ABSTRACT

Design and Synthesis of Potential Serotonin-Selective
Reuptake Inhibitors and Serotonin-Norepinephrine Reuptake Inhibitors
for the Treatment of Depression

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Depression is a common disease characterized by feelings of deep sadness,[1] guiltiness, loss of interest in once pleasurable activities, and thoughts of self-harm among others. Affecting an estimated 121 million people worldwide, depression does not discriminate on the basis of gender, age, culture or social status.

Research shows a diminished concentration of the neurotransmitters serotonin and norepinephrine in the synapses between neurons of depressed patients. For this reason, and with the advent of new and more powerful computational tools, two families of compounds called Serotonin-Selective Reuptake Inhibitors (SSRIs) and Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) have been developed. These compounds block the reuptake process of serotonin and norepinephrine into the presynaptic neuron in order to increase the amount of these neurotransmitters in the synapses.

Although these drugs have been successful in the treatment of depression, the side effects that accompany them are the major reason for patients to abandon treatment

before completion. One of these side effects is sexual dysfunction, which has been associated with the serotonin receptor 2A ($5\text{-}HT_{2A}$).

In this project, a series of compounds based on the molecular structures of fluoxetine (Prozac ®) and 4-[(3-fluorophenoxy)phenylmethyl)piperidine were coupled with chemical entities that are known to possess activity against the $5\text{-}HT_{2A}$ receptor by chemical synthesis. It is anticipated that hybrid molecules will reduce the sexual dysfunction side effect without significantly affecting the antidepressant activity. Since serotonin is known to act as a growth factor, the synthesized compounds were tested for activity against several human cancer cell lines.

A second project involved progress towards the synthesis of a new compound designed to selectively target the tumor microenvironment. This compound incorporates structural features of both combretastatin A4 and the bioreductive agent tirapazamine.

Certain members of the combretastatin series of natural products demonstrate tumor cytotoxicity and damage tumor vasculature. Since many solid tumors are hypoxic, bioreductive entities such as tirapazamine are viable treatment agents.

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Reuptake Inhibitors and Serotonin-Norepinephrine Reuptake Inhibitors
for the Treatment of Depression

by

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A Dissertation

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DEDICATION

To my parents

Ernesto and María Esthela

To my brothers

Jesús and Ernesto

To my wife Mónica and our three little angels

Gerardito, Netito and Ale.

CHAPTER ONE

Introduction

Definition. The World Health Organization (WHO) defines mental health as "a state of well-being in which every individual realizes his or her own potential, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community."[3]

Ranked as the leading cause of disability worldwide and affecting an estimated 120 million people worldwide,[3] depression, formally called major depression, major depressive disorder or clinical depression, is an illness defined by the World Health Organization, the National Institute of Mental Health (NIMH), and the Mayo Clinic as one of the most common and serious medical conditions in the world that affects the body, mood, the way people eat and sleep, the perception people have about things as well as one's self-perception. These problems can become chronic, usually requiring long-term treatment, and significantly impairing an individual's ability to cope with daily life.[3, 4]

Depression does not discriminate on a basis of age, ethnic background, socioeconomic status, or gender. Studies show that women are twice more prone to suffer from depression than men.[1] Weissman and coworkers suggest to interpret these data with caution since, culturally, men are expected to be emotionally stronger than women – a crying man is regarded as weak. So, it is very difficult to really know whether depression is truly less common among men or they are just less likely to recognize, acknowledge and seek help from a professional mental health care provider.[4, 5]

The NIMH reports that in focus groups to assess depression awareness, men described symptoms that can be associated with depression – headaches, increased or decreased appetite, and chronic pain – without becoming conscious that they are suffering from the illness.[4] Unfortunately, in our "macho" culture – worldwide – men have the tendency to think that going to a mental health professional or mental health clinic will imprint them with a stigma of weakness if their friends, family or anybody else find out, losing their respect and/or standing in the community.[4] Surprisingly, these feelings were higher in urban areas and among people with higher levels of education.[3]

At its worst, depression can lead to suicide. The WHO estimates that nearly 800,000 people commit suicide every year. Of these, 86% live in low- and middle-income countries.[3] Fortunately, depression can be successfully treated in most cases[3, 4] with medication and/or psychotherapy.[3, 4]

Types of Depression[4]

Patients suffering from depressive disorders can experience one or several forms of depression. The three most common types are:

- *Major depressive disorder,* also called major depression, is characterized by a combination of symptoms which interfere with the ability to work, study, sleep, eat, and enjoy pleasurable activities. Major depression is disabling and as a result, it prevents the person from functioning normally.

- *Dysthimic disorder,* or dysthimia is very similar to major depressive disorder. The difference is the severity of the symptoms, being less severe than those of major depression. Because of the diminished severity of symptoms, compared

to major depression, dysthimia may not disable a person, but it may still prevent people from functioning normally or feeling well.

- *Minor depression* is not very common. The symptoms are less severe than those of dysthymia and, therefore, less severe than those of major depression lasting for shorter periods of time.

Other forms of depression have minor differences with the ones just described or may develop under very specific circumstances. Regrettably, scientists have not reached an agreement on how to characterize and define these forms of depression. Included in this category are:

Psychotic depression. The NIMH describes this as a severe depressive illness accompanied by some form of psychosis.

Seasonal affective disorder (SAD). This type of depression is usually felt during winter time when there is less natural sunlight. These feelings are generally relieved during spring and summer times.[5]

Bipolar disorder also known as manic-depressive illness is a disorder of the brain characterized by cycling mood changes: severe highs (mania) and lows (depression) with episodes of normal mood often observed in between. Although dramatic and rapid switches between these highs and lows have been observed in patients, these changes are generally gradual affecting energy, activity levels, and the ability to perform everyday responsibilities.

Symptoms of Depression.[4]

Not every individual suffering from depression experiences every symptom. Whereas some patients may experience only some of the symptoms other may suffer

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many of them. In addition, the severity, frequency and duration of symptoms vary with each person and over time.

In general, symptoms for depression can be divided into three families: psychological, behavioral, and physical symptoms.[6] Psychological symptoms include but are not limited to: depressed mood, anxiety/nervousness, reduced concentration, inability to enjoy things, reduced libido, low self-esteem, feelings of worthlessness, thoughts of death or suicide, thoughts of hurting other people. Behavioral symptoms include but are not limited to: crying spells, avoidance of anxiety-producing situations, reduced productivity, social withdrawal, substance use/abuse, self-sacrifice/victimization, suicide attempts/gestures, and violent/assaultive behaviors. Finally, physical symptoms include but are not limited to: fatigue, insomnia/hypersomnia, eating disorders, weight gain/loss, sexual problems, headaches, muscle tension, gastrointestinal irritation, and heart palpitations.

Illnesses that Often Co-Exist with Depression[4]

Depression usually does not come by itself. Frequently it goes together with other illnesses that may come first, provoke or be a consequence of it. These co-existing illnesses also need to be appropriately diagnosed and treated along with depression.

Such illnesses include:[4]

- *Anxiety disorders such as post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), panic disorder, social phobia and generalized anxiety disorder.* PTSD is a condition that results when an individual experiences a terrifying event such as a violent assault, a natural disaster, an accident, terrorism or war.[4] Symptoms of depression are commonly observed among survivors of a stressful event but

appear to be more intense in those with PTSD. For example, survivors from World War II with PTSD account for more depressive symptoms than those with no PTSD. The tendency is akin for Vietnam veterans. In a study from 1998, Shalev and co-workers found evidence that at one month after the traumatic event 44.4% of people suffering from PTSD also suffered from major depression. This frequency remained constant across time, since at four months after the traumatic event, 43.2% of the patients showed comorbidity between major depression and PTSD.[7]

- *Alcohol and other substance abuse or dependence.* This is another disease that may also co-exist with depression. Conway and coworkers have shown that, unfortunately, the comorbidity of mood disorders and substance abuse is spreading rapidly among the U.S. population.[88]

- *Heart disease, stroke, cancer, HIV/AIDS, diabetes and Parkinson's disease.* People who suffer from depression in addition to other serious medical illness frequently have more severe symptoms of both, depression and the co-existing medical condition. It is more difficult for them to get adapted to their illness and their health care costs are, as expected, higher than those who do not have a co-existing condition.[6] Fortunately, the treatment of depression also helps improve the treatment of the co-existing illness.[9]

Causes of Depression

To date, researchers in the field still are not certain about a single cause for depression. What is known about it is that a combination of factors, including genetic, biochemical, environmental, and psychological play an important role in developing the illness.{{{}} Although depression can run in families, suggesting a genetic link, it can also

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occur in people without any history as well.[4] Trauma, loss of a beloved one, a difficult relationship, or any stressful situation are common triggers of depressive episodes.[10]

Depressive illnesses are disorders of the brain. Magnetic resonance imaging (MRI) and positron emission tomography (PET) techniques show that the brains of people suffering from depression look different than those of healthy people.

Figure 1 shows a PET scan of the brain activity in depressed vs. non-depressed people. In this figure, the parts of the brain regulating mood, thinking, sleep, appetite and behavior do not work normally.[4] In comparing the size of the two brains, it is evident that healthy people's brains are bigger than that of depressed people. The difference between both brains is, approximately, 2.3 mL in average.[11] All these abnormalities in brain function have been linked to an unbalance of important molecules called neurotransmitters. However, **the reason why** this unbalance occurs has not been determined[4] (Figure 1).

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                          ┌─────────┐
                          │  Brain  │
                          └─────────┘
                ┌──────────────┴──────────────┐
          ┌─────────┐                    ┌───────────┐
          │ Healthy │                    │ Depressed │
          └─────────┘                    └───────────┘
         ┌─────┴─────┐                  ┌──────┴──────┐
  ┌────────────┐ ┌──────────┐   ┌────────────┐ ┌──────────┐
  │ Very active│ │  Volume  │   │Low activity│ │  Volume  │
  │            │ │~ 1400 cm³│   │            │ │~1397.7cm³│
  └────────────┘ └──────────┘   └────────────┘ └──────────┘
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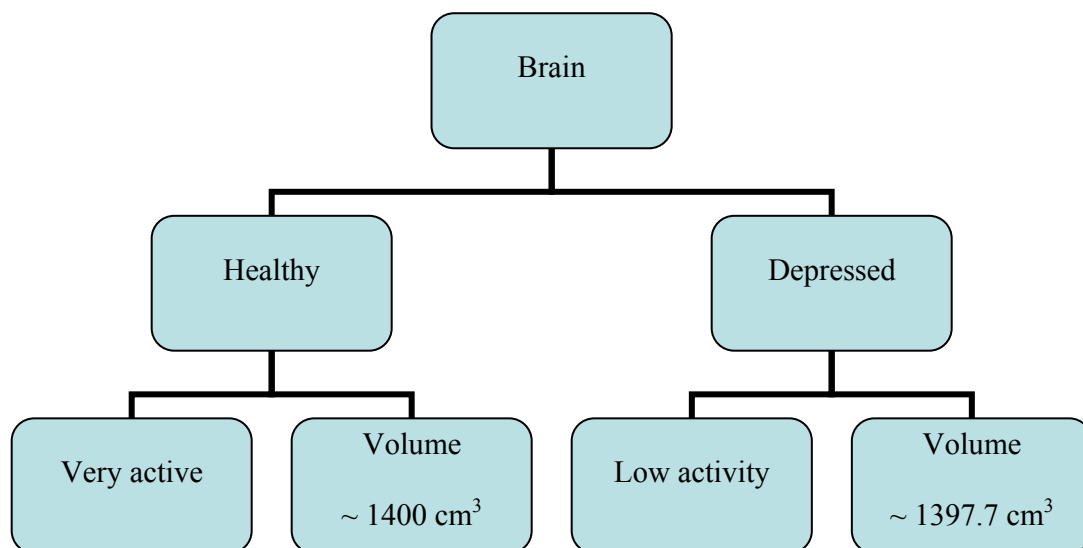
Figure 1. Comparison between brains of depressed and non-depressed people.

Figure **2** shows normal vs. abnormal processes. Serotonin, a neurotransmitter, is released from the pre-synaptic neuron into the synaptic cleft. From the synaptic cleft, it
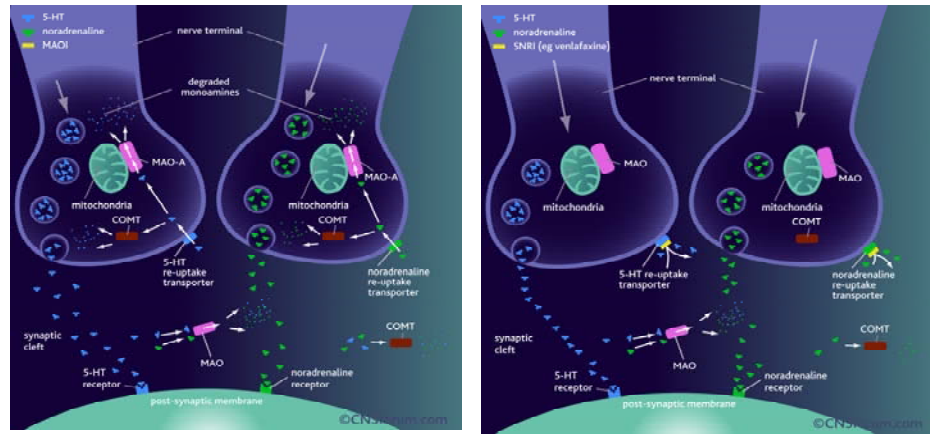
Figure 2.   Normal (left) and abnormal (right) processes of the brain that cause depression[12]

reaches its target – a serotonin receptor – in the post-synaptic neuron. Once it has activated the receptor on the post-synaptic neuron, serotonin is released again in the synaptic cleft in order to be reuptaken by the pre-synaptic neuron via the serotonin transporter (SERT) to be re-utilized (Figure 2, left). However, sometimes for some still unknown reason, the amount of serotonin initially released into the synaptic cleft is smaller than it should be (Figure 2, right) causing depression.

Women and depression. The Center for Disease Control (CDC) reports that, for the years 2005 – 2006, depression was more common among women (6.7 %) than it was in men (4 %).[12] The CDC attributed this fact to biological, life cycle, hormonal and psychosocial factors unique to women. It is a known fact that hormones directly affect the brain chemistry that controls emotions and mood. This is the reason why women are

especially susceptible to depression after giving birth, during the transition into menopause or during their ovulation and menstruation.[4]

Men and depression. Men typically experience depression in a different way than women do, and usually have different ways of coping with it. Whereas women are more likely to experience feelings of sadness, worthlessness and/or excessive guilt, men are more likely to experience fatigue, irritability, loss of interest in once-pleasurable activities and sleep disturbances. Also, men are more prone than women to turn to alcohol or drugs or to become frustrated, discouraged, irritable, angry and sometimes abusive. Some men become "workaholics" in order to avoid talking about their feelings with family or friends, or can engage in reckless, risky behavior. Interestingly, although more women attempt suicide, more men die by suicide in the United States.[4, 12]

Older adults and depression. Depression in the elderly may be more complicated to detect since persons in this age group may show different, less obvious symptoms that can also be interpreted as physical ailments due to their age. Some examples include heart disease, stroke or cancer that may cause depressive symptoms. Also, it is possible that, due to medical conditions, they may be taking medications whose side effects contribute to depression. The good news for them is that the symptoms of depression can improve upon treatment with antidepressants, psychotherapy, or a combination of both.[4,13]

Depression in children and adolescents. Health care providers are taking more seriously the risk of depression in children since data shows that depression during childhood often persists, reappears and continues into adulthood. This is especially true

if the symptoms of depression go untreated. Moreover, childhood depression is generally a good predictor of more severe illnesses in adulthood.

Whereas younger children suffering from depression usually refuse to go to school, pretend to be sick, cling to a parent, or worry that a parent may die, older children may sulk, get in trouble at school, be negative and irritable, and feel misunderstood. In older children, these symptoms may be viewed as normal as they grow up into teen age. This is why it may be difficult to diagnose a teenager with depression.[4]

Diagnosis of Depression

Due to the fact that some medications and certain medical conditions, like viruses or thyroid disorders, can cause symptoms very similar to those of depression, it is suggested to visit a doctor for a complete physical examination which may include:

- Measuring height and weight, checking vital signs such as heart rate, blood pressure and temperature, listening to the heart and lungs, and examining the abdomen.

- Lab tests, which may include a complete blood count, screening for alcohol and drugs, and thyroid function tests.

- Psychological evaluation where the patient will be asked about any family history of depression or history of symptoms such as when they started, how long they have lasted, how severe they are, and thoughts of suicide or self-harm.

Once diagnosed, the patient can be treated with a number of methods which will depend on the severity of the symptoms and the possible coexistence of other serious medical conditions. The most common treatments are medication and psychotherapy.[4, 14]

When the symptoms are mild or moderate, a primary health care provider can treat depression. If the symptoms are severe, a qualified mental health care provider, such as a psychiatrist, psychologist or social worker is a better option.[14] It is important to work together with a doctor or therapist because that way both the patient and the health care provider, can make important decisions on treatment options, personal preferences, insurance coverage, affordability, treatment side effects, etc.

Antidepressants

Drugs known as *antidepressants* work to normalize the neurotransmitters serotonin, norepinephrine and dopamine in the brain. The molecular structures of these neurotransmitters are shown in figure **3**.

Serotonin   Norepinephrine   Dopamine
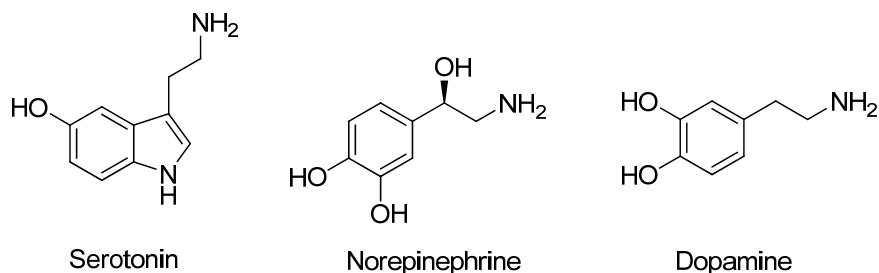
Figure 3. Important neurotransmitters in depression.[16]

Over the years, several families of antidepressants have been developed. The most popular ones are called *selective serotonin reuptake inhibitors* (SSRIs). The most common SSRIs are depicted in figure **4**:

Fluoxetine (Prozac®)　　　Citalopram (Celexa®)　　　Sertraline (Zoloft®)
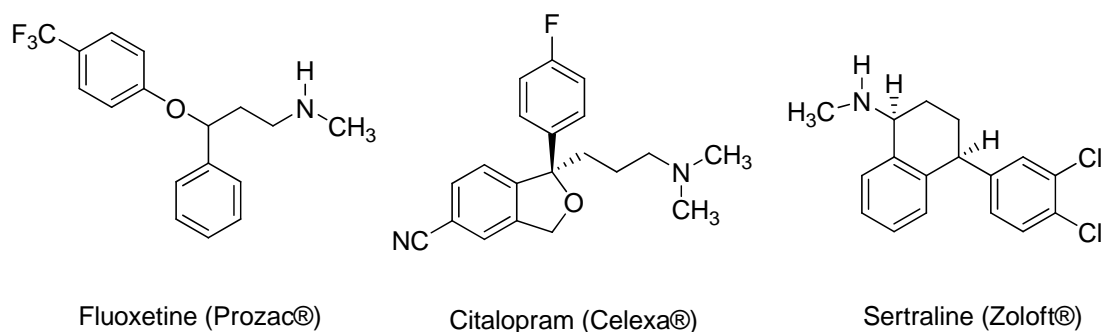
Figure 4. Commonly prescribed SSRIs.[16]

Also common are the *serotonin norepinephrine reuptake inhibitors* (SNRIs) which include, among others Venlafaxine, Devenlafaxine, and Duloxetine (Figure **5**):

Venlafaxine (Effexor®)　　　Desvenlafaxine (Pristiq®)　　　Duloxetine (Cymbalta®)
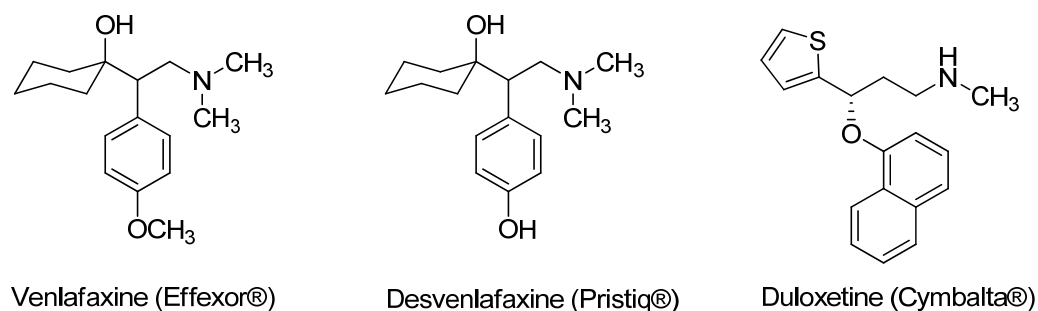
Figure 5. Commonly prescribed SNRIs.[16]

Other families of antidepressants include the *tricyclic antidepressants* (Figure 6) and the *monoamine oxidase inhibitors* (Figure 7)

Clomipramine (Anafranil®)　　　Protriptyline (Vivactil®)　　　Imipramine (Tofranil®)
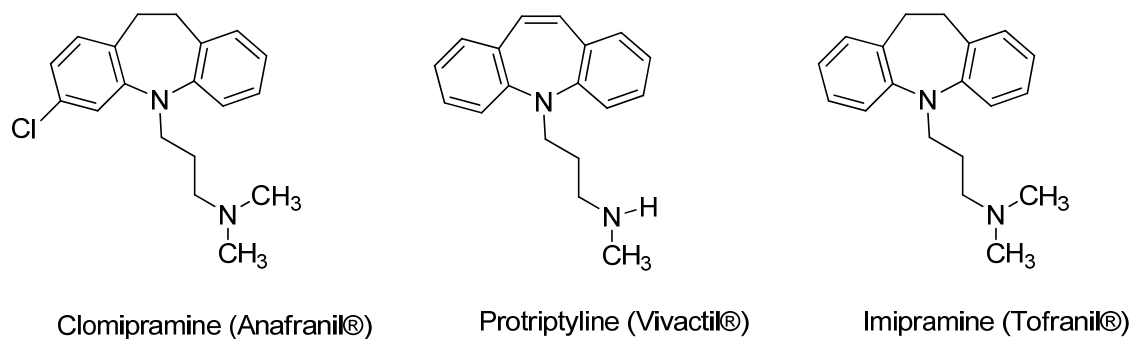
Figure 6. Commonly prescribed TCIs[16]

Isocarboxazid (Marplan®)   Phenelzine (Nardil®)   Tranylcypromine (Parnate®)
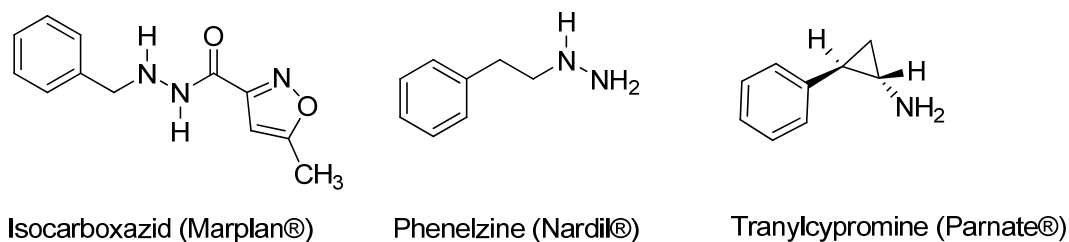
Figure 7. Commonly prescribed MAOIs[16]

Of all these, SSRIs and SNRIs are the most popular among health care providers due to their fewer side effects. However, medications affect every individual in a different way. As a result, for some patients, TCAs or MAOIs may be the best choice. In this way, if one medication does not work, people should be open to try another one.

On the other hand, regardless of the type of antidepressant being taken, it is important to take regular doses, usually once a day, for several weeks before starting to feel some improvement. Even if feeling better, it is not recommended for a patient to stop the medication by him/herself in order to avoid a relapse of the depression and/or withdrawal effects. If the symptoms become chronic, the patient may need to stay on the medication for life.

Side Effects of Antidepressants.[4, 15]

Antidepressants may cause mild side effects which generally are not long term. These include:

SSRIs and SNRIs: headache, nausea, insomnia and nervousness, agitation and sexual problems such as reduced sex drive, erectile dysfunction, delayed ejaculation or inability to have an orgasm.

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Tricyclic antidepressants: dry mouth, constipation, bladder problems, sexual problems similar to those of SSRIs and SNRIs, blurred vision and drowsiness during the day.

Antidepressant Mechanisms

The mode of action of all known antidepressants consists of directly affecting the activity of one or more of the neurotransmitter systems serotonin, norepinephrine, and dopamine. In an attempt to better understand antidepressants, they have been classified according to their chemical structure, historical sequence or presumed mechanism of action.[16]

To date, four classes of antidepressants are recognized: monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin – norepinephrine reuptake inhibitors (SNRIs).[16]

Monoamine oxidase inhibitors (MAOIs). Monoamine oxidase is an enzyme that metabolizes dopamine, serotonin and norepinephrine. Two subtypes called MAO-A and MAO-B are currently known. Of these, MAO-A metabolizes serotonin and norepinephrine, and MAO-B metabolizes dopamine.[16]

Initially intended for the treatment of tuberculosis, MAOIs were found to have antidepressant activity when administered to patients with comorbid depression, experienced improvement in their mood.[16]As expected, the inhibition of MAO enzymes results in increased availability of the corresponding neurotransmitters in the central nervous system achieving an antidepressant effect and in the peripheral nervous system where it can affect blood pressure.[16] Hence, interactions of MAOIs with certain foods or drugs can result in hypertension.[16] At therapeutic doses, MAOIs are generally well

tolerated because of their extremely low affinity for other active sites in the body. However, the risk of developing hypertension and the strict dietary restrictions limit these drugs to last option in most care practices.[16]

Tricyclic antidepressants (TCAs). Initially intended for the treatment of psychosis, TCAs were found to have poor antipsychotic activity but good antidepressant effects.[16] Some of the drugs that belong to this family, act by blocking the reuptake of serotonin (SERT) whereas others block the reuptake of norepinephrine (NET).[16] This blocking process causes the concentration of serotonin or norepinephrine to increase in the synaptic cleft leading to an antidepressant effect.

Other targets of TCAs include:[16]

- The histamine H_1 receptor, making them effective in the treatment of allergic conditions and as hypnotics. However, activity against the histamine receptors in the central nervous system (CNS) has been linked to sedation, weight gain, and increment of CNS depressant effects from other drugs.

- Muscarinic acetylcholine receptors, where TCAs cause memory impairment, increased heart rate, dry mouth, blurred vision, constipation and urinary retention.

- α_1-adrenergic receptors where usual TCAs side effects include orthostasis, dizziness, and syncope.

- Sodium channels in the heart where TCAs show similar effects as antiarrhythmic medication. In high doses, TCAs may cause heart block and acceleration of the heart rate which could lead to fatal arrhythmias.

Serotonin-selective reuptake inhibitors (SSRIs).[16] SSRIs are a class of drugs named after their mechanism of action. Like TCAs, SSRIs inhibit the reuptake of serotonin. However, the difference with TCAs is that SSRIs have a greater affinity for the SERT than for any other central or peripheral receptor system, as well as improved tolerability and safety in overdose.

The side effects associated with these drugs have already been described in this chapter and are usually dose-dependent with sexual dysfunction being the one that has caught the most attention.

Since loss of sexual interest is also a symptom of depression, it is important for health care providers to recognize when this loss of sexual drive is due to the illness or to a side effect of SSRIs. In this way, restoration of premorbid libido should be expected within four to eight weeks of treatment. If disinterest continues or another sign of sexual dysfunction, like anorgasmia, is shown by the patient after this time framework, then SSRI-induced side effect can be suspected.

Serotonin-norepinephrine reuptake inhibitors (SNRIs).[16] The mechanism of action of SNRIs is very similar to that of TCAs. SNRIs inhibit both the SERT and the NET but, unlike TCAs, the drugs in this family have very low affinity for other neurotransmitter systems. The affinity for the serotonin and norepinephrine systems also varies: at lower therapeutic doses, SNRIs mostly bind to the SERT, while at higher doses, the inhibition of NET becomes more equal to that of SERT.

As can be expected, the side effects of SNRIs at low therapeutic doses are very similar to those shown by SSRIs (nausea, sexual dysfunction, insomnia, etc.). At higher

doses, these side effects become more evident and the onset of noradrenergic side effects – such as hypertension, tremor, and diaphoresis – can be detected.

FDA warning on antidepressants. In 2004 the U.S. Food and Drug Administration (FDA) urged the drug companies to adopt a "black box" warning label on all antidepressant medications regarding suicidal tendencies. After reviewing data, it was found that 4% of 4,400 children taking antidepressants had thought or attempted suicide (thankfully, no suicides occurred), versus 2% of those receiving placebos. In 2007, the warning was extended to include young adults up to 24 years of age. The warning stresses the frequent monitoring of patients of all ages, especially during the initial weeks of treatment. Also, the families and caregivers should be told about the importance of this close monitoring and report any changes to the health care provider.[4, 15]

Psychotherapy.[4] In treating depressed people, it is vital for patients to talk about their tribulations and their feelings with the mental health care provider. This psychotherapy – or "talk therapy" – can be short-term (10 to 20 weeks) or longer-term, depending on the individual needs of every person. When it comes to depression, two main types of psychotherapy have proven to be effective:

- *Cognitive-behavioral therapy (CBT)* is a psychotherapeutic approach that aims to solve problems concerning dysfunctional emotions, behaviors and cognitions through a goal-oriented, systematic procedure consisting of learning new ways of thinking and behaving.[4, 17]

- *Interpersonal therapy (IPT)* is a short-term psychotherapy that is based on building interpersonal skills. Depression usually has an interpersonal

component, even though it may not be caused by interpersonal events. In other words, it affects both relationships and roles in those relationships. This type of therapy focuses on interpersonal events that seem to be most important in the onset and/or maintenance of depression.[4, 18]

Drug-resistance is a potentially major problem in the treatment of many illnesses and depression is not an exception. For patients with extreme cases like these, there is also hope. Three methods have been developed for effective therapy in drug-resistant depression:

Vagus nerve stimulation (VNS) is a neurological procedure that sends electrical impulses into the brain in an attempt to improve chronic depression symptoms. In this type of therapy, an electronic device, called a pulse generator, is surgically implanted in the patient's chest. A wire threaded under the skin connects the pulse generator to the left vagus nerve in the neck sending electrical signals that affect mood centers in the brain improving depression symptoms (Figure **8**).[14]

Electroconvulsive therapy (ECT) is a type of therapy consisting in producing electrically induced seizures in anesthetized patients for therapeutic purposes. Although ECT is very effective in the treatment of major depression, it has been associated with significant side effects: 1 in 10,000 risk of death, short-term confusion, memory disturbance, disorientation and headaches.[14, 15]

Transcranial magnetic stimulation (TMS) is a non-intrusive and well-tolerated method to stimulate neurons in the brain. In this technique, a large electromagnetic coil is placed on the scalp near the forehead. An electromagnet sends electric currents that

stimulate nerve cells in the region of the brain involved in mood regulation. This way, brain activity can be triggered with minimal discomfort.[14, 15]
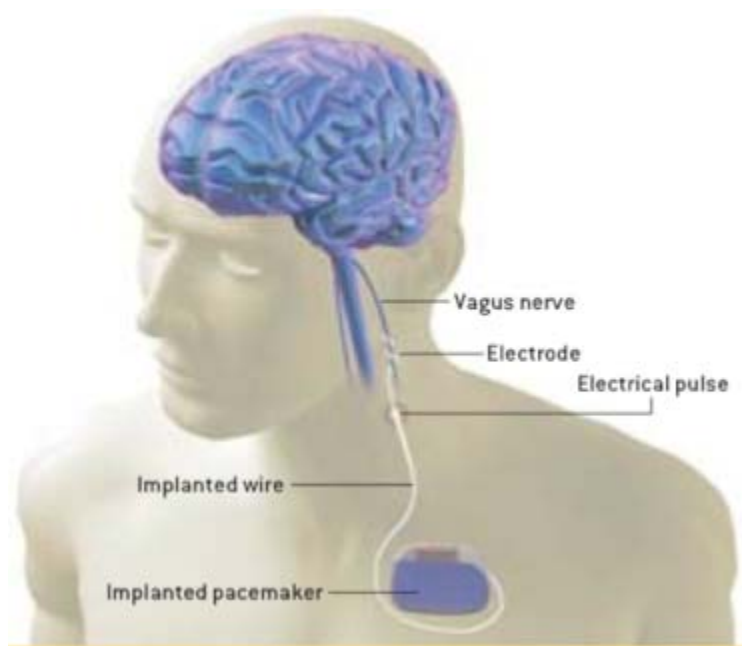
Figure 8. Vagus nerve stimulation.[15]

Drug Design Rationale

The main idea of this project is to develop new potential treatment agents that are active against both the SERT (Chapter 2) and the 5-HT$_{2A}$ receptor (Chapter 3) in an attempt to diminish the secondary side effect of sexual dysfunction common to SSRIs without significantly affecting their antidepressant activity. In order to achieve this goal, several molecules based on the chemical structure of fluoxetine (Prozac®) have been designed and prepared by chemical synthesis. These molecules incorporate chemical entities that have been successfully employed in the treatment of SSRI-induced sexual dysfunction. It is proposed that the coupling of these two chemical entities with activity against the SERT and the 5-HT$_{2A}$ receptor will accomplish the objective (Figure 9).[19, 20]

Affinity to 5-HT$_{2A}$

Affinity to SERT

A = C, N

n = 0, 1

X = F, CF$_3$
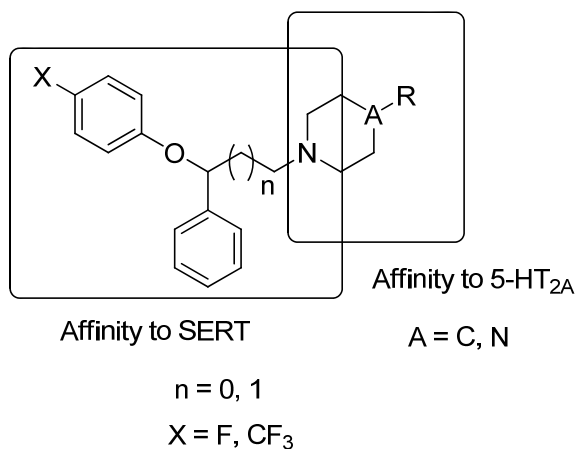
Figure 9. Design rationale of novel compounds based on the chemical structure of fluoxetine for the treatment of depression.

Another set of molecules have been prepared based on the structure of the compound 4-[(3-fluorophenoxy)phenylmethyl]piperidine (Figure 10).
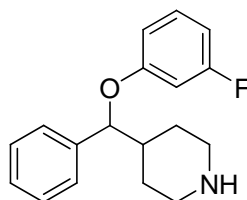
Figure 10. Chemical structure of 4-[(3-fluorophenoxy)phenylmethyl]piperidine.[22]

This compound, still under development, has shown to be more active against the serotonin transporter than fluoxetine itself, but also shows activity against the norepinephrine transporter[21] which also plays an important role in depression (see Chapter 3). The design paradigm for the new compounds is shown in Figure 11.

Due to the fact that both moieties are secondary amines, and that it would be difficult to put them together in a similar manner to their fluoxetine counterparts, it was decided to use a carbonyl group as a linker, giving rise in this way to unsymmetrical ureas. (Figure 11)
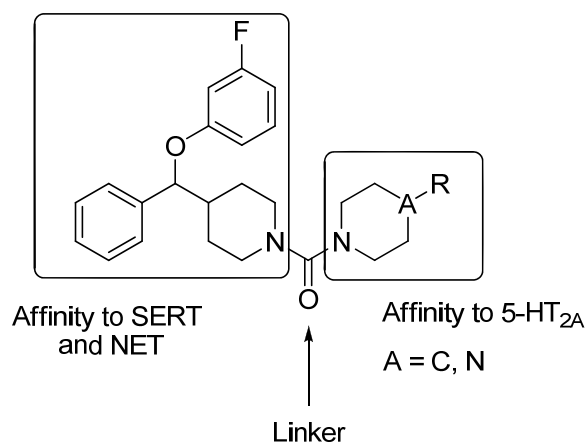
Figure 11. Design rationale of novel compounds based on the chemical structure of the compound 4-[(3-fluorophenoxy)phenylmethyl]piperidine for the treatment of depression.

CHAPTER TWO

The Transporters

The Serotonin Transporter

The serotonin transporter (SERT, 5-HTT) is a 630 aminoacid integral membrane protein that regulates extracellular levels of serotonin in the brain by transporting it back into nerve cells after release from the receptors and, by this means, terminates the transmitters' action at extracellular receptor sites. They represent the first step in the process of transmitter recycling. (Figure 12).[22-25]
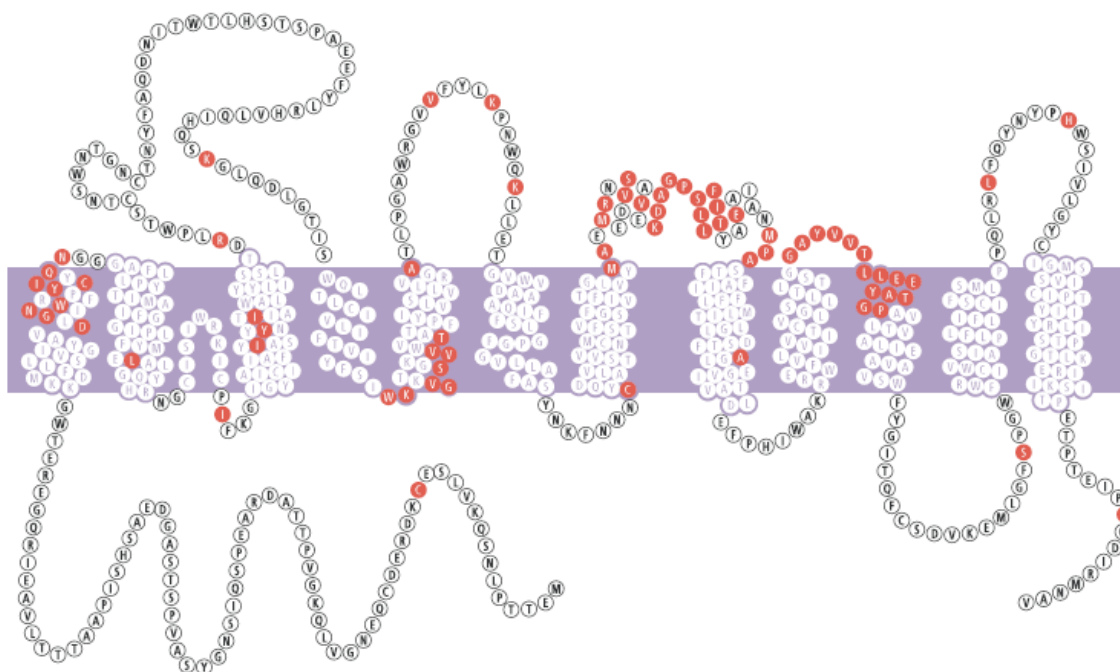
Figure 12. The serotonin transporter (SERT)[23]

The process catalyzed by this protein is complex and involves both symport and antiport transport (Figure 14). A possible mechanism to explain this process requires the binding of 5-HT together with Na^+ and Cl^-. Only after all three solutes are bound is the

transporter able to undergo a series of conformational changes that close off access to the extracellular medium and expose the binding site to the cytoplasm (right side of figure 14). In the next step, 5-HT, Na^+ and Cl^- are released into the cytoplasmic side of the cell and K^+ binds to the empty active site of the transporter (lower left). Once K^+ is bound, the SERT undergoes another series of conformational changes that close off its cytoplasmic side and expose the binding site to the extracellular medium. Dissociation of K^+ completes the cycle[22] (Figure 13).

In order to understand the mechanism of a transporter like this, knowledge of four properties of the protein are required:[22]

1. The nature of the binding site. This determines how the transporter can selectively transport one substrate and not another. In the case of SERT, where ions are co-transported with substrate, the relative positioning of substrate and ion binding sites may be critical for coupling.

2. The pathways through which substrate and ions pass in their movement from one side of the membrane to the binding site and from the binding site to the other side of the membrane. These need to be tightly coupled so that they are not both open simultaneously.

3. Conformational changes that close access from one side of the membrane and open access to the other side are required for translocation to occur.

4. Control of conformational changes so that they occur only when the appropriate ligands are bound at the binding sites.

The SERT is the target of a variety of compounds that are used to treat clinical depression and anxiety. These drugs include TCAs and SSRIs.[22, 23] Their mode of action

is, primarily, by blocking the SERT causing an elevation of the extracellular concentration of serotonin enhancing, in this way, neurotransmission.[26] The action of these drugs supports the indoleamine hypothesis of depression, which suggests that major depression results from a deficiency of available or inefficient serotonin. Researchers observe that depletions of serotonin from certain regions of the brain such as the hypothalamus, amygdala, and cortical areas involved in cognition and other processes, can have a great impact in contributing to depression.[27]
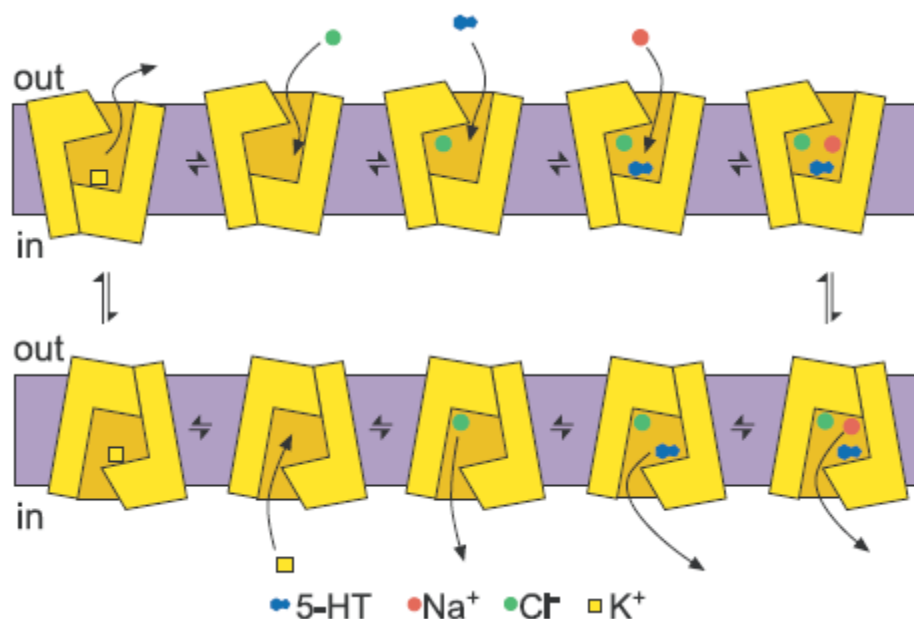
Figure 13 Possible mechanism of serotonin transport[23]

As previously mentioned, the reason why some people show a smaller amount of serotonin released into the synaptic cleft is not clearly known. In their research, Caspi and coworkers found that the SERT gene can have a long and a short allele with the promoter region (the portion of the gene that controls how often the translated region is *read*) being the difference between the two. In their study, the group shows that people with short alleles are more prone to depressive episodes (figure 14):[28]
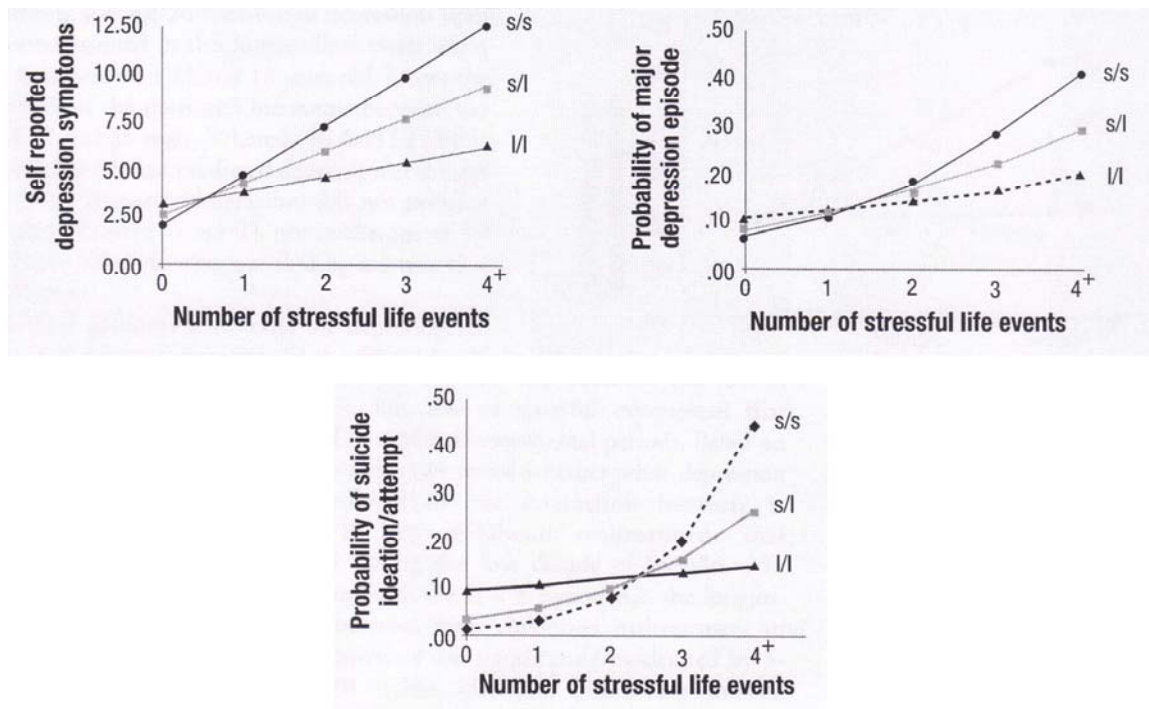
Figure 14. A short allele in the 5-HTTLPR is associated with depression[29]

Even though this is a great breakthrough in the search for an actual reason for the causes of depression, the question has not yet been answered: What happens at the biochemical level that causes the SERT gene to have a shorter promoter region? Or what biochemical events trigger this shortage of the promoter region? To find an answer to these questions, and therefore, an optimum treatment for depression is not an easy task due to the extremely high complexity of the central nervous system. For example, in the case of the SERT, there is evidence that shows that 5-HT input into the thalamus is an important facilitator of appetite.[29] Serotonergic neurons projecting into the suprachismatic (SCN) nucleus of the anterior hypothalamus help regulate circadian rhythms (sleep-wake cycles and body temperature among others). The serotonin system also controls the cycle of alternating periods of REM and non-REM sleep.   The neurotransmission via serotonin is also necessary for affiliative behavior (making friends

24

or finding a boyfriend or girlfriend), as well as the expression of goal-directed behaviors. Interestingly, wild animals showing lower levels of 5-HT (measured by cerebrospinal fluid 5-hydroxyindoleacetic acid, 5-HIAA), show a more impulsive behavior, have more scars from fighting, and generally have lower rankings on social dominance hierarchies than do animals with higher basal levels of 5-HT.[29] These facts explain all the symptoms shown by depressed people (see Chapter 1).

Clearly, this shrinkage in the promoter region is not a natural process because that would imply depression to be something common in the elderly, which is not true.[29]

The Norepinephrine Transporter (NET)

In the 1960s, after many years of research on the subject, data showed that norepinephrine, a catecholamine, is a key component in affective disorders. These data demonstrate that norepinephrine circuits are not normal in affective disorders and affect concentration, attention, memory, arousal states, and sleep regulation.[30]

In the mid-1980s, SSRIs were found to be effective in the treatment of depression. This, along with the theory that enhancement of serotonin neurotransmission might be a final pathway for antidepressants, caused researchers to focus almost entirely on understanding the role serotonin in causing depression.[31]

One of the facts that made researchers turn their attention back into the role of norepinephrine in depression was that neurotransmitter depletion studies showed that the therapeutic effects of SSRIs could be temporarily reversed by rapid serotonin depletion but not by depletion of norepinephrine. In the same way, the therapeutic effects of a norepinephrine inhibitor could be temporarily reversed by depletion of norepinephrine but not by depletion of serotonin.[31]

Delgado and Moreno carried some depletion studies in healthy humans and their data support the hypothesis that antidepressants may work through a number of mechanisms, serotonin and norepinephrine being just two of them. However, a simple change in the concentration of the monoamine in the synaptic cleft between neurons is definitely not enough to alleviate depression. In other words, if the impairment responsible for depression is due to the inability of some neurons to appropriately respond to serotonin and norepinephrine, then a small increment in the concentration of any of these neurotransmitters would not alleviate depression immediately. Similarly, a small lowering of the amount of any of these two neurotransmitters would not worsen depression, as was observed by this group.[31]

It is a well known fact that people under antidepressants take several weeks to show some improvement in their mood. Delgado and Moreno[31] suggest that antidepressants may act not only by temporarily blocking the reuptake process, but also by modifying the ability of postsynaptic neurons to react to monoamines, accounting in this way for the delay. The group reports that their results are in agreement with a model in which depression is a result of dysfunction in areas that are controlled by monoamine systems such as the frontal cortex, hippocampus/amygdala, and basal ganglia (see Figure 15). These areas of the brain are known to be especially sensitive to the effects of stress. This could account for the adverse impact of life stress on depression. The possibility exists that these abnormalities occur at the subcellular level: the G-protein coupling, the second or third messenger systems, or even gene transcription to mention some examples.
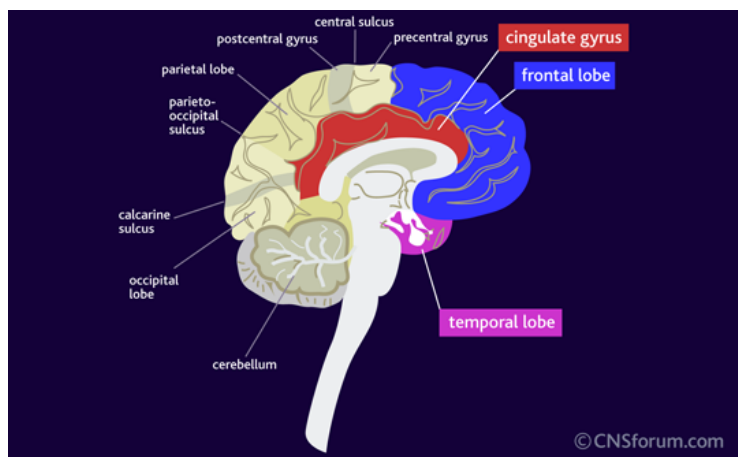
Figure 15. Areas of the brain affected by depression.[12]

On the other hand, Dubini and co-workers[32] found that patients treated with reboxetine (Figure 16), a selective norepinephrine reuptake inhibitor, showed better improvement in social functioning as well as better response to treatment than patients treated with fluoxetine (an SSRI). Although these differences could be just the ability of norepinephrine or serotonin to control the particular type of dysfunction that leads to depression in a particular individual, it illustrates the importance of developing new drugs that are selective to these transporters[32] while reducing the side effects as much as possible.[33]
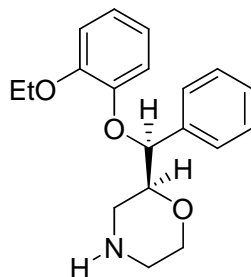
Reboxetine

Figure 16. Chemical structure of reboxetine.[16]

CHAPTER THREE

Serotonin Receptors

G-Proteins

Heterotrimeric G-proteins comprise a family of signal-coupling proteins that control a wide range of physiological processes including neurotransmission, chemotaxis, perception of light, smell, and taste among others, by carrying signals from activated membrane receptors to effector enzymes and channels.[34, 35]

The correct name of these proteins is *GTP-binding protein* because of the fact that they bind to GTP (guanosine triphosphate). To date, two types of G-proteins are known: heterotrimeric and monomeric. G-protein coupled receptors (GPCRs), *e.g.*, serotonin receptors (except 5-HT$_3$), are joined to heterotrimeric G-proteins.[36] This means that when serotonin binds to a receptor, this receptor activates a G-protein which, in turn, stimulates an enzyme that produces a second messenger.[37]

Receptor-activated G-proteins are found on the surface of the cell. They consist of associated subunits called G_α and $G_{\beta\gamma}$. Four different types of G_α subunits are known: $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q/11}$, and $G_{\alpha s12/13}$. Although they behave differently in the recognition of effectors, they share a similar mechanism of activation.

Figure 17 shows the effector (serotonin, in our case) approaching and binding the GPCR (1). When the effector binds the GPCR, in the cytosolic side of the cell the G_α subunit releases GDP and binds GTP (2), causing the G_α subunit to dissociate from the $G_{\beta\gamma}$ dimmer (3). These units activate or inhibit target proteins depending on the type of G-protein (4).Upon hydrolysis of GTP to GDP, a second messenger is produced and the

G_α subunit becomes inactive (5). Finally, the G_α subunit re-associates with the $G_{\beta\gamma}$ subunit and becomes ready to re-start a new cycle (6).[37, 39, 40]
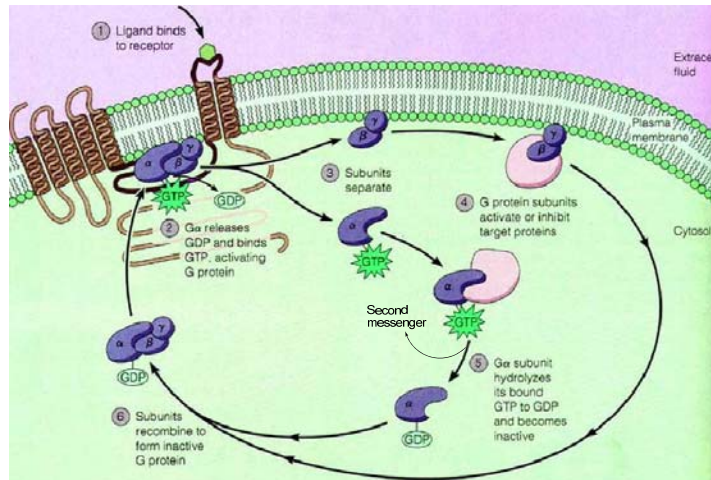
Figure 17. Mechanism of activation of G-proteins by G-protein coupled receptors.[41]

As with many other proteins, G-proteins are divided into families; each one with a different physiological response and function. Of these, the $G_i\alpha$ subtype is known to mediate physiological response of neurotransmitters by inhibiting the synthesis of cAMP, closing Ca^{2+} ion channels or opening K^+ ion channels. On the other hand, the $G_q\alpha$ subtype is also known to mediate physiological response of neurotransmitters by increasing the synthesis of phosphoinositide as well as regulating the concentration of intracellular Ca^{2+} ions.[39, 40]

Serotonin Receptors

Serotonin, 5-hydroxytryptamine (5-HT), belongs to the family of monoamine neurotransmitters which are differentiated by a chemical pattern embracing a basic amino group separated from an aromatic moiety by a two carbon aliphatic chain. See Figure 18.[41]

29
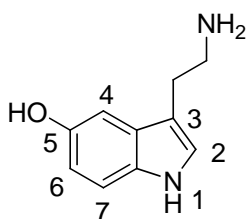
Serotonin, 5-HT

Figure 18. Numbering system in serotonin.[42]

Its biosynthesis in mammals consists of two enzymatic steps and its metabolism is performed by monoamine oxidase A (MAO-A). This process is depicted in Scheme 1.
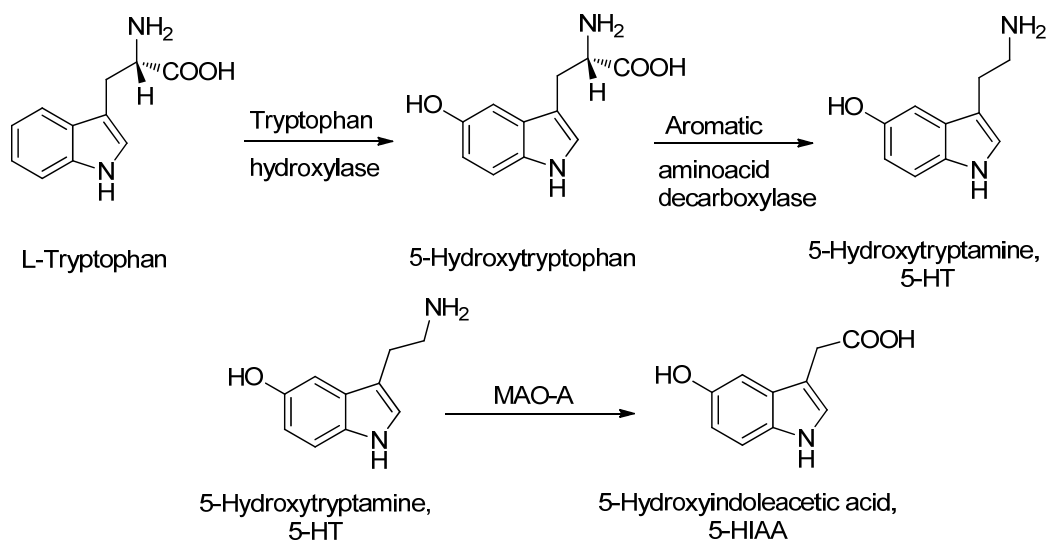
Scheme.1. Enzymatic biosynthesis and metabolism of serotonin.[42]

In the brain, the axon terminals are in charge of the biosynthesis of serotonin.[42] Once synthesized, it is released into the synapse where it diffuses to reach and activate postsynaptic receptors (see Figure 1, Chapter 1) which belong to the family of the G-protein coupled receptors, except for the 5-HT$_3$ receptor. Then, it is removed from the synapse by a specialized protein called serotonin reuptake protein or serotonin transporter

(SERT) which drives the free serotonin back into the neuron terminal to repeat the cycle.[42]

The receptors had to be classified in order to simplify their study. Initially, this classification included two groups that were called "D" and "M." The "D" receptors generally controlled the contraction of various types of smooth muscle and could be antagonistically and irreversibly blocked by dibenzyline (for this reason "D")[42] (Figure 19).
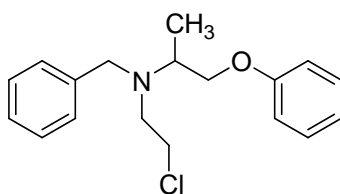
Figure 19. Chemical structure of dibenzyline[16]

On the other hand, the "M" receptor controlled the depolarization of cholinergic nerves and was blocked by morphine (figure 20).[41, 42, 43, 44] As other types of serotonin binding sites in the brain were identified that demonstrate strong affinity for LSD as well as 5-HT were identified, the "M" receptor was re-named 5-HT$_1$. Another type of receptor that shows a high affinity for the antagonist spiperone was named 5-HT$_2$. Eventually, several subtypes were found for these two receptors.[41]

Morphine

Lisergic acid diethyamide (LSD)
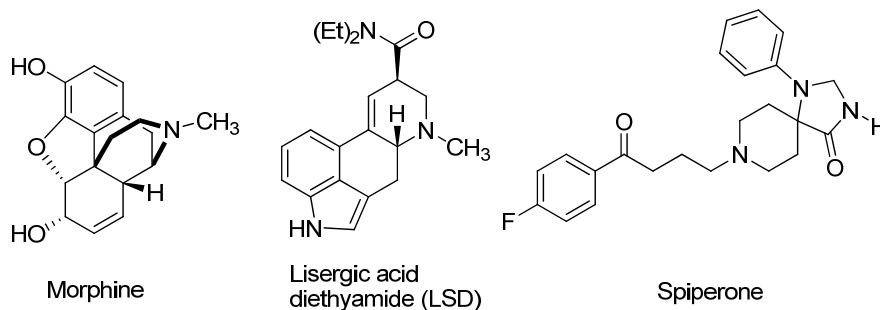
Spiperone

Figure 20. Chemical structure of morphine, spiperone, and LSD[16]

31

A third type of receptor, clearly different from either 5-HT$_1$ or 5-HT$_2$, was found. Interestingly, it is a ligand-gated ion channel. This discovery surprised the scientific community since all of the 5-HT receptors were thought to belong to the G-protein coupled receptors family.[45, 41] As research continued, more serotonin receptors were identified, cloned and characterized. To date, fourteen receptors have been fully characterized and classified as follows:[41]

$G_q\alpha$-Coupled Receptor Types

*5-HT$_{2A}$.* Initially cloned and characterized in 1990,[46] this receptor is of particular interest due to its role in normal brain function. Psychoactive substances known as psychedelics (*e.g.,* LSD and mescaline) appear to utilize this receptor as a key target in the mode of action for their hallucinogenic effects.[46-48] A conclusive proof of this fact was found by Vollenweider and coworkers. In their experiments, this group found that the hallucinogenic effect of psilocybin in healthy human volunteers was blocked by the antagonist ketanserin, which is known to selectively bind to the 5-HT$_{2A}$ receptor in humans.[49] (See figure 21).
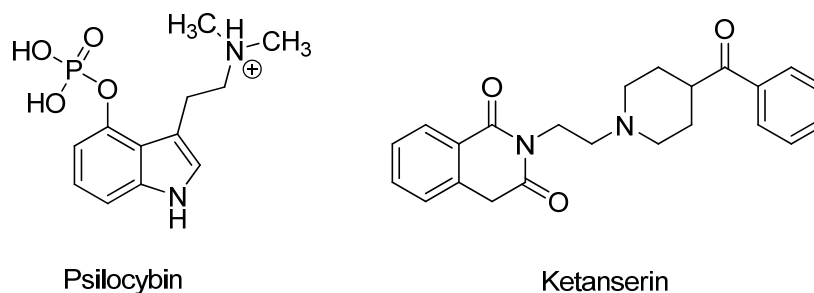

Psilocybin   Ketanserin

Figure 21. Chemical structures of psilocybin and ketanserin[16]

Extensive experimental work has resulted in mapping of the 5-HT_{2A} receptor, identifying heterogeneously distributed densities with very high concentrations in several cortical areas such as frontal, parietal, temporal and occipital lobes, the anterogenual cortex and the entorhinal area.[41] Activation of the 5-HT_{2A} receptor leads to membrane depolarization and the closing of potassium channels in several brain areas.[41]

The 5-HT_{2A} receptor was also found in several types of cardiovascular tissues where it controls a variety of functions including arterial vasoconstriction and pulmonary hypertension in rats.[50, 51] Finally, in the ganglia and spinal cord, 5-HT_{2A} activation produces analgesia and blockade produces hyperalgesia.[51] The 5-HT_{2A} receptor has also been implicated in controlling other functions such as addiction,[52] anxiety,[53] appetite,[54] cognition,[54] imagination,[54] learning,[54] memory,[54] mood,[54] perception,[54] sexual behavior, sleep,[55] thermoregulation,[56] and vasoconstriction.[57]

5-HT$_{2B}$. This receptor seems to be the only serotonin receptor that is necessary for life. Knockout of the gene that codes for 5-HT_{2B} is lethal in mice and produces severe embryonic and neonatal heart defects.[41, 58] High levels of this receptor are found in the liver, kidneys, stomach fundus and gut. Moderate levels are found in the lung and cardiovascular tissues and low expression levels in the brain, where it has been associated with vulnerability to drug abuse.[41] Also found in the cochlea and inferior colliculus, it is thought to be involved in age-related hearing loss.[41, 59]

5-HT$_{2C}$. This receptor is highly expressed in the choroid plexus and widely distributed throughout the brain in regions that are involved in food intake which include the nucleus of the solitary tract, dorsomedial hypothalamus, and the paraventricular

hypothalamic nucleus. The 5-HT$_{2C}$ receptor is also known to be involved in body weight regulation and obesity.[60]

$G_s\alpha$-Coupled Receptor Types

5-HT$_4$. Cloned and characterized in 1995,[61] the human 5-HT$_4$ receptor can be found in both the central nervous system (CNS) and the peripheral nervous system (PNS). In the CNS it is highly expressed in the basal ganglia, cortex, hippocampus and substantia nigra.[62, 63] Animal models show that some agonists to these receptors can effectively enhance learning and memory. On the other hand, knockout mice show normal behavior under standard environmental conditions, but abnormally low locomotor activity and hypophagia are displayed under novelty and stress conditions.[64, 65]

In the PNS, 5-HT$_4$ receptor plays an important role in gastrointestinal function. Its stimulation facilitates the release of acetylcholine and relaxation of the colon.[66] For this reason, agonists of this receptor have been used for the treatment of both constipation and constipation-predominant irritable bowel syndrome (IBS).[41]

5-HT$_6$. Cloned and characterized in 1993,[67, 68] this receptor is expressed almost exclusively in the mammalian CNS with the highest levels of expression found in the striatum, nucleus accumbens, cortex, and olfactory tubercle as well as the hippocampus, thalamus, amygdale, hypothalamus, and cerebellum.[41, 69] The fact that this receptor is restricted to the cortical areas of the CNS, suggests that it plays a role in higher order cognitive processes – skills involving analysis, evaluation and synthesis (creation of new knowledge).[41]

5-HT$_6$ receptor seems to regulate a wide variety of neurotransmitters. Research has shown that blocking this receptor enhances cholinergic neurotransmission and, in animal models, facilitates learning and memory processes.[70] Interestingly, this receptor is not expressed on cholinergic neurons.[71] It has also been shown that antagonists to this receptor increase glutamate release in cortex, whereas agonists diminish glutamate release in the hippocampus.[41] Some drugs acting on these receptors alter dopamine, GABA and norepinepherine levels. However, the mechanism through which this receptor regulates each of these neurotransmitters remains unknown. All these effects on neurotransmission have made this receptor an attractive target for the treatment of cognitive deficits in Alzheimer's disease and schizophrenia.[72]

5-HT$_7$. This receptor was cloned and characterized in 1993.[67] It has been mapped in the hypothalamus,[73] thalamus,[73] hippocampus,[73] and cortex[73] as well as in peripheral blood vessels, where it is involved in smooth muscle relaxation,[66] and in the circular smooth muscle of the human colon.[66] However, no effects on muscle relaxation have been described in this tissue.[66]

Unfortunately, limited information about the 5-HT$_7$ receptor is available, again, due to the lack of selective agonists specific for this receptor, since known agonists of 5-HT$_7$ also show high agonistic activity against the 5-HT$_{1A}$ receptor.[74] However, 5-HT$_7$ receptor may play an important role in regulating sleep, circadian rhythms, and the overall mood of an individual as well as the regulation of body temperature.[41]

G$_i$α-Coupled Receptor Types

5-HT$_{1A}$. This was the very first serotonin receptor to be cloned and characterized. Because of this, a significant amount of research has been done on its physiological function and its role as a potential drug target for the treatment of anxiety,[41] schizophrenia,[41] Parkinson's disease[41] and addictions[41] among many others. It is also known that this receptor is involved in the control of appetite,[75] blood pressure,[75-77] cardiovascular function,[78] heart rate,[76, 77] impulsivity,[79] memory,[79-81] mood,[82] nausea,[8384] penile erection,[84] pupil dilation,[85] respiration[86], sexual behavior,[87] sleep,[88] sociability,[89] thermoregulation[90] and vasoconstriction.[91]

The 5-HT$_{1A}$ receptors can be found both, pre- and postsynaptically in the brain. At either location, the activation leads to neuronal hyperpolarization[41] which is an inhibition of the transmission of nerve impulses by the opening of potassium and/or chloride ion channels. This allows chloride ions to go in and potassium ions to go out, thus, increasing the membrane potential in a process called inhibitory postsynaptic potential (IPSP). By means of this process, any excitatory signal that reaches the neuron is counteracted.[38]

Postsynaptic 5-HT$_{1A}$ receptors are highly expressed in the hippocampus, septum and the entorhinal cortex.[41]

5-HT$_{1B}$. This receptor was initially thought to be absent in humans but present in rodents. However, cloning and sequencing experiments proved that it was also present in humans.[41]

Studies show that this receptor mediates serotonin-induced constriction in human cerebral arteries, functions as an autoreceptor to modulate serotonin release from axon

terminals, is a key regulator of aggression,[92] is involved in learning and memory processes,[93] plays a role in ejaculatory control[94] and is involved in the response to addictive drugs such as alcohol and cocaine.[95, 96][41] It is known that agonists to this receptor decrease alcohol consumption. However, cocaine effects may be augmented.

5-HT$_{1B}$ receptors are presynaptic and are localized on axon terminals.[41] Studies on rat brain show high levels of this receptor in the basal ganglia, in particular the globus palidus and substantia nigra.[41] Moderate amounts were found in superior colliculus, enteropeduncular nuclei, and periaqueductal grey. Low levels were found in the cerebral cortex, hypothalamus, amygdala, and dorsal horn of the spinal cord.[41]

5-HT$_{1D}$. This receptor was first cloned and characterized by Hamblin and Metcalf in 1991.[97] These researchers found that this receptor is most highly expressed in the basal ganglia and substantia nigra. The 5-HT$_{1D}$ receptor is of particular interest due to the fact that it is a target for antimigraine drugs such as sumatriptan (Figure 22). Although it is not clear whether migraines are primarily a vascular or a neurological dysfunction, it has been seen that plasma levels of serotonin decrease during migraine attacks.[41] Carotid vasodilatation has also been observed. On the other hand, it has also been noted that an intravenous infusion of serotonin can bring migraine to an end.[41] The 5-HT$_{1D}$ receptor has also been implicated in cerebral vasoconstriction as well as in neurogenic inflammatory response.[41]
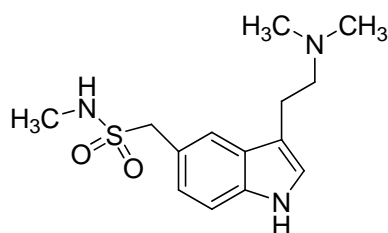
Sumatriptan

Figure 22. Chemical structure of sumatriptan[16]

5-HT$_{1E}$ This receptor has been found in some species such as humans and guinea pigs, but not in others like rats or mice.[41] The 5-HT$_{1E}$ receptor has been found in the hippocampus, but the highest densities were measured in subiculum and entorhinal cortex.[41]

Because of the areas of the brain where this receptor is found, it is thought that it may play an important role in cognition and memory processes.[41] Unfortunately, no specific ligands for the 5-HT$_{1E}$ receptor are currently known. For this reason no information is available about its role in normal brain physiology.[41]

5-HT$_{1F}$ This receptor became important in recent years as a target for newer antimigraine drugs with fewer side effects than sumatriptan. Such side effects include coronary vasoconstriction, among others.[41] The 5-HT$_{1F}$ receptor has been found in high levels in the globus pallidus, substantia nigra, cortex, putamen and hippocampus as well as neural and vascular tissues.[41] Research has shown that the 5-HT$_{1F}$ receptor plays an important role in cerebrovascular functions and dural inflammation.[41]

5-HT$_5$ Two isoforms of this receptor have been cloned and identified: 5-HT$_{5A}$ and 5-HT$_{5B}$. Of these, only 5-HT$_{5A}$ has been detected in humans, rats and mice, whereas the 5-HT$_{5B}$ isoform has only been found in rats and mice as well, but not in humans.[41] In

humans, the 5-HT$_{5A}$ isoform of this receptor has been found essentially in the CNS where high densities have been detected in the olfactory bulb, neocortex, and medial hebenula. Extensive distribution in these areas suggests that the 5-HT$_{5A}$ receptor is involved in higher cortical and limbic functions. In addition, the high expression in the cerebellum may be a sign of the importance of the role of the 5-HT$_{5A}$ receptor in mediating the effects of serotonin on cerebellar functions.[41]

Unfortunately, both the 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors have not been extensively studied, primarily due to the lack of selective ligands. Although some agonist ligands to these receptors such as LSD (see Figure 20) and 5-carboxamidotryptamine (5-CT) (see Figure 23) show very high affinity (5-CT has higher affinity than 5-HT itself) these ligands are nonspecific and bind to a wide variety of serotonin and other monoamine receptors. Such is the case of LSD.[41]
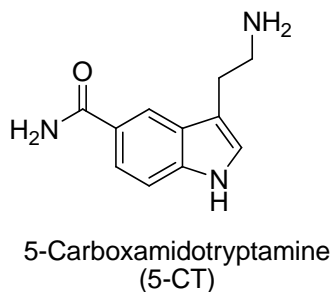

5-Carboxamidotryptamine
(5-CT)

Figure 23. Chemical structure of 5-carboxamidotryptamine (5-CT).[16]

Although more research is needed in order to increase our knowledge on the 5-HT$_{5A}$ receptor, brain localization and functional studies suggest the implication of this receptor in the control of cicardian rythms, mood, and cognitive behavior.[41]

Ligand-Gated Ion Channel

5-HT₃. Contrary to all other serotonin receptors which are G-protein coupled receptors (GPCRs), the $5\text{-}HT_3$ receptor is a cation-selective ion channel of the same superfamily of ligand-gated ion channels that includes the acetylcholine nicotinic receptor, the anion-selective $GABA_A$ receptor, and the glycine receptor.[41]

Although the $5\text{-}HT_3$ receptor can be found in the peripheral nervous system, the highest expression of this receptor is in the central nervous system, particularly in the spinal trigeminal nerve nucleus, area postrema, and solitary tract nucleus.[98, 99] All these areas are known to be critical for emesis (vomiting). Because of this, several antagonists to this receptor have been developed for the treatment of chemotherapy-induced emesis.[41] Expression of the $5\text{-}HT_3$ receptor in forebrain areas suggests that it may play an important role in cognitive processes as well.[41]

Serotonin Receptors and Cancer

Serotonin is a well known neurotransmitter, hormone and mitogenic (mitosis-inducing) factor that regulates a large number of physiological activities in different cells. Although its receptors have been implicated in the regulation of several psychiatric and neurological disorders, specific subtypes have recently been associated with tumor growth.[100]

Prostate cancer cell lines PC-3, DU-145, and LNCaP were used to test the effect of several serotonin receptor antagonists, of which pindobind (Figure 24), a $5\text{-}HT_{1A}$ antagonist, showed the most effectiveness as an antiproliferative agent towards these cell lines in vitro. In addition, radioligand studies show serotonin binding sites on each one

of the cell lines studied.[101] This supports the presence of serotonin receptors on prostate cancer cell lines and suggests a role of serotonin in prostate cancer cell growth.[101] Serotonin receptors 1A, 1B, 1D, 2A, 2B, and 2C were detected in human bladder cancer tissue cells (HT1376), and serotonin was observed to cause a dose-dependent proliferation in these cells. On the other hand, the 5-HT$_{1A}$ antagonist NAN-190 hydrobromide, and the 5-HT$_{1B}$ antagonist SB224289 hydrochloride (Figure 25), both showed dose-dependent inhibition on the growth of HT1376 cells. Other serotonin receptor subtype antagonists had no significant inhibitory effect on cell growth.[102]
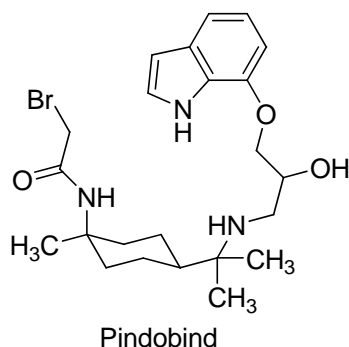

Pindobind

Figure 24. Chemical structure of pindobind.[16]
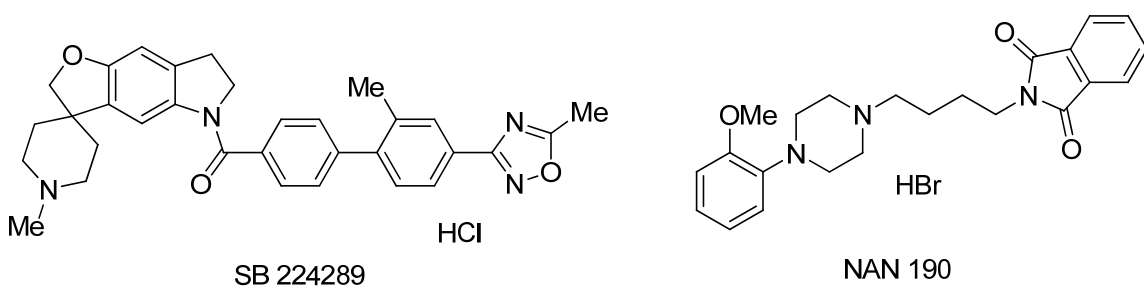

SB 224289

NAN 190

Figure 25. Chemical structure of SB 224289 and NAN 190[16]

As previously mentioned, the 5-HT$_{2A}$ receptor is not only found in the CNS, but is widely distributed in peripheral tissues where it controls a number of physiological

effects. In rats, it has been described to be coupled to several mitogenic signal transduction pathways.[100] For this reason, it has been considered as potentially oncogenic. In a study conducted by Sonier and coworkers, the 5-HT$_{2A}$ receptor (mRNA and protein) was found to be expressed in MCF-7 breast cancer cells. These data suggest that serotonin is a key factor in the proliferation of this cell line *via*, at least in part, the 5-HT$_{2A}$ receptor.[103]

As can be seen from the above information, certain types of cancer are potentially linked to specific serotonin receptors. The combination of this observation with other characteristics of cancer cells (hypoxia, slightly lower pH, non-mature cells, etc.) may open new avenues in the search for new and more effective treatments for cancer.

CHAPTER FOUR

Results and discussion

The synthesis of demethylcyproheptadine was initially performed via a McMurry coupling according to Scheme 2:
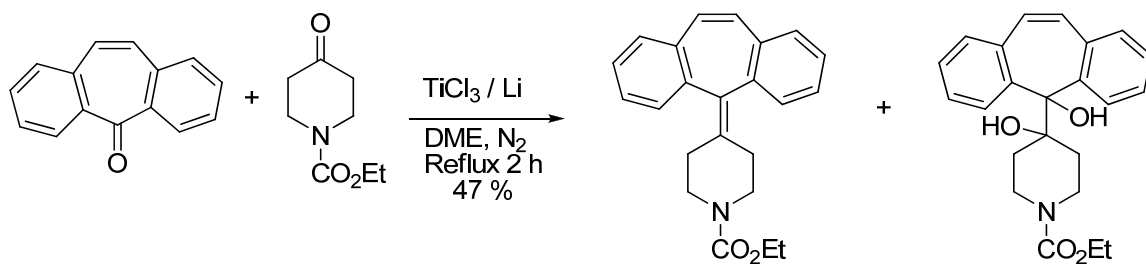
Scheme 2. McMurry coupling for the synthesis of the cyproheptadine moiety[114]

The accepted mechanism for this coupling is as follows:

Scheme 3. Mechanism of the McMurry coupling.[115]

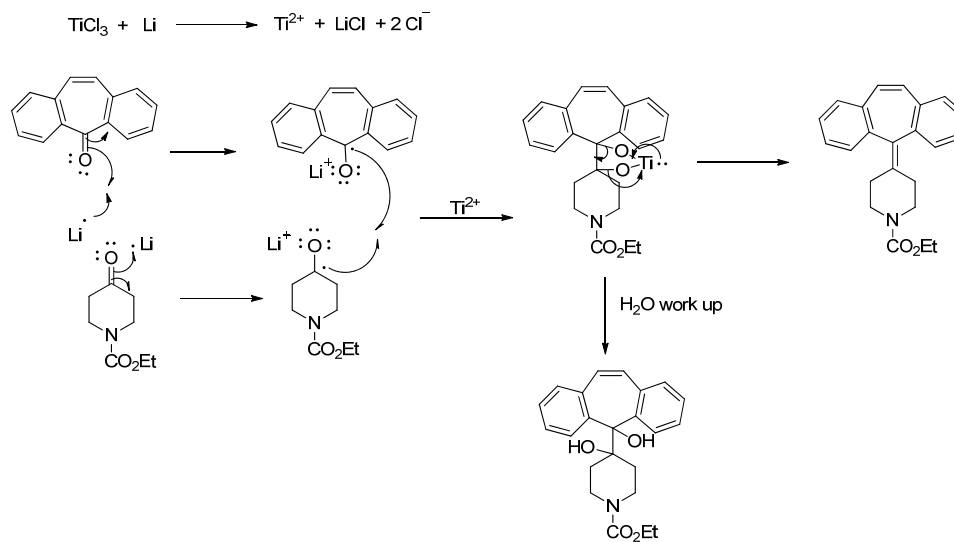The McMurrry coupling for the synthesis of the cyproheptadine moiety, although with an acceptable yield, represented a potential threat due to the use of the unstable titanium (III) chloride (fumes are released and it turns black on contact with air) as well as the use of metallic lithium.

These factors led us to review the literature where it was found that cyproheptadine is commercially available as the hydrochloride sesquihydrate salt. In order to obtain a moiety that could be coupled to another moiety with activity against the SERT, the N-methyl group had to be removed. This was achieved by forming the ethyl carbamate by reacting cyproheptadine with ethyl chloroformate. Carbamates are well known protective groups for amines. Therefore, they can be easily removed. In our case, the ethyl carbamate was hydrolyzed with potassium hydroxide:
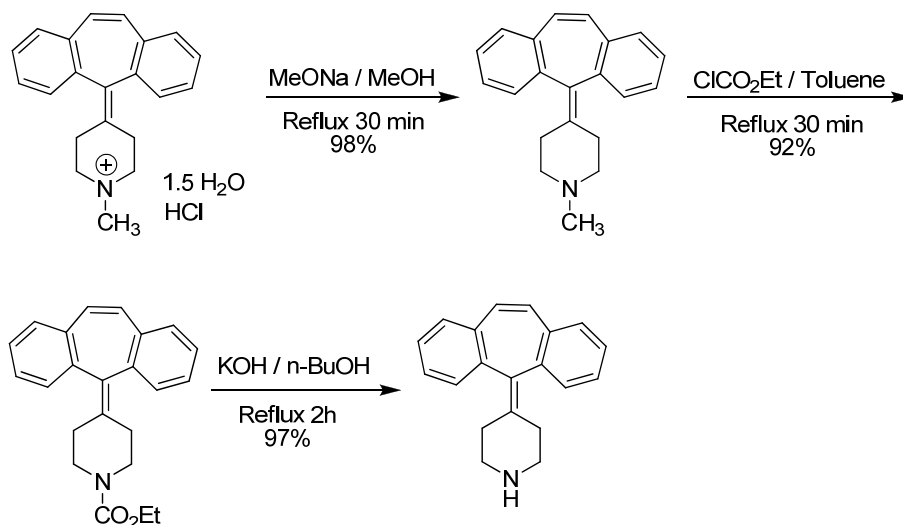
Scheme 4. Demethylation of cyproheptadine.[116]

Once the free amine was obtained, the next step was the synthesis of fluoxetine derivatives bearing the cyproheptadine moiety:

44
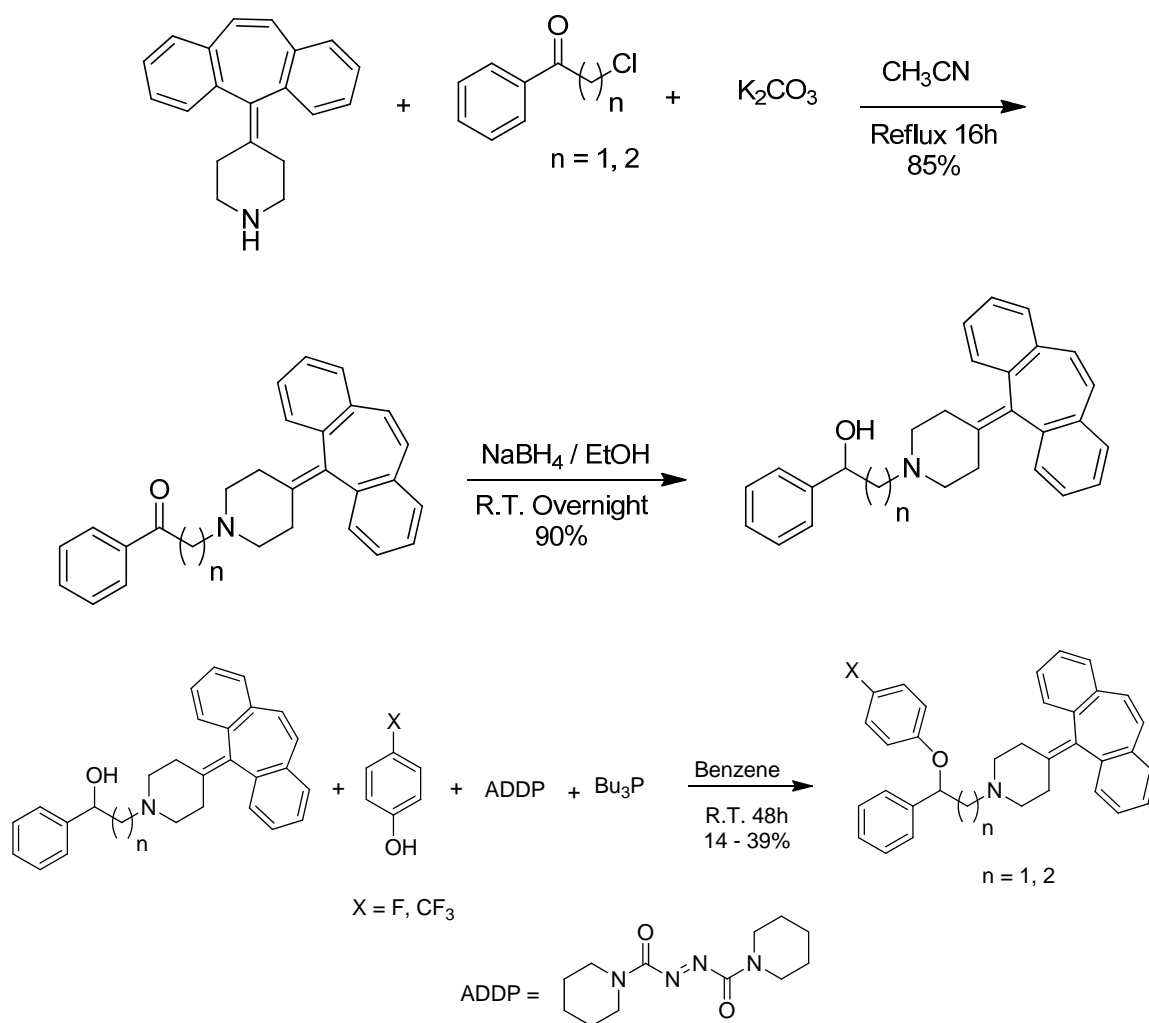
Scheme 5. Synthesis of fluoxetine derivatives coupled with the cyproheptadine moiety.

A possible mechanism for the formation of the ethyl carbamate is shown in Scheme 6:
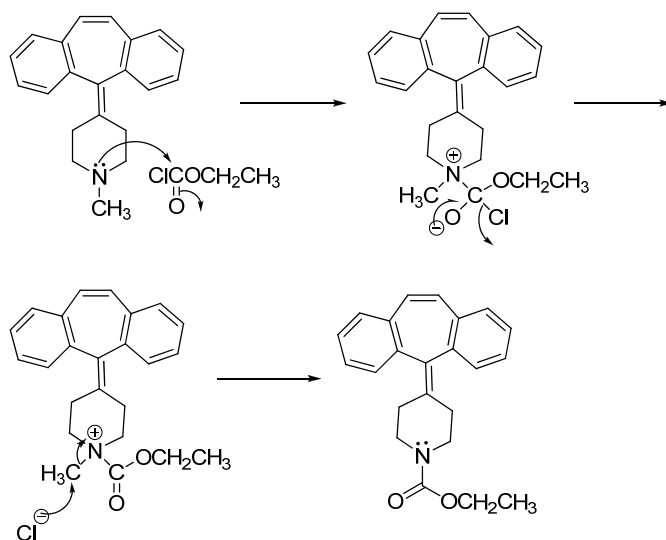
Scheme 6. Possible mechanism for the demethylation of cyproheptadine

The final step involved the removal of the carbamate protective group by means of an alkaline hydrolysis with potassium hydroxide. A possible mechanism is:
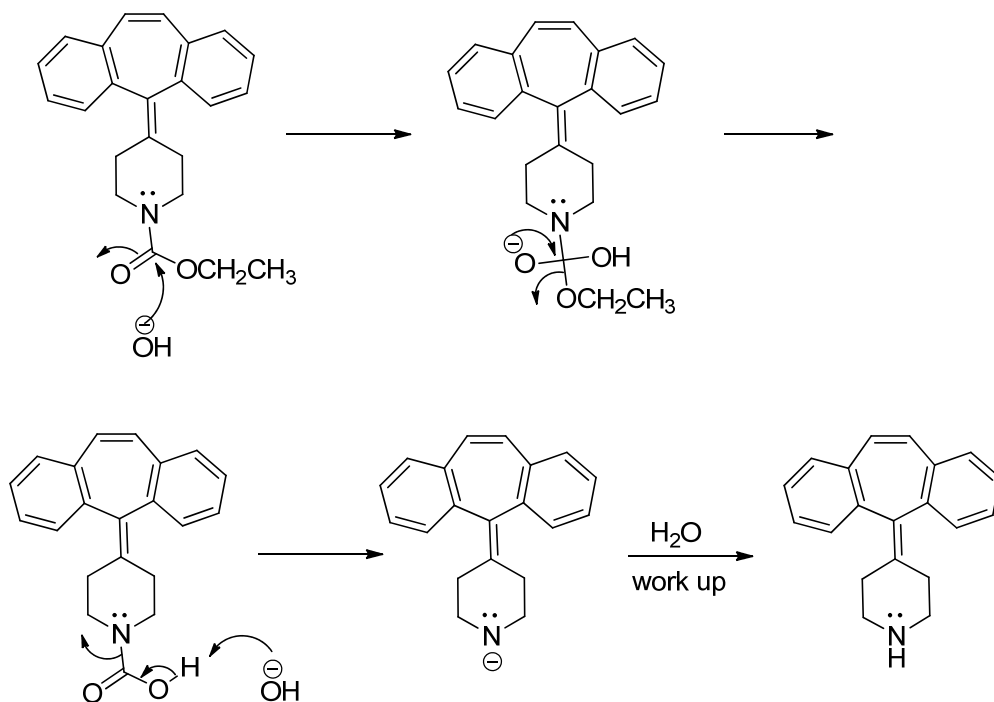
Scheme 7. Possible mechanism of the alkaline hydrolysis of ethyl carbamate.

Desmethylcyproheptadine was reacted with 2-chloroacetophenone and 3-chloropropiophenone to yield the respective aminoketones. These aminoketones were subsequently reduced with sodium borohydride to afford a racemic mixture of the corresponding alcohols. The accepted mechanism for the reduction with sodium borohydride is:
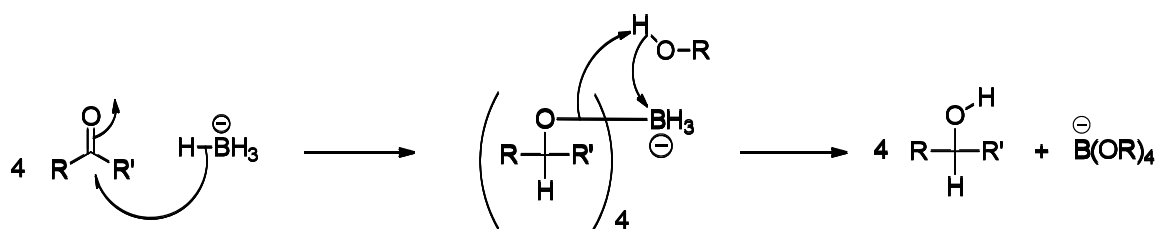
Scheme 8. Mechanism of a carbonyl reduction by sodium borohydride[117]

Another compound of interest in this research project is the demethylated analog of mirtazapine. (Figure 26)
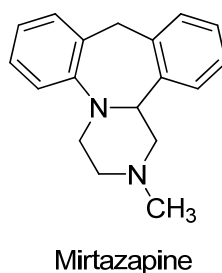
Mirtazapine

Figure 26. Chemical structure of mirtazapine.[16]

The proposed synthetic route for this analog is depicted in scheme 9. Due to the failure of this synthetic route at the reduction step with sodium borohydride, other synthetic routes were attempted to synthesize this analog; however, none of them were successful. These attempts are shown in scheme 10.
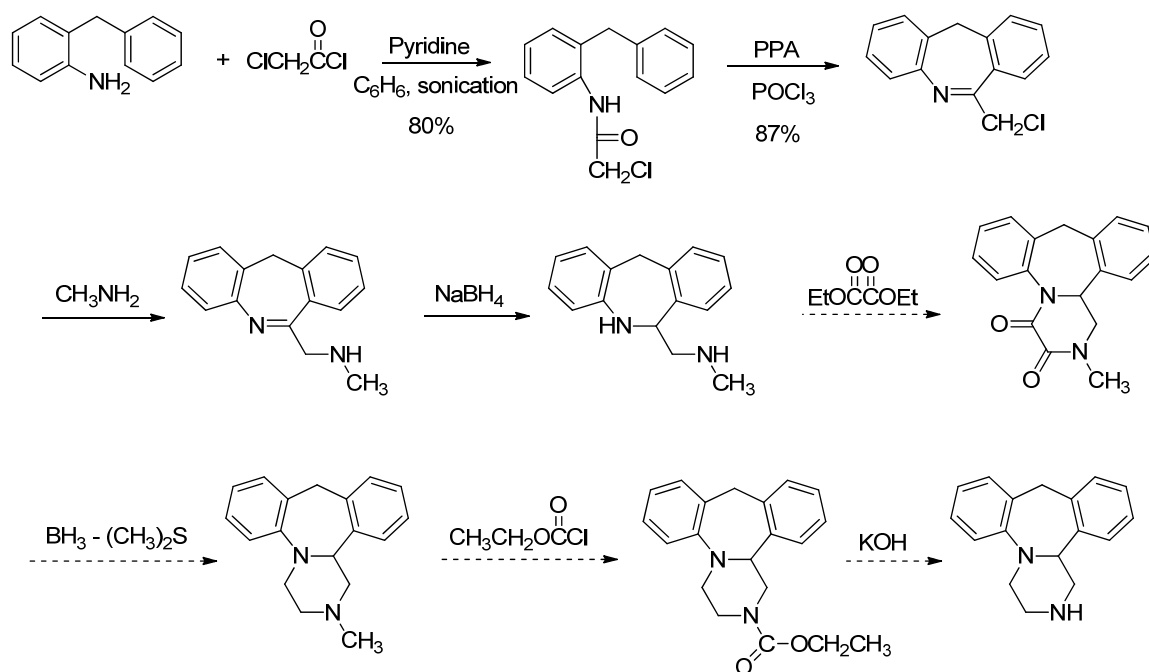
Scheme 9. Proposed synthesis of mirtazapamine.[118]
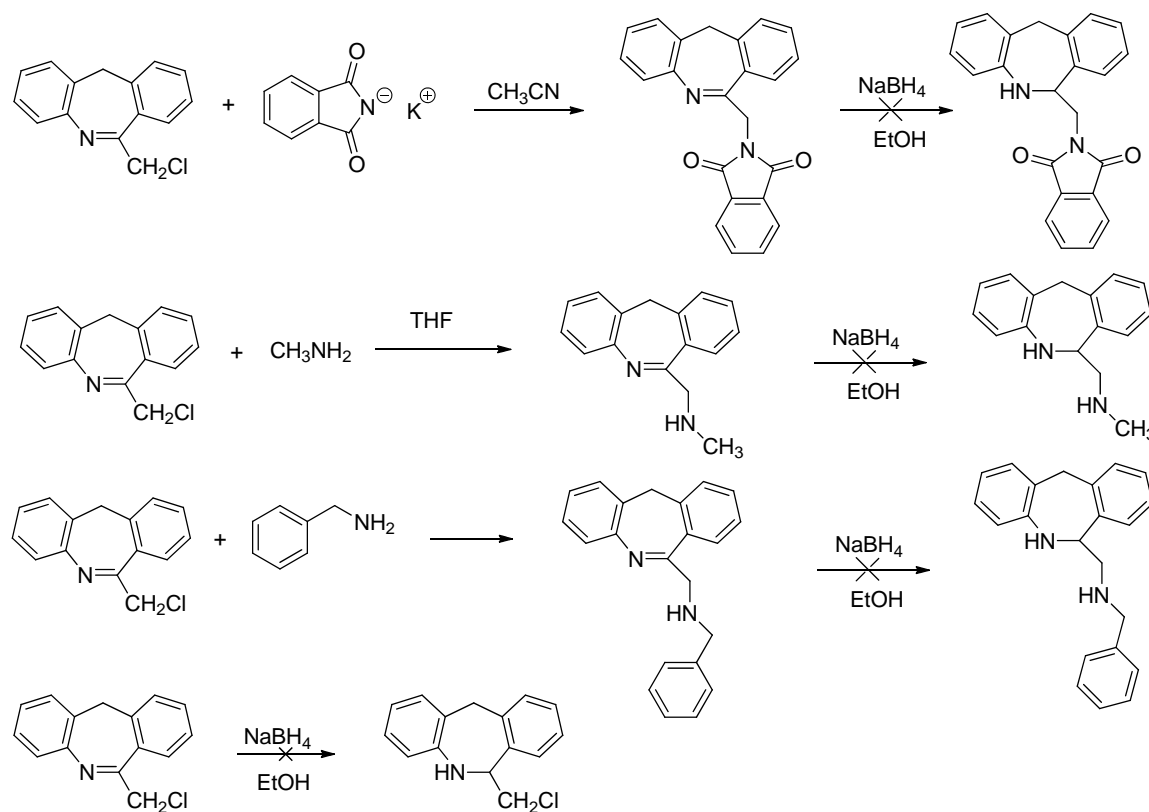
Scheme 10. Other attempted synhetic routes for the synthesis of mirtazapine.

Another attempted synthetic route was through a Beckmann rearrangement as shown in scheme 11:
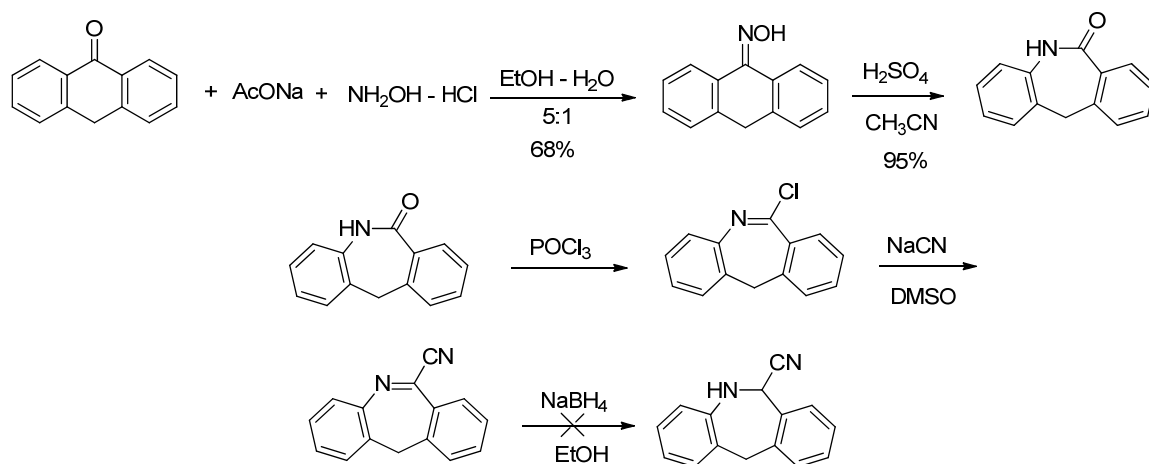
Scheme 11. Attempts to synthesize mirtazapine via a Beckmann rearrangement.

The accepted mechanism for the Beckmann rearrangement is depicted in scheme 12:[2]
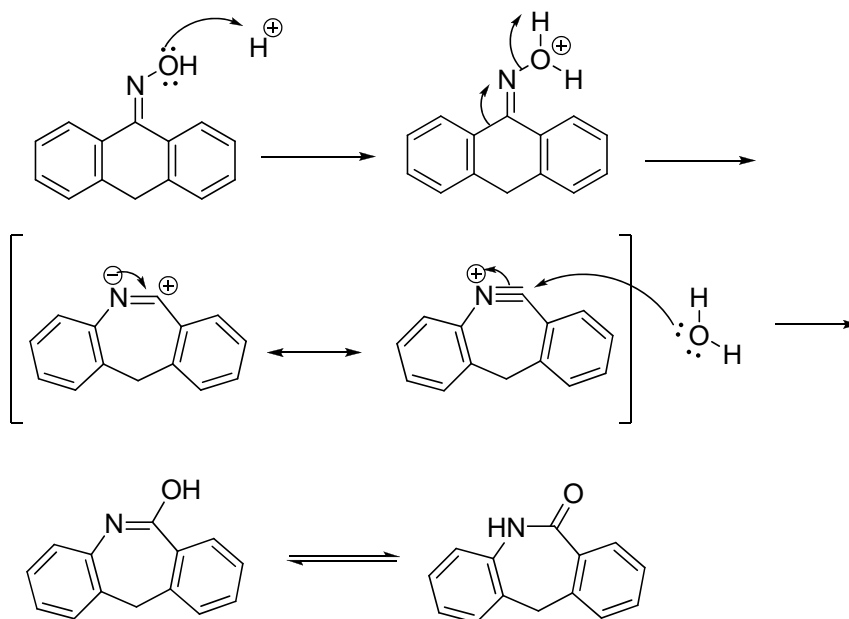
Scheme 12. Mechanism of the Beckmann rearrangement.[115]

Literature reports state that the imine intermediates right before reduction with sodium borohydride are very unstable and have to be reduced immediately after their formation.[104] Although several attempts were made with each one of the methodologies shown, all of these were unsuccessful.

The compound 4-[(3-fluorophenoxy)phenylmethyl)piperidine was reported to be more active than fluoxetine itself against the SERT, but also, it showed important activity against the NET[21] (see chapter 3). The synthesis of this compound was as depicted in scheme 13.:

In method A, a Mitsunobu coupling worked very well with old ADDP. However, the reaction never worked with new ADDP despite several attempts using different reaction conditions such as sonication and DIAD instead of ADDP. Method B was an alternative to this problem with satisfactory results.

Scheme 13. Synthesis of 4-[(3-fluorophenoxy)phenylmethyl)piperidine[22]

The accepted mechanism to the Mitsunobu coupling using ADDP is depicted in scheme 14:
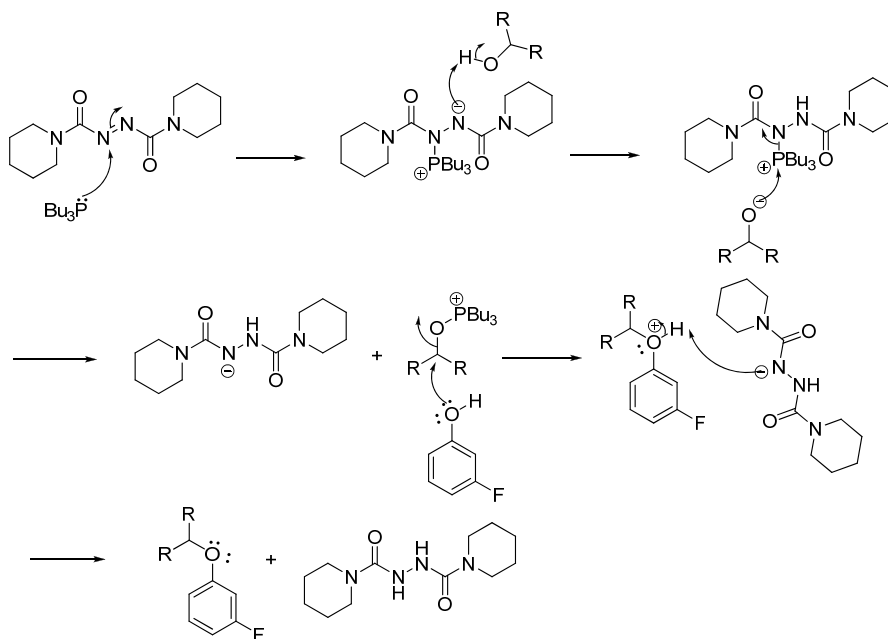
Scheme 14. Mechanism of the Mitsunobu coupling.[115]

The compound 4-[(3-fluorophenoxy)phenylmethyl]piperidine was treated with 1,1'-carbonydiimidazol (CDI) in an attempt to obtain an intermediate that would be stable enough to be stored in order to be used later in the formation of ureas [105] (Scheme 15). However, this reaction did not work for our case:
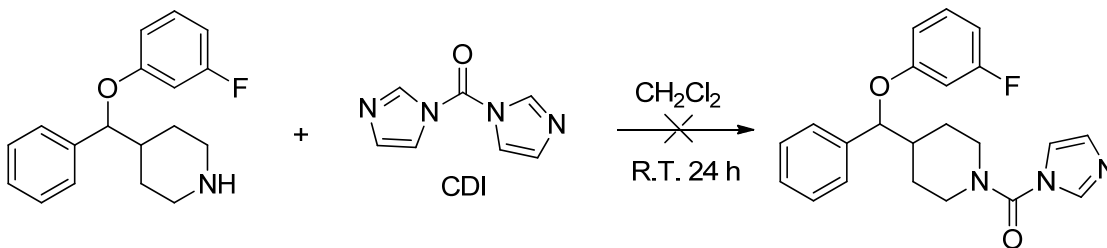
Scheme 15. Formation of a stable intermediate for the facile synthesis of ureas.

The chosen alternative route for this process was the use of triphosgene. The general reaction for this process is shown in scheme 16.
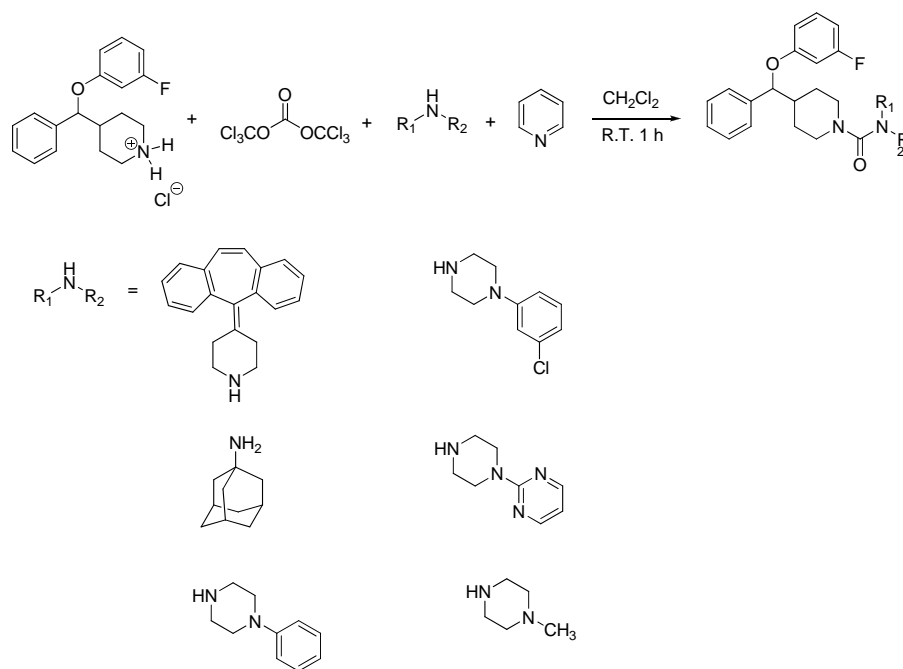
Scheme 16. Synthesis of unsymmetrical ureas using triphosgene.[125]

The proposed synthetic route of unsymmetrical ureas did not work for any of the proposed chemical structures depicted in scheme 16 except for 1-adamantaneamine. The accepted mechanism for reactions with triphosgene is shown in scheme 17.

As already stated in Chapter one, the compounds prepared by chemical synthesis are intended to possess activity against the SERT, the NET, and the 5-HT$_{2A}$ receptor. For this reason, these proteins were chosen to test the compounds.

The protocol designed by Dr. Bryan Roth (Psychoactive Drug Screening Program, University of North Carolina at Chapel Hill)[122] consists of a radioligand assay for all three proteins.

Basically, cells expressing the recombinant proteins of interest are treated with different concentrations of the compounds to be tested as well as with a reference compound. After a certain period of time, the cells are washed and treated with different concentrations of radioligand. After another period of time, the cells are rinsed and radioactivity is measured. The higher the radioactivity, the less amount of tested compound bound to the proteins of interest.
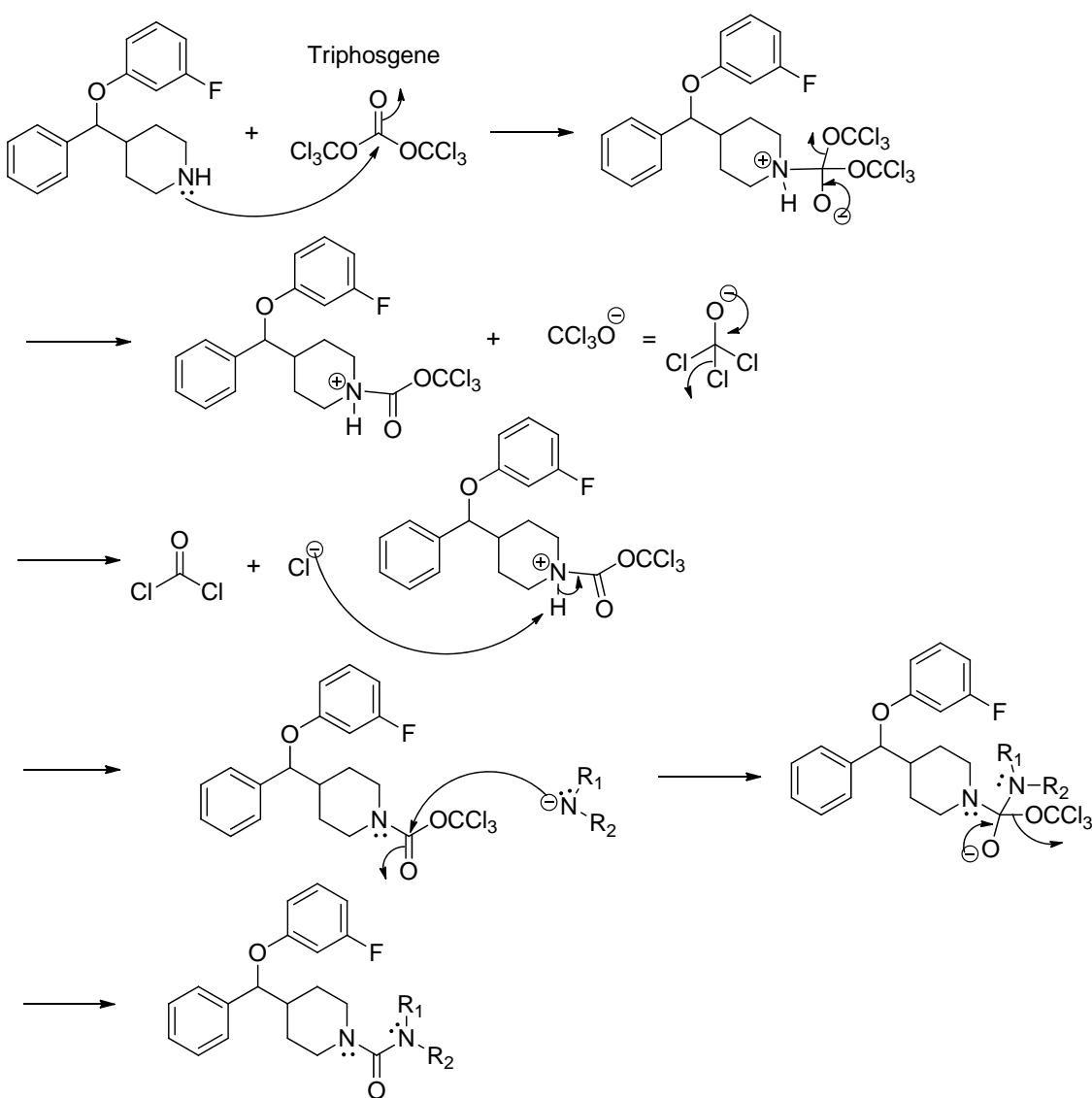
Scheme 17. Mechanism of formation of unsymmetrical ureas using triphosgene[120]

CHAPTER FIVE

Materials and Methods

All chemicals used for chemical synthesis as well as solvents used for flash chromatography were obtained from commercial sources including Sigma Aldrich, Acros Chemicals and Alfa Aesar and were used without any further purification. Reactions involving anhydrous conditions were performed in oven-dried glassware at least overnight. Reactions were monitored by thin layer chromatography using Merck Kieselgel 60 F_{254} glass backed plates. The plates were visualized using a multiband 254/365 UV lamp Spectroline Model ENF-240C. Flash chromatography was performed on an Isolera Four® equipment from Biotage with pre-packed cartridges.

NMR spectra were recorded in a Bruker DPX-300 spectrometer operating at 300 MHz for proton, 75 MHz for carbon and 282 MHz for fluorine and Varian AS 500 spectrometer operating at 500 MHz for proton, 125 MHz for carbon and 470 MHz for fluorine. The NMR spectra were recorded using $CDCl_3$ (0.03% TMS as reference) or DMSO-d_6 with no TMS. Chemical shifts are expressed in ppm (δ). NMR patterns are reported as singlets (s), doublets (d), triplets (t), quartets (q), and combinations of these as well as multiplets (m). Processing of NMR spectra was performed using MestReNova 6.1.1 software (Mestrelab Research Co).

4-Dibenzo[a,d]cyclohepten-5-ylidene-piperidine-1-carboxylic acid ethyl ester (McMurry coupling)[106] To a slurry of titanium (III) chloride (2.87 g, 18.6 mmol) in dry DME (30 mL) under nitrogen atmosphere was added lithium pieces (0.45 mg, 65 mmol). The mixture was refluxed for two hours and then allowed to return to room temperature. A mixture of suberone (0.48 g, 2.32 mmol) and N-carbethoxy-4-piperidone (0.40 g, 2.32 mmol) in dry DME (5 mL) was added and the reaction mixture was stirred at room temperature for four hours and then refluxed for 16 hours. After allowing to return to room temperature, the reaction mixture was diluted with hexanes (30 mL) and filtered through a pad of florisil. The organic layer was washed with a saturated aqueous solution of potassium carbonate. The aqueous layer was extracted with methylene chloride, dried over magnesium sulfate, and the organic solvent was evaporated under reduced pressure and the product was purified by flash chromatography using a mixture of hexanes and ethyl acetate (7:3). The product was obtained as a white solid in 47 % yield.

4-Dibenzo[a,d]cyclohepten-5-ylidene-piperidine-1-carboxylic acid ethyl ester[107] To a well-stirred solution of sodium methoxide in methanol (4.75 mL of 25% - 30% solution w/w) in dry methanol (14 mL) was added cyproheptadine hydrochloride sesquihydrate (2.50 g, 7.10 mmol). The mixture was heated at reflux for 30 minutes, and then allowed to return room temperature. The solvent was evaporated and the residue was extracted with diethyl ether and dried over magnesium sulfate. After filtration, the diethyl ether was reduced under reduced pressure and the extract was dissolved in dry toluene (11 mL). Ethyl chloroformate (12 mL, 42.7 mmol) was added dropwise and the reaction

mixture was subjected to ultrasound for 50 minutes, The mixture was washed with hydrochloric acid (10%), dried over magnesium sulfate and the solvent was removed under reduced pressure. The product was obtained as a white solid in 92% yield.

^{1}H-NMR (300 MHz, CDCl$_3$): δ (ppm): 7.28 (m, 10H), 6.91 (s, 2H), 4.11 (q, J = 7.0 Hz, 2H), 3.61 (m, 2H), 3.08 (ddd, J = 14.5, 8.9, 3.9 Hz, 2H), 2.19 (m, 4H), 1.23 (t, J = 7.1 Hz, 3H).

4-(5H-Dibenzo[a,d]cyclohepten-5-ylidene)piperidine (Desmethylcyproheptadine)[107] A mixture of 4-dibenzo[a,d]cyclohepten-5-ylidene-piperidine-1-carboxylic acid ethyl ester (5.48 g, 15.88 mmol) and potassium hydroxide (2.67 g, 47.64 mmol) were dissolved in n-butanol (20 mL). The mixture was heated at reflux for two hours and allowed to return to room temperature after which, the n-butanol was distilled under reduced pressure. The reaction mixture was diluted with water and extracted with toluene. The organic layer was washed with water, dried over sodium sulfate and, after filtration, the solvent was removed under reduced pressure. The product was obtained as a white solid in 97% yield. ^{1}H-NMR (300 MHz, CDCl$_3$): δ (ppm): 7.25 (m, 9H), 6.91 (s, 2H), 2.87 (ddd, J = 16.0, 11.2, 5.8 Hz, 2H), 2.65 (ddd, J = 11.9, 8.3, 3.6 Hz, 2H), 2.27 (ddd, J = 13.3, 8.2, 4.2 Hz, 2H), 2.07 (ddd, J = 13.4, 6.1, 3.7 Hz, 2H), 1.45 (br. s, 1H).

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-propanone

A mixture of desmethyl cyproheptadine (1.62 g, 5.94 mmol) and potassium carbonate (0.96 g, 6.95 mmol) in dry acetonitrile (325 mL) was stirred at room temperature under nitrogen atmosphere. After stirring for 15 minutes, 3-chloropropiophenone (1.00 g, 5.93 mmol) was added and heated at reflux for 16 hours. The mixture was allowed to return to room temperature and the solvent was removed under reduced pressure. Water was added to the reaction mixture and extracted with dichloromethane and dried over sodium sulfate. After filtration, the solvent was removeded under reduced pressure. The product was obtained as a white solid in 94% yield.

^{1}H-NMR (500 MHz, DMSO): δ (ppm): 7.94 (d, J = 7.5 Hz, 2H), 7.40 (m, 7H), 7.22 (m, 4H), 6.91 (s, 2H), 3.15 (t, J = 8.0 Hz, 2H), 2.79 (t, J = 8.0 Hz, 2H), 2.62 (m, 2H), 2.35 (m, 2H), 2.19 (m, 4H).

^{13}C-NMR (125 MHz, DMSO): δ (ppm): 199.49, 139.32, 137.17, 135.73, 135.00, 135.59, 133.28, 131.21, 128.18, 128.70, 128.41, 128.25, 127.96, 126.46, 55.47, 53.25, 36.69, 30.33.

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-propanol

A   well-stirred   solution   of   3-(4-dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-propanone (1.67 g, 4.10 mmol) in dry methanol (12 mL) at 0 °C under nitrogen atmosphere, was added sodium borohydride (0.44 g, 11.6 mmol) in portions over 30 minutes. After bubbling ceases, the reaction mixture was stirred at room

temperature overnight. Then the solvent was evaporated under reduced pressure and the reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate. After filtration, the solvent was evaporated under reduced pressure. The product was obtained in 93% yield.

^{1}H-NMR (500 MHz, CDCl$_3$): δ (ppm): 7.33 (m, 8H), 7.21 (m, 5H), 6.91 (s, 2H), 4.92 (dd, J = 7.5, 4.0 Hz, 1H), 2.76 (dt, J = 11.0, 4.0 Hz, 1H), 2.62 (m, 2H), 2.48 (m, 1H), 2.38 (m, 2H), 2.22 (m, 3H), 2.22 (m, 3H), 2.05 (t, J = 9.0 Hz, 1H), 1.80 (m, 1H).

^{13}C-NMR (125 MHz, CDCl$_3$): δ (ppm): 145.23, 139.21, 139.19, 135.14, 134.98, 134.97, 134.03, 131.20, 128.66, 128.65, 128.44, 128.40, 128.04, 128.03, 127.04, 126.56, 125.75, 75.82, 57.19, 55.75, 55.03, 34.10, 30.35, 30.26

4-Dibenzo[a,d]-5-cycloheptenylidene-1-[3-(4-fluorophenoxy)-3-phenylpropyl] piperidine

To a well-stirred solution of 3-(4-dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-propanol (1.22 g, 3.00 mmol) in anhydrous benzene (100 mL) under nitrogen atmosphere at room temperature was added 4-fluorophenol (0.37 g, 3.30 mmol) and ADDP (0.83 g, 3.30 mmol). When complete dissolution was achieved, tributyl phosphine (0.83 mL, 3.30 mmol) was added dropwise. After stirring for 48 hours at room temperature, 100 mL of hexanes was added and the salts of PBu$_3$O were filtered. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography (hexanes: ethyl acetate, 7:3). The product was obtained in as a brown solid (0.21 g, 0.42 mmol, 14% yield).

^{1}H-NMR (300 MHz, CDCl$_3$): δ (ppm): 7.28 (m, 17H), 6.91 (s, 2H), 4.92 (dd, *J* = 6.8, 4.8 Hz, 1H), 2.77 (dt, *J* = 10.8, 4.9 Hz, 1H), 2.65 (m, 2H), 2.43 (m, 3H), 2.23 (m, 3H), 1.82 (dt, *J* = 4.8, 2.8 Hz, 2H), 1.70 (m, 1H).

^{13}C-NMR (125 MHz, CDCl$_3$): δ (ppm): 160.61, 145.16, 139.13 (d, *J* = 6.0 Hz), 135.06, 134.98 (d, *J* = 11.5 Hz), 133.97, 131.14, 128.58, 128.35 (d, *J* = 15.0 Hz), 127.97, 127.00, 126.49, 125.68, 75.68, 57.06, 55.65, 54.94, 45.90, 44.75, 34.04, 30.26, 26.03, 25.54, 24.25.

^{19}F-NMR (282 MHz, CDCl$_3$): δ (ppm): -123.89 (s).

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-trifluoromethylphenoxy) propyl]piperidine

To a well-stirred solution of 3-(4-dibenzo[a,d]-cyclohepten-5-ylidene-1-piperidinyl)-1-phenyl-1-propanol (1.00 g, 2.40 mmol) in anhydrous toluene (100 mL) under nitrogen atmosphere at room temperature, was added α,α,α-trifluoro-p-cresol (0.44 g, 2.70 mmol) and ADDP (0.68 g, 2.70 mmol).   After complete dissolution, tributylphosphine (0.67 mL, 2.70 mmol) was added dropwise. The mixture was stirred for 48 hours at room temperature after which, hexanes (100 mL) was added and the mixture was filtered to remove the tributylphosphine oxide. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography with hexanes - ethyl acetate (7:3) and was obtained as a brown solid (0.29 g, 0.54 mmol, 22% yield).

^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 7.33 (m, 8H), 7.23 (m, 6H), 6.98 (br. s, 1H), 6.91 (s, 2H), 4.92 (dd, *J* = 11.0, 7.5 Hz, 1H), 2.76, (dt, *J* = 16.0, 9.0 Hz, 1H), 2.63,

(m, 2H), 2.43 (m, 2H), 2.65 (m, 2H), 2.20 (m, 2H), 2.06 (td, J = 16.0, 5.0 Hz, 1H), 1.81 (dt, J = 9.5, 8.0 Hz, 2H)

^{13}C NMR (125 MHz, CDCl$_3$) δ (ppm): 160.80, 141.19, 139.24, 135.90, 134.89, 133.38, 131.10, 128.78, 128.60, 128.30, 127.86, 127.85, 127.77, 126.79 (q, J = 248.3 Hz), 126.34, 125.95, 125.61, 122.87 115.87, 78.86, 54.36, 36.18, 30.30.

^{19}F NMR (282 MHz, CDCl$_3$) δ (ppm): -61.29.

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-ethanone

To a well-stirred solution of desmethylcyproheptadine (3.72 g, 13.6 mmol) in dry acetonitrile (40 mL) under nitrogen atmosphere, was added anhydrous potassium carbonate (2.63 g, 19.0 mmol). After stirring for 15 minutes at room temperature, 2-chloroacetophenone was added and the mixture was heated at reflux for 16 hours, allowed to return to room temperature and quenched with water. A liquid-liquid extraction was performed with methylene chloride and the organic layer was washed with brine, dried over magnesium sulfate and, after filtration, the methylene chloride was evaporated under reduced pressure obtaining a white solid in 85% yield. The ^{1}H NMR of the crude product shows a singlet at 3.77 ppm.

2-(4-dibenzo[a,d]cyclohepten-5-ylidene-1-piperidinyl)-1-phenyl ethanol

To a well-stirred solution of 2-(4-dibenzo[a,d]cyclohepten-5-ylidene-1-piperidinyl)-1-phenyl ethanone (4.54 g, 11.6 mmol) in dry ethanol (35 mL) at 0 ºC under nitrogen atmosphere, was added sodium borohydride (1.10 g, 29.0 mmol) in portions over 30 minutes. After bubbling ceases, the solution was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and water was added to

quench the reaction. The product was extracted with methylene chloride and the organic layer was dried over magnesium sulfate. After filtration, the methylene chloride was evaporated under reduced pressure and a white solid was obtained in 90 % yield.

^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 7.37(m, 8H), 7.29, (m, 4H), 6.96 (s, 2H), 4.73 (dd, *J* = 9.0, 4.8, 1H), 2.89 (dt, *J* = 10.5, 4.5 Hz, 1H), 2.45, (m, 6H), 2.24 (m, 3H).

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-trifluoromethylphenoxy)-2-phenyl ethyl] piperidine

To a well-stirred solution of 2-(4-dibenzo[a,d]cyclohepten-5-ylidene-1-piperidinyl)-1-phenyl ethanol (1.00 g, 2.50 mmol) in anhydrous toluene (100 mL) under nitrogen atmosphere at room temperature, was added α,α,α-trifluoro-p-cresol (0.89 g, 5.50 mmol) and ADDP (1.38 g, 5.50 mmol). After complete dissolution, tributylphosphine (1.10 mL, 5.50 mmol) was added dropwise. The mixture was stirred for 48 hours at room temperature, after which hexanes (100 mL) was added and the mixture was filtered to remove the tributylphosphine oxide. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography with hexanes - ethyl acetate (7:3) and was obtained as a brown solid (0.31 g, 0.58 mmol, 23% yield)

^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm): 7.29 (m, 16.0H), 6.92 (s, 2H), 4.69 (dd, *J* = 9.9, 4.2 Hz, 1H), 2.85 (dt, *J* = 10.5, 4.5 Hz, 1H), 2.42 (m, 6H), 2.20 (m, 3H)

^{13}C NMR (75 MHz, CDCl$_3$) δ (ppm): 142.42, 139.25, 135.39, 134.99, 133.94, 131.20, 128.63, 128.54, 128.44, 128.03, 128.01, 127.67, 126.53, 126.03, 115.72, 77.65, 77.22, 76.80, 69.06, 66.19, 55.81, 54.75, 30.45, 30.34.

^{19}F NMR (282 MHz, CDCl$_3$) δ (ppm): -61.39 (s).

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-fluorophenoxy)-2-phenyl ethyl]piperidine.

To a well-stirred solution of 2-(4-dibenzo[a,d]cyclohepten-5-ylidene-1-piperidinyl)-1-phenyl ethanol (1.00 g, 2.50 mmol) in anhydrous toluene (100 mL) under nitrogen atmosphere at room temperature, was added 4-fluorophenol (0.61 g, 5.50 mmol) and ADDP (1.38 g, 5.5 mmol). After complete dissolution, tributylphosphine (1.10 mL, 5.50 mmol) was added dropwise. The mixture was stirred for 48 hours at room temperature, after which hexanes (100 mL) was added and the mixture was filtered to remove the tributylphosphine oxide. The solvent was evaporated under reduced pressure and the product was purified by flash chromatography with hexanes - ethyl acetate (7:3) and obtained as a brown solid (0.48 g, 0.99 mmol, 39% yield).

^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 7.33 (m, 9H), 7.22 (m, 6H), 6.91 (s, 2H), 4.68 (dd, *J* = 10.5, 3.5 Hz, 1H), 2.83 (dt, *J* = 10.5, 5 Hz, 1H), 2.53 (m, 1H), 2.39 (m, 5H), 2.18 (m, 3H).

^{13}C NMR (125 MHz, CDCl$_3$) δ (ppm): 142.46, 139.27, 139.26, 135.43, 135.01, 133.94, 131.23, 131.23, 128.67, 128.65, 128.57, 128.47, 128.46, 128.06, 128.04, 127.68, 126.56, 126.05, 77.55, 77.29, 77.04, 69.08, 66.24, 55.83, 54.77, 30.47, 30.37.

^{19}F NMR (470 MHz, CDCl$_3$) δ (ppm): -125.85 (sept, *J* = 4.7 Hz)

1-Acetylpiperidine-4-carbonyl chloride

To a solution of phosphorous pentachloride (1.46 g, 7.00 mmol) in dry dichloromethane (20 mL) was added 1-acetylpiperidin-4-carboxylic acid (1.00 g, 5.80 mmol). After stirring for four hours at room temperature, the solid was filtered, washed with dry dichloromethane and dried under vacuum overnight. The product, a white solid, was obtained in 94 % yield.

1-(4-Benzoyl-1-piperidinyl)methanone

To a solution of aluminum chloride (3.90 g, 29.2 mmol) in dry benzene (17 mL) was added 1-acetylpiperidine-4-carbonyl chloride (1.04 g, 5.50 mmol) slowly. The mixture was heated at reflux for two hours, allowed to return to room temperature, and poured into crushed ice. After extracting with methylene chloride, it was washed with brine, dried over magnesium sulfate and filtered. The solvent was then removed under reduced pressure and the obtained solid was heated at reflux with 6N hydrochloric acid (40 mL), allowed to return to room temperature and extracted with diethyl ether. The aqueous layer was made basic with 10% NaOH and extracted with diethyl ether again. The organic layer was washed with brine and dried over magnesium sulfate and the solvent was evaporated under reduced pressure. The product was obtained in 40% yield overall.

^{1}H-NMR (300 MHz, CDCl$_3$): δ (ppm): 7.94 (dt, *J* = 6.3, 1.2 Hz, 2H), 7.53 (tt, *J* = 7.5, 1.2 Hz, 1H), 7.46 (tt, *J* = 6.3, 1.2 Hz, 2H), 3.39 (tt, *J* = 11.1, 3.6 Hz, 1H), 3.17 (dt,

J = 12.5, 3.3 Hz, 2H), 2.75 (dt, J = 12.0, 2.7 Hz, 2H), 2.02 (br. s, 1H), 1.84 (d, J = 12.5 Hz, 2H), 1.69 (dt, J = 12.0, 3.9 Hz, 2H).

^{13}C-NMR (75 MHz, CDCl$_3$): δ (ppm): 202.53, 135.93, 132.87, 128.63, 128.21, 46.06, 44.01, 29.70.

1- tert-butylcarboxylate-4-piperidinyl phenyl ketone

To a well-stirred solution of 4-piperidinyl phenyl ketone (4.30 g, 22.8 mmol) in water (23 mL) was added di-tert-butyl dicarbonate (6.00 g, 27.4 mmol). The reaction mixture was stirred at room temperature for 30 minutes and then extracted with methylene chloride. The organic layer was dried over magnesium sulfate, filtered, and the methylene chloride was evaporated under vacuum. The product was obtained as a white solid in 98% yield.

^{1}H-NMR (500 MHz, CDCl$_3$): δ (ppm): 7.94 (dd, J = 8.3, 1.0 Hz, 1H), 7.64 (m, 1H), 7.57 (tt, J = 8.3, 1.5 Hz, 1H), 4.16 (br. s, 2H), 3.41 (tt, J = 11.0, 3.5 Hz, 1H), 2.90 (br. s, 2H), 1.83 (m, 2H), 1.70 (m, 2H), 1.46 (s, 8H).

^{13}C NMR (126 MHz, CDCl$_3$) δ (ppm): 202.05, 154.69, 135.85, 133.09, 131.03, 129.30, 128.73, 128.23, 124.76, 85.15, 79.58, 77.30, 77.05, 76.80, 43.47, 28.43.

1-Phenyl-1-(4-piperidinyl-1-tert-butyl carboxylate) methanol

To a well-stirred solution of 1- tert-butylcarboxylate-4-piperidinyl phenyl ketone (6.55 g, 22.6 mmol) in dry ethanol (70 mL) at 0 °C under nitrogen atmosphere was added sodium borohydride (2.14 g, 56.6 mmol) in portions over 30 minutes. After bubbling ceases, the reaction mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and water was added to quench the reaction. Finally,

the product was extracted with methylene chloride, dried over magnesium sulfate, filtered, and evaporated under reduced pressure. The product was obtained as a solid in 87 % yield

^{1}H-NMR (500 MHz, CDCl$_3$): δ (ppm): 7.34 (m, 2H), 7.28 (m, 3H), 4.36 (dd, J = 7.5, 2.5 Hz, 1H), 4.13 (br. s, 1H), 4.04 (br. s, 1H), 2.65 (br. s, 1H), 2.56 (br. s, 1H), 2.08 (d, J = 3 Hz, 1H), 1.95 (dt, J = 13.0, 2.5 Hz, 1H), 1.73 (m, 1H), 1.43, (s, 9H), 1.26 (m, 2H), 1.14 (td, J = 12.5, 4.5 Hz, 1H)

^{13}C NMR (125 MHz, CDCl$_3$) δ (ppm): 154.78, 142.99, 128.37, 127.74, 126.55, 79.27, 78.54, 77.28, 77.03, 76.77, 43.47, 28.45.

4-[(3-fluorophenoxy)phenyl methyl]piperidine. Method A

1-Phenyl-1-(4-piperidinyl-1-tert-butyl carboxylate) methanol (6.00 g, 20.5 mmol) was dissolved in dry THF (100 mL) under nitrogen atmosphere. To this solution, 3-fluorophenol (1.90 mL, 20.5 mmol) and ADDP (5.20 g, 20.5 mmol) were added. Upon complete dissolution, tributyl phosphine (5.00 mL, 20.5 mmol) was added dropwise. After stirring at room temperature for 3 hours, the Bu$_3$PO salts were filtered and the solvent was removed under vacuum. The product was purified by flash chromatography on silica gel using a mixture of hexanes and ethyl acetate (7:3). The rf of the product was 0.58. Finally, the product was dissolved in methylene chloride (40 mL) and trifluoroacetic acid (2.00 mL) was added. The mixture was stirred for 15 hours at room temperature after which it was washed with 10 % NaOH and brine. The organic layer was dried over magnesium sulfate, filtered, and the solvent was removed under reduced pressure. The product was obtained as a brown oil in 39 % yield overall.

4-[(3-fluorophenoxy)phenyl methyl]piperidine. Method B

To a well-stirred suspension of sodium hydride (0.25 g, 10.4 mmol) in dry DMSO (12.0 mL) under nitrogen atmosphere, was added 1-phenyl-1-(4-piperidinyl-1-tert-butyl carboxylate) methanol (2.50 g, 8.60 mmol). After stirring at room temperature for 30 minutes, potassium benzoate (1.30 g, 8.00 mmol) was added. The reaction mixture was stirred at room temperature for another 30 minutes, after which 1,3-difluorobenzene (1.03 mL, 10.5 mmol) was added slowly keeping the temperature below 20 ºC by means of a water bath. The reaction mixture was stirred for 15 hours at 65 °C, after which it was allowed to return to room temperature, quenched with water and brine, and extracted with methylene chloride. The organic layer was dried with magnesium sulfate and, after filtration, the methylene chloride was removed under reduced pressure. The residue was dissolved in a 1:1 mixture of methanol and 10% aqueous hydrochloric acid and was heated at reflux for 1 hour. After allowing the reaction mixture to return to room temperature, the mixture of solvents was removed under reduced pressure and the residue was dissolved in water and extracted with methylene chloride. The organic layer was dried with magnesium sulfate, filtered, and methylene chloride was removed under reduced pressure. The product was obtained as the hydrochloride salt as a white, sticky solid in 79% yield overall.

^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 9.50 (br s, 2H), 7.31 (m, 5H), 7.08 (td, *J* = 8.3, 6.8 Hz, 1H), 6.55 (m, 3H), 4.80 (d, *J* = 7.5 Hz, 1H), 3.56 (d, *J* = 12.9 Hz, 1H), 3.46 (d, *J* = 12.8 Hz, 1H), 2.85 (td, *J* = 12.8, 3.0 Hz, 1H), 2.76 (td, *J* = 12.9, 3.1 Hz, 1H), 2.30 (d, *J* = 13.9 Hz, 1H), 1.99 (m, 1H), 1.86 (m, 2H), 1.49 (d, *J* = 13.9 Hz, 1H).

^{13}C NMR (126 MHz, CDCl$_3$) δ (ppm): 163.35 (d, J = 245.5 Hz), 159.09 (d, J = 10.9 Hz), 138.41, 130.09 (d, J = 10.0 Hz), 128.85, 128.41, 126.62, 111.54 (d, J = 2.8 Hz), 107.97 (d, J = 21.2 Hz), 103.72 (d, J = 24.8 Hz), 83.40, 77.28, 77.03, 76.77, 43.91, 43.75, 41.38, 25.35, 25.30.

^{19}F NMR (470 MHz, CDCl$_3$) δ(ppm): -111.56 (dt, J = 10.6, 7.9 Hz).

Synthesis of Ureas[108]

N-(1-adamantanyl)-4-[(3-fluorophenoxy)phenylmethyl]piperidine-1-methanone

To a solution of triphosgene (0.11 g, 0.37 mmol) in dry methylene chloride (2.0 mL) was added a solution of 4-[(3-fluorophenoxy)phenylmethyl]piperidine hydrochloride (0.32 g, 1.00 mmol) and pyridine (0.2 mL, 2.2 mmol) dissolved in dry methylene chloride (3.5 mL) over 30 – 40 minutes. The reaction mixture was stirred for 20 minutes, at room temperature.   Then, a solution of amine 2 (1.00 mmol) dissolved in dry methylene chloride (2.0 mL) was added in one portion. After stirring for an additional 20 minutes, the methylene chloride was removed under reduced pressure to dryness; the residue was dissolved in ethyl acetate and washed two times with an aqueous solution of potassium hydrogen sulfate (10%), sodium bicarbonate (5%), and brine.   The organic layer was dried over magnesium sulfate and, after filtering, the methylene chloride was removed under reduced pressure under reduced pressure.   The product was purified by flash chromatography using hexane – ethyl acetate (7:3) and was obtained as a white solid. (0.115 g, 0.25 mmol, 25% yield).

^{1}H NMR (500 MHz, CDCl$_3$): δ(ppm) 7.28 (m, 5H), 7.07 (m, 1H), 6.55 (m, 3H), 4.79 (d, J = 6.7 Hz, 1H), 4.21 (s, 1H), 3.91 (m, 2H), 2.69 (td, J = 12.8, 2.2 Hz, 1H), 2.61

(td, *J* = 12.8, 2.9 Hz, 1H), 2.08 (s, 3H), 1.98 (s, 7H), 1.66 (s, 6H), 1.36 (m, 3H), 1.24 (m, 1H).

^{13}C NMR (125 MHz, CDCl$_3$) δ (ppm): 163.39 (d, *J* = 245.0 Hz), 159.60 (d, *J* = 10.9 Hz), 139.21, 130.00 (d, *J* = 10.0 Hz), 128.54, 127.91, 126.65, 111.57 (d, *J* = 2.8 Hz), 107.53 (d, *J* = 21.3 Hz), 103.56 (d, *J* = 24.7 Hz), 84.16, 51.17, 44.16, 43.90, 43.27, 42.42, 36.50, 29.63, 28.21.

^{19}F NMR (470 MHz, CDCl$_3$) δ (ppm): -111.73 (dt, *J* = 10.7, 7.9 Hz).

CHAPTER SIX

Cancer

Definition. The term "cancer" makes reference to any one of the large number of diseases (more than 100) characterized by the uncontrolled division of abnormal cells that have the ability to infiltrate and destroy normal body tissue, as well as the ability to spread to other parts of the body through the blood and lymph systems (metastasis).[14, 109]

[3, 14]Leading health organizations such as the WHO, the NIH (through its National Cancer Institute) and the Mayo Clinic, set cancer as the second leading cause of death worldwide, only after heart disease.[3, 14, 109] However, survival rates have been raising for many types of cancer thanks to improvements in screening and treatment.[109]

Most cancers are named after the organ or type of cell where they start. In this way, cancer that begins in the lungs is called lung cancer; cancer that begins in basal cells, of the skin, is called basal cell carcinoma.[109] As can be seen from these examples, the types of cancer are as many as the different types of organs or cells are in the body.

Symptoms[14]

As can be expected, the symptoms will vary depending on the tissue being affected. However, some general symptoms associated with, but not specific, to cancer include: fatigue, fever, lump thickening that can be felt under the skin, pain, weight changes, including unintended loss or gain, skin changes, such as yellowing, darkening or redness of the skin, sores that will not heal, or changes to existing moles, changes in

bowel or bladder habits, persistent cough, difficulty swallowing, hoarseness, and persistent indigestion or discomfort after eating, among others.

Causes[14, 109]

The cell is the body's basic unit of life. The DNA within these cells contains a series of instructions that control the growth and division of the cells as needed to keep the body healthy. The DNA in normal cells often gets damaged; however, most of these damages (mutations) can be repaired. Cells that have irreparable mutations or just become old die (a process called apoptosis) and are replaced by new cells.

However, sometimes when these mutations are not repaired they can affect the normal cell growth and division causing cells to live beyond their normal cell life span and forming new cells when the body does not need them. These extra cells form a mass of tissue that receives the name of tumor. (See figure 27).
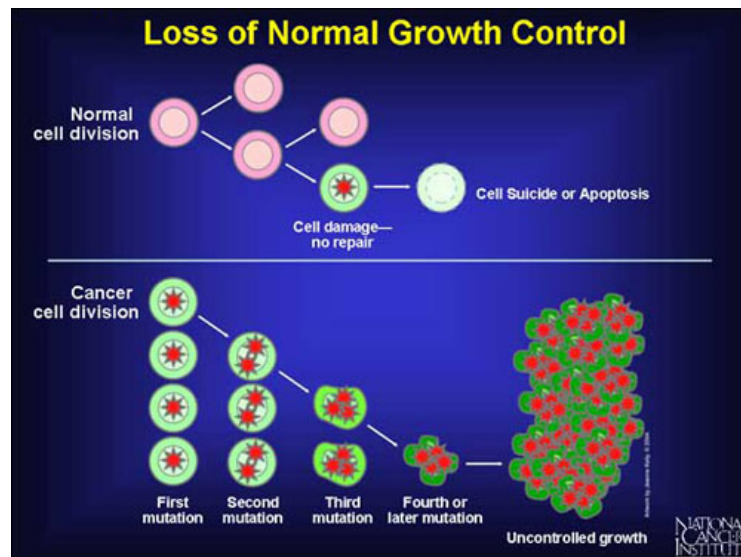
Figure 27. Normal and abnormal cell growth.[109] Artwork originally created for the National Cancer Institute. Reprinted with permission of the artist, Jeanne Kelly. Copyright 2009.

Tumors can be divided into benign or malignant. Benign tumors are not cancerous, can often be removed, usually they do not come back, and its cells do not show metastasis.[109] On the other hand, malignant tumors are cancerous. Its cells can invade vicinal tissue and spread to other parts of the body.[109] It is important to note that although the formation of a tumor is a characteristic of most types of cancer, not all types of cancer show this particularity. Leukemia – cancer of the blood, bone marrow and spleen – is an example of cancer that does not form tumors.[14, 109]

The initial cell damage shown in Figure **28** is just the beginning of the process by which cancer develops. Research suggests that a number of changes are needed within a cell in order to develop cancer. These include:[109]

- *An initiator* that causes the initial genetic mutation. This initial genetic mutation can be caused by internal factors in the human body such as hormones, viruses and chronic inflammation or an inherited genetic mutation. Also, there may exist external factors that influence these mutations such as UV light from the sun or carcinogen chemicals in the environment.

- *A promoter* that causes a rapid cell growth. This could lead to an accumulation of cells, like in a tumor.

- *A progressor* that causes cancer to become more aggressive and spread to surrounding tissue and other parts of the body. Without this progressor, the tumor would remain benign and, therefore, localized.

The individual's genetic information, internal factors in his/her body, lifestyle and environment all contribute to the development of cancer or help complete the process once it has started.[14]

<p align="center">*Risk Factors* [14, 109]</p>

- *Age.* Cancer can be diagnosed at any age. However, it can take decades to develop. When a tumor is detected, it already has 100 million to 1 billion cancer cells, and the original mutation may have originated 5 or 10 or more years ago.

- *Tobacco use.* Cigarette smoking has been linked to cancers of the lung, oral cavity, esophagus, bladder, kidney, pancreas, stomach, cervix, and acute myelogenous leukemia. The National Cancer Institute estimates that 30% of all cancer deaths in the United States are due to cigarette smoking.

- *Infections.* Oncogenic strains of the human papilloma virus have been linked with cancers in the cervix, penis, vagina, anus, and oropharynx. Hepatitis B and hepatitis C viruses have been linked to liver cancer, Epstein-Barr virus has been found to produce Burkitt lymphoma, and Helicobacter pylori has been linked to gastric cancer.

- *Radiation.* Exposure to UV and ionizing radiation are well established causes of cancer. Solar UV radiation is the main cause of non-melanoma skin cancers, which are the most common malignancies in human populations. Ionizing radiation has enough energy to remove electrons from the molecules of living cells. The chemical species formed can react with other molecules in

the cell. At low doses, the cells repair the damage rapidly. At moderate doses, the cells may suffer a permanent change or die, due to their inability to repair the damage. But the possibility exists that the cells do not die and produce more abnormal cells when they divide leading to cancer or other abnormalities such as birth defects.

Complications[14]

Some common complications of cancer and its treatment include:

- *Paraneoplastic syndromes.* This is an unusual immune system reaction to cancer consisting in healthy cells being attacked by the patient's immune system. Usual symptoms of these syndromes include fever, difficulty walking and seizures.

- *Metastasis.* As cancer develops, it usually spreads to other organs of the body. As a general rule, cancer that has spread may be managed, but can not be cured.

- *Cancer recurrence.* Cancer survivors are at risk of a recurrence depending on the type of cancer. In order to reduce this risk, the health professional may develop a follow-up plan after treatment.

Treatment[14]

A number of cancer treatments are available depending on the type and stage of cancer, the individual's general health and his/her preferences. In this sense, it is important to talk to the health care provider so the patient and the doctor can discuss the benefits and risks of each treatment and determine the best option for the patient. The

patient must be aware that the sooner the cancer is found and treatment begins, his/her chances to survive increase.

In general, cancer treatment therapies can be divided into:

- *Primary treatment* is oriented to remove the cancer from the individual's body or kill the cancer cells. The usual primary cancer treatment for the most common types of cancers is surgery. However, if the cancer type is particularly sensitive to radiation therapy or chemotherapy, any one of these two may become the primary treatment.

- *Adjuvant therapy* is a therapy aimed at killing any remaining cancer cells after primary treatment. Common adjuvant therapies include chemotherapy, radiation therapy, and hormone therapy.

- *Palliative care* helps to relieve the side effects of cancer and its treatment in order to help the patient to maintain a quality of life during and after cancer treatment.

As for treatment techniques, the most common ones include:

- *Surgery.* Its goal is to remove as much of the cancer as possible.

- *Chemotherapy.* Consists of the use of drugs to kill cancer cells.

- *Radiation therapy.* The use of high-powered energy beams to kill cancer cells. There are two types: external beam radiation which uses a machine outside the patient's body and brachytherapy in which the radiation source is surgically implanted inside the patient's body.

- *Blood stem cell transplant.* Also known as bone marrow transplant. Bone marrow is the material inside bones that makes blood cells from blood

stem cells. These blood stem cells can come from the patient or from a donor.

- *Biological therapy.* Cancer can pass undetected because the immune system does not recognize it as a foreign entity. Biological therapy aims to help the immune system to recognize and fight cancer.

- *Hormone therapy.* Hormones play an important role in some types of cancer, such as breast and prostate. Hormone therapy aims to kill cancer cells by removing those hormones.

- *Targeted drug therapy.* This therapy targets specific abnormalities in cancer cells that allow them to survive.

Prevention[14, 109]

To date, no certain way to prevent cancer is known. However, in order to reduce the risk for cancer, health care providers suggest the following:

- *Avoid the risk factors already mentioned in this chapter.*

- *Healthy diet.* To date, there is no known certain way to prevent cancer. However, research suggests that the ingestion of fruit and non-starchy vegetables may help protect against cancer. Unfortunately, the results in this matter differ depending on the type of study design. Whereas case-control and cohort studies show a statistical association between diet and cancer development, randomized trials show little or no relationship.

- *Alcohol.* The American Institute for Cancer Research in its second expert report has regarded alcohol as a "convincing" cause of cancers of the mouth,

pharynx, larynx, esophagus, colorectum in men; and breast and probably liver and colorectal cancer in women.[110]

- *Physical activity.* The American Institute of Cancer Research in its second expert report suggests that regular, sustained physical activity protects from colon cancer and female-related cancers and, probably, protects against postmenopausal breast and endometrium cancers. The report also suggests that physical activity also protects against cancers for which the risk is increased by overweight, weight gain, and obesity.[110]

- *Schedule for cancer screening exams.* It is important to talk to the health care professional about what types of cancer screening exams are best for each individual based on his/her risk factors.

<center>*Anti-Vascular Tumor Therapy*</center>

A fundamental issue in tumor growth, progression and metastasis is the formation of new blood vessels from existing vasculature. This process is called *angiogenesis.*[111] Although angiogenesis was first described in 1863, it was not until 1971 that the inhibition of this process as a strategy in the fight against cancer was suggested.[111]

As opposed to conventional chemotherapy, the target of anti-vascular tumor therapy is the vessels that feed the tumor and not the tumor cells themselves.[111] In general, anti-vascular therapy can be divided into *anti-angiogenesis* which prevents the formation of new blood vessels that feed the tumor and *vascular targeting* which aims on the selective destruction of already existing blood vessels that feed the tumor causing the cancer cells to eventually die because of malnourishment.[111]

In the area of vascular targeting, many compounds have been synthesized with different approaches. In general, these compounds belong to any of these categories:[111]

- *Ligand-directed vascular targeting agents* generally use antibodies, specific peptides or growth factors complexed with cytotoxic molecules. Common targets of these agents include VEGF receptors and cell adhesion molecules (VCAM, E-selecting) among others.

- *Cationic liposome based vascular targeting therapy* is based on the fact that cationic liposomes selectively target tumor endothelium. It has been demonstrated that tumor cells express several negatively charged molecules including phosphatidylserine,[100][112]and hyperglycosylated and hypersilylated membrane proteins.[8] Thus the selective targeting could be mediated by charge interaction of cationic liposomes with the more negatively charged cell surface of angiogenic endothelial cells relative to normal endothelial cells.[111]

- *Small molecule vascular targeting agents* are molecules that bind and destabilize the protein tubulin causing rapid changes in endothelial cell shape, from flat to rounded cells. This change in the morphology of the endothelial cells causes the blood vessel to become occluded, with the consequence of shutting down the nutrient supply to the tumor and waste removal from it. Tumor cells supported by affected vessels die due to lack of nutrients.

The combretastatin series of compounds belong to the small molecule class of vascular targeting agents. These compounds were discovered by Prof. Pettit from Arizona State University in the African bush *Combretum caffrum.[113]* (Figure 28)
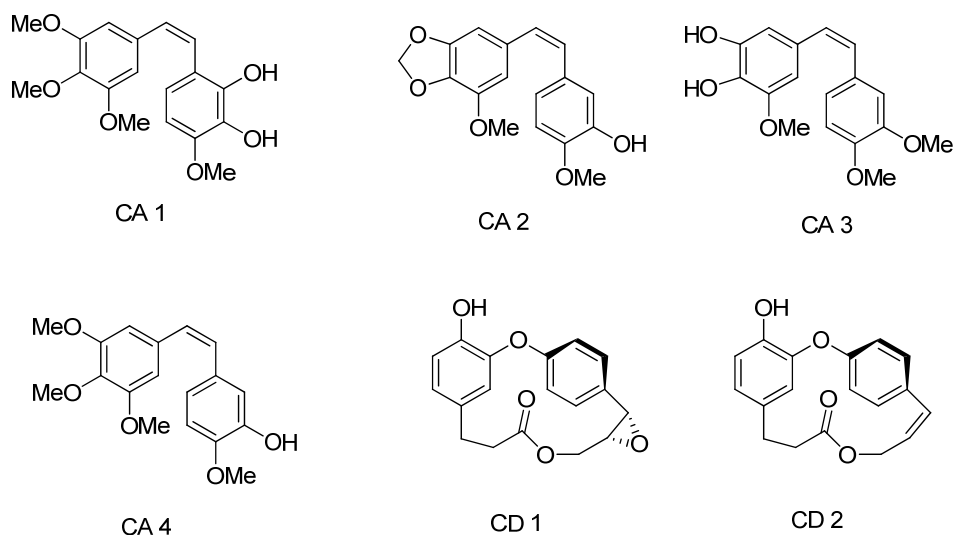
Figure 28. Chemical structures of some combretastatins.[32]

Biorreduction Therapy

The development of a blood network that provides oxygen and nutrients is crucial for the growth of a solid tumor.[114] The fact that tumor cells divide up to 50 times faster than normal cells[111] causes the formation of immature blood vessels which, in turn, results in low or null delivery of oxygen into the tumor.[114] In this way, cells that are approximately 70 to 150 microns away from a feeding blood vessel become chronically hypoxic, and therefore, a significant problem since it is estimated that these cells are 2 to 3 times more resistant to radiotherapy as well as significantly less sensitive to chemotherapeutic agents.[114]

In an effort to eradicate hypoxic cells, scientists have developed a series of compounds that become activated under these hypoxic conditions by certain reductases. The compounds that belong to this family are called *bioreductive*. Some characteristics of bioreductive prodrugs include:[114]

- Must have minimal toxicity in normal (oxic) cells.

- In hypoxic cells, they should be transformed by reductases into a cytotoxin. This process should be inhibited by oxygen.

- The product should covalently bind to DNA with enough affinity to produce cytotoxicity and low enough to diffuse to vicinal hypoxic cells.

Tirapazamine, 3-amino-1,2,4-benzotriazine-1,4-dioxide, is one of the most successful bioreductive prodrugs that selectively eradicates hypoxic cells in solid tumors.[115-118]

In its unique mechanism of action, tirapazamine is reduced by certain reductases to initially form a radical anion which is back oxidized by molecular oxygen in normal cells. In hypoxic cells, however, the radical anion can undergo homolytic cleavage and release hydroxyl radicals. The actual chemical species causing DNA damage has not been determined. Whereas some authors state that the hydroxyl radicals are the ones that cause this DNA double strand cleavage, others state that it is the radical anion formed after the one electron initial reduction the one that causes DNA damage.[115-118]
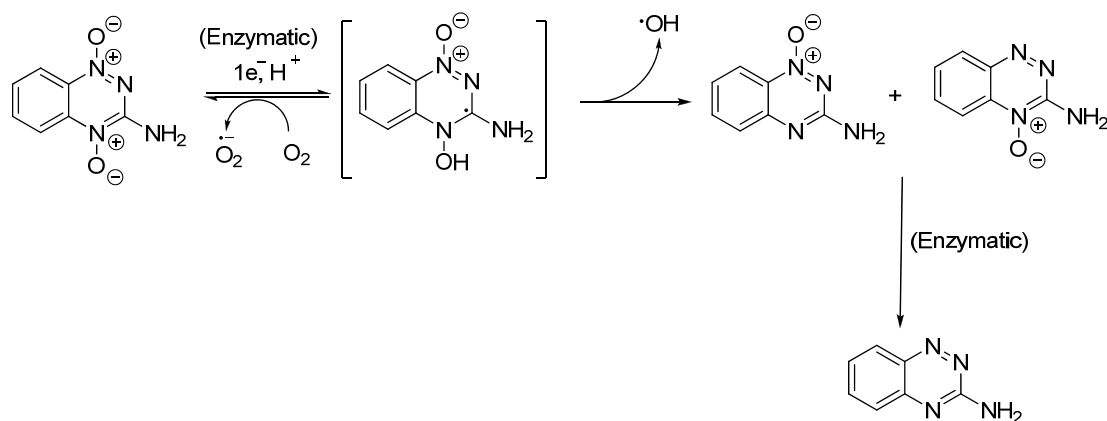
Scheme 18. Mechanism of activation of tirapazamine[112]

Rationale of Drug Design

Although combretastatin A4 has proven to be very effective in eradicating solid tumors – vascular shutdown occurs within 30 to 60 minutes after application – vascular targeting agents preferentially destroy blood vessels in the center of the tumor. This leaves a rim of cancer cells in the tumor periphery that survive treatment and are able to metastasize and, therefore, infect new organs developing new tumors.[111] The elimination of this rim of cancer cells could be achieved by a tirapazamine moiety integrated into the chemical structure of the combretastatin type A moiety. (See figure 29):
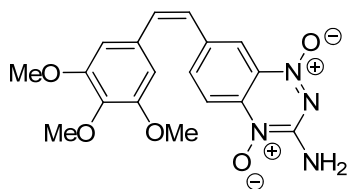
Figure 29. Integrated bioreductive – vascular disrupting agents for the treatment of cancer.

Serotonin and Cancer

Serotonin is a well known monoamine neurotransmitter. Its action consists of controlling a wide range of physiological activities in different cells by binding to multiple receptor subtypes.[100] However, studies suggest that serotonin also stimulates the synthesis of DNA in a synergistic action with growth factors PDGF, EGF and FGF in cultured bovine pulmonary artery smooth muscle cells.[119] In addition, Dizeyi and coworkers show that serotonin has a direct effect in the growth of prostate carcinoma cells and suggest the potential use of serotonin-uptake inhibitors as effective drugs for the treatment of prostate cancer.[100]

CHAPTER SEVEN

Results and Discussion

*Synthesis of an Integrated Bioreductive-Vascular
Disrupting Agent for the Treatment of Cancer*

The chemical synthesis of a compound comprising the chemical structure of the

combretastatin A series integrated with that of tirapazamine (see Figure 30) was

attempted.
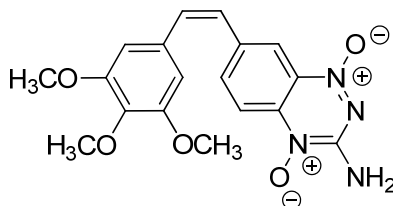
Figure 30. Integrated molecule bearing the combretastatin A series and the tirapazamine
moiety for the treatment of cancer.

Initially, the synthesis of the tirapazamine analog bearing a methyl group at the 7-

position was performed as shown in scheme 19:
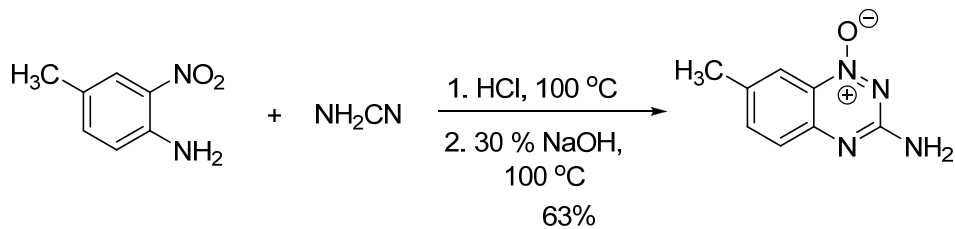
Scheme 19[2] Synthesis of the benzotriazine-1-oxide precursor of tirapazamine.[121]

A possible mechanism for this transformation is:
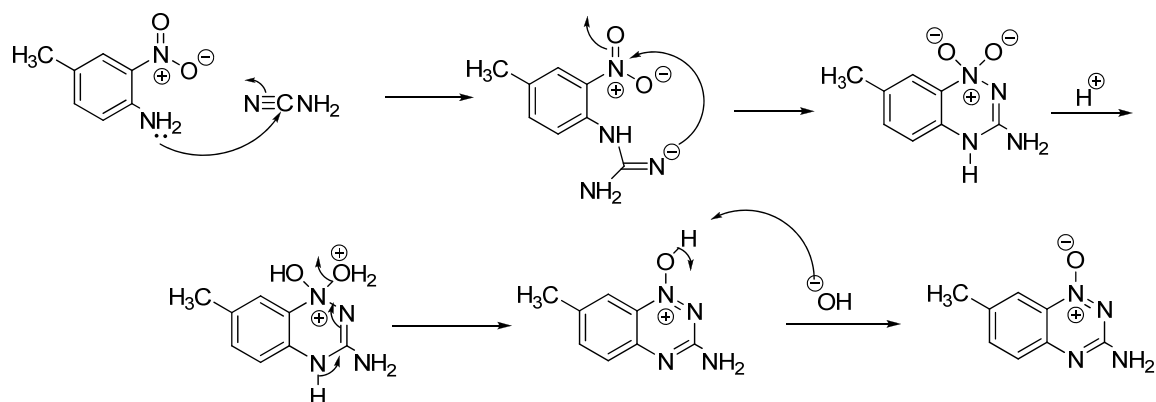
Scheme 20. Possible mechanism for the formation of 3-amino-7-methyl-1,2,4-benzotriazine-1-oxide

The proposed synthetic route for the integrated molecule is depicted in scheme 21.
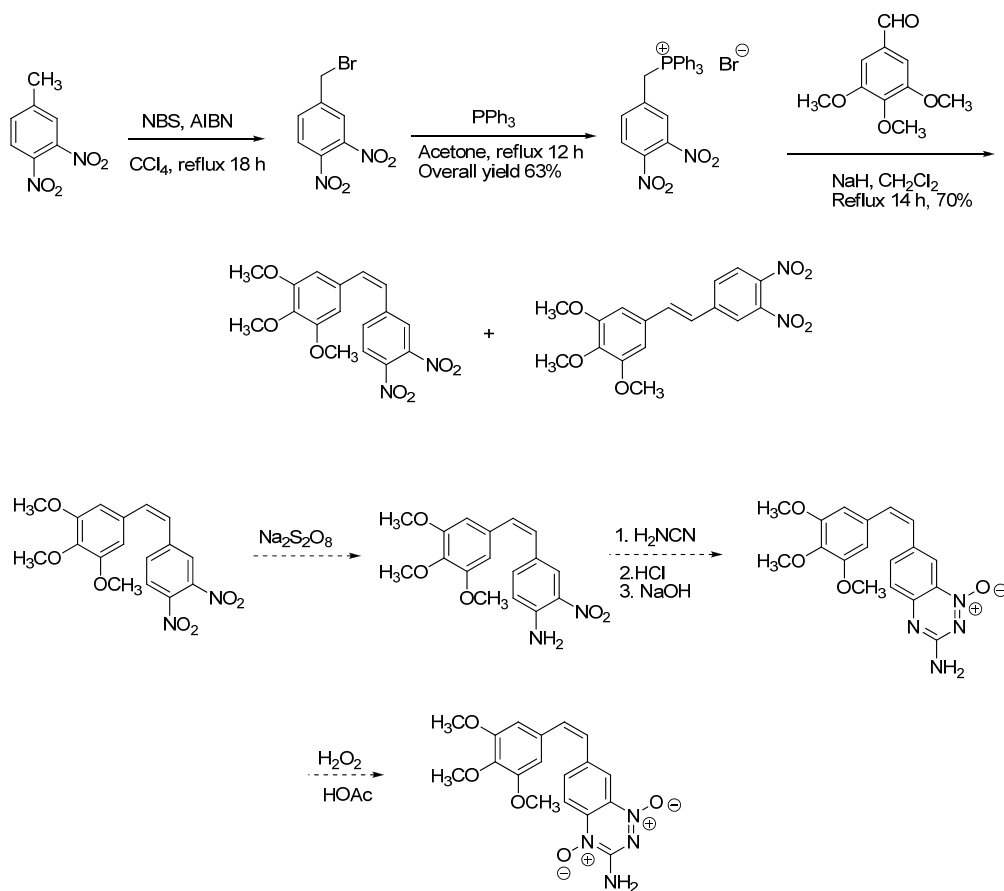
Scheme 21. Proposed synthetic route for the integrated bioreductive-vascular disrupting agent for the treatment of cancer.

Unfortunately, the separation of the cis and trans isomers by column chromatography could not be achieved due to the presence of two nitro groups in the molecule.

Results
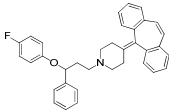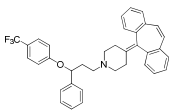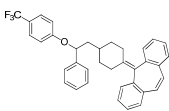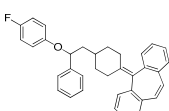
Cancer. The activity for the synthesized molecules is reported as GI_{50}, which is defined as the concentration of a compound at which a growth inhibition of 50% is achieved.[8]

The compounds were tested at Dr. Mary Lynn Trawick's laboratory – Department of Chemistry and Biochemistry, Baylor University, Waco, TX – by Dr. Tracy E. Strecker (postdoctoral fellow) for activity against cancer cell lines DU145 (prostatic cancer),[9] SKOV-3 (ovarian cancer),[10] and NCI-H460 (human lung cancer).[11]

The results are as depicted in Table 1.

Although the activity of the compounds is high compared to that of the standard, the results show a possible implication of the serotonin system in the growth of these cancer cell lines and are encouraging to continue doing research in this area.

Table 1. Activity against cancer lines of bifunctional SERT – 5-HT$_{2A}$ compounds.

Compound	GI$_{50}$ (μM)		
	DU145	**SKOV-3**	**NCI-H460**
Doxorubicin	0.0209	0.000848	0.0269
	11.658	12.462	10.656
	11.730	13.65	13.315
	4.362	13.412	21.160
	3.673	14.185	17.944

CHAPTER EIGHT

Materials and Methods

All chemicals used for chemical synthesis as well as solvents used for flash chromatography were obtained from commercial sources including Sigma Aldrich, Acros Chemicals and Alfa Aesar and were used without any further purification. Reactions involving anhydrous conditions were performed in oven-dried glassware at least overnight. Reactions were monitored by thin layer chromatography using Merck Kieselgel 60 F_{254} glass backed plates. The plates were visualized using a multiband 254/365 UV lamp Spectroline Model ENF-240C. Flash chromatography was performed using silica gel ZEOprep 60HYD 40 – 63 μm.

NMR spectra were recorded in a Bruker DPX-300 spectrometer operating at 300 MHz for proton, 75 MHz for carbon and 282 MHz for fluorine and Varian AS 500 spectrometer operating at 500 MHz for proton, 125 MHz for carbon and 470 MHz for fluorine. The NMR spectra were recorded using $CDCl_3$ (0.03% TMS as reference) or DMSO-d_6 with no TMS. Chemical shifts are expressed in ppm (δ). NMR patterns are reported as singlets (s), doublets (d), triplets (t), quartets (q), and combinations of these as well as multiplets (m). Processing of NMR spectra was performed using MestReNova 6.1.1 software (Mestrelab Research Co).

Synthesis of 3,4,5-trimethoxy-3',4'-dinitro stilbene

7-Methyl-3-amino-1,2,4-benzotriazine-1-oxide

In a round bottomed flask was mixed together 4-methyl-2-nitroaniline (0.65 g, 4.30 mmol) and cyanamide (0.92 g, 22.0 mmol). The reaction mixture was heated at 100 ˚C while stirring until a red melt is formed and then allowed to return to room temperature. Concentrated hydrochloric acid (5.0 mL) was added carefully and the reaction mixture was stirred until the exotherm subsides and then heated again at 100 ˚C for two hours. The reaction mixture was allowed to return to about 50 ˚C and sodium hydroxide (7.50 M, 50.0 mL) was added carefully and heated to 100 ˚C for one hour. The reaction mixture was allowed to return to room temperature and was diluted with water (100 mL). The precipitate formed was filtered, washed with water, diethyl ether (2 x 10 mL) and was dissolved in hot iso-propanol. The precipitate was filtered again and dried. The product was obtained as a yellow solid in 63 % yield (0.47 g).

^{1}H-NMR (500 MHz, DMSO-d6), δ (ppm): 7.93 (s, 1H), 7.63 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.44 (d, *J* = 9.0 Hz, 1H), 7.21 (s, 2H), 2.41 (s, 3H).

^{13}C-NMR (125 MHz, DMSO-d6), δ (ppm): 160.44, 147.73, 138.20, 135.28, 129.98, 126.12, 118.85, 21.17.

3,4-Dinitrobenzyl bromide

To a well stirred solution of 3,4-dinitrotoluene (4.55 g, 2.50 mmol) in carbon tetrachloride (50 mL) was added AIBN (0.41 g, 2.5 mmol) and NBS (4.45 g, 25.0 mmol). The reaction was heated at reflux for 18 hours. After that, water was added and the product was extracted with methylene chloride. The organic layer was dried over

magnesium sulfate and the solvent was evaporated under reduced pressure. The product was obtained as a brown solid and was directly used into the next reaction.

3,4-Dinitrobenzylphosphonium bromide

A mixture of non-pure 3,4-dinitrobenzylbromide (7.16 g) and triphenylphosphine (7.19 g, 27.4 mmol) under nitrogen atmosphere was dissolved in anhydrous acetone (50 mL). The mixture was refluxed for 12 hours and allowed to return to room temperature. The solid was filtered and washed with acetone and cold hexanes. The product was obtained as a white solid in 11 % overall yield (1.45 g).

^{1}H-NMR (300 MHz, DMSO-d_6), δ (ppm): 8.18 (d, J = 9.0 Hz, 1H), 7.81 (m, 20H), 5.50 (d, J = 18.0 Hz, 2H)

^{13}C-NMR (75 MHz, DMSO-d_6), δ (ppm): 141.97 (d, J = 4.3 Hz), 141.84 (d, J = 3.7 Hz), 136.98 (d, J = 5.3 Hz), 136.36 (d, J = 8.3 Hz), 135.93 (d, J = 2.9 Hz), 134.60 (d, J = 10.2 Hz), 130.79 (d, J = 12.7 Hz), 127.95 (d, J = 5.2 Hz), 126.78 (d, J = 3.0 Hz), 31.16, 28.15 (d, J = 47.7 Hz).

^{31}P-NMR (121 MHz, DMSO-d_6), δ (ppm): 24.15.

3,4,5-Trimethoxy-3',4'-dinitro stilbene

To an oven-dried flask was added pure sodium hydride (0.26 g, 11.1 mmol) along with dry methylene chloride (7.0 mL). While stirring under nitrogen atmosphere, solution of 3,4-dinitrobenzylphosphonium bromide (1.45 g, 2.8 mmol) dissolved in methylene chloride (7.0 mL) was added. After stirring for 10 minutes, a solution of 3,4,5-trimethoxybenzaldehyde (0.54 g, 2.8 mmol) in methylene chloride (7.0 mL) was added and the reaction mixture was stirred at room temperature for 14 hours. Water was

added to quench the unreacted sodium hydride and the reaction mixture was extracted with methylene chloride. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. Flash column chromatography (hexanes – ethyl acetate 7:3) afforded a mixture of red crystals and yellow oil (0.70 g, 70% yield) which could not be separated.

^{1}H-NMR (300 MHz, DMSO-d6), δ (ppm): 7.88 (m, 1H), 7.77 (d, J = 1.7 Hz, 1H), 7.61 (dd, J = 8.4, 1.7 Hz, 1H), 6.89 (d, J = 12.1 Hz, 1H), 6.54 (d, J = 12.2 Hz, 1H).

CHAPTER NINE

Conclusions and Future Directions

The research presented herein centers the attention on the development of bifunctional compounds with activity against the SERT and 5-HT$_{2A}$ receptor and activity against the SERT, NET and 5-HT$_{2A}$ receptor. Due to the fact that serotonin is also known to function as a growth factor, these compounds were also tested for activity against several cancer cell lines that included DU-145, SKOV-3 and NCI-H460. Although the compounds showed activity against these cancer cell lines, more research is needed to determine whether the compounds tested had an effect on the serotonin system of these cancer cell lines or their action is exerted on some other system totally different than the serotonin system.

From the organic chemistry point of view, several conclusions can be reached:

- Compounds with potential activity against SERT, NET and 5-HT$_{2A}$ receptor were synthesized using organic chemistry procedures.

- Compounds synthesized possess activity against cancer cell lines DU-145, SKOV-460 and H-460.

- Synthetic routes were fairly straightforward with minimum protection-deprotection reactions.

- Mitsunobu coupling was a very useful synthetic tool for the preparation of fluoxetine derivatives.

- S$_N$Ar was very useful in the preparation of 4-[(3-fluorophenoxy)phenylmethylpiperidine with excellent yields.

Future directions in this area could include:

- Synthesis of a new and larger library of compounds possessing both base structures, fluoxetine and 4-[(3-fluorophenoxy)phenylmethyl]piperidine.

- Pursue QSAR studies on the most potent compounds against the SERT, NET and 5-HT$_{2A}$ receptor for both enantiomers.

- Fine-tune the chemical structures based on the QSAR studies.

In the cancer area, the target compound could not be obtained due to the difficulty of purification of the dinitro compound by flash chromatography. Starting with the *tert*-butylcarbamate protected derivative of 4-methyl-2-nitroaniline would make the Wittig reaction difficult to accomplish. This is due to the fact that the amino group in the aniline moiety still has an acidic proton that could interfere with the strong base used for the reaction.

The chemical preparation of this compound represents a challenge to the synthetic organic chemist. It is known that under certain oxidizing conditions, para-methoxy stilbenes can undergo some sort of rearrangement to yield benzophenones.[113]

From the synthetic point of view, the Wittig reaction probed to be a very useful tool for the chemical preparation of stilbenes. However, the separation of cis- and trans-isomers could be difficult to achieve. In order to improve the yield towards the desired stilbene, the formation of a triple bond and further controlled reduction to a cis- double bond is suggested.

APPENDIX A.

NMR Spectra of Selected Compounds

4-Dibenzo[a,d]cyclohepten-5-ylidenepiperidine-1-carboxylic acid ethyl ester. ^{1}H NMR (CDCl₃) 300 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidenepiperidine. ^{1}H NMR (CDCl₃) 300 MHz

3-(4-Dibenzo[a,d]-5-cycloheptenylidene-1-piperidinyl)-1-phenyl-1-propanone
^{1}H NMR (CDCl₃) 500 MHz

3-(4-Dibenzo[a,d]-5-cycloheptenylidene-1-piperidinyl)-1-phenyl-1-propanone
^{13}C NMR (CDCl₃) 125 MHz

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-propanol
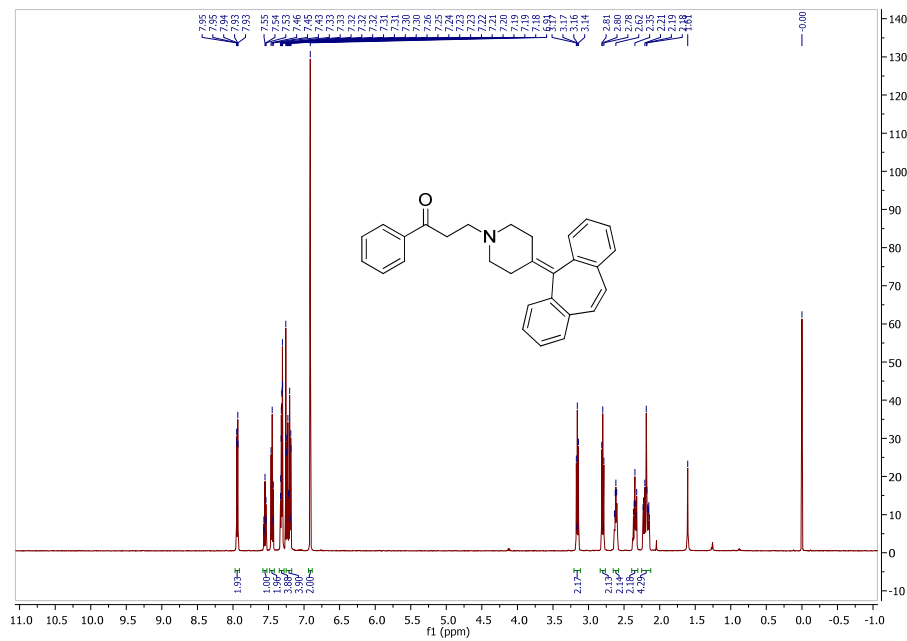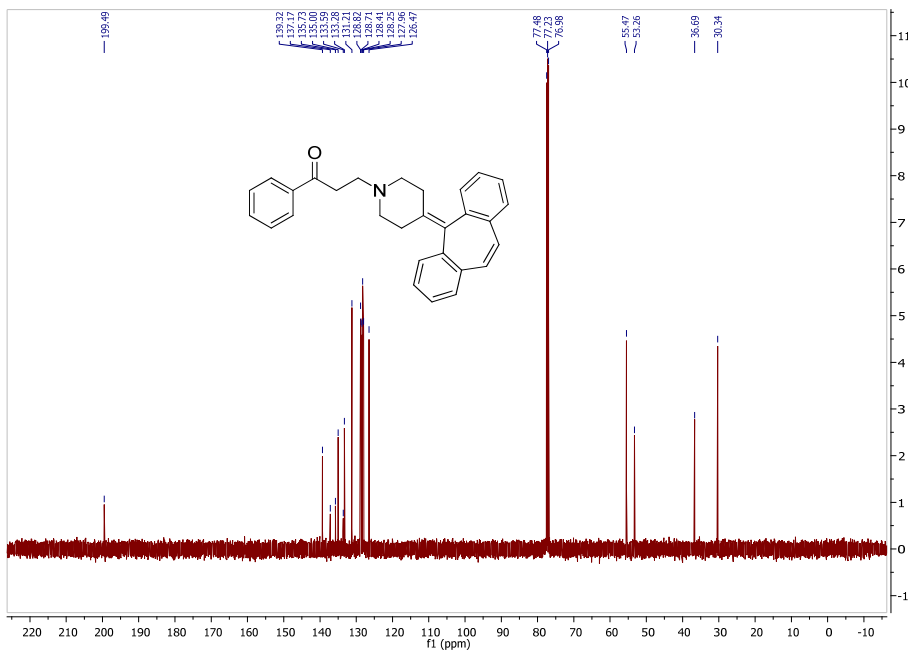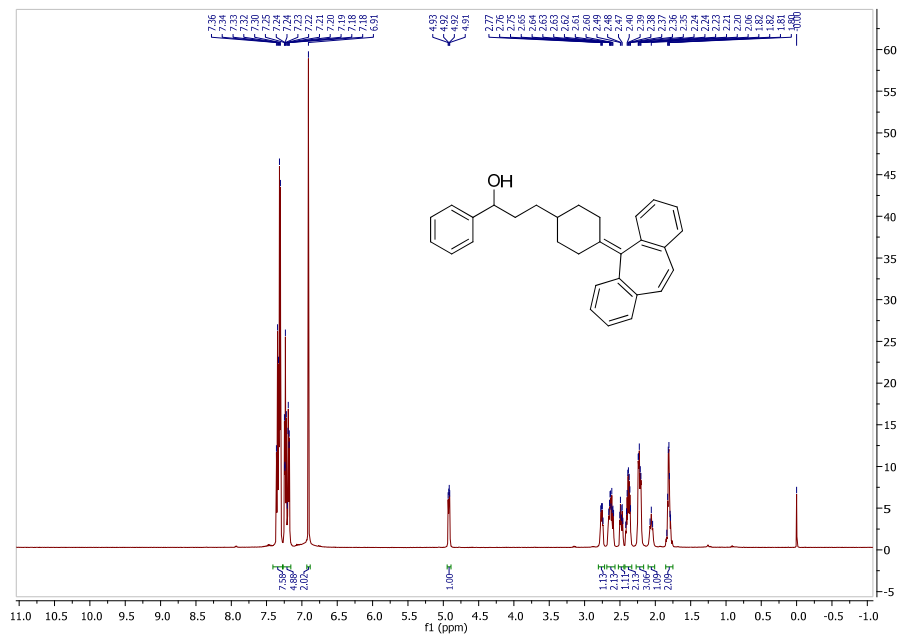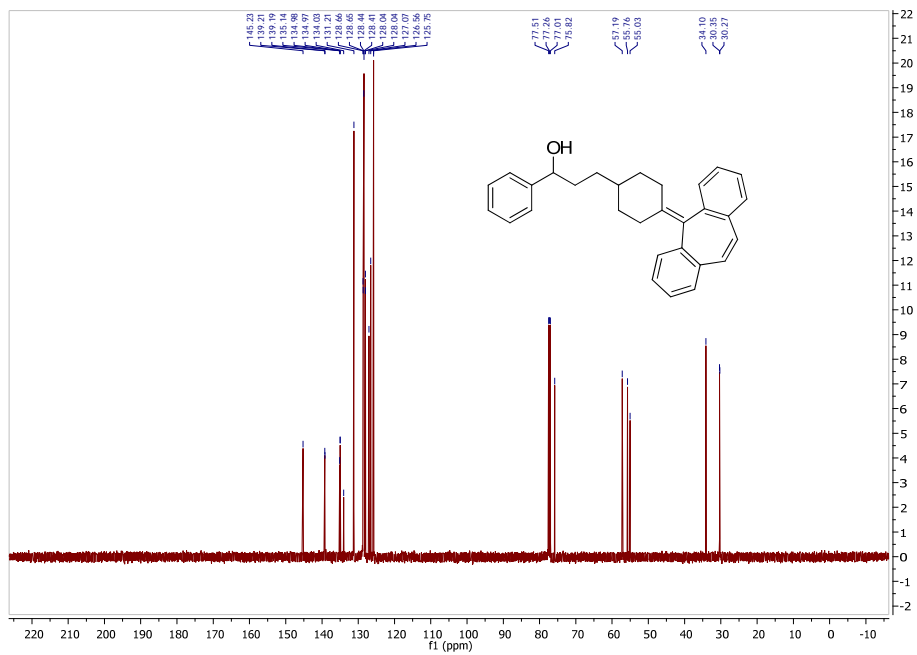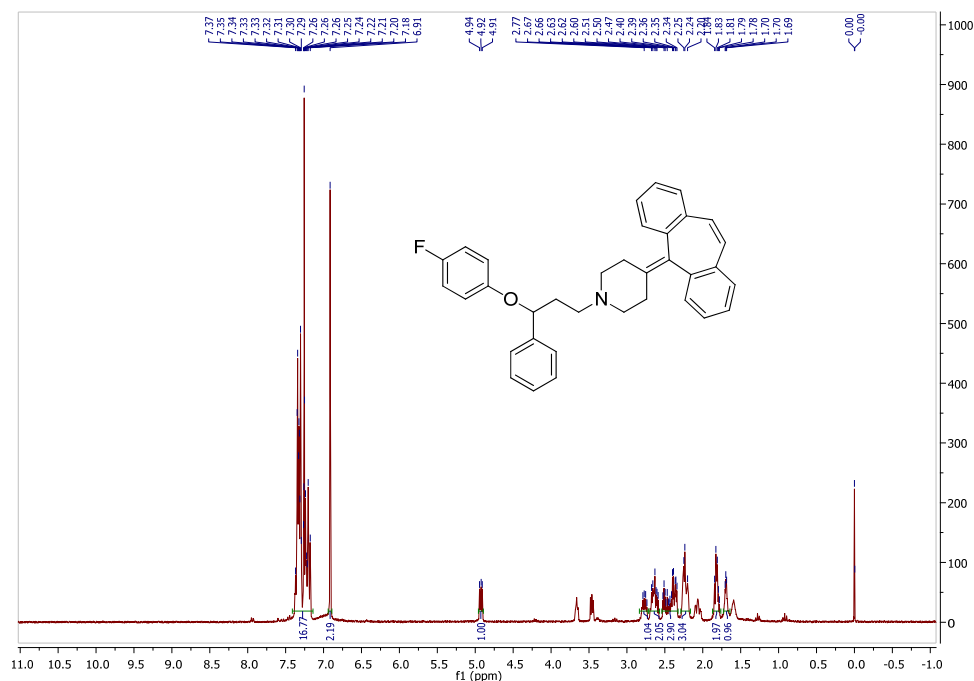^{1}H NMR (CDCl₃) 500 MHz

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-propanol
^{13}C NMR (CDCl₃) 125 MHz

4-Dibenzo[a,d]-5-cycloheptenylidene-1-[3-(4-fluorophenoxy)-3-phenyl propyl]piperidine
^{1}H NMR (CDCl₃) 300 MHz

4-Dibenzo[a,d]-5-cycloheptenylidene-1-[3-(4-fluorophenoxy)-3-phenyl propyl]piperidine
^{13}C NMR (CDCl₃) 75 MHz

4-Dibenzo[a,d]-5-cycloheptenylidene-1-[3-(4-fluorophenoxy)-3-phenyl propyl]piperidine
^{19}F NMR (CDCl₃) 282 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-trifluoromethylphenoxy)   propyl piperidine. ^{1}H NMR (CDCl₃) 300 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-trifluoromethylphenoxy)   propyl piperidine. ^{13}C NMR (CDCl₃) 75 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-trifluoromethylphenoxy)   propyl piperidine. ^{19}F NMR (CDCl₃) 282 MHz

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-ethanone   (crude product). ^{1}H NMR (CDCl₃) 300 MHz

2-(4-dibenzo[a,d]cyclohepten-5-ylidene-1-piperidinyl)-1-phenyl ethanol
^{1}H NMR (CDCl₃) 300 MHz
4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-fluorophenoxy)-2-phenyl   ethyl piperidine. ^{1}H NMR (CDCl₃) 500 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-fluorophenoxy)-2-phenyl   ethyl piperidine. ^{13}C NMR (CDCl₃) 125 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-fluorophenoxy)-2-phenyl   ethyl piperidine. ^{19}F NMR (CDCl$_3$) 470 MHz

1-(4-Benzoyl-1-piperidinyl)methanone. ^{1}H NMR (CDCl$_3$) 300 MHz

1-(4-Benzoyl-1-piperidinyl)methanone. ^{13}C NMR (CDCl$_3$) 75 MHz

1- tert-butylcarboxylate-4-piperidinyl phenyl ketone. ^{1}H NMR (CDCl$_3$) 500 MHz

1- tert-butylcarboxylate-4-piperidinyl phenyl ketone. ^{13}C NMR (CDCl$_3$) 125 MHz

1-Phenyl-1-(4-piperidinyl-1-tert-butyl carboxylate) methanol.
^{1}H NMR (CDCl$_3$) 500 MHz

1-Phenyl-1-(4-piperidinyl-1-tert-butyl carboxylate) methanol.
^{13}C NMR (CDCl$_3$) 125 MHz

4-[(3-fluorophenoxy)phenylmethyl]piperidine hydrochloride.
^{1}H NMR (CDCl$_3$) 500 MHz

4-[(3-fluorophenoxy)phenylmethyl]piperidine hydrochloride.
^{13}C NMR (CDCl$_3$) 125 MHz

4-[(3-fluorophenoxy)phenylmethyl]piperidine hydrochloride.
^{19}F NMR (CDCl$_3$) 470 MHz

N-(1-adamantanyl)-4-[(3-fluorophenoxy)phenylmethyl]piperidine-1-methanone
^{1}H NMR (CDCl$_3$) 500 MHz

N-(1-adamantanyl)-4-[(3-fluorophenoxy)phenylmethyl]piperidine-1-methanone
^{13}C NMR (CDCl$_3$) 125 MHz

N-(1-adamantanyl)-4-[(3-fluorophenoxy)phenylmethyl]piperidine-1-methanone
^{19}F NMR (CDCl$_3$) 470 MHz

7-Methyl-3-amino-1,2,4-benzotriazine-1-oxide. ^{1}H NMR (DMSO-d$_6$) 500 MHz

7-Methyl-3-amino-1,2,4-benzotriazine-1-oxide. ^{13}C NMR (DMSO-d$_6$) 125 MHz

3,4-Dinitrobenzylphosphonium bromide. ^{1}H NMR (DMSO-d$_6$) 300 MHz
3,4-Dinitrobenzylphosphonium bromide. ^{13}C NMR (DMSO-d$_6$) 75 MHz

3,4-Dinitrobenzylphosphonium bromide. ^{31}P NMR (DMSO-d$_6$) 121 MHz

3,4,5-Trimethoxy-3',4'-dinitro stilbene. ^{1}H NMR (DMSO-d$_6$) 300 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidenepiperidine-1-carboxylic acid ethyl ester.
^{1}H NMR (CDCl$_3$) 300 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidenepiperidine.
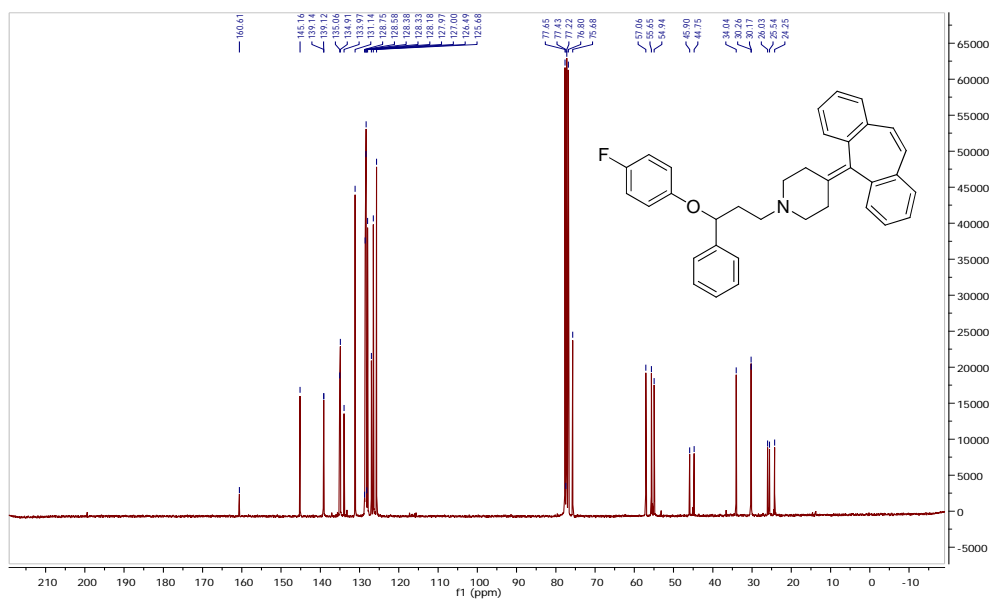## ^{1}H NMR (CDCl$_3$) 300 MHz

3-(4-Dibenzo[a,d]-5-cycloheptenylidene-1-piperidinyl)-1-phenyl-1-propanone
^{1}H NMR (CDCl$_3$) 500 MHz

3-(4-Dibenzo[a,d]-5-cycloheptenylidene-1-piperidinyl)-1-phenyl-1-propanone
^{13}C NMR (CDCl$_3$) 125 MHz

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-propanol
^{1}H NMR (CDCl$_3$) 500 MHz

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-propanol
^{13}C NMR (CDCl$_3$) 125 MHz

4-Dibenzo[a,d]-5-cycloheptenylidene-1-[3-(4-fluorophenoxy)-3-phenyl propyl]piperidine
^{1}H NMR (CDCl$_3$) 300 MHz

4-Dibenzo[a,d]-5-cycloheptenylidene-1-[3-(4-fluorophenoxy)-3-phenyl propyl]piperidine
^{13}C NMR (CDCl$_3$) 75 MHz

4-Dibenzo[a,d]-5-cycloheptenylidene-1-[3-(4-fluorophenoxy)-3-phenyl propyl]piperidine
^{19}F NMR (CDCl$_3$) 282 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-trifluoromethylphenoxy) propyl piperidine. ^{1}H NMR (CDCl$_3$) 300 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-trifluoromethylphenoxy) propyl piperidine. ^{13}C NMR (CDCl$_3$) 75 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-trifluoromethylphenoxy) propyl piperidine. ^{19}F NMR (CDCl$_3$) 282 MHz

3-(4-Dibenzo[a,d]-5-cyclohepteneylidene-1-piperidinyl)-1-phenyl-1-ethanone (crude product). ^{1}H NMR (CDCl$_3$) 300 MHz

2-(4-dibenzo[a,d]cyclohepten-5-ylidene-1-piperidinyl)-1-phenyl ethanol
^{1}H NMR (CDCl$_3$) 300 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-fluorophenoxy)-2-phenyl ethyl piperidine. ^{1}H NMR (CDCl$_3$) 500 MHz

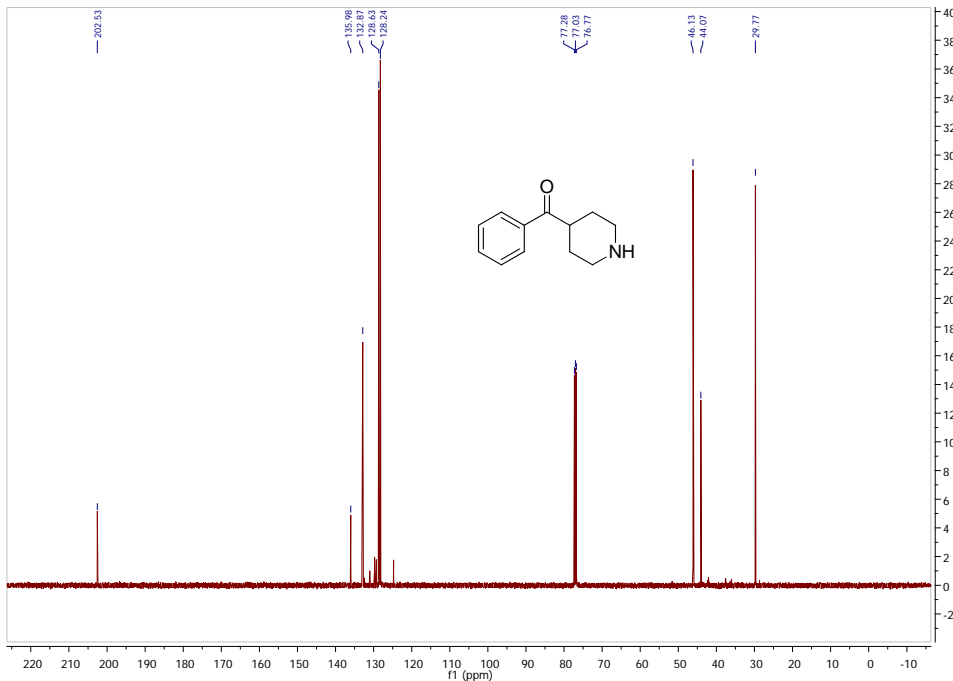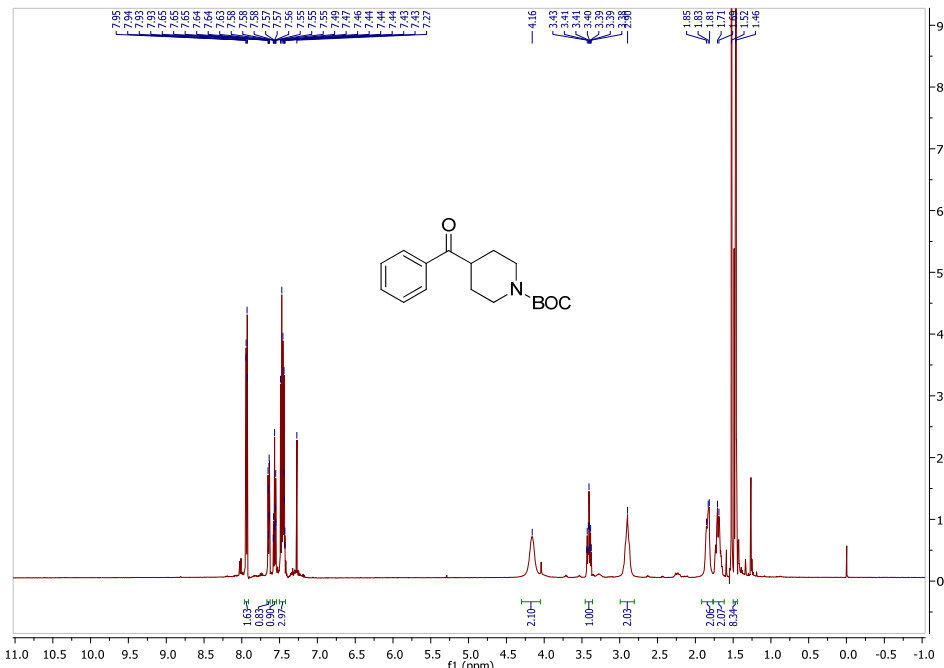4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-fluorophenoxy)-2-phenyl ethyl piperidine. ^{13}C NMR (CDCl$_3$) 125 MHz

4-Dibenzo[a,d]cyclohepten-5-ylidene-1-[3-phenyl-3-(4-fluorophenoxy)-2-phenyl ethyl piperidine. ^{19}F NMR (CDCl$_3$) 470 MHz
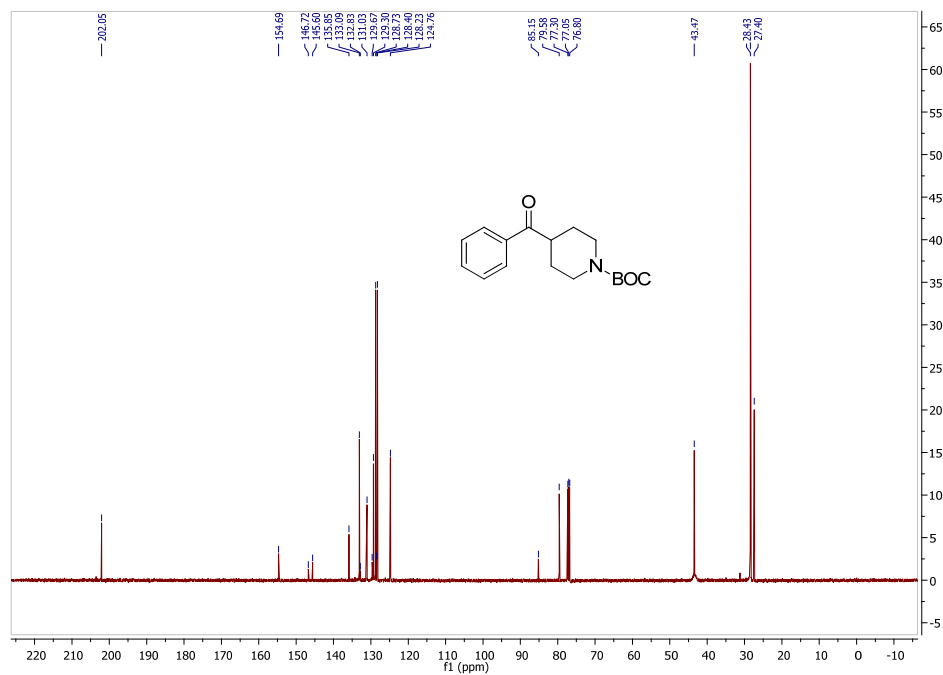

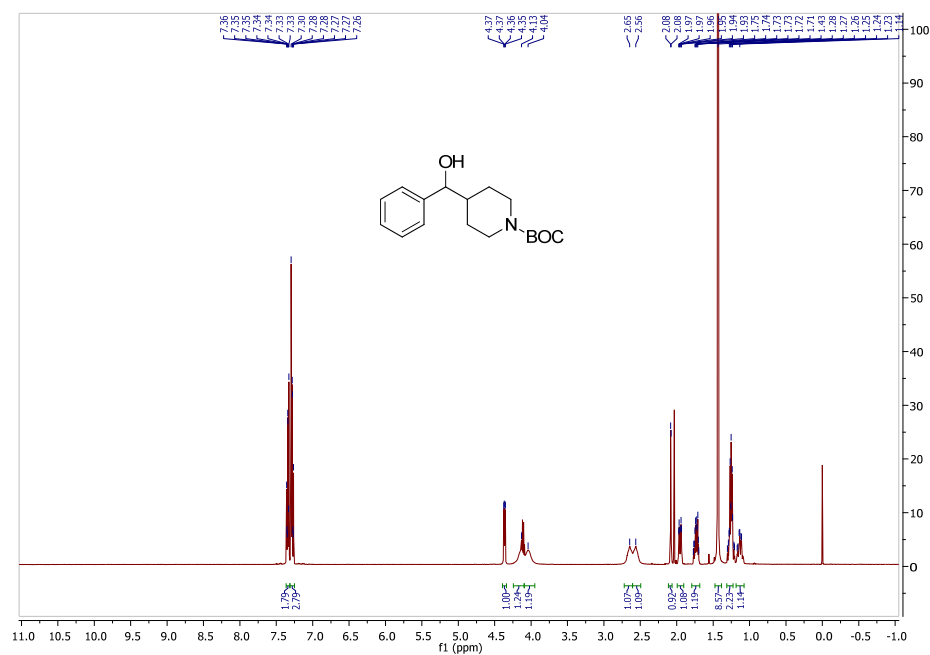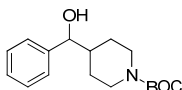1-(4-Benzoyl-1-piperidinyl)methanone. ^{1}H NMR (CDCl$_3$) 300 MHz
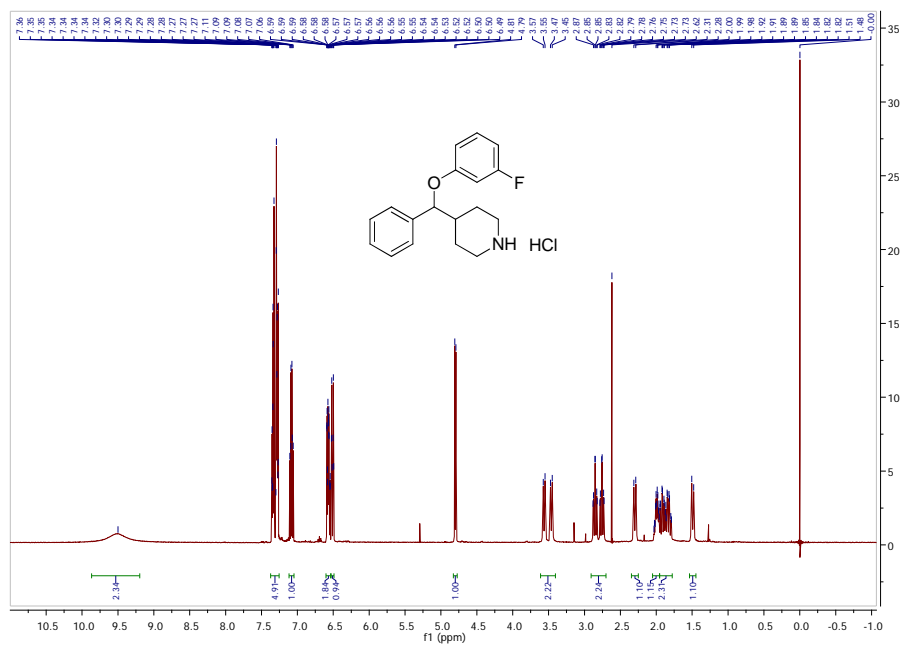
1-(4-Benzoyl-1-piperidinyl)methanone. ^{13}C NMR (CDCl$_3$) 75 MHz

1- tert-butylcarboxylate-4-piperidinyl phenyl ketone. ^{1}H NMR (CDCl$_3$) 500 MHz

1- tert-butylcarboxylate-4-piperidinyl phenyl ketone. ^{13}C NMR (CDCl$_3$) 125 MHz

1-Phenyl-1-(4-piperidinyl-1-tert-butyl carboxylate) methanol.
^{1}H NMR (CDCl$_3$) 500 MHz

1-Phenyl-1-(4-piperidinyl-1-tert-butyl carboxylate) methanol.
^{13}C NMR (CDCl$_3$) 125 MHz

4-[(3-fluorophenoxy)phenylmethyl]piperidine hydrochloride.
^{1}H NMR (CDCl$_3$) 500 MHz

4-[(3-fluorophenoxy)phenylmethyl]piperidine hydrochloride.
^{13}C NMR (CDCl$_3$) 125 MHz

4-[(3-fluorophenoxy)phenylmethyl]piperidine hydrochloride.
^{19}F NMR (CDCl$_3$) 470 MHz

N-(1-adamantanyl)-4-[(3-fluorophenoxy)phenylmethyl]piperidine-1-methanone
^{1}H NMR (CDCl$_3$) 500 MHz

N-(1-adamantanyl)-4-[(3-fluorophenoxy)phenylmethyl]piperidine-1-methanone
^{13}C NMR (CDCl$_3$) 125 MHz

N-(1-adamantanyl)-4-[(3-fluorophenoxy)phenylmethyl]piperidine-1-methanone
^{19}F NMR (CDCl$_3$) 470 MHz

7-Methyl-3-amino-1,2,4-benzotriazine-1-oxide. ^{1}H NMR (DMSO-d$_6$) 500 MHz

7-Methyl-3-amino-1,2,4-benzotriazine-1-oxide. ^{13}C NMR (DMSO-d$_6$) 125 MHz

3,4-Dinitrobenzylphosphonium bromide. ^{1}H NMR (DMSO-d$_6$) 300 MHz

3,4-Dinitrobenzylphosphonium bromide. ^{13}C NMR (DMSO-d$_6$) 75 MHz

3,4-Dinitrobenzylphosphonium bromide. ^{31}P NMR (DMSO-d$_6$) 121 MHz

3,4,5-Trimethoxy-3',4'-dinitro stilbene. ^{1}H NMR (DMSO-d_6) 300 MHz

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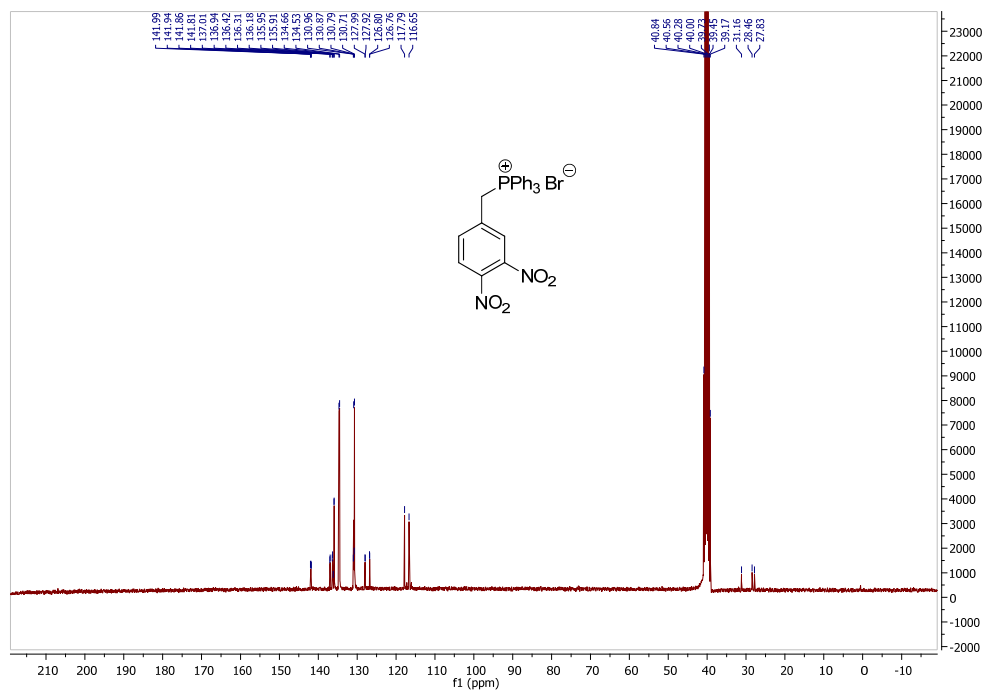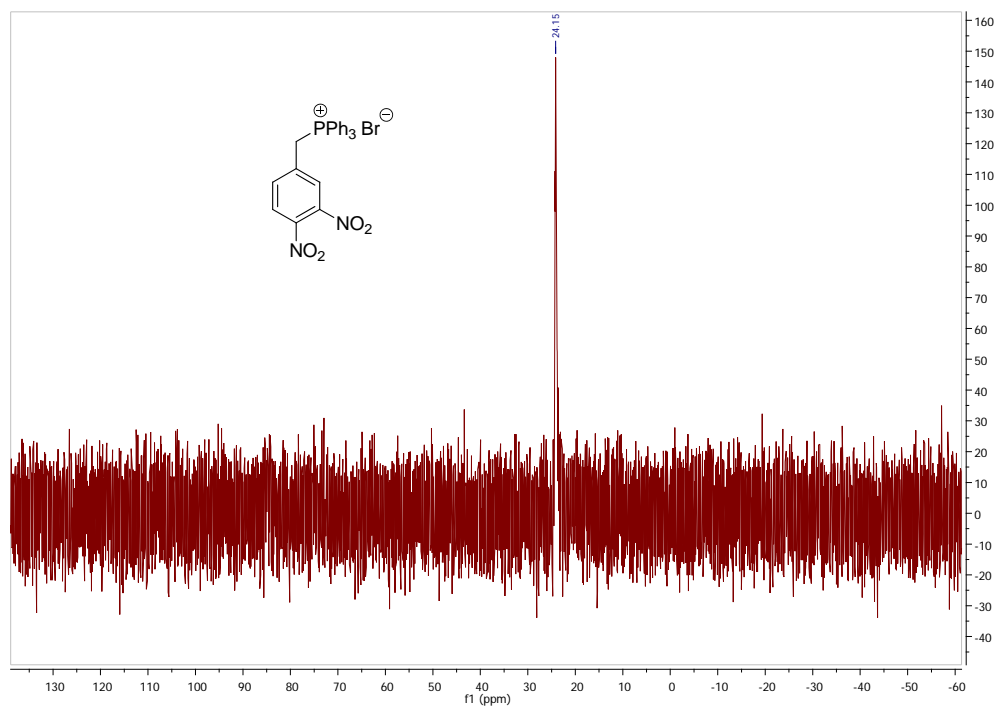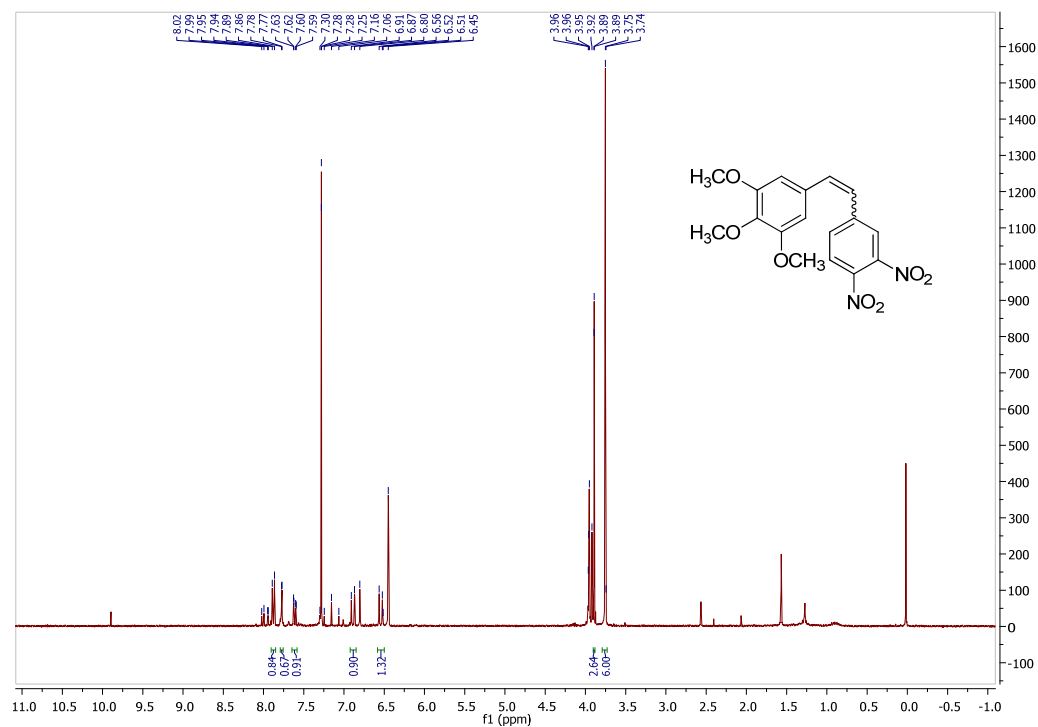(5) Rohan, K. J.; Lindsey, K. T.; Roecklein, K. A.; Lacy, T. J. *J Affect Disorders* **2004**, *80*, 273-283.

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