

# CHAPTER 1

## Introduction:

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### 1.1 Title:

#### 1.1.1 Short title:

Can certain FDA approved drugs be repurposed as monoamine oxidase inhibitors for the treatment of Parkinson's disease?

#### 1.1.2 Expanded title:

To repurpose drugs as pharmacological agents in the treatment of Parkinson's disease, virtual drug libraries of FDA approved drugs may be screened for drugs that inhibit monoamine oxidase.

### 1.2 Introduction and overview:

#### 1.2.1 Parkinson's disease:

Parkinson's disease (PD) is the most common age related neurological disorder after Alzheimer's disease. PD occurs globally in all ethnic groups and affects both sexes approximately equally with a slightly higher incidence in men. PD is a progressive disorder with a mean age at onset of 55. The incidence increases markedly with increasing age, increasing from 20/100 000 overall, to 120/100 000 at age 70. However there are cases of young onset PD, in which the symptoms start at a much younger age (Dauer & Przedborski, 2003).

The symptoms are progressive and before the introduction of levodopa therapy, the mortality rate for people with PD was three times higher than that of the general population. Although levodopa has improved symptomatic relief, patients with PD tend to live shorter than the general population. Most patients also suffer from considerable motor disability within 5-10 years after the symptoms start, even with pharmacological treatment (Dauer & Przedborski, 2003).

The classic triad of major signs of PD is made up of tremor, rigidity and akinesia (Lang & Lozano, 1998). Clinically, any disease or substance that causes striatal damage or striatal

dopamine deficiency can cause the symptoms of PD, known as Parkinsonism, but idiopathic PD is responsible for approximately 80% of cases (Dauer & Przedborski, 2003).

In humans the pars compacta of the substantia nigra contains approximately 450 000 dopaminergic neurons (Lang & Lozano, 1998). In PD the pigmented neurons of the substantia nigra, locus coeruleus and other brain stem dopaminergic neurons are lost (Dauer & Przedborski, 2003). The loss of the substantia nigra neurons, which project into the caudate nucleus and putamen, depletes dopamine in these areas (Eidelberg & Pourfar, 2011).

At the onset of symptoms, approximately 80% of the putamenal dopamine and 60% of the dopaminergic neurons in the substantia nigra have already been lost (Aminoff, 2009). By acting on dopamine 2 receptors, the dopaminergic neurons in the substantia nigra normally inhibit the output of the neurons that release gamma-aminobutyric acid (GABA) in the corpus striatum. In idiopathic Parkinsonism the depleted striatal dopamine results in excessive excitatory effects of GABAergic neurons in the striatum, which leads to the classical symptoms of PD. The symptoms of PD may thus be alleviated by conserving dopamine in the striatum. Since the monoamine oxidases (MAOs) catabolize dopamine in the brain these enzymes have become targets for the design of anti-parkinsonism drugs (Aminoff, 2009).

### **1.2.2 Monoamine oxidase:**

The MAOs are enzymes that are present on the outer mitochondrial membrane of both neuronal and non-neuronal cells (Yamada & Yasuhara, 2004). There are two types of MAO in the nervous system: type A which is responsible for the deamination of noradrenalin, serotonin, dopamine, adrenalin and tyramine; and type B that selectively deaminates dopamine, tyramine,  $\beta$ -phenylethylamine and benzylamine (Aminoff, 2009).

MAO inhibitors are mainly used in psychiatric disorders such as depression and in neurological disorders such as PD and Alzheimer's disease. Selective MAO-A inhibitors mainly affect neurotransmitters that are important in depression and anxiety. These drugs increase the availability of the neurotransmitters at the nerve terminals. Reversible selective MAO-A inhibitors have a markedly better safety profile than non-selective, irreversible inhibitors. In combination with dietary tyramine, both selective and non-selective irreversible inhibitors of MAO-A have been known to cause the so-called 'cheese reaction', a hypertensive crisis (Yamada & Yasuhara, 2004).

In the human brain, approximately 75% of all the MAO enzyme activity is due to type B. Therapeutic MAO inhibitors used in PD are exclusively selective for type B. These include (*R*)-deprenyl, a selective irreversible inhibitor of MAO-B at normal doses. (*R*)-Deprenyl increases the basal dopamine levels in the nigrostriatal dopamine input pathway and prolongs the anti-parkinsonism effect of levodopa (Yamada & Yasuhara, 2004). It may also be beneficial in patients experiencing a mild 'on-off' syndrome. (*R*)-Deprenyl is usually used as an adjuvant in levodopa therapy because it has a small effect when used alone. Another MAO-B inhibitor, rasagiline, is more potent MAO-B inhibitor than (*R*)-deprenyl, and can be used in the early symptomatic treatment of PD (Aminoff, 2009).

As mentioned above, because dopamine is preferentially deaminated by MAO-B in the brain, MAO-B inhibitors should increase the basal central dopamine levels in patients with PD. MAO-B inhibitors should also prolong the effect of levodopa therapy by prolonging the time that dopamine levels are elevated in the brain (Yamada & Yasuhara, 2004). Inhibition of MAO-B, however, also may have a neuroprotective effect due to the fact that the dopamine oxidation step catalyzed by MAO-B, forms hydrogen peroxide as a by-product. The generation of hydrogen peroxide and its associated reactive oxygen species can cause neuronal damage by enhancing oxidative stress. This may eventually lead to neuronal death. (*R*)-Deprenyl and rasagiline have also been found to prevent Parkinsonism induced by neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Yamada & Yasuhara, 2004).

### **1.2.3 Pharmacophore modelling:**

This study will be using a receptor-orientated pharmacophore based *in silico* screening method to identify possible United States Food and Drug Administration (FDA) approved drugs that show promise as MAO inhibitors. This method is a computational process in which a ligand can be fitted into a pharmacophore model representing the binding and active sites of a receptor or an enzyme. This allows the interaction between the binding site of the protein and the docked inhibitor to be examined. When used together with structure-activity relationship studies, critical structural features that are required for optimal activity towards a protein target can be identified and these key structural features can be used to identify drugs that show promise as MAO inhibitors. Once the drugs have been identified, the most promising compounds will be tested *in vitro* to determine their actual efficacies as MAO inhibitors.

#### 1.2.4 Polypharmacology:

According to Medina-Franco *et al.* (2013), in the previous decades drug discovery efforts have focussed on identifying single, selective drugs that target a single mechanism in accordance to the lock and key model proposed by Erlich. This has been largely motivated by the reductionist view of systems biology aided by an increasing understanding of biological processes at a molecular level. This process has been aided further by high-throughput screenings (HTS) with a validated target which allowed scientists to screen combinatorial libraries for biologically active compounds in a relatively short amount of time. Despite the *in vitro* success of HTS, the resulting hits often lacked activity *in vivo*. Computational *structure-based* drug design has also centred on the single-target approach. Drug design at the single molecular target level ignores other processes that are inevitably connected through complex networks with higher levels of the hierarchical nature of biological systems and the approach is not as effective as expected from the lock and key model.

There is growing evidence for the concept of polypharmacology, where clinical effects are observed due to the interaction of single or multiple drugs with multiple targets. In drug design the focus should shift from a single key that opens a lock to a master key that can open many locks to attain the desirable clinical effect. However the master key should not operate on anti-target locks that are responsible for adverse effects. There are different levels of polypharmacology that can lead to both positive and/or negative effects. These levels are dependent on the dose of the drugs. At therapeutic doses the drug may have a positive clinical effect due to its interaction with multiple targets, but at higher doses it could have interactions with anti-targets that lead to adverse effects (Medina-Franco *et al.*, 2013).

Drug repurposing is a direct application of polypharmacology. It is an increasingly popular approach to speed up the drug discovery process by identifying a new clinical use for an existing approved drug. A closely related concept is drug rescue, where drugs that failed to reach clinical trials due to a lack of efficacy for their original indication are repurposed. Researchers working with cancer or rare, neglected diseases actively look to reposition compounds that are already approved for other indications. Drug repurposing occurred in many cases due to chance, but there are ongoing efforts to conduct drug repurposing systematically (Medina-Franco *et al.*, 2013).

Examples of drugs that have been repurposed by clinical observation or a logical connection between the disease's pathophysiology and the drug's target include: sildenafil, that was

originally developed as an antihypertensive drug but it is now used for the treatment of erectile dysfunction; losartan, also developed for hypertension that is approved for the treatment of Marfan syndrome; and thalidomide, approved as a sedative drug and an antiemetic for the treatment of morning sickness in the 1950's that was repurposed for the treatment of leprosy and multiple myeloma. An alternative approach to repurposing is screening drugs in cell based models of the disease. This method found that the antibiotic ceftriaxone could be used in the treatment of amyotrophic lateral sclerosis and the antihistamine astemizole could be used for the treatment of malaria (Huang *et al.*, 2012).

### 1.3 **Hypothesis:**

Among the thousands of drugs that are approved by the FDA in the United States each year, there are drugs that can be repurposed as pharmacological agents that inhibit MAO for the treatment of PD.

### 1.4 **Rationale:**

Despite the large amount of chemical databases available, current experimental data is not sufficient to fill in all the possible relations between chemical and target spaces. It would take a large amount of time and resources to cover all the possible chemical-target associations, therefore computational approaches such as docking, similarity screening and pharmacophore modelling are used to select compounds for further investigation. Computational approaches either identify new ligands for known targets (through virtual screening) or new targets for known ligands (target fishing) (Medina-Franco *et al.*, 2013). Receptor-orientated pharmacophore-based *in silico* screening is a powerful tool for the fast screening of vast numbers of compounds for interactions with a given protein (Lee *et al.*, 2007).

The current therapies used in PD are insufficient. Because MAO-B inhibitors offer both symptomatic relief and potential protection against neurodegeneration, selective MAO-B inhibitors are desirable. Furthermore both selective MAO-A and non-selective inhibitors can be useful in management of depression and other disorders, and therefore identifying new MAO inhibitors is desirable from a therapeutic perspective.

The identification of commonly used drugs that can inhibit MAO is also important from a toxicological perspective. Irreversible MAO inhibitors, especially non-selective inhibitors,

have been linked to the so-called 'cheese reaction' in combination with certain foods, and serotonin syndrome in combination with certain other drugs. Therefore, identifying MAO inhibition as a potential side effect of commonly used drugs can be life-saving.

Developing a new drug is costly and time consuming. Before a new compound can become commercially available, it first has to undergo preclinical development and then clinical safety testing in animals. After the drug is found to be safe and effective in animals, it undergoes extensive clinical trials in humans to confirm its safety and efficacy before it can be approved by the FDA and reach consumers. It takes years before a new drug reaches the market. By repurposing drugs that are already registered with the FDA, a new therapy can reach the market in a relatively short time since no preclinical development is necessary.

### 1.5 **Objectives:**

Main objective: Virtual libraries of the FDA's approved drugs and EPA's maximum daily dose databases will be screened for drugs that may inhibit the MAOs. For this purpose pharmacophore models of the MAOs will be constructed. Drugs that are hits in the modelling study will be purchased and evaluated *in vitro* as MAO inhibitors. Compounds with good activities may subsequently be repurposed for the therapy of PD.

- In this study the Discovery Studio<sup>®</sup> 3.1 modelling software will be used to predict whether drugs in a virtual library may act as MAO inhibitors. For this purpose a *structure-based* pharmacophore approach will be followed. Compounds which are hits will be evaluated *in vitro* as inhibitors of MAO. The *in vitro* activity of the compounds identified as MAO inhibitors will be determined by using the commercially available MAO enzymes with the MAO-A/B mixed substrate, kynuramine, as substrate. Kynuramine is oxidized by MAO-A and MAO-B to ultimately yield 4-hydroxyquinoline, a metabolite which fluoresces ( $\lambda_{\text{ex}} = 310 \text{ nm}$ ;  $\lambda_{\text{em}} = 400 \text{ nm}$ ) in alkaline media (Strydom *et al.*, 2010). Using fluorescence spectrophotometry, the formation of 4-hydroxyquinoline can be readily measured. The inhibitor potencies will be expressed as IC<sub>50</sub> values.
- To evaluate the reversibility of inhibition of promising MAO inhibitors, the recovery of enzyme activities after the dilution and dialysis of the enzyme-inhibitor complexes will be examined.

- Sets of Lineweaver-Burk plots will be generated for promising inhibitors in order to determine whether these inhibitors interact with the MAO enzymes in a competitive manner.
- Drugs which are found to be potent inhibitors of MAO may thus be repurposed for the therapy of PD.