Carbazole Based Multifunctional Dopamine Agonists And Related Molecules As Potential Symptomatic And Disease Modifying Therapeutic Agents For Parkinson’s Disease

Asma S.mohamed Elmabruk
Wayne State University, Fc9851@wayne.edu

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CARBAZOLE BASED MULTIFUNCTIONAL DOPAMINE AGONISTS AND RELATED MOLECULES AS POTENTIAL SYMPTOMATIC AND DISEASE MODIFYING THERAPEUTIC AGENTS FOR PARKINSON’S DISEASE

by

ASMA ELMABRUK

DISSERTATION

Submitted to the Graduate School of Wayne State University, Detroit, Michigan in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

2018

MAJOR: PHARMACEUTICAL SCIENCES

Approved By:

Advisor

Date

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DEDICATION

This work is dedicated to the spirit of my father, to my mom, my husband, my siblings, my children, and my friends who have always been there for me.
ACKNOWLEDGMENTS

It is such a great honor and pleasure for me to have the privilege to work with all the lab crews in order to accomplish this mission. I am very delighted to get the opportunity to convey my gratitude to all the members who assisted with this research.

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1.1. Parkinson’s Disease.

Parkinson’s disease (PD) is a chronic, progressive, neurodegenerative disorder that affects 2-3% of the population with onset age more than 65 years old (J. Z Igmond and E. B Urke 2018; Arora and Fletcher 2013). The neurological hallmarks of PD are the depletion of dopamine in the basal ganglia with an extensive loss of dopaminergic neurons in the midbrain of the substantia nigra pars compacta (SNpc) accompanied by accumulation of an intraneuronal presynaptic filamentous protein called Lewy bodies (LBs). LBs may be responsible for triggering the degeneration of the dopaminergic neuron (Figure 1) (Dauer and Przedborski 2003; Sulzer 2007). The loss of SNpc neurons found to be responsible for the main symptoms of PD. In the 1960 researchers discovered that the pathological features of PD appear when the patient losses 75% of neuromelanin, a dark colored pigment formed within the dopamine and noradrenaline neurons of the SNpc and the locus coeruleus in the human brain (Dauer and Przedborski 2003; Michel, Hirsch, and Hunot 2016).

PD was first described by the general physician James Parkinson in London in 1817 in his classic monograph “An Essay on the shaking palsy” (Dauer and Przedborski 2003; Hurwitz 2014). Early in the 19th century Charcot gave credit to James for his discovery and named the disease “Maladie de Parkinson’ (Jankovic 2007). Charcot also diagnosed another type of PD (slowing in movement) and distinguished from muscle weakness which Parkinson discovered earlier. In 1919 Parkinson’s patients were recognized with losing cells in the substantia nigra. After this discovery, in 1957; Carlson
and his colleagues in Sweden realized dopamine as a putative neurotransmitter. In 1960 Ehringer and Hornykiewicz noticed that patient with PD has a low level of dopamine in the striatum (Hurwitz 2014) (Goetz 2011).

There are several symptoms linked to the development of PD. These symptoms classified into motor and non-motor symptoms. There are four cardinal signs associated with the development and progression of PD. The first sign is rest tremor, involuntary movement, which considered as an early sign of the disorder and the most common warning for having PD. 30% of PD people do not have tremor as a first indicator and appear later (Goetz 2011). The second motor symptom of PD is akinesia without motion, bradykinesia or hypokinesia means slow motion and this happens at an initial stage of the disease (slowness and difficulty of movement). Rigidity is the third motor sign of PD, the muscle stiffness movement caused by increased muscle tone, and it may appear in any part of the body (Schapira 2009a). Rigidity may associate with pain, and at the later stage rigidity may affect the whole body and reduce the ability to move. The fourth common motor indicator of PD is the postural instability, the impaired balance and frequency of falls that may lead to secondary bone fracture (Poewe et al. 2017). Some patients do not show all the cardinal manifestations of PD; there may be only one or two symptoms. Other clinical features that are associated with PD include secondary motor symptoms such as hypomimia, dysphagia, micrographia, shuffling, dystonia, glabellar reflexes. The most common non-motor symptoms in PD include anxiety, depression, dementia and sleep disturbance (Jankovic 2007; Poewe et al. 2017). There are several medications to treat PD, the current therapy relieves only the symptoms (Tanzi 2005).
Studies showed that people with PD are more susceptible to depression (Jinling Liu 2013). Researchers also reported that depression is more prevalent in PD patients than in the general population (Cui et al. 2017). It is unclear if depression is an independent risk factor for PD or if it is a primary sign of the neurodegenerative disease (Cui et al. 2017). Researchers suggested that the onset of depression in the elderly people could be one of the risk factors in PD (Zhu, van Hilten, and Marinus 2016). Antidepressant medications also found to increase the chance of developing PD (Reference?). Although the pathogenesis of PD found to be multifactorial, the mechanism of the disease remains unknown (Moore et al. 2005).

![Schematic diagram showing the normal and the diseased nigrostriatal pathways](image)

**Figure 1.** Schematic diagram showing the normal and the diseases nigrostriatal pathways (Sulzer 2007).

The selective vulnerability of the nigral neurons in the PD neurodegenerative process could be explained by the sensitivity of these neurons to a specific stressful such as high physiological levels of excitation and intracellular Ca$^+$ loads, genetic background, preexisting conditions (e.g., diabetes), aging and predisposing factors; such as chronic
consequences of lesions, injuries from previous infections, and chronic consequences of stress and environmental toxins (Figure 2) (McKee et al. 2009) (Saxena and Caroni 2011).

Several stressful factors such as individual biases, local environmental factors, stress susceptibility, and sensitivity to misfolding-prone proteins cause specific neurodegenerative disease (NDDs). Neuronal connectivity and excitability may have a significant role in determining the intrinsic sensitivity to stress causing dysfunction and decease to the neurons. The loss of the neurons could be due to the accumulation of toxic protein species that could spread to other exposed cells (Arora and Fletcher 2013).

Figure 2: Schematic of How Gradually Increasing Stress in Affected and Selectively Vulnerable Neurons May Underlie the Etiology and Progression of NDDs (Saxena and Caroni 2011).

1.2. Prevalence of PD.

PD is the second most neurodegenerative disease in the worldwide after Alzheimer’s disease (AD). The lifetime risk of the disorder is 2% (Schapira 2009a). PD is an age-related disease, it affects around 2% of the population over the age of 60 and 5% of people over age 85 making aging the most significant risk factor for PD. The failure of cellular compromised mechanisms and the accumulation of age-related somatic cells due
to aging contribute to dopamine neuron demise in the PD rather than the disease onset (Hindle 2010; Collier, Kanaan, and Kordower 2011). Minor cases of PD are due to genetic mutation (Zhang et al. 2018). Over 1 million people in the united states alone are affected by this disabling disorder (Schapira ; Schapira 2009a). The prevalence of PD by geographical area was seen only in patients ages 70 to 79 years old (Pringsheim et al. 2014). PD attacks 50% more men than women. The incidence of occurrence of PD in the world reported up to 190 per 100,000 people (Ratner and G. Feldman 2004). According to the American Parkinson's Disease Association, the incidence of PD ranges from 8.6-19 per 100,000 people (Benjamin C.L. Lai 2001). Approximately 50,000 new cases are diagnosed in the U.S. annually. The prevalence of undiagnosed PD cases is expected to be about 3-4 million people or 1.10%.

The socioeconomic factors can easily affect the prevalence of the disorders, both direct and indirect cost for the treatment of PD can influence the incidence, severity, and course of progression pose a significant burden on those who suffer from it (Céu Mateus 2013). The national economic burden of PD exceeds $14.4 billion in 2010. The indirect cost of loss of productivity estimated to be $6.3 billion (MD 2013). The frequency of PD is a predicted to double or triple according to the current demographic trends as the size of elderly population grows. Most cases of PD are sporadic, the familial instances estimated around 1-2 % (Fernandez-Espejo 2004). The cost related to the treatment of PD in the United States for individual patient each year is staggering. The cost of Medication for a single patient average $2,500 a year and the cost of Curative surgery can be up to 100,000 dollars per patient. Such projections give an incentive for the need of innovative treatment for PD.
1.3. Factors implicated in the pathogenesis of the disease:

Although age is the most substantial risk factor involved in the development and progression of PD, numerous factors found to be implicated and contribute to its pathogenesis. These factors could include but not limited to oxidative, and nitrate stress, protein aggregation, genetics, inflammation, and environmental factors (J. Z Igmund and E. B Urke 2018). Protein aggregation, mitochondrial dysfunction, and oxidative stress are considered the most common pathological risk factors (Samluk, Chroscicki, and Chacinska 2018). The process of the interrelated events of these factors causes the degeneration of neurons (Jenner and Olanow 2006). It was not determined if PD is a single disorder with common pathogenic causes or it is a group of disease with different pathological mechanisms (Jenner and Olanow 2006). PD is considered to be a sporadic disorder like other neurodegenerative diseases, but in rare cases, it can be linked to familial genetic factors. These genetic mutations contribute to the pathogenesis of the dopaminergic neuron death (Przedborski 2005).

Based on current data, it appears that the loss of dopaminergic neurons results from the convergence of several pathogenic factors (Przedborski 2005; Shoichet MS 2008). Although several studies have been done to understand the mechanism of neuronal death in PD, several critical questions remain unanswered (Heman-Ackah et al. 2013; Warner and Schapira 2003). Oxidative stress happens due to some etiologic illnesses which increases the production of ROS with consequent compromization of the protective mechanism or the repair system (Rahal et al. 2014). Understanding of the pathogenesis of PD which distinguishes the factors that initiate the disease and the
factors lead to the progression and the development of the illness would be helpful to develop new treatment agents (Jenner and Olanow 2006).

1.3.1. Mitochondrial dysfunction and oxidative stress.

The intracellular membrane-enclosed organelles found in most eukaryotic cells is the Mitochondria, it plays many vital roles in these cells such as pyruvate oxidation, Krebs cycle, metabolism of proteins, lipids, hormones, and, many other functions. The most crucial role is the generation of energy such as the production of adenosine triphosphate (ATP) which is utilized by the mitochondrial electron-transport chain and the oxidative-phosphorylation system (Perier and Vila 2011). This evidence explained the mitochondria as a place where oxidative phosphorylation takes place, and it is an essential source for reactive oxygen species (ROS) (Rezende Figueira et al. 2012).

Recent studies showed that Mitochondria is involved either directly or indirectly in the pathogenesis of PD (Jaimes 2013; Rimessi et al. 2016). Mitochondrial programmed cell death found to be related to the diminishing of neurons in the SN, the protein involved in familial PD also found to be associated with mitochondria. The alteration of mitochondrial DNA with aging is also linked to PD (Jayaraj et al. 2013). Defects or abnormalities in mitochondria include elevated ROS and decreased ATP levels, especially in the complex I, can lead to neurodegeneration (Vila, Ramonet, and Perier 2008; Jayaraj et al. 2013).

As a regular function of mitochondria, the molecular oxygen is reduced to water at complex IV of the ETC during the process of oxidative metabolism. Other redox centers at a site of electron leak in complex I of the ETC may reduce non-enzymatically a small fraction of oxygen to superoxide (O$_2^-$) and H$_2$O$_2$ (Turrens 2003). Any deficiency in
complex-I can enhance the production of reactive oxygen species (ROS). Neurotoxin causing Parkinsonian syndrome found to inhibit mitochondrial respiration. Studies showed that several neurotoxic agents, such as rotenone, paraquat, and MPP + can have different mechanisms inside dopaminergic neurons in the mitochondria, and produce toxicity: by concentrating inside the mitochondria and causing toxicity, interacting with the cytosolic enzymes or uptaking via the vesicular monoamine transporters (VMAT) into the synaptic vesicles (Dauer and Przedborski 2003). MPP+ inhibits complex I, augments ROS production, and decreases the synthesis of ATP (Dauer and Przedborski 2003). Local ROS can also damage complex I (Wim Mandemakers 2007). The pathogenic role of mitochondria is still unclear (Haddad and Nakamura 2015). Glucolipotoxicity is considered the causative risk factor in mitochondrial dysfunction; the other aspects are the mutation of mitochondrial DNA, the exposure to an environmental toxin, the high-calorie intake and the homeostasis unbalance (Jha et al. 2017). Moreover, researchers found a link correlated between the mitochondrial dysfunction and the oxidative stress in PD (Martins Branco et al. 2010; Hastings 2009). Mitochondrial dysfunction leads to the deficit in energy supply and generation of oxidative stress. Exposure of the mitochondria to DA oxidized products resulted in mitochondrial respiration dysfunction occurs due to the imbalance in the antioxidant mechanisms (Figure 3) (Müller et al. 2010; Shahul Hameed 2011).

The two mechanisms generated in the mitochondria for PD states: (1) Primary mitochondrial dysfunction involved in the generation of endogenous or exogenous toxic metabolites in dopaminergic neurons. (2) The secondary mitochondrial dysfunction happens due to genetic mutation toxicity (Exner et al. 2012; Hastings 2009). Besides DA,
its metabolite products may enhance the degeneration of neurons in PD patients (Martins Branco et al. 2010). Although several factors have contributed to the development and pathogenesis of PD, oxidative stress has been found to have a significant implication for the production of free radical (Siegfried Kösel 1999; Shankar J. chinta 2008). Reactive nitrogen species such as NO and its metabolite peroxynitrite (PN) may also play a primary role in the etiology of PD. NO is known to inhibit complexes I and IV of the mitochondrial electron transport chain (Shankar J. chinta 2008).

**Figure 3.** Schematic representation of MPP+ intracellular pathways (Müller et al. 2010).

**1.3.1.1. Enzymatic and auto-oxidative DA metabolism.**

Studies showed that presence of dopamine, decreased antioxidants (ex; low glutathione level), and increase of iron level are the primary causes of the dopaminergic neurons death due to the induction of ROS generation (Chinta and Andersen 2008). The selectivity of the demise of DA neurons at the SNpc may belong to oxidative stress (Blesa et al. 2015). Dopamine (DA) is a monoamine compound released by neurons and function as a neurotransmitter. DA undergoes auto-oxidation to form dopamine quinones, and free radicals (Figure 4). Dopamine is synthesized in the brain from tyrosine which undergoes hydroxylation by thyroxin hydroxylase to DOPA and then decarboxylation to Dopamine (Figure 4) (Meiser, Weindl, and Hiller 2013). Dopamine, therefore, is stored in synaptic
vesicles in the presynaptic region after uptake by the vesicular monoamine transporter 2 (VMAT2) (Wimalasena 2011).

**Figure 4.** Dopamine synthesis in neurons (Meiser, Weindl).

The excess amount of DA which is not stored in the VMAT2 would undergo hydroxylation by MAO, this process called auto-oxidation (Wimalasena 2011; Chinta and Andersen 2008). Auto-oxidation of dopamine may be amplified in the first stages of the disorder (Zhou, Huang, and Przedborski 2008). Monoamine oxidase is an enzyme involved in the degradation of primary, secondary and tertiary amine such as catecholamines. Monoamine oxidase, specifically, MAO-B catalyzes the oxidative deamination of dopamine in the nerve terminal (substantia nigra and striatum) to form the oxidized dopamine (dopamine-quinone, dopamine-semiquinone and neuromelanin), and free radicals (hydrogen peroxide), H$_2$O$_2$ is the common product in this process (Jenner 2003).

In mitochondria, catecholamines including dopamine get metabolized enzymatically by MAO-B and MAO-A, flavin based protein, to produce hydrogen peroxide, superoxide, and hydroxyl radical (Jr. 2012). Moreover, oxidation of dopamine by MAO mainly by MAO-B produces 3,4-dihydroxyphenylacetaldehyde and anionic semiquinone flavin. Quinones and semiquinones are also as toxic as hydrogen peroxide to the cells as they can bind to DNA, lipids, and proteins in the cells (**Figure 5**). The anionic semiquinone Flavin interacts with oxygen to make superoxide which attacks DNA (Jr. 2012). Autoxidation in PD is a mechanism of cell loss leading to apoptosis (programmed cell
Dopamine oxidation by MAO found to be the dominant risk factor involved in the progression of PD (Meiser, Weindl, and Hiller 2013). Studies showed that the level of MAO-B enzyme increases with aging that would increase H$_2$O$_2$ production in the glial cells, H$_2$O$_2$ can cross into the nearby dopaminergic cells, and influence the dopaminergic neuron (Hussain et al. 2018). It is understood that oxidative stress plays a specific role in the degeneration of dopaminergic neurons in PD (Dias, Junn, and Mouradian 2013). The autoxidation of dopamine and its regulation are essential factors in determining the loss of the dopaminergic neurons, which are characterized in humans by the presence of neuromelanin (Figure 6) (Olanow 1999).

\[
\text{Dopamine (QH$_2$)} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{MAOB}} \text{Semiquinones} + \text{QH}^- + \text{QNO} + \text{H}_2\text{O}_2
\]

**Figure 5.** Enzymatic oxidation of DA by MAO-B, yielding toxic metabolites.

**Figure 6.** Auto-oxidative metabolism of DA.

Nitric oxide (NO) plays a vital role in cell functions through the signal transduction pathway (Zhang, Dawson, and Dawson 2006). It also involves in the pathogenesis of the neurodegenerative diseases. While the mechanism of implication of NO in PD is not fully understood, studies showed that NO might cause neuronal death, DNA damage, protein aggregation, and misfolding. In the substantia nigra of Parkinson’s patients, high level of nitric oxide (NO) was measured (Aquilano et al. 2008). Nitric oxide (+NO) is produced either from the inducible form of nitric oxide synthase (iNOS) or from the neuronal form
(nNOS). NO then participates in the cascade of events which leads to the degeneration of dopamine-containing neurons (Figure 7) (Ahlawat et al. 2014).

Figure 7. Proposed pathway to form quinones from free radical nitric oxide.

Homeostasis of iron is essential in the normal functioning of the nervous system. Human iron metabolism is the set of chemical reactions maintaining human homeostasis of iron at both systemic and cellular level to maintain the redox-signaling proteins functions in neuron cells and the neuronal survival (Willis and Sandyk 1992; Liu et al. 2017). Iron-mediated Fenton reaction potentially reacts with dopamine and produces a toxic hydroxyl radical which ultimately increases the oxidative stress that can damage the cells, the loosely bound iron Fe\(^{3+}\) or iron bound to neuromelanin gives the reduced form Fe\(^{2+}\) (Jenner 2003). Since the substantia nigra is rich in iron, it makes the area susceptible to free radical production in the presence of melanin (Hwang 2013). Which explains the significant role of iron in oxidative stress in PD brain. When the iron content of the SN is higher in the PD brain, it enhances the conversion of H\(_2\)O\(_2\) to hydroxyl radical via Fenton reaction. (Figure 8) (Pichler et al. 2013).

Figure 8. Oxidative Stress: Increased Iron Content.

1.3.1.2. Compromised antioxidant defense pathways
Oxidative stress has strongly been linked to the pathogenesis of PD due to the production of \( \text{H}_2\text{O}_2 \) and free radicals (Hwang 2013). Numerous neuroprotective strategies have been identified to attenuate ROS in the dopaminergic neurons, one of these strategies is the use of Fruits and antioxidants to reduce the damage caused by the free radicals. Vitamins C, E, and GSH are also essential antioxidants in the body that can be reutilized by antioxidant lipoic acid (Liu et al. 2017). Lipoic acid is another example of antioxidants in human cells, its mechanism of action to protect neurons against both oxidative stress and inducers that cause mitochondrial dysfunction (Ahmadinejad et al. 2017). The antioxidants mechanism is achieved by increasing the GSH generation or diminishing the lipid peroxide level in the brain which results in elevating the production of ATP and improvement of the motor function as a result of neuroprotection (Abramov 2012). Other examples of antioxidants are; glutathione peroxidase, catalase, and superoxide dismutase. The functions of these systems are scavenging of ROS, preventing ROS formation and repairing damage caused by ROS (Borut Poljsak 2013). In case of PD, the level of \( \text{H}_2\text{O}_2 \) elevated due to the reduction of glutathione and catalase enzymes levels in the Substantia Nigra region of PD patients (Mythri et al. 2011).

An appropriate amount of antioxidant is essential for the normal functioning of the cells. (Figure 9). Antioxidants scavenge the reactive oxygen species, prevents their formation as well as repairs the damage caused by them (Liu et al. 2017; Drummond et al. 2017). The antioxidant system is a complicated system consisting of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), glutathione, glutathione reductase, glucose-6-phosphate dehydrogenase (Chinta and Andersen 2008). Superoxide dismutase (SOD) catalyzes the conversion of two molecules of
superoxide to $\text{H}_2\text{O}_2$ and $\text{O}_2$, then catalase and glutathione peroxidase convert $\text{H}_2\text{O}_2$ to $\text{H}_2\text{O}$ (Stanley and Gerald 1992; Liu et al. 2017). The glutathione redox cycle should function well in cells to prevent the degeneration of neurons caused by reactive oxygen species (Schafer and Buettner 2001).

**Figure 9.** Oxidative stress: reduced antioxidant level (Lu 1999).

### 1.3.2. Protein aggregation.

Several genetic and environmental factors have been implicated in protein misfolding and aggregation (Soto 2003). Abnormal protein aggregation is a crucial feature in aging and several neurodegenerative diseases in which cells lose their ability to handle the misfolded protein (Takalo et al. 2013). Abnormal formation and aggregation of intracytoplasmic rounded inclusion or extracellular aggregates named Lewy bodies (LBs) are considered one of the pathologic hallmarks of PD (Takalo et al. 2013). $\alpha$-synuclein (ASN) is the primary component of Lewy bodies, and it is highly expressed in the mammalian brain. Although the exact physiological function of $\alpha$-synuclein is still unclear, it is found to be involved in the pathophysiology of PD. $\alpha$-synuclein, a protein consists of 140 amino acids, belongs to the synuclein family of helically folded tetramer that resists
aggregation (Figure 10) (Spinelli et al. 2014). The synuclein family includes α-synuclein, β-synuclein, and γ-synuclein. Although all three members of the synuclein family are neuronal proteins, only α-synuclein is implicated in neurodegenerative diseases. (Figure 10) (Spinelli et al. 2014; Chan et al. 2012; Xu and Chan 2015).

**Figure 10.** α-synuclein protein domain structure. α-synuclein protein is composed of three distinct regions: (1) an amino acid terminal, from 1-60 is the amphipathic region which has a positive charge and contains Apolipoprotein which gives α-helical structure on the membrane binding, (2) a central hydrophobic region called NAC (non-Aβ component), residue of 61-95, responsible for oligomerization and fibrillization and gives a β-sheet structure, and (3) a carboxyl terminal, the rest of the protein 96-140 is the acidic feature region, which has a negative charge, and this part is unstructured (Xu and Chan 2015).

In the normal state, alpha-synuclein is unfolded and exists as a monomer. Under the pathological condition, the protein undergoes misfolding, and also with aging, the cells lose the ability to handle misfolded protein. Mutant α-synuclein is more prone to aggregation and causes cell death in PD (Chan et al. 2012). Genetic abnormalities and environmental factors may accelerate the process (Irwin, Lee, and Trojanowski 2013). In normal condition, the cells have a specific system to reverse and prevent the misfolding of proteins; this system includes chaperones, ubiquitin proteasomes, and phagosome-lysosome. In pathological conditions, the systems are overwhelmed by oligomeric species of α-syn (Irwin, Lee, and Trojanowski 2013). However, it is not known which pathogenic species of α-syn is toxic to neurons. Studies showed that the synthetic α-syn fibrils
precipitation in neurons alone could transmit PD between neurons to cause cell death (E. Burke et al. 1999).

**Figure 11.** Misfolded alpha-synuclein proteins. ASN in pathological conditions is misfolded into abnormal beta-sheets (dimers, trimers, and oligomers) instead of alpha-helices, that further aggregate and bend to form protofibrils, the protofibrils then further precipitate to form fibrils (amyloid fibrils), and eventually transformed into Lewy bodies. Deposition of α-synuclein protein in Lewy bodies is a hallmark for PD diagnosis. (Lee, V.M-Yet al, 2006)

### 1.3.3. Genetic factors.

Genetic mutations or variants are potential biomarkers in the diagnosis and identification of persons at the risk of PD (Jankovic 2007; Klein and Westenberger 2012). Although the cause of dopaminergic degeneration in idiopathic PD is unclear, most cases of PD are sporadic (Fernandez-Espejo 2004) in nature. Genetic factors are usually associated with familial PD (Zhang et al. 2018). Although several genes have been associated with PD, genetic influences are responsible for only rare cases (Massano and Bhatia 2012). The estimated incidence of familial PD is 1-2%. Genetic components play a dominant role in the pathophysiology of the disorder with early and late onset, and also involved in the nigrostriatal loss of the dopaminergic neurons (Massano and Bhatia 2012; Klein and Westenberger 2012). Family history with PD leads to increase in susceptibility to develop the syndrome (Ratner and G. Feldman 2004). Also variant genes such as SNCA over-expression and parkin gene mutation have been involved in the pathogenesis.
of PD; they accounted for a small fraction of the overall the frequency of the disease (Dick et al. 2007).

1.3.4. **Environmental factors.**

Environmental factors are other pathogenic aspects connected to the etiology of PD, such as pesticides, herbicides, and insecticides (Olanow 1999; Ratner and G. Feldman 2004). In the absence of identified genetic causes, environmental factors appear to have a role in the cause of the disease. In another word; people who develop PD due to exposure to the environmental influences are less likely to have a family history of the disorder (Hancock et al. 2008). According to the epidemiologic studies, exposure to environmental toxicants is related to increase the risk of PD. Example of environmental toxins are; pesticides, solvents, metals, and other pollutants (Goldman 2014). Nigral degeneration may occur by binding of oxidative stress and other metabolites to glutathione which happens through the exposure to the industrial toxic chemicals (Figure 11) (Ratner and G. Feldman 2004). The amount of pesticide and the degree of exposure that may lead to the development of PD still unknown, and the identification of the agent that causes the disorder is challenging (Dick et al. 2007). In necropsy studies; the level of the organochlorine pesticide found to be high in patients with PD (Dick et al. 2007).
Figure 11. Factors contributed in the pathogenesis of PD.

1.4. Therapeutic strategies in PD.

Several significant advances have been made to understand the etiology, pathology and clinical phenomenology of PD that have underlined the development of symptomatic treatment and also the possibility to extend the intervention of medications that might stop or slow the progression of the disease (Schapira 2009b). This section explains the different treatment strategies that could be used to treat Parkinson’s patients. According to researchers, two types of treatment could be applied: symptomatic and neuroprotective treatments.

1.4.1. Symptomatic therapy.

Understanding of the clinical manifestation in PD is necessary for the diagnosis of the disease to choose the proper medication and treatment (Oscar Bernal-Pacheco 2012). From the symptomatic treatment point of view, enormous progress has been made in the treatment of PD over the past decades; Levodopa remains the gold standard in controlling the symptoms of PD. Before choosing the first line medication for PD, the level
of impairment and the precise diagnosis must be done (Jankovic and Aguilar 2008). The treatment plan for each PD patient should depend on each individual, the medications alleviate the symptoms in Parkinson’s patients (Chen et al. 2016). Drugs that produce symptomatic relief in PD act either by elevating the regional dopamine levels such as levodopa, monoamine oxidase (MAO) Inhibitors, catechol-o-methyl-transferase (COMT) inhibitors or by stimulating dopamine receptors as dopamine agonists (DA), inhibiting the effect of cholinergic afferents (anticholinergics), or inhibiting glutaminergic NMDA receptors (amantadine) (Figure 12, 13) (Chen et al. 2016; Jankovic and Aguilar 2008; 'Parkinson Disease: Neurologic Pathways & Drug Targets' 2015).

1.4.1.1. **Levodopa Therapy.**  

L-Dopa is a DA precursor (L-3,4-dihydroxyphenylalanine), it was introduced in the late 1957 to reverse Parkinson-like akinesia. In 1967 it was reported that oral administration of L-DOPA improved the rigidity and akinesia in PD. The improvement was significant but with short duration of action (McDowell and Lee 1970). Levodopa is the first pharmacological approved drug to treat PD, and considered the gold standard in PD treatment (Nagal and Singla 2012). Although, L-dopa reduces the motor symptoms of PD; it does not show any effect on the non-motor symptoms nor halt the progression of the disease (Mercuri and Bernardi 2005). Levodopa is given in combination with carbidopa (DOPA decarboxylase inhibitor) to prevent the premature conversion of L-DOPA into dopamine in the peripheral nervous system (PNS). This combination reduces the side effect of L-dopa on the periphery cardiovascular effects such as nausea and vomiting as well as and increases its pharmacological effects on the CNS. Levodopa in
the CNS is converted by the DOPA-decarboxylase enzyme to DA (Nagal and Singla 2012). Long-term use of levodopa causes irreversible dyskinesia and motor fluctuations

1.4.1.2. Dopamine Agonist.

Dopamine agonists act directly on dopamine receptors at the postsynaptic region. Many physicians recommended the use of dopamine agonist as a first-line of treatment in PD. Besides, giving dopamine agonist as monotherapy, it is also used as adjuncts therapy to levodopa. For example, bromocriptine and pergolide have been used with levodopa to reduce its motor complications (Mercuri and Bernardi 2005). Dopamine agonist is used in PD to delay the need for levodopa; such delay is recommended to delay or prevent levodopa-induced complications. Pramipexole, a dopamine agonist, was given to PD patients in combination with levodopa, to reduce the severe side effects associated with levodopa (Amos D. Korczyn 2004; Murata 2009). Although this combination reduces the motor complication of levodopa, it causes increased somnolence and hallucination. Pramipexole and ropinirole are other examples of dopamine agonists, and they are expected to have a lower risk of complications than other dopamine agonists because of non-ergolines. Pramipexole found to be safe and effective in the early stage treatment of PD when used as monotherapy (Jankovic and Aguilar 2008). Ropinirole has also exhibited to be safe in a specific dose and effective in the early PD.

1.4.1.3. Monoamine Oxidase Inhibitors (MAO-Is).

Monoamine oxidase inhibitors are used in the treatment of symptoms of PD. They work by blocking the degradation of dopamine by inhibiting the monoamine oxidase enzyme (Schapira 2011). Current treatments of PD involves the use of selective MAO-B inhibitors that addresses the deficiency of dopamine (Edmondson and Binda 2018). MAO-
Is have also been studied for possible neuroprotective properties as a result of decreasing the oxidative stress (Edmondson and Binda 2018; Schapira 2011). They are used as an alternative therapy to treat PD. Selegiline and safinamide are currently selective MAO-B inhibitors used in the market (Edmondson and Binda 2018). Selegiline was developed to address the symptoms in the early stage of PD (Schapira 2011), it also showed mild antidepressant effect. Studies suggested that selegiline might have neuroprotective properties. Selegiline develops some sympathomimetic side effects such as heartburn, nausea, dry mouth, dizziness, confusion, as well as hallucination (H and S 2006). Safinamide demonstrates glutamatergic antagonist effect, studies showed that selegiline and safinamide might be less effective to treat the motor complication compared with previous PD therapy (Schapira 2011; Blair and Dhillon 2017). When applied as monotherapy, MAO-B inhibitors provide a modest effect, but the motor function was significantly improved, MAO-Is can also delay the demand for levodopa (Teo and Ho 2013). Combination of selegiline or safinamide with dopamine agonist showed significant improvements in the treatment of early stage of PD (Teixeira et al. 2018; H and S 2006). MAO-Is showed good efficacy and less side effect, so they are recommended as monotherapy in the early stage of treatment of PD (Löhle and Reichmann 2011). MAO-Is are used as an add-on to levodopa in the advanced stage of PD, and as monotherapy in the early stage of the disorder (Dézsi and Vécsei 2014). MAO-B inhibitors significantly reduced off-time and were comparable in efficacy to COMT inhibitors (Livia and Laszlo 2017).
1.4.1.4. Catechol-O-methyl transferase inhibitors (COMT-Is)

Catechol-O-methyl transferase COMT is a primary enzyme in the metabolism of catecholamine compounds such as dopamine, norepinephrine (Nissinen and Männistö 2010). Entacapone and tolcapone are examples of COMT-inhibitors, they are given in combination with levodopa to increase its bioavailability and efficacy. They may also be used for individuals who are not tolerant to dopamine agonist and are experiencing wearing off (Rinne, Ulmanen, and Lee 2003). Clinically used COMT inhibitor, Entacapone has been used as an adjunct therapy with levodopa to treat PD who do not suffer from motor fluctuations (Rinne, Ulmanen, and Lee 2003).

1.4.1.5. NMDA glutamate type receptor

To overcome the motor complication side effects associated with previous PD medications, an NMDA-type glutamate receptor antagonist know as amantadine was developed (Chan et al. 2013). Amantadine acts by increasing dopamine release and blocking dopamine reuptake in the presynaptic region (Amos D. Korczyn 2004). Despite no clinical evidence of the effect of Amantadine in the treatment of motor function complication in PD, it is currently used to reduce dyskinesia (Nagal and Singla 2012). Amantadine may also cause a higher risk of psychiatric adverse effects in progressed PD patient (Nagal and Singla 2012; Bédard et al. 2011; M Goldenberg 2008; Chan et al. 2013).
<table>
<thead>
<tr>
<th>DA replacement therapy</th>
<th>Levodopa</th>
<th>Carbidopa</th>
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<tbody>
<tr>
<td>Dopamine agonists</td>
<td>Ropinirole</td>
<td>pramipexole</td>
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<tr>
<td>MAO-inhibitors</td>
<td>Selegiline</td>
<td>Safinamide</td>
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<tr>
<td>COMT inhibitors</td>
<td>Ropinirole</td>
<td>Pramipexole</td>
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<tr>
<td>NMDA-Glutamate type receptor</td>
<td>Amantadine</td>
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**Figure 12.** Chemical structures of pharmacological treatments for PD.
1.4.2. Neuroprotective Therapy.

The idea behind the neuroprotection treatment strategy in PD is to delay or stop the progression of the disease with or without alleviating the symptoms. To develop a novel neuroprotective agent for PD, we need to understand the mechanism and the etiology of the disease. (Zhang, Dawson, and Dawson 2006). Several pathogenic factors have been strongly linked to the death of the neuronal cells in PD. These factors are oxidative and nitrative stress, mitochondrial dysfunction, protein misfolding, excitotoxicity, and inflammation. In PD, the dopaminergic neurons at the SNpc exposed to oxidative stress, which leads to the injury of the neurons. Using neuroprotective agents that will restore the damaged neurons or stop the damages of the neurons would be expected to provide neuroprotective effect (Sarkar, Raymick, and Imam 2016). L-Dopa is used to provide sympathetic relief by increasing the level of dopamine in the synaptic region, but
it could not modify the progression of the disease. Moreover, long-term use of L-dopa administration enhances the neuroprotection of neurons at the SNpc (Calabresi et al. 2015). Several dopamine agonists have been proven to exhibit neuroprotection properties such as pramipexole (Hall et al. 1996).

1.4.2.1. Mechanisms of Neuroprotection.

Since PD is considered as multifactorial, an ideal neuroprotective agent should have the ability to act by various mechanisms to counteract the disease progression. Several strategies have been applied acting on the various possible mechanisms incorporated in agents to act as neuroprotective compounds. Neuroprotection may act by numerous different mechanisms; increase the level of dopamine, reduce the need for L-dopa, activation of DA auto-receptors, antioxidant properties of DA agonists, and also as an antiapoptotic agent (Ossig and Reichmann 2013). The strategy of neuroprotection was proposed to address the deficiency of dopaminergic neurons in the substantia nigra either by using a replacement of dopamine as levodopa or by using MAO-I and COMT-I to prevent the oxidation of dopamine to increase the level of dopamine at the synaptic region and relief the symptoms of PD (Sarkar, Raymick, and Imam 2016). Another strategy of neuroprotection is the use of dopamine agonist to act directly on the dopamine receptor, the example of that is pramipexole which has an antioxidant mechanism, it can bind to the dopamine receptor at the postsynaptic region. Several antioxidant agents could be used to inhibit both oxidation of dopamine and formation of free radicals (Schapira 2009b).
1.5. **Overview of the dopamine receptor system.**

Dopamine neurons and their receptors are known to be implicated in the pathogenesis of PD (Hisahara and Shimohama 2011). Dopamine receptor is classified as a member of biogenic amine receptors belonging to G protein–coupled receptors (GPCRs) family (Butini et al. 2016). Dopamine is one of the monoamine neurotransmitters produced in several areas of the brain including the substantial nigral and the ventral tegmental area. This neurotransmitter activates five types of the G-protein coupled dopamine receptors, and they are classified into two groups: D1 like receptors: D1, D5, and D2 like receptors: D2, D3, D4. The D1 like receptors activate adenylate cyclase leading to increase the cyclic adenosine monophosphate (CAMP) while D2 like receptors inhibits the adenylate cyclase to reduce the production of CAMP (Figure 14) (Levant 1997) Goodman & Gilman's).

![Dopamine Receptor System](image)

**Figure 14.** Dopamine receptor system, (Goodman & Gilman's the pharmacological Basis of therapeutics).

1.6. **The clinical trial of neuroprotection in PD with dopamine receptor agonists.**

To develop a single neuroprotective therapy for Parkinson’s patients, it is essential to have a proper clinical diagnosis to find the common pathogenic factors (Jankovic
2007). In 1970 the first major neuroprotective clinical trial was designed is the DATATOP study. It was designed to test the neuroprotection in Parkinson’s patients using deprenyl (MAO-I) and Tocopherol (vitamin E). Deprenyl is a Type B monoamine oxidase inhibitor used in a dose of 10mg/day; MAO-B metabolizes the catecholamine including dopamine in the brain to its oxidized forms with the production of $\text{H}_2\text{O}_2$. $\text{H}_2\text{O}_2$ reacts with iron to produce hydroxyl radical resulting in oxidative stress. By giving seligiline, it will prevent the oxidation of dopamine and block the formation of ROS (Riederer and Youdim 1986).

$\alpha$-Tocopherol is a biologically active component of vitamin E, its mechanism of action to diminish the effect of lipid peroxidation by trapping free radical (Hassan, Stohs, and Murray 1985). The onset of disability was the primary endpoint as the indication to administrate L-dopa. The results showed that no beneficial effect of tocopherol and no interactions have seen between tocopherol and deprenyl. The endpoint time was determined to calculate the required time for L-dopa treatment. Deprenyl demonstrated a strong and significant effect on delaying the onset of disability required L-dopa treatment. The patients showed improvement in the first three months of treatment, and the motor symptoms became worse after the withdraw of the medication. The purpose of this clinical trial was to test if deprenyl or tocopherol can extend the length of the time before the patient require to administrate levodopa (group 1989).

In conclusion, there is no current treatment available for Parkinson’s patients that would either stop or slow the progression of the disease or restore the dopaminergic neuronal loss to the normal condition. The available therapy of PD in the market is classified as a symptomatic treatment, they only reduce the disorder symptoms to improve the patient life temporarily. By designing a combination therapy of symptomatic
and neuroprotective treatment we might improve the patient quality of life by diminishing or stopping the progression of the disease or restoring the loss of the damaged neurons as well as alleviating the symptoms of the disease. Example of that a combination has dopamine agonist property as well as antioxidant property. Using selegiline in this experiment can diminish the symptomatic symptoms in PD patients, while deprenyl has antioxidant property. The mechanism of action of selegiline is not fully understood. A studied was carried out to understand the exact mechanism of selegiline if its effect is due to its symptomatic influence or it has a neuroprotection outcome. The result of this study showed that selegiline has a neuroprotective effect.
CHAPTER 2

The unmet need to develop symptomatic and disease modifying therapeutics to treat Parkinson’s disease.

Parkinson’s disease is multifactorial in nature arising from the involvement of several pathogenic factors (Kalia and Lang 2015). It has been proposed that treatment of PD with a single target drug may not be adequate due to the complex pathogenesis of the disease process, therefore, multiple medication drugs (MMDs) could be a reasonable treatment approach due to their effectiveness to engage more than one targets at the same time (Youdim and Buccafusco 2005). MMDs is usually composed of two or three different drugs that could target various targets implicated in the pathogenesis of the disease (Morphy and Rankovic 2005). However, multiple medications with different pharmacokinetic, bioavailability and metabolism properties could be challenging and can cause serious problems especially for elderly people and people with chronic disease (Hague, Klaffke, and Bandmann 2005). If a patient has other issues, it could be possible to develop several side effects with MMDs. Another strategy is the combination of different medications in the same formulation to reduce the dosing time regimen of the drug and also improve the patient compliance (Andrea Cavalli 2008).

The new strategy and idea which has become the focus of the researcher lately is the designing and development of single multifunctional molecules (MFM) which can potentially target multiple pathogenic pathways involved in the pathogenesis of the disease (Andrea Cavalli 2008; Van der Schyf 2011). By combining different functional groups in the same molecule to introduce multifunctional properties that could provide a new avenue to treat the complex diseases where several factors are responsible for the
pathogenesis of the disease process. Therefore, by interacting with multiple targets at the same time could provide pharmacokinetic advantages over using MMDs as well as less toxicity and more compliance. (Youdim 2010; Morphy and Rankovic 2005; Bansal and Silakari 2014, JP et al. 2011, Youdim 2010). There is an urgent need for such multifunctional single drug (MFM) to treat PD that could bring a new avenue to address PD (Youdim 2010).

2.1. Previous work on development of multifunctional agents to treat neurodegenerative diseases.

Several studies have been done to develop single molecules with multifunctional activities to treat neurodegenerative diseases. The designing of such MFD was based on combination of two or three pharmacophore structures acting on multiple neuronal and biochemical targets (Youdim and Buccafusco 2005). This new model that addresses the pathogenicity and complexity of PD by using a multi-targeted-single-ligand approach strategy has been introduced and recognized for its potential to offer better outcomes (Van der Schyf and Geldenhuys 2011). Researchers also use the MFM strategy to target neurotransmitter receptors or enzymes within the neurons. For example, examples of such targets are monoamine oxidase, acetylcholinesterase and catechol-O-methyltransferase enzymes which are responsible for degrading catecholamine neurotransmitter, degrading acetylcholine, and for the metabolism of catecholamine neurotransmitters, respectively, (Youdim and Buccafusco 2005; Bansal and Silakari 2014). Examples of the single drug with two or more functional properties are shown in Figure 14 in chapter 1.
2.1.1. Combination of anticholinesterases and muscarinic M₂ receptor antagonism.

JWSUS-C75IX is a bifunctional compound is a potent AChE inhibitor and high affinity muscarinic M₂ receptor antagonist and is designed to be used as a cognitive enhancing drug (Fig 15?) (Van der Schyf, Geldenhuys, and Youdim 2006; Youdim and Buccafusco 2005). This combination exhibited the ability to enhance the release of acetylcholine leading to a decisive mnemonic action, and also showed a better safety profile than drugs that have only AChE inhibition activity by improving the pharmacokinetic properties as well by addressing the potential side effects.

2.1.2. Combination of anticholinesterases and brain-selective MAO inhibitors.

Ladostigil (TV3326) is a combination of two moieties, carbamate (cholinesterase inhibitory activity) and propargyl (a selective inhibitor of the brain MAO) moieties (Fig 15?). The selectivity of Ladostigil to inhibit the central MAO in the brain provides the compound with potential antidepressant activity due to increasing the level of serotonin in the brain. The neuroprotective effects has been attributed to the propargylamine moiety which activates the mitochondrial Bcl₂ family of proteins, protein kinase, C-mitogen-activated protein MAP (PKC-MAP) and the downregulation of Bad and Bax. (Youdim and Buccafusco 2005).

2.1.3. Combination of iron chelating and MAO inhibitors.

HLA20, M30, and M31 are neuroprotective compounds with bifunctional properties for being an iron chelator and MAO inhibitor (Van der Schyf 2011). The propargyl amine possesses neuroprotective activity by inhibiting the MAO enzyme which results in decreasing the production of H₂O₂ and the antioxidant iron-chelator group is present as a 8-hydroxyquinoline derivative. By combining the two pharmacophore groups led to the
development of neuroprotective agents with a potential activity to treat both Alzheimer’s (AD) and PD (Youdim and Buccafusco 2005). HLA20 demonstrated selective inhibition of MAO-B, while M30 was found to be a highly potent inhibitor for both MAO-A and MAO-B (Van der Schyf 2011). Ladostigil is a bifunctional medication used to treat dementia, depression in neurodegenerative diseases, and multifunctional antioxidant iron chelator group (Bansal and Silakari 2014).

2.1.4. Combination of anticholinesterases and NMDA receptor antagonism.

Donepezil (anticholinesterase inhibitor) and Amantadine (NMDA receptor antagonist) are used in a combination to treat dementia in moderate to severe Alzheimer’s disease by enhancing the memory with a potential neuroprotective property (Blanpied, Clarke, and Johnson 2005, Matsunaga, Kishi, and Iwata 2015, Gasparini et al. 2013). The mechanism of action of amantadine is to block the ion channels of NMDA receptor while the mechanism of action of donepezil as a reversible inhibitor is to block the breakdown of acetylcholine by inhibiting the acetylcholinesterase enzyme leading to increasing the level of acetylcholine at the cholinergic synaptic region (Bruno et al. 2012; Gasparini et al. 2013; Blanpied, Clarke, and Johnson 2005). There is no evidence if this combination could treat PD even though it improves the ability of thought process, functioning and behavior (Blanpied, Clarke, and Johnson 2005). By adding a L-dopa to the combination, the compound can be used to treat Lewy body dementia DLB (Youdim and Buccafusco 2005; Blanpied, Clarke, and Johnson 2005).

2.1.5. Combination of MAO-B and COMT inhibitors.

MAO-B inhibitors (selegiline and rasagiline) and COMT inhibitors (entacapone and tolcapone) are used in a combination as multifunctional medications to increase the level
of L-dopa in the brain (Youdim and Buccafusco 2005). MAO oxidase enzyme mainly MAO-B in the brain reduces the level of dopamine in the synaptic region via oxidation. Thus, MAO-B inhibitor prevents the MOA-B from breaking down dopamine. COMT's work by inactivating L-dopa through a methylation which block the conversion of L-dopa to dopamine. Therefore, inhibiting the action of COMT leads to increase the availability of dopamine.

2.1.6. Combination of neuroleptic D<sub>2</sub> receptor antagonist and an SSRI.

Eltropzine a neuroleptic dopamine D<sub>2</sub> receptor antagonist and an SSRI like fluvoxamine, fluoxetine, or citalopram are used in combination as antidepressant and antipsychotic drugs to improve the negative symptoms of schizophrenia as well as to diminish depression, without worsening the extrapyramidal side effects (Bansal and Silakari 2014).

2.1.7. Combination of squalene synthase inhibitor and anti-inflammatory.

A biphenyl morphine derivative (squalene synthase inhibitor) and naproxen (anti-inflammatory) are used in combination as potent anti-atherosclerotic agent. To build the pharmacophore of this compounds, the biphenyl ring of the morphine derivative was replaced with naphthalene part of naproxen to have an MFM which works as squalene synthase inhibitor to diminish the level of cholesterol and triglyceride and has antioxidant activity. (Bansal and Silakari 2014).
2.2. Development of multifunctional ligands to treat PD.

As was mentioned previously, the multifunctional compounds have become the focus of the researchers today to develop medications that may potentially address the multifactorial nature of PD by incorporating appropriate pharmacological activities targeting multiple pathogenic factors implicated in PD. There is an unmet need for multifunctional agents to treat PD because of the complex pathophysiology present in this disorder. Our research group has been working to develop MFD based on the hybrid template that was established earlier in the lab (Dutta, Fei, and Reith 2002). Our molecular template was designed to address several pathogenic factors implicated in the pathogenesis of PD (Johnson et al. 2012). The synthesized lead compounds based on

![Neuroleptic hybrids having both D<sub>2</sub> and serotonin antagonist activities](image-url)
the hybrid template have demonstrated full agonist activities at both D_2 and D_3 receptors; in addition to the high binding and functional activities for both receptors (Johnson et al. 2012). The synthesized compounds were tested in vivo and vitro assays. In vivo studies showed the ability of our lead compounds to penetrate the CNS and reverse the hypolocomotion of reserpinized rats in a PD animal model (Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Santra et al. 2013). The overall goal of designing and developing multifunctional small dopamine agonist molecules is to treat PD symptoms along with slowing or stopping the progression of the disease (Santra et al. 2013; Gogoi et al. 2011). A series of compounds based on the hybrid template structure was developed earlier by our group to target the critical factors involved in the pathogenesis of PD (Dutta, Fei, and Reith 2002; Johnson et al. 2012). The agonist head group was connected via a piperazine linker to other molecular moieties to produce various biological activities (Dutta, Fei, and Reith 2002; Johnson et al. 2012).

2.2.1. Development of multifunctional ligands to treat PD with neuroprotective properties.

Studies have reported that carbazole compounds exert their effects by enhancing the formation of neurons in the subgranular zone of the dental gyrus (Bashir et al. 2015; Głuszyńska 2015). Aminopropyl carbazole compounds P7C3 (Figure 16) was found to protect newborn neurons from apoptosis to enhance neurogenesis by stabilizing the mitochondrial membrane potential. P7C3 was also found to improve the function of the hippocampus, exhibit pro-neurogenic and show potent anti-oxidative activity (Głuszyńska 2015; Yin et al. 2014; Wang et al. 2014; Blaya et al. 2014). Carbazol compounds showed to block the degeneration of the neuronal cells in models of neurodegenerative diseases.
(Yin et al. 2014). P7C3 was modified by introducing a benzophenone and alkyne groups to able the compound to bind to nicotinamide phosphoribosyltransferase (NAMPT) (Wang et al. 2014). It was assumed that the protective properties of this derivative could be due to their ability to activate the phosphoribosyl transferase, which is the rate-limiting step in the salvage of nicotinamide adenine dinucleotide, by converting nicotinamide into nicotinamide adenine dinucleotide (Yin et al. 2014; Loris, Pieper, and Dietrich 2017b). Animal experiments results showed that these derivatives have the ability recover the intracellular levels of NAD which lost due to the administration of doxorubicin (Wang et al. 2014). The lead compound, P7C3-S243 was reported to block the axonal cell death and conserve the normal synaptic activity which correlated with the motor coordination (Yin et al. 2014). It was also reported that these carbazole molecules and their derivatives exhibit the ability to regenerate neurons in the substantial nigra in neurodegenerative diseases.

Studies displayed that P7C3A20 carbazole derivative analogs of P7C3 displayed greater efficacy and affinity than P7C3 (De Jesús-Cortés et al. 2012; Shurubor, Beal, Andersen Julie, et al. 2014). The compound also inhibited the mature neuronal degeneration and, enhanced the perinatal neurogenesis in neurodegenerative and acute injury models (Wang et al. 2014). P7C3A20 also demonstrated to produce significant decline in the atrophy of the cortical, and hippocampal neurons (Loris, Pieper, and Dietrich 2017b). Given the importance of the development of neuroprotection therapies for neurodegenerative disease, different strategies to achieve such goals have become the focus of researchers in this area.
2.2.2. Development of multifunctional ligands with MAOI property as neuroprotective therapeutic agents for PD.

Monoamine oxidase (MAO) is a family of a flavin adenine dinucleotide (FAD)-dependent enzyme responsible for the metabolism of catecholamine neurotransmitter molecules (the biogenic amines: serotonin, norepinephrine, and dopamine) in the outer membrane of the mitochondria in the neurons (Garbis and McElhatton 2007). Two isoforms of MAO enzyme have been discovered: MAO-A and MAO-B. These isoforms differ in their distribution in the body and the substrate they metabolize. Serotonin and norepinephrine are the substrates of MAO-A while benzylamine is the substrate of MAO-B (Strydom et al. 2010). MAO-A and -B inhibitors are used as therapeutic medications in the treatment of neurological diseases. MAO-AI has been used in treatment of Depression, and MAO-BI is used in the treatment of PD. Some MAO-IIs belong to the earlier class of drugs used in the treatment of PD (Strydom et al. 2010). The rationale behind the use of MAOIs in PD is to inactivate the metabolism of the neurotransmitters from being degraded to increase their level within the presynaptic neuron to escape into

Figure 16. Molecular structure of lead carbazole compounds based neuroprotective property.
the synaptic space to the site of action (Mongeau, Blier, and de Montigny 1997; Eisenhofer, Kopin, and Goldstein 2004). MAO-Is can also be classified into selective and non-selective inhibitors. Selective MAO-A inhibitors are effective in treating major depression while selective MAO-B inhibitors are effective in treating Parkinson disease (H and S 2006, Happe 2007; Dinesh et al. 2012). MAO-Is are used as a monotherapy or adjunct therapy to levodopa (L-DOPA) in the treatment of PD (Malco et al. ; Goldenberg 2008). The non-selective MAO-Is have restricted use in the treatment of depression in PD due to their adverse effects (Riederer and Laux 2011). MAO-Is are either reversible or irreversible. Reversible MAO-A inhibitors including tranylcypromine are recommended in the treatment of depression in PD. Selective irreversible MAO-B inhibitors including selegiline and rasagiline are preferred for the treatment of akinesia and motor fluctuations in PD (Finberg 2014; Entzeroth and Ratty 2017, J. Rojas et al. 2015). Although, MAO-Is inhibit the MAO enzyme, they do not inhibit MAO synthesis pathway (H and S 2006). MAO-Is can also inhibit enzymes other than MAO, such as dopamine-β-oxidase, diamine-oxidase, amino-acid decarboxylase and choline dehydrogenase (H and S 2006). The non-selective inhibitory property of MAO-Is appears only with very high doses of the inhibitors, example: tranylcypromine has limited use in the treatment of depression in PD because of its non-selectivity, on the other hand; the selective, reversible MAO-A inhibitors are promoted due to their more straightforward medical management (Finberg 2014). Medications that augment the release and production of serotonin such as tricyclic antidepressants, selective serotonin reuptake inhibitors may cause the serotonin syndrome if they are administered with the MAOIs, even at therapeutic doses ('Antidepressants' ; Michael-Titus, Revest, and Shortland 2010). The toxicity of MAOIs
may be caused by the biogenic amines such as tyramine resulting in hypertensive crises (Santos 1996; M Gardner et al. 1996). MAO enzymes play an important role in the protection of the intestinal and the hepatic systems while by inhibiting the MAO enzymes, the protective role is eliminated due to increase the level of tyramine which releases noradrenaline from the presynaptic vesicles causing a significant effect in increasing the blood pressure (Foley et al. 2000; Noreddine 2016). MAO-Is lose their selectivity if they are given in high dose combination therapies (H and S 2006). Having a MAO-Is with multifunctional activity could be an avenue to overcome the side effects associated with the current MAO-Is, (Morphy, Kay, and Rankovic 2004). Several studies were done in this field in an effort to design and develop MFD with MAO-I activity to treat neurodegenerative diseases specially PD, examples: MT-19, MT-20, KW-6002, and CSC (Figure 17) (Bansal and Silakari 2014; Youdim and Buccafusco 2005).

![Structures of multimodal cholinesterase-monoamine oxidase inhibitor-iron chelator radical scavenger, and MAO-B and adenosine 2A receptor antagonists.](image)

**Figure 17.** Structures of multimodal cholinesterase-monoamine oxidase inhibitor-iron chelator radical scavenger, and MAO-B and adenosine 2A receptor antagonists.
2.2.3. Development of multifunctional ligands to treat depression associated with PD.

According to the national institute of mental health, depression has been defined as a common severe mental disorder that affects the lives of people from the point of feeling, thinking, handling the regular activities including sleeping, eating, and working. People with depression categorized with the loss of pleasure and interest of activity (Slattery and Cryan 2012). Depression affects a high percentage of the population (between 15-20%) in the United States, and around 450 million people in the world (Anxiety and Depression Association of America). Depression can be categorized according to the severity, the period of the disease, and the way it develops under certain circumstances (‘Neurocognitive Disorders’).

According to the World Health Organization (WHO), depression is an enervating disease which classified as the second-most cause of disability worldwide. People who suffer from depression are more susceptible to committing suicide (Gopishetty et al. 2011c). According to the National Alliance of Mental Illness (NAMI), the cause of depression is not fully understood, it could be due to the combination of biological, genetical and environmental factors. Other aspects also could be involved in the implication of depression such as the experiences of trauma in the early age.

Although medications are approved in the market to treat depression, it is estimated that 30-40% of patients do not respond sufficiently to them. The therapies for depression include tricyclic antidepressants (TCA), and monoamine oxidase inhibitors (MAO-I) (Oestergaard and Mølstrup 2011). Tricyclic antidepressant and monoamine oxidase inhibitors medications were the first used as antidepressant drugs in the market.
Some of the examples include imipramine, amitriptyline, nortriptyline, phenelzine, and tranylcypromine (Figure 18) (Lopez-Munoz et al. 2007). However, although antidepressant drugs have been used for the treatment of depression over decades, they were plagued by their nonspecific interactions that cause severe side effects that may be due to their nonspecific binding (Cohen et al. 1982; Sarker et al. 2010; Taylor et al. 2005). The first-generation antidepressant medications were subsequently replaced by selective serotonin reuptake inhibitor (SSRI), selective norepinephrine reuptake inhibitor (SNRI), and dual reuptake inhibitor (SSRI/SNRI) which are currently in use such as fluoxetine, trazodone, and venlafaxine (Figure 18) (Lopez-Munoz et al. 2007; Hansen et al. 2005). The second generation antidepressant drugs were developed to overcome the challenges of the first generation by enhancing the effectiveness and decreasing the undesirable outcomes (Hansen et al. 2005). A significant proportion of individuals suffering from depression continue to suffer from their symptoms under the current therapies. Dual reuptake inhibitors still exhibit slow onset of action, low rate of response and side effects (Stahl et al. 2004). Dopaminergic activity has not been included in the current antidepressant therapy, when dopamine has been implicated strongly in depression (Gartlehner G; Santra, Gogoi, Gopishetty, Antonio, Zhen, Reith, et al. 2012). Preclinical and clinical studies, demonstrated anhedonia as a central component of depression, which develops due to deficit in dopamine activity (Dichter 2010). As the mesolimbic dopamine is associated with reward-related behavior (anhedonia), dopaminergic drug should address anhedonia in depression (Papp, Klimek, and Willner 1994). Therefore, triple uptake inhibitor which includes dopaminergic activity should address the unmet need to treat depression in PD (Sharma, Santra, and Dutta 2015).
Evolution of triple reuptake inhibitors as the next-generation to treat depression would be more efficacious (Liang and Richelson 2008; Sharma, Santra, and Dutta 2015). Triple reuptake inhibitor is a novel approach to develop new generation antidepressant which has been introduced recently to overcome the constraint associated with the current antidepressant drugs (Bruno, Mostafa El, and Pierre 2009). Researchers have found that treating depression in Parkinson’s patients in addition to treating the motor symptoms would improve the overall quality of life (Titova and Chaudhuri 2017). Indeed, researchers have also assumed that people with PD might have a higher number of reuptake pumps for the serotonin, the brain chemical messenger, than normal people (De Jesús-Cortés et al. 2012; 'Depression and Parkinson's disease: a review’ 1992; Marsh 2013; Cummings 1992). Studies showed that Parkinson’s people have high risk of developing depression than normal population. Developing medications to treat...
depression in PD would be a novel approach (Hemmerle, Herman, and Seroogy 2012). Our group has been actively embarked on the development of 3,6-disubstituted and 2,4,5-trisubstituted pyran derivatives targeting monoamine transporters (Santra, Gogoi, Gopishetty, Antonio, Zhen, Reith, et al. 2012). These pyran derivatives showed higher affinity for NET and SERT and moderate affinity for dopamine transporter (DAT) when compared with the piperidine counterparts (Zhang et al. 2005a). One of the explanations of the lower affinity for DAT could be due to the replacement of N-atom in the piperidine analogous by a less basic O-atom (Zhang et al. 2005a). The lead TUIs D-142, D-161 were designed and found to be effective in animal models of depression to show antidepressant-like activity (Santra, Gogoi, Gopishetty, Antonio, Zhen, Reith, et al. 2012; Gopishetty et al. 2011a). D-473 is another TUIs was tested in animal for its penetration to the brain, rat forced swim and locomotor activities. The compound showed good brain penetration and effective activity in rat forced swim test but did not exhibit any locomotion activity (Dutta et al. 2014). D-142 exhibited potent antidepressant activity by inhibiting the reuptake of serotonin, norepinephrine and dopamine, it also showed to diminish the immobility in the mouse tail suspension test (Figure 19) (Dutta et al. 2011).

![Figure 19. Molecular structure of lead pyran-molecule based triple uptake inhibitors.](image-url)
Chapter 3- Hypothesis and Specific aims.

Parkinson’s disease is the second most common neurodegenerative disorders that is considered as an age-related disease affecting a worldwide population (Hindle 2010; Collier, Kanaan, and Kordower 2011). The symptoms of PD are categorized into motor and non-motor symptoms (Visanji and Marras 2015). According to the current Parkinson’s theory (Braak’s hypothesis), the non-motor symptoms may appear early before the motor symptom, resulting in an early diagnosis of the disease that might help to prevent the progression of the disease (Visanji et al. 2013). The existing treatments for PD are classified into monotherapy and multitherapy (a combination of two medications), the monotherapy includes levodopa, monoamine oxidase B (MAO-B), inhibitors and dopamine agonists while the multitherapy includes catechol-O-methyl transferase (COMT) inhibitor with levodopa or dopamine agonists with levodopa (Amos D. Korczyn 2004). However, these medications are only able to improve the symptoms of the disease. Additionally, they produce severe side effects such as dyskinesia and motor fluctuation from long-term therapy with L-DOPA (Dushanova 2012; Sheikh et al. 2012).

3.1. Hypothesis

Parkinson’s disease (PD) is a chronic progressive neurodegenerative disease with multiple pathogeneic factors (Sheikh et al. 2012; Facecchia et al. 2011; Alves et al. 2008). To address the complexity of the disease process, compounds targeting several pathogenic features relevant to PD were developed to offer an advantage to treat the illness over a single targeting drug. The overall goal and hypothesis of this work is to design and develop multifunctional D2/D3 dopamine agonists to treat not only motor dysfunction symptoms in PD but also to provide disease modifying effects to slow the
progression of the disease. By addressing multiple pathogenic factors implicated in the pathogenesis of PD using multifunctional compounds, the progression and the symptomatic aspects of Parkinson’s disease could be potentially reduced or slowed. The proposed compounds are multifunctional dopamine D2/D3 receptors agonists with neuroprotective, antioxidants properties. Carbazole derivatives and MAO inhibitors have been demonstrated to exhibit neuroprotective properties and as such incorporation of these moieties in our multifunctional dopamine agonist template will address not only symptomatic aspect of the disease but also should provide neuroprotective properties (Głuszyńska 2015; Gopishetty et al. 2011b). The other goal of this work is to develop multifunctional small molecules to block the monoamine transporter based on unique pyran template that has been previously developed and established to produce monoamine transporter blocking activity which would not only treat symptomatic aspects of the disease but would also have the potential to treat depression, non-motoric symptom, accompanied with PD as a promising approach for new generation to treat PD (Gopishetty et al. 2011b).

3.2. General aim.

The general aim of this project is to design a library of D2/D3 dopamine agonist compounds by linking D2/D3 agonist moiety to other pharmacophore moieties with different functional activities to produce neuroprotective and antioxidant activities to treat PD. The synthesis of the lead compounds was relied on the hybrid template that previously established and developed to conduct a structure-activity relationship (SAR) study by linking D2/D3 agonist moiety to either novel carbazole moiety or pharmacophore of MAO-inhibitors. Another approach focussed on the development of unique pyran
template that has been designed and established to produce monoamine transporter blocking activity and the lead compounds were found to be efficacious both in vitro and in vivo assays.

3.2. Specific aims.

To accomplish the goal in this project, the following specific aims were proposed:

a. Design and synthesize of novel carbazole based multifunctional dopamine D$_2$/D$_3$ agonists that can potentially exhibit neuroprotective and antioxidant activities. The molecules are designed by linking carbazole moieties with D$_2$/D$_3$ agonist moiety through a piperazine linker based on the hybrid molecular template. A SAR study has been carried out to assess the effect of different molecular attachment of carbazole moiety at different position with the different D$_2$/D$_3$ agonist moieties.

b. Design and synthesis of D$_2$/D$_3$ agonists that can inhibit monoamine oxidase enzyme. The molecules were designed by linking the propargyl containing group to D$_2$/D$_3$ agonist through a piperazine linker based on hybrid molecular template. A SAR study has been carried out to evaluate the effect of MAO inhibiton activity by linking propargyl moiety to the different D$_2$/D$_3$ agonist moieties.

c. Design and synthesis of D$_2$/D$_3$ agonists that could potentially block the monoamine reuptake transporters by incorporating the novel pyran moiety to build the structure-activity relationship study.

3.2.1. In Vitro binding Studies (Radioligand binding assay).

The synthesized compounds have been assessed for their binding affinity and selectivity towards the human D$_2$ and D$_3$ dopamine receptors using in vitro competitive radioligand binding assays.

Based on the binding affinity, the Selected compounds were further evaluated for their functional activity through the *in vitro* functional assay.

3.2.3. *In vitro* neuroprotection study.

Selected lead compounds containing carbazole moiety have been also evaluated for their neuroprotective effect to protect PC12 cells from toxicity induced by 6-hydroxydopamine (6-OHDA) *in vitro* cellular neurotoxin-based model for PD.

3.2.4. *In vitro* Antioxidant study.

In addition to the neuroprotection assay, selected lead compounds containing the carbazole moiety have been assessed for their ability to reduce the reactive oxygen species generated by 6-OHDA in PC12 cells.

3.2.5. *In vitro* Monoamine oxidase inhibitors assay.

Selected lead compounds containing propargyl moiety have been tested for their ability to inhibit MAO enzyme activities using *in vitro* enzymatic assays.

3.2.6. *In vivo* assay with rat model of PD.

Selected lead compounds have been further evaluated for their efficacy and potency to reverse the hypolocomtion in reserpine-treated rat model, a PD animal model for symptomatic activity.
Chapter 4- Result and discussion

4.1. Overview.

This project has three main objectives. The first objective is to design multifunctional D$_2$/D$_3$ agonist molecules with neuroprotection and antioxidant properties to address multiple pathogenic factors of PD. Design of such hybrid molecular template by combining D$_2$/D$_3$ agonist head groups to carbazole moiety that might be suitable to modulate the pathogenic pathway of PD to produce neuroprotective properties. Lead compounds were identified from in vitro receptor binding and functional assays. Based on the D$_2$/D$_3$ binding assay, compounds were selected and subjected to the GTPyS functional assay. Compounds with agonist activity were selected for further in-vitro neuroprotection and antioxidant assays. Subsequently, lead compounds were subjected to in-vivo assay using a well-established Parkinson’s disease (PD) animal model. The second goal is to develop and design a series of multifunctional D$_2$/D$_3$ receptor agonist with monoamine oxidase B (MAO-B) inhibitory activity. The development was based on introduction of a propargyl group into the hybrid template. The third objective is to design and synthesize small molecules as Triple reuptake transporter inhibitors that can have the potential to treat depression, symptomatic aspects and non-motoric symptoms of PD based on the pyran template.

This chapter describes the chemistry involved in generating the library, the data from the in vitro binding and functional receptors assay, neuroprotection and antioxidants activities.
of selected compounds as well as the in vivo efficacy. The details of all the experiments and procedures will be explained in chapter 5.

4.2. Design, Synthesis and Pharmacological Characterization of Carbazole Based Dopamine Agonists as Potential Symptomatic and Neuroprotective Therapeutic Agents for Parkinson’s Disease.

In continuing our work to design multifunctional dopamine D₂/D₃ receptor agonists to address multiple pathogenic factors of PD and the symptomatic aspects involved led us to embark upon a drug discovery approach focused on novel multifunctional dopamine D₂/D₃ agonist molecules. Thus, we have shown from our recent studies that lead molecule like D-512 and D-607 (Figure 20) not only to have the potential to provide robust symptomatic effect but also produced potent neuroprotective effects in various in vitro and in vivo experiments (Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Johnson et al. 2012; Das, Kandegedara, et al. 2017; Das, Rajagopalan, et al. 2017). In our current study, we carried out our structure activity relationship (SAR) study on carbazole based molecules that have previously been identified to have neuroprotective properties (Gluszynska 2015; Wu et al. 2017; Wang et al. 2016; MacMillan et al. 2011). Specifically, our hybrid structure strategy (Figure 21) which combines D₂/D₃ agonist head groups with other moieties that are suitable to modulate the pathogenic pathway of PD, led to development of molecules to validate our proof of concept (Das, Kandegedara, et al. 2017; Biswas et al. 2008; Das et al. 2015; Modi et al. 2014; Luo et al. 2016; Johnson et al. 2012). Previous studies have shown that carbazole containing compounds exert neuroprotective properties by enhancing the formation of neurons in the subgranular zone of the dental gyrus (Pieper et al. 2010; Loris, Pieper, and
Dietrich 2017a). Moreover, scientists have reported that carbazole molecules and their
derivatives exhibit ability to regenerate the neurons in the substantia nigra in
neurodegenerative disease (Wang et al. 2016; Yoon et al. 2013; Loris, Pieper, and
Dietrich 2017a). Based on these findings, we designed and developed a number of
multifunctional molecules by covalently attaching $D_2/D_3$ agonist head groups such as
pramipexole and 5-OH-DPAT to various carbazole moieties through a piperazine linker
(Scheme 1-3).

In our current work, a series of compounds were synthesized, and the selected
compounds were characterized by in vitro binding and GTPγS functional assays to
examine the affinity and potency at both $D_2$ and $D_3$ receptors. The selected compounds
were subjected to further in vitro experiments to evaluate the neuroprotection and
antioxidants properties. In addition to the in vitro evaluation, PD animal model study was
established to assess in vivo activity in the reserpinized rats.

![Molecular structures of dopamine $D_2/D_3$ receptor agonists, and carbazole compounds with neuroprotective properties.](image)

**Figure 20.** Molecular structures of dopamine $D_2/D_3$ receptor agonists, and carbazole compounds with neuroprotective properties.
Figure 21. The hybrid molecule template for multifunctional dopamine D₂/D₃ receptor agonists.

4.2.1. Chemistry involved in the synthesis of carbazole based D2/D3 agonists.

In this study, a series of compounds were synthesized by incorporating the agonist head group (aminotetralin or bioisosteric equivalent) with carbazole functionality via ethylpiperazine linker (scheme 1, 2 and 3). Scheme 1 outlines the syntheses of final compounds (±)-10a, (±)-10b, (±)-10c and (-)-11a, (-)-11b, (-)-11c. A palladium catalyzed coupling of (4-bromophenyl)boronic acid and 1-bromo-2-nitrobenzene afforded 4'-bromo-2-nitro-1,1'-biphenyl (2a), which was then subjected to cyclization in the presence of PPh₃ to get 2-bromo-9H-carbazole (3a) (Kim and Lee 2013) followed by N-protection using di-tert-butyl dicarbonate in the presence of 4-dimethylaminopyridine (4-DMAP) to afford 4a. Palladium-catalyzed cross coupling of 4a with 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazine (Das et al. 2015) in the presence of Cs₂CO₃ and BINAP in toluene under refluxing condition yielded intermediate 5a. The silyl protecting group of compound 5a was removed by treatment with n-Bu₄NF (TBAF) in THF to afford the alcohol 6a, which
on subsequent oxidation in the presence of pyridine-sulfur trioxide yielded the corresponding aldehyde 7a. Reductive amination of the aldehyde with either (±) or (-)-pramipexole in the presence of NaBH(OAc)₃ afforded compounds 8a and 9a, respectively. Finally, the amine protecting t-Boc groups were removed by treatment with trifluoroacetic acid to furnish the final compounds (±)-10a and (-)-11a as TFA salts. The other final compounds (±)-10b, (±)-10c, (-)-11b and (-)-11c were also prepared in a similar fashion as described above, where 3-Bromo-9H-carbazole and 4-Bromo-9H-carbazole were used as the starting materials, respectively.

**Scheme 1.** Synthesis of the carbazole compounds 10a, 10b, 10c, 11a, 11b, and 11c. Reagents and conditions:  a) Pd(PPh₃)₄, 2M K₂CO₃, THF, 90 °C, 12 h; b) PPh₃, 1,2-dichlorobenzene, 170 °C, 12 h; c) (Boc)₂O, 4-DMAP, THF, rt, overnight; d) 1-[2-(tert-butyldimethyl-silylanyloxy)-ethyl]-piperazine, Pd(OAc)₂, BINAP, Cs₂CO₃, toluene, reflux, 24 h; e) n-Bu₄NF, THF, 0 °C to rt, 2 h; f) SO₃·py, CH₂Cl₂·DMSO (2:1), Et₃N, 0 °C to rt, 2 h; g) (±) or (-)-pramipexole, NaBH(OAc)₃, CH₂Cl₂, rt, 48 h; h) CF₃COOH, CH₂Cl₂, 0 °C to rt, 3 h.
**Scheme 2** depicts the syntheses of the final compounds (±)-14a, (±)-14b, (±)-14c and (-)-15a, (-)-15b, (-)-15c. To prepare these compounds, we employed (±) and (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine and reductively alkylated with intermediate aldehydes 7a-7c in the presence of NaBH(OAc)$_3$ as the reducing agent to afford compounds (±)-12a, (±)-12b, (±)-12c, (-)-13a, (-)-13b, and (-)-13c. Finally, demethylation and removal of the amine protecting t-Boc groups were carried out in one step by refluxing with aq. HBr to give the final compounds (±)-14a, (±)-14b, (±)-14c, (-)-15a, (-)-15b, and (-)-15c as HBr salts.

**Scheme 2.** Synthesis of the compounds (±)-14a (D-654), (±)-14b (D-650), (±)-14c (D-655), (-)-15a (D-653), (-)-15b (D-659) and (-)-15c (D-656). Reagents and conditions: a) (±) or (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine, NaBH(OAc)$_3$, CH$_2$Cl$_2$, rt, 48 h; b) 48% aq. HBr, reflux, 5 h.

The synthesis of three more target compounds (±)-20, (-)-21, and (-)-23 are illustrated in **scheme 3**. N-alkylation was first performed by refluxing carbazole with dibromoethane in presence of a mixture of K$_2$CO$_3$, KOH and TBAB to afford 9-(2-bromoethyl)-9H-carbazole 16. Base-catalyzed condensation of 1-(2-((tert-butyldimethylsilyl)-oxy)ethyl)piperazine with intermediate 16 yielded compound 17, which
on TBDMS deprotection in the presence of TBAF in THF afforded alcohol 18 in excellent yield. Alcohol 18 was next oxidized in the presence of pyridine-sulfur trioxide to yield the corresponding aldehyde 19, which was then condensed with (±) or (-)-pramipexole in the presence of NaBH(OAc)$_3$ to afford compounds (±)-20 and (-)-21. These two molecules were converted to their corresponding HCl salts by treatment with ethereal HCl. To prepare compound (-)-23 we employed (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine and reductively alkylated with intermediate aldehydes 19 in the presence of NaBH(OAc)$_3$ as the reducing agent to afford compound (-)-22. Finally, demethylation and removal of the amine protecting t-Boc groups were carried out in one step by refluxing with aq. HBr to give the final compounds (-)-23 as HBr salts. All the final compounds were characterized by $^1$H and $^{13}$C NMR as well as elemental analysis.

Scheme 3. Synthesis of compounds (±)-20 (D-626), (-)-21 (D-637), and (-)-23 (D-689). reagents and conditions:  a) K$_2$CO$_3$, KOH, TBAB, 50 °C, overnight;b) K$_2$CO$_3$, CH$_3$CN, reflux, 24 h;c) n-Bu$_4$NF, THF, 0 °C to rt, 3 h; d) SO$_3$.py, CH$_2$Cl$_2$:DMSO (2:1), Et$_3$N, 0 °C to rt, 2h; e) (±) or (-)-pramipexole, NaBH(OAc)$_3$, CH$_2$Cl$_2$, rt, 48 h.f) (-)-5-methoxy-1,2,3,4-
tetrahydro-naphthalen-2-yl)-propyl-amine, NaBH(OAc)₃, CH₂Cl₂, rt, 48 h; g) 48% aq. HBr, reflux, 5 h.

4.2.2. *In vitro* characterization of the Carbazole Based Dopamine Agonists molecules.

4.2.2.1. Potency and Agonism at DA D₂ and D₃ Receptors.

Our ongoing effort to develop multifunctional compounds for the symptomatic and disease-modifying treatment of PD is based on a hybrid drug design approach in which DA agonist head groups are covalently attached to a variety of moieties capable of producing biological effects which might be important for modulating the pathogenesis of PD (Das, Modi, and Dutta 2015; Dutta, Fei, and Reith 2002; Li et al. 2010; Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Yedlapudi et al. 2016; Das, Rajagopalan, et al. 2017; Das, Kandegedara, et al. 2017). Recently, we have developed compounds such as D-512 (*Figure 20*), which have revealed superior antiparkinsonian effects *in vivo* over a clinically approved drug, ropinirole (Lindenbach et al. 2017). Selected drugs also displayed neuroprotective properties in a myriad of *in vivo* and *in vitro* assays validating our proof of concept and thus, provide a strong support for our multifunctional drug development approach (Lindenbach et al. 2017; Das, Rajagopalan, et al. 2017; Das, Kandegedara, et al. 2017; Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Santra et al. 2013; Yedlapudi et al. 2016; Li et al. 2010). As a part of continuing our work, we have now designed and synthesized a series of molecules where covalent modification of core carbazole moiety at different positions has been incorporated into our hybrid D₂/D₃ agonist template. The rationale of using the carbazole is based on the fact that a novel aminopropyl carbazole P7C3 and its analogue P7C3A20 (*Figure 20*) have shown proneurogenic and
neuroprotective properties in aged rats, stabilized mitochondrial membrane potential and inhibited newborn hippocampal neuron apoptosis (Pieper et al. 2010). Our current study is aimed at investigating the influence of molecular and chemical flexibility of the carbazole fragment when attached to our hybrid template as it relates to D\textsubscript{2}/D\textsubscript{3} receptor binding and functional activity along with neuroprotection potential.

To evaluate receptor binding of the final compounds, a radioligand competition assay was conducted and the binding affinity profiles were compared with that of the reference agent (S)-5-OH-DPAT (Table 1). Binding affinity was determined by inhibition of [\textsuperscript{3}H] spiroperidol binding to rat DA D\textsubscript{2} and D\textsubscript{3} receptors expressed in HEK-293 cells as described by us previously (Biswas et al. 2008). Table 1 summarizes the binding data for analogues that were synthesized. Compounds (±)-10a-c, which incorporate racemic 2-aminothiazole head group and a piperazine ring connected to the different positions of carbazole ring, displayed high affinity for D\textsubscript{3} and low to moderate affinity for D\textsubscript{2} receptors.

When the positions of attachment are at carbon 2 and 3 of the carbazole moiety for compounds 10a and 10b, respectively, both the compounds displayed low affinity for D\textsubscript{2} and high affinity for the D\textsubscript{3} receptors with high selectivity (\(K_i\), D\textsubscript{2} = 902 nM, D\textsubscript{3} = 6.18 nM, D\textsubscript{2}/D\textsubscript{3} = 146 and D\textsubscript{2} = 612 nM, D\textsubscript{3} = 3.12 nM, D\textsubscript{2}/D\textsubscript{3} = 196 for 10a and 10b, respectively). Interestingly, covalent attachment at position 4 of the carbazole ring dramatically improved the affinity for D\textsubscript{2} while that for D\textsubscript{3} receptor remained the same (\(K_i\), D\textsubscript{2} = 76.9 nM, D\textsubscript{3} = 7.8 nM, D\textsubscript{2}/D\textsubscript{3} = 9.86 for 10c). This indicated highest tolerance of the 4-substituted carbazole derivative for interaction with the D\textsubscript{2} and D\textsubscript{3} receptors. As expected, we observed a 2-4-fold improvement in binding affinity when enantiomerically pure aminothiazole moiety was attached to the carbazole as in (−)-11a and (−)-11b compared
to their racemic counterparts ($K_i$, $D_2 = 504$ nM, $D_3 = 3.94$ nM, $D_2/D_3 = 128$ and $D_2 = 135$ nM, $D_3 = 3.80$ nM, $D_2/D_3 = 35$ for (-)-11a and (-)-11b respectively). However, for (-)-11c we did not observe much difference from its racemic version.

Next, we wanted to evaluate the effect of bioisosteric replacement of the aminothiazole moiety with aminotetraline functionality on the receptor binding of target compounds. In corroboration with our previous results (Ghosh et al. 2010a; Das, Kandegedara, et al. 2017). Aminotetraline substituted compounds (±)-14a-c and (-)-15a-c exhibited high affinity at both $D_2$ and $D_3$ receptors. For instance, the aminotetraline analogue (-)-15a has been found to have very high affinity for $D_2$ and subnanomolar preferential affinity for $D_3$ receptor compared to the corresponding thiazolidium counterpart (-)-11a ($K_i$, $D_2 = 71.2$ nM, $D_3 = 0.40$ nM, $D_2/D_3 = 177$ for (-)-15a vs $D_2 = 504$ nM, $D_3 = 3.94$ nM, $D_2/D_3 = 128$ for (-)-11a). Among the three enantiomerically pure isomers (-)-15a-c, which differ only in the substitution positions at the carbazole moiety, positions 2, 3 and 4, showed variable binding affinity at both $D_2$ and $D_3$ receptors ($K_i$, $D_2 = 71.2$ nM, $D_3 = 0.40$ nM for (-)-15a (; $D_2 = 61.6$ nM, $D_3 = 1.94$ nM for (-)-15b and $D_2 = 16.9$ nM, $D_3 = 0.36$ nM for (-)-15c). As discussed before, substitution at the 4-position of the carbazole aromatic ring resulted in compounds 10c, (-)-11c, 14c and (-)-15c) with better $D_2/D_3$ binding affinities in comparison to other isomeric analogues with compound (-)-15c exhibiting the highest affinity among all the molecules, underscoring the importance of positional attachment to the carbazole ring. Finally, the binding affinities were evaluated for another series of compounds in which the piperazine ring of the agonist fragment was appended directly to the carbazole nitrogen atom through a methylene linker. As shown in Table 1, enantiomeric compound (-)-21 displayed relatively
higher binding affinity at $D_2$ and comparable affinity at $D_3$ receptor with moderate selectivity compared to the racemic compound $(\pm)$-20 ($K_i$, $D_2 = 435$ nM, $D_3 = 6.60$ nM, $D_2/D_3 = 65.9$ and $D_2 = 82.6$ nM, $D_3 = 7.18$ nM, $D_2/D_3 = 12$ for 20 and $(\pm)$-21, respectively). This structural modification suggests no significant differences in DA receptor interaction between compounds where the carbazole moiety is attached either at the 2/3 positions of the aromatic ring or through the nitrogen atom; however, a prominent difference exists for compounds where the carbazole nitrogen is sterically free to probably participate in additional receptor interaction (e.g. $(\pm)$-15c vs $(\pm)$-15b and $(\pm)$-21).

**Table 1.** $K_i$ values (nM) for inhibition of $[^3H]$spiroperidol binding (HEK - $D_{2,3}$ cells)$^a$ (cLogP and tPSA values are calculated using ChemDraw)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ (nM)</th>
<th>$D_{2L}$, $[^3H]$spiroperidol</th>
<th>$D_3$, $[^3H]$spiroperidol</th>
<th>$D_{2L}/D_3$</th>
<th>cLogP</th>
<th>tPSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(\pm)$-10a (D-652)</td>
<td>902 ± 132</td>
<td>6.18 ± 0.91</td>
<td>146</td>
<td>4.87</td>
<td>60.13</td>
<td></td>
</tr>
<tr>
<td>$(\pm)$-10b (D-627)</td>
<td>612 ± 92</td>
<td>3.12 ± 0.62</td>
<td>196</td>
<td>4.87</td>
<td>60.13</td>
<td></td>
</tr>
<tr>
<td>$(\pm)$-10c (D-658)</td>
<td>76.9 ± 5.2</td>
<td>7.8 ± 1.17</td>
<td>9.86</td>
<td>4.87</td>
<td>60.13</td>
<td></td>
</tr>
<tr>
<td>(-)-11a (D-651)</td>
<td>504 ± 50</td>
<td>3.94 ± 0.62</td>
<td>128</td>
<td>4.87</td>
<td>60.13</td>
<td></td>
</tr>
<tr>
<td>(-)-11b (D-636)</td>
<td>135 ± 12</td>
<td>3.8 ± 0.38</td>
<td>35.4</td>
<td>4.87</td>
<td>60.13</td>
<td></td>
</tr>
</tbody>
</table>
(-)-11c  92.4 ± 8.5  4.18 ± 0.47  22.2  4.87  60.13
(+)-14a  62.1 ± 7.3  2.85 ± 0.62  21.8  6.12  41.98
(+)-14b  37.8 ± 4.7  1.87 ± 0.41  20.2  6.12  41.98
(+)-14c  29.4 ± 1.3  3.61 ± 0.28  8.13  6.12  41.98
(-)-15a  71.2 ± 9.6  0.400 ± 0.038  177  6.12  41.98
(-)-15b  61.6 ± 3.8  1.94 ± 0.18  31.8  6.12  41.98
(-)-15c  16.9 ± 1.9  0.362 ± 0.032  46.9  6.12  41.98
(+)-20  435 ± 90  6.60 ± 1.13  65.9  5.11
(-)-21  82.6 ± 13.8  7.18 ± 0.86  11.5  5.11

Results are expressed as means ± SEM for 3-6 experiments each performed in triplicate.

On the basis of the binding results, functional activities of the optically active lead compounds for human DA D<sub>2</sub> and D<sub>3</sub> receptors expressed in CHO cells were measured by monitoring stimulation of [<sup>35</sup>S]GTPγS binding in comparison to stimulation by the endogenous ligand DA (Biswas et al. 2008). Comparison with the maximum stimulation ($E_{max}$), produced by the full agonist DA, indicates whether the compound is a full agonist, a partial agonist, or an antagonist. As shown in Table 2, aminothiazole containing compounds (-)-11b and (-)-11c demonstrated moderate potency at both D<sub>2</sub> and D<sub>3</sub> receptors (EC<sub>50</sub> (GTPγS); D<sub>2</sub> = 48.7, D<sub>3</sub> = 0.96 nM and D<sub>2</sub> = 22.2, D<sub>3</sub> = 1.67 nM, respectively), correlating well with binding data. While (-)-11b showed full agonist activity at both D<sub>2</sub> and D<sub>3</sub> receptors ($E_{max}$ = 87-93%), compound (-)-11c revealed partial agonist activity at D<sub>2</sub> and full agonism at D<sub>3</sub> receptor ($E_{max}$ = 57% vs 82%, respectively for D<sub>2</sub> and D<sub>3</sub>). On the other hand, aminotetraline compound (-)-15a displayed very high functional potency (EC<sub>50</sub> (GTPγS); D<sub>2</sub> = 0.87 and D<sub>3</sub> = 0.23 nM) and full agonism ($E_{max}$ = 85-92%).
at both the receptors. Compound (-)-15c was also found to be highly potent and efficacious in stimulating both receptors (EC$_{50}$ (GTP$\gamma$S); D$_2$ = 2.29 and D$_3$ = 0.22 nM; $E_{\text{max}}$ = 74-88%). Neither compounds displayed appreciable selectivity for D$_3$ over D$_2$ (Table 2) and their selectivity for D$_3$ receptor dropped considerably when compared to the binding data (Table 1). We have also calculated the ClogP and tPSA values for all the compounds (Table 1). In general, the values indicate that these compounds should cross the blood brain barrier to produce in vivo CNS efficacy which we observed in case of (-)-11b, (-)-15a and (-)-15c (Table 1). Therefore, our current SAR results of a series of carbazole compounds indicate that the affinity and selectivity for the D$_2$/D$_3$ receptors are governed by the nature of covalent attachment to the carbazole moiety and the structure of agonist binding head group in the hybrid molecule.

Table 2. Stimulation of [${}^{35}$S]GTP$\gamma$S binding to cloned human D$_2$ and D$_3$ receptors expressed in CHO cells$^a$

<table>
<thead>
<tr>
<th>Compound</th>
<th>hCHO-D$_2$</th>
<th>hCHO-D$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[${}^{35}$S]GTP$\gamma$S</td>
<td>[${}^{35}$S]GTP$\gamma$S</td>
</tr>
<tr>
<td>Dopamine (DA)</td>
<td>146 ± 24</td>
<td>100</td>
</tr>
<tr>
<td>(-)-11b</td>
<td>48.7 ± 6.3</td>
<td>87.3 ± 2.1</td>
</tr>
<tr>
<td>(-)-11c</td>
<td>22.2 ± 6.9</td>
<td>56.7 ± 5.1</td>
</tr>
<tr>
<td>(-)-15a</td>
<td>0.87 ± 0.098</td>
<td>85.2 ± 4.7</td>
</tr>
<tr>
<td>(-)-15c</td>
<td>2.29 ± 0.70</td>
<td>73.6 ± 10.1</td>
</tr>
</tbody>
</table>

$^a$EC$_{50}$ is the concentration producing half maximal stimulation. For each compound, maximal stimulation ($E_{\text{max}}$) is expressed as a percent of the $E_{\text{max}}$ observed with 1 mM (D$_2$) or 100 µM (D$_3$) of the full agonist DA ($E_{\text{max}}$, %). Results are the mean ± SEM for 3–6 experiments, each performed in triplicate.
4.2.2.2. Antioxidant assay of the lead compounds.

Cellular antioxidant activity of (-)-11b, (-)-15a and (-)-15c. This experiment detection of reactive oxygen species (ROS) produced by 6-OHDA was carried out by DCFDA assay. 6-OHDA is a widely used toxin that mimics the generation of oxidative stress observed in PD and it induces neurotoxicity via its auto-oxidation and subsequent hydrogen peroxide generation (Blum et al. 2000; Soto-Otero et al. 2000). DCFDA is a non-fluorogenic dye but in the presence of ROS, it is oxidized to produce DCF which produces florescence. 6-OHDA is known to cause cell death in a dose-dependent manner via production of reactive oxygen species. From our previous and current dose-dependent experiment of 6-OHDA, we chose 75 µM 6-OHDA which can induce 40-50% cell death for our study. To examine whether our compounds (-)-11b, (-)-15a and (-)-15c can protect PC12 cells from the ROS produced by the exposure of 75 µM 6-OHDA, the PC12 cells were treated with 6-OHDA after pretreatment with various concentrations of drugs (5, 10, 20 µM) for 24 h, and compared with 6-OHDA treated alone cells. As shown in Figure 3, a well over two-fold increase in ROS was observed in cells treated with 6-OHDA (75 µM) alone compared to the control untreated cells (Figure 22). However, the test compounds could dose dependently decrease the production of ROS induced by 6-OHDA (75 µM) in PC12 cells. In this regard, highest dose of all the three compounds was the most efficacious in producing significant antioxidant effect. Thus at 20 µM, a reduction in 93%, 36% and 76% ROS were induced by (-)-11b, (-)-15a and (-)-15c, respectively. Thus, D-653 was found to be the most potent antioxidant.
Figure 22: Detection of ROS using carboxy-H$_2$DCFDA on relative fluorescence of PC12 cells induced by 6-Hydroxydopamine after pretreatment with different concentrations of D-636 (a), D-653 (b), D-656 (c) respectively. PC12 cells were pretreated with different doses of D-636 (a), D-653 (b), & D-656 (c) for 24 h, the drug containing media was replaced with carboxy-H$_2$DCFDA (20 µm, 2% serum) for 30 min, the carboxy-H$_2$DCFDA containing media was removed, and replaced with fresh media, followed by treatment with 75 µM 6-OHDA, and incubated for 1 h. The PC12 treated with compounds were compared with non-treated cells. Data represents mean ± SDs of three independent experiments in four to six replicates. One-Way ANOVA analysis followed by Turkey’s multiple comparison post doc test was performed. (*P < 0.1, **P < 0.01, ***P < 0.001, and ****P < 0.0001 compared to the 6-OHDA, ####P < 0.0001 compared to the control).

4.2.2.3. Neuroprotection Studies with PC12 cell line.

Neuroprotection Against 6-OHDA-Induced Toxicity. To investigate the multifunctional property of the target molecules, we next embarked on exploring the neuroprotective effect of (-)-11b, (-)-15a and (-)-15c in dopaminergic rat adrenal Pheochromocytoma PC12 cells against 6-OHDA-induced cytotoxicity. Treatment of PC12 cells with 6-OHDA for 24 h resulted in a significant dose-dependent neurotoxicity and the cell viability was significantly decreased to ~ 50% in cells exposed to 75 µM of 6-OHDA and this concentration was used in subsequent in vitro experiments (Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014). In contrast, cells treated with increasing concentrations of either (-)-11b or (-)-15c alone (0.01–30 µM) showed no cell loss at all compared to untreated controls (Figure 23a and 23e, respectively), indicating
the non-toxic profile of the compounds at the doses tested; however, (-)-15a showed some incremental toxicity starting from 20 µM dose (Figure 4c). This is an interesting finding in that a subtle change in the position of aromatic substitution from 2 to 4 in the carbazole moiety resulted in a dramatic improvement in cell survival. The potential neuroprotective effects of (-)-11b, (-)-15a and (-)-15c on 6-OHDA-induced toxicity were evaluated following pre-treatment with the drugs. Thus, when the cells were pre-treated with the test compounds for 24 h followed by exposure to 6-OHDA treatment for another 24 h, the compounds dose-dependently protected the cells from the neurotoxic insult and the greatest protective effect was obtained at concentration of 5 µM for (-)-11b and 20 µM for (-)-15c, both of which increased the cell survival by ~20% compared to 6-OHDA (75 µM) treated alone (Figure 23b and 23f). These data strongly suggest the neuroprotective effect of both the compounds on PC12 cell loss induced by 6-OHDA. Compound (-)-15a also revealed dose-dependent neuroprotection and the highest effect was observed at the dose of 10 µM (15%) but the effect was reversed at higher doses (Figure 4d). This could be due to the fact that the compound is little toxic to the cells at doses ≥20 µM as was seen in the drug-only toxicity experiment (Figure 23c).
Figure 23: Dose dependent effect of D-636, D-653, and D-656 on cell viability of PC12 cells from toxicity induced by 6-Hydroxydopamine after pretreatment with different concentrations of D-636. (a,b, and c) Dose-dependent effect of D-636, D-653, and D-656 respectively. (d,e, and f) PC12 cells were pretreated with different doses of D-636, D-653, and D-656 respectively for 1 h, followed by 75 µM 6-OHDA, and incubated for 24 h. Data represents mean± SDs of three independent experiments in four to six replicates.

4.2.3. In vivo Efficacy of lead molecules from carbazole series compounds.

Reversal of Reserpine-induced Hypolocomotion in Rats by (-)-11b, (-)-15a, (-)-15c and Ropinirole. In vivo evaluation of the compounds (-)-11b, (-)-15a and (-)-15c in PD animal model was next performed. Reserpine induces depletion of catecholamine in the nerve terminals, resulting in a cataleptic condition in rats, which is a well-established animal model for PD (Carlsson, Lindqvist, and Magnusson 1957; Skalisz et al. 2002). Significant inhibition of locomotion of rats was observed 18 h after the administration of reserpine (5 mg/kg, sc), which indicated the development of akinesia (Figure 24). Compounds (-)-11b, (-)-15a and (-)-15c at the dose of 10 µMol/kg, ip, were not only highly
efficacious in reversing akinesia in rats, compared to reserprine treatment alone, but also
demonstrated significant enhancement of locomotion for the entire duration of the study
of 6 h. Among the molecules tested, \((-\text{15a})\) was found to have the highest *in vivo* activity
and this finding correlates nicely with *in vitro* functional assay where the compound
exhibited subnanomolar potency for stimulation of D$_2$/D$_3$ receptors along with full agonist
property. In contrast, treatment with the reference drug ropinirole at the dose of 10
μMol/kg, ip, produced a quick onset of locomotor activation compared to control but with
a much shorter duration of action compared with the test compounds. The mechanism of
the locomotor stimulation in this reserpine model is likely to be mediated by postsynaptic
D$_2$/D$_3$ receptor activation by the compounds.

**Figure 24.** Effect of different drugs on reserpine (5.0 mg/kg, sc, 18h pretreatment)
induced hypolocomotion in rats. Results are expressed as means + SEM for three rats.
Plot represent horizontal locomotor activity at discrete 30 min interval after administration
of D-636 (10 μMol/kg, i.p), D-656 (10 μMol/kg, i.p) and **Ropinirole** (10 μMol/kg, i.p).
Significant effect was demonstrated by ANOVA analysis.
4.3. Design, and synthesis of novel multifunctional dopamine D<sub>2</sub>/D<sub>3</sub> receptors agonists containing a propargyl moiety for potential MAO-B inhibitory activity.

In this section, a series of molecules were synthesized by modifying the hybrid molecule template (Figure 25) as previously done for other compounds (Figure 20). The design of these molecules involved incorporation of the propargyl group that has been reported to show MAO-I activity, into the hybrid structure. The synthesis of these propargyl derived molecules are shown in scheme (4, 5, and 6). In the current template, the piperazine linker was attached to the propargyl group through either phenyl amine or phenyl alkoxy derivatives.

**Figure 25.** The hybrid molecule template for multifunctional dopamine D<sub>2</sub>/D<sub>3</sub> receptor agonists that may potentially inhibit the MAO-B activity.

4.3.1. Synthesis of multifunctional dopamine D<sub>2</sub>/D<sub>3</sub> receptor MAO-B inhibitors:

**Scheme 4** outline the synthesis of two N-propargyl compounds (±)-33 (D-671), and (±)-35 (D-677) in which the piperazine ring as a linker connects to the phenyl ring containing either N- or O-propargyl group. Palladium-catalyzed cross coupling of
commercially available 4-Bromo-benzonitrile 24 with 1-(2-((tert-butyldimethylsilyl)oxy)-ethyl)piperazine (Das et al. 2015) in the presence of Cs$_2$CO$_3$ and BINAP in toluene under refluxing condition yielded intermediate 25. The silyl protecting group of compounds 24 was removed by treatment with $n$-Bu$_4$NF (TBAF) in THF to afford the alcohol 26, which on subsequent oxidation by pyridine-sulfur trioxide yielded the corresponding aldehyde 27. Reductive amination of the aldehyde with (-)-5-(OH)-MPAT in the presence of NaBH(OAc)$_3$ afforded compounds 28. The intermediate 28 was reduced by borane:THF in 1.0 M THF in presence of Conc.HCl and 25 % aq. NaOH to yield the intermediate primary amine 29. The primary amine intermediate 29 was selectively protected by 2-nitrobenzenesulfonyl chloride to give the sulfonamide intermediate 30. The nitrobenzenesulfonamide intermediate 30 was reacted with the commercially available propargyl bromide to get the intermediate 31. Tertiary amine 31 was deprotected by thioglycolic acid to generate the secondary amine 32. Finally, the compound 32 was subjected to either demethylation by refluxing with aq. HBr to give the final compound (±)-33 (D-671), as HBr salt, or to N-methylation with 37% aqueous formaldehyde in a buffer system of NaH$_2$PO$_4$ to get the intermediate 34 which subjected to further O-demethylation by refluxing with 48% HBr to obtain the final compound (±)-35 (D-677) as HBr salt.
Scheme 4. Synthesis of compounds (±)-33 (D-671), and (±)-35 (D-677). Reagents and conditions: a) Pd(OAc)$_2$, BINAP, Cs$_2$CO$_3$, Toluene, reflux, overnight; b) TBAF, THF, 0°C to RT, 3 h; c) SO$_3$py, CH$_2$Cl$_2$:DMSO (2:1), Et$_3$N, 0°C to RT, 2 h; d) (-)-DPAT, NaBH(OAC)$_3$, CH$_2$Cl$_2$, 48 h; e) dryTHF, Borne in THF, 50°C, RT, 0°C, conc. HCl, 25% aq. NaOH; f) THF, nitrobenzylsulfonylchloride, -10°C, Et$_3$N, RT, 1.5 h; g) Propargylbromide, K$_2$CO$_3$, CH$_3$CN, reflux, 24 h; h) DMF, K$_2$CO$_3$, 0°C, thioglycolic acid, 1 h, comp. 30 added; i) 48% aq. HBr, reflux, 5 h; k) 37% aq.CH$_2$=O, NaH$_2$PO$_4$, zinc dust, H$_2$O, 30°C, 48 h. 

Scheme 5 describes the synthesis of the O-propargyl compound (±)-42 (D-678). The commercially available secondary amine intermediate 36 was protected by (Boc)$_2$O to get the tertiary amine Intermediate 37. O-alkylation of compound 37 with the commercially available propargyl bromide to give compound 38. The amine protecting group was removed by treatment with trifluoroacetic acid to get compound 39. The intermediate 39 was refluxed with bromoethanol to produce alcohol 40, which was subsequently oxidized by the Parikh–Doering oxidation to give the corresponding aldehyde intermediate 41. The aldehyde intermediate was then coupled with (±)-5-OH-MPAT to yield the final compound (±)-42 (D-678).
Scheme 5: Synthesis of compound (±)-42 (D-678). Reagents and conditions: a) (Boc)$_2$O, DMAP, THF, 24 h; b) propargyl bromide, K$_2$CO$_3$, CH$_3$CN, reflux, 24 h; c) TFA, CH$_2$Cl$_2$, 0°C, RT, 3 h; d) 2-bromoethanol, K$_2$CO$_3$, CH$_3$CN, reflux, 24 h; e) SO$_3$py, CH$_2$Cl$_2$:DMSO (2:1), Et$_3$N, 0°C to RT, 2 h; f) (±)-DPAT, NaBH(OAc)$_3$, CH$_2$Cl$_2$, 48 h.

Scheme 6 describes the synthesis of compound (−)-49. The commercially available intermediates 1-(4-Nitro-phenyl)-piperazine and 2-bromoethanol were refluxed in acetonitrile to get the alcohol intermediate 44, which was subsequently oxidized by the Parikh–Doering oxidation to give the corresponding aldehyde intermediate 45. The aldehyde intermediate 45 was then coupled with the (−)-5-OH-MPAT to get the intermediate 47. Commercially available propargyl bromide) was used to react with the primary amine intermediate 47 to form the intermediates 48, which was further demethylated by 48% HBr to produce the final compound 49.
**Scheme 6**: Synthesis of compound (-)-49. reagents and conditions: a) 2-bromoethanol, K$_2$CO$_3$, CH$_3$CN, reflux, 24 h; b) SO$_3$py, CH$_2$Cl$_2$:DMSO (2:1), Et$_3$N, 0°C to RT, 2 h; c) (±) or (-)-DPAT, NaBH(OAc)$_3$, CH$_2$Cl$_2$, 48 h; d) 10 mol.% pd/C, H$_2$gas, CH$_3$OH, 20 h; e) Propargylbromide, K$_2$CO$_3$, CH$_3$CN, reflux, 24 h; f) 48% aq. HBr, reflux, 5 h; g) CH$_2$=O, 37% aq. HCHO, NaH$_2$PO$_4$, zinc dust, H$_2$O, 30°C, 48 h; h) 48% aq. HBr, reflux, 5 h.

**4.3.2. In vitro D$_2$/D$_3$ receptor binding and functional assays with multifunctional MAOB-inhibitors.**

Our effort in this work is to develop multifunctional D$_2$/D$_3$ agonists with MAO inhibitory activity to treat and modulate the progression of PD (Das, Modi, and Dutta 2015; Dutta, Fei, and Reith 2002; Li et al. 2010; Shah, Rajagopalan, Xu, Voshavar, Shurubor, Beal, Andersen, et al. 2014; Yedlapudi et al. 2016; Das, Rajagopalan, et al. 2017; Das, Kandegegedara, et al. 2017). In this design DA agonist head groups are covalently attached to a propargyl moiety via a linker (Andrea Cavalli 2008; Naoi et al. 2003; Prins, Petzer, and Malan 2010). The rational of using propargyl moiety was based on the previous research work that propargyl group has monoamine oxidase inhibiton activity. The idea of including a MAO inhibiton moiety in our hybrid structure came from the previously synthesized MAO-I such as (R)-deprenyl which was shown to inhibit the MAO-B, to reduce the formation of H$_2$O$_2$ and dopaldehyde in the brain caused by MAO-B (W. 1992) (Figure 26). Rasagiline (2) is a potent MAO-B inhibitor (H, Aviva, and M 2001),
considered to be useful as an adjuvant therapy to levodopa for the treatment of PD due to its ability to augment dopamine levels in the primate brain (Prins, Petzer, and Malan 2010) (Figure 26).

Figure 26. Chemical Structures of Selegiline, and Rasagiline

Binding affinity of the synthesized compounds was determined by inhibition of $[^3]H$ spiroperidol binding to rat DA D$_2$ and D$_3$ receptors expressed in HEK-293 cells as described by us previously (Biswas et al. 2008). In general, the decrease in the D$_2$ binding affinity of the compound D-677 after methylation of the secondary nitrogen atom directly attached to the propargyl group was observed. The D$_2$ binding affinity of compounds 35 (D-677) decreased compared to its corresponding nonmethylated molecules 32 D-671 ($K_i$, D$_2$ = 38.8 vs. 1,079 nM, Table 3) as well as a reduction in D$_3$ binding affinity (1.30 vs. 99.0 nM, Table 3). The great reduction in binding affinity of the D-677 could be explained by the importance of the hydrogen atom attached to the nitrogen. However, the bioisosteric version of 32-(D-671), compounds 42 (D-678) has exhibited only a slight or no effect in the D$_2$ binding affinity when compared to its bioisosteric D-671 ($K_i$, D$_2$ = 41.2 vs. 38.8 nM, respectively Table 3) as well as slight or no effect in the D$_3$ binding affinity compared with the same molecule ($K_i$, D$_3$=0.85 vs 1.30 nM, respectively Table 3) which indicates tolerance of replacement of N-atom by O-atom for the binding activity to both D$_2$/D$_3$ receptors. The similarity of the binding affinity for D-671, and D-678 might be explained by the presence of the lone pair of electrons on the secondary amine and the
oxygen atom that would be necessary for the binding of these compound to the D$_2$/D$_3$ receptors.

Table 3. Inhibition constants determined by Competition Experiments Assessing [³H]Spiroperidol Binding to Cloned Rat D$_2$L and D$_3$ receptors Expressed in HEK-293 Cells$^a$

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ (nM)</th>
<th>D$_2$L, [³H]spiroperidol</th>
<th>D$_3$, [³H]spiroperidol</th>
<th>D$_{2L}$/D$_3$</th>
<th>cLogP</th>
<th>tPSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-5-OH-DPAT</td>
<td>153 ± 32</td>
<td>2.07 ± 0.38</td>
<td></td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)-33- D-671</td>
<td>38.8 ± 5.2</td>
<td>1.30 ± 0.13</td>
<td></td>
<td>30</td>
<td>4.15</td>
<td>33.19</td>
</tr>
<tr>
<td>(-)-34- D-677</td>
<td>1,079 ± 26</td>
<td>99.0 ± 10</td>
<td></td>
<td>11</td>
<td>4.15</td>
<td>33.19</td>
</tr>
<tr>
<td>(±)-42-D-678</td>
<td>41.2 ± 5.8</td>
<td>0.85 ± 5.0.01</td>
<td></td>
<td>9.86</td>
<td>3.95</td>
<td>41.98</td>
</tr>
</tbody>
</table>

$^a$Results are expressed as means ± SEM. For 3-6 experiments each in triplicate.

Based on the binding results, compounds were selected for further in vitro examination by measuring the stimulation of [³S]GTPγS binding to assess their ability to activate the human dopamine hD$_2$ and hD$_3$ expressed in CHO cells. Dopamine (DA) was used as reference because of its full agonist activity. Both (-)-33- D-671, and (±)-42-D-678 showed a high potency (EC$_{50}$ (GTPγS); D$_2$= 0.932, 1.64, respectively, Table 4), and full agonist activities for D$_2$ receptor with ($E_{\text{max}}$ =97% and 91.3%, Table 4).

Table 4. Stimulation of [³S]GTPγS Binding to Cloned Human D$_2$ and D$_3$ Receptors Expressed in CHO Cells$^a$
<table>
<thead>
<tr>
<th>Compound</th>
<th>hCHO-D₂</th>
<th>hCHO-D₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[³⁵S]GTPγS  EC₅₀ (nM)</td>
<td>[³⁵S]GTPγS  EC₅₀ (nM)</td>
</tr>
<tr>
<td>Dopamine (DA)</td>
<td>146 ± 24</td>
<td>1.95 ± 0.62</td>
</tr>
<tr>
<td>(-)-33- D-671</td>
<td>0.932±0.212</td>
<td>0.078±0.0176</td>
</tr>
<tr>
<td>(±)-42-D-678</td>
<td>1.64±0.30</td>
<td>0.197±0.064</td>
</tr>
</tbody>
</table>

EC₅₀ is the concentration producing half maximal stimulation. For each compound, maximal stimulation (Eₘₐₓ %) is expressed as percent of the Eₘₐₓ observed with 1mM (D₂) or 100 µM (D₃) of the full agonist DA (Eₘₐₓ %). Results are expressed as means + SEM. For 3-6 experiments, each performed in triplicate.

4.3.3. MAO inhibition assay with multifunctional MAO-inhibitors.

A fluorescence-based enzymatic assay was carried out to evaluate the affinity of the synthesized compounds to inhibit the MAO-B activity, it was also used to evaluate the effect of changing the heteroatom group connecting to the propargyl group on the inhibition of monoamine oxidase enzyme. In this experiment, pargyline, was used as the reference compound. Pargyline is known to be a potent and selective MAO-B inhibitor. The experiment started by incubating 25 µM of the test compounds, 15 µg/mL of the MAO-B enzyme with 25 µM kynuramine used as a substrate. The fluorescence of the product 4-hydroxyquinoline was measured as an assay readout. Among the three test compounds, (-)-33- D-671 exhibited higher inhibitor effect on MAO-B than (±)-42-D-678, and less inhibitory effect on MAO-B. This result could be drawn from the effect of the secondary nitrogen connected directly to the propargyl group compared with the oxygen group on the compounds (±)-42-D-678.
These findings suggest that the hydrogen on the nitrogen atom connecting the propargyl group to the phenyl ring may potentially enhance the MAO-B enzyme inhibitory activity. Substitution of the nitrogen with the bioisosterically oxygen could be responsible for reducing the affinity to inhibit the MAO-B in compound (±)-42-D-678 compared to compound (-)-33- D-671. The compound (±)-42-D-678 has less receptor binding affinity than (-)-33- D-671. Moreover, compound (-)-33- D-671 displayed better agonist potency at D₂ receptor activity in the functional receptor assays than (±)-42-D-678. This could suggest that there could be a relation between the D₂/D₃ receptor agonist activities and the enzyme inhibitory activity. This could also shed the point on the requirement of further modifications of the current molecular template compatibility for dopamine D₂/D₃ receptors binding, functional activity, and MAO-B enzyme interaction for the structural preference and conformational requirement.

**Table 5.** The IC₅₀ values for the inhibition of recombinant human MAO-A and MAO-B by compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>MAO-A IC₅₀(µM)</th>
<th>MAO-B IC₅₀(µM)</th>
<th>SI²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pargyline</td>
<td>0.28 ± 0.02</td>
<td>10.54 ± 0.79</td>
<td>37.6</td>
</tr>
<tr>
<td>(-)-33- D-671</td>
<td>71.90±5.805</td>
<td>12.38±2.282</td>
<td>5.80</td>
</tr>
<tr>
<td>(±)-42-D-678</td>
<td>57.07±0.30</td>
<td>24.17±0.064</td>
<td>2.40</td>
</tr>
</tbody>
</table>

²Results are expressed as means ± SEM for three experiments, each performed in triplicate.
²Selectivity index = IC₅₀(MAO-A)/IC 50 (MAO-B).
4.4. Design, development, synthesize and pharmacological studies of monoamine reuptake blockers.

We previously reported pyran based triple reuptake inhibitors (TUIs), which inhibit reuptake of all three monoamine neurotransmitters. TUIs have been implicated for production of higher efficacy in antidepressant action (Zhang et al. 2005a; Zhang et al. 2006). Our group has been working on the development of asymmetric 3,6-disubstituted and 2,4,5-trisubstituted pyran derivative targeting monoamine transporters (Figure 4). These compounds exhibited affinity towards dopamine reuptake transporters and high potency for 5-HT and NE reuptake transporters (Zhang et al. 2006; Zhang et al. 2005b).

To explore the structurally novel molecular template for the monoamine transporters, derivatives of well-established 2,4,5-trisubstituted pyran were synthesized (Figure 27). The goal of this project is to carry out the SAR of pyran based compounds to develop a novel multifunctional pyran molecule template to treat the motor, non-motor symptoms like depression associated with PD.

![Pharmacophore model of pyran derivatives with monoamine transporters molecules.](image)

**Figure 27.** Pharmacophore model of pyran derivatives with monoamine transporters molecules.

In this study, the effect of connecting biphenyl substituents at position 2 on the pyran ring was explored directly without methylene bridge to the pyran ring (Figure 28). It was reasoned that connecting biphenyl groups directly to the pyran template could
increase the binding affinity for the dopamine transporters resulting in compounds with high selectivity and potency.

![Diagram of dopamine transporter binding affinity](image)

**Figure 28.** Initial SAR study on pyran templates (template 1: n=1, and template 2: n=2)

### 4.4.1. Chemistry involved in the syntheses of multifunctional D2/D3 agonist to treat depression in PD.

In this study, a version of the asymmetric trisubstituted pyran derivative precursor was developed as described in the following schemes.

![Chemical reaction schemes](image)

**Scheme 7;** Synthesis of the intermediate racemic expoxides 54. Reagents and conditions: a) allylmagnesiumbromide/Cul/THF,-78°C→RT, overnight; b) NaH/allylbromide/DMF, 1.5h, 0°C→RT; c) Grubb'scatalyst/benzene/reflux,2h; d) mCPBA/CH₂Cl₂,0°C→RT.

Scheme 7 outlines the synthesis of the intermediate racemic expoxides. Commercially available benzophenone 50 was treated with allylmagnesium bromide in the presence of copper iodide at -78°C to obtain alcohol 51. O-Alkylation of 51 with allylbromide in the presence of NaH at 0 °C to give compound 52, which was further converted to pyran derivative 53 via ring metastasis reaction in the presence of 1st
generation Grubbs catalyst (Sturino and Wong 1998). Direct epoxidation of olefin 53 with m-CPBA resulted in formation of low stereoselective trans-and cis-epoxide 54 (Zhang et al. 2005b).

Scheme 8; Synthesis of compound **D-594**. Reagents and conditions: a) NaN$_3$/CH$_3$OH:H$_2$O(8:1),NH$_4$Cl,80°C,24h; b) CH$_3$OH/10%Pd/C,30psi; c) aldehyde/CH$_2$Cl$_2$:CH$_3$OH(3:1), glacialaceticacid/Na(OAC)$_3$BH.

**Scheme 8** describes the synthesis of the target compound **57b (D-594)** starting from epoxide 54. The epoxide ring opening was achieved in the presence of sodium azide (NaN$_3$) followed by the addition of NH$_4$Cl at 80 °C to obtain the racemic azides 55, which was reduced in the presence of 10% Pd-C at 30psi (1 atm) to the corresponding amines 56, which undergo further reductive amination with 4-methoxy-benzaldehyde to obtain the final compounds 57.
Scheme 9 outlines the synthesis of compounds 60a (D-620) and 60b (D-621). 4-methoxyphenylacetonitrile 58 was treated with borane-methyl sulfide complex to obtain 2-(4-Methoxy-phenyl)-ethylamine 59 which reacted with the racemic epoxides 54 to produce compounds 60a (D-620) and 60b (D-621).

Scheme 10. Synthesis of a regioselective epoxide 61. Reagents and conditions: (a) acetonitrile:DME, Na₂B₄O₇.H₂O, Na₂EDTA, Tertbutyl ammonium hydrogen sulfate, Epoxane, RT→10 °C, NaCl ice bath, oxone in aqueous Na₂EDTA 0.004 M and K₂CO₃ added.

Scheme 10 describes the synthesize of enantiomer epoxides 61 in a stereoselective manner. Alkene 53 was carried out via Shi epoxidation catalyst to give optically active cis-epoxide. The regioselectivity of shi epoxidation catalyst to give cis-epoxide was confirmed by NMR experiments. Alkene was treated with expoxone and
sodium ethylene diamine tetraacetic acid in a buffer media of potassium hydrogen monopersulfate, sodium tetraborate, tetabutylammonium hydrogen sulfate and potassium carbonate to give the cis-epoxide regioselectivity. The regioselectivity of the shi epoxidation catalyst to the give cis-epoxide was confirmed by the NMR experiments.

Scheme 11. Synthesis of a regioselective compound D-620: Reagents and conditions: (a) 2-(4-methoxyphenyl) ethan-1-amine/ ethanol was reflux 100 °C under N₂ overnight.

Scheme 11 explains the synthesis of the novel pyran compound D-620 by introducing all the three substituents in a regiospecific manner after opening the epoxide. Cis-epoxide 61 was subjected to a regioselective ring opening in the presence of 2-(4-Methoxy-phenyl)-ethylamine to obtain compound 60a (D-620). The selectivity of the ring opening reaction was directed by nucleophilic attack at the site remote from the endocyclic oxygen atom where carbocationic character is better tolerated, the ring opening will involve high energy twist boat like transition state (Larin, Kochubei, and Atroshchenko 2014; Bagal et al. 2010). The relative stereochemistry of compound 60a was confirmed by NMR experiments.

4.4.2. ¹H NMR spectrum (normal proton NMR, 2-D COSY NMR, ¹H homo decoupling (HMDC), and nuclear overhauser experiments (NOE)) studies for the synthesized compounds.

4.4.2.1. The study of structure of epoxide 61.
Figure 29. The assignment structure of epoxide 61.

The NMR studies were done in C$_6$D$_6$, OCH$_2$ and OCH to assign the chemical shift values of the protons in CDCl$_3$. The assignment of protons was carried out by $^1$H NMR spectrum data (normal proton NMR and 2-D COSY NMR experiments) (Figure 29). In compound 61 the splitting of H-4 at $\delta=2.95$ ppm (assigned from proton nmr and COSY nmr) is a triplet ($J=4.94$). Based on the MM2 minimized 3-D model of cis epoxide, the torsion angle of H1-C-C-H3ax is close to 90°. As explained above, in this cis-isomer no coupling exists (less than 1(0.66)) between H-3ax and H-4. The bt splitting of H-4 is from the coupling with H-3eq and H-1 proton, respectively, this was confirmed by the splitting pattern of H-3ax which is a doublet of doublet ($J=15.8, 0.66$), The coupling interactions originated from the interaction with the H-3eq and slight coupling with H-4 ($J=0.66$, less than 1). The splitting of H-3eq is doublet of doublet, which arises from the coupling of H-3ax and H-4 ($J=15.8, 5.7$), the doublet from the geminal coupling with H-3ax and another doublet from the vicinal coupling with H-4. Furthermore, 2-D COSY NMR study demonstrated the coupling between H-4 and H-3eq and determined coupling between H-4 and H-3ax that verified H-4 is in the same phase as H-3eq.

These findings were further confirmed by $^1$H homo decoupling (HMDC) and nuclear overhauser experiments (NOE) in 600 MHz NMR machine in (CDCl$_3$): (i)
Irradiation of the proton signal at 2.25 ppm (H-1) collapsing br triplet at 2.95 ppm (H-4) into doublet and the doublet of the doublet at 3.25 ppm (H-2ax) into doublet. (ii) Irradiation of the proton signal at 2.95 ppm (H-4) resulted in collapsing the doublet of the doublet at 2.25 ppm (H1) into singlet and the doublet of doublet at 2.42 ppm (H-3eq) into doublet. (iii) Irradiation of the proton signal at 3.25 ppm (H-2ax) collapsing the doublet at 3.85 (H-2eq) and the doublet of the doublet at 2.25 (H-1). (iv) Irradiation of 3.85 ppm (H-2 eq) resulted in collapsing the signal at 3.25 ppm (H-2ax) into doublet which coupled only with H-1 proton.

Further Characterization of the compound 61: It was though that one of the possible reasons that no such interaction was observed, may be due to the existence of a very small coupling constant between H-1 and H-2 proton. As a result, we could detect H-2ax (δ=3.25) as the doublet of the doublet pattern with a big geminal interaction (J = 13.6 Hz) and a small vicinal interaction with H-1 (J = 1.5 Hz). It was also observed that the doublet of the doublet for H1is due to a vicinal coupling interaction with H-4 (J =4.3 Hz) and a small vicinal interaction with H-2ax (J = 1.5 Hz). The coupling constant at J =1.5 showed that H-1 and H-2ax have interaction with H-2eq. Thus, the cis structure of epoxide was confirmed.

4.4.3. In vitro evaluation of the binding affinity and functional potency for the triple reuptake inhibitor compounds.

5. In vitro uptake inhibition studies was carried out with the three designed compounds to test their potencies for the inhibition uptake of [3H]DA, [3H]5-HT and [3H]NE in the cell lines expressing the cloned human monoamine transporters (Table 7). D-594 was evaluated for its the binding affinities at the DAT, SERT, NET in the brain.
by measuring its inhibitory activity for the uptake of [³H] DA, [³H]-5-HT, and [³H]NE, and found to have weak binding affinity for the three transporters. To improve the binding, a modification of the compound was done by expanding the methylene bridge connected to the nitrogen group in position 5 in the pyran template to an ethylene bridge (Scheme 11) to produce compound D-620. The reason for such structural expansion idea was to find whether the expanded designed compound fit in the binding pocket of the transporters at the receptor sites. The intermediate P-methoxybenzyl was used as a substituted group in position 5 to produce compound D-620 that showed a high binding affinity for the dopamine transporter than D-594 and D-621 (Table 7). Moreover, compound D-621 exhibited good binding affinity for the norepinephrine transporters. The conclusion from the binding data in table 7 that D-620 could be considered a dual reuptake transporter inhibitor as it exhibited the ability to inhibit both DAT, and NET.

Table 7. Affinity of drugs at DAT, SERT, and NET in Rat Brain.

<table>
<thead>
<tr>
<th>Compound</th>
<th>DAT uptake, [³H]DA&lt;sup&gt;x&lt;/sup&gt;</th>
<th>SERT uptake, [³H]-5-HT&lt;sup&gt;x&lt;/sup&gt;</th>
<th>NET uptake, [³H]NE&lt;sup&gt;x&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-594</td>
<td>208±30</td>
<td>5,094±864</td>
<td>8,091±678</td>
</tr>
<tr>
<td>D-620</td>
<td>49.2±4.6</td>
<td>993±113</td>
<td>62.6±14.5</td>
</tr>
<tr>
<td>D-621</td>
<td>3,244±637</td>
<td>462±28</td>
<td>1,447±186</td>
</tr>
</tbody>
</table>

For uptake by DAT, SERT, and NET: [³H] DA<sup>x</sup>, [³H]-5-HT<sup>x</sup>, [³H] NE<sup>x</sup> accumulation was measured. Results are the average ± SEM of three to seven independent experimental assays in triplicate.
Chapter 5- Materials and Methods

5.11. Chemistry.

Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise indicated. Dry solvent was obtained according to the standard procedure. All reactions were performed under N₂ atmosphere unless otherwise indicated. Analytical silica gel 60 F254-coated TLC plates were purchased from EMD Chemicals, Inc. and were visualized with UV light or by treatment with phosphomolybdic acid (PMA), or ninhydrin. Flash column chromatographic purification was done using Whatman Purasil 60A silica gel 230–400 mesh. Proton nuclear magnetic resonance (¹H NMR) spectra and carbon nuclear magnetic resonance (¹³C NMR) spectra were measured on Varian 400 and 600 MHz NMR spectrometer with tetramethylsilane (TMS) as an internal standard. The NMR solvent used was either CDCl₃ or CD₃OD unless otherwise indicated. Autopol III automatic polarimeter (Rudolph Research Analytical) was used to record the optical rotations. MEL-TEMP II (Laboratory Devices Inc., U.S.) capillary melting point apparatus was used to record the melting points. Purity of the compounds was determined by elemental analysis and was within ±0.4% of the theoretical value (≥95% purity). Elemental analyses were performed by Atlantic Microlab, Inc., GA, USA.

Procedure A. 4'-Bromo-2-nitro-1,1'-biphenyl (2a).

To a stirring solution of 1-bromo-2-nitrobenzene (2.0 g, 9.9 mmol) and (4-bromophenyl)boronic acid (2.19 g, 10.89 mmol) in THF (25 mL) were added Pd(PPh₃)₄ (0.572 g, 0.50 mmol) followed by 2M K₂CO₃ (5.53 g in 20 mL water) at room temperature. The reaction mixture was stirred at 90 °C for 12 h after which it was cooled and extracted with CH₂Cl₂. The combined organic layer was dried over Na₂SO₄, filtered, and
concentrated in vacuo. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (9:1) as solvent to give compound 2a (2.7 g, 98%).

**1H NMR** (600 MHz, CDCl₃): δ 7.88 (d, J = 8.4 Hz, 1H), 7.64-7.61 (m, 1H), 7.59-7.55 (m, 2H), 7.53-7.50 (m, 1H), 7.42-7.40 (m, 1H), 7.20-7.18 (m, 2H).

2-Bromo-2'-nitro-1',1'-biphenyl (2c).

To a stirring solution of 1-bromo-2-nitrobenzene (2.50 g, 12.37 mmol) and (2-bromophenyl) boronic acid (2.73 g, 13.61 mmol) in THF (30 mL) were added Pd(PPh₃)₄ (0.715 g, 0.61 mmol) followed by 2M K₂CO₃ (5.53 g in 20 mL water) according to procedure A to give compound 2c (2.18 g, 94%).

**1H NMR** (600 MHz, CDCl₃): δ 7.87 (d, J = 8.4 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.40 (d, J = 9 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.14 (t, J = 7.2 Hz, 1H), 7.10 (d, J = 7.2 Hz, 1H), 7.03 (d, J = 7.2 Hz, 2H).

Procedure B. 2-Bromo-9H-carbazole (3a).

Compound 2a and PPh₃ were dissolved in 1,2-dichlorobenzene and the resulting solution was stirred at 170 °C for 12 h after which it was cooled and extracted with CH₂Cl₂/H₂O. The combined organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (9:1) to yield compound 3a (1.57 g, 89%).

**1H NMR** (600 MHz, CDCl₃): δ 8.04 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.62-7.58 (m, 2H), 7.52-7.47 (m, 2H), 7.39 (d, J = 8.4 Hz, 1H).

4-Bromo-9H-carbazole (3c).

Compound 2c and PPh₃ were reacted in 1,2-dichlorobenzene according to procedure B to yield compound 3c (1.25 g, 87%).

**1H NMR** (600 MHz, CDCl₃): δ 8.81 (d,
J = 7.8 Hz, 1H), 7.97 (s, 1H), 7.50 (t, J = 7.2 Hz, 1H), 7.44 (d, J = 7.2 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.25-7.20 (m, 1H).


To a stirring solution of compound 3a (1.5 g, 6.09 mmol) in THF (20 mL) were added (Boc)$_2$O (1.46 g, 6.7 mmol) and DMAP (0.819 g, 6.7 mmol) in THF (20 mL) at room temperature. The reaction mixture was stirred at the same temperature for 12 h. The crude mixture was evaporated under reduced pressure, followed by extraction with EtOAc (3 × 20 mL) in water. The combined organic layer was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 19:1) to yield compound 4a (1.78 g, 84%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 8.48 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.18 (d, J = 7.8 Hz, 1H), 7.11 (t, J = 7.2 Hz, 1H), 6.97 (t, J = 8.10 Hz, 1H), 1.53 (s, 9H).

tert-Butyl 3-bromo-9H-carbazole-9-carboxylate (4b).

3-Bromo-9H-carbazole (3b) (2.0 g, 8.13 mmol) was reacted with (Boc)$_2$O (1.95 g, 8.94 mmol) and DMAP (1.09 g, 8.94 mmol) in THF (20 mL) according to procedure C. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (19:1) as solvent to give compound 4b (2.8 g, ~100%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 8.27 (d, J = 8.4 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 8.08 (d, J = 2.4 Hz, 1H), 7.92 (qd, J = 7.8, 0.6 Hz, 1H), 7.55 (dd, J = 6.6, 2.4 Hz, 1H), 7.49 (td, J = 8.4, 1.2 Hz, 1H), 7.36 (td, J = 8.4, 1.2 Hz, 1H), 1.76 (s, 9H).
**tert-Butyl 4-bromo-9H-carbazole-9-carboxylate (4c).**

Compound 3c (1.5 g, 6.09 mmol) was reacted with (Boc)₂O (1.46 g, 6.7 mmol) and DMAP (0.819 g, 6.7 mmol) in THF (20 mL) according to procedure C. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 19:1) to yield compound 4c (1.78 g, 84%). ¹H NMR (600 MHz, CDCl₃): δ 8.53 (s, 1H), 8.26 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.49-7.46 (m, 2H), 7.36 (t, J = 7.2 Hz, 1H), 1.77 (s, 9H).

**Procedure D. tert-Butyl 2-((tert-butyl)dimethylsilyloxy)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (5a).**

To a mixture of compounds 4a (0.8 g, 2.31 mmol), 1-((tert-butyl)dimethylsilyloxy)-ethyl)piperazine (Das et al. 2015) (1.13 g, 4.62 mmol), BINAP (0.144 g, 0.23 mmol) and Cs₂CO₃ (2.26 g, 6.93 mmol), toluene (15 mL) was added under N₂ atmosphere. The reaction mixture was degassed by bubbling N₂ for 5 min and then Pd(OAc)₂ (0.039 g, 0.17 mmol) was added quickly. The system was degassed again and refluxed for 24 h under inert condition. The reaction mixture was cooled to room temperature, filtered through a pad of celite, washed with dichloromethane and concentrated in vacuum. The crude residue was purified by column chromatography (hexane:EtOAc = 4:1) to afford compound 5a (0.97 g, 82%). ¹H NMR (600 MHz, CDCl₃): δ 8.23 (d, J = 7.8 Hz, 1H), 7.92 (s, 1H), 7.84 (d, J = 7.2 Hz, 1H), 7.81 (d, J = 9.0 Hz, 1H), 7.35 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.00 (dd, J = 9.0, 1.8 Hz, 1H), 3.82 (t, J = 6.6 Hz, 2H), 3.32 (t, J = 4.8 Hz, 4H), 2.74 (t, J = 4.8 Hz, 4H), 2.62 (t, J = 6.6 Hz, 2H), 1.75 (s, 9H), 0.91 (s, 9H), 0.09 (s, 6H).
**tert-Butyl 3-(4-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (5b).**

A mixture of compound 4b (1.2 g, 3.47 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazine (1.27 g, 5.20 mmol), Pd(OAc)$_2$ (0.058 g, 0.26 mmol), BINAP (0.216 g, 0.35 mmol) and Cs$_2$CO$_3$ (3.39 g, 10.4 mmol) in toluene (25 mL) was heated at 110 °C for 24 h according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 4:1) to give compound 5b (1.4 g, 79%). $^1$H NMR (600 MHz, CDCl$_3$): δ 8.27 (d, $J = 8.4$ Hz, 1H), 8.16 (d, $J = 8.4$ Hz, 1H), 7.93 (d, $J = 7.8$ Hz, 1H), 7.48 (d, $J = 2.4$ Hz, 1H), 7.44 (t, $J = 7.8$ Hz, 1H), 7.32 (t, $J = 7.8$ Hz, 1H), 7.13 (dd, $J = 6.6$, 2.4 Hz, 1H), 3.83 (t, $J = 6.6$ Hz, 2H), 3.28 (t, $J = 4.8$ Hz, 4H), 2.77 (t, $J = 4.8$ Hz, 4H), 2.63 (t, $J = 6.6$ Hz, 2H), 1.75 (s, 9H), 0.92 (s, 9H), 0.09 (s, 6H).

**tert-Butyl 4-(4-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (5c).**

A mixture of 4c (2.70 g, 7.80 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazine (3.24 g, 13.25 mmol), Pd(OAc)$_2$ (0.0.13 g, 0.59 mmol), BINAP (0.49 g, 0.78 mmol) and Cs$_2$CO$_3$ (7.62 g, 23.4 mmol) in toluene (30 mL) was heated at 110°C for 24 h according to procedure D. The crude residue was purified by column chromatography (hexane:EtOAc = 4:1) to afford compound 5c (3.24 g, 82%). $^1$H NMR (600 MHz, CDCl$_3$): δ 8.34 (dd, $J = 8.1,4.8$ Hz, 2H), 8.08 (d, $J = 8.4$ Hz, 1H), 7.45 (t, $J = 7.2$ Hz, 1H), 7.41-7.36 (m, 2H), 7.04 (d, $J = 8.4$ Hz, 1H), 3.85 (t, $J = 6.6$ Hz, 2H), 3.33 (d, $J = 5.4$ Hz, 2H), 2.99 (t, $J = 5.4$ Hz, 2H), 2.71 (t, $J = 6.6$ Hz, 4H), 1.75 (s, 9H), 0.91 (s, 9H), 0.10 (s, 6H).
Procedure E. tert-Butyl 2-(4-(2-hydroxyethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (6a).

Into a stirring solution of compound 5a (0.95 g, 1.86 mmol) in THF (10 mL) was added n-tetrabutylammonium fluoride (2.8 mL, 2.8 mmol, 1.0 M solution in THF) at 0 °C. The reaction mixture was then stirred at room temperature for 2 h. THF was evaporated in vacuo, and the residue was diluted with CH₂Cl₂ (25 mL) and washed with a saturated solution of NaHCO₃. The water layer was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give compound 6a (0.595 g, 81%). ¹H NMR (600 MHz, CDCl₃): δ 8.23 (d, J = 7.8 Hz, 1H), 7.94 (s, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 9.0 Hz, 1H), 7.36 (t, J = 7.2 Hz, 1H), 7.30 (t, J = 7.2 Hz, 1H), 7.00 (dd, J = 6.6, 1.8 Hz, 1H), 3.68 (t, J = 5.4 Hz, 2H), 3.34 (t, J = 4.8 Hz, 4H), 2.73 (t, J = 4.8 Hz, 4H), 2.64 (t, J = 5.4 Hz, 2H), 1.76 (s, 9H).

tert-Butyl 3-(4-(2-hydroxyethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (6b).

Compound 5b (1.2 g, 2.35 mmol) in THF (15 mL) was reacted with n-tetrabutylammonium fluoride (4.71 mL, 4.71 mmol, 1.0 M solution in THF) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to yield compound 6b (0.78 g, 84%). ¹H NMR (600 MHz, CDCl₃): δ 8.28 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 2.4 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.13 (dd, J = 6.6, 2.4 Hz, 1H), 3.69 (t, J = 5.4 Hz, 2H), 3.29 (t, J = 4.8 Hz, 4H), 2.76 (t, J = 4.8 Hz, 4H), 2.66 (t, J = 5.4 Hz, 2H), 1.75 (s, 9H).
**tert-Butyl 4-(4-(2-hydroxyethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (6c).**

Compound 5c (3.30 g, 6.47 mmol) was reacted with *n*-tetrabutylammonium fluoride (9.70 mL, 9.70 mmol, 1.0 M solution in THF) in THF (30 mL) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give compound 6c (2.01 g, 80%). **1H NMR (600 MHz, CDCl3):** δ 8.27 (d, *J* = 6.6 Hz, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.47 (s, 1H), 7.43 (t, *J* = 7.2 Hz, 1H), 7.31 (t, *J* = 7.2 Hz, 1H), 7.11 (d, *J* = 9.0 Hz, 1H), 3.69 (t, *J* = 5.4 Hz, 2H), 3.26 (s, 4H), 2.76 (s, 4H), 2.62 (t, *J* = 5.4 Hz, 2H), 1.73 (s, 9H).

**Procedure F. tert-Butyl 2-(4-(2-oxoethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (7a).**

Into a stirring solution of compound 6a (0.30 g, 0.76 mmol) in CH2Cl2 (6 mL) and DMSO (3 mL), was added Et3N (0.74 mL, 5.31 mmol) at 0 °C. The reaction mixture was stirred for 5 min followed by addition of SO3.py complex (0.604 g, 3.79 mmol) at 0 °C. Ice bath was removed, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by addition of water and extracted with CH2Cl2 (3 × 30 mL). The combined organic layer was dried using Na2SO4, and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 3:7) to give aldehyde 7a (0.25 g, 84%). The purified aldehyde was used immediately for next step. **1H NMR (600 MHz, CDCl3):** δ 9.74 (s, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 7.94 (s, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.36 (td, *J* = 7.2, 1.2 Hz, 1H), 7.29 (td, *J* = 7.2, 1.2 Hz, 1H), 6.97 (dd, *J* = 6.6, 1.8 Hz, 1H), 3.36 (t, *J* = 4.8 Hz, 4H), 3.24 (t, *J* = 1.2 Hz, 2H), 2.73 (t, *J* = 4.8 Hz, 4H), 1.75 (s, 9H).

**tert-Butyl 3-(4-(2-oxoethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (7b).**
Compound 6b (0.45 g, 1.14 mmol) in CH₂Cl₂ (10 mL) and DMSO (5 mL), was oxidized using SO₃.py complex (0.905 g, 5.69 mmol) and Et₃N (1.11 mL, 7.96 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc) to yield compound 7b (0.35 g, 78%). ¹H NMR (600 MHz, CDCl₃): δ 9.76 (s, 1H), 8.28 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.12 (dd, J = 6.6, 2.4 Hz, 1H), 3.31 (t, J = 4.2 Hz, 4H), 3.25 (t, J = 1.2 Hz, 2H), 2.74 (t, J = 4.2 Hz, 4H), 1.74 (s, 9H).

tert-Butyl 4-(4-(2-oxoethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (7c).

Alcohol 6c (0.35 g, 0.88 mmol) was oxidized using SO₃.py complex (0.704 g, 4.425 mmol), DMSO (9 mL) and Et₃N (0.86 mL, 6.19 mmol) in CH₂Cl₂ (6 mL) according to procedure F. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 3:7) to give aldehyde 7c (0.31 g, 89%). The purified aldehyde was used immediately for next step. ¹H NMR (600 MHz, CDCl₃): δ 9.78 (s, 1H), 8.33 (d, J = 7.8 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.44-7.40 (m, 2H), 7.35 (t, J = 7.2 Hz, 1H), 7.06 (dd, J = 7.8, 1.8 Hz, 1H), 3.36-3.34 (m, 4H), 3.06 (t, J = 11.1 Hz, 2H), 2.73 (d, J = 11.4 Hz, 2H), 2.71 (d, J = 10.2 Hz, 2H), 1.75 (s, 9H).

Procedure G. tert-Butyl 2-(4-(2-amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)-ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (8a).

Into a stirring solution of racemic N⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (0.058 g, 0.27 mmol) in CH₂Cl₂ (7 mL) was added aldehyde 7a (0.12 g, 0.31 mmol). After the mixture was stirred for 1.5 h, NaBH(OAc)₃ (0.13 g, 0.61 mmol) was added and the mixture was stirred for another 46 h at room temperature. The reaction mixture
was quenched with a saturated solution of NaHCO$_3$ at 0 °C and extracted with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic layer was dried over Na$_2$SO$_4$, and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (CH$_2$Cl$_2$:MeOH = 19:1) to afford compound **8a** (0.06 g, 38%). **$^1$H NMR** (600 MHz, CDCl$_3$): $\delta$ 8.23 (d, $J$ = 8.4 Hz, 1H), 7.92 (s, 1H), 7.85 (d, $J$ = 7.8 Hz, 1H), 7.81 (d, $J$ = 8.4 Hz, 1H), 7.35 (td, $J$ = 7.2, 1.2 Hz, 1H), 7.29 (td, $J$ = 7.2, 1.2 Hz, 1H), 7.00 (dd, $J$ = 6.0, 2.4 Hz, 1H), 4.77 (bs, 2H), 3.33 (t, $J$ = 4.8 Hz, 4H), 3.07 (m, 1H), 2.78–2.68 (m, 8H), 2.60–2.47 (m, 6H), 2.02–2.00 (m, 1H), 1.75 (s, 10H), 1.51–1.47 (m, 2H), 0.90 (t, $J$ = 7.2 Hz, 3H).

**tert-Butyl** 3-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[de]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (8b).

Aldehyde **7b** (0.15 g, 0.38 mmol) in CH$_2$Cl$_2$ (10 mL) was reacted with racemic $N^6$-propyl-4,5,6,7-tetrahydrobenzo[de]thiazole-2,6-diamine (0.073 g, 0.34 mmol) and NaBH(OAc)$_3$ (0.162 g, 0.76 mmol) according to procedure G. Crude product was purified by column chromatography (EtOAc:MeOH = 9:1) to afford compound **8b** (0.065 g, 32%). **$^1$H NMR** (400 MHz, CDCl$_3$): $\delta$ 8.27 (d, $J$ = 8.0 Hz, 1H), 8.16 (d, $J$ = 9.2 Hz, 1H), 7.93 (d, $J$ = 7.6 Hz, 1H), 7.48 (d, $J$ = 2.4 Hz, 1H), 7.44 (t, $J$ = 7.2 Hz, 1H), 7.32 (t, $J$ = 7.2 Hz, 1H), 7.12 (dd, $J$ = 7.2, 2.4 Hz, 1H), 5.12 (bs, 2H), 3.28 (t, $J$ = 4.8 Hz, 4H), 3.07–3.02 (m, 1H), 2.78–2.67 (m, 8H), 2.58–2.45 (m, 6H), 2.00–1.98 (m, 1H), 1.74 (s, 10H), 1.52–1.46 (m, 2H), 0.90 (t, $J$ = 7.2 Hz, 3H).

**tert-Butyl** 4-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[de]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (8c).
Aldehyde 7c (0.15 g, 0.38 mmol) was reacted with racemic \(N^6\)-propyl-4,5,6,7-tetrahydrobenzo[\(d\)]thiazole-2,6-diamine (0.073 g, 0.34 mmol) and NaBH(OAc)\(_3\) (0.162 g, 0.76 mmol) in CH\(_2\)Cl\(_2\) (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH\(_2\)Cl\(_2\):MeOH = 19:1) to afford compound 8c (0.91 g, 41\%). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta \) 8.23 (d, \(J = 6.6\) Hz, 1H), 8.28 (d, \(J = 6.6\) Hz, 1H), 8.06 (d, \(J = 5.4\) Hz, 1H), 7.43 (d, \(J = 5.4\) Hz, 2H), 7.02 (d, \(J = 6.0\) Hz, 1H), 5.03 (s, 2H), 3.10-3.05 (m, 3H), 3.07-2.98 (m, 2H), 2.76-2.69 (m, 4H), 2.62-2.60 (m, 8H), 2.01-2.00 (m, 1H), 1.73 (s, 10H), 1.50-1.46 (m, 2H), 0.90 (t, \(J = 7.2\) Hz, 3H).

(S)-\textit{tert}-Butyl \(2\)-((2-amino-4,5,6,7-tetrahydrobenzo[\(d\)]thiazol-6-yl)\(N\)propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (9a).

Aldehyde 7a (0.125 g, 0.32 mmol) was reacted with (-)-pramipexole (0.06 g, 0.29 mmol) and NaBH(OAc)\(_3\) (0.135 g, 0.64 mmol) in CH\(_2\)Cl\(_2\) (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH\(_2\)Cl\(_2\):MeOH = 19:1) to afford compound 9a (0.067 g, 40\%). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta \) 8.23 (d, \(J = 8.4\) Hz, 1H), 7.92 (s, 1H), 7.85 (d, \(J = 7.2\) Hz, 1H), 7.81 (d, \(J = 8.4\) Hz, 1H), 7.35 (td, \(J = 7.2, 1.2\) Hz, 1H), 7.29 (td, \(J = 7.2, 1.2\) Hz, 1H), 7.00 (dd, \(J = 6.6, 2.4\) Hz, 1H), 4.76 (bs, 2H), 3.33 (t, \(J = 4.8\) Hz, 4H), 3.07 (m, 1H), 2.79-2.68 (m, 8H), 2.61-2.48 (m, 6H), 2.02-2.00 (m, 1H), 1.75 (s, 10H), 1.51-1.48 (m, 2H), 0.90 (t, \(J = 7.2\) Hz, 3H); \([\alpha]_D^{25}\) = -44.0 (c=1.0 in CH\(_2\)Cl\(_2\)).

(S)-\textit{tert}-Butyl \(3\)-((2-amino-4,5,6,7-tetrahydrobenzo[\(d\)]thiazol-6-yl)\(N\)propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (9b).

Aldehyde 7b (0.30 g, 0.76 mmol) was reacted with (-)-pramipexole (0.145 g, 0.69 mmol) and NaBH(OAc)\(_3\) (0.323 g, 1.52 mmol) in CH\(_2\)Cl\(_2\) (15 mL) according to procedure
The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to afford compound 9b (0.158 g, 39%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 8.27 (d, $J$ = 7.2 Hz, 1H), 8.16 (d, $J$ = 8.4 Hz, 1H), 7.93 (d, $J$ = 7.8 Hz, 1H), 7.48 (d, $J$ = 2.4 Hz, 1H), 7.43 (t, $J$ = 7.2 Hz, 1H), 7.32 (t, $J$ = 7.2 Hz, 1H), 7.12 (dd, $J$ = 6.6, 2.4 Hz, 1H), 5.21 (bs, 2H), 3.28 (t, $J$ = 4.8 Hz, 4H), 3.07‒3.02 (m, 1H), 2.78‒2.66 (m, 8H), 2.59‒2.46 (m, 6H), 2.01‒1.99 (m, 1H), 1.74 (s, 10H), 1.52‒1.46 (m, 2H), 0.90 (t, $J$ = 7.2 Hz, 3H).

(S)-tert-Butyl 4-(4-(2-amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (9c).

Aldehyde 7c (0.150 g, 0.38 mmol) was reacted with (-)-pramipexole (0.73 g, 0.54 mmol) and NaBH(OAc)$_3$ (0.162 g, 0.76 mmol) in CH$_2$Cl$_2$ (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH$_2$Cl$_2$:MeOH = 19:1) to afford compound 9c (0.097 g, 43%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 8.32 (d, $J$ = 7.2 Hz, 1H), 8.28 (d, $J$ = 7.8 Hz, 1H), 8.06 (d, $J$ = 7.8 Hz, 1H), 7.43 (d, $J$ = 7.2 Hz, 1H), 7.40-7.35 (m, 2H), 7.30 (d, $J$ = 7.2 Hz, 1H), 4.83 (bs, 2H), 3.33 (d, $J$ = 8.4 Hz, 2H), 3.06 (m, 3H), 2.99 (m, 2H), 2.76‒2.70 (m, 4H), 2.60 (m, 7H), 1.74 (s, 10H), 1.51‒1.48 (m, 2H), 0.90 (t, $J$ = 6.6 Hz, 3H); $[\alpha]$$_D^{25}$ = -27.20 (c=1.0 in CH$_2$Cl$_2$).

Procedure H. $N^6$-(2-(4-(9H-carbazol-2-yl)piperazin-1-yl)ethyl)-$N^6$-propyl-4,5,6,7-tetrahydrobenzo-[d]thiazole-2,6-diamine (10a) (D-652).

To a stirred solution of 8a (0.055 g, 0.09 mmol) in CH$_2$Cl$_2$ (3 mL) at 0 °C, trifluoroacetic acid (3 mL) was added slowly and the reaction mixture was stirred for 3 h at room temperature. Unreacted TFA and solvent were removed under reduced pressure and the obtained TFA salt was washed with ether for several times followed by drying to yield 10a (0.079 g, 90%). $^1$H NMR (600 MHz, CD$_3$OD): $\delta$ 7.99 (d, $J$ = 8.4 Hz, 1H), 7.96
(d, J = 7.8 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.25 (s, 1H), 7.13 (t, J = 7.2 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 3.84 (m, 1H), 3.64–3.55 (m, 5H), 3.48–3.42 (m, 3H), 3.36 (s, 4H), 3.23–3.14 (m, 2H), 3.01–2.99 (m, 1H), 2.84–2.79 (m, 1H), 2.71–2.65 (m, 2H), 2.32–2.31 (m, 1H), 2.02–1.95 (m, 1H), 1.83–1.79 (m, 2H), 1.03 (t, J = 7.2 Hz, 3H);

$^{13}$C NMR (150 MHz, CD$_3$OD): $\delta$ 170.28, 160.68, 145.32, 140.57, 132.69, 125.56, 124.53, 122.50, 121.20, 120.14, 119.84, 119.33, 118.79, 118.29, 117.08, 115.15, 111.37, 110.77, 109.73, 59.75, 52.49, 51.56, 50.08, 22.29, 21.88, 21.39, 18.01, 10.19; Anal. Calcd for C$_{28}$H$_{36}$N$_{6}$S.4CF$_3$COOH: C, 45.77; H, 4.27; N, 8.90. Found: C, 45.71; H, 4.64; N, 8.87.

$N^6$-(2-((4-(9H-carbazol-3-yl)piperazin-1-yl)ethyl)-$N^6$-propyl-4,5,6,7-tetrahydrobenzo [d]thiazole-2,6-diamine (10b) (D-627).

Compound 8b (0.05 g, 0.09 mmol) in CH$_2$Cl$_2$ (2 mL) at 0 °C, was treated with trifluoroacetic acid (2 mL) according to procedure H to obtain the TFA salt of compound 10b (0.052 g, 84%). $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ 8.01 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 1.6 Hz, 1H), 7.41-7.36 (m, 2H), 7.32 (t, J = 7.2 Hz, 1H), 7.16-7.14 (m, 1H), 7.11 (t, J = 7.2 Hz, 1H), 3.34 (s, 1H), 3.19 (s, 4H), 2.98–2.91 (m, 2H), 2.80-2.51 (m, 12H), 2.07–2.04 (m, 1H), 1.81-1.77 (m, 1H), 1.63–1.53 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ 168.63, 144.25, 143.69, 143.37, 140.69, 135.84, 125.07, 123.21, 122.92, 119.50, 118.21, 118.00, 113.45, 110.87, 110.41, 107.90, 58.85, 55.41, 53.24, 53.12, 51.07, 25.37, 24.70, 23.79, 20.35, 10.43; Anal. Calcd for C$_{28}$H$_{36}$N$_{6}$S.2CF$_3$COOH: C, 53.62; H, 5.34; N, 11.73. Found: C, 53.64; H, 5.83; N, 11.34.

$N^6$-(2-(4-(9H-carbazol-4-yl)piperazin-1-yl)ethyl)-$N^6$-propyl-4,5,6,7-tetrahydrobenzo [d]thiazole-2,6-diamine (10c) (D-658).
Compound 8c (0.082 g, 0.14 mmol) was treated with trifluoroacetic acid (3 mL) in CH$_2$Cl$_2$ (3 mL) according to procedure H to furnish the TFA salt of 10c (0.091 g, 90%). $^1$H NMR (600 MHz, CD$_3$OD): δ 8.08 (d, $J = 7.8$ Hz, 1H), 7.45 (d, $J = 7.8$ Hz, 1H), 7.36 (t, $J = 7.8$ Hz, 1H), 7.31 (t, $J = 7.8$ Hz, 1H), 7.22 (d, $J = 7.8$ Hz, 1H), 7.19 (t, $J = 7.2$ Hz, 1H), 6.82 (d, $J = 7.8$ Hz, 1H), 3.94–3.89 (m, 1H), 3.83 (d, $J = 7.2$ Hz, 2H), 3.77–3.59 (m, 8H), 3.29 (d, $J = 1.2$ Hz, 1H), 3.24–3.20 (m, 3H), 3.06–3.04 (m, 1H), 2.92–2.88 (m, 1H), 2.78–2.71 (m, 2H), 2.37–2.36 (m, 1H), 2.11–2.04 (m, 1H), 1.86–1.82 (m, 2H), 1.04 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (150 MHz, CD$_3$OD): δ 170.32, 161.19, 146.42, 141.50, 139.78, 132.81, 125.91, 124.74, 121.78, 121.34, 118.62, 115.64, 111.66, 110.23, 107.39, 59.17, 53.36, 53.25, 52.80, 50.81, 48.60, 48.01, 44.95, 22.51, 22.03, 21.49, 18.34, 9.79; Anal. Calcd for C$_{28}$H$_{36}$N$_6$S, 3CF$_3$COOH, CH$_3$OH, H$_2$O: C, 47.73; H, 5.15; N, 9.54. Found: C, 47.63; H, 4.67; N, 9.23.

(S)-N$_6$-(2-(4-(9H-carbazol-2-yl)piperazin-1-yl)ethyl)-N$_6$-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (11a) (D-651).

Compound 9a (0.065 g, 0.11 mmol) was treated with trifluoroacetic acid (3 mL) in CH$_2$Cl$_2$ (3 mL) according to procedure H to furnish the TFA salt of 11a (0.085 g, 93%). $^1$H NMR (600 MHz, CD$_3$OD): δ 7.99 (d, $J = 8.4$ Hz, 1H), 7.96 (d, $J = 7.8$ Hz, 1H), 7.42 (d, $J = 7.8$ Hz, 1H), 7.33 (t, $J = 7.2$ Hz, 1H), 7.26 (s, 1H), 7.13 (t, $J = 7.2$ Hz, 1H), 7.04 (d, $J = 8.4$ Hz, 1H), 3.86 (m, 1H), 3.65–3.56 (m, 5H), 3.49–3.42 (m, 3H), 3.37 (s, 4H), 3.25–3.16 (m, 2H), 3.02–3.00 (m, 1H), 2.85–2.81 (m, 1H), 2.73–2.66 (m, 2H), 2.34–2.32 (m, 1H), 2.04–1.97 (m, 1H), 1.83–1.80 (m, 2H), 1.03 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (150 MHz, CD$_3$OD): δ 170.29, 160.41, 145.25, 140.58, 132.67, 125.55, 124.55, 122.49, 121.21, 120.15, 119.82, 119.32, 118.82, 118.24, 116.97, 115.06, 111.39, 110.76, 109.70, 59.75,
52.50, 51.55, 50.17, 22.31, 21.89, 21.40, 17.98, 10.18; $[\alpha]_D^{25} = -24.8$ (c=1.0 in CH$_3$OH);

Anal. Calcd for C$_{28}$H$_{36}$N$_6$S.3CF$_3$COOH.2H$_2$O: C, 47.11; H, 5.00; N, 9.70. Found: C, 47.63; H, 4.67; N, 9.23.

$(S)$-$N^6$-(2-(4-(9H-carbazol-3-yl)piperazin-1-yl)ethyl)-$N^6$-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (11b) (D-636).

Compound 9b (0.15 g, 0.25 mmol) was treated with trifluoroacetic acid (8 mL) in CH$_2$Cl$_2$ (8 mL) according to procedure H to furnish the TFA salt of 11b (0.20 g, 96%).

$^1$H NMR (600 MHz, CD$_3$OD): $\delta$ 8.27 (s, 1H), 8.11 (d, $J = 7.2$ Hz, 1H), 7.59-7.54 (m, 2H), 7.49 (d, $J = 8.4$ Hz, 1H), 7.44 (t, $J = 7.2$ Hz, 1H), 7.21 (t, $J = 7.2$ Hz, 1H), 3.96 (m, 1H), 3.80 (s, 4H), 3.57-3.45 (m, 2H), 3.34-3.27 (m, 2H), 3.20-3.10 (m, 7H), 2.97-2.92 (m, 1H), 2.83-2.75 (m, 2H), 2.44-2.42 (m, 1H), 2.17-2.10 (m, 1H), 1.90-1.84 (m, 2H), 1.07 (t, $J = 7.2$ Hz, 3H); $^{13}$C NMR (150 MHz, CD$_3$OD): $\delta$ 170.35, 160.54, 141.04, 139.17, 135.08, 132.80, 126.39, 123.44, 122.29, 119.95, 119.05, 117.60, 111.67, 111.55, 111.45, 110.90, 59.31, 54.18, 53.61, 51.21, 50.61, 22.42, 22.09, 21.50, 17.98, 9.82; $[\alpha]_D^{25} = -17.4$ (c=1.0 in CH$_3$OH); Anal. Calcd for C$_{28}$H$_{36}$N$_6$S.3CF$_3$COOH.H$_2$O: C, 48.11; H, 4.87; N, 9.90. Found: C, 48.25; H, 4.99; N, 9.58.

$(S)$-$N^6$-(2-(4-(9H-carbazol-4-yl)piperazin-1-yl)ethyl)-$N^6$-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (11c) (D-657).

Compound 9c (0.83 g, 0.14 mmol) was treated with trifluoroacetic acid (7 mL) in CH$_2$Cl$_2$ (7 mL) according to procedure H to furnish the TFA salt of 11c (0.085 g, 93%).

$^1$H NMR (600 MHz, CD$_3$OD): $\delta$ 8.07 (d, $J = 7.2$ Hz, 1H), 7.44 (d, $J = 7.8$ Hz, 1H), 7.36 (d, $J = 7.8$ Hz, 1H), 7.31 (t, $J = 7.8$ Hz, 1H), 7.22 (d, $J = 7.8$ Hz, 1H), 7.19 (t, $J = 7.2$ Hz, 1H), 6.81 (d, $J = 8.4$ Hz, 1H), 3.90 (s, 1H) 3.84 (d, $J = 6.6$ Hz, 2H), 3.78-3.71 (m, 4H), 3.56-
3.43 (m, 4H), 3.29 (s, 2H), 3.26-3.21 (m, 3H), 3.06-3.04 (m, 1H), 2.92-2.88 (m, 1H), 2.78–2.71 (m, 2H), 2.36 (bs, 1H), 2.09-2.04 (m, 1H), 1.86–1.82 (m, 2H), 1.04 (t, J = 7.2 Hz, 3H); $^{13}$C NMR (150 MHz, CD$_3$OD): δ 170.32, 161.06, 146.42, 139.77, 132.80, 126.38, 124.29, 122.26, 121.30, 119.075, 118.18, 115.62, 111.61, 110.74, 109.72, 59.72, 58.72, 53.27, 52.76, 50.70, 44.91, 22.99, 22.34, 21.44, 18.30, 10.07, 9.46; [α]$^D_{25}$ = -25.0 (c=1.0 in CH$_3$OH); Anal. Calcd for C$_{28}$H$_{36}$N$_6$S.3CF$_3$COOH: C, 49.16; H, 4.73; N, 10.12. Found: C, 48.93; H, 4.84; N, 9.77.

tert-Butyl  2-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)-piperazin-1-yl)-9H-carbazole-9-carboxylate (12a).

Compound 7a (0.10 g, 0.25 mmol) was reacted with ($\pm$)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.056 g, 0.25 mmol) and NaBH(OAc)$_3$ (0.108 g, 0.51 mmol) in CH$_2$Cl$_2$ (7 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hex:EtOAc = 1:3) to afford compound 12a (0.115 g, 77%). $^1$H NMR (600 MHz, CDCl$_3$): δ 8.23 (d, J = 7.8 Hz, 1H), 7.91 (s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.34 (t, J = 7.2, Hz, 1H), 7.28 (t, J = 7.2 Hz, 1H), 7.08 (t, J = 7.8 Hz, 1H), 6.98 (dd, J = 6.6, 1.8 Hz, 1H), 6.72 (d, J = 7.2, Hz, 1H), 6.64 (d, J = 7.8, Hz, 1H), 3.79 (s, 3H), 3.32 (t, J = 4.8 Hz, 4H), 3.02–2.93 (m, 2H), 2.88–2.85 (m, 1H), 2.79–2.75 (m, 3H), 2.70 (t, J = 4.8 Hz, 4H), 2.56–2.50 (m, 5H), 2.09–2.06 (m, 1H), 1.74 (s, 9H), 1.62–1.55 (m, 1H), 1.53-1.47 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H).

tert-Butyl  3-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (12b).

Compound 7b (0.170 g, 0.43 mmol) was reacted with ($\pm$)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.095 g, 0.43 mmol) and NaBH(OAc)$_3$ (0.183
g, 0.86 mmol) in CH₂Cl₂ (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography with EtOAc to afford compound 12b (0.11 g, 43%). **¹H NMR** (600 MHz, CDCl₃): δ 8.27 (d, J = 7.8 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 2.4 Hz, 1H), 7.43 (t, J = 7.2, Hz, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.12 (dd, J = 6.6, 2.4 Hz, 1H), 7.08 (t, J = 8.4 Hz, 1H), 6.72 (d, J = 7.2, Hz, 1H), 6.64 (d, J = 8.4 Hz, 1H), 3.79 (s, 3H), 3.27 (t, J = 4.8 Hz, 4H), 3.03–2.98 (m, 2H), 2.90–2.87 (m, 1H), 2.81–2.77 (m, 3H), 2.73 (t, J = 4.8 Hz, 4H), 2.59–2.51 (m, 5H), 2.11–2.08 (m, 1H), 1.74 (s, 9H), 1.63–1.57 (m, 1H), 1.55-1.49 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H).

**tert-Butyl 4-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (12c).**

Compound 7c (0.10 g, 0.25 mmol) was reacted with (±)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.055 g, 0.25 mmol) and NaBH(OAc)₃ (0.108 g, 0.50 mmol) in CH₂Cl₂ (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound 12c (0.112 g, 74%). **¹H NMR** (600 MHz, CDCl₃): δ 8.23 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 8.4, Hz, 1H), 8.09 (d, J = 7.2 Hz, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.39 (m, 2H), 7.13 (t, J = 7.8 Hz, 1H), 7.00 (d, J = 7.8 Hz, 1H), 6.70 (q, J = 7.8 Hz, 2H), 3.92 (bs, 2H), 3.79 (s, 3H), 3.58–3.48 (m, 4H), 2.29 (m, 2H), 3.06–3.04 (m, 5H), 2.62 (m, 1H), 2.37 (bs, 1H), 2.09–2.06 (m, 1H), 1.74 (s, 9H), 1.62–1.55 (m, 1H), 1.53-1.47 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H).

**(S)-tert-Butyl 2-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (13a).**

Compound 7a (0.10 g, 0.25 mmol) was reacted with (−)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.056 g, 0.25 mmol) and NaBH(OAc)₃ (0.108 g, 0.50 mmol) in CH₂Cl₂ (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound 13a (0.115 g, 43%). **¹H NMR** (600 MHz, CDCl₃): δ 8.27 (d, J = 7.8 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 2.4 Hz, 1H), 7.43 (t, J = 7.2, Hz, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.12 (dd, J = 6.6, 2.4 Hz, 1H), 7.08 (t, J = 8.4 Hz, 1H), 6.72 (d, J = 7.2, Hz, 1H), 6.64 (d, J = 8.4 Hz, 1H), 3.79 (s, 3H), 3.27 (t, J = 4.8 Hz, 4H), 3.03–2.98 (m, 2H), 2.90–2.87 (m, 1H), 2.81–2.77 (m, 3H), 2.73 (t, J = 4.8 Hz, 4H), 2.59–2.51 (m, 5H), 2.11–2.08 (m, 1H), 1.74 (s, 9H), 1.63–1.57 (m, 1H), 1.55-1.49 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H).
g, 0.51 mmol) in CH₂Cl₂ (7 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **13a** (0.11 g, 74%). **¹H NMR** (600 MHz, CDCl₃): δ 8.23 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.34 (t, J = 7.2 Hz, 1H), 7.28 (t, J = 7.2 Hz, 1H), 7.08 (t, J = 7.8 Hz, 1H), 6.97 (dd, J = 6.6, 1.8 Hz, 1H), 6.71 (d, J = 7.2 Hz, 1H), 6.63 (d, J = 7.8, Hz, 1H), 3.78 (s, 3H), 3.32 (t, J = 4.8 Hz, 4H), 3.02–2.94 (m, 2H), 2.88–2.85 (m, 1H), 2.79–2.74 (m, 3H), 2.70 (t, J = 4.8 Hz, 4H), 2.56–2.50 (m, 5H), 2.08–2.06 (m, 1H), 1.74 (s, 9H), 1.61–1.54 (m, 1H), 1.52–1.47 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H); [α]D²⁵ = -28.6 (c=1.0 in CH₂Cl₂).

**(S)- tert-Butyl 3-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (13b)**.

Compound **7b** (0.140 g, 0.456 mmol) was reacted with (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.070 g, 0.32 mmol) and NaBH(OAc)₃ (0.151 g, 0.71 mmol) in CH₂Cl₂ (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography with EtOAc to afford compound **13b** (0.07 g, 33%). **¹H NMR** (600 MHz, CDCl₃): δ 8.18 (d, J = 6 Hz, 1H), 8.09 (m, 1H), 7.83 (d, J = 7.2 Hz, 1H), 7.38-7.32 (m, 2H), 7.23 (q, J = 9.6, 7.5 Hz, 1H), 7.03 (d, J = 9 Hz, 1H), 6.99 (t, J = 7.8 Hz, 1H), 6.63 (d, J = 6.6, Hz, 1H), 6.55 (d, J = 7.8, Hz, 1H), 3.70 (s, 3H), 3.18 (t, J = 4.2 Hz, 4H), 3.27–3.26 (m, 1H), 3.12-3.10 (m, 2H), 2.93–2.90 (m, 2H), 2.81–2.78 (m, 1H), 2.69-2.64 (m, 5H), 2.48–2.43 (m, 4H), 2.03–2.00 (m, 1H), 1.65 (s, 9H), 1.54–1.47 (m, 1H), 1.45-1.37 (m, 2H), 0.82 (t, J = 7.2 Hz, 3H).

**(S)- tert-Butyl 4-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino)ethyl)piperazin-1-yl)-9H-carbazole-9-carboxylate (13c)**.
Compound 7c (0.10 g, 0.25 mmol) was reacted with (−)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.055 g, 0.25 mmol) and NaBH(OAc)$_3$ (0.108 g, 0.50 mmol) in CH$_2$Cl$_2$ (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound 13c (0.124 g, 82%). $^1$H NMR (600 MHz, CDCl$_3$): δ 8.35-8.30 (m, 2H), 7.07 (d, $J$ = 7.8 Hz, 1H), 7.44-7.41 (m, 1H), 7.41-7.39 (m, 2H), 7.08 (d, $J$ = 7.8 Hz, 1H), 7.04 (d, $J$ = 7.8 Hz, 1H), 6.73 (d, $J$ = 7.2, Hz, 1H), 6.64 (d, $J$ = 8.4 Hz, 1H), 3.79 (s, 3H), 3.53-3.48 (m, 4H), 3.29 (m, 2H), 3.16-3.04 (m, 5H), 2.67-2.60 (m, 1H), 2.03–2.01 (m, 2H), 1.85–1.84 (m, 3H), 1.75 (s, 9H), 1.26-1.24 (m, 3H), 0.92 (t, $J$ = 7.2 Hz, 3H); $[\alpha]_D^{25}$ = −20.3 (c=1.0 in CH$_2$Cl$_2$).

Procedure I. 6-((2-(4-(9H-carbazol-2-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (14a) (D-654).

A mixture of compound 12a (0.10 g, 0.17 mmol) and 48% aqueous HBr (8 mL) was refluxed at 130 °C for 4 h. The reaction mixture was evaporated to dryness, washed with ether followed by vacuo drying to yield HBr salt of 14a (0.095 g, 78%). $^1$H NMR (600 MHz, CD$_3$OD): δ 8.11 (d, $J$ = 8.4 Hz, 1H), 8.03 (d, $J$ = 7.8 Hz, 1H), 7.67-7.62 (m, 1H), 7.46 (d, $J$ = 7.8 Hz, 1H), 7.38 (t, $J$ = 7.2 Hz, 1H), 7.34-7.29 (m, 1H), 7.17 (t, $J$ = 7.2 Hz, 1H), 6.96 (t, $J$ = 7.8 Hz, 1H), 6.67 (d, $J$ = 7.8, Hz, 1H), 6.62 (d, $J$ = 7.8, Hz, 1H), 3.99-3.86 (m, 5H), 3.84–3.72 (m, 5H), 3.68-3.63 (m, 2H), 3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.16-3.08 (m, 2H), 2.71-2.66 (m, 1H), 2.63 (m, 1H), 2.49-2.47 (m, 1H), 2.00–1.93 (m, 3H), 1.07 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (150 MHz, CD$_3$OD): δ 154.74, 140.88, 140.12, 133.27, 129.28, 126.63, 125.85, 122.06, 121.59, 121.12, 120.99, 119.93, 119.78, 119.11, 117.61, 112.08, 110.61, 110.26, 61.26, 52.93, 51.47, 50.73, 50.47, 50.32, 45.16, 29.29, 23.55, 22.35,
18.26, 9.92, 8.31; Anal. Calcd for C₃₁H₃₈N₄O·3HBr: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.50; H, 5.92; N, 7.46.

6-((2-(4-(9H-carbazol-3-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (14b) (D-650).

A mixture of compound 12b (0.08 g, 0.13 mmol) and 48% aqueous HBr (7 mL) was refluxed according to procedure I to yield HBr salt of 14b (0.095 g, 98%). ¹H NMR (600 MHz, CD₃OD): δ 8.58 (s, 1H), 8.14 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 9.0 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.21 (t, J = 7.2 Hz, 1H), 6.94 (t, J = 7.8 Hz, 1H), 6.66 (d, J = 7.8 Hz, 1H), 6.61 (d, J = 7.8, Hz, 1H), 4.30 (s, 4H), 4.11-4.04 (m, 6H), 3.93–3.89 (m, 2H), 3.78 (s, 1H), 3.46-3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.22-3.16 (m, 1H), 3.09-3.05 (m, 1H), 2.72-2.69 (m, 1H), 2.52-2.47 (m, 1H), 2.02–1.94 (m, 3H), 1.07 (t, J = 7.2 Hz, 3H); ¹³C NMR (150 MHz, CD₃OD): δ 154.67, 141.05, 139.88, 133.32, 132.51, 127.22, 126.31, 123.45, 122.11, 121.60, 120.77, 120.15, 119.76, 117.91, 116.92, 112.40, 112.24, 111.96, 111.54, 111.40, 110.53, 60.95, 53.09, 50.47, 49.91, 29.34, 22.81, 22.37, 18.30, 10.26, 9.73; Anal. Calcd for C₃₁H₃₈N₄O·3HBr·2H₂O: C, 48.90; H, 5.96; N, 7.36. Found: C, 49.30; H, 5.86; N, 7.24.

6-((2-(4-(9H-carbazol-4-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (14c) (D-655).

Compound 12c (0.11 g, 0.18 mmol) was refluxed with 48% aqueous HBr (8 mL) according to procedure I to yield HBr salt of 14c (0.097 g, 78%). ¹H NMR (600 MHz, CD₃OD): δ 8.11 (d, J = 7.8 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 7.46-7.43 (m, 1H), 7.37-7.32 (m, 1H), 7.31-7.27 (m, 1H), 7.25-7.19 (m, 1H), 6.95 (t, J = 7.8 Hz, 1H), 6.87 (d, J = 7.8 Hz, 1H), 6.79 (d, J = 6.6, Hz, 1H), 6.61 (d, J = 7.8, Hz, 1H), 4.02 (s, 3H), 3.90–3.85 (m,
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5H), 3.58-3.57 (m, 3H), 3.39 (m, 2H), 3.33-3.29 (m, 2H), 3.29-3.17 (m, 2H), 3.11-3.08 (m, 1H), 2.72-2.70 (m, 1H), 2.48 (m, 1H), 1.99-1.94 (m, 3H), 1.07 (t, J = 7.2 Hz, 3H); $^{13}$C NMR (150 MHz, CD$_3$OD): $\delta$ 154.71, 141.47, 139.70, 133.20, 126.61, 125.85, 124.74, 121.97, 121.81, 121.58, 121.25, 119.96, 118.76, 118.66, 115.57, 112.07, 110.24, 110.20, 107.23 61.40, 52.89, 50.25, 48.57, 47.09, 45.57, 43.81, 29.23, 23.57, 22.30, 18.45, 9.91; Anal. Calcd for C$_{31}$H$_{38}$N$_4$O.3HBr: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.07; H, 5.91; N, 8.17.

(S)-6-((2-(4-(9H-carbazol-2-yl)piperazin-1-yl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (15a) (D-653).

Compound 13a (0.10 g, 0.17 mmol) was refluxed with 48% aqueous HBr (8 mL) according to procedure I to yield HBr salt of 15a (0.10 g, 82%). $^1$H NMR (600 MHz, CD$_3$OD): $\delta$ 8.11 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.62 (m, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.38 (t, J = 7.2 Hz, 1H), 7.34-7.28 (m, 1H), 7.17 (t, J = 7.2 Hz, 1H), 6.96 (t, J = 7.8 Hz, 1H), 6.68 (d, J = 7.8, Hz, 1H), 6.62 (d, J = 7.8, Hz, 1H), 3.99 (s, 3H), 3.93-3.85 (m, 2H), 3.82-3.63 (m, 7H), 3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.17-3.08 (m, 2H), 2.70 (m, 1H), 2.63-2.62 (m, 1H), 2.49-2.47 (m, 1H), 2.01-1.94 (m, 3H), 1.07 (t, J = 7.2 Hz, 3H); $^{13}$C NMR (150 MHz, CD$_3$OD): $\delta$ 154.73, 140.86, 140.14, 133.28, 129.27, 126.62, 125.82, 122.08, 121.60, 121.09, 120.97, 119.93, 119.76, 119.10, 117.58, 112.08, 110.60, 110.26, 72.29, 61.27, 52.94, 51.83, 51.45, 50.78, 50.49, 41.61, 29.31, 23.48, 22.35, 18.26, 9.92, 8.31; $[\alpha]_D^{25}$= -21.5 (c=1.0 in CH$_3$OH); Anal. Calcd for C$_{31}$H$_{38}$N$_4$O.3HBr: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.44; H, 5.93; N, 7.47.

(S)-6-((2-(4-(9H-carbazol-3-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (15b) (D-659).
Compound 13b (0.07 g, 0.12 mmol) was refluxed with 48% aqueous HBr (7 mL) according to procedure I to yield HBr salt of 15b (0.085 g, 97%). $^1\text{H NMR}$ (600 MHz, CD$_3$OD): $\delta$ 8.58 (s, 1H), 8.15 (q, $J$ = 7.8 Hz, 2H), 7.76 (d, $J$ = 6.6 Hz, 1H), 7.61 (d, $J$ = 8.4 Hz, 1H), 7.57 (d, $J$ = 7.8 Hz, 1H), 7.43 (q, $J$ = 7.8 Hz, 1H), 7.21 (d, $J$ = 7.8 Hz, 1H), 6.94 (t, $J$ = 7.2 Hz, 1H), 6.66 (d, $J$ = 7.2 Hz, 1H), 6.61 (d, $J$ = 7.8, Hz, 1H), 4.17 (s, 4H), 3.91-3.85 (m, 6H), 3.87‒3.85 (m, 2H), 3.57 (s, 1H), 3.50‒3.49 (m, 1H), 3.32‒3.28 (m, 2H), 3.17‒3.14 (m, 1H), 3.08-3.05 (m, 1H), 2.69-2.67 (m, 1H), 2.51-2.46 (m, 1H), 1.96–1.93 (m, 3H), 1.48 (t, $J$ = 6.0 Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, CD$_3$OD): $\delta$ 157.19, 151.08, 147.936, 147.513, 138.93, 137.85, 133.55, 132.78, 129.08, 127.16, 126.92, 122.87, 122.74, 121.64, 119.43, 118.42, 117.40, 107.78, 106.90, 106.54, 57.16, 55.21, 51.85, 50.41, 48.10, 40.131, 29.71, 25.60, 23.87, 22.08, 11.92; [$\alpha$]$_D^{25}=$ -22.2 (c=1.0 in CH$_3$OH); Anal. Calcd for C$_{31}$H$_{38}$N$_4$O.4HBr: C, 46.18; H, 5.25; N, 6.95. Found: C, 46.13; H, 5.66; N, 7.59. (S)-6-((2-(4H-carbazol-4-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (15c) (D-656).

Compound 13c (0.112 g, 0.18 mmol) was refluxed with 48% aqueous HBr (9 mL) according to procedure I to yield HBr salt of 15c (0.10 g, 82%). $^1\text{H NMR}$ (600 MHz, CD$_3$OD): $\delta$ 8.09 (d, $J$ = 7.2 Hz, 1H), 7.34 (d, $J$ = 8.4 Hz, 1H), 7.33 (t, $J$ = 7.2 Hz, 1H), 7.26 (dd, $J$ = 4.8, 2.4 Hz, 1H), 7.20 (d, $J$ = 8.4 Hz, 2H), 6.92 (t, $J$ = 7.8 Hz, 1H), 6.72 (d, $J$ = 7.2 Hz, 1H), 6.65 (d, $J$ = 7.2 Hz, 1H), 6.62 (d, $J$ = 7.8, Hz, 1H), 4.02 (s, 3H), 3.90-3.84 (m, 6H), 3.59–3.56 (m, 2H), 3.40 (m, 2H), 3.30-3.28 (m, 2H), 3.19-3.14 (m, 1H), 3.06-3.05 (m, 1H), 2.71-2.68 (m, 1H), 2.47 (bs, 1H), 1.96–1.92 (m, 3H), 1.05 (t, $J$ = 7.2 Hz, 3H); $^{13}\text{C NMR}$ (150 MHz, CD$_3$OD): $\delta$ 154.69, 141.41, 139.73, 133.24, 127.24, 125.30, 124.28, 122.56, 121.57, 121.21, 120.28, 119.63, 119.28, 118.33, 115.52, 112.43, 111.75, 110.77,
109.71, 107.68, 61.27, 52.94, 51.83, 60.95, 52.82, 50.31, 44.47, 43.68, 22.53, 18.97,
9.92, ; [α]D25= -17.8 (c=1.0 in CH3OH); Anal. Calcd for ) C31H38N4O.2HBr. CH2Cl2: C,
52.69; H, 5.80; N, 7.68. Found: C, 52.23; H, 6.18; N, 7.96.

9-(2-bromoethyl)-9H-carbazole (16).

A suspension of carbazole (1.0 g, 5.98 mmol), K2CO3 (1.82 g, 13.16 mmol),
tetrabutylammonium bromide (0.039 g, 0.12 mmol) and KOH (2.25 g, 40.07 mmol) in
dibromoethane (10 mL, 119.61 mmol) was stirred at 50 °C under N2 overnight. The
reaction mixture was filtered off and diluted with CH2Cl2. The organic layer was washed
with water, dried over sodium sulfate, filtered and concentrated. The crude product was
purified by silica gel column chromatography with petroleum ether to afford compound 16
(0.52 g, 32%). 1H NMR (400 MHz, CDCl3): δ 8.10 (d, J = 7.6 Hz, 2H), 7.51-7.42 (m, 4H),
7.29-7.25 (m, 2H), 4.71 (t, J = 7.2 Hz, 2H), 3.68 (t, J = 7.2 Hz, 2H).

9-(2-(4-(2-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)ethyl)-9H-carbazole (17).

A mixture of compound 16 (0.7 g, 2.55 mmol), 1-(2-((tert-butyldimethylsilyl)-
oxy)ethyl)piperazine (0.75 g, 3.06 mmol), and K2CO3 (1.06 g, 7.66 mmol) in acetonitrile
(20 mL) was refluxed for 24 h under inert condition. The reaction mixture was cooled to
room temperature, filtered, washed with EtOAc and concentrated in vacuo. The crude
material was purified by silica gel column chromatography (hexane:EtOAc = 3:2) to give
compound 17 (0.81 g, 73%). 1H NMR (400 MHz, CDCl3): δ 8.09 (d, J = 7.2 Hz, 2H), 7.49-
7.41 (m, 4H), 7.25-7.21 (m, 2H), 4.45 (t, J = 7.2 Hz, 2H), 3.76 (t, J = 6.8 Hz, 2H), 2.76 (t,
J = 7.2 Hz, 2H), 2.68-2.57 (m, 6H), 2.54 (t, J = 6.4 Hz, 4H), 0.90 (s, 9H), 0.06 (s, 6H).

2-(4-(2-(9H-carbazol-9-yl)ethyl)piperazin-1-yl)ethanol (18).
Compound 17 (0.875 g, 2.0 mmol) was treated with n-tetrabutylammonium fluoride (4.0 mL, 4.0 mmol, 1.0 M solution in THF) in THF (12 mL) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 5:1) to give compound 18 (0.525 g, 81%). 1H NMR (400 MHz, CDCl3): δ 8.09 (d, J = 7.2 Hz, 2H), 7.48-7.41 (m, 4H), 7.25-7.21 (m, 2H), 4.44 (t, J = 7.2 Hz, 2H), 3.62 (t, J = 5.4 Hz, 2H), 2.82-2.76 (m, 4H), 2.61-2.55 (m, 8H).

Alcohol 18 (0.30 g, 0.93 mmol) was oxidized using SO3.py complex (0.74 g, 4.64 mmol), DMSO (3 mL) and Et3N (0.90 mL, 6.49 mmol) in CH2Cl2 (6 mL) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give aldehyde 19 (0.23 g, 77%). The purified aldehyde was used immediately for next step. 1H NMR (600 MHz, CDCl3): δ 9.67 (s, 1H), 8.07 (d, J = 7.8 Hz, 2H), 7.47-7.41 (m, 4H), 7.23-7.21 (m, 2H), 4.44-4.42 (m, 2H), 3.38-3.36 (m, 2H), 2.78-2.74 (m, 4H), 2.60-2.56 (m, 6H).

N6-(2-(4-(2-(9H-carbazol-9-yl)ethyl)piperazin-1-yl)ethyl)piperazin-1-yl)ethyl)-N6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (20) (D-626).

Compound 19 (0.14 g, 0.44 mmol) was reacted with (±)-pramipexole (0.083 g, 0.39 mmol) and NaBH(OAc)3 (0.185 g, 0.87 mmol) in CH2Cl2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound 20 (0.115 g, 57%). The compound was converted to HCl salt. 1H NMR (400 MHz, CDCl3): δ 8.07 (d, J = 8.4 Hz, 2H), 7.47-7.39 (m, 4H), 7.23-7.20 (m, 2H), 5.20 (bs, 2H), 4.41 (t, J = 7.2 Hz, 2H), 3.04–2.97 (m, 1H), 2.75 (t, J = 7.2 Hz, 2H), 2.69–2.48 (m, 14H), 2.45 (t, J = 7.2 Hz, 4H), 1.97–1.94 (m, 1H), 1.73-1.63 (m, 1H), 1.49–1.40
(m, 2H), 0.87 (t, J = 7.2 Hz, 3H); \[^{13}\text{C NMR}\] (100 MHz, CDCl\(_3\)) \(\delta\) 166.01, 144.77, 140.29, 125.70, 122.90, 120.39, 118.96, 116.88, 108.54, 58.52, 58.08, 56.12, 53.59, 53.48, 48.28, 40.94, 26.46, 25.82, 25.09, 22.33, 11.85; Anal. Calcd for C\(_{30}\)H\(_{40}\)N\(_6\)S.5HCl.CH\(_3\)OH: C, 50.25; H, 6.61; N, 11.72. Found: C, 50.81; H, 7.00; N, 11.33.

\((S)-N^6-(2-(4-(9H-carbazol-9-yl)ethyl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (21) (D-637).

Compound 19 (0.19 g, 0.59 mmol) was reacted with (-)-pramipexole (0.112 g, 0.53 mmol) and NaBH(OAc)\(_3\) (0.25 g, 1.18 mmol) in CH\(_2\)Cl\(_2\) (12 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound 21 (0.13 g, 47%). The compound was converted to HCl salt. \[^{1}\text{H NMR}\] (600 MHz, CDCl\(_3\)) \(\delta\) 8.07 (d, \(J = 7.8\) Hz, 2H), 7.45 (t, \(J = 7.8\) Hz, 2H), 7.40 (d, \(J = 7.8\) Hz, 2H), 7.22 (t, \(J = 7.8\) Hz, 2H), 5.11 (bs, 2H), 4.41 (t, \(J = 7.2\) Hz, 2H), 3.03‒2.98 (m, 1H), 2.74 (t, \(J = 7.2\) Hz, 2H), 2.69‒2.48 (m, 14H), 2.45 (t, \(J = 7.2\) Hz, 4H), 1.96‒1.94 (m, 1H), 1.71-1.64 (m, 1H), 1.48‒1.41 (m, 2H), 0.87 (t, \(J = 7.2\) Hz, 3H); \[^{13}\text{C NMR}\] (150 MHz, CDCl\(_3\)) \(\delta\) 165.85, 144.91, 140.28, 125.68, 122.89, 120.39, 118.95, 117.00, 108.53, 58.58, 58.08, 56.13, 53.63, 53.52, 48.33, 40.95, 26.52, 25.84, 25.10, 22.35, 11.85; \([\alpha]_D^{25}=-19.8\) (c=1.0 in CH\(_2\)Cl\(_2\)); Anal. Calcd for C\(_{30}\)H\(_{40}\)N\(_6\)S.4HCl.2CH\(_3\)OH: C, 52.89; H, 7.21; N, 11.57. Found: C, 53.16; H, 7.14; N, 11.68.

\{2-[4-(2-Carbazol-9-yl-ethyl)-piperazin-1-yl]-ethyl\}-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (22).

Compound 19 (0.140.0g, 0.435 mmol) was reacted with (-)-DPAT (0.0.095 g, 0.435 mmol) and NaBH(OAc)\(_3\) (0.184 g, 0.87 mmol) in CH\(_2\)Cl\(_2\) (12 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH =...
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4:1) to afford compound 22 (0.09 g, 32.3%). \textbf{1H NMR} (600 MHz, CDCl$_3$): $\delta$ 8.03 (d, $J$ = 7.8 Hz, 2H), 7.42-7.39 (m, 2H), 7.17 (d, $J$ = 7.2 Hz, 2H), 7.05-7.01 (m, 2H), 6.64 (t, $J$ = 7.8 Hz, 2H), 6.59 (t, $J$ = 8.4 Hz, 2H), 4.37 (t, $J$ = 7.8 Hz, 2H), 3.74 (s, 1H), 3.61 (t, J = 6 Hz, 2H), 3.54 (t, $J$ = 5.4 Hz, 2H), 3.46 (t, $J$ = 4.8 Hz, 2H), 3.34 (t, $J$ = 5.4 Hz, 2H), 3.24 (t, $J$ = 5.4 Hz, 2H), 3.03 (d, $J$ = 4.2 Hz, 1H), 3.00 (d, $J$ = 4.2 Hz, 1H), 2.95 (dd, $J$ = 3.6, 1.8 Hz, 2H), 2.92 (d, $J$ = 3.6 Hz, 2H), 2.89 (dd, $J$ = 2.4, 2.4 Hz, 2H), 2.88-2.85 (m, 4H), 2.80 (m, 2H), 2.77 (d, $J$ = 3.0 Hz, 1H), 2.76-2.69 (m, 3H), 2.66-2.64 (m, 2H), 2.02-2.00 (m, 2H), 1.61 (dd, $J$ = 7.8 Hz, 7.2 Hz, 1H), 1.5 (qd, $J$ = 6.6, 5.5 Hz, 2H), 1.42 (dd, $J$ = 6.0, 5.4 Hz, 1H), 0.91 (t, $J$ = 7.8 Hz, 3H), 0.87 (t, $J$ = 7.2 Hz, 3H).

6-([2-[4-(2-Carbazol-9-yl-ethyl)-piperazin-1-yl]-ethyl]-propyl-amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (23) (D-689).

Compound 22 (0.09 g, 0.172 mmol) was dissolved in dryCH$_2$Cl$_2$ and BBr$_3$ was added at -40 °C for 2 h, the reaction mixture was stirred overnight at room temp. The reaction was quenched with an ice water, and the aqueous layer was extracted with CH$_2$Cl$_2$ (3 $\times$ 20 mL). The combined organic layer was dried over Na$_2$SO$_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 8:2) to yield compound 23 (0.025 g, 60%). \textbf{1H NMR} (600 MHz, CD$_3$OD): $\delta$ 8.18 (s, 1H), 7.54 (d, $J$ = 8.4 Hz, 1H), 7.46 (d, $J$ = 7.8 Hz, 2H), 6.94 (t, $J$ = 7.2 Hz, 3H), 6.61 (d, $J$ = 6.6 Hz, 5H), 4.43 (s, 1H), 3.44-3.37 (m, 4H), 3.20-3.18 (m, 4H), 2.99-2.96 (m, 4H), 2.87-2.83 (m, 4H), 2.75-2.74 (m, 2H), 2.64-2.63 (m, 3H), 2.45-2.42 (m, 3H), 2.33–2.32 (m, 4H), 1.79-1.75 (m, 4H), 1.04 (t, $J$ = 7.2 Hz, 3H); \textbf{13C NMR} (150 MHz, CD$_3$OD): $\delta$ 154.74, 154.71, 154.66, 138.77, 132.97, 129.77, 128.78, 126.62, 123.83, 123.55, 122.51, 121.65, 120.70, 119.70, 118.80, 112.99, 112.60, 112.01, 110.56,
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111.43, 111.11, 55.00, 54.67, 54.20, 53.87, 32.19, 31.78, 31.37, 25.94, 25.42, 25.31, 24.81, 22.81, 22.22, 21.44, 21.08, 20.37, 20.20, 19.57, 18.94, 10.75, 10.41, 10.09, 9.79, 9.14; \([\alpha]_D^{25}= -27.2 \, (c=1.0 \, \text{in CH}_3\text{OH})\); Anal. Calcd for C\(_{33}\)H\(_{45}\)Cl\(_3\)N\(_4\)O.3HCl: C, 63.92; H, 7.31; N, 9.04.

4-{4-[2-(tert-Butyl-dimethyl-silanyloxy)-ethyl]-piperazin-1-yl}-benzonitrile (25).

A mixture of 4-Bromo-benzonitrile 24 (1.0g, 5.49 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)ethyl) piperazine (2.015 g, 8.24 mmol), Pd(OAc)\(_2\) (0.0925 g, 0.412 mmol), BINAP (0.342 g, 0.55 mmol) and Cs\(_2\)CO\(_3\) (5.37 g, 16.49 mmol) in toluene (25 mL) was heated at 110 °C for 24 h according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 4:1) to give compound 25 (1.71 g, 90%). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 7.37 (t, \(J=4.8\) Hz, 2H), 6.76 (d, \(J=8.4\) Hz, 2H), 3.70 (t, \(J=6.0\) Hz, 2H), 3.23 (d, \(J=7.2\), 1H), 2.57 (t, \(J=4.2\), 4H), 2.49 (t, \(J=5.4\) Hz, 2H), 1.13 (td, \(J=6, 1.2\) Hz, 2H), 0.89 (s, 9H), 0.08 (s, 6H).

Procedure J. 4-{4-[2-Hydroxy-ethyl]-piperazin-1-yl}-benzonitrile (26).

Into a stirring solution of compound 25 (1.71 g, 4.95 mmol) in THF (50 mL) was added \(n\)-tetrabutylammonium fluoride (7.42 mL, 7.42 mmol, 1.0 M solution in THF) at 0 °C. The reaction mixture was then stirred at room temperature for 5 h. THF was evaporated in vacuo, and the residue was diluted with CH\(_2\)Cl\(_2\) (25 mL) and washed with a saturated solution of NaHCO\(_3\). The water layer was extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with brine, dried over Na\(_2\)SO\(_4\), and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give compound 26 as brown powder (0.880 g, 60.1%). \(^1\)H NMR (600 MHz, CDCl\(_3\)): \(\delta\) 7.48 (dd, \(J = 5.4, 1.8\) Hz, 2H), 6.84 (d, \(J = 5.4, 1.8\) Hz, 2H).
Hz, 2H), 3.67 (t, J = 5.4 Hz, 2H), 3.37(t, J = 2.4 Hz, 4H), 3.33 (t, J = 4.8 Hz, 4H), 2.61 (t, J = 5.4 Hz, 2H).

4-[4-(2-Oxo-ethyl)-piperazin-1-yl]-benzonitrile (27).

Compound 26 (0.880 g, 3.81 mmol) in CH₂Cl₂ (30 mL) and DMSO (15 mL), was oxidized using SO₃-py complex (3.028 g, 19.025 mmol) and Et₃N (3.71 mL, 26.635 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc) to yield compound 27 (0.133 g, 62%). ¹H NMR (600 MHz, CDCl₃): δ 9.60 (t, J=1.2 Hz, 1H), 7.37 (d, J = 7.8 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H), 3.39 (d, J=7.2, 1H), 3.27 (t, J=7.8, 3H), 2.56 (t, J=7.2 Hz, 4H), 1.13 (td, J=6, 1.2 Hz, 2H).

(s)-4-{4-{2-[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl}-benzonitrile (28).

Compound 27 (0.133 g, 0.542 mmol) was reacted with (-)-5-OMe-MPAT (0.107g, 0.488 mmol) and NaBH(OAc)₃ (0.229 g, 1.08 mmol) in CH₂Cl₂ (15 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound 28 (0.454 g, 32.3%). ¹H NMR (600 MHz, CDCl₃): δ 7.44 (dd, J = 5.4, 1.8 Hz, 2H), 7.05 (t, J = 7.8 Hz, 1H), 6.81 (dd, J = 5.4, 1.8 Hz, 2H), 6.68 (dd, J= 4.2, 3 Hz, 1H), 6.62 (dd, J= 4.8, 3 Hz, 1H), 3.77 (s, 3H), 3.29 (t, J= 5.4 Hz, 4H), 2.98 (d, J = 2.4 Hz, 1H), 2.95 (d, J = 6.0 Hz, 1H), 2.80 (dd, J= 4.2, 3 Hz, 1H), 2.71–2.68 (m, 2H), 2.66 (t, J = 7.8 Hz, 1H), 2.52–2.48 (m, 5H), 1.55-1.53 (m, 2H), 1.45 (dd, J= 7.8, 7.2 Hz, 2H), 1.23(t, J= 7.2 Hz, 2H), 0.91 (td, J= 5.4, 2.4 Hz, 2H), 0.87 (t, J = 7.2 Hz, 3H).

Procedure K. 2-[4-{4-Aminomethyl-phenyl}-piperazin-1-yl]-ethyl)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (29).
Into a stirred solution of compound 28 (454 g, 1.05 mmol) in 30 ml dry THF, borane:THF complex in 1.0 M THF (2.102 mL, 2.102 mmol) was added dropwise at R. T. The reaction mixtures was stirred at 50 °C for 1.5 h, cooled to R.T. Water and conc. HCl (2 mL) were added slowly at 0 °C. The solvent was evaporated and 10 ml of 25 % aq. NaOH was added at 0°C. The water layer was extracted with EtOAC (3 × 50 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM:MeOH = 9:1) to give compound 29 (0.880 g, 60.1%). ¹H NMR (600 MHz, CDCl₃): δ 7.47 (d, J = 9.0, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.09-7.06 (m, 1H), 6.88 (d, J= 8.4, 1H), 6.83 (d, J= 9.0 Hz, 1H), 6.69 (d, J= 7.8 Hz, 1H), 6.63 (d, J= 8.4 Hz, 1H), 3.79 (s, 3H), 3.31 (t, J= 5.4 Hz, 2H), 3.19 (t, J = 5.4 Hz, 2H), 2.99 (d, J = 4.8 Hz, 1H), 2.96 (d, J= 3.6 Hz, 1H), 2.93-2.89 (m, 1H), 2.88-2.82 (m, 2H), 2.73-2.70 (m, 3H), 2.64-2.61 (m, 3H), 2.52-2.48 (m, 4H), 2.04 (d, J= 12 Hz, 1H), 1.55 (dd, J= 6.6, 5.4 Hz, 2H), 1.48 (dd, J= 7.8, 7.2 Hz, 2H), 0.88 (t, J= 7.2 Hz, 3H).

Procedure L. N-[4-4-[2-[[5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl]-propyl-amino]-ethyl]-piperazin-1-yl]-benzyl]-2-nitro-benzenesulfonamide (30).

Into a stirring solution of 2-nitrobenzenesulfonyl chloride (0.4614 g, 0.208 mmol) in THF (5 mL), Et 3 N (0.131 mL, 0.937 mmol) was added (S)-4,5,6,7tetrahydrobenzo[d]thiazole-2,6-diamine 29 (0.10 g, 0.229 mmol) at −10 °C, and the resulting suspension was then stirred at room temperature for 1.5 h. The suspension was first filtered to remove precipitated triethylammonium chloride, and the filtrate was concentrated in vacuo (Brown et al. 2009). Water was added, and CH₂Cl₂ (3 × 15 mL) was used to extract the product. The combined organic layer was dried over Na₂SO₄, and
the solvent was removed in vacuo to obtain the intermediate 30. The crude mixture was purified by a silica gel column chromatography (0.134 g, 94%). $^1$H NMR (600 MHz, 123CD 3 OD): $\delta$ 7.24 (d, $J = 0.6$, 1H), 7.18 (d, $J = 8.4$ Hz, 1H), 7.07 (t, $J = 7.8$ Hz, 1H), 6.88 (d, $J = 8.4$ Hz, 2H), 6.70 (d, $J = 7.2$ Hz, 1H), 6.64 (d, $J = 7.2$ Hz, 1H), 3.79 (s, 3H), 3.18-3.16 (m, 3H), 3.02-2.96 (m, 1H), 2.93-2.91 (m, 1H), 2.87 (t, $J = 5.4$ Hz, 1H), 2.85-2.83 (m, 1H), 2.74-2.71 (m, 2H), 2.68 (t, $J = 5.4$ Hz, 1H), 2.65-2.63 (m, 3H), 2.58-2.55 (m, 1H), 2.52-2.51 (m, 3H), 2.34 (d, $J = 1.8$ Hz, 1H), 2.10-2.04 (m, 1H), 1.98-1.89 (m, 2H), 1.61-1.51 (m, 2H), 1.51-1.45 (m, 1H), 0.94-0.91 (m, 2H), 0.89 (s, 3H), 0.07-0.05 (m, 2H).

Procedure M. N-[4-(4-[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl]-piperazin-1-yl)-benzyl]-2-nitro-N-prop-2-ynyl-benzenesulfonamide (31).

Compound 30 (0.134 g, 0.217 mmol), potassium carbonate (0.90 g, 0.651 mmol), and propargyl bromide (0.31 mL, 0.261 mmol) were suspended in acetonitrile (7 mL) and the reaction mixture was heated to approximately 50 °C for 5 h. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was condensed in vacuo. Water was added, and the compound was extracted with EtOAC (3 × 15 mL). The combined organic layer was dried over Na$_2$SO$_4$, and the solvent was removed in vacuo to give the crude product, which was purified by column chromatography using (1:9) MeOH:EtOAC to give intermediate 31 (0.345 g, 60%). $^1$H NMR (600 MHz, 123CD 3 OD): $\delta$ 7.97 (d, $J = 7.8$, 1H), 7.79 (d, $J = 7.8$ Hz, 1H), 7.69-7.59 (m, 2H), 7.24 (dd, $J = 3.6$, 1.2 Hz, 1H), 7.19 (d, $J = 7.8$ Hz, 1H), 7.06 (t, $J = 8.4$, 2H), 6.83 (d, $J = 7.8$ Hz, 1H), 6.71 (dd, $J = 7.8$, 7.8, 2H), 6.64 (d, $J = 7.8$, 1H), 4.48 (s, 1H), 4.20 (s, 1H), 3.98 (s, 1H), 3.79 (s, 3H), 3.34 (d, $J = 1.2$ Hz, 1H), 3.17 (d, $J = 4.2$ Hz, 2H), 3.11 (d, $J = 4.8$ Hz, 2H), 2.99 (d, $J = 5.4$ Hz, 2H).
Hz, 1H), 2.96 (d, J=3.6 Hz, 1H), 2.88-2.85 (m, 1H), 2.87-2.77 (m, 3H), 2.62-2.48 (m, 8H), 2.13-2.11 (m, 1H), 2.080-2.07 (m, 1H), 1.58 (dd, J= 5.4, 4.8 Hz, 2H), 1.51-1.50 (m, 2H), 0.89 (t, J= 7.2 Hz, 3H).

Procedure N. (5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-{2-[4-(4-prop-2-ynylaminomethyl-phenyl)piperazin-1-yl]-ethyl}-amine (32).

Potassium carbonate (0.118 g, 1.364 mmol) was suspended in DMF (6 mL) and the suspension was cooled to 0 °C followed by slow addition of thioglycolic acid (0.070 mL, 0.758 mmol) and propargyl bromide (0.194 ml, 1.63 mmol). The mixture was stirred for 1 h at room temperature. A solution of the intermediate 31 (0.345 g, 0.522 mmol) in DMF (15 mL) was added, the reaction mixture was heated to 50 °C and stirred overnight. The reaction mixture was quenched carefully by adding 1N NaOH at room temperature, and CH₂Cl₂ (4 × 30 mL) was used to extract the product. The combined organic layer was dried over Na₂SO₄, and the solvent was removed in vacuo to give the crude, which was purified by a silica gel column chromatography using EtOAC:MeOH (9:1) to give compound 32 (0.30 g, 64%). ¹H NMR (600 MHz, CDCl₃): δ ppm 7.46 (dd, J = 5.4, 1.8 Hz, 1H), 7.22 (d, J = 1.8 Hz, 1H), 7.07 (d, J = 7.8 Hz, 1H), 6.85 (d, J= 9.0 Hz, 2H), 6.84-6.82 (m, 2H), 6.70 (d, J=7.8 Hz, 1H), 6.64 (d, J=7.8 Hz, 1H), 3.79 (s, 3H), 3.38 (d, J=2.4 Hz, 2H), 3.34-3.30 (m, 3H), 3.19 (d, J= 5.4 Hz, 2H), 2.99 (dd, J= 4.8 Hz, 2H), 2.87-2.84 (m, 2H), 2.76-2.74 (m, 3H), 2.66 (d, J= 4.2 Hz, 2H), 2.62(d, J= 3.6 Hz, 2H), 2.55-2.53 (m, 4H), 2.24 (d, J= 1.8 Hz, 1H), 1.58-1.56 (m, 2H), 1.50-1.49 (m, 2H), 0.89 (t, J=7.2 Hz, 3H).

Procedure O. 6-{Propyl-{2-[4-(4-prop-2-ynylaminomethyl-phenyl)piperazin-1-yl]-ethyl}-amino}-5,6,7,8-tetrahydro-naphthalen-1-ol (33).
Compound 32 (0.050 g, 0.105 mmol) was dissolved in dry CH₂Cl₂ and BBr₃ was added at -40 °C for 2 h. The reaction mixture was stirred overnight at room temp. The reaction was quenched by adding ice water, and the aqueous layer was extracted with CH₂Cl₂ (3 × 15 mL). The combined organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 8:2) to yield the final compound 33 (D-671) (0.025 g, 60%). ¹H NMR (600 MHz, CD₃OD): δ ppm 7.48 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 0.6 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 9.0 Hz, 2H), 6.70 (d, J = 7.8 Hz, 1H), 6.64 (d, J = 7.8 Hz, 1H), 3.31 (d, J = 5.4 Hz, 4H), 3.00-2.99 (m, 1H), 2.99-2.96 (m, 144H), 2.86-2.86 (m, 1H), 2.7-2.70 (m, 3H), 2.64-2.61 (m, 3H), 2.52-2.50 (m, 3H), 1.57-1.54 (m, 2H), 1.49-1.46 (m, 2H), 0.89 (t, J = 7.2 Hz, 3H). ¹³C NMR (150 MHz, CD₃OD): δ 233.87, 229.67, 223.82, 218.35, 210.53, 204.88, 199.54, 196.80, 190.59, 156.53, 156.20, 121.25, 118.65, 108.23, 101.50, 66.22, 55.28, 51.11, 46.77, 42.15, 31.26, 30.02, 29.69, 25.49, 22.68, 14.12, 8.71; [α]D²⁵ = -22 (c = 1.0 in CH₃OH); Anal. Calcd for C₂₈H₄₀N₄O₃·3HBr, 2H₂O: C, 54.21; H, 7.53; N, 8.72. Found: C 54.31; H, 7.29; N, 8.54.

Procedure P. (5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-[2-(4-[(methyl-prop-2-ynyl-aminomethyl]-phenyl]-piperazin-1-yl]-ethyl]-propyl-amine (34).

A mixture of compound 32 (0.7 g, 0.147 mmol), 37% aqueous formaldehyde (6.13 ml, 0.221 mmol), and NaH₂PO₄ (0.176 g, 0.147 mmol) were suspended in (10 mL) water and stirred for 5 min at room temperature. The mixture was refluxed at 30 °C for 48 h. The reaction was quenched by adding water and the crude was extracted with CH₂Cl₂ (3 * 10 mL). The crude material was purified by a silica gel column chromatography (EtOAC: MeOH= 9:1) to give compound 34 (0.51 g, 71%). ¹H NMR (600 MHz, CDCl₃): 7.13-7.10
(m, 2H), 6.86-6.84 (m, 2H), 6.71-6.65 (m, 3H), 3.79 (s, 3H), 3.65-6.63 (m, 1H), 3.53-3.50 (m, 1H), 3.37 (s, 2H), 3.19 (s, 3H), 3.11-3.07 (m, 2H), 3.01-2.98 (m, 4H), 2.76-2.72 (m, 3H), 2.60-2.56 (m, 4H), 2.66 (d, J= 4.2 Hz, 2H), 2.62 (d, J=3.6 Hz, 2H), 2.54-2.49 (m, 5H), 2.24-2.24 (m, 1H), 1.99-1.95 (m, 3H), 1.69-1.63 (m, 2H), 1.54-150 (m, 2H), 1.01 (d, J= 7.2 Hz, 3H), 0.89 (t, J=11.4 Hz, 3H).

6-[(2-(4-{4-[{Methyl-prop-2-ynyl-amino]-methyl}-phenyl]-piperazin-1-yl}-ethyl]-propyl-amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (35).

Compound 34 (0.050 g, 0.105 mmol) was dissolved in dry CH$_2$Cl$_2$ and BBr$_3$ was slowly added at -40 °C for over a period of 2 h. The reaction mixture was stirred overnight at room temp according to procedure O. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 8:2) to yield compound 35 (D-677) (0.020 g, 55%). $^1$H NMR (600 MHz, CD$_3$OD): 7.26-7.22 (m, 1H), 7.09-7.06 (m, 1H), 6.86-6.82 6 (m, 2H), 6.70 (d, J=7.2 Hz, 1H), 6.64 (d, J=7.8 Hz, 1H), 3.39-6.38 (m, 1H), 3.34-3.30 (m, 3H), 63.19 (d, J=4.2 Hz, 2H), 3.01-2.94 (m, 2H), 2.87-2.84 (m, 3H), 2.75-2.74 (m, 3H), 2.66 (d, J= 4.2 Hz, 2H), 2.62 (d, J=3.6 Hz, 2H), 2.54-2.49 (m, 5H), 2.24-2.24 (m, 1H), 2.07-2.02 (m, 1H), 1.58-1.54 (m, 1H), 1.49 (t, J=6.6 Hz, 2H), 0.89 (t, J=11.4 Hz, 3H). $^{13}$C NMR (150 MHz, CD$_3$OD): δ 232.81, 229.21, 222.31, 217.46, 211.65, 202.40, 197.89, 194.32, 190.12, 154.96, 153.01, 150.89, 120.76, 117.30, 106.80, 101.02, 64.57, 53.79, 50.70, 45.77, 41.58, 30.79, 29.92, 27.45, 24.56, 20.94, 12.83, 8.06; $[α]_D^{25}$ = -25 (c=1.0 in CH$_3$OH); Mp. 205-210 °C, Anal. Calcd for C$_{30}$H$_{42}$N$_4$O$._3$HCl, H$_2$O: C, 54.33; H, 7.69; N, 7.20. Found: C 54.05; H, 7.22; N, 7.12.

4-(4-Hydroxy-phenyl)-piperazine-1-carboxylic acid tert-butyl ester (37).
A mixture of 4-Piperazin-1-yl-phenol 36 (1.0 g, 5.49 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)ethyl) piperazine (2.015 g, 8.24 mmol), Pd(OAc)$_2$ (0.0925 g, 0.412 mmol), BINAP (0.342 g, 0.55 mmol) and Cs$_2$CO$_3$ (5.37 g, 16.49 mmol) in toluene (25 mL) was heated at 110 °C for 24 h according to procedure D. The crude material was purified by a silica gel column chromatography (hexane:EtOAc = 4:1) to give compound 25 (1.71 g, 90%). $^1$H NMR (600 MHz, CDCl$_3$): δ 7.24-6.87 (m, 4H), 3.55 (t, J = 1.8 Hz, 4H), 2.94 (s, 2H), 2.86 (s, 2H), 1.46 (s, 9H).

4-(4-Prop-2-ynyloxy-phenyl)-piperazine-1-carboxylic acid tert-butyl ester (38).

Compound 37 (0.134 g, 0.217 mmol), potassium carbonate (0.90 g, 0.651 mmol), and propargyl bromide (0.31 ml, 0.261 mmol) were suspended in acetonitrile (7 mL). The stirring mixture was heated to approximately 50 °C for 5 h according to procedure M. The crude, Procedure I which was purified by column chromatography using (1:9) MeOH:EtOAC to give intermediate 31 (0.345 g, 60%). $^1$H NMR (600 MHz, CDCl$_3$): δ 7.24-6.87 (m, 4H), 4.62 (d, J = 1.8 Hz, 2H), 3.55 (t, J = 1.8 Hz, 4H), 2.94 (s, 2H), 2.86 (s, 2H), 2.49-2.48 (m, 1H), 1.46 (s, 9H).

1-(4-Prop-2-ynyloxy-phenyl)-piperazine (39).

Into a stirring solution of compound 38 (0.110 g, 0.293 mmol) in THF (10 mL) was added n-tetrabutylammonium fluoride (0.127 ml, 0.44 mmol, 1.0 M solution in THF) at 0 °C by following procedure E. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 9:1) to give compound 39 (0.50 g, 78.7%). $^1$H NMR (600 MHz, CDCl$_3$): δ 6.89-6.88 (m, 4H), 4.61 (d, J = 2.4 Hz, 2H), 3.04-3.02 (m, 8H), 2.50 (t, J = 2.4 Hz, 1H).

Procedure Q. 2-[4-(4-Prop-2-ynyloxy-phenyl)piperazin-1-yl]-ethanol (40).
A suspension of 1-(4-Prop-2-ynyloxy-phenyl)-piperazine 39 (0.130 g, 0.601 mmol), potassium carbonate (48.13 g, 348.27 mmol), and 2-Bromo-ethanol (0.017 mL, 0.24 mmol) in acetonitrile (5 mL) was refluxed under N\textsubscript{2} overnight. The reaction mixture was filtered off, and the filtered was evaporated under reduced pressure. The residue was then diluted with ether, washed with water, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated to give alcohol 40 (0.085g, 54.32%). \textsuperscript{1}H NMR (600MHZ, CDCl\textsubscript{3}): δ 6.92-6.87 (m, 4H), 4.61 (d, J = 2.4 Hz, 2H), 3.84-3.82 (m, 2H), 3.34-3.31 (m, 2H), 3.05-3.04 (m, 4H), 3.00 (m, 4H), 2.50 (t, J= 2.4 Hz, 1H).

[4-(4-Prop-2-ynyloxy-phenyl)-piperazin-1-yl]-acetaldehyde (41).

Compound 40 (0.200 g, 0.768 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (10 mL) and DMSO (5 mL), was oxidized using SO\textsubscript{3}.py complex (3.028 g, 19.025 mmol) and Et\textsubscript{3}N (0.611 mL, 3.84 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAC) to yield compound 41 (0.170 g, 85.6%). \textsuperscript{1}H NMR (600 MHz, CDCl\textsubscript{3}): δ 7.84 (s, 1H), 7.00 (dd, J= 6.0, 1.8 Hz, 1H), 6.61-6.59 (m, 1H), 6.17-6.13 (m, 2H), 4.77 (d, J= 0.6 Hz, 1H), 3.92-3.90 (m, 4H), 3.84-3.82 (m, 2H), 3.33-3.31 (m, 4H), 3.26-3.23 (m, 4H).

(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-{2-[4-(4-prop-2-ynyloxy-phenyl)-piperazin-1-yl]-ethyl]-amine (42, D-678).

Compound 41 (0.170 g, 0.658 mmol) was reacted with (±)-5-OMe-MPAT (0.129 g, 0.592 mmol) and NaBH(OAc)\textsubscript{3} (0.278 g, 1.316 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (15 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to obtain compound 42 (D-678) (0.060 g, 32.3%). \textsuperscript{1}H NMR (600 MHz, CDCl\textsubscript{3}): δ 7.24-6.92 (m, 1H), 6.90-6.87 (m, 4H), 6.69 (d, J= 7.8 Hz, 1H), 6.63 (d, J=...
7.8 Hz, 1H), 4.63-4.61 (m, 2H), 3.79 (s, 3H), 3.69-3.68 (m, 1H), 3.52-3.50 (m, 1H), 3.34-3.33 (m, 1H), 3.12-3.05 (m, 4H), 3.03-3.01 (m, 1H), 2.99-2.98 (m, 1H), 2.96-2.95 (m, 1H), 2.85-2.83 (m, 1H), 2.76-2.71 (m, 2H), 2.66-2.63 (m, 3H), 2.53-2.50 (m, 1H), 2.49-2.47 (m, 2H), 0.87 (t, J = 7.8 Hz, 3H). 13C NMR (150 MHz, CD3OD): δ 235.34, 231.04, 224.94, 219.39, 214.29, 207.74, 199.97, 197.85, 195.48, 159.34, 157.36, 153.32, 126.06, 122.76, 110.21, 107.43, 59.20, 55.60, 49.35, 44.67, 34.23, 32.79, 29.98, 28.45, 25.53, 16.93, 14.67, 10.65; [α]D25 = -24 (c = 1.0 in CH3OH); Mp = 195 °C, Anal. Calcd for C29H42N3O2.3HCl, H2O, C, H, N.

2-[4-(4-Nitro-phenyl)-piperazin-1-yl]-ethanol (44).

A mixture of 2-bromoethanol (0.274 mL, 3.86 mmol), 1-(4-Nitro-phenyl)-piperazine 43 (2.0 g, 9.65 mmol), and K2CO3 (4.0 g, 28.95 mmol) in acetonitrile (25 mL) was refluxed for 24 h under inert condition according to procedure Q. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 3:2) to give compound 44 (1.20 g, 49.5%). 1H NMR (600 MHz, CDCl3): δ 6.82-6.80 (m, 2H), 6.50-7.44 (m, 2H), 3.76 (t, J = 6.8 Hz, 2H), 2.76 (t, J = 7.2 Hz, 2H), 2.68-2.57 (m, 4H), 2.54 (t, J = 6.4 Hz, 4H).

[4-(4-Nitro-phenyl)-piperazin-1-yl]-acetaldehyde (45).

Compound 44 (1.20 g, 4.77 mmol) in CH2Cl2 (20 mL) and DMSO (10 mL), was oxidized using SO3.py complex (3.80 g, 23.87 mmol) and Et3N (4.65 mL, 33.43 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc) to yield compound 45 (0.80 g, 67.2%). 1H NMR (600 MHz, CDCl3): δ 6.82-6.79 (d, J = 9.6, 2.4 Hz, 2H), 6.69-6.64 (m, 2H), 4.10 (t, J = 7.2 Hz, 2H), 3.25 (t, J = 1.2 Hz, 2H), 2.74 (t, J = 4.2 Hz, 4H), 2.25 (m, 2H).
(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-{2-[4-(4-nitro-phenyl)-piperazin-1-yl]-ethyl}-propyl-amine (46).

Compound 45 (0.251 g, 1.007 mmol) was reacted with (-)-5-OMe-MPAT (0.197 g, 0.906 mmol) and NaBH(OAc)$_3$ (0.427 g, 2.015 mmol) in CH$_2$Cl$_2$ (15 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound 46 (0.30 g, 65.7%). $^1$H NMR (600 MHz, CDCl$_3$): δ 8.10 (dd, J = 4.8, 2.4 Hz, 2H), 7.08 (t, J = 7.8 Hz, 1H), 6.79 (dd, J = 7.8, 1.8 Hz, 2H), 6.69 (d, J = 7.8 Hz, 1H), 6.64 (d, J = 8.4 Hz, 1H), 3.79 (s, 3H), 3.40 (t, J = 5.4 Hz, 4H), 3.00 (d, J = 4.2 Hz, 1H), 2.79 (d, J = 3.6 Hz, 1H), 2.95-2.93 (m, 1H), 2.85-2.82 (m, 1H), 2.76–2.70 (m, 3H), 2.65–2.60 (m, 4H), 2.53–2.48 (m, 5H), 2.03 (dd, J = 4.2, 3.0 Hz, 4H), 1.59-1.52 (m, 2H), 1.50–1.44 (m, 1H), 0.87 (t, J = 7.2 Hz, 3H).

Procedure R. {2-[4-(4-Amino-phenyl)-piperazin-1-yl]-ethyl}-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (47).

Compound 46 (0.30, 0.661) was dissolved in methanol (15 mL) and the mixture was hydrogenated on a parr apparatus (1 atm) in the presence of 10% Pd-C (0.70 g, 10 wt %) at 30 psi for overnight. Then, the reaction mixture was filtered through a short bed of Celite, and the filtrate was concentrated under reduced pressure on a rotary evaporator. The crude was purified via column chromatography using MeOH:EtOAc (1:10) as the eluent to afford the amine 48 (0.20 g, 71.4%). $^1$H NMR (600 MHz, CDCl$_3$): δ 7.08 (t, J = 8.4 Hz, 1H), 6.80-6.78 (m, 2H), 6.70 (d, J = 7.2 Hz, 1H), 6.64-6.62 (m, 3H), 3.79 (s, 3H), 3.41 (s, 2H), 3.05 (t, J= 4.8 Hz, 4H), 2.99 (dq, J= 3.6, 1.8 Hz, 1H), 2.92 (tq, J= 2.4, 1.8 Hz, 1H), 2.86 (d, J= 2.4 Hz, 1H), 2.83 (d, J= 3.0 Hz, 1H), 2.75-2.71(m,
2H), 2.67-2.63 (m, 4H), 2.55-2.48 (m, 5H), 2.07-2.03 (m, 1H), 1.60-1.55 (m, 2H), 1.51-1.44 (m, 1H), 0.89 (t, J = 7.2 Hz, 3H).

Procedure S. (5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-{2-[4-(4-prop-2-ynylamino-phenyl)-piperazin-1-yl]-ethyl}-amine (48).

Compound 47 (0.109 g, 0.332 mmol), potassium carbonate (0.137 g, 0.995 mmol), and propargyl bromide (0.047 mL, 0.398 mmol) were suspended in acetonitrile (10 mL). according to procedure N. The crude compound was purified by column chromatography using (1:9) MeOH:EtOAC to give compound 48 (0.456 g, 40%). ¹H NMR (600 MHz, CDCl₃): δ ppm 7.08-7.06 (m, 1H), 6.85 (td, J = 3.6, 1.8 Hz, 2H), 6.69 (d, J = 7.8 Hz, 1H), 6.67-6.63 (m, 3H), 3.89 (dd, J = 4.2, 2.4 Hz, 2H), 3.79 (s, 3H), 3.60 (d, J = 6.6 Hz, 1H), 3.98 (dd, J = 13.8, 4.2 Hz, 2H), 2.94-2.88 (m, 1H), 2.85 (d, J = 2.4 Hz, 2H), 2.82 (d, J = 2.4 Hz, 2H), 2.76-2.82 (m, 3H), 2.64-2.63 (m, 4H), 2.53-2.47 (m, 5H), 2.19 (s, 1H), 2.06-2.03 (m, 1H), 1.59 (m, 2H), 1.50-1.44 (m, 2H), 0.88 (t, J = 7.8, 3H).

6-(Propyl-{2-[4-(4-prop-2-ynylamino-phenyl)-piperazin-1-yl]-ethyl}-amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (49).

Compound 48 (0.0456 g, 0.140 mmol) was dissolved in dryCH₂Cl₂ (5 mL) and BBr₃ (0.110 ml, 0.705 mmol) was added at -40 °C for 2 h, the reaction mixture was stirred overnight at room temp according to procedure O. The crude product was purified by silica gel column chromatography (EtOAC:MeOH = 8:2) to yield HBr salt of 49 (0.025 g, 60%). ¹H NMR (600 MHz, CD₃OD): δ 7.12 (d, J = 7.2 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 6.90-6.82 (m, 2H), 6.71 (d, J = 4.8 Hz, 1H), 6.68-6.67 (m, 1H), 4.10 (q, J = 7.2 Hz, 3H), 3.79 (s, 2H), 3.65-3.63 (m, 2H), 3.33 (s, 3H), 3.22-3.00 (m, 7H), 2.79 (s, 1H), 2.59-2.53 (m, 4H), 2.32 (t, J = 7.2 Hz, 1H); 2.27 (t, J = 7.2 Hz, 1H); 2.1-2.03 (m, 3H), 1.61 (t, J =
8.4 Hz, 3H), 1.07 (t, J = 6.0 Hz, 3H). $^{13}$C NMR (150 MHz, CD$_3$OD): $\delta$ 155.43, 142.45, 142.83, 135.09, 128.67, 128.48, 128.49, 124.59, 123.49, 122.48, 121.32, 120.56, 120.34, 120.05, 119.59, 115.69, 113.34, 113.01, 75.30, 65.29, 56.39, 54.89, 54.21, 53.98, 53.30, 46.34, 34.09, 26.40, 24.39, 22.23, 13.41, 11.98; $[^\alpha]D^{25}$ = -26.08 (c=1.0 in CH$_3$OH); Anal. Calcd for C$_{28}$H$_{38}$N$_4$O.3HBr: C, H, N.

Procedure T. 1,1-Diphenyl-but-3-en-1-ol (51).

Into a mixture of benzophenone (50) (5.0 g, 27.40 mmol) in anhydrous THF (50.0 mL), copper iodide (CuI) (0.52 g, 2.74 mmol) at -78 °C under nitrogen was added allyl magnesium chloride in THF (3.39 g, 2 mmol) slowly under stirring condition. The reaction mixture was warmed slowly to room temperature and stirred overnight. The mixture was cooled to 0 °C and sat. NH$_4$Cl and water were added. The organic layer was separated, and the aqueous layer was extracted with EtOAC (3 * 100 ml). The organic layer was combined, dried over Na$_2$SO$_4$ and concentrated in rotary evaporator. The crude product was mostly pure compound 51 (6.14 g, 99%) and used for the next step without further purification. $^1$H NMR (500MHz, CDCl$_3$) $\delta$, 7.42–7.45 (m,4H), 7.27–7.23 (m,4H), 7.18–7.12 (m,2H), 5.68–7.62 (m,1H), 5.20–5.12 (m,2H), 2.58 (S, 1H).

Procedure U. 1,1-Diphenyl-but-3-en-1- (prop-3-en)ol ether (52).

The alcohol 51 (6.14 g, 27.37 mmol) was taken in an oven-dried RB flask equipped with a magnetic stir-bar. Anhydrous DMF (50ml) was then added to the flask via syringe. The solution was cooled to 0 °C, and NaH 60% (13.45 g, 560.42 mmol) was added portionwise. The reaction mixture was stirred for 15-20 min. Next the allyl bromide (18.42, 152.26 mmol) was added dropwise, after 5 min the water bath was removed, and the reaction mixture was stirred at room temperature for 1.5 h. Reaction mixture was cooled
again to 0 °C and the reaction was quenched with ethyl acetate followed by addition of water. The crude was purified by gradient column chromatography using hexan:EtOAC (10:1) to yield compound 52 (7.0 g, 96%). 1H NMR (600 MHz, CDCl3): δ 7.24-7.30 (m, 4H), 7.21-7.19 (m, 4H), 7.13-7.11 (m, 2H), 5.86-5.80 (m, 1H), 5.57-5.50 (m, 1H), 5.29-5.26 (m, 1H), 5.06-5.03 (m, 1H), 4.96-4.94 (m, 1H), 4.91-4.88 (m, 2H), 3.07 (dd, J= 5.4, 1.2 Hz), 2.91-2.79 (m, 2H).

Procedure V. 2,2-Diphenyl-3,6-dihydro-2H-pyran (53).

Into a stirred solution of vinyl ether (52) dissolved in anhydrous benzene under continuous flow of nitrogen at room temperature, 1st generation Grubb’s catalyst (0.44 g, 0.53 mmol) was added. The solution mixture was slowly heated to reflux at 90 °C for 2 h. After the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure on a rotary evaporator. The crude residue was purified by gradient column chromatography using hexane:EtOAC (9.5:0.5) to yield the cyclic olefin 53 (3.25 g, 51.1%). 1H NMR (600 MHz, CDCl3): δ 7.36 (dd, J=7.8, 0.6 Hz, 4H), 7.31-7.29 (m, 4H), 7.24-7.21 (m, 2H), 6.0-5.96 (m, 1H), 5.64 (dt, J=2.4, 1.8, 1H), 4.06-4.04 (m, 2H), 2.82-2.80 (m, 2H). kimkk

Procedure W. 2,2-Diphenyl-7-oxa-bicyclo[4.1.0]heptane (54).

m-CPBA (1.64 g, 9.35 mmol, 50% wt/wt in water), was added portion wise to a solution of alkene 53 (1.50 g, 6.35 mmol) in DCM (15 ml) at 0 °C. The ice bath was removed, and the reaction mixture was stirred at room temperature for 24 h. Next, the reaction mixture was cooled again to 0 °C and quenched with saturated NaHCO3 (100 mL). The organic layer was separated, and the aqueous layer was extracted with additional DCM (2 × 50 ml). The organic layers were combined and washed with brine
100 mL. The organic layer was separated, dried over Na₂SO₄, and concentrated over rotary evaporator. The crude product was purified via gradient silica gel column chromatography using hexane:EtOAC, 8:1 to obtain pure racemic epoxide 54 (wrong structure in the scheme) (0.53 g, 50%). ¹H NMR (600 MHz, CDCl₃): minor portion; δ 7.44-7.38 (m, 4H), 7.30-7.23 (m, 5H), 7.18-7.15 (m, 1H), 4.22-4.16 (m, 1H), 4.07 (dd, J= 6.11, 5.49 Hz, 1H), 3.99-3.94 (m, 1H), 3.37-3.30 (m, 2H), 2.50-2.46 (m, 1H), 2.29 (s, 1H), major portion: δ 7.46-7.44 (m, 2H), 7.38 (t, J= 7.63 Hz), 7.30-7.22 (m, 5H), 7.17-7.14 (m, 1H), 4.11 (J=7.02, 5.88 Hz, 1Hz), 4.04-3.99 (m, 1Hz), 3.94-3.90 (m, 1H), 3.58 (t, J=11.60 Hz, 1H), 3.20 (dd, J= 9.77, 4.27, Hz, 1 H), 2.42 (d, J= 2.13 Hz, 1H), 2.03-1.98 (m, 1H).

Procedure X. 2-Azido-5,5-diphenyl-cyclohexanol (55 a, and b).

Epoxide 54 (0.43 g, 1.70 mmol) was dissolved in MeOH:H₂O (8:1). Sodium azide (0.55 g, 8.52 mmol) and NH₄Cl (0.18 g, 3.40 mmol) were added at once. The mixture was then stirred for 48 h at 80 °C under a continuous flow of N₂, cooled to room temperature, and quenched with water (50 mL). The solution was extracted with EtOAC (3 × 50 mL), the organic layers were combined and washed with brine (25 mL). The organic layer was separated, dried over Na₂SO₄, and concentrated under reduced pressure on a rotary evaporator. The crude product was purified by gradient silica gel column chromatography using Hexan:EtOAC to obtain the racemic azide (55 a and b) (0.23 g, 46 %). ¹H NMR (600 MHz, CDCl₃): δ 7.44-7.43 (m, 4H), 7.30-7.23 (t, 5H), 7.26-7.19 (m, 1H), 4.15-4.12 (m, 1H), 3.56 (dd, J= 8.4, 6.0 Hz, 1H), 3.37-3.30 (m, 2H), 2.97-2.91 (m, 1H), 2.50-2.46 (m, 1H), 2.30-2.26 (m, 1H).
Procedure Y. 2-Amino-5-(1-methylene-but-2-enyl)-5-phenyl-cyclohexanol (56 a, and b).

The azides (55 a, and b) (0.32 g, 0.78 mmol) was dissolved in methanol (5 mL) and the mixture was hydrogenated on a parr apparatus (1 atm) in the presence of 10% Pd-C (0.026 g, 10 wt %) at 30 psi for overnight according to procedure R. The crude was purified via column chromatography using MeOH:DCM (1:10) as the eluent to afford the amine (56a and b) (0.050 g, 50%). 1H NMR (600 MHz, CDCl₃): δ 7.44-7.43 (m, 4H), 7.30-7.23 (t, 5H), 7.26-7.19 (m, 1H), 4.15-4.12 (m, 1H), 3.56 (dd, J= 8.4, 6.0 Hz, 1H), 2.97-2.91 (m, 1H), 1.80–1.48 (m, 2 H), 1.28–1.72 (m, 1 H).

Procedure R. 2-(4-Methoxy-benzylamino)-5,5-diphenyl-cyclohexanol (57a and b).

4-methoxy-benzaldehyde (0.035 g, 0.26 mmol) was dissolved in a mixture of 1,2-dichloromethane (3 mL)/methanol (1 mL) and glacial acetic acid (0.015 µL, 0.26 mmol) was then added. Then, amine (56a and b) (0.107 g, 0.38 mmol) was added and the solution stirred at room temperature for 2 h following which Na(OAc)₃BH (0.0.05 g, 0.24 mmol) was added. The resulting mixture was then stirred at room temperature for 24 hours, cooled to 0 °C, diluted with DCM (12 mL) and quenched by the addition of water (18 mL). The organic layer was separated, and the aqueous layer was extracted with additional DCM (3 × 18 mL). The organic layers were combined, dried over Na₂SO₄, and concentrated under reduced pressure on a rotary evaporator. The crude residue was purified by gradient silica gel column chromatography using DCM:MeOH (10:1) to obtain compounds (57a (D-594) and 57 b) (68 mg, 46%). (c = 1, MeOH). 1H NMR (500 MHz, CDCl₃): δ 7.46-7.44 (m, 2 H), 7.33 (t, J = 7.93 Hz, 2 H), 7.32-7.27 (m, 2 H), 7.23-7.22 (m,
2 H), 7.20-7.18 (m, 2 H), 7.13 (t, J = 7.32 Hz, 2 H), 6.84–6.82 (m, 2 H), 4.1-4.07 (m, 1H), 3.78 (s, 3H), 3.59-3.53 (m, 1H), 3.12-3.07 (m, 2H), 2.76-2.71 (m, 1H), 1.93-1.88 (m, 2H), 13C NMR (100 MHz, CDCl₃): δ 158.7, 147.61, 132.1, 129.2, 128.7, 127.1, 126.63, 113.8, 81.2, 69.3, 69.6, 61.2, 55.2, 55.24, 50.86, 42.4. The free base was converted into corresponding hydrochloride salt. Mp = 250–255 °C. Anal. (C₂₅H₂₈NO₂·HCl) C, H, N. **Procedure Z.** 2-(4-Methoxy-phenyl)-ethylamine (59). 4-methoxyphenylacetanitrile (2.0 g, 13.59 mmol) was dissolved in THF (11 mL) at room temperature and the reaction mixture was cooled to 0 °C followed by addition of boron-methyl sulfide complex (5.74 g, 47.56 mmol). The reaction mixture was stirred for 2 h, then gradually warmed to 40 °C and stirred overnight. The reaction mixture was slowly quenched by adding 2N HCl solution (16 ml), the quenched mixture was stirred for 1 h at 40 °C followed by the addition of conc.NH₄OH (5 ml) then EtOAC, and dibasic sodium phosphate. The organic layer was separated, dried over Na₂SO₄, and concentrated over rotary evaporator to get compound (59). The crude was mostly pure for use in the next step without further purification (0.1 g, 0.396 mmol) ¹H NMR (600 MHz, CDCl₃): δ 7.06–7.05 (m, 2 H), 6.84-6.82 (m, 2 H), 4.21-4.17 (m, 2 H), 3.71 (s, 3 H), 3.54-3.53 (s, 2H). **Procedure Z’.** 2-[2-(4-Methoxy-phenyl)-ethylamino]-5,5-diphenyl-cyclohexanol (60a and b). A mixture of epoxide (0.1 g, 0.396 mmol) and 2-(4-methoxyphenyl) ethan-1-amine (1.198g, 7.92 mmol) in ethanol was refluxed at 100 °C under N₂ overnight. The reaction was quenched with saturated NaHCO₃, and DCM was added. The organic layer was separated, dried over Na₂SO₄, and concentrated in rotary evaporator. The crude product
was purified via gradient silica gel column chromatography using MeOH:DCM (1:10) to get compounds (60a (D-620), and b (D-621)) (0.096 and 0.025 g, 64% and 17%). Major yield: $^1$H NMR (600 MHz, CDCl$_3$): δ 7.45 (d, J= 7.2 Hz, 2H), 7.33 (t, J= 7.5Hz, 2H), 7.28 (d, J= 7.8 Hz, 2H), 7.23 (t, J= 7.8 Hz, 1H), 7.22 (t, J= 7.8 Hz,2H), 7.14 (t, J= 7.5 Hz, 1H), 7.07 (d, J=8.4 Hz, 2H), 6.8 (d, J= 8.4 Hz, 2H), 4.07 (dd, J= 11.50, 4.8 Hz, 1H), 3.77 (s, 3H), 3.50 (td, J= 11.60, 4.1 Hz, 1H), 3.09 (dd, J= 11.60, 4.3 Hz, 1H), 3.05 (t, J= 11.0 Hz, 1H), 2.95 (dt, J= 14.7 Hz, 1H), 2.78 (m, 1H), 2.69 (btw d&t, J= 10.6, 4.8 Hz, 1H), 2.68 (m, 1H), 1.91 (dd, J= 13.8, 4.3 Hz, 1H). $^1$H NMR (600 MHz, CDCl 3): Minor yield: δ 7.67–7.63 (m, 2 H), 7.54-7.52 (m, 2H), 7.46-7.43 (m, 2H), 7.28-7.20 (m, 2H), 7-08-7.01 (m, 2H), 6.83 (d, J= 9.0 Hz, 2H), 6.82-6.78 (m, 2H), 4.06 (dd, J = 7.2, 4.2 Hz, 1 H), 3.78 (s, 3 H), 3.53 (dd, J = 6.6, 6.0 Hz, 2 H),3.42 (dd, J = 6.6, 6.0 Hz, 1 H), 3.09 (dd, J = 9.0, 4.2 Hz, 1 H), 2.78-2.73 (m, 2 H), 2.71-2.69 (m, 2 H), 2.67–2.63 (m, 2 H), 1.91 (dd, J=11.4, 2.4 Hz, 1H), 0.88–0.82 (m, 3 H). $^{13}$C NMR (150 MHz, CDCl$_3$): δ 158.053, 147.646, 142.104, 131.537, 129.550, 128.737, 128.077, 127.219, 127.101, 126.647, 124.858, 113.92, 81.287, 69.264, 64.701, 61.974, 55.226, 48.787, 42.451, 36.038. (60a (D-620), and b (D-621)); Mp = 195 °C. Anal. (C$_{26}$H$_{30}$NO$_3$. HCl) C, H, N and Mp = 200 °C. Anal. (C$_{26}$H$_{30}$NO$_3$. HCl) C, H, N respectively.

Procedure Z”. 2,2-Diphenyl-7-oxa-bicyclo[4.1.0]heptane (61).

The alkene (0.20 g, 0.85 mmol) (53) was dissolved in a mixture of acetonitrile:DME (1:2) (15 mL), A buffer Na$_2$B$_4$O$_7$.H$_2$O (0.01 g, 0.05 mmol) in 0.0004 M aqueous Na$_2$EDTA (?) was added. Tertbutyl ammonium hydrogen sulfate (0.0114 g, 0.034 mmol), and epoxane (ketone) (0.065 g, 0.25 mmol) were then added with stirring. The mixture was cooled to about -10 °C bath temperature by using an NaCl ice bath. A solution of oxone
in aqueous Na$_2$EDTA 0.004 M and a solution of K$_2$CO$_3$ (0.68 g, 4.91 mmol) in water were added dropwise separately over a period of 2 h via droplets to give the chiral epoxide (61) (0.074 g, 35%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 7.31-6.92 (m, 10H), 3.85 (d, $J$ = 13.6 Hz, 1H), 3.25 (dd, $J$ = 13.6, 1.5 Hz, 1H), 2.95 (bt, $J$ = 4.9 Hz, 1H), 2.42 (dd, $J$ = 15.8, 5.8 Hz, 1H), 2.25 (dd, $J$ = 4.3, 1.5 Hz, 1H), 2.20 (dd, $J$ = 15.8, 0.66 Hz, 1H).

2-[2-(4-Methoxy-phenyl)-ethylamino]-5,5-diphenyl-cyclohexanol (60a).

A mixture of chiral epoxide (61) (0.1 g, 0.396 mmol) and 2-(4-methoxyphenyl)ethan-1-amine (1.19 g, 7.92 mmol) in ethanol was refluxed at 100 °C under N$_2$ overnight. The reaction was followed according to procedure Z'. The crude product was purified via silica gel column chromatography using MeOH:DCM (1:10) to get compounds 60a (D-620) (0.096 g, 64%). $^1$H NMR (600 MHz, CDCl$_3$): $\delta$ 7.45 (d, $J$ = 7.2 Hz, 2H), 7.33 (t, $J$ = 7.5 Hz, 2H), 7.28 (d, $J$ = 7.8 Hz, 2H), 7.23 (t, $J$ = 7.8 Hz, 1H), 7.22 (t, $J$ = 7.8 Hz, 2H), 7.14 (t, $J$ = 7.5 Hz, 1H), 7.07 (d, $J$ = 8.4 Hz, 2H), 6.8 (d, $J$ = 8.4 Hz, 2H), 4.07 (dd, $J$ = 11.50, 4.8 Hz, 1H), 3.77 (s, 3H), 3.50 (td, $J$ = 11.60, 4.1 Hz, 1H), 3.09 (dd, $J$ = 11.60, 4.3 Hz, 1H), 3.05 (t, $J$ = 11.0 Hz, 1H), 2.95 (dt, $J$ = 14.7 Hz, 1H), 2.78 (m, 1H), 2.69 (btw d&t, $J$ = 10.6, 4.8 Hz, 1H), 2.68 (m, 1H), 1.91 (dd, $J$ = 13.8, 4.3 Hz, 1H). $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 158.053, 147.646, 142.104, 131.537, 129.550, 128.737, 128.077, 127.219, 127.101, 126.647, 124.858, 113.92, 81.287, 69.264, 64.701, 61.974, 55.226, 48.787, 42.451, 36.038. Mp = 195 °C. Anal. (C$_{26}$H$_{30}$NO$_3$. HCl) C, H, N.

5.2. Evaluation of binding affinity and functional potencies at dopamine D$_2$ and D$_3$ receptors.

Binding affinity was evaluated by inhibition of $[^3]$H spiroperidol (15.0 Ci/mmol, Perkin-Elmer) binding to DA rD$_2$ and rD$_3$ receptors expressed in HEK-293 cells in a buffer
containing 0.9% NaCl to determine the inhibition constants (K_i) of the synthesized compounds (Zhen et al. 2010; Ghosh et al. 2010b). The Cheng–Prusoff equation was used to convert the observed IC_{50} into inhibition constants (K_i) (Zhen et al. 2010). Functional activity of test compounds in activating dopamine hD_2 and hD_3 receptors expressed in CHO cells was measured by stimulation of [^{35}S] GTPγS (1250 Ci/mmol, Perkin-Elmer) binding in comparison to stimulation by the full agonist DA. All these procedures were described by us previously (Biswas et al. 2008; Zhen et al. 2010; Ghosh et al. 2010b).


5.3.1. Drugs and chemicals.

The following commercially available drug was used in the experiment: reserpine hydrochloride (Alfa Aesar). The TFA salt of (-)-11b (D-636) and HBr salts of (-)-15a (D-653) and (-)-15c (D-656) were dissolved in water. Reserpine was dissolved in 20 μL of glacial acetic acid and further diluted with 5.5% glucose solution. The compounds for this study were administered in a volume of 0.1−0.2 mL for subcutaneous administration and 0.5−0.7 ml for interaperitoneal administration into each rat.

5.3.2. Animals.

In rodent studies, animals were male and female Sprague-Dawley rats from Harlan (Indianapolis, IN) weighing 220-225 g unless otherwise specified. Animals were maintained in sawdust-lined cages in a temperature and humidity-controlled environment at 22 ± 1 °C and 60 ± 5% humidity, respectively. A 12 h light/dark cycle was maintained, with lights on from 6:00 a.m. to 6:00 p.m. They were group-housed with unrestricted
access to food and water. All experiments were performed during the light component. All animal use procedures were in compliance with the Wayne State University Animal Investigation Committee, consistent with AALAC guidelines.

5.3.3. Reversal of reserpine-induced hypolocomotion in rats.

The ability of compounds D-636, D-653 and D-656 to reverse reserpine-induced hypolocomotion was investigated according to a reported procedure (McCall et al. 2005). Reserpine (5.0 mg/kg, sc) was administered 18 h before the injection of drug or vehicle. The rats were placed individually in the chambers for 1 h for acclimatization before administration of the test drugs or vehicle. Immediately after administration of drug or vehicle, animals were individually placed in Opto-Varimex 4 animal activity monitor chamber (Columbus Instruments, Ohio, USA) to start measuring locomotor activity. Locomotion was monitored for 6 h. Consecutive interruption of two infrared beams, situated 50 cm apart and 4 cm above the cage floor, in the monitor chamber recorded movement. The data were presented as horizontal activity (HACTV). The effect of individual doses of drugs on locomotor activity was compared with respect to saline treated controls (mean ± SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test. The effect was considered significant if the difference from control group was observed at p < 0.05.


5.4.1. Cell Cultures and Treatments.

PC12 cells (ATCC CRL1721.1, Manassas, VA, USA), a rat adrenal pheochromocytoma cell line, were cultured in T-75 flasks (Greiner Bio One, Frickenhausen, Germany) and maintained in RPMI 1640 medium supplemented with
10% heat inactivated horse serum, 5% fetal bovine serum, 100 U/ml penicillin, and 100 µg/mL streptomycin at 37 °C in 95% air/5% CO₂. Stock solutions of D-636, D-653, and D-656 were prepared in dimethylsulfoxide (DMSO) and stored at -20 °C, a stock solution of 6-Hydroxydopamine (6-OHDA) was stored at -80 °C, a solution of 6-carboxy-2',7'-dichlorodihydroflourescence diacetate (carboxy-DCFDA) was prepared fresh in DMSO before addition. All stock solution were stored for the period of the experiments.

5.4.2. Antioxidant activity studies: Measurement of antioxidant activity:

To determine the effects of D-636, D-653 and D-656 in decreasing reactive oxygen species (ROS) in PC12 cells produced by the neurotoxin 6-OHDA, a quantitative fluorometric ROS assay was performed. PC12 cells were plated at 30,000 cells/well density in 100 µL media in 96-well black plates and incubated at 37 °C under 5% CO₂ atmosphere for 24 h. The cells were treated for 24 h with various concentrations of compounds D-636, D-653 and D-656. Then the drugs containing media were removed and replaced with DCFDA 20 µm for 30 min in an incubator (37 °C, 5% CO₂). The DCFDA containing media was then removed and the cells were washed with PBS buffer to remove the traces of the dye. Fresh culture media was added followed by treatment with 75 µM of 6-OHDA alone for an additional 1 h under the same conditions. After incubation for 1 h, the fluorescence was measured using spectrophotometer fluorescence generated microplate reader (Biotek Epoch, Winooski, VT, USA) at excitation 497 nm and emission at 527nm. Data from at least three experiments were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post hoc test using GraphPad software (version 6, San Diego, CA, USA). The specific fluorescence emission was calculated after subtraction of the DCFDA untreated control cells from the DCFDA
treated cells for both the background and 1h treatment conditions. This was followed by the division of the 1h treatment data by the background activity which was determined from vials before treatment with 6-OHDA, to derive the final data point.

5.4.3. Neuroprotection Studies.

5.4.3.1. Assessment of Cell Viability:

PC12 cells (ATCC CRL1721.1, Manassas, VA, USA), a rat adrenal pheochromocytoma cell line, were cultured in T-75 flasks (Greiner Bio One, Frickenhausen, Germany) and maintained in RPMI 1640 medium supplemented with 10% heat inactivated horse sérum, 5% fetal bovine sérum, 100 U/ml penicillin, and 100 \( \mu \)g/mL streptomycin at 37 \( ^\circ \)C in 95% air/5% CO\(_2\). Stock solutions of D-636, D-653, D-656, and 6-hydroxydopamine (6-OHDA) were prepared in dimethylsulfoxide (DMSO) and aliquots were stored at -20 \( ^\circ \)C and -80 \( ^\circ \)C, respectively. For all experiments assessing neuroprotective effects of the test compounds, PC12 cells were pretreated with indicated concentrations of D-636, D-653 and D-656 for 24 h and then treated with 75 \( \mu \)M 6-OHDA for another 24 h. The control cells were treated with above media containing 0.01% DMSO only.

To determine the neuroprotective effects of D-636, D-653 and D-656 in the presence of neurotoxin 6-OHDA, a quantitative colorimetric MTT assay was performed. PC12 cells were plated at 17000 cells/well density in 100 \( \mu \)L of media in 96-well plates and incubated at 37 \( ^\circ \)C under 5% CO\(_2\) atmosphere for 24 h. Cells were treated with varying concentrations of the test compounds to determine their direct effect on cell viability. Neuroprotection experiments were conducted by treating cells for 24 h with varying concentrations of D-636, D-653 and D-656. Then the drug containing media was
replaced with fresh culture media followed by treatment with 75 µM of 6-OHDA alone for an additional 24 h under the same condition. After incubation for 24 h, 5 mg/mL MTT solution (prepared in Dulbecco's phosphate-buffered saline) was added to the cells (to a final concentration of 0.5 mg/mL) and the plates were further incubated at 37 °C in 95% air/5% CO₂ atmosphere for 3-4 h to produce dark-blue formazan crystals. Afterward, the plates were centrifuged at 450 g for 10 min and the supernatants were carefully removed. Formazan crystals were dissolved by adding 100 µL of methanol:DMSO (1:1) mixture to each well and shaking gently at 400 rpm for 30 min at room temperature on a Thermomix R shaker (Eppendorf, Hamburg, Germany). The absorbance was measured on a microplate reader (Biotek Epoch, Winooski, VT, USA) at 570 nm with background correction performed at 690 nm. Data from at least three experiments were analyzed using GraphPad software (version 6, San Diego, CA, USA). Cell viability was defined as percentage reduction in absorbance compared to untreated controls. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post hoc test.

5.5. Human monoamine oxidase inhibition (hMAO) studies.

This study was done to determine the ability of selected compounds in inhibiting MAO enzymes. A fluorometric screening was carried out according to the protocols with some modifications (Zhengyin et al. 2004; Novaroli et al. 2005; Chimenti et al. 2013). The enzyme activity was determined by measuring the fluorescence generated from the oxidation of kynuramine to 4-hydroxyquinoline (4-HQ) catalyzed by monoamine oxidase enzyme. In this experiment the MAO enzyme source was used containing recombinant hMAO-A and hMAO-B microsomes from insect cells as commercially available in Sigma-
Aldrich. They were stored at −80 °C as pre-aliquoted to avoid repeated freeze-thaw cycles. During experiment preparation, they were liquefied rapidly in a 37 °C water bath and kept on ice until use. In this study, the substrate for both MAO-A and MAO-B was kynuramine, and buffer assay was potassium phosphate (0.1 M, pH 7.4, made isotonic with KCl 20.2 mM). The final volume of the 146 reactions was set to 200 µL. The reference compound for this assay was a pargyline, a known potent MAO-B inhibitor.

5.5.1. Initial hMAO-B inhibition screening

Selected compounds were first examined at a dose of 25 µM for their inhibitory activity against hMAO-B. The final concentration of enzyme and substrate were set at 15 µg/mL and 25 µM, respectively. Stocks of compounds (50 µM) were prepared in DMSO, whose percentage was kept at 0.05% in the final assay reaction mixture. Substrate (50 µL/well) and compound solutions (100 µL/well) were added into a black 96-well plate and pre-incubated at 37 °C for 10 min. The control wells received assay buffer instead of compound solutions. The enzymatic reaction was initiated by the addition of hMAO-B solution (50 µL/well). The reaction mixture was then incubated at 37 °C for 20 min, and subsequently 2N NaOH aqueous solution (75 µL/well) was added to terminate the reaction. The fluorescence of 4-HQ was measured in triplicates using the Synergy Hybrid H1 fluorescence microplate reader (BioTek) at the wavelength pair of 310/400 nm (excitation/emission), and the readings were averaged and normalized with respect to the control. Assays were carried out in three independent experiments.

5.5.2. IC 50 values determination

Compounds, which reduced the hMAO-B activity to near half in the initial screening at 25 µM were considered hit molecules and were tested further for the IC 50 values for
both hMAO-A and hMAO-B to determine their selectivity ratio. Seven doses of test compounds (0-250 µM) were used, and the final concentration of the enzyme was set to 15 µg/mL. The final concentrations of substrate for measuring either hMAO-A or hMAO-B activity were set to 40 µM and 25 µM, respectively. The fluorescence was measured in either duplicates or triplicates, and the IC 50 values were determined from non-linear regression of dose-response curves using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). Assays were carried out in three independent experiments.

**Monoamine reuptake inhibitors studies.**

5.5.3. **Inhibition of monoamine uptake by cloned human biogenic amine transporters in heterologous cells.**

Inhibition of substrate uptake by cloned human transporters was measured with stably transfected human embryonic kidney (HEK) 293 cells as in our previous work (E A Reith et al. 2012). The cell lines were obtained and used in uptake assays as described in the same paper (E A Reith et al. 2012). [3H]DA ([ring 2,5,6- 3 H]dopamine (45.0 Ci/mmol, PerkinElmer, Boston, MA, U.S.A) was used for monitoring DAT and NET, DA was used as a reference because of its excellent substrate for NET (Santra, Gogoi, Gopishetty, Antonio, Zhen, E A Reith, et al. 2012), [3H]5-HT ([1,2- 3H] serotonin (27.9 Ci/mmol, Perkin-Elmer) was the radioligand for monitoring SERT.

Drug stocks contained an additional 0.01% (w/v) bovine serum albumin in order to reduce absorption of drug to the walls of the assay plates. At least five triplicate concentrations of each test compound were studied, spaced evenly around the IC50 value which was converted to Kᵢ with the Cheng-Prusoff equation (Soumava et al. 2012).

5.6. **Statistical analysis**
Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). For all the *in vitro* assays, the data were analyzed by one-way analysis of variance (ANOVA) analysis followed by Tukey’s multiple comparison post hoc test unless otherwise specified. And for the *in vivo* assays, one-way ANOVA analysis followed by Dunnett’s analysis was used. The effect was considered significant if the difference from control group was observed at p < 0.05.
CHAPTER 6

CONCLUSION

Parkinson's disease (PD) is a progressive age-related neurodegenerative disorder that is characterized by the loss of dopamine and the degeneration of the DA neurons in the SNpc of the central nervous system (CNS). The pathogenesis of PD has been identified as multifactorial in nature. Therefore, drugs that target a single biological target, have been found to be insufficient to treat PD (Nagal and Singla 2012). Therefore, it is hypothesized that designing and development of multifunctional molecules having multiple pharmacological properties targeting multiple pathogenic pathways associated with the progression and development of PD should be beneficial as disease-modifying agent for the treatment of PD.

The first main objective represents the development of a novel series of multifunctional dopamine D2/D3 agonists based on carbazole derivatives that can potentially protect the neurons from degeneration in PD. The dopamine D2/D3 receptor agonists pharmacophore were attached to the various carbazole moieties via va linkers to build various hybrid molecules. A series of compounds were synthesized, and characterized in vitro assays which was followed by in vivo assay for selected lead molecules. Compounds (-)-11b, (-)-15a and (-)-15c exhibited high affinity and full agonist activity at both D2 and D3 receptors. In PD animal model, the lead molecules exhibited potent activity and high efficacy in augmenting the locomotor activity with a long duration of action by reversing hypolocomotion in reserpinized rats, which indicated their potential as anti-PD drugs. To glean insight into their possible multifunctional property, the data presented here also shows that both (-)-11b and (-)-15c are neuroprotective in an in vitro
model of dopaminergic PC12 cells treated with the neurotoxin 6-OHDA to demonstrate a significant dose-dependent reduction of toxicity induced by treatment with the neurotoxin 6-hydroxydopamine. Therefore, supports the notion that multifunctional drugs like (-)-11b and (-)-15c have the potential not only to ameliorate motor dysfunction in PD patients but also to modify disease progression by protecting DA neurons from neurotoxic insults in addition to restoring their function. This study will, therefore, shed additional light on the importance of carbazole moiety (Głuszyńska 2015) as a potential molecular component in hybrid drug design approach for developing disease-modifying therapeutics for PD. Further mechanistic studies to ascertain the disease-modifying effects of the compounds are currently underway and will be published in due course.

The second objective was to design and synthesize a series of novel dopamine D₂/D₃ receptor agonists that should selectively inhibit MAO-B activity also. Such molecules were designed by combining the D₂/D₃ receptor agonist fragments with the propargyl group. The designed compounds were evaluated for the binding activity for D₂/D₃ receptors first. In this SAR study, a noticeable reduction in the D₂ binding affinity of the compound (-)-35-(D-677) was observed when the compound underwent methylation of the secondary nitrogen atom directly attached to the propargyl group. A reduction in the D₃ binding affinity was also observed. Conversely, the O-analog version (±)-42 D-678), exhibited similar D₂ binding affinity when compared to (-)-33 D-671 The similarity of the binding affinity for D-(±)-33 D-671, and (±)-42 D-678 might be explained by the presence of the lone pair of electrons on the secondary amine and the oxygen atom that would be necessary for the binding of these compound to the D₂/D₃ receptors possibly via H-bonding interaction. In the case of functional activities, both (-)-33 D-671 and (±)-
42 D-678 produce full agonist activity. It was interesting that the compound (±)-42 D-678 remained full agonist at D2 receptor even after the substitution of the nitrogen atom and also exerted a slight improvement in the potency at the D3 receptor. Compounds were further tested in the in vitro enzymatic assays and compound (−)-33 D-671 was shown to be more active than (±)-42 D-678, demonstrating potent inhibition of the MAO-B.

The determination of IC\(_{50}\) values suggested that both compounds (−)-33 D-671 and (±)-42 D-678 are slightly more selective for MAO-B than MAO-A. Further structural modification and characterization studies are required to improve the selectivity of the current lead compound to inhibit the MAO-B.

Our final goal was to develop novel multifunctional triple reuptake inhibitors (TUIs) to treat the motor, and the non-motor symptoms like depression associated with PD. Our drug development study was based on the modification of the pyran template using different substituted groups. To develop suitable TUIs and to further understand the effect of the structural modifications on the activity profile for the three monoamine transporters, we further expanded our previous SAR studies with 2,3,5-trisubstituted pyran compounds. Compound D-594 was synthesized by introducing biphenyl groups directly to the pyran moiety to evaluate their effect on the profile of the uptake inhibition activity. Compound D-594 exhibited moderate potency at DAT and weak potency at both SERT and NET. To follow up on the SAR studies to improve the binding activity of this compound, further modifications have been made by substituting the methylene bridge with the ethylene bridge to develop compounds D-620, and D-621 which were subjected to in vitro inhibition assays. Compound D-621 exhibited low potency at both DAT and NET transporters with moderate potency at SERT transporter while compound D-620
exhibited balanced potency at both DAT and NET transporters with moderate potency at SERT transporter. Introduction of methylene atom to build the ethylene bridge in general showed increasing the activity for the SERT. Compound D-620, was identified as the lead compound from this SAR study and it exhibited dual reuptake inhibitor activity (DNRI-type) profile. This outcome suggests further study and structural modifications are necessary to develop TUIs with potent activity at three monoamine transporters.
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ABSTRACT

CARBAZOLE BASED MULTIFUNCTIONAL DOPAMINE AGONISTS AND RELATED MOLECULES AS POTENTIAL SYMPTOMATIC AND DISEASE MODIFYING THERAPEUTIC AGENTS FOR PARKINSON’S

by

ASMA ELMABRUUK

August 2018

Advisor: Dr. Aloke Dutta

Major: Pharmaceutical Sciences

Degree: Doctor of Philosophy

Parkinson’s disease (PD) is a progressive neurodegenerative disease that develops from gradual depletion of dopamine (DA) and dopaminergic neurons in the substantia nigra pars compacta (SNc) with the accumulation of intraneuronal proteinaceous matter named as Lewy bodies. The four cardinal symptoms associated with PD are tremor, rigidity, bradykinesia, and postural instability. Although the exact mechanism and etiology of PD are not fully understood, several factors have been implicated in the pathogenesis and progression of PD including protein aggregation, oxidative stress, mitochondrial dysfunction, environmental, and genetic factors.

The current therapy of Parkinson’s disease is categorized into four classes: levodopa, DA agonists, monoamine oxidase inhibitors (MAO-Is), catechol-o-methyl transferase inhibitors (COMT-Is) and Dopamine agonist (DA). Even though these medications are available to treat PD, they only reduce the symptoms and do not slow or stop the disease progression; in addition to developing the severe side effects such as dyskinesia and motor fluctuation with long-term therapy. To overcome the concerns associated with
current PD medications, a new strategy has been adopted by developing multifunctional molecules to target multiple factors implicated in the pathogenesis of the disease that could be beneficial to treat the patients.

The hypothesis of this dissertation is to develop novel multifunctional dopamine D2/D3 agonist molecules with neuroprotective, antioxidants properties to modulate the pathogenic pathway while addressing the symptomatic deficits. Specifically, our hybrid structure strategy which combines D2/D3 agonist head groups to the other moieties that are suitable to modulate the pathogenic pathway of PD, led to development of molecules to validate our proof of concept.

In this project, the structure activity relationship (SAR) study was carried out based on our hybrid structure strategy template that was previously established. Three main objectives were set forward in this project: the first is to design and develop multifunctional molecules by covalently attaching D2/D3 agonist head groups such as pramipexole and 5-OH-DPAT to various carbazole moieties through a piperazine linker. The lead molecules (-)-11b, (-)-15a and (-)-15c exhibited high affinity for both D2 and D3 receptors whereas in GTPγS functional assay, the compounds showed potent agonist activity at both D2 and D3 receptors (EC50 (GTPγS); D2 = 48.7 nM, D3 = 0.96 nM for 11b, D2 = 0.87 nM, D3 = 0.23 nM for 15a and D2 = 2.29 nM, D3 = 0.22 nM for 15c). In PD animal model study, the test compounds exhibited potent in vivo activity by reversing hypolocomotion in reserpinized rats with a long duration of action compared to the reference drug. In a cellular antioxidant assay, compounds (-)-11b, (-)-15a and (-)-15c exhibited potent activity in reducing oxidative stress induced by neurotoxin 6-hydroxydopamine (6-OHDA). Also, in a cell-based PD neuroprotection model, these lead compounds significantly increased
cell survival from toxicity of 6-OHDA, thereby, producing neuroprotection effect. These observations suggest that the lead carbazole-based dopamine agonists are promising multifunctional molecules for a viable symptomatic and disease modifying therapy of PD and should be further investigated. The second objective is to combine D$_2$/D$_3$ agonist head groups with monoamine oxidase inhibitor property. Based on the results from in vitro receptor assays and enzymatic inhibition assay of the generated compounds led to the identification of compounds ($-$)-33 (D-671) and ($\pm$)-42 (D-678) as the lead compounds that demand further modification. The third main objective is to develop novel multifunctional triple reuptake inhibitor based on the modification of the pyran template that was previously established by us to treat the motor, non-motor symptoms like depression associated with PD. The designed compounds were evaluated for their binding affinities for the DAT, SERT, NET in the brain tissue. Based on the results of the affinity data of the initial compounds for the DAT, SERT, NET, cis-isomer compound 60a (D-620) exhibited high affinity for both DAT and NET that could be considered as a dual inhibitor for the monoamine reuptake transporters. According to this finding 60a (D-621) was identified as the lead compound that requires further modification.
## AUTOBIOGRAPHICAL STATEMENT

### Asma Elmabruk

#### Education

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