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Synthesis and SAR study of Meperidine Analogues as Selective Serotonin Reuptake Inhibitors (SSRIs)

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Synthesis and SAR study of Meperidine Analogues as Selective Serotonin Reuptake Inhibitors (SSRIs)

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To my family and friends for all their love and support

Father: Pinzhong Gu

Mother: Jianping Pan

Wife: Yan Wu

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ABSTRACT

Meperidine has been shown to have potent binding affinity for serotonin transporters (SERT) (K_i = 41 nM) and be an inhibitor of serotonin reuptake. Based upon these pharmacological results meperidine has been identified as a lead compound for the development of a novel class of serotonin-selective reuptake inhibitors (SSRIs).

A variety of potent analogues of meperidine have been synthesized and evaluated *in vitro* as potential ligands for the serotonin transporter. Substitutions have been made on the aryl ring, the ester moiety and the piperidine nitrogen of meperidine. Potent analogues of the aryl substituted series that included 4-iodophenyl, 2-naphthyl, 3,4-dichlorophenyl and 4-biphenyl meperidine derivatives were synthesized and chosen for further optimization of the benzyl ester analogues. Benzyl ester analogues included 4-nitro, 4-methoxyl and 3,4-dichloro benzyl analogues and exhibited high potency for serotonin transporters and high selectivity over the dopamine transporter (DAT) and the norepinephrine transporter (NET). Also the *N*-demethylated analogues improve the binding affinity and selectivity for serotonin transporter. The analogue 4-(carboxymethoxybenzyl)-4-(4-iodophenyl) piperidine (**69f**), was found the most potent (K_i=0.6 nM) and selective ligand for serotonin transporter (DAT/SERT >4500; NET/SERT >4500) for the series and has been advanced to *in vivo* evaluation.

Keywords: meperidine, piperidine, serotonin transporter, SSRIs, antidepressant.

INTRODUCTION

Neurotransmitters

Neurotransmitters are endogenous chemicals released from presynaptic neurons to stimulate neighboring neurons, allowing impulses to be passed throughout the nervous system. A nerve impulse arriving at the axon terminal of one neuron stimulates release of a neurotransmitter, which crosses the synaptic cleft to the adjoining neuron's dendrite. (Figure 1).[1]

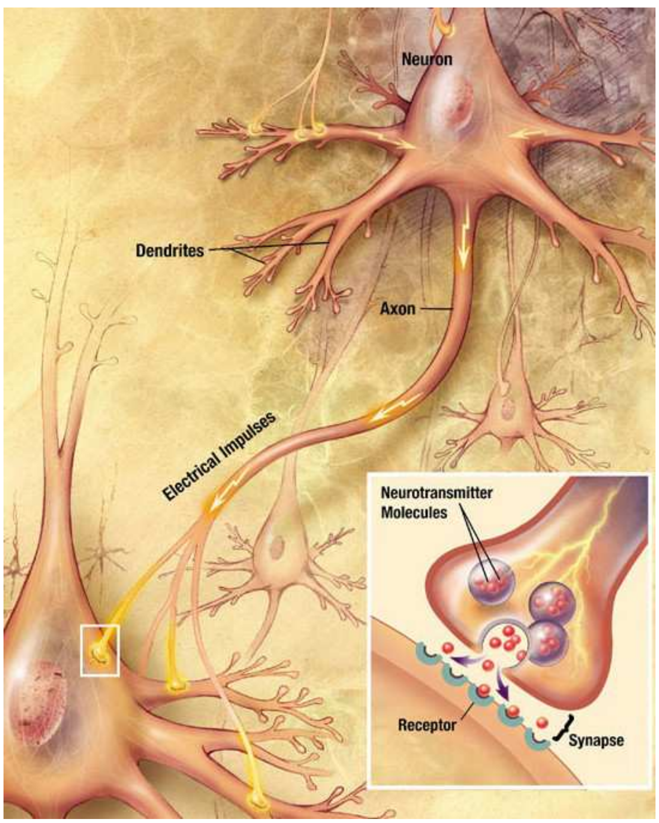


Figure 1. Neuron cell and neurotransmitters[1.]

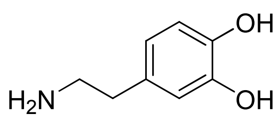
There are many different classifications of neurotransmitters, but most of these neuro-transmitters are divided into following major groups for research purposes:

- **Amino acids**: glutamate,[2] aspartate,[3] serine,[4] γ-aminobutyric acid (GABA),[5] glycine.[6]
- **Monoamines**: dopamine (DA),[7] serotonin (SE, 5-HT),[8] norepinephrine (noradrenaline; NE),[9] epinephrine (adrenaline),[10] histamine,[11] melatonin.[12]
- **Others**: acetylcholine (ACh),[13] adenosine,[14] anandamide,[15] nitric oxide,[16] etc.

All these neurotransmitters are released by specific neurons and showing their different effects on central nervous system. The direct effect of a neurotransmitter is to activate one or more types of receptors. The effect on the postsynaptic cell depends, therefore, entirely on the properties of those receptors. It happens that for some neurotransmitters, the most important receptors have excitatory effects: that is, they increase the probability that the target cell will fire an action potential. For other neurotransmitters, the most important receptors have inhibitory effects. There are, however, other neurotransmitters, such as acetylcholine, for which both excitatory and inhibitory receptors exist,[17,18] and there are some types of receptors that activate complex metabolic pathways in the postsynaptic cell to produce effects that cannot appropriately be called either excitatory or inhibitory.

Monoamine Neurotransmitters

Monoamine neurotransmitters are neurotransmitters that contain one amino group that is connected to an aromatic ring by a two-carbon chain ($-CH_2-CH_2-$). All monoamines are derived from aromatic amino acids like phenylalanine, tyrosine, tryptophan, and the thyroid hormones by the action of aromatic amino acid decarboxylase enzymes.[19] The most important members in this group are dopamine (DA, **1**), serotonin (SE, **2**), and norepinephrine (NE, **3**). Despite their similar structures, they have different roles in neurotransmission and have specific receptor/transporter mechanism.



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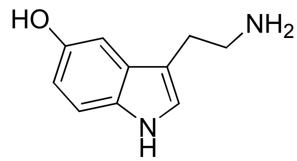

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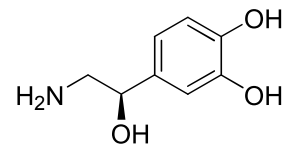

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As mentioned above, the different concentration levels of these monoamines will enhance or weaken the receptor binding activities and so lead to different levels of stimulation. The releasing and reuptake actions of these monoamines are illustrated in Figure 2.[20]

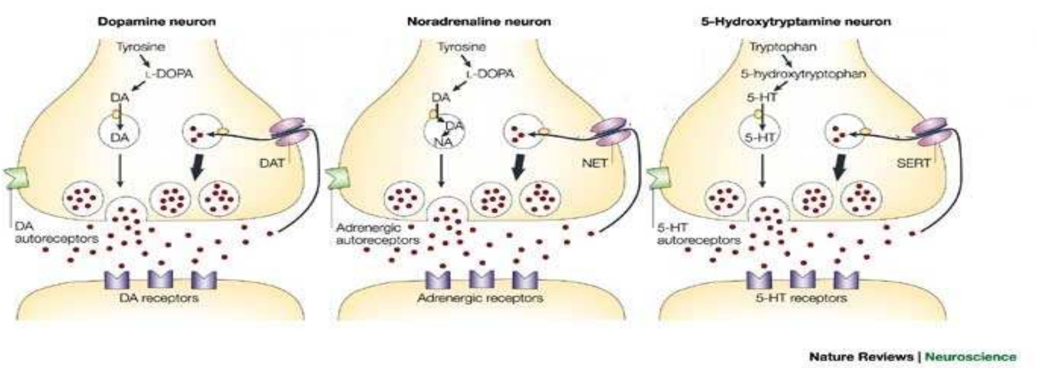


Figure 2. Monoamine transporters and reuptake action[20]

In the synaptic cleft, some the released monoamines are bound to the receptors on postsynaptic membranes, which can initiate an action potential. However, the remaining concentration of unbound monoamine will be recovered by their transporters and sent back to presynaptic vesicles waiting the next wave of releasing impulses. In addition some of monoamine will be metabolized and degraded by specific enzymes like monoamine oxidases.

Most Central Nervous System (CNS) medications are working on neurotransmitter systems due to their specific drug targeting. For example, Parkinson's disease is at least in part related to failure of dopaminergic cells in deep-brain nuclei. Also, Cocaine, for example, blocks the reuptake of dopamine back into the presynaptic neuron, leaving the neurotransmitter molecules in the synaptic gap longer. A brief comparison of the major drugs targeting these neurotransmitter systems is listed as Table 1.

Table 1. Monoamines and their Drug Targeting

Monoamines	Physiological Effect	Drug Targeting
Dopamine (DA)	Reward system , Motor system, Cognition , etc.	Cocaine Addiction, Parkinson , Alzheimer,
Serotonin (SE)	Mood, Satiety, Body temperature, Sleep, etc.	Depression, Psychotics, Migraine.
Norepinephrine (NE)	Reward system, Arousal, also hormone, etc.	Attention-deficit/ hyperactivity disorder, depression and hypotension

One important method to modulate the extracellular (cleft) level of monoamine is by blocking the reuptake route by inhibiting the transporter proteins. Monoamine transporters are the proteins on the presynaptic membrane that transfer the free monoamines from synaptic cleft back to the presynaptic cells. This procedure is also called "reuptake". Monoamine transporters are specific and highly selective. As in Figure 2, the dopamine transporters (DAT) only reuptake dopamine and the serotonin transporters (SERT) and norepinephrine transporters (NET) only reuptake the corresponding monoamine. Drugs that bind to these transporters either physically block of allosterically modulate the transporter protein structure to prevent the re-uptake of the corresponding monoamine. This leads to sustained extracellular concentration of the monoamine and continued excitation of the monoamineric system. Not until the drug/inhibitor is removed from the system, does system return to the "normal" state.

Monoamine Transporters and Their Genes

Dopamine transporters (DAT), as well as serotonin transporters (SERT) and norepinephrine transporters (NET), belong to the same solute carrier family of Na^+ or Cl^- dependent neurotransmitter transporters, and are also known as neurotransmitter sodium symporters (NSSs) family 2.A.22, which include serotonin autoreceptors, glycine, γ-aminobutyric acid (GABA), and other amino acid neurotransmitters. [21]

In 1990s, express cloning of these transporters was successfully fulfilled from various sources including human, monkey, rat, mouse and cow.[22-29] The gene for human DAT is officially termed the Solute Ligand Carrier Family 6A, Member 3 (SLC6A3), with the number 3 indicating that it was the third gene in the family of neurotransmitter transporter genes to be cloned, after the γ-aminobutyric acid (GABA) and norepinephrine transporters.[30] The dopamine transporter gene is located on chromosome 5p15, with a region of over 64 kilobase (kb) long and comprising 15 exons encoding a protein of 630 amino acid.[31,32] The human serotonin transporter (SLC6A4) spans 37.8 kb on chromosome 17q11.2, and is composed of fourteen exons encoding a protein of 630 amino acids.[33,34] The norepinephrine transporter, with the gene symbol SLC6A2, consists of 14 exons spanning 45 kb chromosome 16 locus 16q12.2.31. The human NET is a 617 amino acid (aa) protein which shows 66% overall identity in amino acid sequence with the human DAT and 48% identity with the human SERT.[34-37] These gene code locations on human chromosome are illustrated in Figure 3.

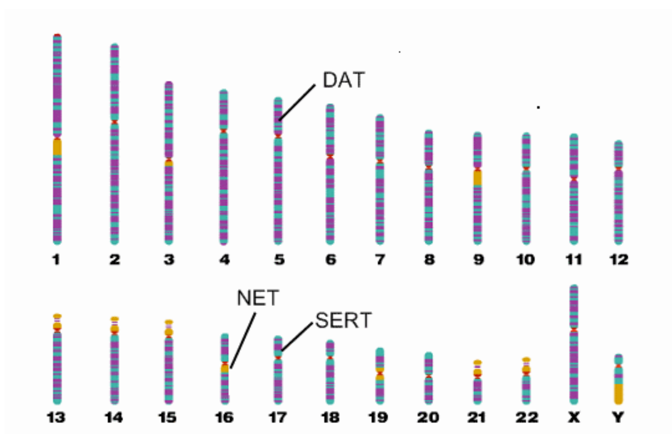


Figure 3.Human chromosome with locations of DAT, SERT and NET

Serotonin Transporter: Homology and Structure

Although the human serotonin transporter gene was sequenced and cloned a decade ago, the tertiary structure of serotonin transport protein is still unclear due to limited quantities of dissolved membrane proteins for even initial studies. This challenge comes from its nature to easy decompose and aggregate.[38-40]

So far the only available structural 3-D models for serotonin transporter are bacterial transporter homology such as lactose permease,[39] the glycerol-3-phosphate transporter,[38] and the adenine nucleotide exchanger in mitochondria.[41] Among these three bacterial homologies, extensive studies of the LacY permease of *Escherichia coli* and other major facilitator superfamily (MFS) carriers were focused. Despite the fact that these proteins share 20% similarities with human serotonin transporter, they do not belong to the same family as SERT and their topology might therefore be different. Indeed, some important residues for ligand binding are missed in LacY- based SERT model.[41-43]

Recently, more and more homology studies are focus on the bacterial leucine transporter (LeuT) from *Aquifex aeolicus*, which belongs to the same transport as SERT and shares 20–25% identity in primary sequence and 40 to 45% similarity with human neurotransmitter transporters with the human neurotransmitter transporters.[44,45]

Hydrophobicity analysis of these transporters amino acid sequences revealed the same presence of 12 transmembranes (TMs) spanning domains, consisting primarily of α-helical structure. The TMs are linked through intracellular and extracellular loops (ILs and ELs), with the N- and C-termini facing the cytoplasm.[44] (Figure 4). The second extracellular loop (EL2, between TM3 and TM4) is characteristic of this transporter family, being the largest connecting loops and containing extensive gylcosylation and disulfide bonding.[46] Many residues predicted by the initial topological predictions to lie in hydrophilic loops were demonstrated to be accessible from the appropriate side of the membrane.[30,47] These studies extensively utilized cysteine scanning mutagenesis of internal and external loops and transmembrane domains. Topology Studies generated from the crystal structure of LeuT detected various reactive sites on both ELs and ILs, especially on EL3 and EL4.[30, 48-50]

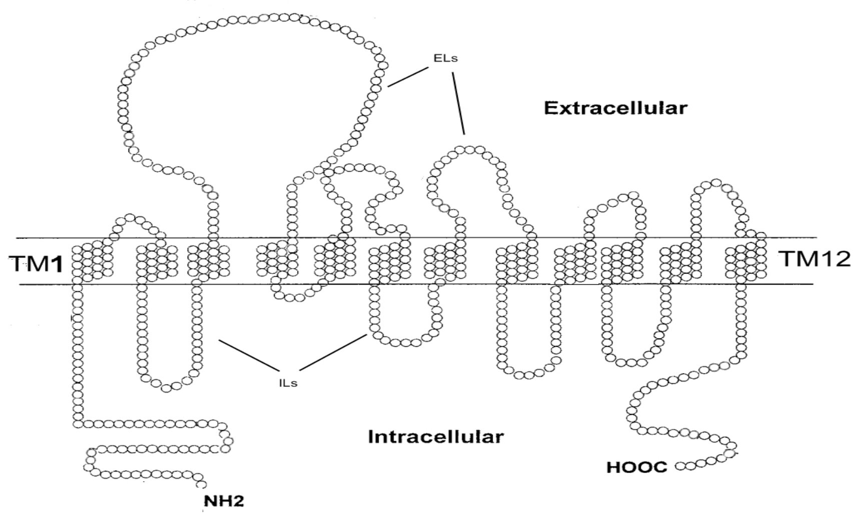


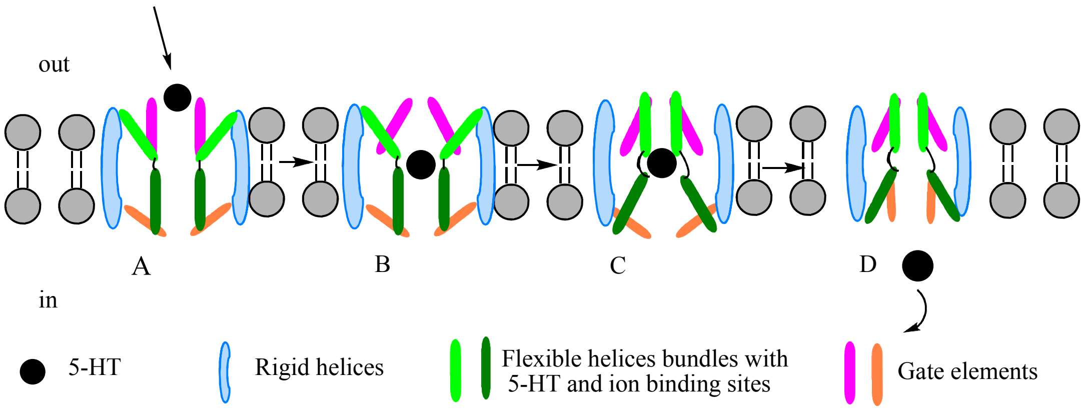
Figure 4.Basic Structure of Monoamine Transporters (illustration only)

Despite the major advance in our understanding provided by the structure of LeuT, many aspects of SERT structure remain unresolved. The difference in ion coupling between SERT and LeuT must be reflected in differences in the structure of the substrate binding site. In addition, there are three regions of SERT where the structure of LeuT provides little or no information. The first of these is EL2, which is much longer in SERT than in LeuT. The second is that SERT has much longer NH2- and COOH-terminal regions than does LeuT, which contain specific domains with potential interaction with the intracellular face of the central region of SERT.[51]

Mechanism of Serotonin Transporters

One generalized mechanism for transport is based on the well-known alternate access model.[52,53] In this model, transporters are believed to function by alternately exposing a substrate binding site to the cytoplasmic and extracellular faces of the plasma membrane. The model allows solutes to be transported from one side of the membrane to the other, but more importantly, it provides a mechanism for a transmembrane concentration difference of one solute to be utilized as a driving force to generate a concentration difference for another solute. The two main mechanisms for this process, named symport and antiport by Mitchell et al., couple the movement of two solutes moving in the same direction across the membrane or in opposite directions, respectively.[54] In symport, two (or more) solutes bind to the transporter in one conformation, and are later released to the opposite side of the membrane from another conformation. In antiport, binding of one solute facilitates conversion of the transporter to the form facing the opposite side of the membrane. After dissociation of the first solute, binding of a second solute allows the reverse conformational change leading to dissociation of the second solute on the side of the membrane where the first solute originated.

In 2008, A more improved model of mechanism is been proposed, which is based on different homology conducted among the families of neurotransmitter sodium symporters [NSS; glutamate transporter (GltPh) and leucine transporter (LeuT)], solute sodium symporters [SSS; sodium galactose transporter (vSGLT)], and nucleobase cation symporters [NCS1; benzyl-hydantoin transporter (Mhp1)].[43,55-59] Although belonging to three evolutionarily distinct protein families with no primary amino acid sequence similarity and different substrate specificities, all four transporters share a similar structure and suggest transporter structure consists of two V-shaped domains (with five TMs each), intertwined in an antiparallel topology. A substrate binding site is located in a nearly identical position at two broken helices, one from each domain. The ion binding sites necessary for energy coupling are located close to the substrate binding. Hence, there are three conformations caught in among these four homologies (see Figure 5): "outward-facing open" (LeuT,[57] Mhp1,[58]),"outward-facing occluded" (vGltPh,[43,55] LeuT,[57] Mhp1[58]) and "inward-facing occluded" (vSGLT[56]). A fourth conformation, corresponding to an inward-facing open can only be hypothetical. This creates a sequence of action to explain how the transporter works inside itself.



Substrate-induced structural conformations of gated switch transporter
A) The outward-facing open conformation is ready to accept the 5-HT and ion particle.
B) The outward-facing occluded conformation with5-HT bounded and external gate closed.
C) Closure of the external gate drives the inward-facing occluded conformation.
D) The hypothetically inward-faceing conformation open the gate for 5-HT and ion release.

Figure 5. "Rock-switch" mechanism for serotonin transporters (illustration only)

In both open and occluded outward-facing conformations of LeuT and Mhp1, the intracellular gate is closed by a considerable protein mass. Similarly, in the occluded inward-facing conformation of vSGLT, a considerable helical mass closes the extracellular gate. This implies that there are two kinds of sequential conformational changes (see the figure). The first involves specific gating amino acids or parts of helices located over and below the substrate binding site, and the second involves a more massive movement of transmembrane domains, which leads to alternating inward- or outward-facing hydrophilic vestibules.

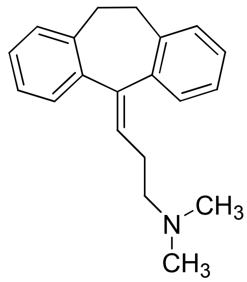
There is still some crucial details concerning transporter function and mechanism that remain unclear, especially how the ion coupling and what is the gating protein. A series of transporter structures with different bound substrates will be significant to explore these unknown regions.[59]

Serotonin Hypothesis and Antidepressants

Depressive disorders are one of the most common illnesses in modern society. Every year, 9.5 percent of the population, or about 18.8 million American adults, suffer from a depressive illness.[60] It involves human body, mood, and thoughts and is not a “passing blue mood” or a weakness of personal character. Depression not only brings the patient a negative effect on mood and motive, but also causes pain and weakens the immune system leading to other infectious diseases.

Although there are many factors can cause depression in varying degrees, biologically, depressive disorders are a disease caused by the disrupted serotonergic pathway between the neurons.[61] The monoamine hypothesis postulates that the deficit of serotonin is responsible for the corresponding features of depression. Most antidepressant medications increase the levels of one or more of the monoamine neurotransmitters like serotonin, norepinephrine and dopamine in the synaptic cleft between neurons in the brain, while some medications affect the monoamine receptors directly.

Tricyclic antidepressants (TCAs) were first discovered in the early 1950s and were subsequently introduced later in the decade.[62] They are heterocyclic chemical compounds used primarily as antidepressants. The TCAs are named after their chemical structure, which contains three rings of atoms, like Amitriptyline (**4)**.[63] The tetracyclic antidepressants (TeCAs), which contain four rings of atoms, are also a closely related group of antidepressant compounds [e.g., Amoxapine (**5)**]. [64]



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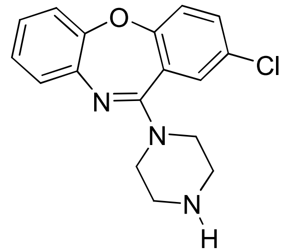

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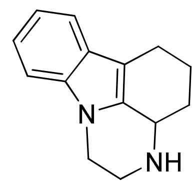
The drawbacks of the TCAs are their side effects. These include drowsiness,[65] restlessness,[66] anxiety attacks,[67] urinary problems like urinary retention,[67] irregular cardiac rhythm and other effects.[68] Also TCAs overdose is a significant cause of fatal drug poisoning.[69] The severe morbidity and mortality associated with these drugs is well documented due to their cardio-vascular and neurological toxicity. Most of side effects are due to their poor binding selectivity for the serotonin transporter (SERT) over other transporters like the dopamine transporter and the norepinephrine transporter.[65-67]

Monoamine oxidase inhibitors (MAOIs,) are another class of antidepressant drugs prescribed for the treatment of depression.[70] MAOIs act by inhibiting the activity of monoamine oxidase, thus preventing the breakdown of monoamine neurotransmitters and thereby increasing their availability. The early MAOIs inhibited monoamine oxidase irreversibly. When they react with monoamine oxidase, they permanently deactivated these enzymes, and the enzyme would function until it had been replaced by the body. This process would take about two weeks. A few newer MAOIs, which are reversible inhibitors of MAO-A (RIMA), notably Pirlindole (**6**),[71] are able to detach from the enzyme to facilitate usual catabolism of the substrate. The level of inhibition in this way is governed by the concentrations of the substrate and the MAOIs.



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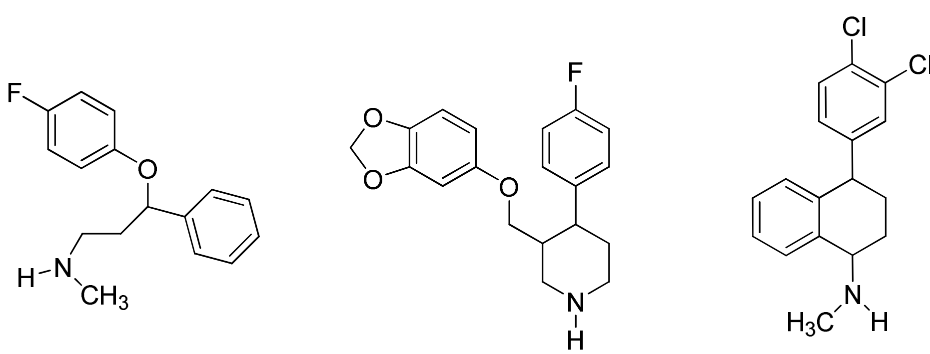
Since MOAIs have no selectivity on reducing the breakdown of most monoamines, they have a higher risk of serotonin syndrome or a hypertensive crisis.[72] Also tyramine is broken down by MAO-A, therefore inhibiting its action may result in excessive build-up of it, so diet must be monitored for tyramine intake. Due to potentially lethal dietary and drug interactions, MAOIs had been reserved as a last line of defense, used only when other classes of antidepressant drugs have failed.

The Serotonin-Selective Reuptake Inhibitors

Serotonin-selective reuptake inhibitors (SSRIs) were discovered in the 1980's as a new class of drugs useful for treatment of depression.[73] The SSRIs widely replaced tricyclic antidepressants (TCAs, e.g. amitriptyline **4**) and monoamine oxidase inhibitors (MAOIs, e.g. pirlindole **6**) as new medications for the treatment of depression. [73-77]

As antidepressants the SSRIs selectively inhibited the reuptake of the neurotransmitter serotonin (5-HT) resulting in the increased concentration of extracellular serotonin in the synapse. This leads to the therapeutic effect by remediating the disrupted serotonergic pathway that is manifested by depression.[74-76] As a result of their selective actions on serotonergic mechanisms the SSRIs have much improved tolerability, a broader therapeutic index and increased safety towards overdose than TCAs and MAOIs.[78-80] In addition to the treatment of depression, some SSRIs have been recognized as possessing therapeutic value for the treatment of a variety of central nervous system (CNS) disorders and disease-states. These include Panic Disorder,[81,82] Post-Traumatic Stress Disorder,[83] Social Phobia,[84] Obsessive-Compulsive Disorder,[85] Pre-Menstrual Dysphoric Disorder, [86] Anorexia,[87] Bulimia[76,83] and Schizophrenia.[88]

There are five main SSRI-classed drugs (Figure 3) currently on the market and widely prescribed for a variety CNS mediated illnesses. [74-76] The most widely prescribed SSRI for the treatment of depression is fluoxetine (**7**, Prozac®).[89] The SSRIs paroxetine (**8**, Paxil®), sertraline (**9**, Zoloft®), citalopram (**10**, Celexa®), fluvoxamine (**11**, Luvox®) have all been shown to be effective in the treatment of depression. However, due to differences in metabolism (cytochrome P450 enzymes) and secondary pharmacology these SSRIs can exhibit varied pharmacological profiles among patients.



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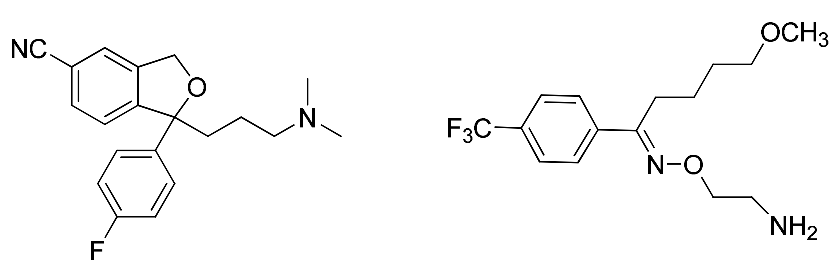


10 **11**

Figure 6. Currently Marketed SSRIs

Most SSRIs exhibit high selectivity for the serotonin transporter over the dopamine transporter (DAT) while somewhat less selective at the norepinephrine transporter (NET). The SSRI citalopram (**9**) is the most selective compound as measured by the in vitro serotonin (5-HT), norepinephrine (NE) and dopamine (DA) reuptake inhibition (Table 2).[78] However selectivity does not necessarily correlate with efficacy. Fluoxetine (**7**) is generally considered to be the most efficacious SSRI but is 94-fold less selective (NE/5-HT) than citalopram (**9**) and ten-fold less selective (NE/5-HT) than paroxetine (**7**).[90]

Table 2. SSRIs Inhibition of Monoamine Neurotransmitters[a]

SSRI	5-HT (K_i,nM)	NE (K_i,nM)	DA (K_i, nM)	NE/5-HT	DA/5-HT
Fluoxetine(**7**)	8	250	1,300	31	163
Paroxetine(**8**)	0.2	60	5,400	300	27,000
Citalopram(**10**)	2	5800	>10,000	2,900	>5,000
Sertaline(**9**)	3	220	440	73	147

[a]Derived from data cited in reference [79].

Mechanism of Action of SSRIs

The SSRIs elicit their pharmacological effects by selectively blocking the serotonin recovery system of serotonergic neurons (Figure 4).[91] SSRIs bind to the serotonin transporter located on the pre-synaptic terminal. The normal function of the serotonin transporter is to remove serotonin from the synapse and terminate the serotonergic action. The serotonin transporter recovers the serotonin where it is stored in the pre-synaptic terminal until the next neurochemical event. By binding to the SERT, the SSRIs block the recovery mechanisms of the

SERT thus leading to increased extracellular concentrations of serotonin in the synapse thus enhancing post-synaptic serotonin receptor activation. The increased serotonergic transmission then remedies the disruption of the serotonin pathways associated with depression and other psychiatric disorders.

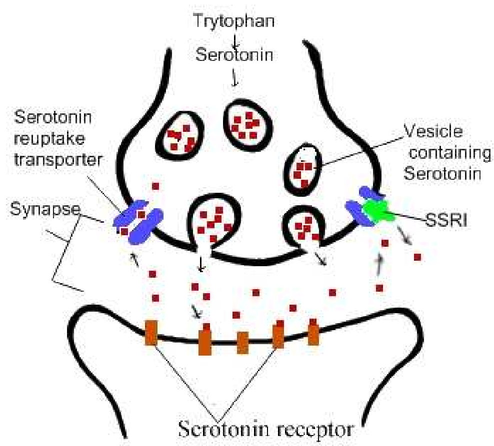


Figure 7. Illustrated mechanism of SSRIs activity.

Although the extracellular serotonin level can be simply increased by selective inhibition mechanism, it is puzzling that whereas inhibitor block reuptake immediately, alleviation of symptom usually requires at least 2-4 weeks.[92,93] Many studies reveal this limitation is partially due to serotonin autoreceptors. Autoreceptor is a receptor located also on presynaptic nerve cell terminals and serves as a part of a feedback loop in signal transduction. For serotonin neuron cell, the major autoreceptors are 5-HT_{1A} and 5-HT_{1B}, which are both sensitive to local serotonin concentration with different extent. Studies show when autoreceptor 5-HT_{1A} is activated as serotonin extracellular level enhanced by SSRIs, it gives negative feedback to the neuron cell and decrease release of serotonin from presynaptic cell. While there may be little basal endogenous tone at the 5-HT_{1B} autoreceptor sites.[94-97] Also research works reveal combined treatment with an SSRI and an autoreceptor antagonist provide a more rapid, and perhaps more efficient means of enhancing 5-HT neurotransmission.[98,99] While, jointly blocking 5-HT_{1A} and 5-HT_{1B} autoreceptors has proven without effect on basal 5-HT output, arguing against the possibility that a blockade of the former would be offset by an action arising from increased activation of the latter, and vice versa.[95, 100] Detail mechanism of these autoreceptors still unclear and need further study.

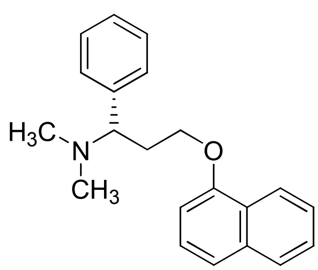
The Disadvantages of SSRIs

Despite many advances in SSRI therapies, as many as 30-40% of patients treated for depression with these drugs typically do not respond.[74-77] In addition, nearly 50% of those patients that do respond never fully achieve complete remission of their depressive symptoms. Moreover, many patients using SSRIs experience adverse side effects. These side effects include sexual dysfunction,[85] increased anxiety, gastrointestinal effects (nausea)[101] and insomnia.[76] Many patients also terminate their medication regiment due to slow-onset of action of the SSRIs.[102] In clinical environments it can take 2-6 weeks of continuous SSRI administration before antidepressant activity can be observed. In late 2004 media attention was given to a proposed link between SSRI use and juvenile suicide.[103,104]

Given the plethora of adverse side effects associated with currently available SSRIs there clearly remains a need for new SSRI-based drugs with higher efficacy and fewer adverse side effects that could be used for treatment of a variety of psychiatric illnesses.

New Targets for Drug Development

The SSRIs such as fluoxetine (**7**) and paroxetine (**8**) are currently among the most widely prescribed drugs for the treatment of depression.[75-77] However; recently several new approaches have been explored as potential avenues for the treatment of depression and related disorders. One approach has been to target the development of serotonin autoreceptor (5-HT_{1A}, 5-$HT_{1B/1D}$, 5-HT_{2A}) antagonists.[105,106] Co-administration of a 5-HT_{1A} antagonist with an SSRI would then lead to shorter induction periods. While there is some evidence that the 5-HT_{1A} antagonist/SSRI therapies have enhanced the clinical efficacy of the co-SSRI the results do not clearly mandate the abandonment of current SSRI-based therapies. In addition, several dual acting 5-HT_{1A} antagonist/SSRI compounds have been identified. The Lilly compound dapoxetine (**12** in Figure 5) has exhibited perhaps the greatest potential as a dual action ligand for the treatment of depression and is under investigation.[107]



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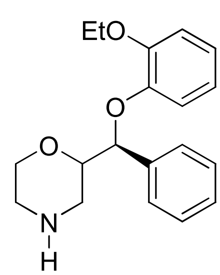

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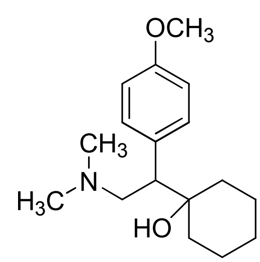

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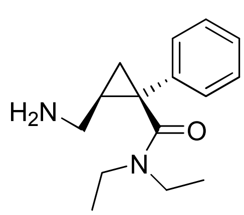

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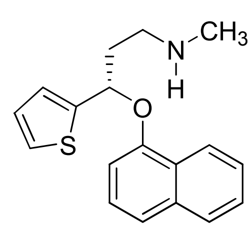

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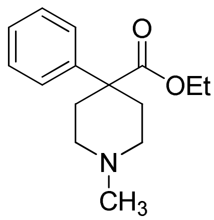
Figure 8. Other Antidepressant Drugs

A second approach has been to target compounds with dual SSRI and norepinephrine reuptake inhibition (NRI) pharmacological profiles.[108,109] It is believed that the efficacy of the dual action compounds is achieved via a synergistic effect on the serotonergic and norepinephrinergic systems. Compounds such as reboxetine (**13**),[110] venlafaxine (**14**),[111] milnacipran (**15**)[112] and duloxetine (**16**)[113] all exhibit slightly improved onset of action rates with diminished adverse side effects. However, these dual acting SSRI/NRI agents are generally no more efficacious and sometimes less efficacious than currently prescribed SSRIs.

Meperidine

Meperidine (**17**), commercially marketed under the name Demerol®, was first synthesized in 1939 at an IG Farben laboratory as an antimuscarinic agent.[114] It was discovered that it possessed analgesic effects similar to morphine, by acting as an atypical agonist at the μ-opioid receptor (K_i = 920 nM).[115] For much of the 20th century, meperidine was the opioid of choice for many physicians; in 1983 60% of doctors prescribed it for acute pain and 22% for chronic severe pain.[116] In 1990s, there are multiple reports of a serotonin syndrome observed in patients treated with meperidine.[117,118] This serotonin syndrome which is characterized by rigidity, confusion, nausea, diarrhea and coma, has been described in both animals and humans.[119,120] The following bioassay proved that it has potent binding affinity for the serotonin transporter (K_i = 413 nM) and be an inhibitor of serotonin reuptake.[121] Meperidine also exhibits weak affinity for the dopamine transporter with biphasic reuptake inhibition of [^{3}H]dopamine [IC_{50} = 0.61 ± 2.2 nM (22% of total inhibition)] when examined in a chopped tissue rather than synaptosomal preparations.[115] Further, the concentration response curve of dopamine reuptake inhibition exhibited a plateau at approximately 20% inhibition over a broad range of low concentrations of meperidine. The maximal inhibition of dopamine reuptake produced by meperidine at low concentrations (20%) was also consistent with the maximal inhibition associated with the high-affinity binding component (18%) of the selective dopamine transporter ligand [^{3}H]WIN 35,428 (**18**).[115] However, structural modification of meperidine has been shown to effectively eliminate dopaminergic activity relative to its serotonergic pharmacological profile.[115]



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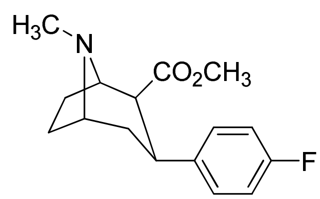

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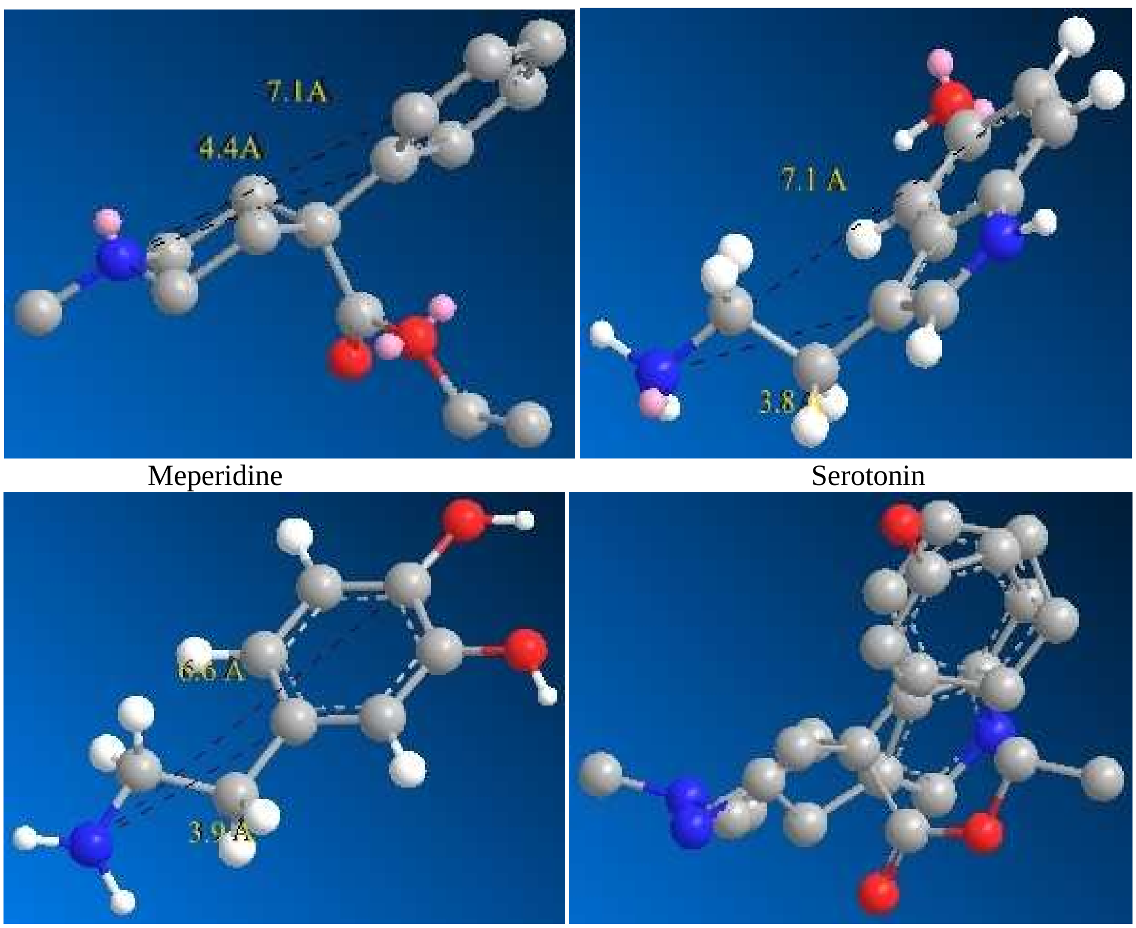
Meperidine Hypothesis

Meperidine (**17**) exhibits high affinity for the serotonin transporter and relative poor affinity for the dopamine transporter (K_i = 18,000 nM).[115] All the facts observed directed our attention to compare the structure of meperidine, serotonin and dopamine to find their structural similarities. (Figure 6) From a comparison of geometry-optimized structures, meperidine was found share a high similarity with serotonin. The distance between amine nitrogen atom to aryl ring was determined to be 4.4-7.1Å for meperidine.[122] (see Table 3) This was very similar to that calculated for serotonin, 3.8-7.1Å. An overlay of meperidine and serotonin exhibits a good match for the ring systems and the amino groups, while there is less of a structural alignment with dopamine. This can partially explain why meperidine shows binding differences for the serotonin transporter over the dopamine transporter.

Based upon these pharmacological results and the structural comparison, meperidine has been identified as a lead compound for the development of a novel class of SSRIs. It was envisaged that through structural modification of meperidine increased serotonin transporter selectively over the dopamine transporter and the norepinephrine transporter can be achieved. Structure-activity studies of meperidine analogues at monoamine transporters will lead to identification of the necessary functionality for high serotonin transporter selectivity and potent serotonin reuptake inhibition, but also improve our understanding of the protein structure of these transporters.



Meperidine　　Serotonin

Dopamine　　Overlay of Meperidine and Serotonin

Figure 9. Structural comparison of Meperidine, Serotonin and Dopamine

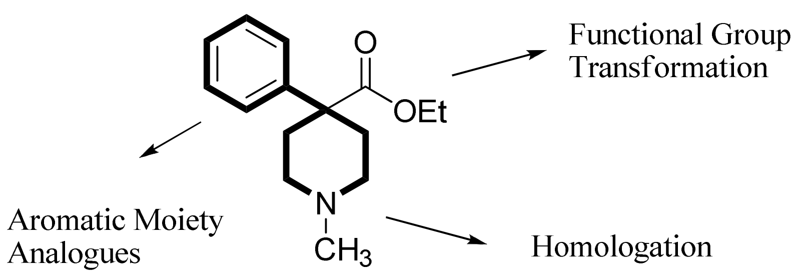
Table 3. Geometry features comparison of meperidine, serotonin and dopamine

Compound	N-Ar Distance (Å)[122]
Meperidine	4.4-7.1
Serotonin	3.8-7.1
Dopamine	3.9-6.6
Average	4.1-6.9

Lead Modification

As our lead compound, Meperidine is chemically constructed by attaching a phenyl ring to the 4-position of the piperidine ring, and also requires an ethyl carboxylic ester on the 4-position. Since the carbon-nitrogen ring scaffold of meperidine was determined to be highly important for molecular recognition at serotonin transporters from the previous structural comparison,[30,121] the lead modification will focus on the sub-structures shown in Scheme 1.

Scheme 1



Meperidine (**17**)

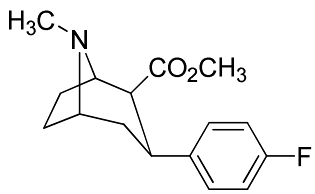
The first modifications will focus on optimizing the aromatic ring system for transporter affinity. The different ring moieties with various substituted groups will be examined for serotonin transporter, dopamine transporter and norepinephrine transporter binding affinity and selectivity. Also the μ-opioid affinity will be re-examined, since any analgesic effects from meperidine is unwanted in SSRI development.

The second modification will focus on the functional group transformations at the ester moiety. Initially, the ester group will be modified to explore the structure-activity relationships at of the alkyl group. Secondly, new functional groups will be explored that could lead to reduced lipophilicity and enhanced bioavailabity.

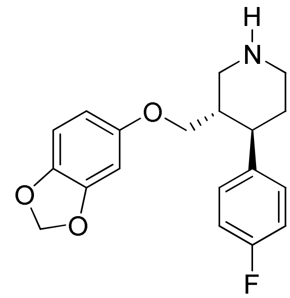
The last optimization is focused on the amino group which is the most important factor in monoamine transporter recognition. Different substituted N-groups will be introduced that do not change the basicity of nitrogen atom, but effect the steric environment around the nitrogen atom as well as the piperidine ring conformation.

Transporter Binding Affinity and Inhibition Constant

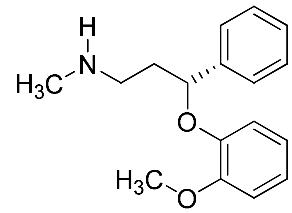
The potency of compounds for the monoamine transporters are typically given as IC_{50} or K_i value. These values indicate the affinity or ease with which the compound binds to the specific transporter; the lower the value, the higher the affinity. Therefore, a compound that exhibits high affinity for a transporter binds to that transporter at low concentrations. The compounds are compared to a bound radiolabeled ligand that exhibits high affinity for the transporter, such as [^{3}H]WIN35,428 (**18**) for the dopamine transporter, [^{3}H]paroxetine (**8**) for the serotonin transporter and [^{3}H]nisoxetine (**19**) for norepinephrine transporter.



WIN 35,425 (**18**)



Paroxetine (**8**)



Nisoxetine (**19**)

The IC_{50} is the concentration at which the compound is needed to displace 50% of the radiolabeled ligand. The inhibition constant (K_i) was derived by Cheng and Prusoff in 1973 and is shown in equation (1).[123]

$$K_i = \frac{IC_{50}}{\frac{[^3\text{Hsub}]}{K_d} + 1} \qquad (1)$$

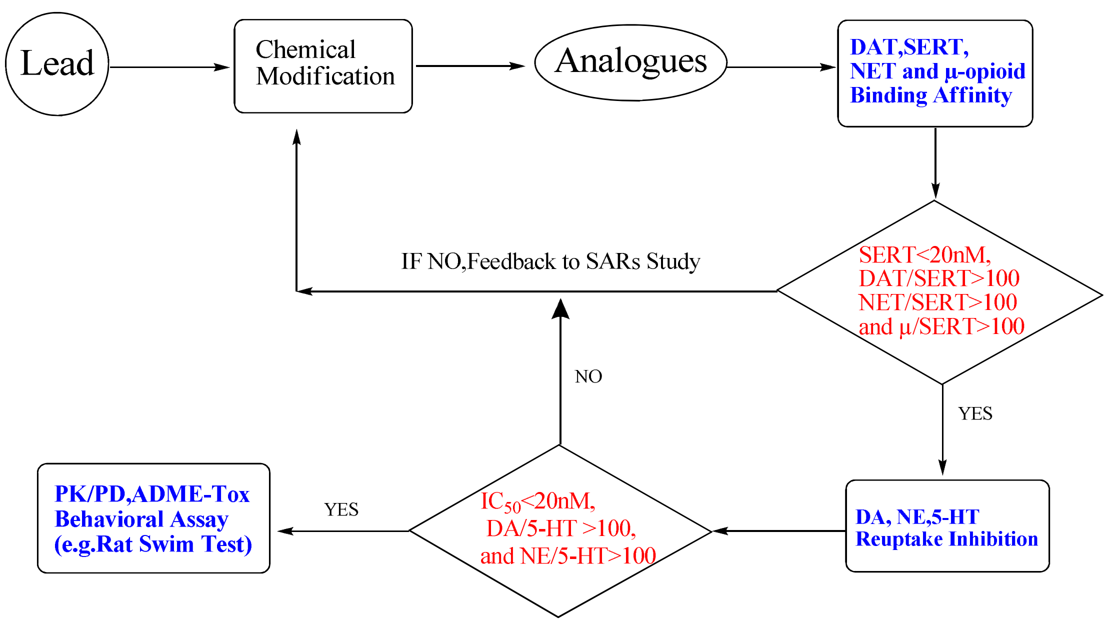
[^{3}Hsub] is the concentration of the radiolabeled ligand or substrates, and K_d is the dissociation constant of the radiolabeled ligand previously determined for specific transporter. By taking the concentration of radiolabeled ligand and the dissociation constant for that ligand into account in addition to the IC_{50} value for the compound being tested, the values are more comparable between laboratories.

General Hierarchy of Screening

Our general screening flow for this project is illustrated as Scheme 2. The analogues from each modification step will be screened for the candidates with potency for serotonin transporter

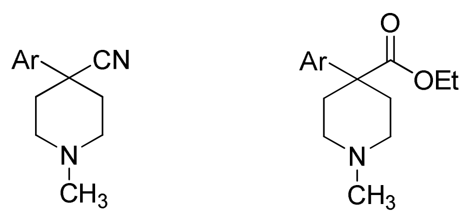
(SERT K_i<20nM) and selectivity over the dopamine, norepinephrine transporters (DAT, NET) and μ-opioid receptor (>100 times), all the analogues will complete the inhibition assays with the radiolabeled ligands in vitro. Then, the new candidates will be sent to next screening level of in vitro assays to determine the serotonin, dopamine and norepinephrine reuptake inhibition. The most promising compounds from these assays will then be advanced to behavioral assays and pharmacokinetic studies.

Scheme 2



Preliminary Structure-Activity Relationships of Meperidine Analogues

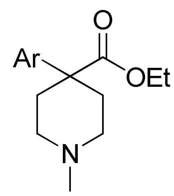
Novel derivatives of meperidine (**17**) were synthesized and the binding affinities were compared for the dopamine and serotonin transporter,[30] as well as the μ-opioid receptors. The substituted aryl ring group on the piperidine system was explored for the meperidine system with both the nitrile (**20a-f**) and ethyl ester moieties (**21a-f**) at the 4-position of the piperidine ring.[121,124]



20a-f **21a-f**

The *in vitro* binding affinities of the aryl derivatives (**21a-f**) of this series are listed in Table 4. In general, these compounds were more potent than the corresponding nitrile derivatives (**20a-f**) at the both transporters.[124] The binding affinities for μ-opioid receptor were measured against [^{3}H]DAMGO ([*D*-Ala-*N*-Me-Phe-Gly-ol]enkephalin). The selectivities of each compound for the serotonin transporter relative to dopamine transporter and μ-opioid receptor are listed in Table 5.

Table 4. The *In Vitro* Binding Data at Dopamine Transporter (DAT), Serotonin Transporter (SERT), and μ-Opioid Receptor for 4-Aryl-Substituted Meperidines.

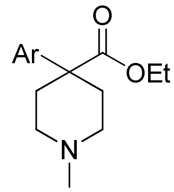


21a-f

Cpd	Ar[a]	[^{3}H]WIN 35,428 (DAT) K_i(nM)[b]	[^{3}H]Paroxetine (SERT) K_i(nM)[b]	[^{3}H]DAMGO (μ) K_i(nM)[b]
17	Ph	17,800±2,670	413±44	920
21a	4-F-Ph	10,700±2250	308±26	1,470
21b	4-Cl-Ph	4,100±1270	277±40	4,410
21c	4-I-Ph	3,250±195	21.0±2.4	2,350
21d	3,4-Cl_2-Ph	125±15	19.0±2.6	2,040
21e	2-Naphthyl	1140±380	7.20±0.53	2,030
21f	4-Me-Ph	12,400±5,210	1,610±110	2,670

[a] All compound were tested as HCl salt. [b] All values are the mean±SEM of three experiments performed in triplicate.

Table 5. The Binding Selectivity on Serotonin Transporter over Dopamine Transporter and μ-Opioid Receptor for 4-Aryl-Substituted Meperidines.



21a-f

Cpd	Ar	DAT/SERT	μ/SERT
17	Ph	43	2.2
21a	4-F-Ph	34	4.8
21b	4-Cl-Ph	14	16
21c	4-I-Ph	154	111
21d	3,4-Cl_2-Ph	6.7	109
21e	2-Naphthyl	162	281
21f	4-Me-Ph	7.7	1.7

In general, the aryl-substituted meperidine analogues were more selective for the serotonin transporter than the dopamine transporter, following the same trend as meperidine (**17**). In particular, the 4-iodophenyl **21c**, 3,4-dichlrorophenyl **21d** and 4-naphthyl **21e** were highly selective for serotonin transporter.[124] All of the analogues were less selective for the μ-opioid receptor than meperidine. These results prompted interest in further studies of meperidine analogues to selectively inhibiting serotonin reuptake.

Other modifications were made on the ester group at the 4-position of the meperidine scaffold. These analogues were synthesized by Rhoden *et al.* via the synthetic route illustrated in

Scheme 3.[125] The *in vitro* binding affinities of the piperidine derivatives (**22** - **28**) of this series are listed in Table 5.

Scheme 3

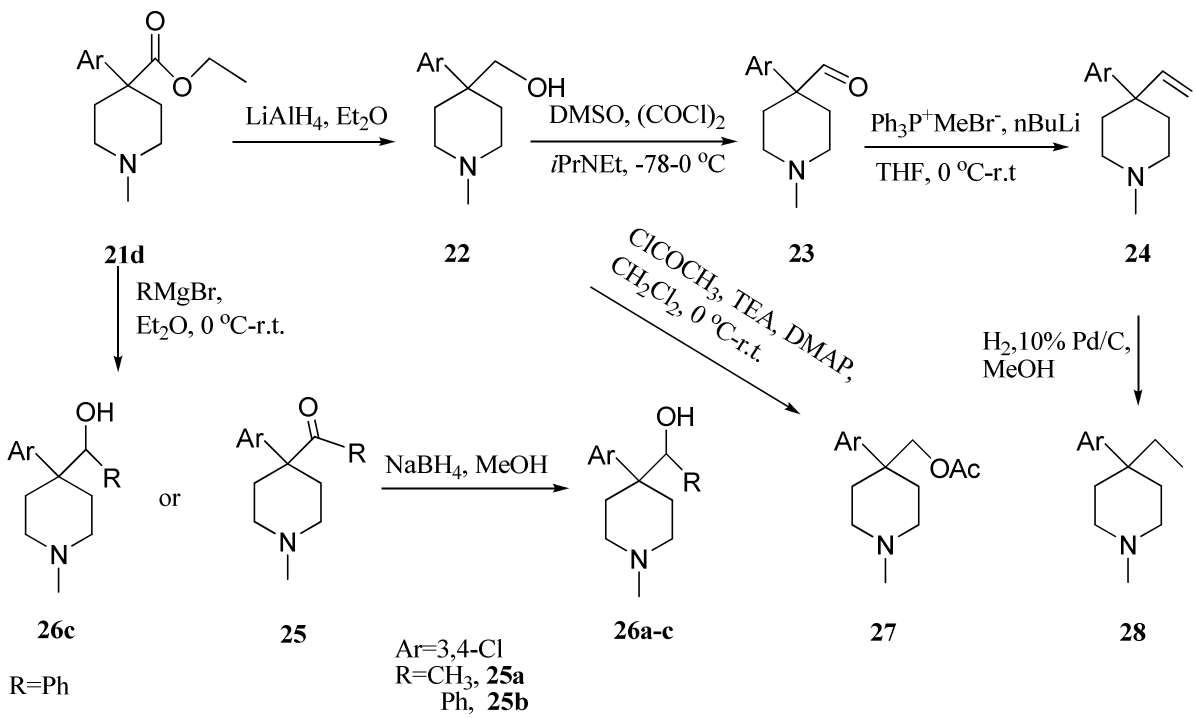
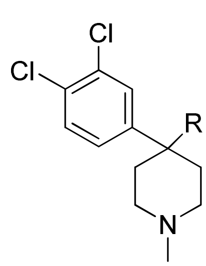


Table 6. Binding Data at Serotonin Transporters (SERT) and Dopamine Transporter (DAT) for 4-(3,4-Dichlorophenyl) 4-Substituted Meperidines.



22-28

Cmpd	R	[^{3}H]WIN 35,428 (DAT) K_i(nM)[a]	[^{3}H]Paroxetine (SERT) K_i(nM)[a]	DAT/SERT
21d	$CO_2CH_2CH_3$	125±15	18.7±2.6	6.7
28	CH_2CH_3	163±38	9.1±1.6	18
24	$CH{=}CH_2$	192±31	11.4±0.5	17
25b	COPh	253±21	200±18	1.3
25a	$COCH_3$	367±35	35±13	10
27	CH_2OCOCH_3	633±91	38±6	17
26a	$CHOHCH_3$	670±39	17±1	36
26c	$CHOH(C_6H_5)_2$	4590±330	NT	NT
22	CH_2OH	3310±210	83±3	40

[a] All values are the mean ± SEM of three experiments performed in triplicate. NT (Not tested).

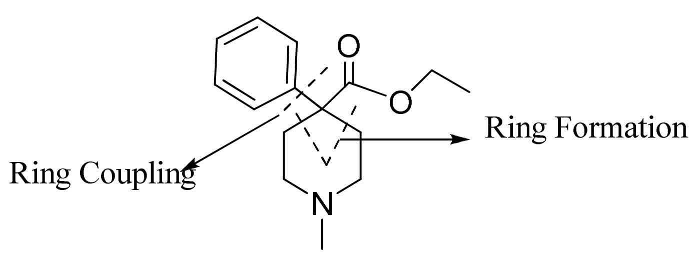
The results of the dopamine and serotonin binding affinities of the various functional group transformations indicated that the ethyl ester moiety of **21d** is not necessary for high affinity binding to the serotonin transporter. Replace most of the ester moiety with short rigid chain showed no improvement on binding affinity at the serotonin transporter. Chain and branch alcohols increased selectivity to some extent, due to decreased dopamine transporter affinity. An extra electron donor or H-bond acceptor on the 4-position is not favored by the dopamine transporter, but still tolerated by the serotonin transporter. These preliminary results provided helpful SAR information and pointed out that the ester functional group itself can be maintained for the further modification, since it can be easily converted into other derivatives via transesterification, reduction and hydrolysis.

Synthesis of Meperidine

Meperidine (**17**) was synthesized in 1939 at an IG Farben laboratory as an anti-muscarinic agent.[114] After its analgesic effect was discovered, meperidine and its derivatives have been synthesized using several different methods. Each of the methods has drawbacks due to limited substitution potential, highly toxic reagents, number of synthetic transformations, low total yields or production of highly toxic intermediates. A synthesis which minimizes the use of toxic reagents and intermediates and allows facile substitutions of wide variety moieties with better total yields on the meperidine system is ideal for obtaining a full spectrum of compounds for structure-activity studies at the dopamine and serotonin transporters.

Meperidine consists of one benzene ring, one piperidine ring and one ester functional group. By examining the structure, two different synthetic routes can be identified by retro-synthetic analysis as illustrated in Scheme 4. The first route is to the close the piperidine by dialkylation of α-carbon of phenyl nitrile; the other way, which seems more straightforward, is coupling two ring systems with an organometallic agent and specific catalyst.

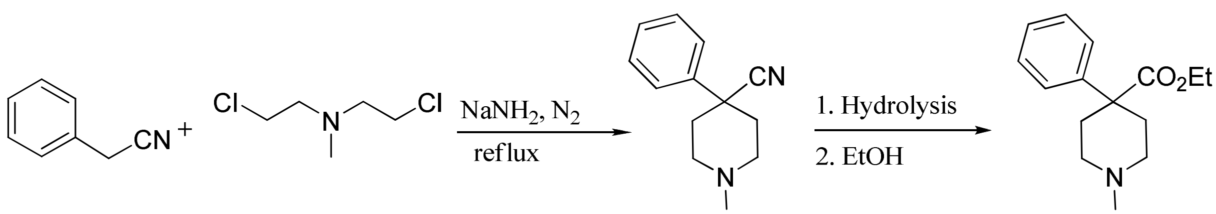
Scheme 4



Meperidine (**17**)

The most widely used synthesis, via Route I, involves the reaction of bis(β-haloalkyl)amines, in particular mechlorethamine (**30**). However, **30** is considered a highly toxic cancer suspect agent.[125,126] This synthesis of meperidine (**17**) was patented by Otto Eisleb in 1939 (Scheme 5).[125] Other amines can be synthesized as the hydrochloride salt to provide various substitutions on the nitrogen; however the amine compounds are severe vesicants and must be handled with caution.[127] The amines are reacted with phenylacetonitrile(**29**) and sodium amide to give the piperidine ring. The nitrile can be hydrolyzed with acid and esterified with ethanol via Dean Stark Trap to give the meperidine compound.[125,126]

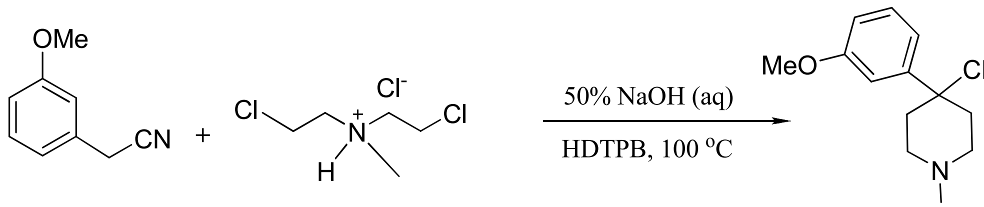
Scheme 5



29 **30** **20** **21**

One variation of the synthesis eliminates the necessity of sodium amide by using sodium hydroxide and a phase transfer catalyst, such as hexadecyltributylphosphonium bromide (HDTPB) was employed as illustrated in Scheme 6. The hydrochloride salt of the amine **32** (R = methyl, ethyl, n-butyl, t-butyl, and phenyl) and m-methoxyphenylacetonitrile (**31**) in 50% sodium hydroxide reacted to give the meperidine nitrile (**33**).[127,128] The disadvantage of phase transfer catalyst is that the total yield is highly dependent upon the stirring efficiency, which usually causes the reaction mixture to foam up during the course of the reaction.

Scheme 6

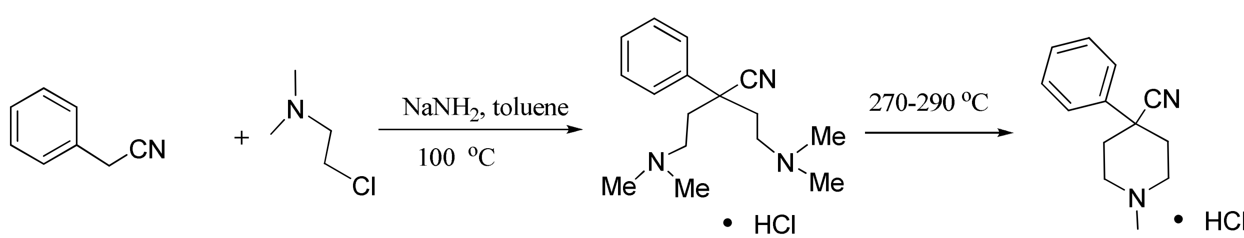


31 **32** **33**

HDTPB: $CH_3(CH_2)_{15}\overset{+}{P}[(CH_2)_3CH_3]_3Br^-$

In 1952, Blicke *et al.* reported a different method to obtain the piperidine ring system of meperidine (Scheme 7).[129] Phenylacetonitrile(**29**), sodium amide and β-dimethylaminoethyl chloride (**34**) were reacted to form *α*,*α*-di-(β-dimethylaminoethyl)-α-phenylacetonitrile (**35**). Heating **35** at high temperature (270-290°C) furnished the meperidine nitrile hydrochloride (**20**).[129] This method limits *N*- substitution based on the nature of the ring-closing step.

Scheme 7

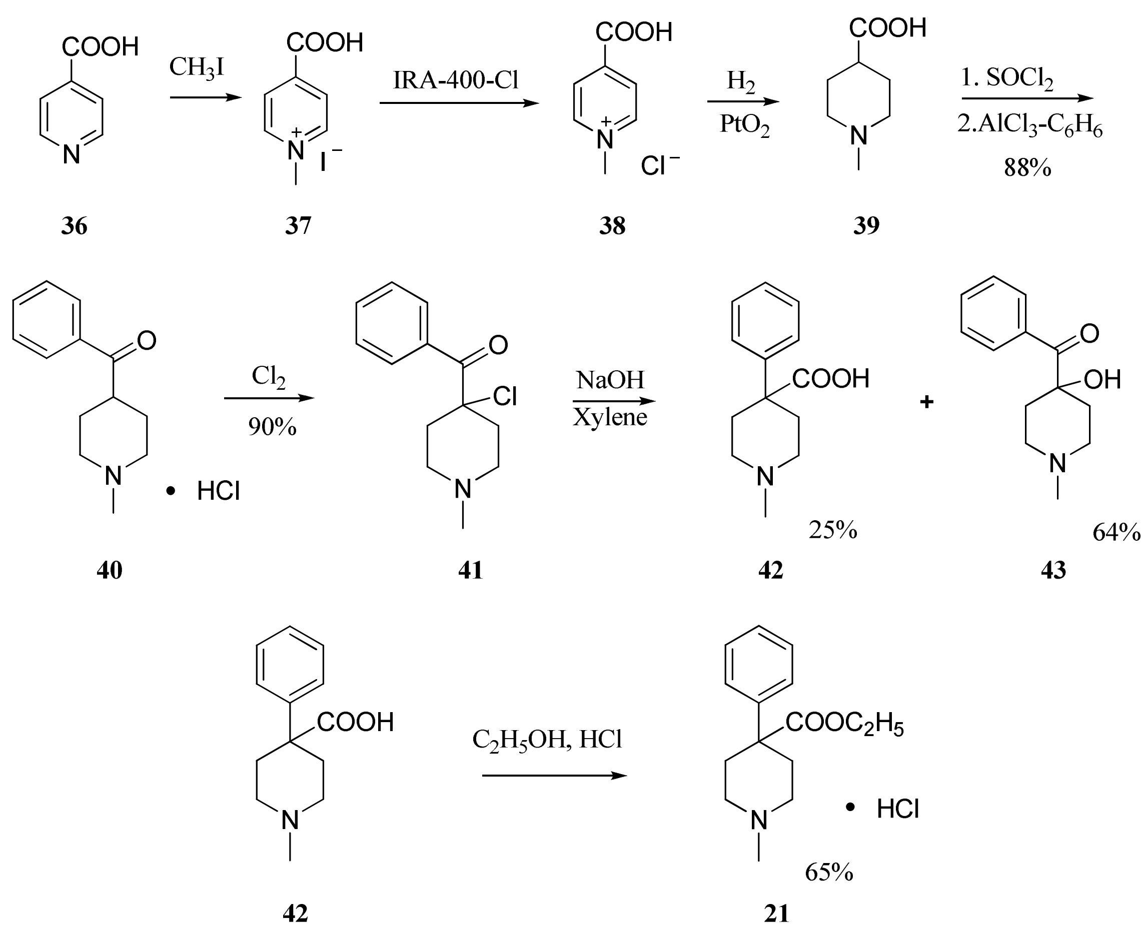


29 **34** **35** **20**

Another approach employed by Smisson *et al.* was to use a quasi-Favorskii rearrangement (Scheme 8).[130] This method is considerably longer and limited in the manner of facile substitutions of various moieties including aryl substitutions and nitrogen substitutions.

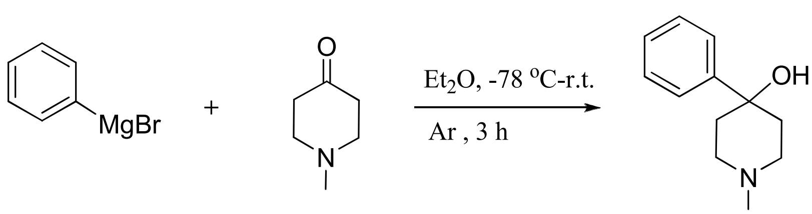
Isonicotinic acid (**36**) was methylated with methyl iodide followed by the use of an ion-exchange column to give **38**. Hydrogenation afforded the piperidine ring (**39**), which was then reacted with thionyl chloride, to give the acid chloride. The acid chloride was converted into 1-methyl-4-benzoyl-piperidine hydrochloride (**40**) via the Friedel-Crafts reaction with aluminum trichloride and benzene. Chlorination afforded the di-substituted piperidine ring (**41**). Treatment with sodium hydroxide and xylenes gave a mixture of the ketone (**42**) and 1-methyl-4-phenyl-4-piperidingcarboxylic acid (**43**). Esterification of **43** using hydrochloric acid and ethanol gave meperidine hydrochloride (**21**) in an overall yield lower than10%.[130]

Scheme 8

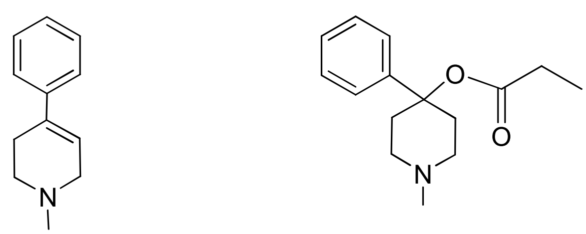


Also a more straightforward method via Route II, is direct coupling the aromatic ring with the piperidine via Grignard method (Scheme 9). The Grignard reagent phenylmagnesium bromide was reacted with 1-methylpiperidin-4-one (**45**) and afforded a 30% yield of target product 1-methyl-4-phenylpiperidin-4-ol (**46**), and a 25% yield of the byproduct 1-methyl-4-phenyl-1,2,5,6-tetra-hydropyridine (MPTP, **47**), because the tertiary alcohol is liable to dehydration under acidic conditions if the reaction temperature rises above -30 °C. MPTP has been found to produce Parkinson's-like symptoms, making this synthetic sequence and the handling of the intermediate compound quite unattractive.[131-133] One case in particular involved the development of Parkinson-like symptoms in a 37-year old chemist who had worked with the compound for eight years.[134] Also in 1982, seven people were diagnosed with Parkinson-like symptoms after having used 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP, **48**), an illegal recreational drug, contaminated with MPTP. Eventually the motor symptoms of two of the seven patients were successfully treated.[135]

Scheme 9



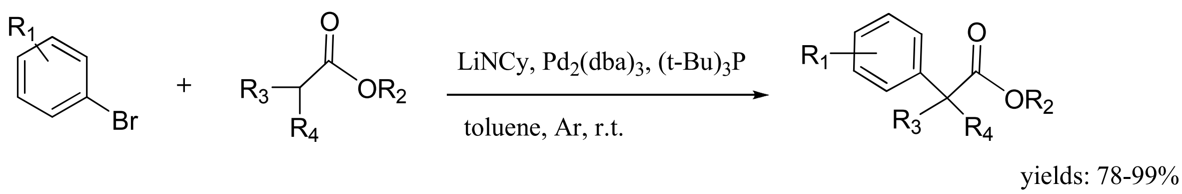
44 **45** **46**



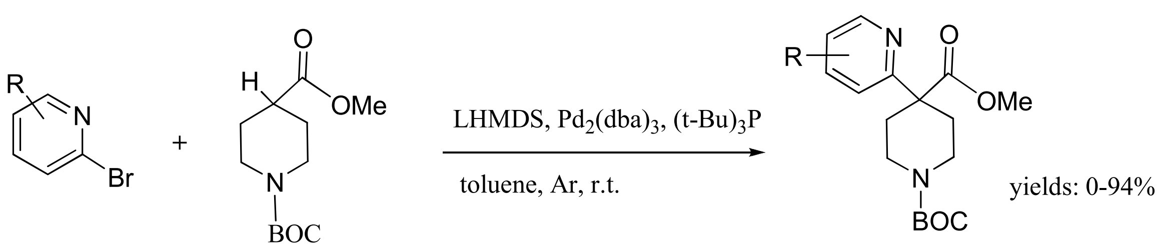
MPTP (**47**) MPPP (**48**)

In 2002, Hartwig *et al.* publish a new coupling method for α-arylation, which connect aryl ring directly to the α-carbon of activated esters or ketones.[141] (Scheme 10) According to the published paper, the yields are highly depend on the start materials as well as optimized base ligand pair and optimized solvents. In 2007, similar work was published from from a Pfizer laboratory.[142] In their publish paper, only N-BOC protected piperidines, with electron-deficient pyridine halides were applied with variable yields.

Scheme 10



49 **50** **51**

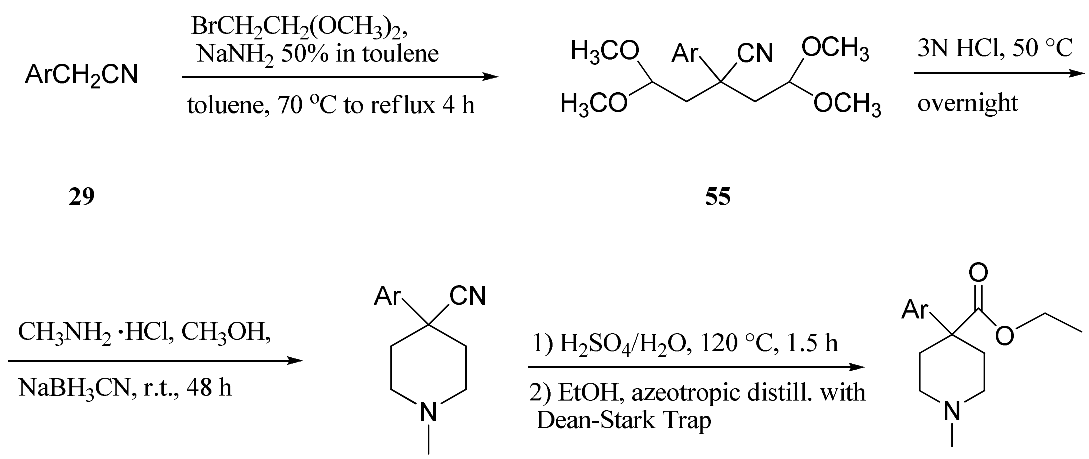


52 **53** **54**

Acetal Pathway for Meperidine Synthesis

Since the direct coupling route was determined to be low yielding and produced an inevitably toxic by-product and alternative approach was investigated. In 1999, a new synthesis pathway was designed and developed by Lomenzo, *et al.* (Scheme 11).[121] This synthesis provided a facile method of incorporating various aryl substitutions on the meperidine scaffold, while keeping toxic reagents and intermediates to a minimum. Improvements to the synthesis were achieved to make it more efficient for large scale. Specifically, the ring closure step was targeted.

Scheme 11



Objectives and Specific Aims

Based on the previous SAR studies of meperidine analogues, we have clearly identified a new class of serotonin transporter selective ligands (**21c,d,e**).[121] These preliminary studies demonstrated the potential for the development of meperidine—based SSRIs with diminished μ-opioid receptor affinity. The serotonin transporter binding affinity data obtained for these meperidine derivatives (**21c,d,e**) indicate that serotonin transporter reuptake inhibition should be both potent and selective over dopamine transporter and norepinephrine transporter. [121]

Although structural modifications on norepinephrine reuptake inhibition (NRI) have yet to be determined, NRI will probably not be significant since the meperidine analogues generally exhibited very poor affinity for the norepinephrine.[121] Moreover, the dopamine uptake inhibition may be selectively diminished by simple structural modifications of the aryl group and ester group.

We realized that the preliminary SAR was rather limited in scope and far from optimized. Nevertheless based on the serotonin transporter selectivity observed for these compounds, we were encouraged that a novel meperidine-based SSRI could be developed with therapeutic value for the treatment of a variety of psychiatric disorders.

The objective of the proposed research is to synthesize and evaluate the preclinical biological and behavioral effects of novel compounds targeted for the serotonin transporter. These compounds are designed to further elucidate the structure-activity relationships of serotonin transporter ligands as well as provide leads toward the development of new therapeutic SSRIs for the treatment of depression and related psychiatric disorders. To achieve these objectives the proposed areas of research are described in the following sections.

Specific Aim I: To design, synthesize and evaluate the 4-aryl-4·carboethoxy-piperidines as potential SSRIs.

A series of 4-aryl-4-carboethoxy-piperidines are to be synthesized. The initial focus of the proposed study will be to complete the SAR study described in the Introduction Section. This will require the synthesis of the analogues (**21c,d,e**) for evaluation of the norepinephrine affinity, monoamine reuptake inhibition (dopamine, serotonin, and norepinephrine), as well as μ-opioid receptor affinity. In addition to these analogues, the SAR of the aryl ring of meperidine will be explored further by the preparation of a variety of novel 4-aryl meperidine derivatives (**21**). It has been shown that increased lipophilicity on the aryl ring of 3-phenyl tropane derivatives led to enhanced serotonin transporter selectivity over dopamine transporter and norepinephrine transporter.[136-140] Since the SAR of the meperidine analogues has been shown to parallel the SAR of the 3-phenyltropanes many of the target compounds have aryl substitution patterns that have been shown to favor the serotonin transporter over the dopamine transporter and the norepinephrine transporter.

Specific Aim 2: To design, synthesize and evaluate 4-aryl-4-substituted meperidine derivatives as potential SSRIs.

A series of 4-aryl-4-substituted piperidine analogues that possess a variety of functional groups at the 4-position of 4-aryl-piperidines identified in Specific Aim 1 will be synthesized. The focus of this study will be to optimize the SAR of the ester moiety to enhance SERT selectivity over DAT and NET. As described in the Preliminary Results Section, replacing the ester group with other function groups did not enhance SERT selectivity relative. Therefore, for potent 4-aryl-carboethoxypiperidine derivatives (**21**) in Specific Aim 1, a series of ester analogues will be prepared. The initial focus of this study will be the preparation of benzyl esters.

Specific Aim 3: To design, synthesize and evaluate N-Demethylated 4-aryl-4-substituted meperidine derivatives as potential SSRIs.

Based on early SAR studies of N-substituted meperidine analogues, substituent group on N-atom will exhibit serotonin transporter affinity dramatically decreased due to increased steric hindrance and rigid amino conformation. However, some N-demethylated analogues (**21**) exhibit higher serotonin transporter selectivity over dopamine transporter and norepinephrine transporter.[125] Hence, N-demethylated analogues, which combine with optimized functional groups from aim 1 and 2, will be explored.

RESULTS AND DISCUSSION

Since the discovery of the analgesic effects of meperidine, various methods for the synthesis of meperidine and meperidine derivatives have been reported. [127-134,121] As we introduced and compared in previous section, several of the methods involve the use of toxic reagents or intermediates.[125-129,131-134] Other syntheses either involved a number of synthetic transformations or were limited in potential substitutions. To meet the objectives of this project, a synthesis that involved user- friendly reagents, the potential for incorporating a wide variety of substitutions and efficient in nature to produce a large number of analogues as well as large scale production of select analogues would be desirable. The synthesis reported by Lomenzo *et al.*[121] meets several of these requirements: 1) toxic reagents and intermediates are kept to a minimum; 2) various substitutions can be afforded in different steps of the synthesis and; 3) the five-step sequence can provide useful quantities from commercially available starting materials. As illustrated in Scheme 10, the synthetic sequence begins with dialkylation of an arylacetonitrile derivative **29** with bromoacetaldehyde dimethyl acetal to furnish the bisacetal **55**. Acid hydrolysis of **55** gives the dialdehyde and concomitant reductive amination provides the nitriles **20**. Hydrolysis of the nitriles **20** gives the carboxylic acid, followed by esterification with an alcohol to afford various esters **21**.

Attempted Alternative Meperidine Synthesis

Early experiments in our lab using mechlorethamine were successful to close the ring with good efficiency providing the meperidine derivatives in 50% yields. However, in order to avoid the toxic start materials, diethanolamine (**56**) was used as the starting material instead of mechlorethamine in Scheme 12, following the tosylation to convert both hydroxyl and amino groups into a sulfonate and sulfamide groups, respectively. The tosylated amine **57** should decrease the nitrogen basicity and nucleophilicity and lead to lower toxicity than that of mechlorethamine. However, when the following ring closure step was conducted with sodium amide, initially only monoalkylated phenyl acetonitriles (**59**) were obtained. Different bases like sodium hydride, lithium diisopropylamide and *n*-butyllithium were tested in parallel with different solvent systems, temperatures, reaction times and starting material concentrations (Table 7). Unfortunately the major product was consistently found to be mono-alkylated phenyl acetonitrile (**59**), and only <10% of the piperidine nitrile (**58**) was afforded. The carbon anion is observed after addition of base, but the alkylation stopped after one side chain was attached. Extended reaction times were unsuccessful and only the mono-alkylated nitrile (**59**) was observed after heating for 2 days, even in diluted systems. Also the further attempts to form the piperidine rings by using purified mono-alkylated nitrile (**59**) and base-promoted ring-closing conditions to effect the ring closure were unsuccessful and afforded low yields (<10%) of piperidine nitrile (**58**). Unreacted starting materials were observed and a mixture of intractable material was observed. The failure to close this ring was possibly due to the sulfamide group, which results in increased ring strain and making it difficult for the other side chain to be attacked by nitrile α-carbon anion.

Scheme 12

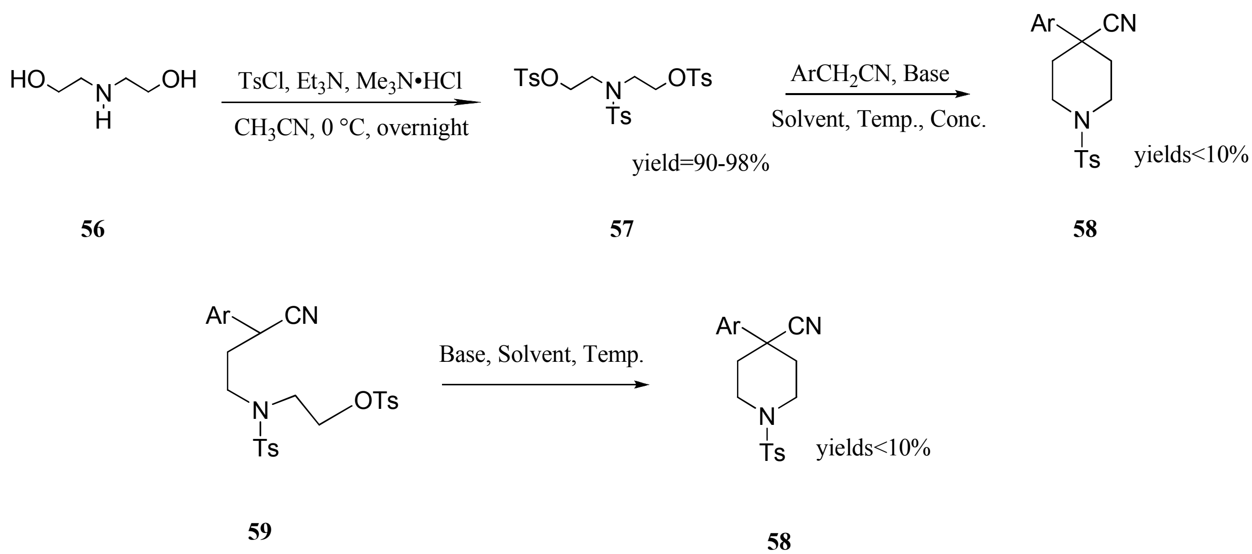
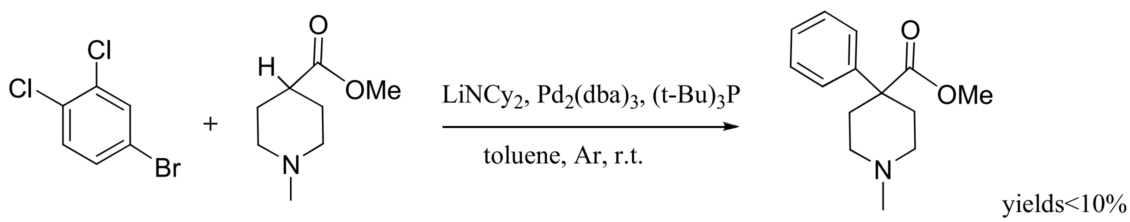


Table 7.Various conditions for ring closing step of **57** and **59**

Base	Solvent	Temp. (°C)	Conc. (M)
$NaNH_2$	Toluene/Ether	-23°C-r.t, then reflux	0.1M 0.2M 0.3M
NaH	THF/Toluene	0°C-R.T, then reflux	
LDA	CH_3CN/THF	-78°C-r.t then reflux	
n-BuLi	THF	0°C-r.t, then reflux	

According to the published method from Hartwig's group,[141] a couple of trails were carried out as illustrated Scheme 12. The procedure was carried out in glove box under a dry Argon atmosphere. A solution of the ester **61** (1.1 mmol) in toluene (2 mL) was added to a vial containing 1.3 equiv of $LiNCy_2$ (1.3 mmol). The solution was stirred for 10 min before it was transferred to a screw-capped vial containing a catalytic amount of $Pd_2(dba)_3$ and the aryl halide (1.0 mmol). Finally, P(*t*-Bu)$_3$ was added from a 0.5 M toluene stock solution. The vial was fitted with a PFTE septum and removed from the glove box. The reaction mixture was stirred at room temperature for 24 h. The target product methyl 1-methyl-4-phenyl-4-carboxymethylpiperidine (**62)** was observed by GC-MS however with low yields (< 10%), the homo-coupling byproduct and unreacted ester were also present. The result can possibly explained by the low reactivity of phenyllithium and the potential catalyst poisoning by basic piperidine nitrogen. This analysis was supported by the research work from a Pfizer laboratory.[142] Only N-BOC protected piperidines, with electron-deficient pyridine halides were applied with variable yields.

Scheme 12



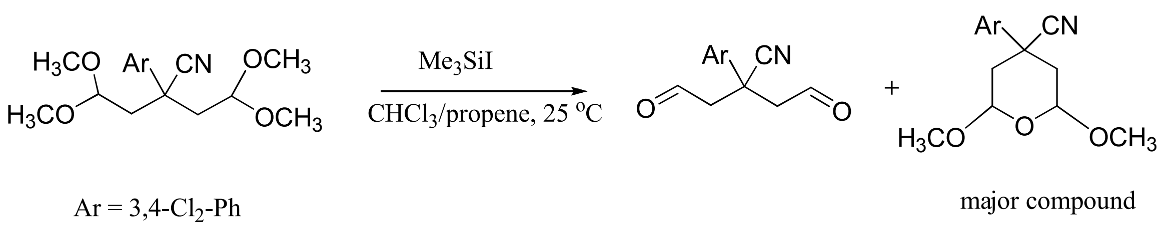
60 **61** **62**

Attempted Modification of Ring Closure Step

Since our attempts to develop new method for synthesis meperidine were successful. Attention focused on the optimization of acetyl synthetic pathway. It was evident that the synthesis shown in Scheme 11 would require increased efficiency for large-scale synthesis of the target aryl-substituted meperidines in order to obtain the desired gram quantities for behavioral studies. In particular, the ring closure step, which typically gave the piperidine ring products in moderate yields (30-50%), was targeted.

A search of the literature revealed that the deprotection of acetals using trimethylsilyl iodide afforded the corresponding aldehydes.[143] Previous attempts of using this deprotection method to obtain the dialdehyde product **63** from the diacetal compound **55** (Scheme 14). Propene was bubbled into chloroform followed by addition of iodotrimethylsilane and the diacetal **55**. (The use of the propene was necessary to eliminate the hydrogen iodide formed from the reaction of iodotrimethylsilane with moisture in the air. The resulting product, isopropyl iodide, could then be easily removed in vacuo.) The solution was allowed to stir for up to 75 minutes at room temperature. No evidence of the dialdehyde product **63** was detected by thin layer chromatography. It was determined by ^{1}H NMR and MS that the major product was actually a substituted pyran **64** obtained in 77% yield. It is thought that the proximity of the diacetals allows for favorable intramolecular ring closure to form a pyran ring upon formation of an intermediate hemiacetal.

Scheme 14

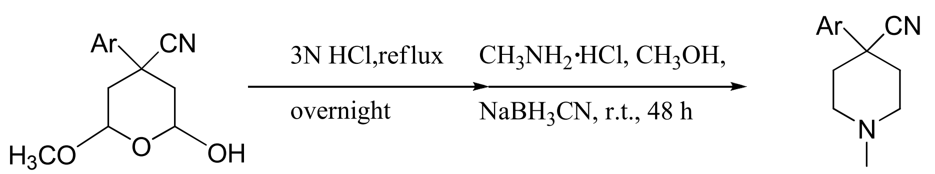


55 **63** **64**

Purified byproducts of the original deprotection/reductive amination method revealed a similar pyran ring system **65** had been formed in the hydrolysis of **55** (Scheme 10). The pyran **65** was obtained in 33% yield with a 35% yield of the desired nitrile **20**. With trails of different conditions and reactant amounts, the optimized yield of 50% can be achieved with fresh made 3N HCl for hydrolysis and three equivalents of methylamine HCl for the reductive amination.

Attempts to hydrolyze the pyran **65** revealed that it was necessary to increase the reaction temperature to reflux to afford the desired dialdehyde. Subsequent reductive amination furnished the piperidine ring **20** in only 30% yield (Scheme 15). Presumably the higher reaction temperature required for the hydrolysis also led to hydrolysis of the nitrile moiety as well, leading to the low overall yield of **20.**

Scheme 15



Ar = 3,4-Cl_2-Ph

30% yield

65 **20**

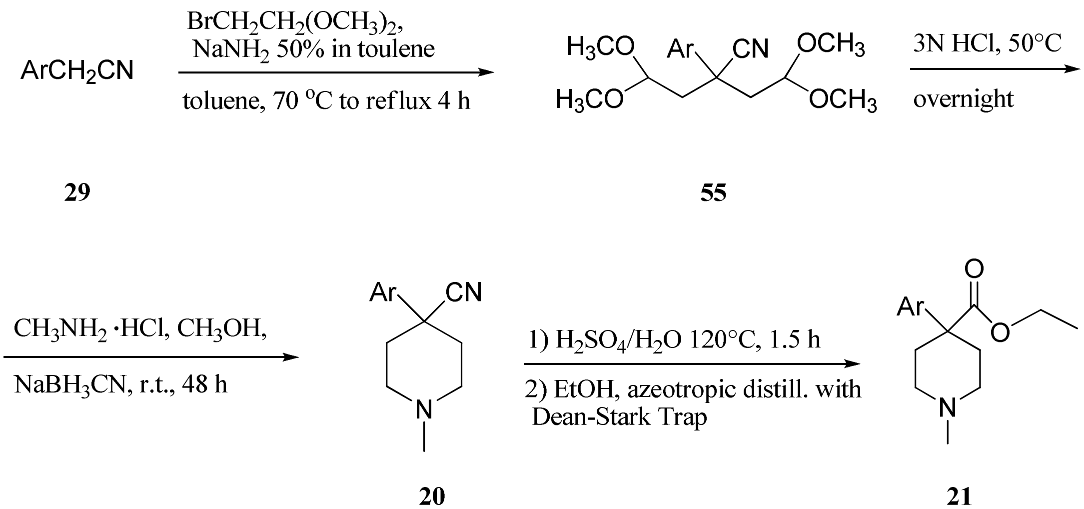
At this point it was determined that the original synthesis using the acetyl pathway could be scaled up enough to obtain gram quantities of the desired meperidine derivatives despite the modestly efficient ring closing sequence. The proximity of the diacetal moieties led to intramolecular ring-closure competition using the iodotrimethylsilane deprotection method and would more than likely be problematic using other deprotection methods as well. Alternatively, harsh conditions for the hydrolysis of the diacetals increased the risk of by-products such as hydrolysis of the nitrile. Performing the initial hydrolysis at 80 °C gave multiple products by thin layer chromatography and it can be assumed that use of a stronger acid would also give multiple products. Also treating the diacetal nitrile with strong acid and higher temperatures to make the dialdehyde with a carboxylic acid, or even straight to esterification step was assumed to be problematic due to too many possible byproducts like amino acid, amide and dehydrated pyrans, which will diminish the overall yield and add potential difficulty to the work up and purification steps.

Synthesis of Aryl-substituted Meperidine Derivatives

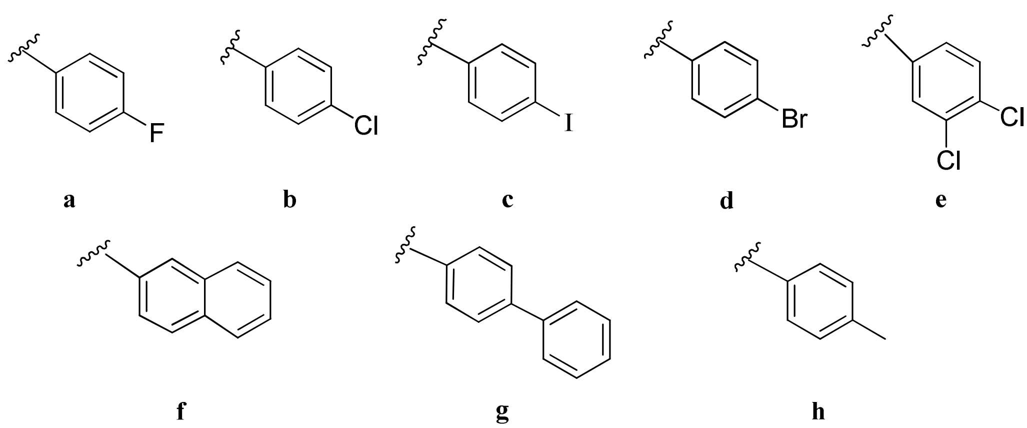
A series of aryl-substituted meperidine derivatives were chosen to be synthesized and tested for in vitro binding affinity. These compounds were chosen for their high affinity or selectivity for the serotonin transporter relative to meperidine. The desired variations of aryl moieties could be obtained as illustrated in Scheme 16.

The dialkylation of 2-aryl acetonitriles **29a-h** with sodium amide and bromoacetaldehyde dimethyl acetal in dry toluene gave the corresponding diacetals **55a-h** in good yields (65-80%). The diacetals **55a-h** were then deprotected via acid hydrolysis using 3N hydrochloric acid at 50 °C followed by reductive amination with methylamine hydrochloride and sodium cyano-borohydride in dry methanol to obtain the piperidines **20a-h**. This two-step process afforded the 4-aryl-4-cyano piperidines **20a-h** in moderate overall yields (30-50%). The nitriles **20a-h** were converted into the corresponding ethyl esters **21** first by hydrolysis with aqueous sulfuric acid at 120 °C to obtain the carboxylic acid, followed by addition of excess ethanol to the reaction mixture. Azeotropic distillation of the ethanol/water afforded the ethyl esters **21a-h** in good overall yields (50-80%).

Scheme 16



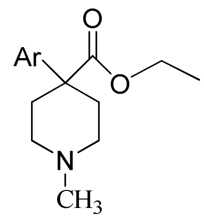
Ar =



Biological Studies of Dopamine Transporter, Serotonin Transporter, Norepinephrine Transporter and μ-opioid Binding Affinities of Aryl-substituted Meperidine Derivatives (21)

The binding assays were performed by Dr. Sari Izenwasser at the University of Miami School of Medicine. The binding affinities for monoamine transporters and μ-opioid receptor are reported as K_i values and listed in Table 8 and Table 9.

Table 8. The *In Vitro* Binding Data at Dopamine Transporter (DAT), Serotonin Transporter (SERT), Norepinephrine Transporter (NET) and μ-Opioid Receptor for 4-Aryl-Substituted Meperidines.

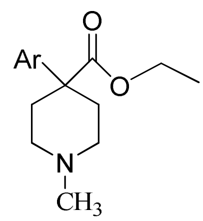


21a-h

Cpd	Ar	[^{3}H]WIN 35,428 (DAT) K_i(nM)[b]	[^{3}H]Paroxetine (SERT) K_i(nM)[b]	[^{3}H]Nisoxetine (NET) K_i(nM)[b]	[^{3}H]DAMGO (μ) K_i(nM)[b]
17	Ph	17,800±2,670	413±44	NT	920
21a	4-F-Ph	10,700±2250	308±26	(71.9)[d]	1,470
21b	4-Cl-Ph	4,100±1270	277±40	601,000±45,900	4,410
21c	4-I-Ph	3,250±195	21.0±2.4	519,000±51,100	2,350
21d	4-Br-Ph	6,601±137.6	48.6±6.1	NT	NT
21e	3,4-Cl_2-Ph	125±15	19.0±2.6	74,500±5,100	2,040
21f	2-Naphthyl	1140±380	7.20±0.53	71,100±9,700	2,030
21g	4-Ph-Ph	6,800±3,000	43.0±8.0	4,820±632	4,390
21h	4-Me-Ph	12,400±5,210	1,610±110	(57.6)[d]	2,670

[a] All compound were tested as HCl salt. [b] All values are the mean±SEM of three experiments performed in triplicate. [c] Percent inhibition at highest dose tested (100μM). [d] Percent inhibition at highest dose tested (10 μM). NT, Not tested.

Table 9. The Binding Selectivity on SERT over DAT, NET and μ-Opioid Receptor for 4-Aryl-Substituted Meperidine Compounds.

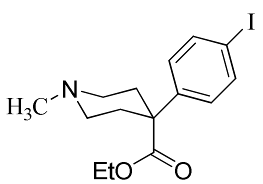
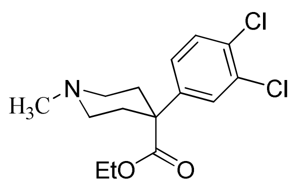
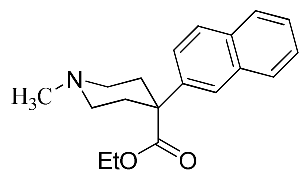
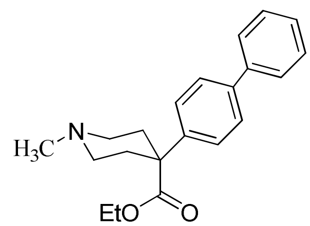


21a-h

Cpd	Ar	DAT/SERT	NET/SERT	μ/SERT
17	Ph	43	--	2.2
21a	4-F-Ph	34	--	4.8
21b	4-Cl-Ph	14	2,170	16
21c	4-I-Ph	154	24,700	111
21d	4-Br-Ph	136	--	--
21e	3,4-Cl_2-Ph	6.7	3,920	109
21f	2-Naphthyl	162	9,880	281
21g	4-Ph-Ph	158	112	102
21h	4-Me-Ph	7.7	--	1.7

In general, the substituted aryl ring improved the serotonin transporter binding potency and selectivity, except compound **21h**, which bearing 4-methyl group as an electron-donating substituent. For the analogues containing an electron-withdrawing substituent, the serotonin transporter binding affinity improved with larger substituent, such as 4-I-phenyl (**21c**), 3,4-Cl_2-phenyl (**21e**), 2-naphthyl (**21f**), and 4-biphenyl (**21g**) analogues. Also the μ-opioid receptor binding affinity was dramatically diminished by larger substituent. For norepinephrine transporter binding, these meperidine analogues show poor affinities and can be considering not relative.

The results of this SAR study gave us the picture that there must be a large hydrophobic pocket at serotonin transporter that can accommodate a large substituent on the aryl ring (e.g. analogues **21c,e,f,g).** The 3-D models of these candidates are shown below to illustrate these ring features (Figure 10).

21c **21e** **21f** **21g**

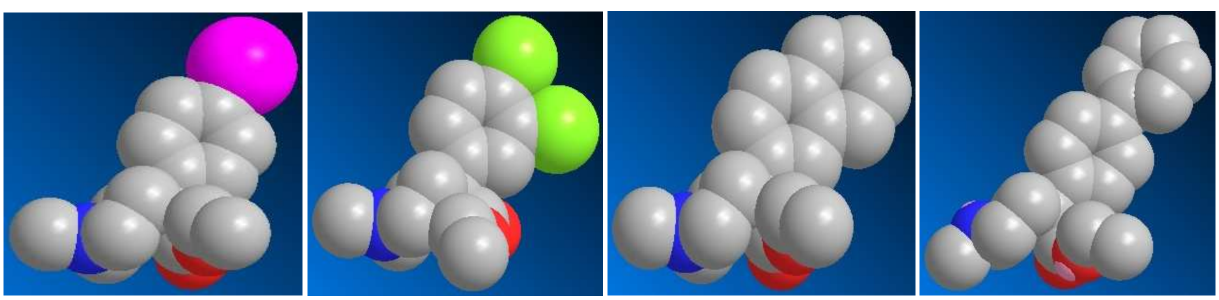


Figure 10. Structure comparison of Meperidine Analogues (**21c,e,f,g**).

Also the bioavailability properties of these analogues are also summarized in Table 10. Both partition coefficient (ClogP) and topological polar surface area (tPSA) data are calculated and compared.[144] CLogP and tPSA are important index for predict a drug's possibility to pass through cell membranes and blood brain barriers. Usually the tPSA should be lower than 140 angstroms square ($Å^2$) for the drug penetrate cell membranes and lower than 60 angstroms square to pass the blood-brain barriers, [145] also the drug candidate with ClogP <5 will be able to pass the blood-brain barriers ,according to Lipinski's rule of five.[146]

Table 10. CLog P and tPSA properties of meperidine analogues **21c, e, f, g**

Cmpd.No.	CLogP	Topological Polar Surface Area,[144] (Å^2)
21c	3.35	29.54
21e	3.53	29.54
21f	3.40	29.54
21g	4.11	29.54

Among of those aryl rings, 4-I- phenyl (**21c**), 3,4-Cl_2-phenyl (**21e**), 2-naphthyl (**21f**),and 4-biphenyl (**21g**) analogues, which exhibit high potency and selectivity, will serve a new leads to the next level of structure modifications. Also all the analogues present good bioavailability with low CLogP and tPSA prediction. The 3,4-Cl_2-phenyl derivative **21e w**as selected as the primary lead for further optimization due to the relative ease it could be synthesized and the availability of gram quantities.

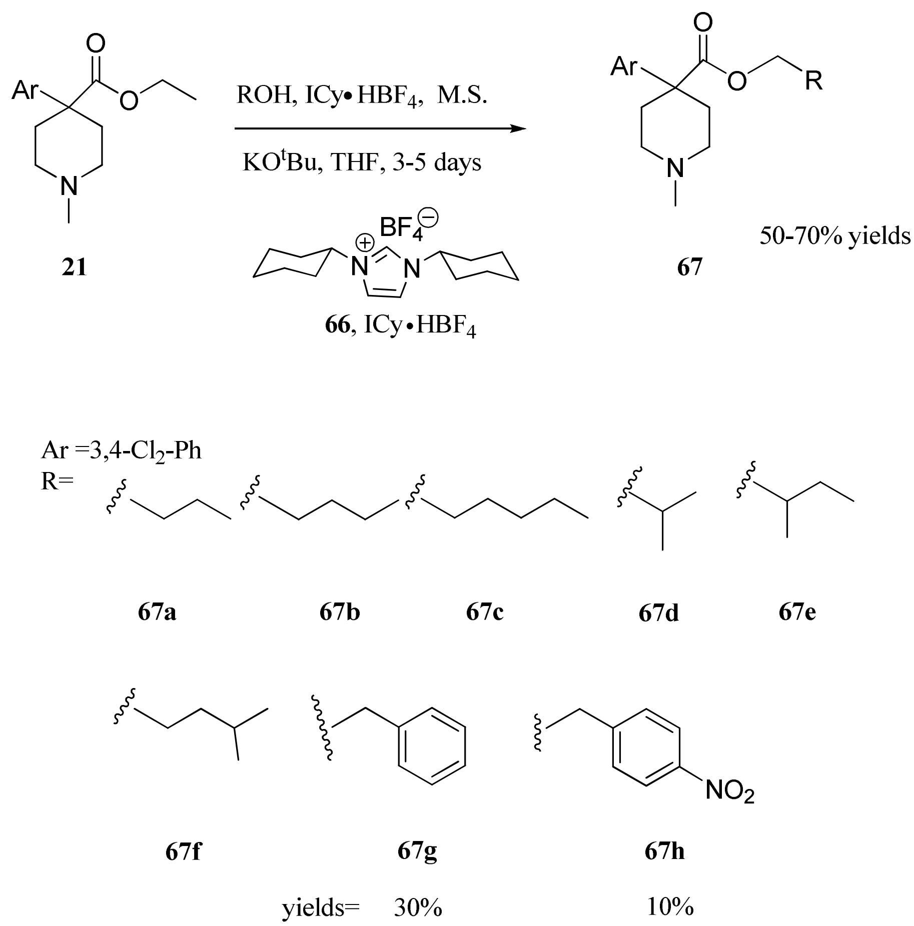
Synthesis of Substituted Benzyl 1-methyl-4-aryl-4-carboalkoxy-piperidine

Based on the earlier work of Rhoden *et al*., the binding data of 4-aryl-4-substituted meperidine analogues exhibited no positive effects on serotonin transporter binding and selectivity.[125] In other words, modification of the ester moiety into other functionalities did not significantly improve serotonin transporter binding compared to the ester moiety. Hence, our next task focused on different esters possessing different alkyl groups and different substituted benzyl group.

Due to the high boiling points of some alkyl alcohol or crystalline nature of the substituted benzyl alcohols, the hydrolysis and esterification process in Scheme 10 were not successful. The N-heterocyclic carbene catalyst work from the laboratories of Professor Steve Nolan appeared to be well suited for this transformation. We choose to apply this novel trans-esterification method illustrated as Scheme 16, to obtain our desired ester analogues.[147-149]

Based upon the literature, the 1,3-bis(cyclohexane) imidazol-2-ylidene (ICy) N-hetero-cycliccarbene catalyst was identified as the best catalyst for the 3,4-dichlorophenyl meperidine system. Using a fluoroboric acid salt of ICy **66** the N-heterocycliccarbene catalyst was form in situ by adding potassium *tert*-butoxide (9.5 mol%), and activated molecular sieves. The reaction was performed in tetrahydrofuran at room temperature for 1-3 days and gave the desired esters **67a-f** with isolated 50% yield or 70% by GC. The low isolated yield is due to the similar polarity of both esters **21** and **67**. All of the products show the same spot on TLC plate, it could only be separated with long thin column using SiO_2 and 10% MeOH in $CHCl_3$.

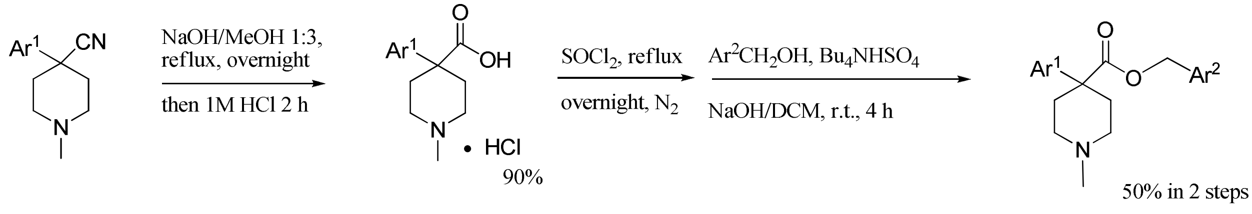
Scheme 17



While the transesterification method worked well for the formation of alkyl esters, further application of this method for some substituted benzyl alcohols like 4-nitro, 4-methoxy, 4-phenyl and 3, 4-dichloro groups gave low yields (10% to 30%) of the corresponding benzyl esters. Even with prolonged reaction times of up to 5-7 days and higher catalyst loading (5%-10%), the yields did not improve. The source of the low yield is presumably due to the lower nucleophilicity of the oxygen of the substituted benzyl alcohols.

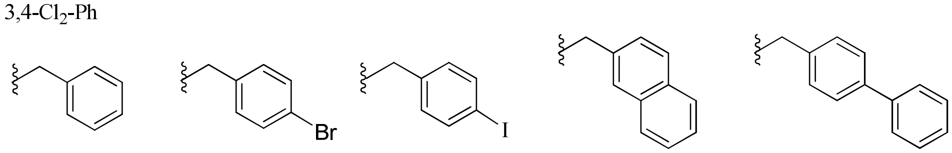
Generally problems encountered during transesterification arise from equilibration.[150] There is plethora of methods to bias the equilibrium to the product side. Comparing to several other strategies and attempts, the acid chloride pathway as illustrated in Scheme 18 gave moderate overall yields, about 55%, with easy purification of the final ester compound. In this route, basic hydrolysis of the piperidine nitriles (**20**) was chosen because of higher yields, easy purification and avoiding potential piperidine ring opening. Released ammonia gas NH_3 can be detected by wet pH paper as a monitor for reaction progress. The 4-aryl-4-carboxylic acid piperidine analogues (**43**) were afforded by recrystalization as the HCl salt, with 92% yield. Thionyl chloride was used to convert the carboxylic acids into the corresponding acid chlorides. Employing thionyl chloride for this step required careful handling and waste control, due to its corrosive nature and irritating smell. To this end we designed a simple but effective device to absorb and HCl fumes produced by the reaction. After removing all the thionyl chloride at 100 °C, the mixture was cooled down and dried by a continuous flow of N_2. The following esterification employed a heterogeneous system of aqueous NaOH and dichloromethane. Tetra-butylammonium bisulphate was used as a phase transfer catalyst and the esterification gave the product **67** in 50% yields for the two step process. Other phase transfer agents like 18-crown-6 ethers and tetra-butyl ammonium bromide were also explored for this reaction, but gave significantly lower yields of the desired benzyl esters.

Scheme 18

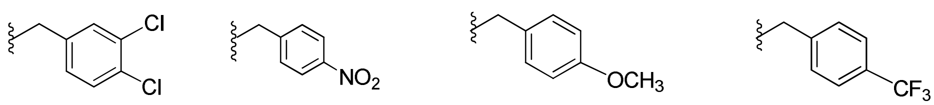


20 **43** **67**

Ar^1 = 3,4-Cl_2-Ph
Ar^2 =



67g **67h** **67i** **67j** **67k**

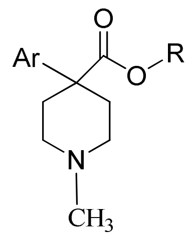


67l **67m** **67n** **67o**

Biological Studies of Dopamine Transporter, Serotonin Transporter, and Norepinephrine Transporter Binding Affinities of 4-Aryl-4-carboalkoxy meperidine Derivatives (67)

The binding assays were performed by Dr. Sari Izenwasser at the University of Miami School of Medicine. The binding affinities for monoamine transporters and μ-opioid receptor are reported as K_i values and are listed in Table 11.

Table 11. The *In Vitro* Binding Data at Dopamine Transporter (DAT), Serotonin Transporter (SERT) for 4-Aryl-4-Substituted Meperidines.

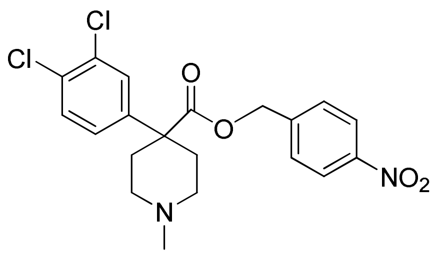


67a-n

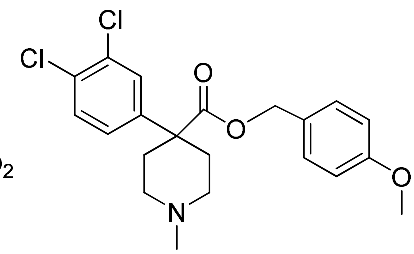
Cmpd[a]	Ar	R	[^{3}H]WIN 35,428 (DAT) K_i(nM)[b]	[^{3}H]Paroxetine (SERT)K_i(nM)[b]	DAT/ SERT
21e	3,4-Cl_2-Ph	CH_2CH_3	125±15	19±2.6	6.6
21i	3,4-Cl_2-Ph	CH_3	383±32	15±1.1	26
67a	3,4-Cl_2-Ph	$CH_2CH_2CH_3$	449±94	16±0.7	27
67b	3,4-Cl_2-Ph	$CH_2CH_2CH_2CH_3$	864±114	16±2.5	54
67d	3,4-Cl_2-Ph	$CH(CH_3)_2$	271±51	43±7.0	6.3
67e	3,4-Cl_2-Ph	$CH(CH_3)CH_2CH_3$	283±11	44±4.7	6.4
67g	3,4-Cl_2-Ph	$CH_2C_6H_5$	917±138	9.2±3.1	100
67h	3,4-Cl_2-Ph	$CH_2C_6H_5$-4-Br	2563±47.8	7. 0±0.9	366
67i	3,4-Cl_2-Ph	$CH_2C_6H_5$-4-I	3471±237.8	13.2±1.8	262
67j	3,4-Cl_2-Ph	CH_2-naphthyl	4913±525.2	9.9±3.7	496
67k	3,4-Cl_2-Ph	$CH_2C_6H_5$-4-phenyl	5629±508	10.9±2.1	516
67l	3,4-Cl_2-Ph	$CH_2C_6H_5$-3,4 Cl_2	5,230±152	4.3±0.5	1,215
67m	3,4-Cl_2-Ph	$CH_2C_6H_5$-4-NO_2	1,530±334	1.0±0.10	1,530
67n	3,4-Cl_2-Ph	$CH_2C_6H_5$-4-OMe	2792±626.4	1.7±0.2	1642

[a]All compound were tested as HCl salt. [b] All values are the mean±SEM of three experiments performed in triplicate.

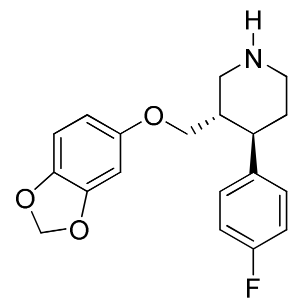
From the above data, the benzyl ester congeners generally exhibited better serotonin transporter affinity and selectivity than alkyl esters. Among the benzyl ester congeners, substituted benzyl ring with 3,4-Cl **67l**, 4-NO_2 **67m**, 4-OMe **67n** exhibited higher potency and selectivity.



67m



67n



Paroxetine (**8**)

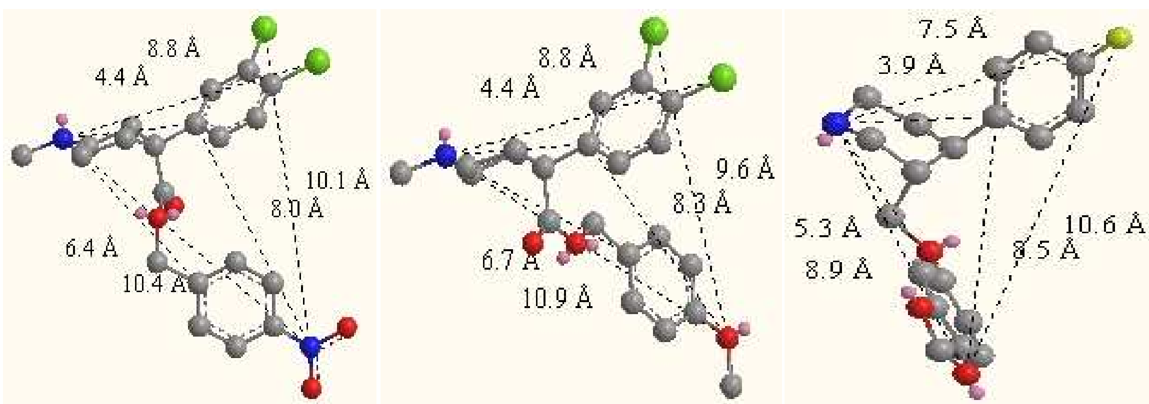


Figure 11. Structure comparison of meperidine analogues **67m**, **67n**, and Paroxetine

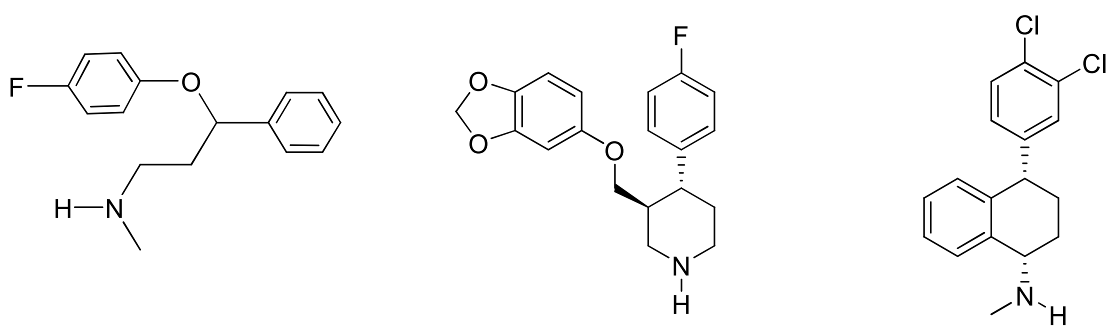
Table 12. Structual summary of meperidine analogues **67m**, **n**, and Paroxetine[122]

Compound	N-Ar^1 Distance (Å)	N-Ar^2 Distance (Å)	Ar^1-Ar^2 Distance (Å)
67m	4.4-8.8	6.4-10.4	8.0-10.1
67n	4.4-8.8	6.7-10.9	8.3-9.6
Paroxetine	3.9-7.5	5.3-8.9	8.5-10.6

From the SAR studies, a second aromatic ring appears important for SERT recognition. In addition, a strong H-bond acceptor substituent on the benzyl ester is favored. As illustrated in Figure 11 and Table 12, the computational comparison **67n, 67m** and Paroxetine revealed that these molecules share similar structural geometry features. The aryl groups of the ester lies in the same domain as the benzodioxole group of paroxetine.

Synthesis of *N*-Demethylated Benzyl 1-methyl-4-aryl-4carboalkoxy-piperidine

The previous SAR studies from our group showed that the N-substituted meperidine analogues exhibit diminished serotonin transporter binding affinity.[125] However the N-demethylated piperidine analogue showed slight improvement of serotonin transporter binding affinity and apparently better selectivity. It was noteworthy, that most current market SSRIs possesses a secondary amine group Figure 12.

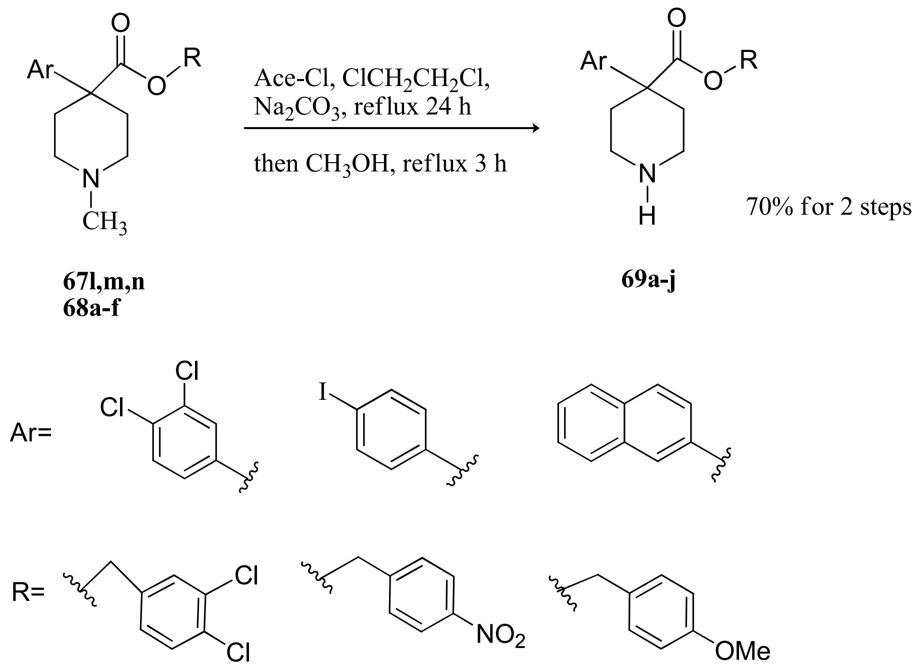


(±)Fluoxetine (**7**) (-)Paroxetine (**8**) Sertraline (**10**)

Figure 12. Secondary amines in current market SSRIs.

Based on the above analysis, all the potent candidates that had passed previous screening would be demethylated using our established method (Scheme 19). The target compounds are the combination of previous screening candidates, 3,4-Cl_2-phenyl, 4-I-phenyl and 2-naphthyl, combined with the three potent benzyl esters groups, 4-OMe benzyl, 4-NO_2 benzyl and 3,4-dichloro benzyl.

Scheme 19



68a: Ar= 4-I-Phenyl;R=3,4-Cl_2-Benzyl
68b: Ar= 4-I-Phenyl;R=4-NO_2-Benzyl
68c: Ar= 4-I-Phenyl;R=4-OMe-Benzyl
68d: Ar=2-Naphthyl;R=3,4-Cl_2-Benzyl
68e: Ar= 2-Naphthyl;R=4-NO_2-Benzyl
68f: Ar= 2-Naphthyl;R=4-OMe-Benzyl

Biological Studies of DAT, SERT, and NET Binding Affinities of Benzyl 4-Aryl- 4-Carboxylate N-Substituent Piperidine Derivatives (69a-j)

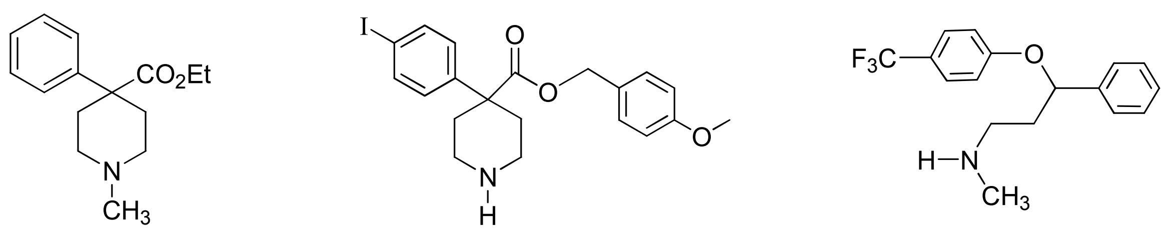
The binding assays were performed by Dr. Sari Izenwasser at the University of Miami School of Medicine. The binding affinities for monoamine transporters are reported as K_i values for the N-demethylated analogues are listed in Table 13.

Table 13. The *In Vitro* Binding Data at Dopamine Transporter (DAT), Serotonin Transporter (SERT), and Norepinephrine Transporter (NET) for 4-Aryl-4-carbobenzyloxy piperidines (**68l-n**,**69a-j**).

Cpd[a]	Ar	X	R	DAT K_i(nM)[b]	SERT K_i(nM)[b]	NET K_i(nM)[b]	DAT/ SERT	NET/ SERT
68l	3,4-Cl_2-Ph	3,4 Cl_2	CH_3	5,230±152	4.3±0.5	NT	1,215	-
69a	3,4-Cl_2-Ph	3,4 Cl_2	H	2,892±715	30.2±4.5	11,930 ±1060	96	395
68m	3,4-Cl_2-Ph	4-NO_2	CH_3	1,530±334	1.0±0.10	NT	1,530	-
69b	3,4-Cl_2-Ph	4-NO_2	H	1,295±263	3.7±1.2	10,190 ±701	350	2754
68m	3,4-Cl_2-Ph	4-OCH_3	CH_3	2,792±626	1.7±0.2	299.9 ±29	1642	176
69c	3,4-Cl_2-Ph	4-OCH_3	H	1,264±142	4.5±0.9	12,109 ±931	280	2690
69j	3,4-Cl_2-Ph	4 CF_3	H	2,244±200	46.7±17	7,902 ±896	48	196
21c	4-I-Ph	--	H	3,500±321	21.6±1.6	NT	162	--
69d	4-I-Ph	3,4-Cl_2	H	4,342±1089	5.9±2.7	6,059 ±1598	736	1027
69e	4-I-Ph	4-NO_2	H	1,777±265	5.9±2.0	2,069 ±322	301	351
69f	4-I-Ph	4-OCH_3	H	2,925±626	0.6±0.2	2,731 ±698	4875	4552
21f	2-Naphthyl	--	H	710±138	2.5±0.8	NT	284	--
69g	2-Naphthyl	3,4-Cl_2	H	3,212±311	26.5±2.4	16,557 ±4034	121	625
69h	2-Naphthyl	4-NO_2	H	732±132	2.0±0.5	937±7	366	469
69i	2-Naphthyl	4-OCH_3	H	889±463	2.9±0.2	2,349 ±127	307	810

[a]All compounds were tested as oxylate salts. [b]All values are the mean±SEM of three experiments performed in triplicate. NT means not test.

From above data, we observed N-demethylated meperidine analogues (**69)** generally exhibited improved serotonin transporter affinity and selectivity. Analogue **69f** showed the highest potency and selectivity in the meperidine derivatives, which exhibited sub-nanomolar binding affinity and great than 4500 times selectivity for the serotonin transporter. Compared to the our initial lead compound, meperidine (**17**), compound **69f** is 700 times more potent and 100 times more selective for the serotonin transporter (Figure 13). Also comparing to the marketed drug Prozac®, [fluoxetine (**7**)], **69f** still shows at least ten-fold greater potency and 30-fold increased selectivity over dopamine transporter binding and 150 fold over norepinephrine transporter. This compound will be advanced to *in vivo* behavioral studies for evaluating as a potential antidepressant.



Meperidine (**17**)

SERT K_i=413 nM
DAT/SERT=43

69f

SERT K_i=0.6 nM
DAT/SERT=4875
NET/SERT=4552

Fluoxetine (**7**)

SERT K_i=8 nM
DAT/SERT=163
NET/SERT=31

Figure 13. Comparison of potency and selectivity of analogue **69f** with meperidine and fluoxetine.

Design of Next Generation SSRIs

Although we have done the optimization and obtained a promising candidate for in vivo behavioral tests, we always keep "Lipinski's Rule of Five" for blood brain barrier permeability in mind throughout our design and development.[146] The rule of five is the basic guideline to evaluate druglikeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely to pass through the blood-brain barriers in humans. Over the past decade Lipinski's profiling tool for druglikeness has led to further investigations by scientists to extend profiling tools to lead-like properties of compounds in the hope that a better starting point in early discovery can save time and cost.re According to Lipinski's rules, a CNS active drug has no more than one violation of the following criteria:

1. Not more than 5 hydrogen bond donors.
2. Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms).
3. A molecular weight under 500 daltons (better from 160-480).
4. An octanol-water partition coefficient log P of less than 5.

Drug candidate **69f** possesses a molecular weight of 451.30 and CLogP value of 4.686, which are close to the boundary lines of the Rule of Five (illustrated in Figure 14). Hence, lowering the molecular weight and CLogP of next the candidate should lead to improved blood brain permeability and potential efficacy. Also the ester functional group in **66f** is liable to hydrolysis that might lead to a short pharmacological half-life. The half-life is an important pharmaco-kinetic parameter in drug development, which indicate how long the drug will maintain its pharmacological activity in human body. Here we designed *N*-benzyl-1-(1-methyl-4-phenylpiperidin-4-yl) derivatives as the next generation SSRIs. With the replacement of ester group by less metabolically liable amino group, the half life is assumed to be extended while the amino group still participates as potential H-bond acceptor/donor and provides chain flexibility. In addition, the conversion of diamine to dihydrochloride salt, the diamine congeners would be expected to exhibit much improved water solubility.

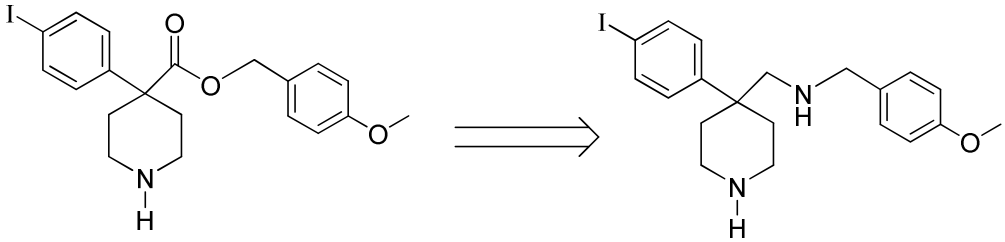
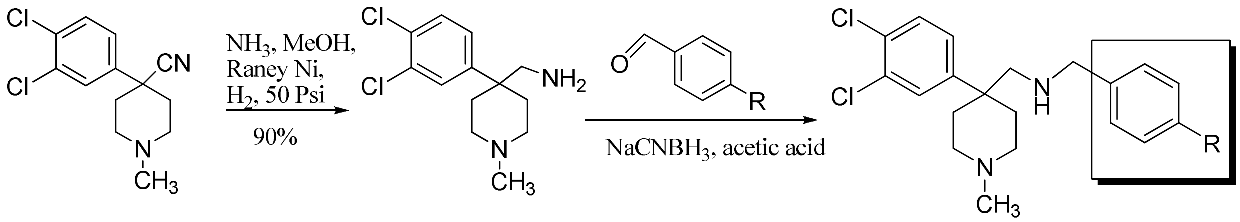


Figure 14. New design of next generation SSRIs based on analogue **69f**

Synthesis of *N*-benzyl-1-methyl-4-aryl-4-methylamine Derivatives (71)

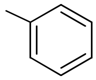
The synthesis of a *N*-benzyl piperidine analogues was achieved from piperidine nitriles as shown in Scheme 20. The first reduction step used Raney Ni in a saturated NH_3/methanol solution in a hydrogen atmosphere (50 psi) and afford the primary amine **70** in 90% yield. The subsequent reductive amination gave the N-alkylated secondary amines **71** in 65-70% yields. The target compounds were converted in the oxalate salts and submitted for in vitro testing at dopamine transporter, serotonin transporter and norepinephrine transporter. The results of these bioassay studies will be reported elsewhere.

Scheme 20

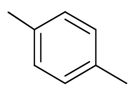


20d **70** **71**

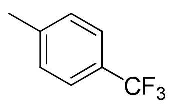
Benzyl amine analogues, **68 a-i**



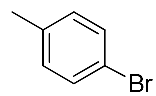
71a



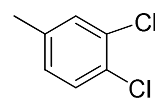
71b



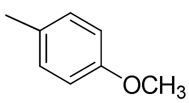
71c



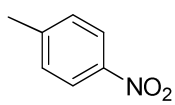
71d



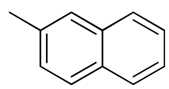
71e



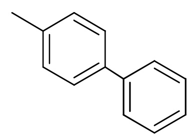
71f



71g



71h



71i

CONCLUSIONS

In conclusion, an efficient synthesis for derivatives of meperidine was developed to obtain a variety of meperidine derivatives as ligands for the serotonin transporter. This synthesis allowed for large-scale (gram) quantities of potent aryl-substituted derivatives of meperidine to be synthesized, as well as allow the facile incorporation of various substitutions and transformations on the meperidine system in order to future explore the structure-activity relationships at the serotonin transporter. The synthesis also minimized the use of toxic reagents and intermediates to provide a safe procedure to prepare the target compounds.

From the *in vitro* binding affinity studies, the substituted aryl ring generally improved the serotonin transporter binding potency and selectivity. Only compound **21h**, bearing 4-methyl group as an electron-donating substituent, exhibited diminished potency relative to meperidine. For the analogues containing an electron-withdrawing substituent, the serotonin transporter binding affinity improved with larger substituents, such as 4-I-phenyl (**21c**), 3,4-Cl_2-phenyl (**21e**), 2-naphthyl (**21f**), and 4-biphenyl (**21g**) analogues. Also the μ-opioid receptor binding affinity was dramatically diminished by larger substituents. All these ring systems indicate a hydrophobic pocket at the serotonin transporter that can hold an aromatic ring system like the 4-iodophenyl or biphenyl rings.

Substituted benzyl esters exhibit higher potency and selectivity than the corresponding alkyl esters. The high affinity of these benzyl esters indicates that a second ring system is quite important for serotonin transporter recognition. Among the benzyl ester congeners, a substituted benzyl ring with a 3,4-Cl_2 (**67l**), 4-NO_2 (**67m**), 4-OCH_3 (**67n**) group exhibited the highest potency and selectivity in this series.

The *N*-substitution of piperidine analogues decrease both the binding affinity at the serotonin transporter and the dopamine transporter. Substituents larger than a methyl group decreased the potency. Presumably, the steric hindrance around the nitrogen atom blocks the nitrogen terminal for dopamine transporter and serotonin transporter recognition. Alternatively, *N*-demethylated meperidine analogues (**69**) generally exhibited improved serotonin transporter affinity and selectivity. Compound **69f**, the methoxylbenzyl-4-iodophenyl piperidine ester analogue, showed the highest selectivity and potency of the compounds prepared in this study with sub-nanomolar binding affinity (K_i = 0.6 nM) and selectivity at the serotonin transporter greater than 4500 times that of the dopamine transporter and the norepinephine transporter. This compound **69f** will be advanced to *in vivo* bioassays and animal behavioral tests.

Based upon the structure-activity studies, the pharmacophore of the meperidine analogues can be summarized in Figure 15. The substituted phenyl ring at 4-position is required for SERT binding recognition. Here, lipophilic groups are favored with the 4-iodo substiution preferred. The second aryl ring system of the benzyl esters is also crucial for high binding potency. Substitution on this aryl ring is favored by the electron-rich 4-methoxy group. Finally a secondary amine terminal leads to improved serotonin transporter selectivity.

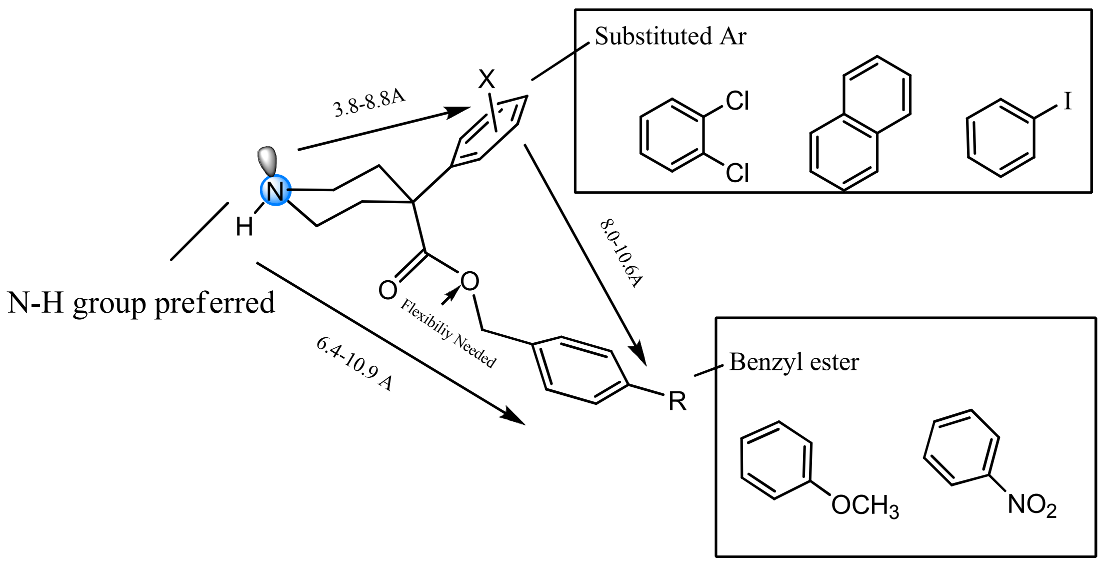


Figure 12. Meperidine analogue pharmacophore for SERT Binding.

A series of N-benzyl piperidine analogues were synthesized as the next generation of SSRIs, based upon the ester analogues. These amine congeners are expected to maintain the high potency and selectivity with lower molecular weight, better bioavailability and extended half-life relative to ester analogues. The results of these bioassay studies are still undergoing and the binding data will be reported elsewhere.

EXPERIMENTAL SECTION

All chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI. unless otherwise noted. Anhydrous THF, CH_2Cl_2 and CH_3OH were purchased from Mallinkrodt Baker. Toluene and Et_2O (Drisolv®, EMD Chemicals) were purchased from VWR International. Chromatography refers to flash column chromatography on silica gel (Sorbent Technologies Silica Gel 60Å, 230-400 mesh, 32-63 µm Standrard Grade). Petroleum ether refers to pentanes with a boiling point range of 30-60 °C. Reported melting points are uncorrected and were determined using a Hoover Mel-Temp apparatus. NMR spectra were recorded on a Varian-Gemini 400 MHz spectrometer. Chemical shifts are reported as δ values with tetramethylsilane (TMS), employed as the internal standard. Elemental analyses were obtained from Atlantic Microlabs, Inc. Norcross, GA.

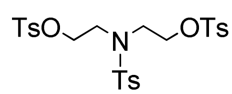
General Procedure A-1. Preparation of Hydrochloride Salts.

Some of the compounds were converted into the hydrochloride salts for biological testing, as well as for storage and handling purposes. The base (50-100 mg) was dissolved in a minimum amount of diethyl ether (1-2 mL) and added to a saturated ethereal solution (10 mL) of anhydrous hydrogen chloride. The hydrochloride salts crystallized and were washed with Et_2O (3 x 2 mL) and purified by trituration with Et_2O and ethyl acetate. Fractional moles of water could not be prevented, despite vigorous drying (110 °C, 1h) under vacuum (0.01 mm Hg). All compounds were homogeneous on thin-layer chromatography ($CHCl_3$/CH_3OH/NH_4OH, 90:9:1).

General Procedure A-2. Preparation of Oxalic Acid Salts.

Some of the final compounds were converted into the oxalic acid salts for biological testing, as well as for storage and handling purposes. The base (50-100 mg) was dissolved in a minimum amount of anhydrous THF (1-2 mL) and added to a saturated THF solution (10mL) of anhydrous oxalic acid. The salts crystallized and were washed with anhydrous THF (1 x 2 mL) and then washed with Et_2O (3 x 2 mL) and purified by trituration with Et_2O and ethyl acetate. Fractional moles of water could not be prevented, despite vigorous drying (110 °C, 1h) under vacuum (0.01 mm Hg). All compounds were homogeneous by thin-layer chromatography ($CHCl_3$/CH_3OH /NH_4OH, 90:9:1).

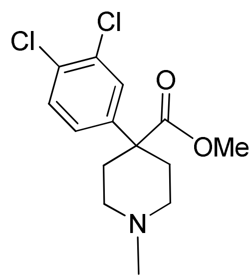
2,2'-(Tosylazanediyl)bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (57). [151]



TsCl (33.3 mmol) in dry toluene (30 mL) was added to a stirred suspension of diethanolamine (9.51 mmol), Et_3N (4.0 mmol) and Me_3N•HCl (0.2 mmol) in toluene (10 mL) at 0-5 °C, and the mixture was stirred overnight. Water (20 mL) was added to the mixture, which was extracted with EtOAc (3 x 25 mL). The organic layers were combined and washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica-gel column chromatograph (SiO_2, hexane: EtOAc, 1:1) to give **57** as white solid (4.7 g, 91%

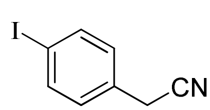
yield).[1]H NMR ($CDCl_3$) δ 7.77-7.75 (d, J = 8.4 Hz, 3H), 7.63-7.61 (d, J = 8.4 Hz, 2H), 7.37-7.35 (d, J = 8.0 Hz, 4H), 7.31-7.29 (d, J = 8.0 Hz, 3H), 4.11 (t, J = 8Hz, 4H), 3.38 (t, J = 6 Hz, 4H), 2.46 (s, 6H), 2.43 (s, 3H).

Synthesis of meperidine using Hartwig-Buchward method (62).



The procedure was carried out in glove box under a dry Argon atmosphere. A solution of the ester **61** (173 mg, 1.1 mmol) in toluene (2 mL) was added to a vial containing 1.3 equiv of $LiNCy_2$ (250 mg, 1.3 mmol). The solution was stirred for 10 min before it was transferred to a screw-capped vial containing a catalytic amount of $Pd_2(dba)_3$ (1.15 mg, 0.002 mmol)and the aryl halide **60** (225.9 mg, 1.0 mmol). Finally, P(*t*-Bu)$_3$ (0.002 mmol, 0.5 µl) was added from a 0.5 M toluene stock solution. The vial was fitted with a PFTE septum and removed from the glove box. The reaction mixture was stirred at room temperature for 24 h. The target product methyl 1-methyl-4-phenyl-4-carboxymethylpiperidine (**62)** was observed by GC-MS with low yields (< 10%), m/z: found [ESI]: [MH^+] 301.55, $C_{14}H_{17}Cl_2NO_2$ requires [MH^+] 302.06.

(4-Iodophenyl) Acetonitrile (29c).[152]

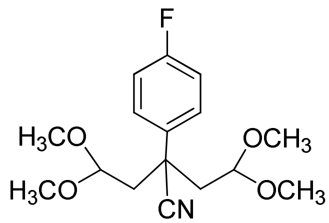


A mixture of KCN (2.7 g, 41 mmol) and deionized water (4.0 mL) was heated in an oil bath to dissolve the KCN. A solution of the 4-iodobenzyl bromide (9.8 g, 33 mmol) in MeOH (190 mL) was added to the reaction flask via addition funnel and allowed to stir at reflux for 4 h. The reaction mixture was allowed to cool to room temperature. The salts were filtered, and the filtrate was partially concentrated under reduced pressure. The resulting residue was purified by bulb-to-bulb distillation. The impurities were collected from 80-100 °C and the product was collected from 100-120 °C. This compound was obtained as a white solid (4.8 g, 60% yield). mp 55-56 °C (lit.: mp 56-57 °C). ^{1}H NMR ($CDCl_3$) δ 7.71 (d, *J*= 8.4 Hz, 2H), 7.09 (d, *J* = 8.4Hz, 2H), 3.70 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 138.1, 129.7, 129.5, 117.2, 93.5, 23.2.

General Procedure B. Dialkylation of 2-Aryl Acetonitriles 29a-h.[121]

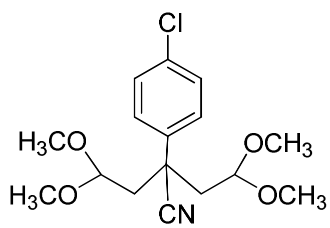
To a 100 mL round bottom flask fitted with a condenser, containing 2-aryl acetonitrile **29** (5.4 mmol) and bromoacetaldehyde dimethyl acetal (13 mmol) in anhydrous toluene (7 mL) under an atmosphere of nitrogen, a suspension of 50% $NaNH_2$ (50% by wt in toluene,1 equiv.) was added in portions. The reaction was allowed to stir at 70 °C to reflux for 15 min before the next portion of $NaNH_2$ was added. This was repeated until the mono-substituted product was no longer visible using thin layer chromatograph (Et_2O/ hexanes, 1:1). The reaction mixture was cooled to room temperature and quenched with deionized water. The aqueous layer was extracted with ether (4 x 25 mL). The combined organic fractions were dried (Na_2SO_4) and filtered. The solvent was removed under vacuum and the residue was purified by silica-gel column chromatography using a gradient of 25% Et_2O/hexanes to 50% Et_2O/hexanes to afford products **55a-h**.

2-(4-Fluorophenyl)-2-(2,2-dimethoxy-ethyl)-4,4-dimethoxy-butyronitrile (55a).



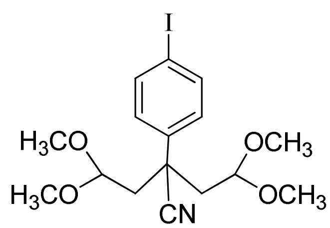
This compound was obtained from General Procedure B, as an orange solid (12 g, 66% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (m, 2H), 7.10 (m, 2H), 4.27 (dd, *J* = 4.8, 4.2 Hz, 2H), 3.23 (s, 6H), 3.17 (s, 6H), 2.40 (dd, *J* = 14.2, 6.6 Hz, 2H), 2.14 (dd, *J* = 4.0, 14.4 Hz, 2H), ^{13}C (101 MHz, $CDCl_3$) δ 133.6, 127.7, 121.1, 115.7, 101.9, 53.6, 53.1, 43.7, 41.4.

2-(4-Chlorophenyl)-2-(2,2-dimethoxy-ethyl)-4,4-dimethoxy-butyronitrile (55b).



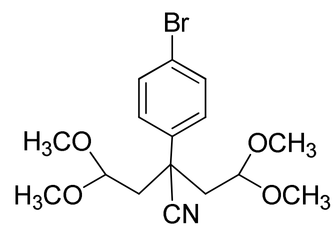
This compound was obtained from General Procedure B, as an orange solid (14 g, 66% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.40 (m, 3H), 4.27 (m, 2H), 3.32 (s, 6H), 3.17 (s, 6H), 2.40 (dd, *J* = 6.8, 14.4 Hz, 2H), 2.13 (dd, *J* = 4.0, 14.4 Hz, 2H), ^{13}C (101 MHz, $CDCl_3$) δ 136.4, 133.7, 128.9, 127.4, 120.8, 101.9, 53.5, 53.1, 43.4, 41.5, *Anal*. Calcd. for $C_{16}H_{22}NO_4Cl$: C, 58.62; H, 6.76; N, 4.27. Found: C, 58.70; H, 6.75; N, 4.19.

2-(2,2-Dimethoxyethyl)-2-(4-iodophenyl)-4,4-dimethoxy-butyronitrile (55c).



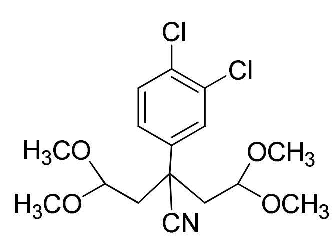
The compound was obtained from General Procedure B, as an orange solid (6.9 g, 64% yield). mp 73-75 ^{o}C. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.8 Hz, 2H), 7.22(d, *J* = 8.4 Hz, 2H), 4.27 (m, *J* = 4.0 Hz, 2H), 3.32 (s, 6H), 3.17 (s, 6H), 2,40 (dd, *J* = 7.2, 14.4 Hz, 2H), 2.13 (dd, *J* = 4.4, 14.6 Hz, 2H), ^{13}C (101 MHz, $CDCl_3$) δ 137.8, 137.6, 127.8, 120.7, 101.7, 93.3, 53.5, 53.1, 43.2, 41.3, *Anal*. Calcd. for $C_{16}H_{22}NO_4I$: C, 45.84; H, 5.29; N, 3.34. Found: C, 46.11; H, 5.24; N, 3.33.

2-(4-Bromophenyl)-2-(2,2-dimethoxy-ethyl)-4,4-dimethoxy-butyronitrile (55d).



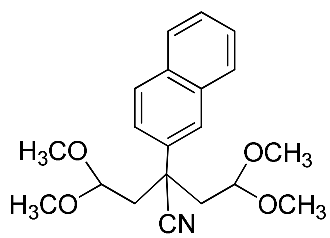
This compound was obtained from General Procedure B, as an orange solid (14 g, 66% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.8 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 4.27 (m, 2H), 3.32 (s, 6H), 3.17 (s, 6H), 2,40 (dd, *J* = 6.4, 14.4 Hz, 2H), 2.13 (dd, *J* = 4.0, 14.4 Hz, 2H), ^{13}C (101 MHz, $CDCl_3$) δ 136.4, 133.7, 128.9, 127.4, 120.8, 101.9, 53.5, 53.1, 43.4, 41.5, *Anal*. Calcd. for $C_{16}H_{22}NO_4CI$: C, 58.62; H, 6.76; N, 4.27. Found: C, 58.70; H, 6.75; N, 4.19.

2-(3,4-Dichlorophenyl)-2-(2,2-dimethoxyethyl)-4,4-dimethoxybutyronitrile (55e).



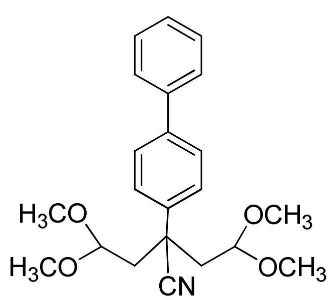
This compound was obtained from General Procedure B, as orange oil (7.9g, 81% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* =2.4 Hz, 1H), 7.49 (d, *J* =8.4 Hz, 1H), 7.31 (dd, *J* = 2.4, 8.4 Hz, 1H), 4.30 (m, *J* =4.4 Hz, 2H), 3.33 (s, 6H), 3.19 (s, 6H), 2,40 (dd, *J* = 6.4, 14.4 Hz, 2H), 2.13 (dd, *J* = 4.4, 14.4 Hz, 2H), ^{13}C (101 MHz, $CDCl_3$) δ 137.8, 138.3, 133.0, 132.1, 130.6, 128.1, 125.4, 120.4, 101.8, 53.6, 53.3, 43.3, 41.5. *Anal*. Calcd. for $C_{16}H_{21}NO_4Cl_2$: C, 53.05; H, 5.84; N, 3.87. Found: C, 52.87; H, 5.77; N, 3.77.

2-(2,2-Dimethoxyethyl)-4,4-dimethoxy-2-naphthalen-2-yl-butyronitrile (55f).



This compound was obtained from General Procedure B, as orange oil (7.9 g, 76% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.87 (m, 3H), 7.52 (m, 3H), 4.28 (dd, *J* = 4.0, 7.0 Hz, 2H), 3.31 (s,6H), 3.13 (s,6H), 2.50 (dd, *J* = 7.2, 14.2 Hz, 2H), 2.26 (dd, *J* = 4.0, 14.6 Hz, 2H). ^{13}C (101 MHz, $CDCl_3$) δ 135.0, 133.1, 132.6, 128.9, 128.3, 127.5, 126.7, 126.6, 125.6, 122.9, 121.3, 102.1, 53.7, 53.2, 43.5, 42.1. *Anal*. Calcd. for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08. Found: C, 69.75; H, 7.22; N, 4.03.

2-Biphenyl-4-yl-2-(2,2-dimethoxyethyl)-4,4-dimethoxy-butyronitrile (55g).

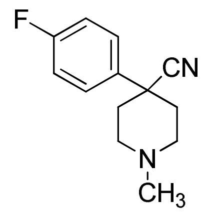


This compound was obtained from General Procedure B, as a yellow solid (9.4 g, 82% yield). mp 50-52^{o}C. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.60 (m, 4H), 7.55 (m, 2H), 7.44 (m, 2H), 7.35 (m, 1H), 4.33 (q, *J* = 4.0 Hz, 2H), 3.34 (s, 6H), 3.18 (s, 6H), 2,46 (dd, *J* = 6.8, 14.4 Hz, 2H), 2.21 (dd, *J* = 4.0, 14.4 Hz, 2H), ^{13}C (101 MHz, $CDCl_3$) δ 140.5, 139.7, 136.7, 128.7, 127.5, 127.2, 126.8, 126.2, 121.1, 101.9, 53.4, 52.9, 43.4, 41.5, 13.9. *Anal*. Calcd. for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79. Found: C, 71.62; H, 7.38; N, 3.80.

General Procedure C. Reductive Amination of Diacetal Nitriles 55a-h.[121]

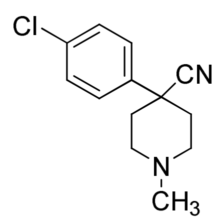
To a three neck 100 mL round-bottom flask containing 3 N HCl (160 mL) at 50 °C was added the diacetal **6** (10 mmol) and the mixture was allowed to stir overnight. The acid mixture was allowed to cool down to room temperature and then extracted with EtO_2 (300 mL). The ethereal layer was washed with saturated $NaHCO_3$ (150 mL) and dried (Na_2SO_4). The Et_2O was removed under reduced pressure. The resulting residue was dissolved in dry methanol (52 mL) and then the methylamine hydrochloride (25 mmol) was added, followed by the addition of $NaBH_3CN$ (9.4 mmol), and the mixture was allowed to stir for 48 h under an atmosphere of nitrogen. The methanol was removed under reduced pressure and the residue was treated with saturated $NaHCO_3$ solution (160 mL) and the mixture extracted with Et_2O (3 x 100mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was purified by chromatography ($CHCl_3/CH_3OH$, 39:1) to afford **20a-h**, respectively.

4-(4-Fluorophenyl)-1-methyl-piperidine-4-carbonitrile (20b).



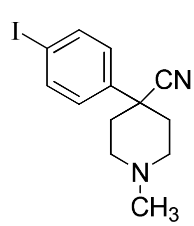
This compound was obtained from General Procedure C, as an orange solid (2.1 g, 30% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (m, 2H), 7.09 (m, 2H), 2.96 (m, 2H), 2.45 (m, 2H), 2.39(s, 3H), 2.09 (m, 4H). ^{13}C (101 MHz, $CDCl_3$) δ 162.2, 135.9, 127.3, 121.7, 115.8, 52.6, 45.9, 41.6, 36.7.

4-(4-Chlorophenyl)-1-methyl-piperidine-4-carbonitrile (20b).



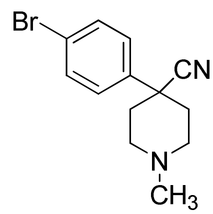
This compound was obtained from General Procedure C, as an orange solid (2.1 g, 30% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* =7.6 Hz, 2H), 7.37 (d, *J* = 9.2 Hz, 2H), 2.96 (m, 2H), 2.48 (m, 2H), 2.38(s, 3H), 2.09 (m, 4H). ^{13}C (101 MHz, $CDCl_3$) δ 138.6, 133.9, 129.1, 126.9, 121.4, 52.5, 45.9, 41.7, 36.4.

4-(4-Iodophenyl)-1-methyl-piperidine-4-carbonitrile (20c).



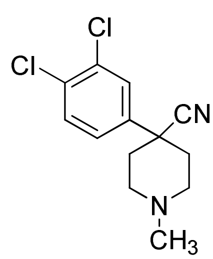
This compound was obtained from General Procedure C, as a yellow oil (1.9 g, 30% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.8 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 2.97 (d, J = 14.8 Hz, 2H), 2.48 (m, 2H), 2.39 (s, 3H), 2.09 (m, 4H). ^{13}C (101 MHz, $CDCl_3$) δ 139.8, 138.1, 127.5, 121.4, 93.7, 52.6, 46.0, 42.0, 36.4.

4-(4-Bromophenyl)-1-methyl-piperidine-4-carbonitrile (20d).



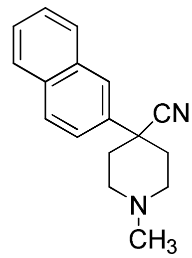
This compound was obtained from General Procedure C, as an orange solid (2.1 g, 30% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55-7.54 (d, J = 8.4 Hz, 2H), 7.39-7.37 (d, J = 8.8 Hz, 2H), 3.04-3.01 (d, J = 6.0 Hz, 2H), 2.55 (m, 2H), 2.43(s, 3H), 2.19-2.09 (m, 4H). ^{13}C (101 MHz, $CDCl_3$) δ 138.6, 133.9, 129.1, 126.9, 121.4, 52.5, 45.9, 41.7, 36.4.

4-(3,4-Dichlorophenyl)-1-mehtyl-piperidine-4-carbonitrile (20e).



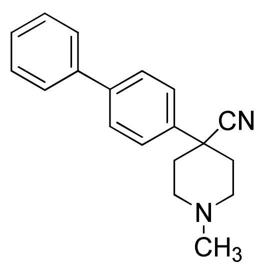
This compound was obtained from General Procedure C, as an orange oil (2.2 g, 39%). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, J = 2.0 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.31 (dd, J = 2.4, 8.4 Hz, 1H), 2.95 (m, 2H), 2.46 (m, 2H), 2.07 (m, 4H), ^{13}C (101 MHz, $CDCl_3$) δ 140.2, 133.2, 132.4, 130.9, 125.0, 121.0, 52.4, 45.8, 41.6, 36.3.

1-Methyl-4-naphthalen-2-yl-piperidine-4-carbonitrile (20f).



This compound was obtained from General Procedure C, as an orange oil (2.3 g, 35% yield). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 1.6 Hz, 1H), 7.85 (m, 3H), 7.57 (dd, J = 2.0, 8.4 Hz, 1H), 7.50 (m, 2H), 2.99 (d, J = 12.0, 2H), 2.53 (m, 2H), 2.40 (s, 3H), 2.22 (m, 4H). ^{13}C (101 MHz, $CDCl_3$) δ 137.3, 133.1, 132.7, 128.9, 128.1, 127.5, 126.6, 126.5, 124.6, 123.3, 121.9, 52.7, 46.0, 42.3, 36.5.

4-Biphenyl-4-yl-1-methyl-piperidine-4-carbonitrile (20g)

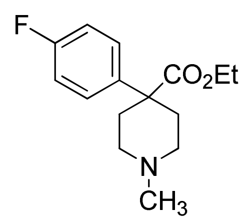


This compound was obtained from General Procedure C, as an orange oil (2.2 g, 40%). ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.55 (m, 6H), 7.44 (m, 2H), 7.35 (m, 1H), 2.98 (d, J = 12.6 Hz, 2.51 (m, 2H), 2.40 (s, 3H), 2.16 (m, 4H). ^{13}C (101 MHz, $CDCl_3$) δ 141.0, 140.1, 139.1, 128.8, 127.6, 127.0, 126.0, 121.9, 52.7, 46.0, 41.9, 36.6.

General Procedure D. Esterification of Nitriles 20a-h.[121]

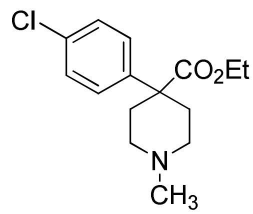
The nitrile **30** (5.2 mmol) in an aqueous solution of sulfuric acid (6.5 mL H_2SO_4:H_2O,1:1) was heated in an oil bath at 120 °C for 1.5 h. The flask was then equipped with a Dean-Stark Trap and excess alcohol was added. The water was azeotropically removed over 4.5 h and alcohol was added as needed. The reaction was heated to reflux overnight and then allowed to cool to room temperature. The alcohol was removed under reduced pressure. The flask was then cooled in an ice bath and the acid was neutralized to pH 10 with 1 N NaOH. The aqueous layer was extracted with Et_2O (3 x 75 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was purified by chromatography ($CHCl_3$/CH_3OH, 39:1) to afford esters **21a-h,** respectively.

4-Carboethoxy-(4-fluorophenyl)-1-methyl-piperidine (21a).



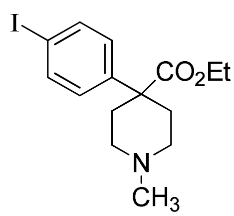
This compound was obtained from General Procedure D as a white solid (1.0 g, 70% yield), and converted into a hydrochloride salt (General Procedure A-1), which was obtained as a white solid, mp 137-138 ºC. ^{1}H NMR (free base): δ 7.36 (m, 4H), 7.00 (m,2H), 4.12 (q, *J* = 6.8 Hz, 2H), 2.78 (br s, 2H), 2.57 (d, *J* = 12.8 Hz, 2H), 2.26 (s, 3H), 2.13 (t, *J* = 11.2 Hz, 2H), 1.94 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 3H), ^{13}C NMR (free base): δ 174.1, 161.7, 138.7, 127.5, 115.3, 60.8, 53.4, 48.1, 46.2, 34.0, 14.0.

4-Carboethoxy-(4-chlorophenyl)-1-methyl-piperidine (21b).



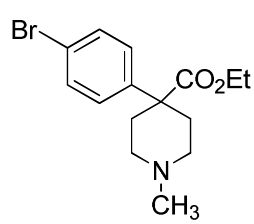
This compound was obtained from General Procedure D as a white solid (1.1 g, 70% yield), and converted into a hydrochloride salt (General Procedure A-1), which was obtained as a white solid, mp 168-169 ºC. ^{1}H NMR (free base): δ 7.31 (m, 4H), 4.12 (q, *J* = 6.8 Hz, 2H), 2.77 (br s, 2H), 2.56 (d, *J* = 12.8 Hz, 2H), 2.27 (s, 3H), 2.13 (t, *J* = 11.2 Hz, 2H), 1.93 (t, *J* = 10.4 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 3H), ^{13}C NMR (free base): δ 174.0, 141.5, 132.9, 128.6, 127.3, 60.9, 53.4, 48.3, 46.2, 33.6, 14.0.

4-Carboethoxy-(4-iodophenyl)-1-methyl-piperidine (21c).



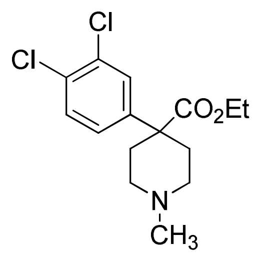
This compound was obtained from General Procedure D as a white solid (1.1 g, 68% yield), and converted into a hydrochloride salt (General Procedure A-1), which was obtained as a white solid, mp 247-249 ºC. ^{1}H NMR (free base): δ 7.65 (d, *J* = 8.8 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.12 (m, *J* = 7.2 Hz, 2H), 2.78 (d, *J* = 8.4 Hz, 2H), 2.54 (d, *J* = 13.2 Hz, 2H), 2.27 (s, 3H), 2.14 (t, *J* = 10.8 Hz, 2H), 1.94 (t, *J* = 10.4 Hz, 2H), 1.18 (t, *J* = 7.6 Hz, 3H), ^{13}C NMR (free base): δ 173.7, 137.5, 127.8, 92,7, 60.9, 53.2, 48.3, 46.1. 33.6, 14.0. *Anal*. Calcd. for $C_{15}H_{20}NO_2I\cdot HCl$: C, 43.98; H, 5.17; N, 3.42. Found: C, 44.10; H, 5.23; N, 3.43.

4-(4-Bromophenyl)-4-carboethoxy-1-methyl-piperidine (21d).



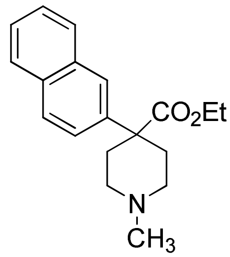
This compound was obtained from General Procedure D as a white solid (1.1 g, 80% yield), and converted into a hydrochloride salt (General Procedure A-1), which was obtained as a white solid, mp 247-249 ºC. ^{1}H NMR (free base): δ 7.47 (d, *J* = 8.8 Hz, 2H), 7.26 (d, *J* = 8.8 Hz, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 2.78 (br s, 2H), 2.54 (d, *J* = 12.4 Hz, 2H), 2.26 (s, 3H), 2.13 (t, *J* = 11.2 Hz, 2H), 1.93 (t, *J* = 11.2 Hz, 2H), 1.18 (t, *J* = 7.2 Hz, 3H).

4-Carboethoxy-(3,4-dichlorophenyl)-1-methyl-piperidine (21e).



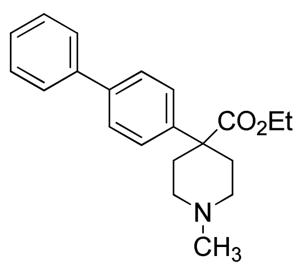
This compound was obtained from General Procedure D as an off-white solid (1.2 g, 70% yield), and converted into a hydrochloride salt (General Procedure A), which was obtained as a white solid, mp 195-196 ºC. ^{1}H NMR (free base): δ 7.47 (d, *J* = 2.0 Hz, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.23 (dd, *J* = 2.4, 8.4 Hz, 1H), 4.14 (m, *J* = 6.8 Hz, 2H), 2.80 (d, *J* = 8.4 Hz, 2H), 2.55 (d, *J*= 12.8 Hz, 2H), 2.28 (s, 3H), 2.16 (t, *J* = 11.6 Hz, 2H), 1.94 (m, *J* = 11.2 Hz, 2H), 1.20 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (free base): δ 173.4, 132.6, 131.2, 130.4, 128.2, 125.4, 61.2, 53.2, 48.2, 46.0, 33.6, 14.0. *Anal*. Calcd. for $C_{15}H_{19}NO_2Cl_2$: HCl: C, 51.08; H, 5.72; N, 3.97. Found: C, 51.23; H, 5.78; N, 4.00.

4-Carboethoxy-1-methyl-4-naphthalen-2-yl-piperidine (21f).



This compound was obtained from General Procedure D as an off-white solid (1.2 g, 32% yield) and converted into a hydrochloride salt (General Procedure A), which was obtained as a white solid, mp 191-193 ºC. ^{1}H NMR (free base): δ 7.80 (m, 4H), 7.55 (dd, *J* = 2.0, 8.6 Hz, 1H), 7.44 (m, 2H), 4.12 (m, *J* = 6.8 Hz, 2H), 2.83 (d, *J* = 9.2 Hz, 2H), 2.70 (d, *J* = 12.4 Hz, 2H), 2.28 (s, 3H), 2.21 (t, *J* = 11.6 Hz, 2H), 2.10 (m, 2H), 1.16 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (free base): δ 174.3, 133.3, 132.3, 128.1, 128.0, 127.3, 126.0, 125.9, 124.6, 124.0, 60.8, 53.5, 48.7, 46.2, 33.9, 14.0. *Anal*. Calcd. for $C_{19}H_{23}NO_2\cdot HCl$: C, 68.36; H, 7.25; N, 4.20. Found: C, 68.27; H, 7.30; N, 4.14.

4-Biphenyl-4-carboethoxy-1-methyl-piperidine (21g).



The compound was obtained from General Procedure D as a yellow solid (1.1 g, 80% yield) and converted into a hydrochloride salt (General Procedure A), which was obtained as a white solid, mp 196-198 ºC. ^{1}H NMR (free base): δ 7.54 – 7.28 (m, 9H), 4.10 (q, *J* = 6.8 Hz, 2H), 2.78 (d, *J* = 9.2 Hz, 2H), 2.61 (d, *J* = 12.8 Hz, 2H), 2.24(s, 3H), 2.15 (t, *J* = 11.2 Hz, 2H), 2.01 (t, *J* = 11.2 Hz, 2H), 1.14 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (free base): δ 174.3, 140.5, 139.8, 128.7, 127.3, 127.2, 127.0, 126.2, 60.9, 53.5, 48.5, 46.3, 33.9, 14.0. *Anal*. Calcd. for $C_{21}H_{25}NO_2\cdot HCl\cdot {}^1/_4H_2O$: C, 69.21; H, 7.73; N, 3.84; Found: C, 69.47; H, 7.25; N, 3.80.

General Procedure E. Transesterification of Ethyl Ester of 21e. [124]

A flask containing oven-dried molecular sieves (500 mg) was charged with ICy·HCl (10 mol %), potassium *t*-butoxide (9.5 mol %), and THF (1 mL). The ethyl ester (1.0 mmol) and alcohol (1.5 mmol) were dissolved in THF (0.5 mL) and added to the reaction mixture via cannula addition. The reaction was allowed to stir at room temperature and was monitored by TLC ($CHCl_3$:MeOH, 19:1). The mixture was filtered and the solvent removed under reduced pressure. The residue was purified using column chromatography to afford compounds **67a-h.**

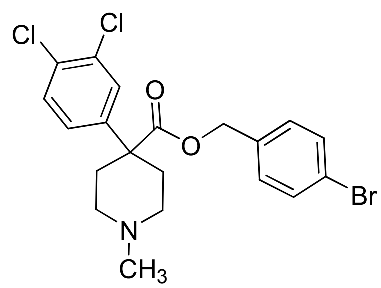
General Procedure F. Hydrolysis of Nitriles 20c,e,f .[152]

A solution of the nitrile (5.2 mmol) and 25 % (wt) NaOH (34 mL) in methanol (100 mL) was stirred at reflux overnight, then cool down to room temperature. The mixture was reduced to half the volume under reduced pressure and extracted with Et_2O (3 x 30 mL). The aqueous layer was cooled to 0 °C, then acidified to pH = 2 with 1M HCl solution and then extracted with ethyl acetate (3 x 75 mL). A white suspension was formed and white solid was filtered by vacuum filtration. The solid was recrystallized from H_2O/MeOH (4/1, v/v) to afford product **67g-o** as white crystals.

General Procedure G. Esterification of Carboxylic Acids 43g-o. [153]

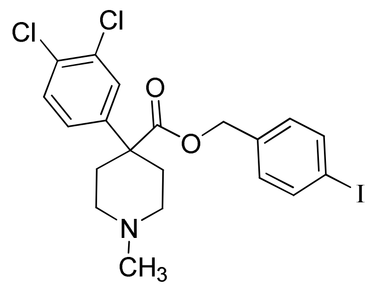
Thionyl chloride (20 mL) was transferred to a 100 mL round-bottom flask fitted with a condenser and filled with carboxylic acid **43g-o** (1.85 mmol). The mixture was heated to reflux overnight with stirring under N_2. Excess thionyl chloride was removed by distillation with absorption device (Sat.NaOH solution). A solution of alcohol (1.85 mmol) and Bu_4NHSO_4 (0.36 mmol) in CH_2Cl_2 (20 mL) was transferred to the residue. The mixtures were cool to -5 °C then 5% NaOH (3mL) was added. Stirring was continued at -5 °C for 1 h and then the mixture was allowed to warm room temperature. The reaction was monitored by TLC every 30 minutes until the starting material was consumed. The organic layer was separated, washed with water, dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by column chromatography (MeOH/$CHCl_3$ 2.5:98) gave ester **67g-o** as white solids.

4-Bromobenzyl-4-(3, 4-dichlorophenyl)-1-methylpiperidine-4-carboxylate (67h).



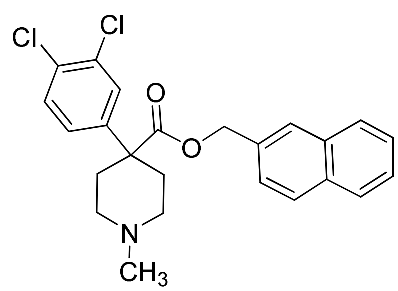
Compound was obtained from General Procedure G as a white solid (411 mg, 50% yield) and converted into the HCl salt (General Procedure A-1), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.41 (m, 3H), 7.38-7.36 (d, *J* = 8.0 Hz, 1H), 7.18-7.15 (m, 1H), 7.07 – 7.05 (d, *J* = 8.0 Hz, 1H), 5.04(s, 2H), 2.76-2.74 (d, *J* = 8.4 Hz, 2H), 2.55-2.52 (d, *J* = 12.0 Hz, 2H), 2.24 (s, 3H), 2.13-2.07 (t, *J* = 12.0 Hz, 2H), 1.94 (t, *J* = 10.0 Hz, 2H). *Anal.* Calcd. for $C_{20}H_{20}BrCl_2NO_2 \cdot HCl \cdot 1/2H_2O$: C, 47.78; H, 4.41; N, 2.79. Found: C, 47.70; H, 4.47; N, 2.73.

4-Iodobenzyl-4-(3, 4-dichlorophenyl)-1-methylpiperidine-4-carboxylate (67i).



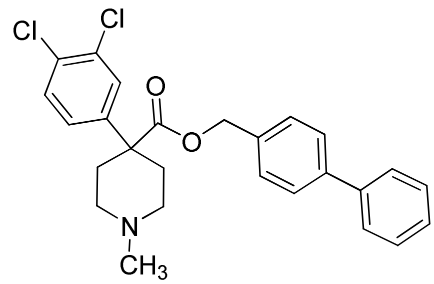
Compound was obtained from General Procedure C as a white solid (450 mg, 50% yield) and converted into the HCl salt (General Procedure A-1) to afford white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.63 (m, 2H), 7.40-7.36 (m, 2H), 7.18-7.15 (m, 1H), 6.93 – 6.91 (d, *J* = 8.0, 2H), 5.03(s, 2H), 2.76-2.74 (d, *J* = 8.8, 2H), 2.55-2.52 (d, *J* = 12.0, 2H), 2.24 (s, 3H), 2.11 (t, *J* = 10.4, 2H), 1.94 (t, *J* = 10.8, 2H). *Anal.* Calcd. for $C_{20}H_{20}Cl_2INO_2 \cdot HCl \cdot H_2O$: C, 43.00; H, 4.15; N, 2.51. Found: C, 43.01; H, 4.02; N, 2.39.

2-Naphthyl-4-(3, 4-dichlorophenyl)-1-methylpiperidine-4-carboxylate (67j).



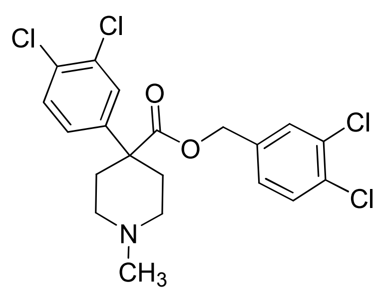
Compound was obtained from General Procedure C as a white solid (385 mg, 50% yield) and converted into the HCl salt (General Procedure A-1), to afford white foam. ^{1}H NMR (free base): δ 7.84– 7.74 (m, 2H), 7.40-7.36 (m, 2H), 7.60 (s, 1H), 7.51-7.45(m,2H), 7.35-7.33 (d, *J* = 8.0 Hz,1H) 7.27 (s,1H) 7.20-7.17 (dd, *J* = 2.0,2.0 Hz,1H), 5.27(s, 2H), 2.76-2.74 (d, *J* = 5.6 Hz, 2H), 2.59-2.56 (d, *J* = 12.0 Hz, 2H), 2.23 (s, 3H), 2.14 (t, *J* = 10.8 Hz, 2H), 1.95 (t, *J* = 10.8 Hz, 2H). *Anal*. Calcd. for $C_{24}H_{23}Cl_2NO_2 \cdot HCl \cdot 1.5H_2O$: C, 58.61; H, 5.53; N, 2.85. Found: C, 58.37; H, 5.50; N, 2.83.

4-Biphenyl-4-(3, 4-dichlorophenyl)-1-methylpiperidine-4-carboxylate (67k).



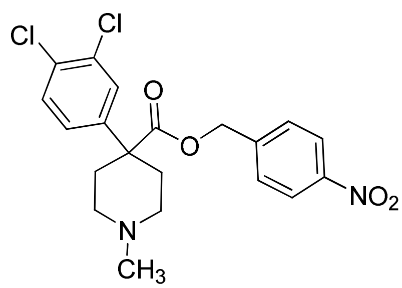
Compound was obtained from General Procedure C as a white solid (410 mg, 51% yield) and converted into the HCl salt (General Procedure A-1), to afford a white foam ^{1}H NMR (free base): δ 7.59– 7.53 (m, 4H), 7.46-7.43 (dd, *J* = 6.8, 2.0 Hz, 3H), 7.38-7.36 (d, *J* = 8.8 Hz, 2H), 7.27-7.25(d, *J* = 8.0 Hz, 2H), 7.21-7.18 (dd, *J* = 2.4, 6.4 Hz, 1H), 5.15(s, 2H), 2.78-2.76 (d, *J* = 8.0 Hz, 2H), 2.59-2.56 (d, *J* = 12.8 Hz, 2H), 2.25 (s, 3H), 2.15 (t, *J* = 10.8 Hz, 2H), 1.96 (t, *J* = 10.8 Hz, 2H). *Anal.* Calcd. for $C_{26}H_{25}Cl_2NO_2 \cdot HCl \cdot H_2O$: C, 61.37; H, 5.55; N, 2.75. Found: C, 61.33; H, 5.48; N, 2.84.

3,4-Dichlorobenzyl 4-(3,4-dichlorophenyl)-1-methylpiperidine-4-carboxylate (67l).



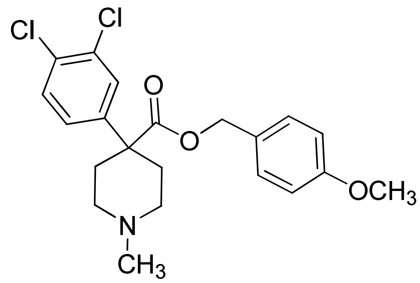
Compound was obtained from General Procedure G as an off-white solid (400 mg, 50% yield) and converted into the HCl salt (General Procedure A-1), which was obtained as a white solid, mp 182-183 °C. ^{1}H NMR (free base):^{1}H NMR (400 MHz, $CDCl_3$) 7.40 (dd, *J* = 2.4, 8.4 Hz, 2H), 7.36(s, 1H), 7.18 (dd, *J* = 2.0, 2.1 Hz, 2H), 7.00 (dd, *J* = 2.0, 2.0 Hz, 1H) 5.04 (s, 2H), 2.83 (s, 2H), 2.58-2.55 (d, *J* = 12.8 Hz, 2H), 2.30 (s, 3H), 2.20-2.18 (dt, *J* = 8.0 Hz, 2H), 2.06-2.03 (d, *J* = 10.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 141.3, 135.9, 133.1, 132.7, 131.8, 130.8, 130.0, 128.5, 127.3, 125.6, 65.5, 53.3, 48.6, 46.3, 33.7. *Anal.* Calcd. for $C_{20}H_{19}NO_2Cl_4 \cdot HCl$: C, 49.67; H, 4.17; N, 2.90. Found: C, 49.44; H, 4.27; N, 2.84.

4-Nitrobenzyl 4-(3,4-dichlorophenyl)-1-methylpiperidine-4-carboxylate (67m).



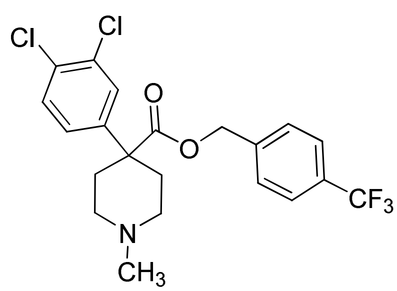
Compound was obtained from General Procedure G as a yellow solid (410 mg, 47% yield) and converted into a HCl salt (General Procedure A-1), which was obtained as an orange solid, mp 187-189 °C. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, *J* = 8.8 Hz, 2H), 7.40 (dd, *J* = 2.4, 8.4 Hz, 2H), 7.29 (d, *J* = 8.8 Hz, 2H), 7.13 (dd, *J* = 2.4, 2.4 Hz, 1H), 5.17 (s, 2H), 2.74 (s, 2H), 2.56-2.53 (d, *J* = 12.4 Hz, 2H), 2.24 (s, 3H), 2.15-2.10 (dt, *J* = 10.8 Hz, 2H), 2.00-1.98 (d, *J* = 11.2 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 147.9, 142.8, 135.5, 133.1, 131.9, 130.8, 128.6, 125.6, 124.0, 65.7, 53.3, 48.6, 46.3, 33.7. *Anal.* Calcd. for $C_{20}H_{20}N_2O_4Cl_2 \cdot HCl \cdot 1/4H_2O$: C, 51.74; H, 4.67; N, 6.03. Found: C, 51.74; H, 4.75; N, 5.83.

4-Methoxylbenzyl 4-(3,4-Dichlorophenyl)-1-methylpiperidine-4-carboxylate (67n).



Compound was obtained from General Procedure G as a white solid (410 mg, 51% yield) and converted into the HCl salt (General Procedure A-1), to afford a white foam ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 2.3, 1H), 7.34 (dd, *J* = 8.4, 4.1 Hz, 1H), 7.20 – 7.16 (m, 1H), 7.13 (dt, *J* = 17.3, 8.2 Hz, 2H), 6.91 – 6.77 (m, 2H), 5.04 (s, 2H), 3.80 (s, 3H), 2.75-2.73 (d, *J* = 9.2 Hz, 2H), 2.54-2.51 (d, *J* = 12.6 Hz, 2H), 2.23 (s, 3H), 2.12-2.10 (m, 2H), 1.92 (d, *J* = 10.8 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6, 159.6, 132.9. 131.5, 130.6, 130.2, 128.5, 127.8, 125.6, 118.74, 114.1, 67.1, 55.9, 55.5, 53.4, 48.6, 46.3, 33.9. *Anal.* Calcd. for $C_{21}H_{23}Cl_2NO_3 \cdot HCl \cdot H_2O$: C, 54.50; H, 5.66; N, 3.03. Found: C, 52.37; H, 5.60; N, 3.03.

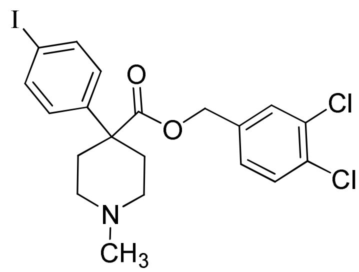
4-Trifluromethyl-(3,4-dichlorophenyl)-1-methylpiperidine-4-carboxylate (67o).



Compound was obtained from General Procedure C as a white solid (400 mg, 48% yield) and converted into the oxalic salt (General Procedure A-2) to afford a white foam ^{1}H NMR (free base): (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.0 Hz, 2H), 7.39 (m, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.18 (dd, *J* = 2.0 Hz 1H), 5.15 (s, 2H), 2.80 (s, 2H), 2.56 (d, *J* = 12.8 Hz, 2H), 2.28 (s, 3H), 2.17 (t, *J*

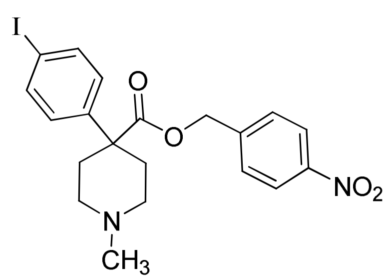
= 10.8 Hz 2H), 2.01(t, *J* = 10.8 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 139.5, 135.4, 133.1, 130.8, 128.5, 128.3, 125.8, 125.8, 125.7, 125.6, 66.3, 53.2, 48.5, 46.2, 33.6.

3,4-Dichlorobenzyl 4-(4-iodophenyl)-1-methylpiperidine-4-carboxylate (68a).



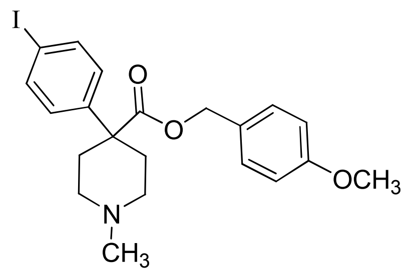
Compound was obtained from General Procedure G as a white solid (500 mg, 48% yield) and converted into the oxalic acid salt (General Procedure A-2), to afford a white solid form. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.60 (m, 2H), 7.43 – 7.32 (m, 1H), 7.18 – 7.03 (m, 3H), 6.95 (dt, *J* = 16.2, 8.1 Hz, 1H), 5.03 (s, 2H), 2.93 – 2.67 (m, 2H), 2.55 (d, *J* = 12.9 Hz, 3H), 2.26 (m, 3H), 2.20 – 2.09 (m, 2H), 2.01 (d, J = 10.0 Hz, 2H).

4-Nitrobenzyl 4-(4-iodophenyl)-1-methylpiperidine-4-carboxylate (68b).



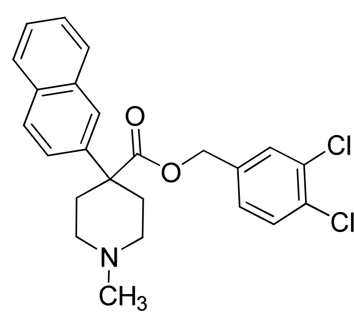
Compound was obtained from General Procedure G as a white solid (400 mg, 45% yield) and converted into the oxalic salt (General Procedure A-2), to afford white foam. ^{1}H NMR (free base):^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 – 8.07 (m, 2H), 7.69 – 7.58 (m, 2H), 7.33 – 7.19 (m, 2H), 7.17 – 7.02 (m, 2H), 5.22 – 5.09 (m, 2H), 2.75 (s, 2H), 2.58-2.55 (d, *J* = 12.0, 2H), 2.31 – 2.20 (m, 3H), 2.19 – 2.06 (m, 2H), 2.01 (d, *J* = 11.0, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 173.7, 137.9, 128.3, 123.9, 123.3, 118.5, 115.5, 93.2, 65.4, 53.4, 46.4, 36.4, 33.7.

4-Methoxylbenzyl 4-(4-iodophenyl)-1-methylpiperidine-4-carboxylate (68c).



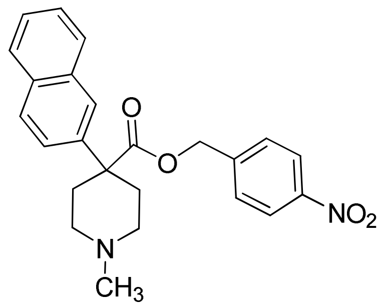
Compound was obtained from General Procedure G as a white solid (440 mg, 50% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam ^{1}H NMR (free base):^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.56 (m, 2H), 7.19 – 6.98 (m, 4H), 6.89 – 6.74 (m, 2H), 5.08 – 4.97 (m, 2H), 3.85 – 3.75 (m, 3H), 2.73 (s, 2H), 2.55-2.53 (d, *J* = 12.8 Hz, 2H), 2.20 (d, *J* = 19.7 Hz, 3H), 2.15 – 2.00 (m, 2H), 1.94 (d, *J* = 11.0 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 159.8, 137.8, 130.1, 128.2, 127.9, 114.0, 93.0, 66.8, 55.5, 53.4, 48.7, 46.3, 33.7.

3,4-Dichlorobenzyl 4-(naphthalen-2-yl)-1-methylpiperidine-4-carboxylate (68d).



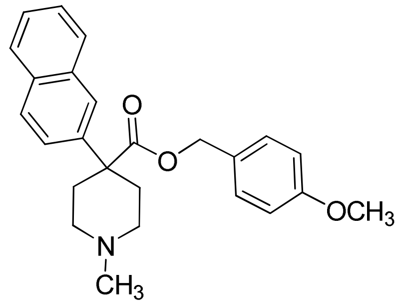
Compound was obtained from General Procedure G as a white solid (380 mg, 48% yield) and converted into the oxalic salt(General Procedure A-2), to afford a white foam ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.70 (m, 4H), 7.55 – 7.43 (m, 2H), 7.29 – 7.19 (m, 2H), 7.14 (d, *J* = 1.9 Hz, 0H), 7.00 – 6.86 (m, 1H), 5.03 (s, 2H), 2.81 (s, 2H), 2.72-2.70 (d, *J* = 8.0 Hz, 2H), 2.43 – 2.31 (m, 4H), 2.29 (s, 3H), 2.00 (d, *J* = 17.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 136.1, 133.5, 132.8, 132.7, 130.6, 129.8, 128.7, 128.4, 127.7, 127.2, 126.5, 126.5, 125.1, 124.0, 65.7, 53.4, 48.9, 46.1, 33.6.

4-Nitrobenzyl 4-(naphthalen-2-yl)-1-methylpiperidine-4-carboxylate (68e).



Compound was obtained from General Procedure G as a white solid (360 mg, 48% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.91 (m, 2H), 7.79 (m, 4H), 7.50 (m, 3H), 7.18 (d, *J* = 8.6 Hz, 2H), 5.15 (m, 2H), 2.85 – 2.72 (m, 4H), 2.30 (s, 3H), 2.27 – 2.24 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.2, 147.7, 143.2, 133.5, 132.6, 128.6, 128.3, 128.3, 127.7, 126.6, 126.6, 125.2, 124.1, 123.8, 65.3, 53.6, 49.2, 46.5, 33.9.

4-Methoxylbenzyl 4-(naphthalen-2-yl)-1-methylpiperidine-4-carboxylate (68f).

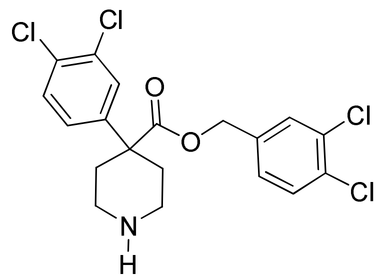


Compound was obtained from General Procedure C as a white solid (350 mg, 50% yield) and converted into the oxalic salt (General Procedure A-2) to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 – 7.74 (m, 4H), 7.52 – 7.49 (m, 1H), 7.46 – 7.44 (m, 2H), 7.12 – 7.10 (d, J = 8.0 Hz,2H), 6.78 – 6.75 (m, 2H), 5.05 (s, , 2H), 3.75 (s, 3H), 2.80 (s, 2H), 2.71– 2.68 (d, J = 12.0 Hz, 2H), 2.26 (s, 3H), 2.18 – 2.12 (m, 4H). ^{13}C NMR (101 MHz, CDCl3) δ 174.20, 159.8, 133.5, 132.6, 130.2, 128.5, 128.4, 128.0, 127.6, 126.4, 126.3, 125.1, 124.1, 114.0, 66.9, 55.5, 53.3, 48.9, 45.8, 33.4.

General procedure H. *N*-Demethylation of Benzyl 4-Aryl-1-methyl-piperidine Analogues (67,68).[124]

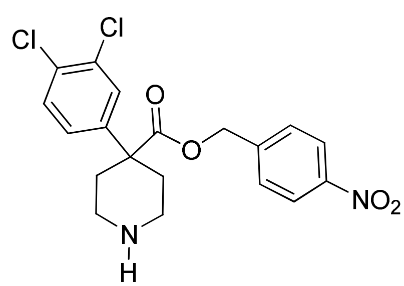
A solution of **65** (6.1 mmol), sodium bicarbonate (9.1 mmol) and 1-chloro-ethylchloroformate (52 mmol) in 1,2-dichloroethane (27 mL) was heated to reflux under an atmosphere of nitrogen for 48 h. The mixture was filtered to remove any solids and the solvent was removed under reduced pressure. Methanol (115 mL) was added and the mixture was heated to reflux for 3 h. The solvent was removed under reduced pressure. Chloroform was added, washed with 1.8 N NaOH (30 mL) and water (30 mL), and then dried (Na_2SO_4). The crude product was purified by column chromatography (SiO_2, $CHCl_3$/CH_3OH, 12:1) to afford the normeperidine analogues **69a-j**.

3,4-Dichlrobenzyl 4-(3,4-dichlorophenyl) piperidine-4-carboxylate (69a).



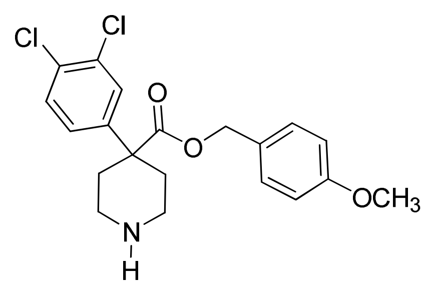
Compound was obtained from General Procedure H as a white solid (173 mg, 70% yield) and converted into the oxalic salt(General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, *J* = 14.3, 8.3, 2H), 7.32 (d, *J* = 1.9 Hz, 1H), 7.17 – 7.06 (m, 2H), 6.98 (dd, *J* = 8.2 Hz, 1.6, 1H), 5.06 (s, 2H), 3.44 (d, *J* = 12.8 Hz, 2H), 3.04 (t, *J* = 11.7 Hz, 2H), 2.68 (d, *J* = 14.4 Hz, 2H), 2.42 – 2.21 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 151.1, 139.9, 134.9, 132.9, 131.2, 130.9, 130.2, 128.0, 127.5, 125.1, 66.4, 55.9, 48.1, 41.9, 34.6. *Anal.* Calcd. for $C_{19}H_{17}Cl_4NO_2 \cdot C_2H_2O_4 \cdot 1/2H_2O$: C, 47.39; H, 3.79; N, 2.63. Found: C, 47.60; H, 3.83; N, 2.74.

4-Nitrobenzyl 4-(3,4-dichlorophenyl) piperidine-4-carboxylate (69b).



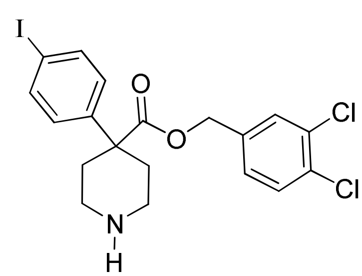
Compound was obtained from General Procedure H as a white solid (163 mg, 69% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.5 Hz, 2H), 7.45 – 7.36 (m, 2H), 7.32 (d, *J* = 8.7 Hz, 2H), 7.24 – 7.15 (m, 1H), 5.19 (s, 2H), 3.07 (d, *J* = 12.7 Hz, 2H), 2.77 (t, *J* = 11.5 Hz, 2H), 2.54 (d, *J* = 13.1 Hz, 2H), 2.39 (s, 1H), 1.99 – 1.78 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 147.9, 142.8, 142.8, 133.1, 131.8, 130.8, 128.6, 128.4, 125.5, 123.9, 65.7, 49.5, 43.9, 34.6. *Anal.* Calcd. for $C_{19}H_{18}Cl_2N_2O_4 \cdot C_2H_2O_4$: C, 50.52; H, 4.04; N,5 .61. Found: C, 50.60; H, 4.15; N, 5.33.

4-Methoxylbenzyl 4-(3,4-dichlorophenyl) piperidine-4-carboxylate (69c).



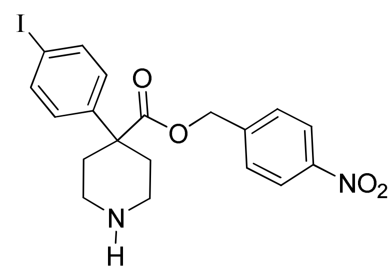
Compound was obtained from General Procedure H as a white solid (160 mg, 71% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.29 (m, 2H), 7.11 (t, *J* = 10.2 Hz, 4H), 6.83 (d, *J* = 8.5 Hz, 2H), 5.06 (s, 2H), 3.80 (s, 3H), 3.43 (s, 2H), 2.98 (s, 2H), 2.65 (d, *J* = 13.1 Hz, 2H), 2.29 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6, 159.9, 143.2, 132.9, 131.5, 130.6, 130.2, 128.4, 127.7, 125.5, 114.0, 67.1, 55.5, 49.4, 44.0, 34.6. *Anal.* Calcd. for $C_{20}H_{21}Cl_2NO_3 \cdot C_2H_2O_4 \cdot 0.1H_2O$: C, 54.36; H, 4.81; N, 2.88. Found: C, 54.70; H, 4.91; N, 2.87.

3,4-Dichlorobenzyl 4-(4-iodophenyl) piperidine-4-carboxylate (69d).



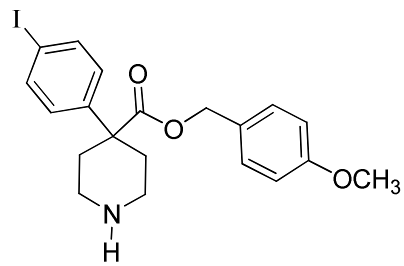
Compound was obtained from General Procedure H as a white solid (196 mg, 70 % yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.13 (d, *J* = 1.8 Hz, 1H), 7.09 (d, *J* = 8.5 Hz, 2H), 6.96 (dd, *J* = 8.2, 1.8 Hz, 1H), 5.02 (s, 2H), 3.07 (s, 2H), 2.79 (d, *J* = 6.9 HZ, 2H), 2.53 (s, 2H), 1.87 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 142.3, 138.0, 136.1, 132.9, 132.5, 131.1, 130.7, 129.8, 128.1, 127.2, 93.4, 65.3, 49.6, 43.94, 34.6. *Anal.* Calcd. for $C_{19}H_{18}Cl_2INO_2 \cdot C_2H_2O_4$: C, 43.47; H, 3.47; N, 2.41. Found: C, 43.92; H, 3.59; N, 2.39.

4-Nitrobenzyl 4-(4-iodophenyl) piperidine-4-carboxylate (69e).



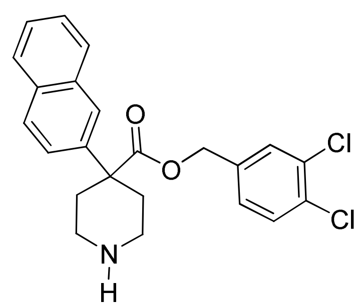
Compound was obtained from General Procedure H as a white solid (186 mg, 72% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.7 Hz, 2H), 7.66 (t, *J* = 5.5 Hz, 2H), 7.33 – 7.22 (m, 2H), 7.11 (d, *J* = 8.6 Hz, 2H), 5.17 (s, 2H), 3.06 (d, *J* = 12.5 Hz, 2H), 2.77 (t, *J* = 11.6 Hz, 2H), 2.54 (d, *J* = 12.6 Hz, 2H), 2.16 – 1.49 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 143.1, 138.0, 128.4, 128.1, 123.9, 104.5, 93.2, 65.4, 49.7, 44.1, 34.6. *Anal.* Calcd. for $C_{19}H_{19}IN_2O_4 \cdot C_2H_2O_4$: C, 45.34; H, 3.80; N, 5.04. Found: C, 46.44; H, 4.15; N, 4.69.

4-Methoxylbenzyl 4-(4-iodophenyl) piperidine-4-carboxylate (69f).



Compound was obtained from General Procedure H as a white solid (168mg, 73% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.6 Hz, 2H), 7.11 (d, *J* = 8.6 Hz, 2H), 7.03 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 5.03 (s, 2H), 3.80 (s, 3H), 3.33 – 3.08 (m, 2H), 2.99 – 2.70 (m, 2H), 2.72 – 2.43 (m, 2H), 2.28 – 1.95 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3, 159.9, 138.0, 137.7, 130.2, 129.9, 128.0, 127.9, 127.5, 114.2, 114.0, 93.4, 67.3, 55.5, 49.8, 33.7. *Anal.* Calcd. for $C_{20}H_{22}INO_3 \cdot C_2H_2O_4$: C, 48.81; H, 4.47; N, 2.59. Found: C, 49.50; H, 4.71; N, 2.52.

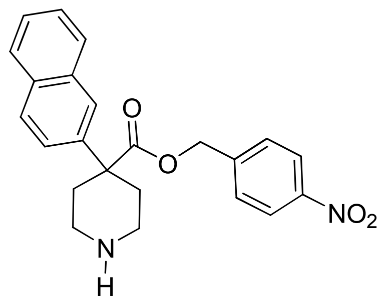
3,4-Dichlorobenzyl 4-(naphthalen-2-yl) piperidine-4-carboxylate (69g).



Compound was obtained from General Procedure H as a white solid (180 mg, 71% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.74 (m, 4H), 7.54 – 7.41 (m, 3H), 7.25 – 7.17 (m, 1H), 7.17 – 7.06 (m, 1H), 6.98 – 6.85 (m, 1H), 5.09 – 4.94 (m, 2H), 3.10 (t, *J* = 22.6 Hz, 2H), 2.87 (dd, *J* = 25.4, 14.1 Hz, 3H), 2.68 (d, *J* = 13.2 Hz, 2H), 2.16 – 1.93 (m, 2H). ^{13}C NMR (101

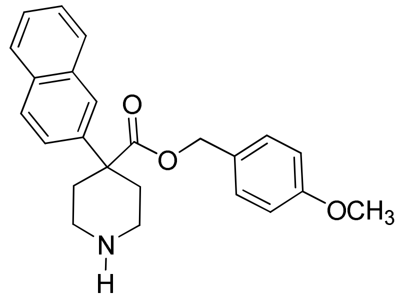
MHz, $CDCl_3$) δ 174.2, 139.7, 136.2, 133.5, 132.8, 132.7, 132.4, 130.6, 129.8, 128.7, 128.4, 127.7, 127.2, 126.6, 126.55, 126.5, 125.1, 123.9, 65.2, 49.9, 44.1, 34.6. *Anal.* Calcd. for $C_{23}H_{21}Cl_2NO_2 \cdot C_2H_2O_4$: C, 59.53; H, 4.60; N, 2,78. Found: C, 59.53; H, 5.02; N, 2.60.

4-Nitrobenzyl 4-(naphthalen-2-yl) piperidine-4-carboxylate (69h).



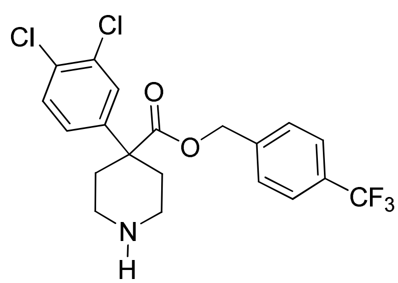
Compound was obtained from General Procedure H as a white solid (160 mg, 73% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.92 (m, 2H), 7.85 – 7.77 (m, 2H), 7.76 – 7.71 (m, 1H), 7.71 (s, 1H), 7.55 – 7.46 (m, 2H), 7.42 – 7.34 (m, 1H), 7.20 – 7.07 (m, 2H), 5.30 – 5.07 (m, 2H), 3.54 – 3.36 (m, 2H), 3.16 (t, J = 11.1 Hz, 2H), 2.84 (t, J = 14.3 Hz, 2H), 2.52 (t, J = 10.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 147.9, 142.3, 133.4, 132.8, 129.2, 128.6, 128.4, 128.3, 127.7, 127.1, 127.0, 125.2, 123.9, 123.2, 65.9, 48.6, 42.0, 30.5. *Anal.* Calcd. for $C_{23}H_{22}N_2O_4 \cdot C_2H_2O_4 \cdot 1/2H_2O$: C, 61.34; H,5.15; N,5.72. Found: C, 61.85; H, 5.04; N, 5.68.

4-Methoxylbenzyl 4-(Naphthalen-2-yl) piperidine-4-carboxylate (69i).



Compound was obtained from General Procedure H as a white solid (150 mg, 72% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.66 (m, 4H), 7.54 – 7.40 (m, 3H), 7.17 – 7.05 (m, 2H), 6.85 – 6.69 (m, 2H), 5.05 (s, 2H), 3.75 (s, 3H), 3.12-3.05 (m, 2H), 2.84 (s, 2H), 2,67 (s, 2H), 2.03 (t, *J* =10.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 159.8, 140.3, 133.5, 132.6, 130.1, 128.5, 128.4, 128.1, 127.6, 126.4, 126.2, 124.9, 124.2, 114.0, 66.8, 55.4, 49.9, 34.8. *Anal.* Calcd. for $C_{24}H_{25}NO_3 \cdot C_2H_2O_4 \cdot 1/3H_2O$: C, 66.32; H, 5.91; N, 2.97. Found: C, 66.34; H, 5.95; N, 2.93.

4-Trifluromethylbenzyl 4-(3,4-dichlorophenyl) piperidine-4-carboxylate (69j).



Compound was obtained from general method H as a white solid (180mg, 72% yield) and converted into the HCl salt (General Procedure A-1), to afford a white foam. ^{1}H NMR (free base): 1H NMR (400 MHz, CDCl3) δ 7.67 – 7.50 (m, 2H), 7.46 – 7.35 (m, 2H), 7.27 (t, J = 9.1 Hz, 2H), 7.17 (td, J = 8.2, 2.4 Hz, 1H),5.04 (s, 2H), 3.22 – 2.93 (m, 2H), 2.89 – 2.66 (m, 2H), 2.53 (d, J = 13.2 Hz, 2H), 2.35 – 2.14 (m, 1H), 1.99 – 1.72 (m, 2H).^{13}C NMR (101 MHz, CDCl3) δ 173.5, 142.9, 139.6, 133.0, 131.7, 130.7, 128.4, 128.3, 125.8, 125.8, 125.7, 125.7, 125.5, 122.8, 66.2, 49.5, 44.1, 43.9, 34.8, 34.8, 29.9. *Anal.* Calcd. for $C_{20}H_{18}Cl_2F_3NO_2 \cdot HCl \cdot 1/3H_2O$: C, 50.60; H, 4.18; N, 2.95. Found: C: 50.65; H, 4.27; N, 2.92

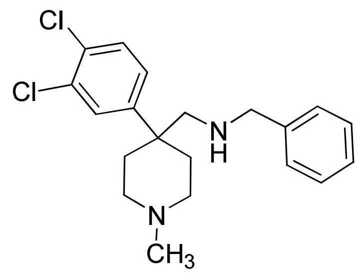
General procedure I. Reduction of 4-(3,4 Dichlorobenzyl) Piperidine Nitrile (20d).[154]

In a pressure reaction bottle were placed nitrile **20d** (1.73g, 6.3mmol)**,** anhydrous methanol (40 mL), and Raney Ni (1.97 g). Anhydrous NH_3 was bubbled through the solution until saturated. The reaction bottle was sealed and fixed to Parr Hydrogenation apparatus. After shaking at room temperature overnight under an atmosphere of H_2 (50 psi), the reaction mixture was filtered through a layer of celite and the filtrate was concentrated under reduced pressure to yield **70** (1.53 g, 97%) as a white solid. The product was dried overnight under vacuum and used in subsequent reactions with no further purification.

General Procedure J. Reductive Amination of 4-(3,4-Dichlorobenzyl)-piperidines (70).[155]

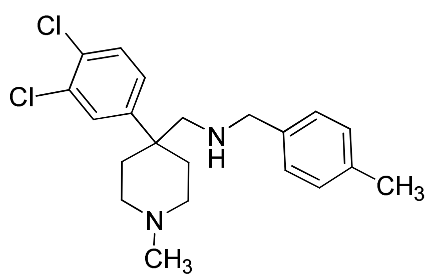
The amine **70** (0.82 mmol), benzaldehyde (0.82 mmol), $NaBH_3CN$ (51.4 mg, 0.818 mmol) and glacial acetic acid (51.4 mg, 0.82mmol) were combined in a 100 mL round-bottom flask, with CH_2Cl_2 (30 mL) and mixture was allowed to stir overnight at room temperature. The mixture was then treated with saturated Na_2CO_3 solution and extracted with CH_2Cl_2 (3 x 25 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was purified by chromatography ($CHCl_3/CH_3OH$, 9:1) to afford **71a-h**, respectively.

***N*-Benzyl-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71a).**



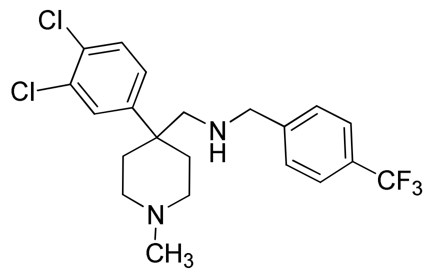
Compound was obtained from General Procedure J as a white solid (203 mg, 70% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.38 (m, 2H), 7.30 – 7.26 (m, 2H), 7.25 – 7.22 (d, *J* = 8.8 Hz 1H), 7.16 – 7.13 (m, 3H), 3.64 (s, 2H), 2.64 (s, 2H), 2.48 (s, 2H), 2.20 (s, 3H), 2.16-2.12 (d, *J* = 14.0 Hz, 4H), 1.94-1.88 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 140.6, 132.8, 130.5, 130.2, 129.6, 128.5, 128.0, 127.1, 126.9, 54.1, 52.1, 46.4, 40.4, 33.9. *Anal.* Calcd. for $C_{20}H_{24}Cl_2N_2 \cdot 1.5C_2H_2O_4 \cdot H_2O$: C, 53.50; H, 5.66; N, 5.42. Found: C, 53.54; H, 5.74; N, 5.13.

***N*-(4-Methylbenzyl)-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71b).**



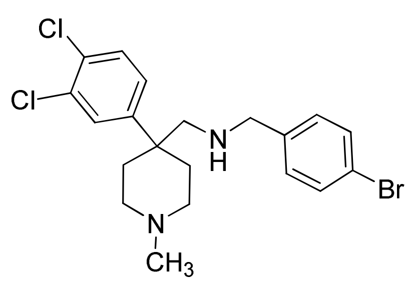
Compound was obtained from General Procedure J as a white solid (200 mg, 70% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.38 (m, 2H), 7.15 – 7.13 (m, 1H), 7.08 – 7.06 (d, *J* = 8.0 Hz, 2H), 7.02 – 7.00 (d, *J* = 8.0 Hz, 2H), 3.59 (s, 2H), 2.63 (s, 2H), 2.49 (s, 2H), 2.31 (s, 3H), 2.20 (s, 3H), 2.15-2.11 (d, *J* = 15.2 Hz, 4H), 1.91 (dd, *J* = 16.3, 6.4 Hz, 2H). ^{13}C NMR (101 MHz, CDCl3) δ 137.5, 136.6, 132.7, 130.4, 130.2, 129.6, 129.2, 127.9, 126.9, 53.9, 52.1, 46.4, 40.4, 33.9, 21.3. *Anal.* Calcd. for $C_{21}H_{26}Cl_2N_2 \cdot C_2H_2O_4 \cdot 2H_2O$: C, 54.87; H, 6.41; N, 5.56. Found: C, 54.51; H, 6.38; N, 4.45.

***N*-(4-Trifloromethylbenzyl)-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71c).**



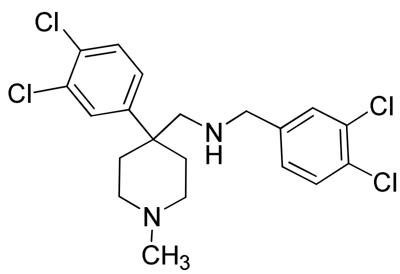
Compound was obtained from General Procedure J as a white solid (258 mg, 72% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.51 (d, *J* = 8.0 Hz, 2H), 7.41 – 7.39 (m, 2H), 7.28 – 7.26 (d, *J* = 8.0 Hz, 2H), 7.16 – 7.14 (d, *J* = 8.0 Hz, 2H), 3.68 (s, 2H), 2.62 (s, 2H), 2.48 (s, 2H), 2.20 (s, 3H), 2.16-2.13 (d, *J* = 12.1 Hz, 4H), 1.91 (dd, *J* = 16.6, 6.6 Hz, 2H). ^{13}C NMR (101 MHz, CDCl3) δ 144.7, 132.8, 130.5, 130.3, 129.6, 128.2, 126.9, 125.4, 125.4, 125.3, 53.6, 52.0, 46.4, 40.5, 33.9. *Anal.* Calcd. for $C_{21}H_{23}Cl_2F_3N_2 \cdot C_2H_2O_4 \cdot 1.5H_2O$: C, 50.37; H, 5.15; N, 5.11. Found: C, 50.90; H, 5.10; N, 5.11.

***N*-(4-Bromobenzyl)-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71d).**



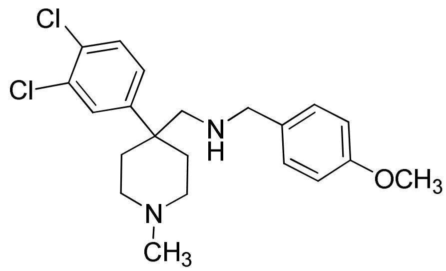
Compound was obtained from General Procedure J as a white solid (247 mg, 70% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.36 (m, 4H), 7.15 – 7.12 (d, *J* = 8.5, 2.3 Hz, 1H), 7.03 – 7.00 (d, *J* = 8.4 Hz, 2H), 3.57 (s, 2H), 2.60 (s, 2H), 2.48 (s, 2H), 2.20 (s, 3H), 2.15-2.11 (d, *J* = 14.3 Hz, 4H), 1.89 (dd, *J* = 16.4, 6.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.6, 132.8, 131.5, 130.5, 130.3, 129.7, 129.6, 126.9, 120.8, 53.4, 52.0, 46.4, 40.4, 33.9. *Anal.* Calcd. for $C_{21}H_{23}BrCl_2N_2 \cdot C_2H_2O_4 \cdot H_2O$: C, 48.02; H, 4.95; N, 5.09. Found: C, 48.17; H, 5.02; N, 4.54.

***N*-(3,4-Dichlorobenzyl)-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71e).**



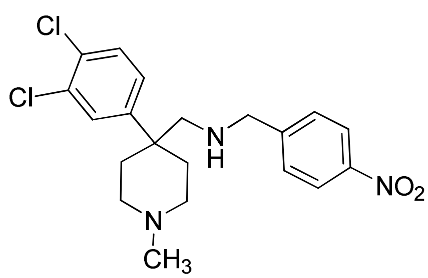
Compound was obtained from General Procedure J as a white solid (253mg, 72% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.36 (m, 2H), 7.29 – 7.26 (m, 1H), 7.21-7.20 (d, *J* = 2.0 Hz, 1H), 7.14-7.11 (dd, *J* = 6.0, 2.4 Hz, 1H), 6.96-6.93 (dd, *J* = 6.0, 2.4 Hz, 1H), 3.55 (s, 2H), 2.58 (s, 2H), 2.45 (s, 2H), 2.17 (s, 3H), 2.13-2.09 (d, *J* = 14.0 Hz, 4H), 1.90-1.84 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.0, 132.8, 132.5, 130.8, 130.5, 130.4, 129.8, 129.6, 127.3, 126.9, 52.8, 52.0, 46.4, 40.4, 33.8. *Anal.* Calcd. for $C_{20}H_{22}Cl_4N_2 \cdot C_2H_2O_4 \cdot H_2O$: C, 48.91; H, 4.85; N, 5.19. Found: C, 49.05; H, 5.01; N, 4.55.

N-(4-Methoxylbenzyl)-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71f).



Compound was obtained from General Procedure J as a white solid (243 mg, 70% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.37 (m, 2H), 7.14 – 7.12 (dd, *J* = 6.4, 2.0 Hz, 1H), 7.05-7.03 (d, *J* = 8.0 Hz, 2H), 6.80-6.78 (d, *J* = 8.0 Hz, 2H), 3.78 (s, 3H), 3.55 (s, 2H), 2.62 (s, 2H), 2.49 (s, 3H), 2.21 (s,3H), 2.15-2.11 (d, *J* = 14.0 Hz, 4H), 1.93-1.87 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.7, 132.7, 132.6, 130.4, 130.2, 129.5, 129.1, 126.9, 113.8, 55.5, 53.5, 52.0, 46.3, 40.4, 33.8. *Anal.* Calcd. for $C_{21}H_{26}Cl_2N_2O \cdot C_2H_2O_4 \cdot H_2O$: C, 55.09; H, 6.03; N, 5.59. Found: C, 55.24; H, 5.96; N, 5.56.

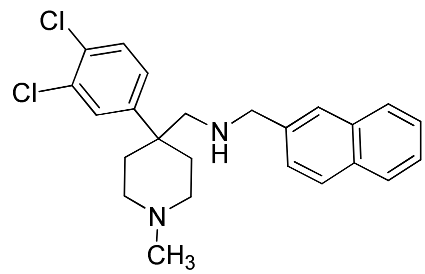
N-(4-Nitrobenzyl)-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71g).



Compound was obtained from General Procedure J as a white solid (167 mg, 67% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 8.03 (d, *J* = 4.0 Hz, 2H), 7.36-7.34 (m,2H), 7.29 –

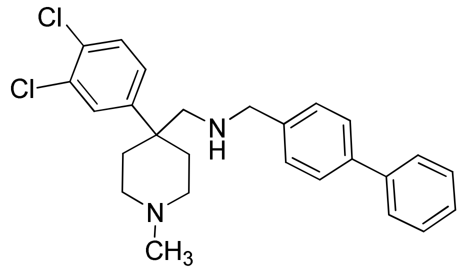
7.26 (m,2H), 7.14-7.11 (m, 1H), 3.68 (s, 3H), 2.59 (s, 2H), 2.43 (s, 2H), 2.15 (s,3H), 2.09 (m, 4H), 1.88-1.83 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.5, 147.1, 132.8, 130.5, 130.3, 129.6, 128.6, 126.9, 123.6, 53.3, 52.0, 46.4, 40.5, 33.8. *Anal.* Calcd. for $C_{20}H_{23}Cl_2N_3O_2 \cdot C_2H_2O_4 \cdot H_2O$: C, 51.17; H, 5.27; N, 8.14. Found: C, 51.14; H, 5.04; N, 7.69.

***N*-(2-Naphthyl)-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71h).**



Compound was obtained from General Procedure J as a white solid (258mg, 68% yield)and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81-7.91 (m, 1H), 7.76-7.74 (d, *J* = 8.0, 2H), 7.54 (s, 1H), 7.46 – 7.43 (m, 2H), 7.42 – 7.38 (m, 2H), 7.27-7.26 (m, 2H), 7.16-7.14 (dd, *J* = 8.5, 2.2, 1H), 3.80 (s, 2H), 2.68 (s, 2H), 2.49 (s, 2H), 2.19 (s, 3H), 2.16-2.13 (m, 4H), 1.95-1.92 (m, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 138.0, 133.5, 132.8, 132.7, 130.5, 130.3, 129.6, 128.2, 127.9, 127.0, 126.4, 126.3, 126.2, 125.7, 54.2, 52.1, 46.4, 40.5, 34.0. *Anal.* Calcd. for $C_{24}H_{26}Cl_2N_2 \cdot C_2H_2O_4 \cdot 1.75H_2O$: C, 58.38; H, 5.94; N, 5.24. Found: C, 58.38; H, 5.89; N, 4.59.

***N*-(4-Biphenyl)-1-(4-(3,4-dichlorophenyl)-1-methylpiperidin-4-yl)methanamine (71i).**



Compound was obtained from General Procedure J as a white solid (263mg, 70% yield) and converted into the oxalic salt (General Procedure A-2), to afford a white foam. ^{1}H NMR (free base): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61-7.59 (m, 2H), 7.53-7.51 (d, *J* = 8.0, 2H), 7.46 – 7.40 (m, 4H), 7.36 – 7.32 (m, 1H), 7.23-7.21 (m, 2H), 7.19-7.17 (m, 1H), 3.69 (s, 2H), 2.69 (s, 2H), 2.51 (s, 2H), 2.22 (s, 3H), 2.19-2.15 (m, 4H), 1.97-1.94 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.2, 140.0, 139.7, 132.8, 130.5, 130.2, 129.6, 129.0, 128.7, 128.4, 127.4, 127.3, 127.3, 126.9, 53.8, 52.1, 46.4, 40.5, 34.0.*Anal.* Calcd. for $C_{26}H_{28}Cl_2N_2 \cdot C_2H_2O_4 \cdot 2H_2O$: C, 59.47; H, 6.06; N, 4.95. Found: C, 59.35; H, 5.95; N, 4.47.

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APPENDIX

Experimental Procedures

for

Binding Assays for the Dopamine, Serotonin and Norepinephrine Transporters

Dr. Sari Izenwasser and coworkers at University of Miami, School of Medicine performed the *in vitro* binding assays for the dopamine, serotonin and norepinephrine transporters.

[^{3}H] WIN 35,428 Binding Assay.

Male Sprague-Dawley rats (200-250 g,Taconic, Germantown,NY) were decapitated and their brains removed to an ice-cooled dish for dissection of the caudate-putamen.the tissue was homogenized in 30 volumes of ice-cold modified Krebs-HEPES buffer (15 mM HEPES, 127Mm NaCl, 5mM KCl,1.2 mM $MgSO_4$,2.5 mM $CaCl_2$, 1.3 mM NaH_2PO_4,10 mM glucose, pH adjusted to 7.4) using a Teflon/glass homogenizer and centrifuged at 20000g for 10 min at 4℃. The resulting pellet was then washed two more times by re-suspension in ice-cold buffer and centrifugation at 20000g for 10 min at 4℃. Fresh homogenates were used in all experiments. Binding assays were conducted in modified Krebs-HEPES buffer on ice, essentially as previously described. The total volume in each tube was 0.5 mL, and the final concentration of membrane after all additions was approximately 0.3% (w/v) corresponding to 150-300 g of protein/sample. Increasing concentrations of the drug being tested were added to triplicate samples of membrane suspension. Five minutes later, [^{3}H] WIN 35,428 (final concentration 1.5 nM) was added and the incubation was continued for 1h on ice. The incubation was terminated by the addition of 3 mL of ice-cold buffer and rapid filtration through Whatman/GF/B glass fiber filter paper (presoaked in 0.1 BSA in water to reduce nonspecific binding) using a Brandel Cell Harvester (Gaitherburg, MD). After filtration, the filters was washed with three additional 3 mL washes and transferred to scintillation vials. Absolute ethanol (0.5 mL) and Beckman Ready Value Scintillation Cocktail (2.75 mL) were added to the vials which were counted the next day at an efficiency of about 36%. Under these assay conditions, an average experiment yielded approximately 6000 dpm total binding per sample and approximately 250 dpm nonspecific binding. Nonspecific binding was defined as binding in the presence of 100 μM cocaine. K*i* values were derived from 14 point competition assays using increasing concentrations of unlabeled compounds (0.05 nM to 100 μM) against 1.5 nM [^{3}H] WIN 35 428. Data were analyzed with Graphpad Prism software (San Diego, Califonia).

[^{3}H]Paroxetine Binding Assay.

Brains from male Sprague-Dawley rats weighing 200-225 g (Taconic Labs) were removed, and midbrain was dissected and rapidly frozen. Membranes were prepared by homogenizing tissues in 20 volumes (w/v) of 50 mM Tris containing 120 mM NaCl and 5 mM KCl (pH 7.4 at 25 °C), using a Brinkman Polytron (setting 6 for 20 s) and centrifuged at 50,000g for 10 min at 4 °C. The resulting pellet was re-suspended in buffer, recentrifuged, and re-suspended in buffer to a concentration of 15 mg/mL. Ligand binding experiments were conducted in assay tubes containing 4.0 mL buffer for 90 min at room temperature. Each tube contained 0.2 nM [^{3}H]paroxetine (New England Nuclear, Boston MA)and 1.5 mg midbrain tissue (original wet weight). Nonspecific binding was determined using 1 µM citalopram. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments Gaithersburg, MD). The filters were washed twice with 5 mL cold buffer and transferred to scintillation vials. Beckman Ready Safe (3.0 mL) was added, and the vials were counted the next day using a

Beckman 6000 liquid scintillation counter (Beckman Coulter Instruments, Fullerton, CA). Data were analyzed by using GraphPad Prism software (San Diego, CA).

[^{3}H]Nisoxetine Binding Assay.

Frontal cortex of male Sprague-Dawley rats was removed and frozen. Membranes were prepared by homogenizing tissues in 50 mM Tris (120 mM NaCl, 5 mM KCl; pH 7.4 at 25 °C) and centrifuging (50 000g for 10 min at 4 °C. The resulting pellet was then washed and centrifuged two more times. The final pellet was re-suspended to a concentration of 80 mg/mL (original wet weight). Assays were conducted in the above Tris buffer. Volume totaled 0.5 mL with tissue concentration of 8 mg/tube. [^{3}H]Nisoxetine (specific activity 80 Ci/mmol; final concentrated 0.5 nM, New England Nuclear, Boston, MA) was added and the incubation continued for 1 h on ice. Incubations were terminated by rapid filtration through Whatman GF/B filters, presoaked in 0.05% polyethylenimine (PEI). Nonspecific binding was defined using 1 M desipramine. For these assays, an initial screen was conducted to assess displacement of nisoxetine at a concentration of from 0.01 nM to 100 µM of the unknown compound. If there was greater than 50% displacement of nisoxetine, a K_i value was determined in subsequent studies.

VITA

Xiaobo Gu was born in Shanghai, China on December 7, 1977. He received his B.S. degree from East China University of Science and Technology in Shanghai, China in 2000. He worked as a chemist for Shanghai Research and Development Center of Polymeric Materials from June 2000 to January 2003. He then came to United States to continue his education at University of New Orleans. In 2005, his life was interrupted by Hurricane Katrina, and he was relocated in Midland, Michigan for six months. Under the supervision of Professor Trudell, he went on to complete his Doctor of Philosophy in Synthetic Medicinal Chemistry in May 2010.