt Im2 version 4.62 imaging software (Intracellular Imaging Inc., Cincinnati, OH).

A standard curve was used to derive experimental $[Ca^{2+}]_i$ values. The standard curve was generated by using various concentrations of Ca^{2+} (Calcium Calibration Buffer Kit; Invitrogen, Carlsbad, CA)in the presence of indicator dye Fura-2 free acid (Invitrogen, Carlsbad, CA). During each experiment, background fluorescence was estimated for a region without cells, and this value was automatically subtracted from the measured emission of each channel. The ratios of cell emissions were compared to the CB-64D, a prototypic σ_2 agonist,(Crawford and Bowen, 2002) curve and both the ratio and $[Ca^{2+}]_i$ were displayed.

5.4 RESULTS AND DISCUSSION

5.4.1 Opioid and Sigma Receptor Binding

All of the tested compounds exhibited low to negligible affinity (1,100->10,000 nM) for opioid receptors (μ, δ, and κ). Among the tested compounds, compound **43** displayed the highest affinity for the σ_1 receptor (4.6 nM), with the greatest selectivity for the σ_1 receptor when compared to the σ_2 receptor (σ_1/σ_2 244). Reduction of a double bond on the cinnamyl group to give **44** decreased affinity at σ_1 and σ_2 receptors (59 nM and 3840 nM, respectively). Introduction of a phenylpropyl group on the oxygen position of compound **46** led to compound **47**, which displayed higher selectivity for σ_1 (compared to **46**) with 84 nM affinity. In contrast, introduction of a phenylpropyl group on the oxygen position of compound **49** to give compound **50** resulted in dramatic decreases in both σ_1 and σ_2 receptor affinities (compared to **49**), exhibiting low affinity at σ_1 (793 nM) receptors and negligible affinity at σ_2 receptors (>10,000 nM). In agreement with previous reports (Maeda et al., 2002a), increasing the chain length from phenethyl (**46**) to phenylpropyl (**49**) gave rise to higher σ_1 and σ_2 affinities as indicated by **49**. These results further suggest that the phenylpropylamines are required for σ_2 activity. Surprisingly, compounds **72** and **73**, which closely resemble AC927, exhibited low to negligible affinities for both σ_1 and σ_2 receptors, suggesting that the position of the phenylpropyl substituent, which tends to increase the affinity (Maeda et al., 2002a), is not optimal for σ receptor binding. Additionally, compounds **64** and **66** displayed little to no affinity at σ receptors. Compounds **46** and **49** were selected, as they produced no affinity for the opioid

receptors, to undergo further *in vivo* testing in order to determine the compounds' ability to block cocaine-induced convulsions.

Table 5.1 Opioid and Sigma Binding Affinity Data

	Opioid Binding			Sigma Binding		
	K_i± SEM (nM)			K_i± SEM (nM)		Selectivity
Compd	μ[a]	δ[b]	K[c]	σ_1[d]	σ_2[e]	σ_2/σ_1
43	4200 ± 135	>10,000	ND[f]	4.6 ± 0.2	1123 ± 29	244
44	2340 ± 74	>10,000	ND[f]	59 ± 2	3840 ± 803	65
46	>10,000	ND[f]	ND[f]	116 ± 8	223 ± 6	2
47	1640 ± 41	>10,000	>10,000	84 ± 15	566 ± 31	7
49	>10,000	ND[f]	ND[f]	18.6 ± 0.9	6.7 ± 0.3	0.4
50	3090 ± 61.9	6210 ± 774	ND[f]	793 ± 76	>10,000	> 13
64	>10,000	ND[f]	ND[f]	2180 ± 331	>10,000	> 5
66	1110 ± 91	6390 ± 206	>10,000	8360 ± 1048	>10,000	> 1
72	>10,000	ND[f]	ND[f]	4110 ± 357	>10,000	> 2
73	>10,000	ND[f]	ND[f]	258 ± 14	>10,000	> 39
AC927*				30 ± 2	138 ± 18	5

[a]μ = Displacement off [^{3}H]DAMGO
[b]δ = Displacement of [^{3}H]DPDPE
[c]κ = Displacement of [^{3}H]U69,593
[d]σ_1 = Displacement of [^{3}H](+)-pentazocine
[e]σ_2 = Displacement of [^{3}H]DTG in presence of (+)-pentazocine
[f]ND = not determined
* Citations reference previously known compounds and results ref. (Maeda et al., 2002b)

5.4.2 Cocaine-Induced Convulsions

Results demonstrate that pretreatment of Swiss Webster mice with compound **46** led to dose-dependent attenuation of the convulsive effects following 70 mg/kg dose of cocaine (Figure 5.3A). Fisher's exact tests showed a statistically significant ($p < 0.05$) reduction in cocaine-induced convulsions at a 30 mg/kg dose of compound **46**. As expected, pretreatment of mice with compound **49** resulted in more significant attenuation of cocaine-induced convulsions at a lower dose than **46** (Figure 5.3B), presumably due to its

high affinity for the σ receptors. Fisher's exact tests revealed that a significant reduction in convulsions was obtained with 1 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.01$) doses of compound **49**. These results show that the newly synthesized compound **49**, an analog of AC927, dose dependently produced significant reductions in cocaine-induced convulsions. These results suggest that compounds **46** and **49** may in part produce their protective effects through the σ receptors.

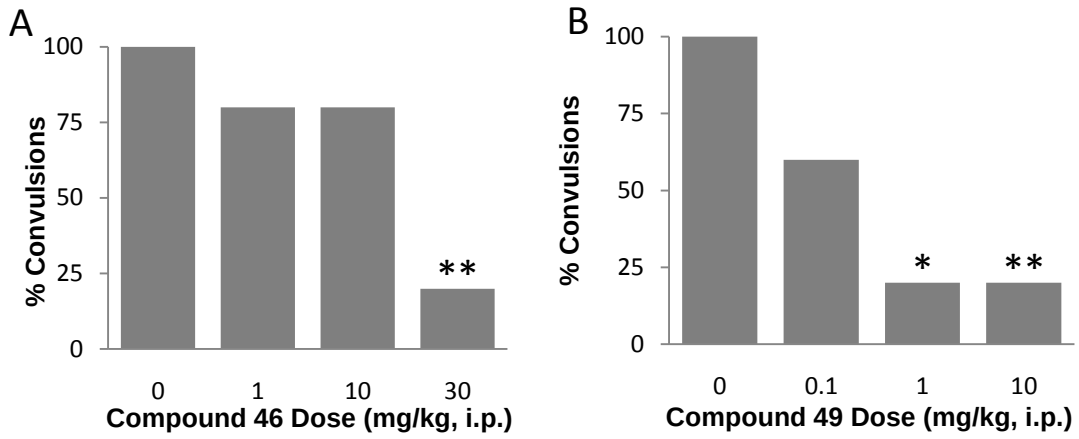


Figure 5.3 Cocaine-induced convulsions. A: pretreatment of Swiss Webster mice with compound **46** (0-30 mg/kg i.p.), followed by a convulsive dose of cocaine (70 mg/kg i.p.) produces a dose-dependent reduction in the convulsive effects of cocaine. B, pretreatment of Swiss Webster mice with compound **49** (0-10 mg/kg i.p.), followed by a convulsive dose of cocaine (70 mg/kg i.p.) produces a dose-dependent reduction in the convulsive effects of cocaine. *, $p < 0.05$; **, $p < 0.01$; Fisher's exact test.

5.4.3 $[Ca^{2+}]_i$ Measurements

Intracellular Ca^{2+} plays an important role in multiple cellular processes such as growth, cell differentiation, and cellular stimulus-secretion coupling.(Clapham, 1995; Simpson et al., 1995; Berridge et al., 1998) Additionally, the release of intracellular Ca^{2+} can lead to apoptosis in several cell types (Berridge et al., 1998). Previous studies have shown that σ_2 agonists can activate the release of Ca^{2+} from the intracellular storage system and produce cell death upon continued exposure (Vilner et al., 1995). The human SK-N-SH

neuroblastoma cells which express both σ_1 and σ_2 receptors (Vilner and Bowen, 2000), were utilized to determine the effect of compounds **46** and **49** on intracellular Ca^{2+} levels. Figure 5.4 displays time versus calcium concentration for compounds **46**, **49**, and CB-64D the prototypical σ_2 agonist, at a single dose of 30 μM. Slight increases in $[Ca^{2+}]_i$ was observed following injection of the tested compounds **46** and **49** at 26 seconds. Compound **46** produced a larger Ca^{2+} signal as compared to **49**. However, the peak levels of the tested compounds were much lower compared to that produced by CB-64D. Their inability to produce intracellular Ca^{2+} release suggests that both compounds **46** and **49** are producing antagonist like effects upon binding to the σ_2 receptors. To validate that the compounds are indeed antagonists, in the future studies, compounds **46** and **49** will be tested for their ability to attenuate the signal produced by CB-64D.

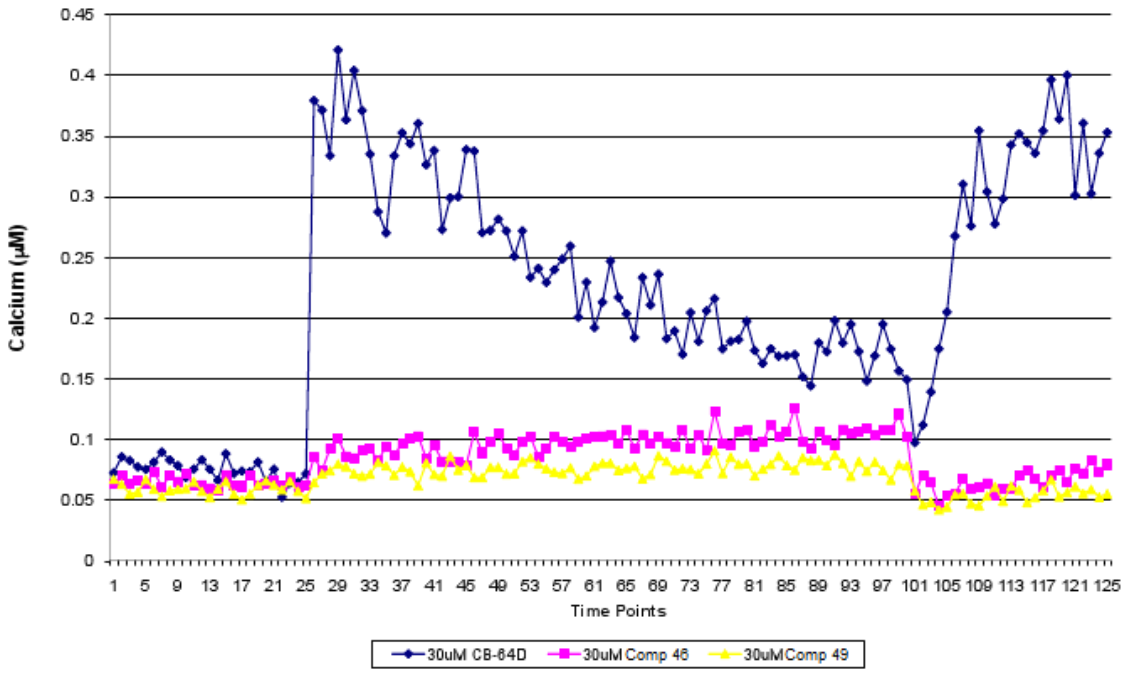


Figure 5.4 Effect of compounds **46**, **49**, and CB-64D on $[Ca^{2+}]_i$ in human SK-N-SH neuroblastoma cells.

5.5 CONCLUSION

Among the tested compounds, **43** produced the highest selectivity and binding affinity for the σ_1 receptor, even higher than that produced by the reported AC927.(Maeda et al., 2002b) Results from binding assays indicate that both compounds **46** and **49** show significant preference for the σ receptors over the opioid receptors. In agreement with previous results,(Maeda et al., 2002b) increasing the chain length from phenethyl (**46**) to phenylpropyl (**49**) increased affinity at both σ receptor subtypes. σ Receptor ligands **46** and **49** produced low $[Ca^{2+}]_i$ signals, suggesting that these compounds act as σ_2 antagonists. Intriguingly, compound **49** produced significant reduction in cocaine-induced convulsions with 1 mg/kg ($p < 0.05$) and 10 mg/kg ($p < 0.01$) doses of compound, while compound **46** had significant ($p < 0.05$) reduction in cocaine-induced convulsions at a 30 mg/kg dose. These results provide further evidence of σ involvement in the actions of cocaine and identifies compound **49** as a viable lead compound for the development of cocaine treatment.

5.6 EXPERIMENTAL

Compounds discussed in this Chapter were prepared using standard methods or following novel synthetic routes as discussed in Chapter 3. All reagents and solvents were purchased from Sigma Aldrich Inc. (St. Louis, MO) and used without further purification. All reactions were carried out under an atmosphere of nitrogen. Thin layer

chromatography was performed on silica 60 F_{254} plated (Analtech, Inc., Newark, DE). All compounds were purified using standard techniques (crystallization, etc.) and characterized using standard spectroscopic methods such as ^{1}H NMR (Varian Inova 500 MHz) and MS (ThermoFinnigan LCQ Classic, Waltham, MA). Melting points were determined using Mel-Temp (Laboratory Devises, Cambridge, MA) apparatus. Elemental analysis was performed by Atlantic Microlabs (Norcross, GA).

***trans*-2-(Cinnamyloxy)-*N,N*-dimethylcyclohexanamine** (**43**, **UMB400**) Yield 10% (0.18 g); ^{1}H NMR (D_2O) δ 7.52 (d, 3.56 Hz, 2H), δ 7.42 (t, 7.33 Hz, 2H), δ 7.33-7.38 (m, 1H), δ 6.73-6.79 (m, 1H), δ 6.36-6.44 (m, 1H), δ 4.36-4.41 (m, 1H), δ 4.20-4.26 (m, 1H), δ 3.65-3.72 (m, 1H), δ 3.15-3.22 (m, 1H), δ 2.80 (s, 6H), δ 2.38-2.44 (m, 1H), δ 2.07 (d, 5.86 Hz, 1H) δ 1.87 (d, 6.07 Hz, 1H), δ 1.76-1.81 (m, 1H), δ 1.41-1.51 (m, 1H), δ 1.19-1.38 (m, 4H); MS ESI m/z = 260 (M+ H^+); Anal. ($C_{19}H_{31}NO$ $(C_2H_2O_4)_1$) C, H, N.

***trans*-*N,N*-Dimethyl-2-(3-phenylpropoxy)cyclohexanamine** (**UMB401**, **44**) Yield 7% (0.092 g); ^{1}H NMR (D_2O) δ 7.41 (t, 7.67 Hz, 2H), δ 7.26-7.33 (m, 3H), δ 3.69-3.76 (m, 1H), δ 3.47-3.59 (m, 2H), δ 3.11-3.19 (m, 1H), δ 2.68-2.94 (m, 8H), δ 2.28-2.35 (m, 1H), δ 2.04-2.11 (m, 1H), δ 1.91-1.99 (m, 2H), δ 1.89 (d, 6.79 Hz, 1H), δ 1.75-1.81 (m, 1H); MS ESI m/z = 262 (M+ H^+); Anal. ($C_{19}H_{29}NO$ $(C_2H_2O_4)_2$) C, H, N.

***trans*-2-(methyl(phenethyl)amino)cyclohexanol (46 ,UMB408)** Yield 70% (3.04 g); ^{1}H NMR (D_2O) δ 7.44 (t, 7.23 Hz, 2H), δ 7.35-7.40 (m, 3H), δ 3.74-3.86 (m, 1H), δ 3.51-3.64 (m, 1H), δ 3.38-3.46 (m, 1H), δ 3.01-3.31 (m, 3H), δ 2.98 (s, 1H), δ 2.83 (s, 2H), δ

2.01-2.15 (m, 2H), δ 1.82-1.90 (m, 1H), δ 1.71-1.79 (m, 1H), δ 1.21-1.55 (m, 4H); MS ESI m/z = 234 (M+ H^+); Anal. ($C_{15}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

***N*-Methyl-*N*-phenethyl-2-(3-phenylpropoxy)cyclohexanamine (47 ,UMB404)** Yield 6% (0.14 g); ^{1}H NMR (D_2O) δ 7.33-7.47 (m, 6H), δ 7.26-7.33 (m, 4H), δ 3.59-3.69 (m, 3H), δ 3.39-3.48 (m, 1H), δ 3.25-3.32 (m, 1H), δ 3.16-3.24 (m, 1H), δ 3.09-3.15 (m, 1H), δ 2.94-3.00 (m, 2H), δ 2.80-2.84 (m, 1H), δ 2.59-2.68 (m, 2H), δ 2.27-2.33 (m, 1H), δ 2.01-2.14 (m, 1H), δ 1.73-1.90 (m, 4H), δ 1.44-1.56 (m, 1H), δ 1.11-1.39 (m, 4H); MS ESI m/z = 352 (M+ H^+); Anal. ($C_{24}H_{33}NO$ $(C_2H_2O_4)_1$) C, H, N.

***trans*-2-(Methyl(3-phenylpropyl)amino)cyclohexanol (49, 414)** Yield 52.4% (1.26 g); ^{1}H NMR (D_2O) δ 7.41 (t, 7.41 Hz, 2H), δ 7.30-7.30 (m, 3H), δ 3.71-3,79 (m, 1H), δ 3.05-3.34 (m, 3H), δ 2.91-3.00 (m, 1H), δ 2.88 (s, 1H), δ 2.70-2.83 (m, 4H), δ 2.00-2.20 (m, 3H), δ 1.89-1.96 (m, 1H), δ 1.79-1.88 (m, 1H), δ 1.70-1.78 (m, 1H), δ 1.21-1.48 (m, 4H); MS ESI m/z = 248 (M+ H^+); Anal. ($C_{16}H_{25}NO$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$) C, H, N.

***trans*-*N*-Methyl-2-(3-phenylpropoxy)-*N*-(3-phenylpropyl)cyclohexanamine (50, UMB415)** Yield 33% (0.29 g); ^{1}H NMR (D_2O) δ 7.23-7.32 (m, 5H), δ 7.14-7.24 (m, 5H), δ 3.54-3.62 (m, 1H), δ 3.40-3.47 (m, 1H), δ 3.18-3.26 (m, 1H), δ 2.54-2.75 (m, 5H), δ 2.42-2.51 (m, 1H), δ 2.28-2.38 (m, 3H), δ 2.06-2.14 (m, 1H), δ 1.84-1.93 (m, 2H), δ 1.72-1.83 (m, 3H), δ 1.63-1.72 (m, 2H), δ 1.53-1.60 (m, 1H), δ 1.04-1.30 (m, 4H); MS ESI m/z = 366 (M+ H^+); Anal. ($C_{25}H_{35}NO$ $(C_2H_2O_4)_1$) C, H, N.

***N,N*-Dimethyl-1-(8-(3-phenylpropoxy)-1,4-dioxaspiro[4.5]decan-8-yl)methanamine (64, UMB410)** Yield 65% (0.5 g); ^{1}H NMR (D_2O) δ 7.34-7.39 (m, 2H), δ 7.24-7.33 (m, 3H), δ 4.02 (s, 4H), δ 3.42 (t, 6.48 Hz, 2H), δ 3.32 (s, 2H), δ 2.92 (s, 6H), δ 2.75 (t, 7.34 Hz, 2H), δ 1.90-1.99 (m, 4H), δ 1.77 (t, 13.10 Hz, 2H), δ 1.65-1.72 (m, 2H), δ 1.57 (t, 13.10 Hz, 2H); MS ESI m/z = 334 (M+ H^+); Anal. ($C_{20}H_{31}NO_3$ $(C_2H_2O_4)_1$ $(H_2O)_{0.25}$) C, H, N.

***N,N*-Dimethyl-1-(3-(3-phenylpropoxy)-2,3,4,4a,9,9a-hexahydro-1H-carbazol-3-yl) methanamine (66, UMB412)** Yield 43% (0.12 g); ^{1}H NMR (D_2O) δ 7.48 (t, 8.24 Hz, 2H), δ 7.10-7.24 (m, 5H), δ 6.98-7.04 (m, 2H), δ 3.52-3.58 (m, 1H), δ 3.50-3.52 (m, 1H), δ 3.38-3.45 (m, 1H), δ 3.07-3.14 (m, 1H), δ 2.90-3.02 (m, 7H), δ 2.74-2.86 (m, 3H), δ 2.50-2.64 (m, 3H), δ 2.28-2.35 (m, 1H), δ 1.93-2.01 (m, 1H), δ 1.70-1.88 (m, 1H); MS ESI m/z = 365 (M+ H^+); Anal. ($C_{24}H_{32}N_2O$ $(C_2H_2O_4)_1$) C, H, N.

1-Methyl-3-(3-phenylpropoxy)piperidine (72, UMB386) Yield 40% (0.81 g); ^{1}H NMR (D_2O) δ 7.41 (t, 7.46 Hz, 2H), δ 7.27-7.35 (m, 3H), δ 3.89 (s, 1H), δ 3.59-3.68 (m, 1H), δ 3.52-3.58 (m, 2H), δ 3.46 (d, 6.22 Hz, 1H), δ 3.07-3.14 (m, 1H), δ 3.01 (t, 12.73 Hz, 1H), δ 2.90 (s, 1H), δ 2.84 (s, 2H), δ 2.68-2.77 (m, 3H), δ 1.88-2.06 (m, 4H), δ 1.53-1.63 (m, 2H); MS ESI m/z = 234 (M+ H^+); Anal. ($C_{15}H_{23}NO$ $(C_2H_2O_4)_1$) C, H, N.

3-(cinnamyloxy)-1-methylpiperidine (73, UMB387) Yield 31% (0.62 g); ^{1}H NMR (D_2O) δ 7.50 (d, 3.84 Hz, 2H), δ 7.32-7.47 (m, 3H), δ 6.71-6.77 (m, 1H), δ 6.34-6.46 (m, 1H), δ 4.22-4.34 (m, 2H), δ 4.05 (s, 1H), δ 3.59 (d, 6.53 Hz, 1H), δ 3.44 (d, 6.15 Hz, 1H),

δ 3.12 (d, 6.53 Hz, 1H), δ 3.02 (t, 12.11 Hz, 1H), δ 2.83-2.92 (m, 3H), δ 1.97-2.12 (m, 2H), δ 1.78-1.88 (m, 1H), δ 1.58-1.68 (m, 1H); MS ESI m/z = 232 (M+ H^+); Anal. ($C_{18}H_{26}NO$ $(C_2H_2O_4)_1$ $(H_2O)_?$) C, H, N.

Chapter 6. Conclusions and Future Studies

6.1 INTRODUCTION

Patients experiencing severe to moderate pain are frequently exposed to opioid therapy (Zieglgansberger et al., 1995; Stein et al., 2003). While opioid analgesics are most effective at relieving pain, they are often accompanied by respiratory depression, chronic opioid induced constipation (McNicol et al., 2003; Benyamin et al., 2008), tolerance, and dependence (Kieffer and Evans, 2002). Though the extent of some side effects can be treated with additional medications (Klaschik et al., 2003; Yuan et al., 2005; Lavine, 2008), there is an urgent need for the development of pharmacotherapies with reduced side effects.

The focus of this dissertation is to develop opioid analgesics that display functional selectivity. Our studies challenged the existing skeleton via the development of μ-opioids lacking the A-ring, traditionally considered essential for opioid activity (Casy and Parfitt, 1986). Removal of the A-ring will allow the skeleton to adopt an alternative binding mode with the receptor thereby causing alternate receptor trafficking (Ignatova et al., 1999) and post receptor mechanism all of which are involved in the development of adverse effects commonly associated with opioid therapy (Kieffer and Evans, 2002). The importance of the phenolic A-ring is derived from a belief that the A-ring mimics the tyrosine residue on enkaphalin (Andersson et al., 1995), which strongly suggests that the removal of this tyrosine mimetic in the morphinan class would result in total loss in affinity for opioid receptors. In order to overcome the loss in activity, extremely potent opioids were considered for the development of a novel opioid skeleton. It is evident

from previous studies that 14-phenylpropyloxymorphinans achieve most optimal potency (Schutz et al., 2003), suggesting that the 14-phenylpropyloxy group has a major effect on receptor binding and is responsible for the dramatic increase in potency. It was therefore hypothesized that opioid activity could be achieved in the presence of a phenylpropyloxy group and a basic amine.

6.2 *N*-substituent optimization

The overall goal of this research was to delineate the minimal structural requirements for high affinity at the μ receptor. Accordingly, a series of phenylpropyloxyethylamines was synthesized as described in Chapter 2 and analyzed for opioid receptor binding affinity. Differing *N*-substituents were evaluated to develop SAR for the novel series of compounds. Using the CSP approach, we validated that the aromatic moiety coming off the oxygen does not mimic the A-ring. Moreover, we found that the phenylpropyloxyethylamines are capable of binding to the μ opioid receptor possessing a fairly weak affinity while maintaining negligible affinity for κ and δ receptors. Based on the molecular modeling and opioid binding studies, we have identified the optimal *N*-substituent as the *N*-phenethyl, with 1680 nM affinity for the μ opioid receptor.

6.3 Ring-constrained phenylpropyloxyethylamines

To determine the bioactive conformation, and aid in future modeling studies, constrained rings B, C, and D were re-introduced back into the system iteratively. In agreement with our hypothesis, compounds lacking a phenylpropyl group (*trans*-2-

(methyl(phenethyl)amino)cyclohexanol and *trans*-2-(methyl(phenylpropyl)amino) cyclohexanol) were not capable of binding to the opioid receptors indicating that the phenylpropyloxy group is essential for binding activity. Binding studies showed that the B-ring analog containing the *N,N*-dimethyl substituent, produced the highest affinity of 2340 nM, while the C- and D-ring analogs were fully inactive. Further optimization was achieved by combining the B-ring with the optimal *N*-substituent, phenethyl, to give *N*-methyl-*N*-phenethyl-2-(3-phenylpropoxy)cyclohexanamine which had 1640 nM affinity at μ receptors. The interaction with the μ opioid receptor was greatly improved when the C-ring analog was modified to contain an indole group, (*N,N*-dimethyl-1-(3-(3-phenylpropoxy)-2,3,4,9-tetrahydro-1H-carbazol-3-yl)methanamine) resulting in 1110 nM affinity for the μ opioid receptor. Additionally, this compound produced weak affinity (6400 nM) for δ and negligible affinity (>10,000) for κ opioid receptors. These results indicate that *N,N*-dimethyl-1-(3-(3-phenylpropoxy)-2,3,4,9-tetrahydro-1H-carbazol-3-yl)methanamine is a viable lead compound for optimization studies. Future studies will include efficacy (% stimulation) and potency (EC_{50}) determination using the GTPγS assay. Antinociception will be assessed by the gold standard tail-flick (TF), hot-plate (HP), and paraphenylquinone abdominal-stretching (PPQ) assays.

6.4 Phenylpropyloxyethylamines containing multiple rings

It is anticipated that the inactivity of the D-ring analog was a result of flawed D-ring conformation. To further investigate, we proposed to synthesize the B/D ring analog. Numerous attempts have been made to re-introduce the B- and D- ring systems to determine the bioactive conformation and aid in future modeling studies. Although

compounds leading up to the cyclization step have been successfully synthesized, the cyclization step proved to be difficult for all of the attempted methods. The methodology explored in this dissertation will aid in the future synthesis of phenylpropyloxyethylamines.

The ultimate goal of this research is to develop ring-constrained phenylpropyloxyethylamine analogs that will enhance future modeling studies and aid in the design of improved opioid analgesics. To achieve this goal, future studies will continue toward the development of phenylpropyloxyethylamines containing polycyclic ring systems mimicking the B/D ring system. Additionally, compounds containing ring systems B/C and C/D will be synthesized and optimization of the final product will be achieved by introducing the optimal *N*-substituent determined as *N*-phenethyl.

6.5 Sigma receptor antagonist

This project investigated the effect of ring-constrained phenylpropyloxyethylamines on σ receptors. Though the compounds discussed here were initially synthesized as opioid ligands, their close resemblance to AC927 prompted our decision to further investigate the partial opioid structures at the two established σ receptors subtypes (σ_1, σ_2). Among the tested compounds, *trans*-2-(cinnamyloxy)-N,N-dimethylcyclohexanamine produced the highest selectivity and binding affinity for the σ_1 receptor, even higher than that produced by the reported AC927 analog (Maeda et al., 2002b). Results from binding assays indicate that both compounds *trans*-2-(methyl(phenethyl)amino)cyclohexanol and *trans*-2-(methyl(3-phenylpropyl)amino) cyclohexanol are sigma selective ligands. In

agreement with previous results (Maeda et al., 2002b), increasing the chain length from phenethyl (*trans*-2-(methyl(phenethyl)amino)cyclohexanol) to phenylpropyl (*trans*-2-(methyl(3-phenylpropyl)amino)cyclohexanol) increased affinity at both the σ receptors while showing preference for the σ_2 receptors. σ Receptor ligands *trans*-2-(methyl(phenethyl)amino)cyclohexanol and *trans*-2-(methyl(3-phenylpropyl)amino) cyclohexanol produced low $[Ca^{2+}]_i$ signals, indicating that the compounds are either antagonists or weak partial agonists. Perhaps the most interesting finding was that *trans*-2-(methyl(3-phenylpropyl)amino)cyclohexanol produced significant reduction in cocaine-induced convulsions. (need to improve rationale for pharmacology conclusions) These results provide further evidence of σ involvement in the actions of cocaine and identifies compound *trans*-2-(methyl(3-phenylpropyl)amino) cyclohexanol as a viable lead compound for further anti-cocaine studies.

6.6 CONCLUSION

The focus of this thesis dissertation is determining the minimal structural requirements for high affinity at the μ opioid receptor. Accordingly a series of phenylpropyloxyethylamines, novel opioid analogs lacking the A-ring have been synthesized and pharmaologically evaluated. Opioid binding studies showed that the optimal *N*-substituent is the *N*-phenethyl, specifically the 2-(cinnamyloxy)-*N*-methyl-*N*-phenethylethanamine analog, which has an affinity of 1680 nM for μ opioid receptors. Subsequently, rings B, C, and D from the morphine skeleton were systematically re-introduced as ring-constrained analogs. Binding studies showed that the B-ring analog

containing the *N*,*N*-dimethyl substituent produced the highest affinity of 2340 nM, while the C- and D-ring analogs were fully inactive. Furthermore, by combining the B-ring with the optimal *N*-substituent, phenethyl, we were able to achieve 1640 nM affinity at μ. Moreover, upon introduction of an indole group into the C-ring analog, *N*,*N*-dimethyl-1-(3-(3-phenylpropoxy)-2,3,4,9-tetrahydro-1H-carbazol-3-yl)methanamine, the affinity was increased to 1110 nM, which represents a viable lead compound for optimization studies.

Additional studies investigated the effect of ring-constrained phenylpropyloxyethylamines on σ receptors. Binding studies showed that *trans*-2-(cinnamyloxy)-*N*,*N*-dimethylcyclohexanamine produced the highest selectivity and binding affinity for the σ_1 receptor, even higher than that produced by the reported AC927 (Maeda et al., 2002b). Most interestingly, *trans*-2-(methyl(3-phenylpropyl)amino) cyclohexanol, a high affinity σ_2 preferring antagonist, showed significant reduction in cocaine-induced convulsions. These results provide further evidence of σ involvement in the actions of cocaine and identifies compound *trans*-2-(methyl(3-phenylpropyl)amino) cyclohexanol as a viable lead compound for further anti-cocaine studies.

Appendix A. The effect of the pyridyl nitrogen position in pyridylpiperazine sigma ligands.

A.1 INTRODUCTION

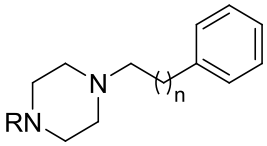
The continued growth in the abuse of methamphetamine necessitates the urgent development of pharmacotherapies. No pharmacotherapies for methamphetamine abuse currently exist and efforts have mainly focused on the development of therapies for the dopaminergic systems (Gundlach et al., 1986; Booth and Baldessarini, 1991; Bastianetto et al., 1995; Weiser et al., 1995). Our studies have utilized the fact that methamphetamine interacts with sigma receptors (Itzhak, 1993; Nguyen et al., 2005) and sigma antagonists attenuate both the stimulant and neurotoxic effects of methamphetamine. Although sigma receptors were first thought to be a subtype of opioid receptors, they are now considered to be a unique class of receptors (Martin et al., 1976) comprised of two subtypes, σ_1 and σ_2 (Quirion et al., 1992). σ_1 Receptors have been cloned (Prasad et al., 1998; Mei and Pasternak, 2001) and are involved in intracellular signaling, synaptic transmission, modulation of inositol phosphates, protein kinases, and calcium (Morin-Surun et al., 1999; Hayashi et al., 2000; Hayashi and Su, 2001; Guitart et al., 2004; Bermack and Debonnel, 2005). In addition, σ_1 antagonists reduce the convulsive, lethal, locomotor stimulatory and rewarding actions of cocaine in mice (Romieu et al., 2000; Matsumoto and Mack, 2001; Matsumoto et al., 2001; Matsumoto et al., 2002; Romieu et al., 2004). σ_2 Receptors have not yet been cloned; however they appear to be comprised of heterodimers and are smaller in size compared to σ_1 (Hellewell and Bowen, 1990; Moebius et al., 1993; Hellewell et al., 1994). Further studies have demonstrated that σ_1 selective antagonists reduce the stimulant effects of methamphetamine, while AC927 (*N*-phenethylpiperidine), a mixed σ_1 and σ_2 antagonist, attenuates the locomotor stimulant and neurotoxic effects of methamphetamine in mice (Nguyen et al., 2005; Matsumoto et

al., 2007b). A selective σ_2 antagonist is therefore urgently required to further study the relationship between σ_2 antagonism and methamphetamine neurotoxicity.

Truly selective σ_2 antagonists continue to be the goal of several research groups (Xu et al.; Mesangeau et al., 2008; Chu et al., 2009). One of the major disadvantages of the current σ_2 antagonists is their ability to bind to the dopamine receptors, opioid receptors, and N-methyl-D-aspartate (NMDA) receptors (Matsumoto, 2007). Recent studies showed that CM156 (3-(4-(4-cyclohexylpiperazin-1-yl)butyl)benzo[*d*]thiazole-2(3*H*)-thione) exhibits better affinity for the sigma receptor however, it has poor metabolic stability (Xu et al.). Studies performed previously by our laboratory have showed that *N*-(2-pyridyl)piperazines not only have the tendency to favor σ_2 receptors but they also favor sigma receptors over opioid and NMDA receptors with low affinity for the dopamine receptor.(Maeda et al., 2002a; Matsumoto et al., 2007a) Specifically, compound **5**, 1-(2-Phenylethyl)-4-(2-pyridyl)piperazine, produced protective actions against cocaine induced convulsions which provides evidence that compound **5** is an antagonist (Matsumoto et al., 2003; Matsumoto et al., 2007a). Moreover, 1-(3-phenylpropyl)-4-(2-pyridyl)piperazine, **6**, has 17-fold preference for the σ_2 receptor, over σ_1 (Maeda et al., 2002a). In an effort to design a pharmacophore for selective σ_2 antagonism in this series, we have investigated the effect of pyridyl nitrogen position and chain length in the phenylalkylpiperazinepyridine series.

A.2 CHEMISTRY

Compounds **1-4** (Figure 1) were prepared by the alkylation of the corresponding halogenated alkyl phenyls with the appropriate pyridinylpiperazine in the presence of K_2CO_3 in DMF at room temperature. and purified as oxalate salts from methanol (Maeda et al., 2002a). All salt targets were characterized using NMR and MS and all elemental analyses of salts were within ±0.4%.



1 R = 4-pyridyl, n = 1
2 R = 4-pyridyl, n = 2
3 R = 3-pyridyl, n = 1
4 R = 3-pyridyl, n = 2
5* R = 2-pyridyl, n = 1
6* R = 2-pyridyl, n = 2

Figure 1. Phenylalkylpiperazinepyridines

*Reported in reference ref. (Maeda et al., 2002b)

A.3 PHARMACOLOGY

In vitro competition binding assays were preformed as follows. Preparation of rat brain membrane and binding assays for the σ_1 and σ_2 receptor were performed as previously described in detail (Matsumoto et al., 1995; Matsumoto et al., 2008). In brief, σ_1 receptors were labeled with 5 nM [^{3}H](+)-pentazocine. The σ_2 receptors were labeled with 3 nM [^{3}H]di-o-tolylguanidine (DTG) in the presence of 300 nM (+)-pentazocine to block σ_1 receptors. Nonspecific binding was determined in the presence of 10 µM

haloperidol. Ten concentrations of each sigma compound (0.1–10,000 nM) were used in the assays. The compounds were incubated for 120 min at 25°C to measure their ability to displace the radioligands from their binding sites. Termination of the reaction was achieved through rapid vacuum filtration over glass fiber filters which were previously soaked in 1% polyethyleneimine for at least 45 min. K_i values were calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

A.4 RESULTS AND DISCUSSION

All compounds possessed affinity at both σ_1 and σ_2 receptors (Table 1). As shown previously, (2-pyridyl)piperazines (**5**,**6**) favored σ_2 receptors (Maeda et al., 2002a), while (3-pyridyl)piperazines (**3**,**4**) and (4-pyridyl)piperazines (**1,2**) showed preference for σ_1 receptors. Similar binding affinities were achieved by the (4-pyridyl)piperazine compounds (**1**,**2**) independent of the chain length, whereas the phenylpropyl linker in both (3-pyridyl)piperazine and (2-pyridyl)piperazine resulted in higher affinity for both σ_1 and σ_2 receptors. All new compounds showed significantly lower affinity for σ_2 receptors than our lead compound **6**.

Table1. Binding affinities of phenylalkylpiperazinepyridines **1-6** at sigma receptors.

	K_i (nM)±SEM		Selectivity
Cmpds	σ_1[a]	σ_2[b]	σ_1/σ_2
1	41.8 ± 5.9	69.7 ± 6.3	0.60
2	34.2 ± 2.8	84.0 ± 5.9	0.41
3	97.2 ± 6.9	440 ± 20	0.22
4	21.2 ± 2.3	110.0 ± 8.6	0.19
5[*]	326 ± 41.2	119 ± 3.8	2.7
6[*]	82.9 ± 0.21	4.91 ± 0.77	16.9

*Citations reference previously known compounds and results ref. (Maeda et al., 2002b)
[a] Displacement of [^{3}H](+)-pentazocine
[b] Displacement of [^{3}H]DTG in presence of (+)-pentazocine

A.5 CONCLUSION

In summary, binding affinity studies showed that the (3-pyridyl)piperazines and (4-pyridyl)piperazines have lower affinity for σ_2 receptors, than the previously reported lead compound **6**. Moreover, both new series lost σ_2 selectivity, indicating that (2-pyridyl)piperazines are optimal for the development of highly selective σ_2 ligands.

A.6 EXPERIMENTAL

All reagents and solvents were purchased from Sigma Aldrich Inc. unless stated otherwise and used without further purification. All reactions were carried out under an atmosphere of nitrogen. Thin layer chromatography was performed on silica 60 F_{254} plated (Analtech, Inc., Newark, DE). All compounds were purified using standard

techniques (crystallization, etc) and characterized using standard spectroscopic methods such as ^{1}H NMR (Varian Inova 500 MHz) and MS (ThermoFinnigan LCQ Classic, Waltham, MA). Melting points were determined using Mel-Temp (Laboratory Devises) apparatus. Elemental analysis was performed by Atlantic Microlabs (Norcross, GA).

1-(2-Phenylethyl)-4-(4-pyridyl)piperazine (1). To obtain target **1,** 1-(4-pyridyl)piperazine (1eq.) was reacted with with phenethylbromide (1eq.) 2 in the presence of K_2CO_3 (10 eq.) in DMF (20 mL/g). The reaction mixture was allowed to stir overnight at room temperature. After completion by TLC, the reaction was quenched with H_2O and extracted with Et_2O. The combined organic layers were washed with brine solution and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was purified from methanol and oxalic acid to produce oxalate salt. Yield 76%; mp 120-121°C; ^{1}H NMR (D_2O) δ 8.11 (d, 3.79 Hz, 2H), δ 7.36 (t, 7.44 Hz, 2H), δ 7.29 (m, 3H), δ 7.10 (d, 3.94 Hz, 2H), δ 3.94 (m, 4H), δ 3.48 (m, 6H), δ 3.08 (t, 7.55 Hz, 2H); MS ESI m/z = 268 (M+ H^+); Anal. ($C_{17}H_{21}N_3$ $(C_2H_2O_4)_{1.5}$) C, H, N.

1-(3-Phenylpropyl)-4-(4-pyridyl)piperazine (2) was prepared through alkylation of 1-(4-pyridyl)piperazine with phenylpropylbromide following the method described above. Yield 37%; mp 180-181°C; ^{1}H NMR (D_2O) δ 8.11 (d, 3.76 Hz, 2H), δ 7.33 (t, 7.14 Hz, 2H), δ 7.24 (t, 7.03 Hz, 3H), δ 7.08 (d, 3.70 Hz, 2H), δ 4.32 (m, 2H), δ 3.67 (m, 2H), δ 3.51 (m, 2H), δ 3.15 (m, 4H), δ 2.69 (t, 7.56Hz, 2H), δ 2.05 (m, 2H); MS ESI m/z = 282 (M+ H^+); Anal. ($C_{18}H_{23}N_3$ $(C_2H_2O_4)_2$ $(H_2O)_1$) C, H, N.

1-(2-Phenylethyl)-4-(3-pyridyl)piperazine (3) was prepared through alkylation of 1-(3-pyridyl)piperazine with phenethylbromide following the method described above. Yield 12%; mp 178-179°C; ^{1}H NMR (D_2O) δ 8.30 (d, 1.43 Hz, 1H), δ 8.13 (d, 2.54 Hz, 1H), δ 8.03 (m, 1H), δ 7.80 (m, 1H), δ 7.36 (t, 6.90 Hz, 2H), δ 7.30 (d, 7.29 Hz, 3H), δ 3.98 (m, 2H), δ 3.68 (m, 3H), δ 3.46 (m, 3H), δ 3.35 (m, 2H), δ 3.09 (t, 7.58 Hz, 2H); MS ESI m/z = 268 (M+ H^+); Anal. ($C_{17}H_{21}N_3$ $(C_2H_2O_4)_{1.5}$ $(H_2O)_{.5}$) C, H, N.

1-(3-Phenylpropyl)-4-(3-pyridyl)piperazine (4) was prepared through alkylation of 1-(3-pyridyl)piperazine with phenylpropylbromide following the method described above. Yield 30%; mp 94-96°C; ^{1}H NMR (D_2O) δ 8.29 (d, 8.29 Hz, 1H), δ 8.13 (d, 2.45 Hz, 1H), δ 8.03 (m, 1H), δ 7.81 (m, 1H), δ 7.34 (t, 7.27 Hz, 2H), δ 7.254 (t, 7.68 Hz, 3H), δ 4.00 (m, 2H), δ 3.67 (m, 2H), δ 3.30 (m, 2H), δ 3.17 (m, 4H), δ 2.70 (t, 7.47 Hz, 2H), δ 2.06 (m, 2H); MS ESI m/z = 282 (M+ H^+); Anal. ($C_{18}H_{23}N_3$ $(C_2H_2O_4)_2$ $(H_2O)_{1.5}$) C, H, N.

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