Deanship of Graduate Studies

Al-Quds University

Synthesis, Characterization and *in Vitro* Kinetic Study of Dopamine Prodrugs

Yahya Fu'ad Rasheed Khawaja

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Prepared By:

Yahya Fu'ad Rasheed Khawaja

B.Sc., Pharmacy, Philadelphia University, Jordan.

Supervisor

Prof. Dr. Rafik Karaman

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Prepared by: Yahya Fu'ad Rasheed Khawaja

Registration No.: 21312021

Supervisor: Prof. Dr. Rafik Karaman

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The names and signatures of the examining committee members are as follows:

1- Head of Committee: Prof. Dr. Rafik Karaman **Signature:**.....

2- Internal Examiner: Dr. Hatem Hejaz **Signature:**.....

3- External Examiner: Dr. Nasr Shraim **Signature:**.....

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Dedication

This thesis is dedicated to my parents who sacrificed a lot for me to be what I am now. I am very grateful for their love, support and prayers.

Yahya Khawaja

Declaration

I certify that the thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Signed: ……..

Yahya Fu'ad Rasheed Khawaja

Date: December 21, 2016

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Abstract:

Parkinson patients have insufficient dopamine in specific regions of the brain, so attempts have been made to replenish the deficiency in the dopamine. Dopamine itself doesn't cross blood brain barrier, but its precursor, levodopa (LD) is actively transported into the CNS and is converted to dopamine in the brain. The bioavailability of LD is less than 10% with only 1% of administered oral levodopa penetrates the brain. Large doses of levodopa are required because much of the drug is decarboxylated to dopamine in the periphery, resulting in side effects that include nausea, vomiting, cardiac arrhythmias, and hypotension. To minimize the conversion to dopamine (DA) outside the central nervous system (CNS), LD is usually co- administered with peripheral inhibitors of amino acid decarboxylase (carbidopa and benserazide). In spite of that, other central nervous side effects such as dyskinesia, on-off phenomenon and end-of-dose deterioration still remain.

Based on DFT calculations a novel dopamine prodrugs for the treatment of Parkinson's disease that can improve the overall biopharmaceutical profile of the current medications to enhance effectiveness and to ease the use of the medications were synthesized, characterized, *in vitro* intra-conversion to their parent drugs and *in silico* pharmacokinetics and toxicity prediction were also studied. The synthesized dopamine prodrugs have a carboxylic group as a hydrophilic moiety and a hydrocarbon skeleton as a lipophilic moiety, where the combination of both groups ensures a moderate hydrophilic lipophilc balance value. The potential prodrugs are expected to give better bioavailability than the parental drug owing to improved absorption. Furthermore, these prodrugs are believed to be more effective than L-dopa because the latter undergoes decarboxylation in the periphery before reaching the blood– brain barrier. Additionally, the synthesized prodrugs can be used in different dosage forms (I.V., S.C., tablets, and others) because of their potential solubility in organic and aqueous media. For dopamine **ProD 1** the experimental t½ values in 0.1N HCl, buffer pH 2.2, buffer pH 5.5 and buffer pH 7.4 were 60.3 hours, 54.66 hours, 99.93 hours and 138.13 hours, respectively. Dopamine **ProD 2** was readily converted in 0.1N HCl, buffer pH 2.2, pH 5.5 and pH 7.4 with half -life time (t½) of 48.34 hours, 54.22 hours, 131.98 hours and 193.42 hours, respectively. Finally, in silico predicting of physiochemical parameters, ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties, oral bioavailability and BBB permeability for the synthesized prodrugs were studied. The results revealed that no prodrug had a high risk of toxicity, and all the prodrugs showed good pharmacokinetic properties. Moreover, all synthesized dopamine prodrugs were found to obey Lipinski's rule of five.

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Abbreviations

ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
BBB	blood brain barrier
CDS	chemical delivery systems
$CHCl_3$	Chloroform
CNS	central nervous system
COMT	catechol-O-methyltransferase
DA	dopamine
DFT	Density functional theory
DOPH	2-Amino- N-[2-(3,4-dihydroxy-phenyl)-ethyl]-3-phenyl-propionamide
GLUT1	glucose transporter
GSH	glutathione
HCL	Hydrochloride
HLB	hydrophilic lipophilic balance
^{1}H –NMR	Nuclear magnetic resonance spectroscopy
HPLC	High performance Liquid Chromatography
IV	Intravenous
JPRC	Jordan Center For Pharmaceutical Research
KBr	potassium bromide
LAT1	large neutral amino acid transporter
LC-MS	Liquid Chromatography- Mass Spectroscopy
LD, L-dopa	levodopa
LRRK2	leucine-rich repeat kinase 2
MAO-B	Monoamine oxidase type B
MCT	monocarboxylic acid transporter
M.P	Melting point
MPTP	1-Methyl-4-phenyl-1,2,3,6- tetrahydropyridine
MRDD	Max. recommended daily dose
m/z	Mass-to-Charge ratio
NaH	Sodium hydride
NaOH	Sodium hydroxide
PD	Parkinson's disease
PDA	Photo diode array
PDDP	N-3,4-bis(pivaloyloxy)-dopamine-3-(dimethylamino)propanamide
PINK1	PTEN-induced putative kinase 1
PPB	Plasma protein binding
Ppm	part per million
PRKN	parkin
PSA	polar surface area
SC	Subcutaneous
SLNs	polymeric solid lipid nanoparticles
SNCA	alpha-synuclein
THF	tetrahydrofurane

TLC	Thin layer chromatography
$t_{1/2}$	half-life

Introduction

Chapter one

1. Introduction

1.1 Parkinson’s disease

1.1.1 Symptoms of Parkinson’s disease

Parkinson's disease (PD) was first described by English physician Dr. James Parkinson in 1817 [1,2]. Parkinson's disease is classically defined as a progressive, idiopathic, neurodegenerative disease associated with four fundamental motoric signs: akinesia/bradykinesia, rest tremor, cogwheel rigidity and postural instability. A resting tremor is the first symptom in 70% of Parkinson's disease patients [3-5]. Moreover, parkinson’s disease can cause a wide range of non-motor symptoms such as depression, loss of sense of smell, gastric problems, cognitive changes and many others [6].

1.1.2 Incidence and prevalence of Parkinson’s disease

According to the Parkinson's Disease Foundation, Parkinson's disease affects about 1 million people in the United States [7-9] and more than 10 million people worldwide [7,10,11]. About 60,000 people are diagnosed each year in the United States [7,9]. Although the disease itself is not fatal, the complications caused by Parkinson’s are the 14th leading cause of death in the United States [12]. The disorder occurs in all races, but Parkinson's is somewhat more prevalent among Caucasians [13]. Men were more likely than women to have Parkinson’s disease [14].
The average age of diagnosis is around 60, but approximately 5-10% of people with the condition develop "young-onset" Parkinson's disease before reaching age 50 [15].

1.1.3 Causes of Parkinson’s disease

Although the exact cause of PD remains unknown, most cases are hypothesized to be a result of multiple factors acting together, including ageing, genetic susceptibility, and environmental exposures [16].

1.1.3.1 Environmental risk factors

A number of environmental factors have been associated with an increased risk of Parkinson's including:pesticide exposure, living in rural areas (in industrialized countries), and drinking well water [17]. Conversely, certain environmental exposures seem to lessen the risk of Parkinson's disease, like cigarette smoking and the intake of caffeine [18]. 1-Methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP) is the only toxic agent that has been directly linked to development of parkinsonism [19].

1.1.3.2 Genetics

PD traditionally has been considered a non-genetic disorder; however, around 15% of individuals with PD have a first-degree relative to who has the disease [5]. Mutations in specific genes have been conclusively shown to cause PD. These genes code for alpha-synuclein (SNCA), parkin (PRKN), leucine-rich repeat kinase 2 (LRRK2 or dardarin), PTEN-induced putative kinase 1 (PINK1), DJ-1 and ATP13A2 [20,21].In most cases, people with these mutations will develop PD.

1.1.4 Pathology of Parkinson's disease

The pathologic hallmark of the Parkinson's disease result from reduced activity of dopamine-secreting cells caused by cell death in the pars compacta region of the substantia nigra [22]. The other major neuropathological symptom of PD is the existence of Lewy bodies which is formed mainly by an abnormal accumulation of the protein α-synuclein bound to ubiquitin in the damaged cells [23]. The leading cause of neuronal death is not known. Proteosomal along with mitochondrial dysfunction are possible causes [24].

1.1.5 Treatment of Parkinson's disease

1.1.5.1 Anticholinergic drugs

These medications were the first available pharmacological agents used in the treatment of Parkinson's disease. They block the effect of acetylcholine, another brain chemical, to re-balance its levels with dopamine. They include benztropine, biperidine and benzhexol. Nowadays, all these drugs are rarely used because of the relatively modest benefits that they provide compared with their side effect profile [25].

1.1.5.2 Dopamine replacement therapy

Levodopa (LD, L-dopa), coupled with carbidopa, a peripheral decarboxylase inhibitor (PDI), remains the gold standard of symptomatic treatment for Parkinson disease. Carbidopa inhibits the decarboxylation of levodopa to dopamine in the systemic circulation, allowing for greater levodopa distribution into the central nervous system [26]. Levodopa provides the greatest antiparkinsonian benefit for motor signs and symptoms, with the fewest adverse effects in the short term; however, long-term treatment leads to involuntary movements and response fluctuations which add to the complexities of later disease-management. In addition, preclinical evidence suggests that levodopa is toxic to dopaminergic neurons. Once fluctuations and dyskinesias become problematic, they are difficult to resolve [26,27].

1.1.5.3 Dopamine agonists

Dopamine agonists have a complex pharmacology acting directly on post- and presynaptic dopamine receptors to mimic the effects of endogenous dopamine (DA). They include ergot derivatives such as bromocriptine, lisuride, pergolide, and cabergoline and other agents which do not possess the ergot structure such as pramipexole and ropinirole [28].

Initially, dopamine agonists were believed to be effective only as adjunct therapy to L-dopa [29]. A few years later, they are accepted as primary treatment in early PD with a delay in the development of dyskinesia [30].The use of DA agonist in the early PD provide moderate symptomatic benefit and delay the introduction of L-dopa [31]. However, DA agonists are not as effective as L-dopa in the later stages of PD, and they still produce many of the dopaminergic side effects associated with L-dopa use, such as nausea, vomiting, hypotension, hallucinations, and psychosis. [32].

1.1.5.4 MAO-B Inhibitors

Monoamine oxidase (MAO) type B is an enzyme that naturally breaks down several chemicals in our brain, including dopamine. By blocking this enzyme, the breakdown of the chemical messenger dopamine within the brain will be prevented and therefore prolong the action of levodopa [33]. There is considerable laboratory evidence that MAO-B inhibitors do exert antioxidant and antiapoptotic activity in experimental models, which may potentially translate into long-term clinical neuroprotective effect [34].

Two MAO-B inhibitors, selegiline and rasagiline, are currently approved for the symptomatic improvement of early Parkinson's disease and to reduce off-time in patients with more advanced Parkinson's disease and motor fluctuations related to levodopa [35].

1.1.5.5 COMT inhibiters

When peripheral decarboxylation is inhibited by carbidopa or benserazide, the main metabolic pathway of levodopa is O-methylation by catechol-O-methyltransferase (COMT). Entacapone and tolcapone are new potent, selective COMT inhibitors. They have the ability to block the COMT enzyme from converting levodopa into a useless form (3-O-methyldopa), thus making more levodopa in the brain available and helping to reduce PD symptoms. Dopaminergic and gastrointestinal effects are the main adverse effects of the COMT inhibitors [36].

1.1.5.6 Amantadine

Amantadine hydrochloride was originally used as an antiviral for the treatment of influenza, but was coincidentally has been shown to improve the symptoms of Parkinson's disease [37]. Amantadine is a weak antagonist of the NMDA-type glutamate receptor, increases dopamine

release, and blocks dopamine reuptake [38]. It was reported that amantadine given as adjuvant to levodopa can markedly improve motor response complications [39].

1.2 Dopamine

Dopamine is a natural neurotransmitter that needs to turn on the five dopamine receptors in the brain, D_1, D_2, D_3, D_4 and D_5 to function through the CNS. Dopamine is produced in several areas of the brain, including the substantial nigra and the ventral tegmental area. Also is considered a neurohormone as dopamine is secreted from the gland of hypothalamus and its main function as a hormone is to inhibit the release of prolactin from the anterior lobe of the pituitary [40]. Dopamine production in human body is one step during the catecholamine biosynthesis that starts from phenylalanine to tyrosine, levodopa and then dopamine (Figure 1). This cascade is accomplished and catalyzed with the aid of three enzymes. The rate limiting enzyme is tyrosine hydroxylase, which can then be inhibited by the catecholamine neurotransmitters to keep the proper regulation of dopamine levels all over the body [41]. Dopamine plays several and variant roles among the body, including CNS, circulatory, renal, digestive and immune systems. Its role in the CNS is the cornerstone in controlling the body's movement, motivation, emotion and the feel of pleasure. Dopamine can be supplied as a medication that acts on the sympathetic nervous system, producing effects such as increased heart rate and blood pressure [40].

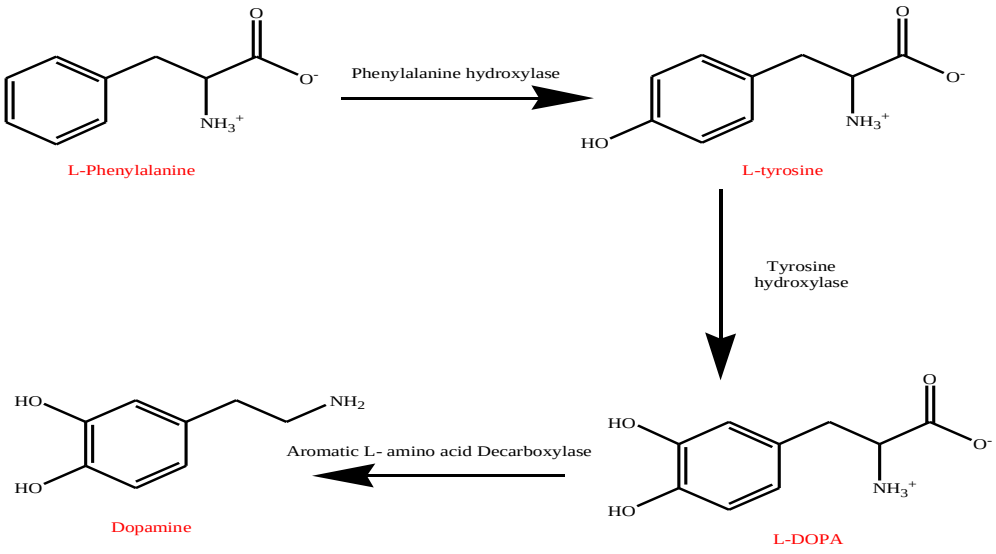


Figure 1 : Production of dopamine inside human body

Dopamine deficiency as a result of the loss of dopamine secreting neurons in the substantial nigra in the brain combined with the formation of Lewybodies (intracytoplasmic proteinaceous inclusions of fibrillar-synuclein) causes impaired movement and tremor, a neurodegenerative

disorder called Parkinson disease (PD) [42]. However, giving the PD patient external dopamine as a treatment is not a choice, as dopamine is a water-soluble drug that does not satisfy the characteristics of a substance that can enter the brain. This substance must penetrate the highly selective and lipophilic membrane that protects the brain, the blood brain barrier (BBB) [40].

1.3 Prodrug approach

1.3.1 The principle of prodrug approach

Many therapeutic drugs have adverse properties that may become pharmacological, pharmaceutical or pharmacokinetics barriers in the clinical drug application [43]. Development of new chemical entities with desirable efficacy and safety can be achieved to overcome the undesirable physicochemical, biological and organoleptic properties of some existing drugs. However, this is a challenging, expensive and time consuming process that requires very expensive phase I, II and III clinical trials [44]. So that, it becomes much more feasible to modify and improve the properties of already existing drugs through exploring the prodrug approach which may represent a life-cycle management opportunity [43].

Historically, the term prodrug was first introduced in 1958 by Albert [45]. Prodrugs are pharmacologically inactive chemical derivatives of a drug molecule that converted to its active form by enzymatic and/or chemical transformation within the body [46]. In both drug discovery and development, prodrugs have become an established tool for improving physicochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically active agents [47].

Approximately, 10% of all worldwide marketed medications can be categorized as prodrugs, 20% of medicines that were approved between 2000 and 2008 were prodrugs and when focusing on 2008 alone, 33% of all approved small-molecular-weight drugs were found to be prodrugs. Therefore, nowadays the interest in prodrug approach become increasingly popular and successful in pharmaceutical industries [48,49].

Prodrugs can be classified according to two major criteria, chemical classes (carrier-linked prodrugs, bioprecursors, sit-specific chemical delivery systems, etc.) and mechanism of activation (enzymatic versus nonenzymatic, activation by oxidation, reduction or hydrolysis, catabolic versus anabolic reaction) [50].

1.3.2 Prodrug activation

Successfully designed prodrug should be converted to its parent drug. Generally, activation process involves metabolism by enzymes distributed throughout the body. The most important enzymes in the bioconversion of prodrugs are hydrolytic enzymes such as esterases and amidases. However, non-hydrolytic enzymes, including all cytochrome P450 enzymes, are able

to catalyze the bioconversion of ester and amide-based prodrugs [51]. Enzymes accelerate the rate of chemical reactions that the substrate (drug) might undergo in physiological environment. The rate constants for a large majority of enzymatic reactions is 10^{10} to 10^{18} fold the non-enzymatic reactions [52].

Prodrugs that are designed to be activated by natural enzymes such as esterases and amidases may be tackled by a premature hydrolysis during the absorption phase in enterocytes of gastrointestinal tract, this might produce more polar and less permeable prodrug which results in a decreased bioavailability [52], while if the prodrug is activated by cytochrome P450 enzymes which are responsible for 75% of the enzymatic metabolism of prodrugs, a genetic polymorphisms might persist and then lead to variability in prodrug activation and thus affect the efficacy and safety of designed prodrugs [53]. Thus, it might be difficult to predict the bioconversion rate of the enzymatic hydrolysis of the prodrug and hence a difficulty in predicting their pharmacological or toxicological effects. Moreover, the rate of hydrolysis is not always predictable, and bioconversion can be affected by various factors such as age, health conditions and gender [54-56].

Modern computational methods based on molecular orbital and molecular mechanics methods are explored for the design of innovative prodrugs for drugs containing hydroxyl, phenol, or amine groups. For example, mechanisms of some enzyme models that have been utilized to understand enzyme catalysis have been recently investigated by Karaman's group and used for the design of some novel prodrug linkers [57-60]. The traditional prodrug approach was focused on altering various physiochemical parameters, whereas the modern computational approach, considers designing prodrugs through attaching appropriate linkers with drugs having poor bioavailability which upon exposure to physiological environments release the parent active drugs in a programmable (controlled) manner resulting in an improvement of their bioavailability. With the possibility of designing prodrugs with different linkers, the release rate of the parent active drugs can be controlled [61].

1.3.3 Applications of prodrug approach

1.3.3.1 Improving solubility and dissolution rate of drugs

Poor solubility of a drug will be a major issue when dissolution of the drug from a dosage form is the rate limiting step [62]. It has been reported that about 35-40 % of drug discovery compounds have poor aqueous solubility [63]. There are numerous formulation approaches, such as salt formation and solubilizing excipients, have been used to overcome this barrier. Prodrugs offer an alternative tool to increase the aqueous solubility of the parent drug molecule by attaching ionizable or polar neutral groups, such as phosphates, amino acids, or sugar moieties [64-66]. Enzymes such as phosphatases, esterases, glucosidase, amidases or peptidases in plasma or other tissues can then breakdown the molecules into its active form. Fosphenytoin is a good

example of a prodrug which by the addition of a phosphate group has improved the aqueous solubility of phenytoin by a factor of 7,000 fold [67].

1.3.3.2 Enhancing permeability and absorption

For drug to be transported to its site of action it should pass through several lipid membranes; therefore, membrane permeability has a considerable influence on drug efficacy [68]. Prodrug strategies are most commonly employed to promote membrane permeation and either oral or topical absorption by increasing drug lipophilicity via masking polar and ionizable groups within a drug molecule [69]. A hydrophilic hydroxyl, thiol, carboxyl, phosphate, or an amine group on the parent drug can be converted to more lipophilic alkyl or aryl esters, and these prodrugs are readily converted to the parent drugs via hydrolysis catalyzed by esterase enzyme [70,71]. An example of this type of prodrug is oseltamivir which is an ethyl ester prodrug and undergoes rapid conversion by carboxylesterase to its parent drug. The bioavailability of the more lipophilic oseltamivir is almost 80%, whereas the corresponding value for free carboxylate is as low as 5%. [67].

Another method to increase the oral absorption is to design prodrugs, which have structural features similar to substrates that are absorbed by carrier-mediated transport [67]. Enalapril is an example of an ester prodrug which improves the bioavailability from 3% (active drug) to 40%. The ethyl ester moiety increases lipophilicity and is also a substrate of the PEPT1transporter [72].

1.3.3.3 Changing the Distribution Profile

Selective delivery of drugs to particular tissues can increase their therapeutic activity and decrease their side effects. For decades, attempts have been made to achieve site-selective drug delivery by utilizing different macromolecular strategies and nanotechnologies. Unfortunately, these methods lack clinical success. Therefore, scientists have focused their interests on other approaches. Today, the prodrug approach is one of the most promising site-selective drug delivery strategies which exploit target cell- or tissue- specific endogenous enzymes and transporters [25]. One example is the prodrug capecitabine which is metabolized initially in the liver and subsequently in tumor cells to form the anticancer agent 5-fluorouracil [72].

1.3.3.4 Protecting from Rapid Metabolism

The first pass metabolism in the gastrointestinal tract and liver may greatly reduce the oral drug bioavailability [73]. Sublingual or buccal administration and modified or controlled release formulations has been formed to bypass this problem [74]. The prodrug approach can also protect the rapid metabolic breakdown of the drug and thereby increase its oral bioavailability by masking the metabolically labile functions [75].

1.3.3.5 Taste masking

Bitterness of the drug is the major reason for patient incompliance. In order to eliminate the bitter taste of a drug and hence increasing its efficacy, the prodrug approach can be used either by decreasing the drug solubility in saliva or by masking the functional group that is responsible for the drug's binding to the taste receptors located on the tongue [76].

1.3.4 Prodrug approaches for the CNS delivery

One of the major difficulties in terms of drug delivery to the central nervous system (CNS) is the inability of many therapeutical compounds to pass through the blood-brain barrier (BBB) [77]. BBB is a highly selective permeable barrier that is formed by brain endothelial cells, which are connected by tight junctions. BBB has no fenestrations, exhibit very low pinocytic activity and are surrounded by astrocyte foot processes that are part of its structure and mediate its permeability [78].The BBB is necessary to protect the central nervous system from potentially harmful chemicals while selectively facilitating the transport of essential molecules into the CNS and maintaining an optimal stable environment for brain function [79].

Due to its unique properties, passage across the BBB often becomes the main limiting factor for the delivery of potential CNS drugs into the brain parenchyma. In fact, it is estimated that more than 98% of small-molecular weight drugs and practically 100% of large-molecular weight drugs developed for the CNS diseases do not readily cross the BBB [80].

The penetration into the BBB can be achieved by one of the three main ways. First, a drug will diffuse freely through the membrane if it obeys Lipinski's rule of five, suggested and applied since 1997 [81]. Lipinski suggests that for any substance to penetrate the lipophilic membranes all over the body passively, it must have these properties, a molecular mass less than 500, a lipophilicity expressed as log p not greater than 5, no more than 5 hydrogen bond donors and a number of hydrogen accepting groups less than 10. Furthermore, lipid soluble (lipophilic) molecules with a molecular mass under 400–600 Da can go through the BBB by means of a passive diffusion mechanism [81,82]. Secondly, essential nutrients including glucose, amino acids and nucleosides can be transported actively by carriers (carrier-mediated transport). Yet, proteins and peptides are thirdly facilitated to be transport either by specific receptors (receptor mediated endocytosis) or by electrostatic interactions with endothelial membrane (adsorptive-mediated transcytosis) [83-86].

Enhancement of drug permeability to BBB can be achieved by different approaches: 1) administration of the drug into CNS directly, 2) disruption of the BBB temporarily, thus enhancing its permeability, 3) prodrug strategy by chemical alteration of drug and 4) Formulation strategies using colloidal carriers such as polymeric solid lipid nanoparticles (SLNs), dendrimers, polymeric micelles, and liposomes [87].

The prodrug strategy is widely used to optimize physicochemical properties that allow for passive diffusion via the transcellular route or to insert structural features necessary to serve as a substrate for one of the endogenous influx transport systems[88].

1.3.4.1 Lipophilic prodrugs

Lipophilic prodrugs can be achieved using the lipidization approach or that of chemical delivery systems (CDS). A very simple approach to increase the CNS entry of hydrophilic molecules would be via reversibly masking the polar functionalities within such compounds. This is referred to as a lipidization of molecules. Although the lipidization with lipophilic drug analogues often results in diminished therapeutic effect by enhanced toxicity or decreased activity of the drug, the lipidization through prodrugs offers a probability to enhance CNS delivery of polar drugs. However, this approach does not usually produced effective therapeutic outcomes [77,89]. The CDS approach requires multiple steps bioactivation for conversion to active drugs. It captures the drug inside the brain by converted the prodrug into a more hydrophilic derivatives after crossing CNS. Thus reduce the efflux of drug from the CNS and provide a sustained release for it [90,91].

1.3.4.2 Carrier-mediated prodrugs

This approach based on attaching the parent drug to an endogenous transporter substrate for example amino acids, glucose, and other hexoses, which can recognize the prodrug as its own substrate and enable it to enter the CNS. Then, bioconversion to parent drug occur [92]. Most widely used transport systems in prodrugs approach are glucose transporter (GLUT1),large neutral amino acid transporter(LAT1), monocarboxylic acid transporter (MCT) and peptide transport systems [93,94].

1.4 Problem statement

Patients with Parkinson's disease have insufficient dopamine in specific regions of the brain, so attempts have been made to replenish the deficiency in the dopamine [95]. Unfortunately, peripherally administered (outside of the central nervous system) dopamine is not effective because it cannot cross the blood brain barrier. The reason for its inability to cross the BBB has to do with at least two influencing factors. The first is that dopamine is a hydrophilic molecule which is expected to exist primary in the ionized forms (Figure 2) in a physiologic environment of pH 7.4 (blood circulation) resulting in a greater degree of difficulty in crossing cell membranes. The second is the absence of a transporter for dopamine to pass the blood brain barrier into the brain [96].

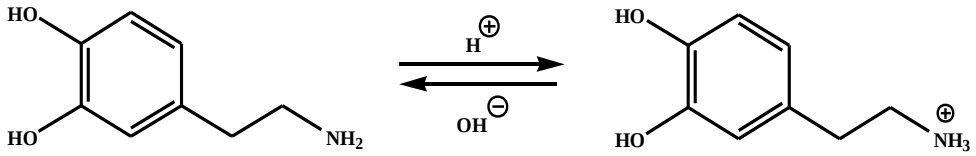


Figure 2 : Ionized form of dopamine at physiological environment

However, the precursor to dopamine, LD (Figure 3), was and still the best choice of treatment for this disease. LD is able to get into the brain via a large neutral amino acid carrier or L (leucine) system [97]. Once LD gets inside the brain it can then be metabolized by dopa decarboxylase or amino acid decarboxylase to form dopamine within the dopaminergic neurons within the substantia nigra [98].

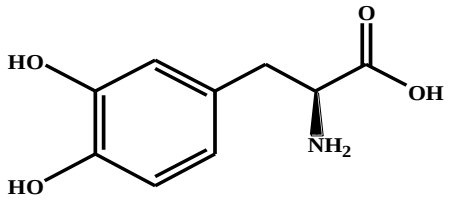


Figure 3: Chemical structure of Levodopa

Because much of the drug is decarboxylated to dopamine in the periphery, high doses of LD are required, resulting in side effects that include nausea, vomiting, cardiac arrhythmias, and hypotension [99]. These drawbacks of LD are the known reason of disability in PD patients [100, 101]. They can be explained according to this manner: In the normal brain the basal ganglia always maintained to satisfy the brain needs of dopamine for motor control and others, but LD oral administration have a low bioavailability of 10% with only 1% of LD reaching the brain. This is due to the erratic gastrointestinal metabolism the drug faces before it attaches to the l-amino acid carrier that transports it actively through the duodenum where it enters the blood stream intact [102-107]. With the co-administration of either carbidopa or benserazide, an increase of LD bioavailability by two-fold was observed with only 5% to 10% of administered LD enters the brain [108,109]. As a result, lessened amounts of dopamine put the brain under fluctuations that are hard to accommodate [110,111]. To minimize the conversion to DA outside the CNS, LD is usually given in combination with peripheral inhibitors of amino acid decarboxylase such as carbidopa and benserazide. In spite of that, other central nervous side effects such as dyskinesia, on-off phenomenon and end-of-dose deterioration still remain [112].

The main factors responsible for the poor bioavailability and the wide range of inter- and intra-patient variations of plasma levels are the LD physicochemical properties such as low water and lipid solubility which resulted to unfavorable partition, and the high susceptibility to chemical and enzymatic degradation [113]. Starting from these considerations the prodrug approach has been applied to dopamine in order to overcome its metabolism problems and to improve its bioavailability.

1.5 Thesis Objectives

1.5.1 General objectives

The main objective of this study is to synthesize new prodrugs for the treatment of Parkinson's disease that have the potential for higher bioavailability than the current medications when given in different dosage forms.

For achieving this goal, the dopamine prodrugs physiochemical properties must have the following: (i) to be soluble in physiochemical environment; (ii) to have a moderate hydrophilic lipophilic balance (HLB) value; (iii) to provide upon chemical cleavage a safe and non-toxic by-products.

By achieving these requirements, the following objectives may be fulfilled: (i) a high disposition of the prodrug into the body tissues; (ii) the capability to use the anti-Parkinson's drug in different dosage forms; (iii) a chemically driven sustained release system that release the dopamine in a controlled manner; and (iv) a drug with a high bioavailability and efficient pharmacokinetic properties.

1.5.2 Specific objectives of our work are

1. To synthesize dopamine prodrugs for the treatment of PD that has the potential for higher bioavailability than the current medications by using different linkers.
2. To characterize the proposed prodrugsusing several analytical techniques.
3. To perform *in vitro* kinetic studies for the synthesized dopamine prodrugs at different pHs mimicking the physiological media.
4. To predict oral bioavailability, pharmacokinetics, BBB permeability and toxicity of dopamine prodrugs using in silico computational software.

1.6 Research Questions

1. Would it be possible to link dopamine to the linkers via a chemical synthesis?
2. Would the synthesized prodrugs be capable of *in vivo* releasing the parent drug (dopamine) in a sustained release?
3. Does the synthesized prodrug have physiochemical properties which could lead to a good pharmacokinetic properties and a high bioavailability?
4. Does the synthesized prodrug have the capability to be used in different dosage forms ?
5. Does the synthesized prodrug have the ability to cross BBB ?
6. Does the synthesized prodrug have the drug-like properties ?

Literature Review

Chapter two

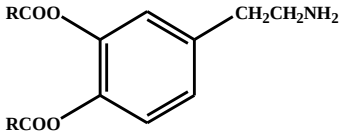
Literature Review

Several attempts have been made to overcome the patient's symptoms of PD, therefore enhancing his lifestyle and the ability to live normally. In this section, I am going to discuss the different dopamine prodrug approaches which were done by medicinal chemists to enhance its bioavailability.

2.1 Lipophilic Dopamine prodrugs

2.1.1 Ester dopamine prodrugs

Dopamine have poor permeation across the BBB and other cell membranes due to its complete ionization at physiological pH. So it cannot be used for PD [114]. To overcome these problems, Casagrande et al. and Borgman et al. have prepared a number of lipophilic 3,4-O-diesters prodrugs of DA (54-58) (Figure 4) as a latent lipophilic derivatives of DA to be used in the therapy of parkinsonism, hypertension and renal failure [114,115]. But the results showed that O-acetylation was not enough to provide entry into CNS while preservation intrinsic dopaminergic activity and N-alkylation of the DA molecule are also required.



1. R = CH_3
2. R = $CH(CH_3)_2$
3. R = $C(CH3)_3$
4. R = C_6H_5
5. R = C_2H_5O

Figure 4 : A series of lipophilic 3,4-O diesters dopamine pro-drugs.

2.1.2 Chemical delivery systems

Chemical delivery systems (CDSs) have been established to enhance the permeation of DA to central nervous system. These prodrug devices have been prepared by joining DA with a pyridinium/dihydropyridine redox carrier. A dihydropyridinium-type CDS is lipophilic enough to cross the membrane of CNS by passive transport and then undergoes an enzymatic oxidation to an ionic pyridinium precursor, this lead to locked compounds in the CNS [116]. (CDS) used also for brain-enhanced delivery of neurotransmitters, steroids, anticonvulsants, antibiotics, antiviral, anticancer, neuropeptides and their analogs [116-118].This carrier enables the prodrug

to cross BBB and then be oxidized to a quaternary precursor that is retained in the CNS, to provide a DA in a sustained release form (Figure 5).

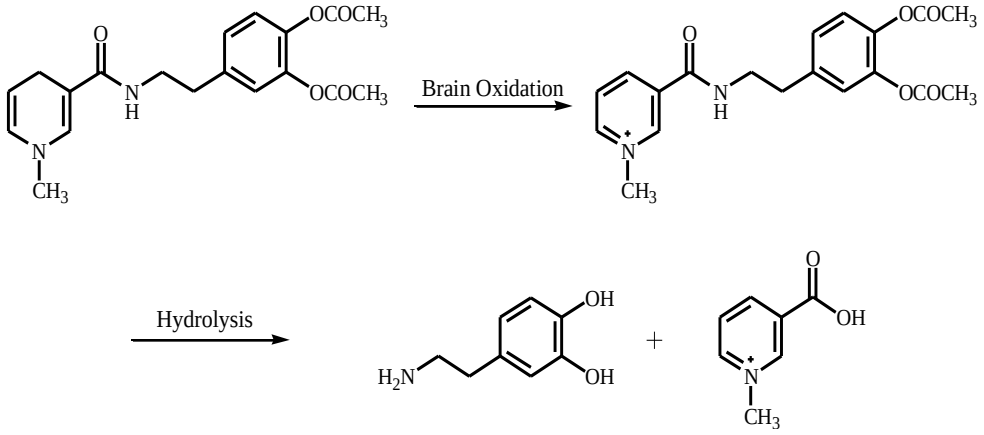


Figure 5 : Dopamine delivery from pyridinium/dihydropyridine redox carrier system.

The use of the dihydropyridine is actually restricted due to instability of its 5,6-double bond, which undergoes air-oxidation and/or hydration. This oxidation/hydration reaction yields 6-hydroxy-1,4,5,6-tetrahydropyridine, which does not undergo enzymatic oxidation in vivo to give the corresponding quaternary pyridinium salt [7]. In order to overcome this problem, Carelli et al. suggest an interconvertible tetrahydrobipyridine/pyridinium salt (Figure 6) by irreversible dimerization of two pyridinyl radicals accomplish by one-electron electro-chemical reduction of pyridinium salts as nicotin-amide coenzymes or their models. In contrast with monomeric dihydropyridines, the tetrahydrobipyridines are more stable and easily oxidized back to the compound pyridinium salts by chemical oxidants or by oxygenase and peroxidase enzymes [118].

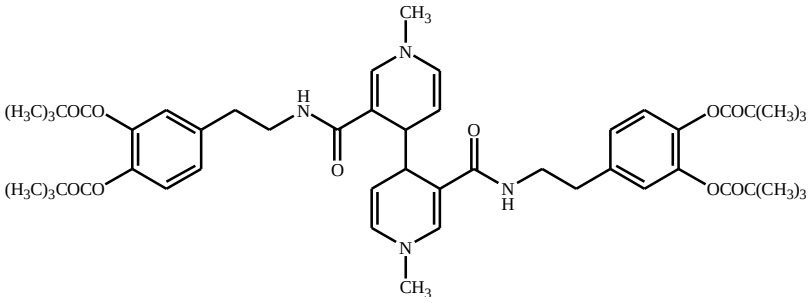


Figure 6 : Chemical structure of tetrahydrobipyridine.

2.2 Carrier-mediated prodrugs

2.2.1 Peptide transport-mediated prodrugs

2-Amino-N-[2-(3,4-dihydroxy-phenyl)-ethyl]-3-phenyl-propionamide (DOPH), an amide prodrug of DA, has been earlier proposed by Giannola et al. (Figure 7) [119]. It is synthesized by condensation of dopamine with a neutral amino acid to be able to interact with the BBB endogenous transporters and easily enter the brain. (DOPH) has the capacity to be slowly cleaved by cerebral enzyme (t½ 460 min) and produce free dopamine in the brain, but it undergoes a rapid hydrolysis in human plasma (t½ 28 min). Chemical stability studies on DOPH showed that no DA release occurred in the gastrointestinal tract and the prodrug was able to pass through a simulated intestinal mucosal membrane.

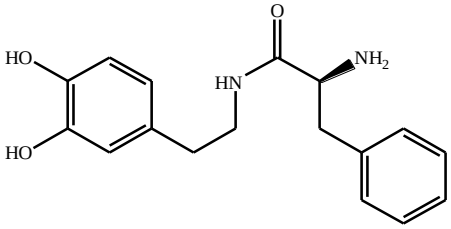


Figure 7 : Chemical structure of 2-amino-N-[2-(3,4-dihydroxy-phenyl)-ethyl]-3-phenyl-propionamide (DA-PHEN)

In another study and in attempt to enhance BBB permeability of dopamine, More and Vince focused on the glutathione uptake transporters that are located on the luminal side of the BBB. The broad substrate specificity displayed by these transporters provides vast opportunity for rational prodrug design. The design of glutathione transporter targeted prodrug (Figure 8) involved three components: the carrier, glutathione (GSH), the active drug, and a suitable linker for conjugation of the carrier with the drug molecule. The prodrug in (Figure 9) in which the dopamine is covalently linked via amide bond to glutathione (GSH) showed high affinity for the GSH transporter at the BBB, released dopamine at the active site and possessed a good stability balance between the periphery and brain [120].

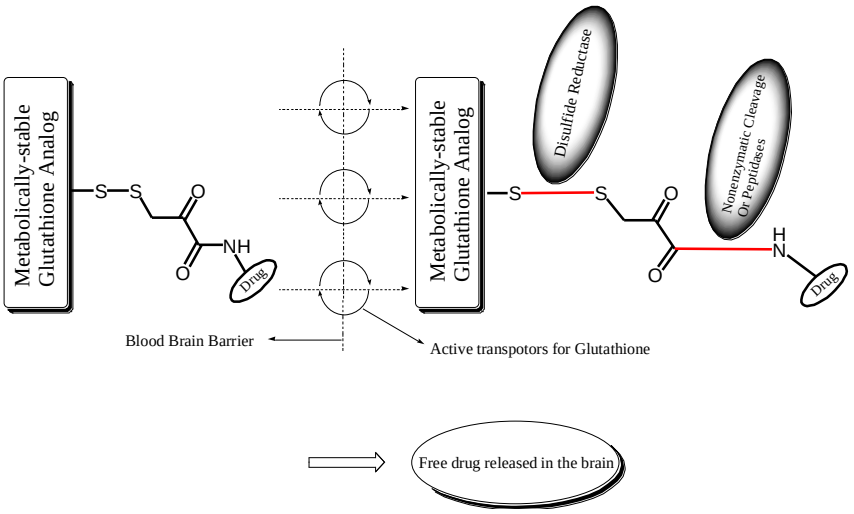


Figure 8: A rational for a prodrug design

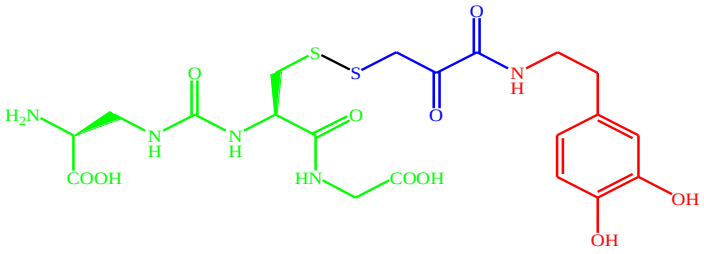


Figure 9: Chemical structure of the anti-Parkinson's prodrug of dopamine. Shown in green is the carrier, metabolically stable glutathione analogue; in blue is the linker, mercaptopyruvic acid, and in red is the active drug moiety.

N-3,4-bis(pivaloyloxy)-dopamine-3-(dimethylamino)propanamide (PDDP) (Figure 10), a brain specific derivative of dopamine, was designed and prepared, which consists of a brain targeted ligand, N,N-dimethyl amino group, and two dipivaloyloxy groups for lipophilic modification. Tissue distribution, brain bioavailability, and therapeutic efficacy of PDDP were evaluated and compared with L-DOPA and another brain dopamine prodrugs without N,N-dimethyl amino group which showed a more marked accumulation in rats brain microvascular endothelial cells than brain dopamine prodrugs through an active transport process. Following IV administration, the concentration of PDDP in the CNS was 269.28- and 6.41-folds higher than that of L-DOPA and brain dopamine prodrugs at 5 min, respectively. Therefore, PDDP would be a promising drug candidate that can be applied for targeted PD treatment [121].

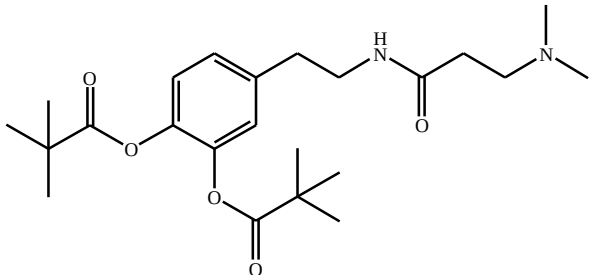


Figure 10 : Chemical structure of N-3,4-bis(pivaloyloxy)-dopamine-3-(dimethylamino)propanamide (PDDP)

2.2.2 GLUT1 carrier-mediated prodrugs

With the aim of overcoming the problem of the low BBB permeability of dopamine, a novel glycosyl derivatives of dopamine were synthesized which have the ability to be transported by GLUT1. Fernandez and coworkers described the synthesis and biological activities of several glycosyl derivatives of dopamine by conjugating sugar with dopamine through a succinyl linker, carbamate bond, glycosidic and ester bonds. They linked the amino group of dopamine to the C-6 , C-3 and C-1 of the sugar through a succinyl linker (compounds 6-8 in Figure 11) or a carbamate bond (compounds 9-13 in Figure 11). In another series, the sugar was linked to the phenolic groups of dopamine through a glycosidic bond (compounds 14 and 15 in Figure 11) and ester bonds(compounds 16-18 in Figure 11). The affinity of the these prodrugs for glucose carrier GLUT-1 using human erythrocytes was also tested [122,123]. When incubated with the brain extracts, the nature of the bond that links DA with glucose affected the rate in which the prodrug releases dopamine. The glycosyl conjugates substituted at the C-6 position of the sugar were more potent inhibitors of glucose transport in contrast to that of C-1 and C-3 substituted derivatives. From the studied compounds, the carbamate derivatives 9, 11 and 12 were the prodrugs of choice, in particular compound 9, which showed the best affinity for GLUT-1, even with higher affinity than glucose itself [124,125].

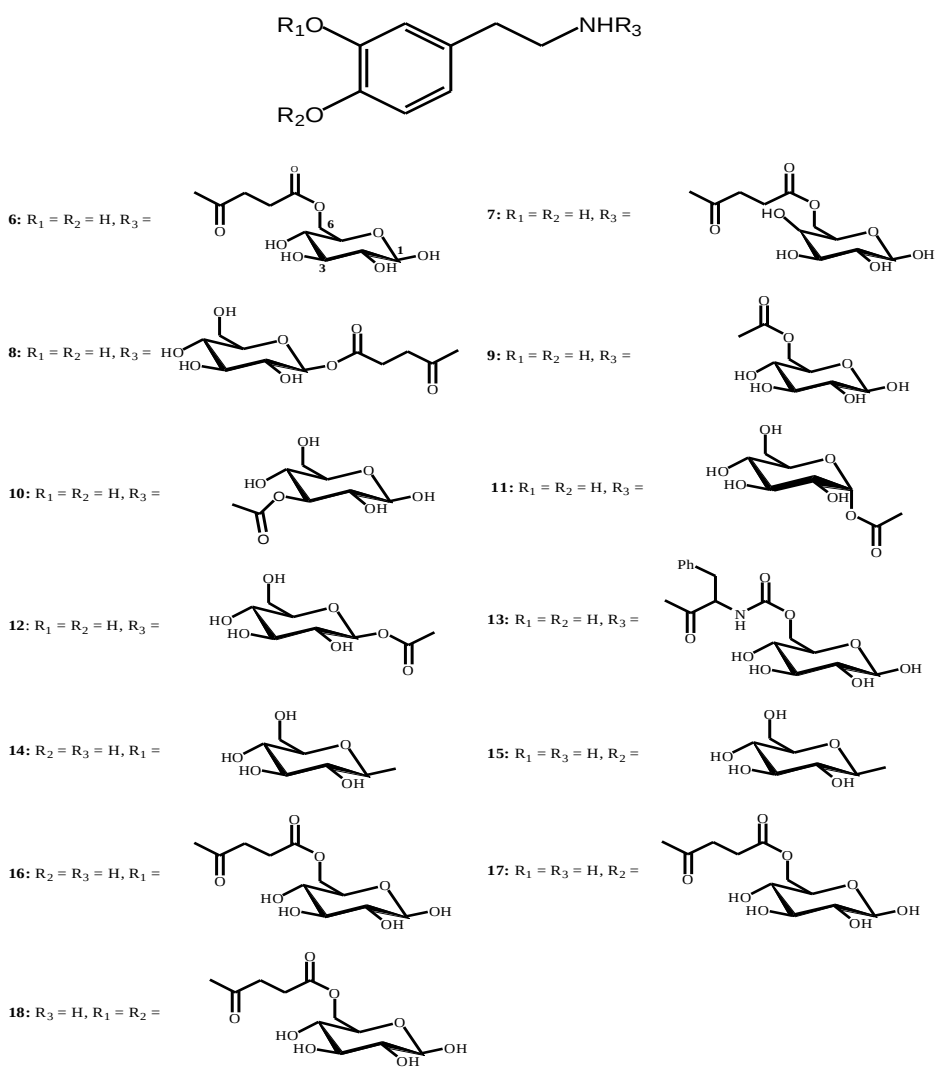


Figure 11: Glycosyl dopamine derivatives.

In another study, Bonina et al. and Ruocco et al. have prepared dopamine glycoside prodrugs by attaching DA to C-3 position of glucose (19 in Figure 12) and to C-6 of galactose (20 in Figure 12) by a succinyl spacer. Pharmacological studies showed that these two prodrugs were found to be more active than LD in reversing reserpine-induced hypo-locomotion in rats.

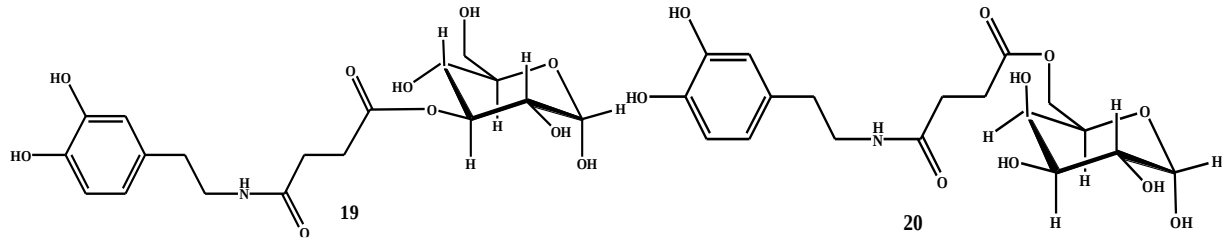


Figure 12 : Chemical structures of glycosuccinyl-derivatives of dopamine.

2.3 Enzyme Model

Despite that some success has been obtained using the different strategies by which prodrugs of dopamine were used to supply dopamine in adequate concentrations and sustained release manner, the prodrugs chemical approach involving enzyme catalysis has many limitations related to many intrinsic and extrinsic factors that can affect the process. For example, the activity of many prodrug-activating enzymes may be varied due to genetic polymorphisms, age-related physiological changes, or drug interactions, causing variation in clinical effects [126-130].

Karaman's group has explored a number of intra-molecular processes to gain insight into enzyme catalysis, toward the development of prodrug linkers that can be covalently attached to commonly used drugs which could have the potential for higher bioavailability over existing medications and would be chemically, and not enzymatically, be converted to release the active drugs in a controlled manner [131-166], by using ab-initio and density functional theory (DFT) molecular orbital methods.

Recently they have been designed a number of dopamine prodrugs to be used in the treatment of Parkinson's disease with a higher bioavailability than the current medication. These designed prodrugs have the following physicochemical features :(i) owning moderate hydrophilic lipophilic balance (ii) soluble in physiological environment (iii) deliberate dopamine in a controlled manner, and (iv) undergo chemical cleavage to nontoxic by-products [96].

They explored the proton transfer reaction in some of Kemp's acid amide derivatives 21-31 (Figure 13) by using enzyme models as potential linkers to be linked to amine-drugs [153]. Based on the DFT calculations on proton transfer mechanism of these acid amides, two dopamine derivatives were proposed. As shown in (Figure 14), ProD 32 and ProD 33 have a carboxylic group as a hydrophilic moiety and the rest of the prodrug as a lipophilic moiety, where the combination of both moieties secures a moderate HLB. Furthermore, at physiological pH in the blood circulation the expected predominant form of dopamine is the ionized form while its prodrug 32 and prodrug 33 are predicted to exist in the ionic and free acid forms. So, ProD 32 and ProD 33 may have a higher bioavailability than dopamine due to improved

absorption. Also, the designed prodrugs can be used in many dosage form (e.g. enteric coated tablets) because they are predicted to be soluble in organic and aqueous media due to the ability of the carboxylic group to be converted to the corresponding carboxylate anion in physiological environments of pH 5.0-7.4 (intestine and blood circulation).

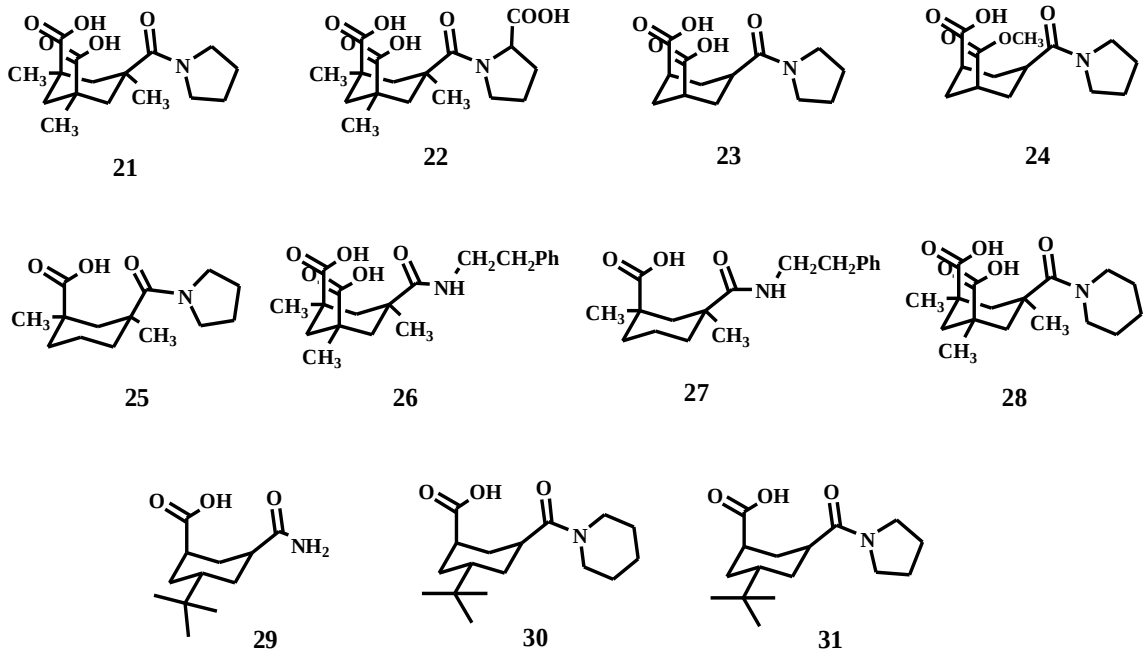


Figure 13 : Chemical structures of Kemp's acid amides 21-31.

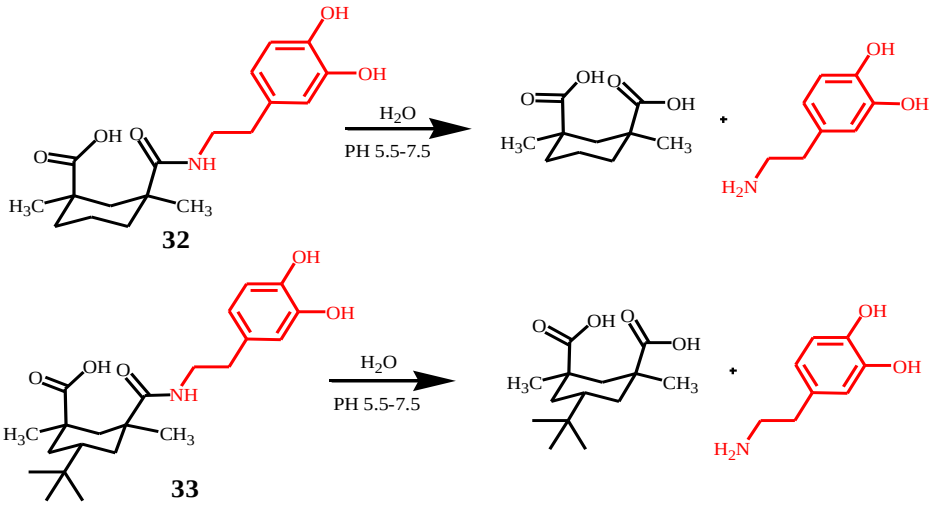


Figure 14 : Dopamine prodrugs, ProD 32- ProD 33.

Experimental

Chapter Three

Experimental Part

This chapter consists of three major parts. The first one is the synthetic part which concerns with the reactions, reagents, solvents and materials used, the second is the Kinetic studies part which describes the specific preparations and analysis used to investigate dopamine prodrugs hydrolysis in different pH solutions using the HPLC instrument and the third part is *in silico* predicting of physiochemical parameters, ADMET (absorption, distribution, metabolism, excretion and toxicity) properties, oral bioavailability and BBB permeability for the synthesized prodrugs.

3.1 Part one

3.1.1 Chemicals and Instrumentation.

3.1.1.1 Reagents

1,2-cyclohexanedicarboxylic anhydride, hexahydro-4-methylphthalic anhydride, sodium hydride (NaH), thin layer chromatography (TLC), glacial acetic acid (>99.8%) and pure standard of dopamine are all purchased from Sigma Aldrich.

3.1.1.2 Solvents

High purity chloroform ($CHCl_3$), tetrahydrofurane (THF) and diethyl ether (> 99%), were used directly from the bottles and all were purchased from Sigma Aldrich. Distilled water was obtained from a distillatory device available at Karaman's lab. HPLC grade solvents of methanol, and water were purchased from J.T. Paker.

3.1.1.3 Instrumentation and substance identification

Chemical hazards fuming hood, vacuum pumps, hotplates, available at Karaman's Lab in the Faculty of Pharmacy, Al-Quds University. FTIR, pH meter and rotary evaporator are available at Al-Quds University. HPLC was done at Al-Quds University and at Jerusalem Pharmaceutical Company in Ramallah. ^{1}H-NMR and LC/MS were taken at the Hebrew University and at Jordan Center For Pharmaceutical Research (JPRC) respectively.

3.1.1.3.1 Melting point determination by capillary method.

Capillary method is commonly used in chemistry labs to determine the melting points of solid substances. This technique is easy and requires a small amount of the material. It is performed by introducing a small amount of the solid into a one end sealed capillary tube, which is then fixed into a thermometer, then dipped into an oil bath. Heating of the oil bath should be done slowly

and gently, to ensure uniform heating of the sample and the thermometer. Then, the temperature range over which the sample starts to melt is recorded to be as the melting point of the material.

3.1.1.3.2 High performance Liquid Chromatography HPLC.

HPLC from Waters 2695 (Israel, Shimadzu corp. Japan), and waters Micromass® Masslynx ™ detector with Photo diode array (PDA) (Waters 2996: Israel). Data acquisition and control were carried out using Empower ™ software (Waters: Israel).
Analysis was done using C_{18}, 4.6 mm x125 mm, 5 µm particle size, protected by XBridge® C18 guard column. Micro filters 0.45µm porosity were used (Acrodisc® GHP, Waters). The C-18(1 gm) cartridges 6cc single use for laboratory use, were purchased form Waters Company (Milford, MA, USA).

Dopamine ProD 1 intraconversion analysisin 0.1N HCl, buffer pH 2.2 and buffer pH 5.5 were done at Al-Quds University by using HPLC device with 10 cm column length, while at Jerusalem Pharmaceutical company, the column length was 15 cm for dopamine **ProD 1** in buffer pH 7.4, and for dopamine **ProD 2** in 0.1N HCl, buffer pH 2.2, buffer pH 5.5 and buffer pH 7.4.

3.1.1.3.3 pH meter

pH values were recorded by pH meter model HM-30G: TOA electronics ™ was used to measure pH values for prepared buffers.

3.1.1.3.4 FT-IR

All infrared spectra (FTIR) were obtained from KBr (potassium bromide) matrix(4000–400 cm^{-1}) using a Perkin Elmer Precisely, Spectrum 100, FT-IR spectrometer.

3.1.1.3.5 Nuclear magnetic resonance spectroscopy (^{1}H -NMR)

All ^{1}H-NMR spectra were conducted using the 400 MHz Varian NMR spectrometer. The experimental samples was run in CD_3OD. ^{1}H-NMR experiments are stated in parts per million (ppm) downfield of TMS.

The following symbols used for ^{1}H-NMR peak investigation: chemical shift (δ ppm), multiplicity (s =singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constant (Hz). All ^{1}H-NMR data were analyzed by MestReNova Software.

3.1.1.3.6 Liquid Chromatography- Mass Spectroscopy (LC-MS)

HPLC–MS/MS Shimadzu prominence high performance liquid chromatography system (Shimadzu corp. Japan) was employed to record LC/MS measurements, at Jordan Center For Pharmaceutical Research (JPRC).

3.1.2 Preparation of dopamine prodrugs

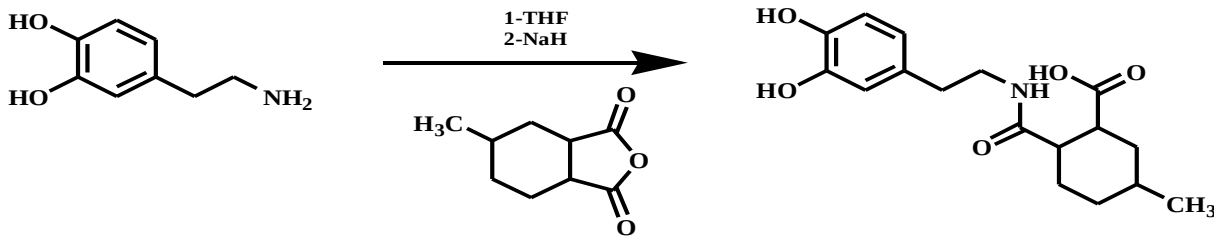
In a 250 ml round-bottom flask, 1.53g of dopamine (10 mmol) was dissolved in THF (100 ml) followed by an addition of 1 equivalent of sodium hydride (0.24g) to the reaction and stirred at room temperature for 2 hours, then 3.5 equivalent of hexahydro-4-methylphthalic anhydride (5.06 ml) or 1,2 cyclohexanedicarboxilic anhydride (5.4g) was added to the reaction. The flask was air-tightened and closed with a flexible rubber stopper. The reaction was left over night at room temp for 5 days and monitored by TLC to ensure reaction completion using chloroform and methanol (1:3) system as an eluent. When the reaction was completed, 10 ml of water was added drop wise to destroy the excess of NaH.

The solvent was evaporated by the rotary evaporator and the resulting precipitate was washed three times with diethyl ether then filtered. Evaporation was done to the filtrate to yield a brown product. The product was characterized by M.P, H-NMR, FTIR and LC-MS.

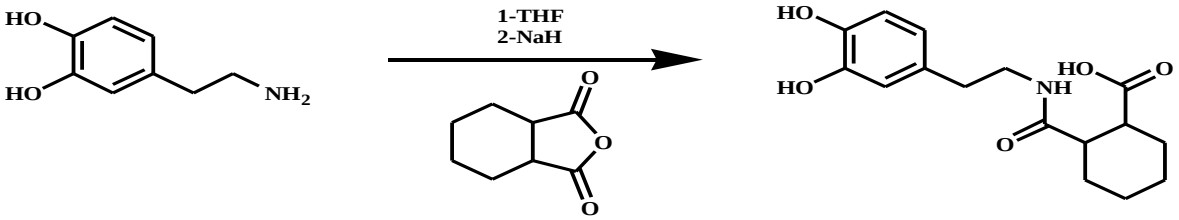
Reaction of dopamine with hexahydro-4-methyl phthalic anhydride or 1,2 cyclohexane dicarboxilic anhydride provided dopamine ProD 1 and ProD 2,respectively with yield 90%, 95%, respectively (Schemes 2 and 3). M.P. 180^{o}C and178^{o}C, respectively.

Dopamine **ProD 1,** ^{1}H-NMR δ (ppm) CD_3OD - 0.96 (CH3-CH-CH2, d), 1.43 (CH3-CH-CH2-CH2, m), 1.76 (CH3-CH-CH2-CH), t), 1.91 (CH3-CH-CH2-CH2-CH, m), 2.25 (NH-CH2-CH2, t), 2.46 (HOOC-CH-CH2-CH-CH3, m), 2.98 (NH-C=O-CH, m), 3.32 (NH-CH2-CH2, t), 6.68 (aromatic, M). IR (KBr/ν_{max} cm^{-1}) 1693 (C=O), 3395 (NH), 2800-3000 (OH). LC-MS (positive mode) *m*/*z* 322.1 $(M+1)^{+}$.

Dopamine **ProD 2,** ^{1}H-NMR δ (ppm) CD_3OD-1.4 (q, 2H, HN-C=O-CH-CH2-CH2), 1.74 (m, 2H, COOH-CH-CH2-CH2-CH2), 1.81 (m, 2H, HN-C=O-CH-CH2-CH2-CH2), 2.2 (m, 2H, COOH-CH-CH2-CH2), 2.65 (m, 1H, COOH-CH), 2.85 (t, 2H, HN-CH2-CH2-Ar), 3.2 (m, 1H, COOH-CH-CH-CH2), 3.6 (t, 2H, HN-CH2-CH2), 6.55 (m, 3H, Aromatic). IR (KBr/ν_{max} cm^{-1}) 1692 (C=O), 3396 (NH), 2800-3000 (OH). LC-MS (positive mode) *m*/*z* 308.2 $(M+1)^{+}$.



Scheme 1. Dopamine ProD 1;Synthesis scheme for the formation of dopamine hexahydro-4-methyl phthalate reaction



Scheme 2. Dopamine ProD 2;Synthesis scheme for the formation of dopamine 1,2 cyclohexane dicarboxilic reaction

3.2. Part Two

3.2.1 Kinetic Methods

3.2.1.1 Buffer Preparation

Potassium dihydrogen phosphate (6.8 g) were dissolve in 900 ml water for HPLC, the pH of buffer pH 2.2 was adjusted by diluted o-phosphoric acid and water was added to a final volume of 1000 ml. The same procedure was done for the preparation of buffers pH 5.5 and 7.4, however, the required pH was adjusted using 1 N NaOH. 0.1N HCl was prepared by diluting 8.5 ml of hydrochloric acid with water to 1000 ml.

Intra-conversion of 500 ppm dopamine **ProD 1** and dopamine**ProD2**solutions, in 0.1N HCl, buffer pH 2, buffer pH 5.5 or buffer pH 7.4, to its parent drug, dopamine, was followed by HPLC at a wavelength of 247 nm. Conversion reactions were run mostly at 37 ˚C.

3.2.1.2 Calibration curve

A stock solution of dopamine **ProD 1**or **ProD 2** (100 ml) with a final concentration of 500 ppm were prepared by dissolving 50 mg from each prodrug in 100 ml methanol. The following diluted solutions were prepared from the stock solution: 100, 200, 300 and 400 ppm. To construct a calibration curve for dopamine and dopamine **ProD1-2**, 5 calibrants (100, 200, 300, 400 and 500 ppm) were prepared. Then, 20 µl of each solution was injected to the HPLC apparatus using 250 mm x 4.6 mm, 5 µm C18 XBridge ® column. Methanol and deionized water (0.1% v/v acetic acid) with a ratio of 60:40 was used as mobile phase with a flow rate of 1 ml min^{-1} and UV detection at a wavelength of 247 nm [167].
Peak area vs. concentration of the pharmaceutical (ppm) was then plotted, and R^2 of the plot was recorded.

3.2.1.3 Preparation of standard and sample solution

A 500 ppm of standard dopamine was prepared by dissolving 50 mg of dopamine in 100 ml of 0.1N HCl, buffer pH 2.2, buffer pH 5.5 or buffer pH 7.4 and then each sample was injected into HPLC to detect the retention time of dopamine.

A 500 ppm of dopamine **ProD 1** and **ProD 2** was prepared by dissolving 50 mg of dopamine **ProD 1** and **ProD 2** in 100 ml of 0.1N HCl, buffer pH 2.2, buffer pH 5.5 or buffer pH 7.4 then each sample was injected into HPLC to detect the retention time of dopamine **ProD 1** and **ProD 2**.
The progression of the reaction was followed by monitoring the disappearance of prodrug and the appearance of the parent drug (dopamine) with time.

3.3 Part three

3.3.1 Prediction of drug-likeness and *in silico* ADMET studies

In silico prediction methods represent an alternative approach and aim to rationalize the preclinical drug development, thus enabling the reduction of the associated time, costs and animal experiments [168].

3.3.1.1 BBB permeability prediction

Predicting blood-brain barrier (BBB) permeability is essential to drug development, as a molecule cannot exhibit pharmacological activity within the brain parenchyma without first investigating this barrier.

In silico BBB permeability were predicted using Online BBB predictor (Prof Xiang-Qun (Sean) Xie, Pitt.edu) at http://www.cbligand.org/BBB/ for the synthesized prodrugs. This uses

AdaBoost and SVM combining with 4 different fingerprints to predict if a compound can pass the BBB(BBB+) or cannot pass the BBB(BBB-).

3.3.1.2 In silico prediction of physicochemical parameters and ADMET properties

In silico pharmacokinetics and toxicities were predicted for the two dopamine prodrugs since ADMET (absorption, distribution, metabolism and excretion and toxicity) can be a major cause of failure of drug candidates during the later phases of drug development.

Two software were used to calculate the physiochemical properties and to predict ADMET. The first one is ACD/Lab software (Advanced Chemistry Development Inc, Ontario, Canada) [169], which can predict physicochemical, ADME, and toxicity properties from structure of the compound. The second software is an online webserver, admetSAR [170] which provides a number of ADMET values for a certain chemical structure to be encoded as SMILES (simplified molecular input line entry specification). AdmetSAR is a knowledge based tool comprising of ADMET related properties taken from wide range literature which are further used to predict properties of unknown compounds.

Results and Discussion

Chapter Four

Results and Discussion Part

We have successfully obtained two antiparkinson's prodrugs of dopamine with two different linkers. They were characterized by melting points, FT-IR, ^{1}H-NMR and LC-MS analytical techniques, to guarantee pure dopamine prodrugs that are expected to give better bioavailability than the parent drug owing to improved absorption and are capable of releasing the parent drug in a sustained release manner.

4.1Prodrugs characterization using different analytical techniques

4.1.1Melting point, FT-IR, NMR and LC-MS analysis of dopamine ProD 1

1) Melting point of dopamine **ProD 1** was 180 ˚C.

2) IR (KBr/ν_{max} cm^{-1}) 1693 (C=O), 3395 (NH), 2800-3000 (OH).

3) ^{1}H-NMR δ (ppm) CD_3OD - 0.96 (CH3-CH-CH2, d), 1.43 (CH3-CH-CH2-CH2, m), 1.76 (CH3-CH-CH2-CH), t), 1.91 (CH3-CH-CH2-CH2-CH, m), 2.25 (NH-CH2-CH2, t), 2.46 (HOOC-CH-CH2-CH-CH3, m), 2.98 (NH-C=O-CH, m), 3.32 (NH-CH2-CH2, t), 6.68 (aromatic, M).

4) The product molecular formula is $C_{17}H_{23}NO_5$ (yield 90%). LC-MS (positive mode) *m*/*z* 322.1 $(M+1)^+$ (Figure 15d**)**.

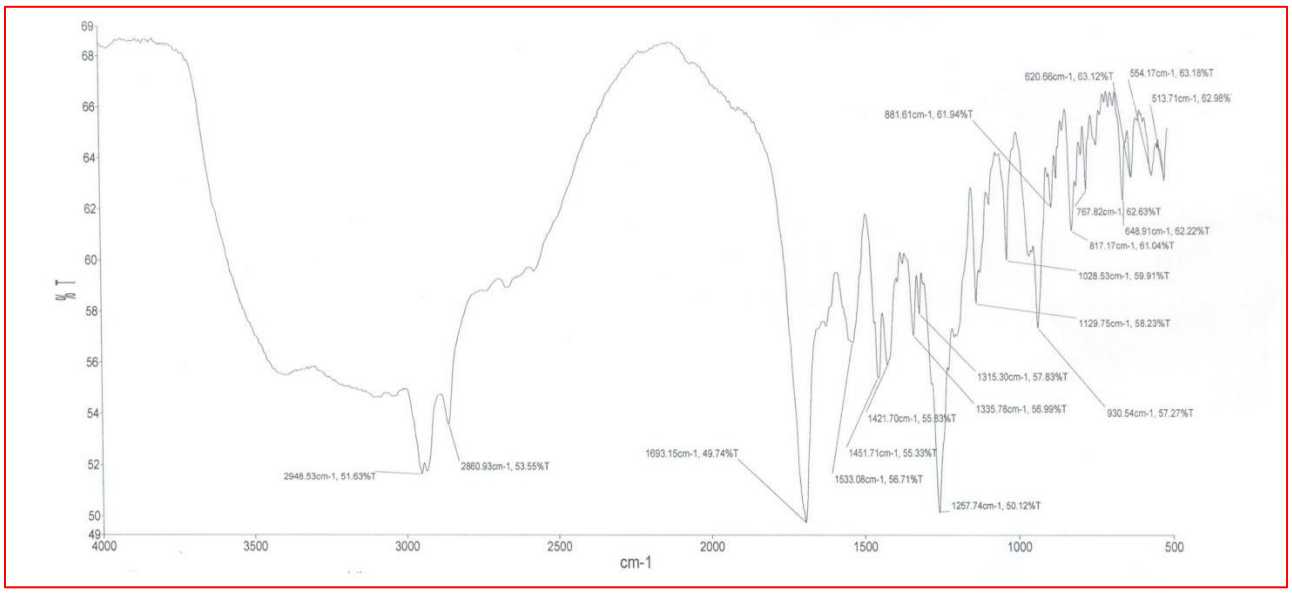


Figure 15 a: FT-IR spectrum of dopamine ProD 1 (500-4000 cm^{-1}).

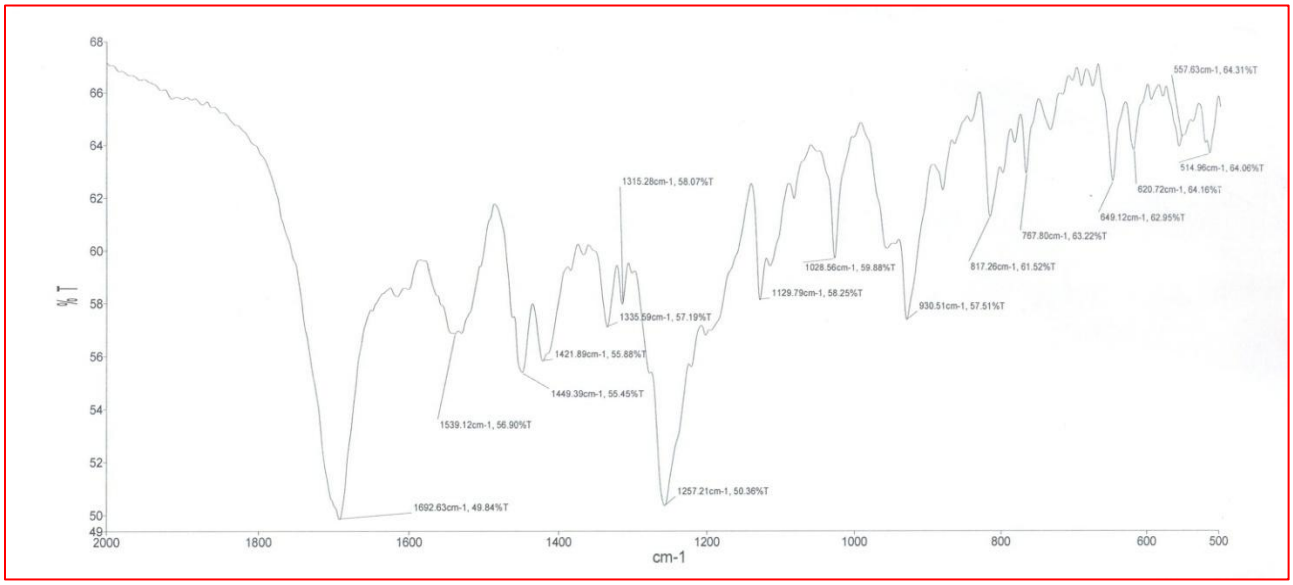


Figure 15 b: FT-IR spectrum of dopamine ProD 1 (500-2000 cm^{-1}).

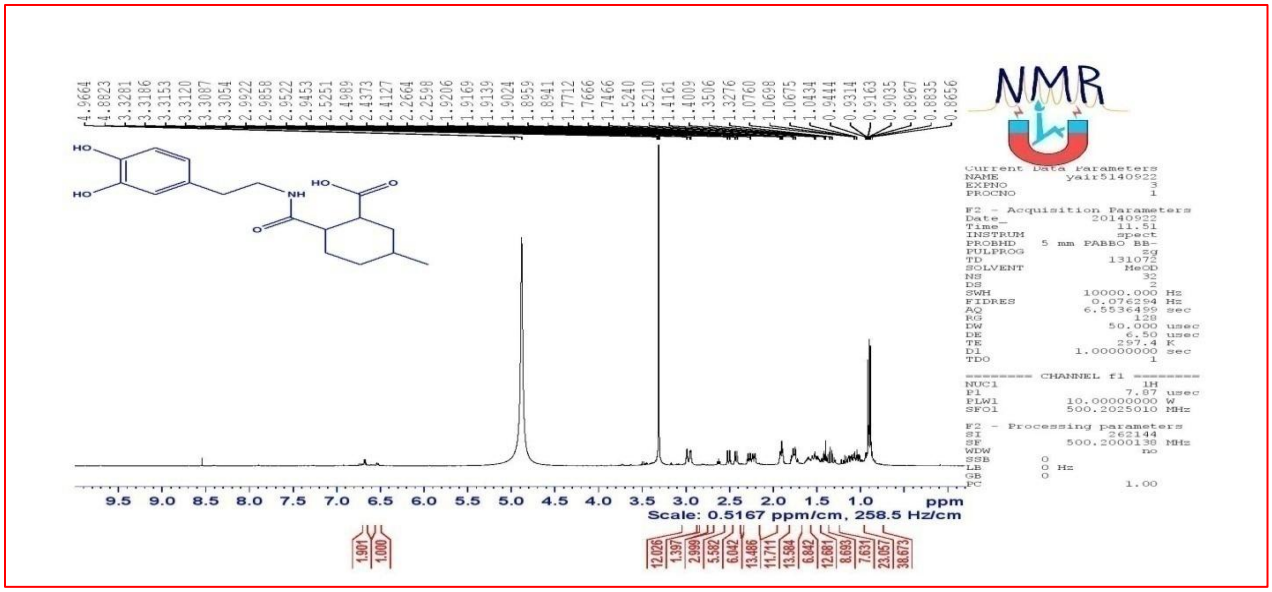


Figure 15 c: ^{1}H-NMR spectrum of dopamine ProD 1.

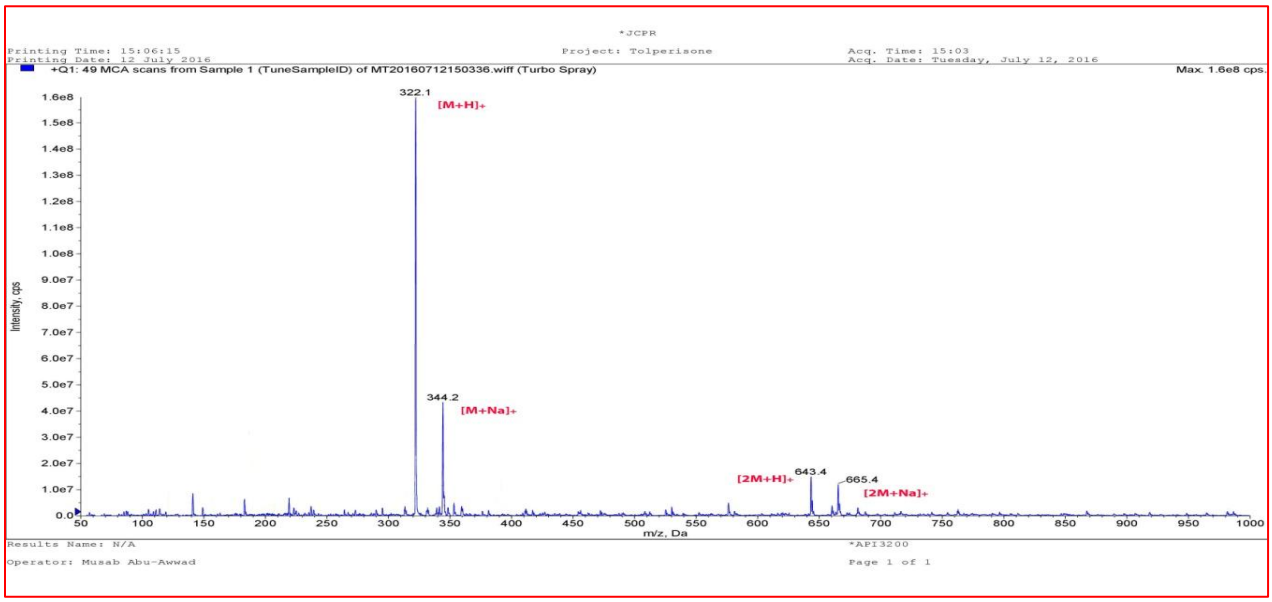


Figure 15 d: LC-MS spectrum of dopamine ProD 1.

4.1.2 Melting point, FT-IR, NMR and LC-MS analysis of dopamine ProD 2

1) Melting point of dopamine **ProD 2** was 178 ˚C.

2) IR (KBr/ν_{max} cm^{-1})1692 (C=O), 3396 (NH), 2800-3000 (OH).

3) ^{1}H-NMR δ (ppm) CD_3OD - 1.4 (q, 2H, HN-C=O-CH-CH2-CH2), 1.74 (m, 2H, COOH-CH-CH2-CH2-CH2), 1.81 (m, 2H, HN-C=O-CH-CH2-CH2-CH2), 2.2 (m, 2H, COOH-CH-CH2-CH2), 2.65 (m, 1H, COOH-CH), 2.85 (t, 2H, HN-CH2-CH2-Ar), 3.2 (m, 1H, COOH-CH-CH-CH2), 3.6 (t, 2H, HN-CH2-CH2), 6.55 (m, 3H, Aromatic).

4) The product molecular formula is $C_{16}H_{21}NO_5$ (yield 95%). LC-MS (positive mode) *m/z* 308.2 (M+1) (Figure 16 d).

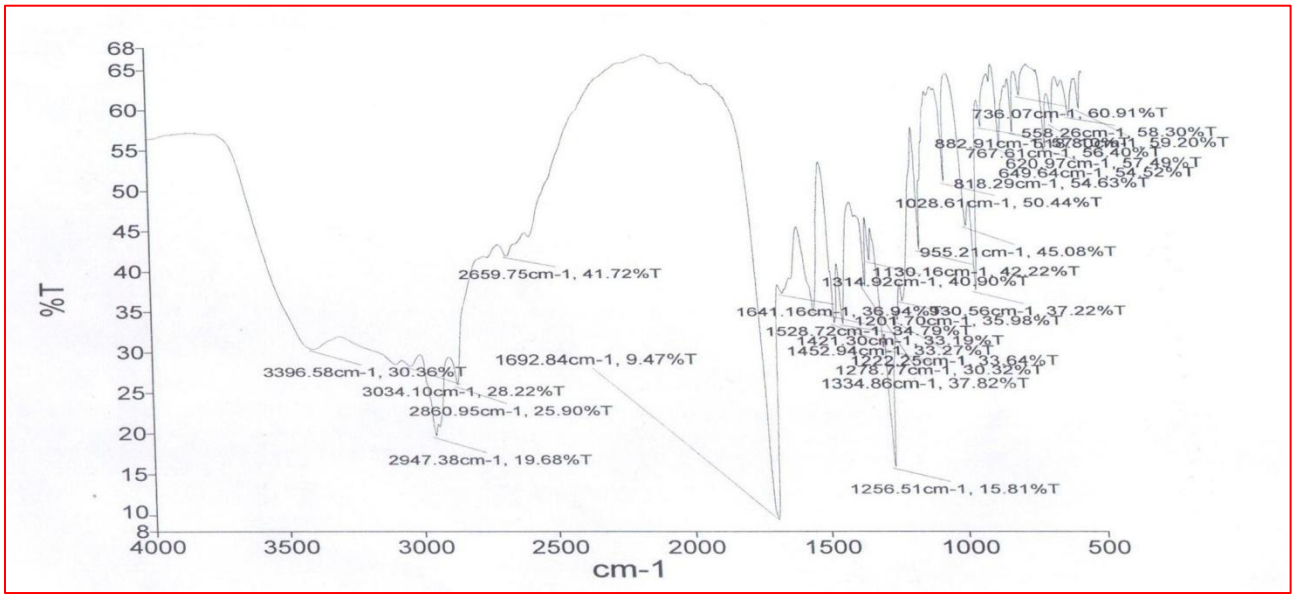


Figure 16 a: FT-IR spectrum of dopamine ProD 1 (500-4000 cm^{-1})

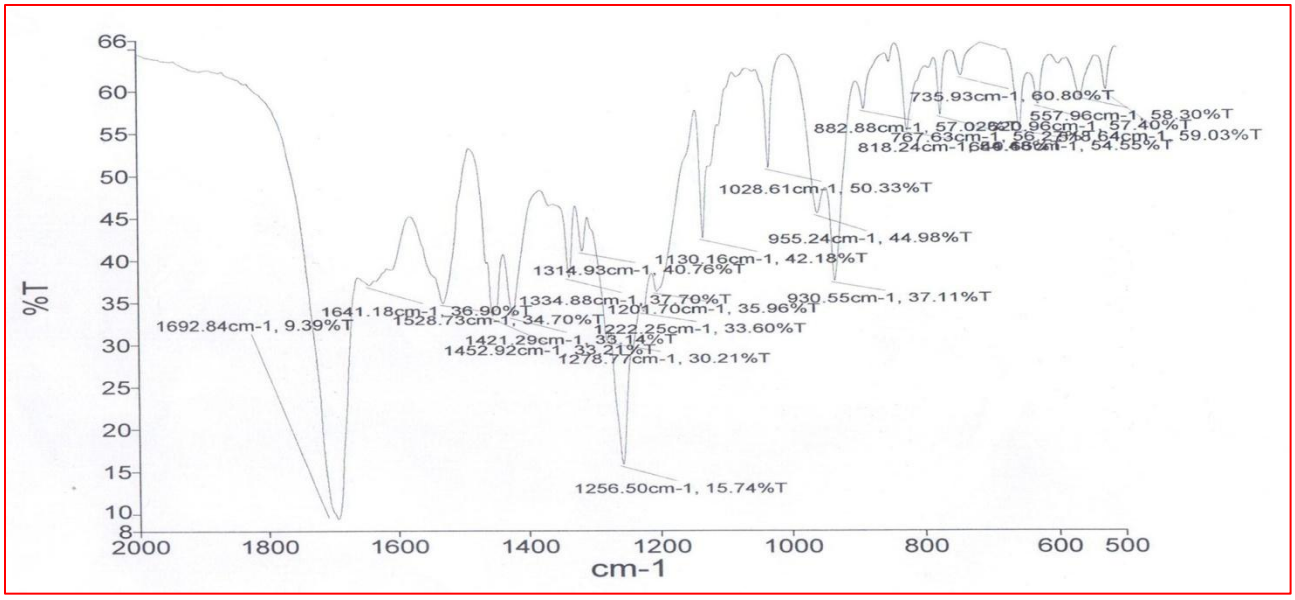


Figure 16 b: FT-IR spectrum of dopamine ProD 1 (500-2000 cm^{-1})

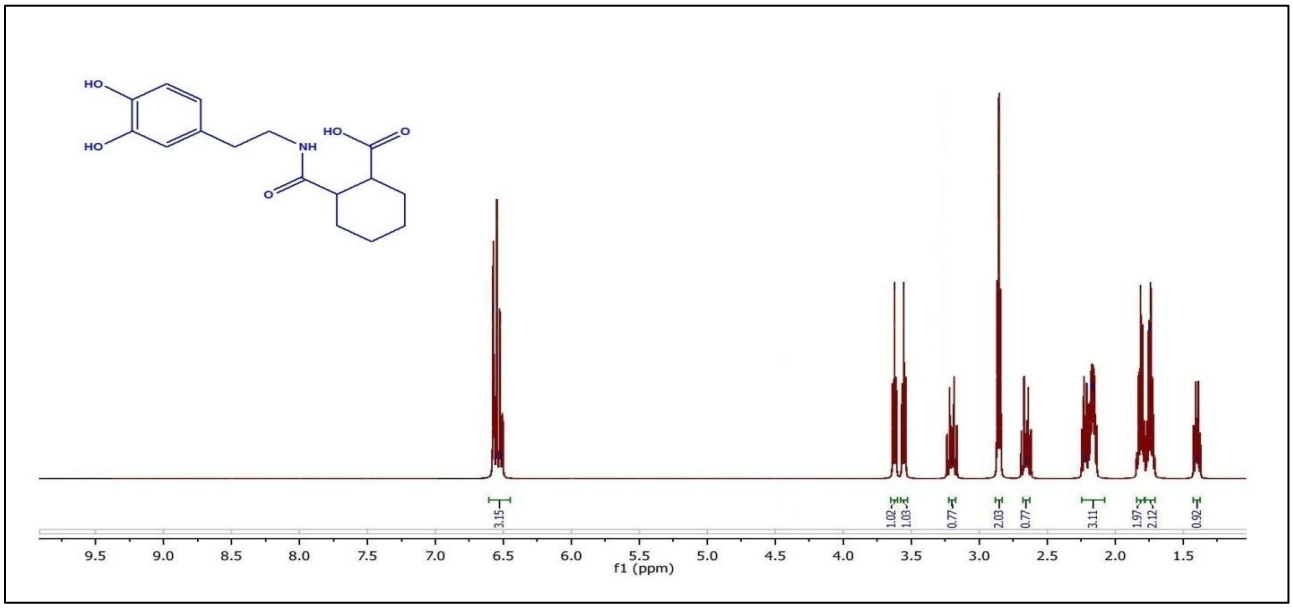


Figure 16 c: ^{1}H-NMR spectrum of dopamine ProD 2

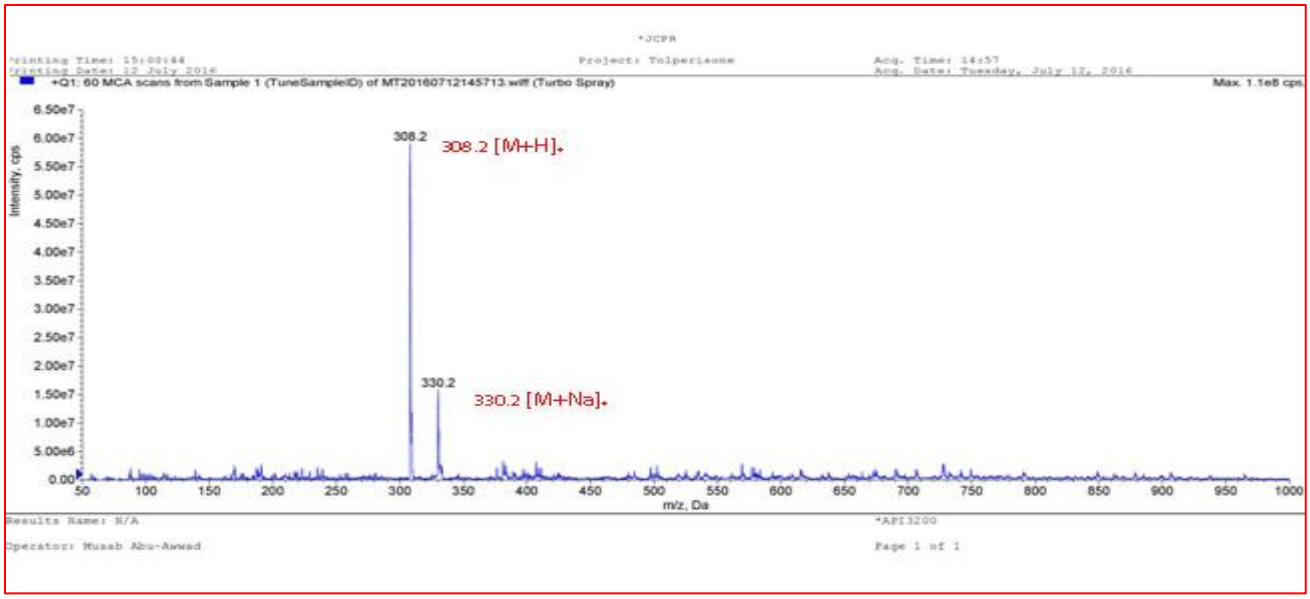


Figure 16 d. LC-MS spectrum of dopamine ProD 2.

4.1.3 Melting point, FT-IR, and NMR analysis of dopamine standard

1) Melting point of dopamine was 128 ˚C.

2) IR (KBr/ν_{max} cm^{-1}) 1600-1620 (NH bending), 3345 (OH).

3) ^{1}H-NMR δ (ppm) CD3OD: 2.55 (t, 2H, H2N-CH2-CH2), 2.75 (t, 2H, H2N-CH2-CH2), 6.6 (m, 3H, Aromatic).

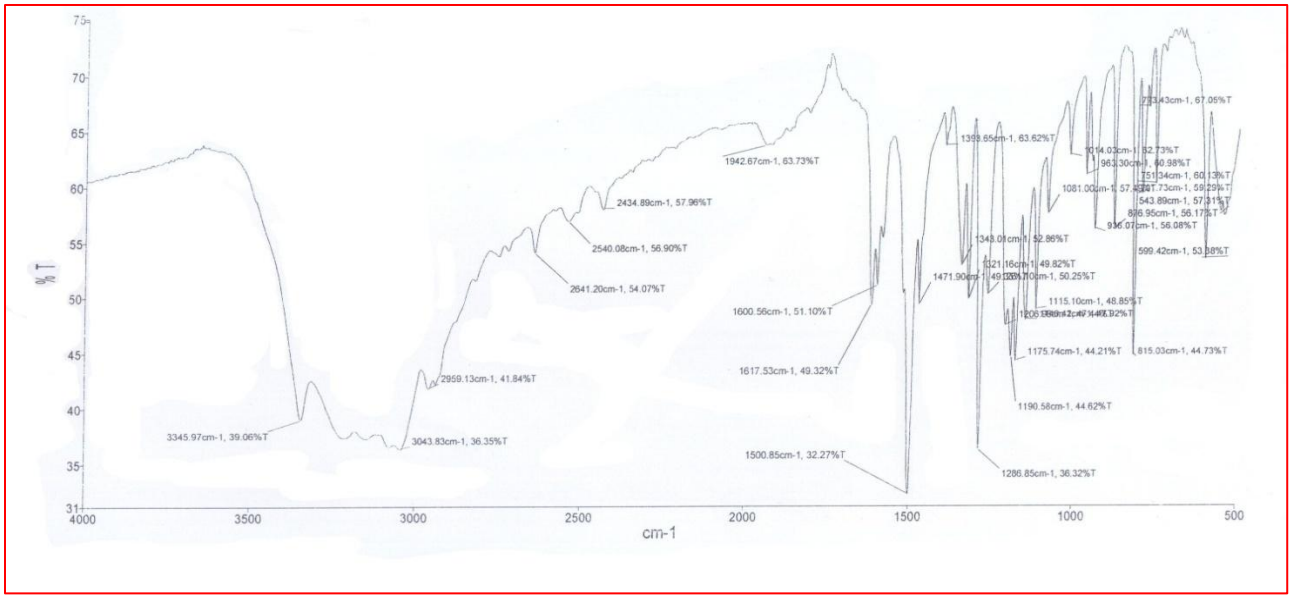


Figure 17 a: FT-IR spectrum of dopamine (500-4000 cm^{-1})

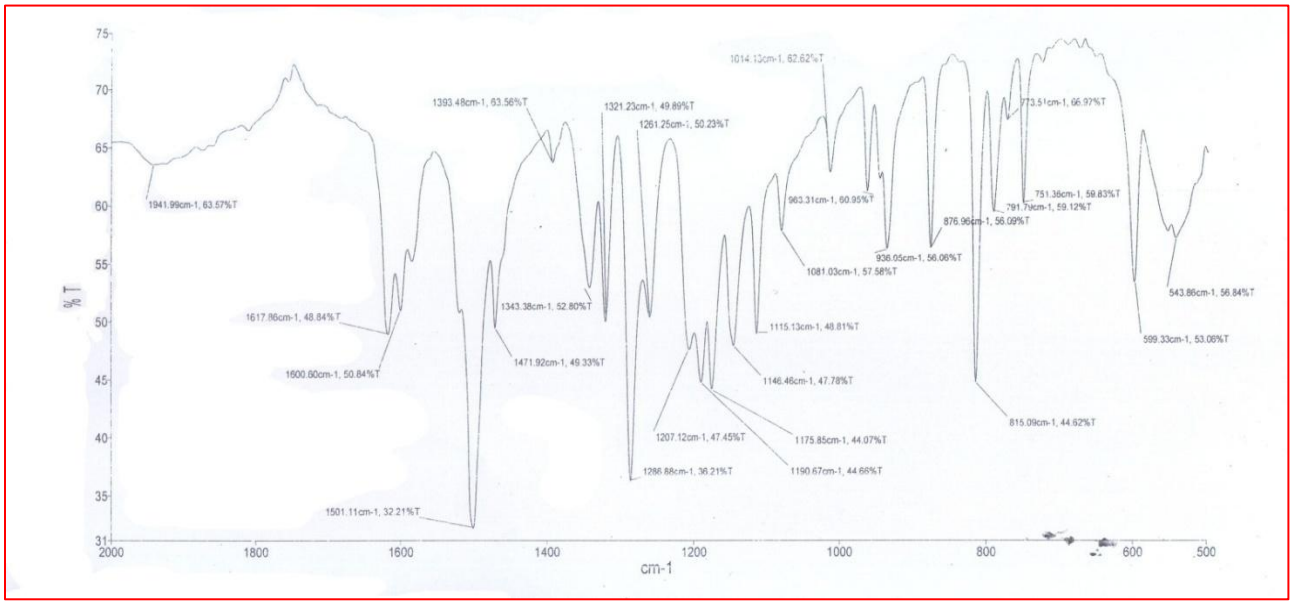


Figure 17 b: FT-IR spectrum of dopamine (500-2000 cm^{-1})

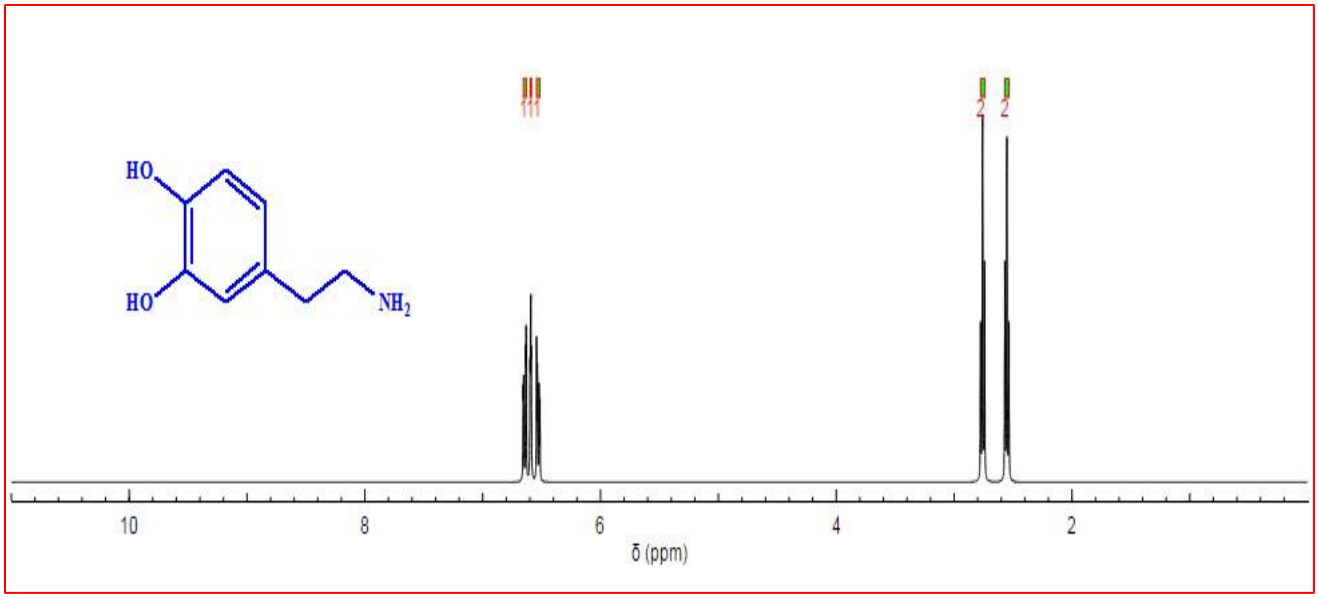


Figure 17 c: ^{1}H-NMR spectrum of dopamine

4.2 calibration curves of dopamine prodrugs

The calibration curves were obtained by plotting peak area versus concentration. As shown in Figure 18excellent linearity with regression (R^2) of 0.997 and 0.999 for dopamine **ProD 1** and **ProD2** was obtained, respectively.

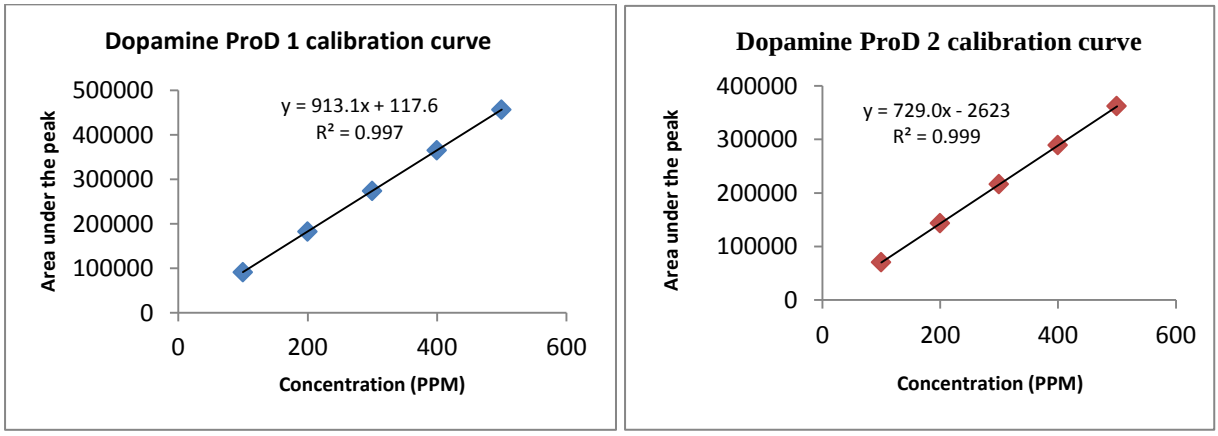


Figure 18: Calibration curves for dopamine ProD 1 and ProD 2

4.3 Hydrolysis studies

The kinetics of the acid-catalyzed hydrolysis studies were carried out in aqueous buffer in the same manner as that done by Kirby on Kirby's enzyme model **1-9** [73]. This is in order to explore whether the prodrug hydrolyzes in aqueous medium and to what extent or not, suggesting the fate of the prodrug in the system. Acid-catalyzed hydrolysis kinetics of the synthesized dopamine **ProD 1** and **ProD 2** were studied in four different aqueous media, 0.1N HCl, buffer pH 2.2, buffer pH 5.5 and buffer pH 7.4 (Table 1 and 2). Under the experimental conditions the target compounds hydrolyzed to release the parent drug as evident by HPLC analysis, at constant pH and temperature the reaction displayed strict first order kinetics as the k_{obs} was fairly constant and a straight plot was obtained on plotting ln concentration of residual prodrug vs. time. The rate constant (k_{obs}) and the corresponding half-lives ($t_{1/2}$) for dopamine prodrugs in the different media were calculated from the linear regression equation correlating the ln concentration of the residual prodrug vs. time. The 0.1N HCl and pH 2.2 were selected to examine the intra-conversion of the dopamine prodrug in pH as of stomach, because the meanstomach pH of adult is approximately 1-3. In addition, buffer pH 5.5 mimics the beginning of the small intestine pathway. pH 7.4 was selected to examine the intra-conversion of the tested prodrugs in the blood circulation system. At pH 5.0-7.4 the carboxylic group in prodrugs **ProD 1** and **ProD 2** will equilibrate with the corresponding carboxylate form. Subsequently, the free acid form will undergo proton transfer reaction (rate limiting step) after being transferred through the membrane to yield dopamine and the inactive linker as a byproduct. The proposed prodrugs **ProD 1- ProD 2** will be exploited for oral use in the form of enteric coated tablets. It is well known, that enteric coated tablets are stable at a high acidic pH found in the stomach, but break down rapidly at a less acidic pH. For example, the enteric coated tablets will not dissolve in the acidic juices of the stomach (pH ~3), but they will be dissolved in the higher pH (above pH 5.5) present in the small intestine. In the intestine, prodrugs **ProD 1- ProD 2** will exist in the acidic and ionic forms where the equilibrium constant for the exchange between both forms is dependent on the pKa of the given prodrug. The experimental determined pK_as for **ProD-1-ProD 2** linkers are in the range of 5.0-6.0. Therefore, it is expected that the pK_as of the corresponding prodrugs will be in the same range. Since the pH for the small intestine lies in the range of 5.0-7.5, the calculated unionized (acidic) /ionized ratio will be 40-50%. Although the percentage of the acidic form is not significantly high, we anticipate that prodrugs undergoing an efficient proton transfer (rate limiting step) to yield dopamine and Kemp's carboxylic acid by-products and will have the potential to be effective prodrugs. In the blood circulation (pH 7.4), the calculated acidic form for those prodrugs is around 10- 30% and it is expected that the rate for delivering the parent drug will be reduced.

Amidase specific activation was done for these two dopamine prodrugs in Finland, the results revealed that **ProD 1**and **ProD 2** have found to be non-specific for amidase in the first 24 hours.

Table 1: The observed *k* value and $t_{1/2}$ for the intraconverion of dopamine**ProD 1**in 0.1N HCl, pH 2.2, pH 5.5 and pH 7.4.

Medium	k_{obs} **(h^{-1})**	$t_{½}$ **(h)**
0.1N HCl	0.0115	60.30
Buffer pH 2.2	0.0126	54.66
Buffer pH 5.5	0.0069	99.93
Buffer pH 7.4	0.005	138.13

Table 2: The observed *k* value and $t_{1/2}$ for the intraconverion of dopamine**ProD 2**in 0.1N HCl, pH 2.2, pH 5.5 and pH 7.4.

Medium	k_{obs} **(h^{-1})**	$t_{½}$ **(h)**
0.1N HCl	0.0143	48.34
Buffer pH 2.2	0.0128	54.22
Buffer pH 5.5	0.0052	131.98
Buffer pH 7.4	0.0036	193.42

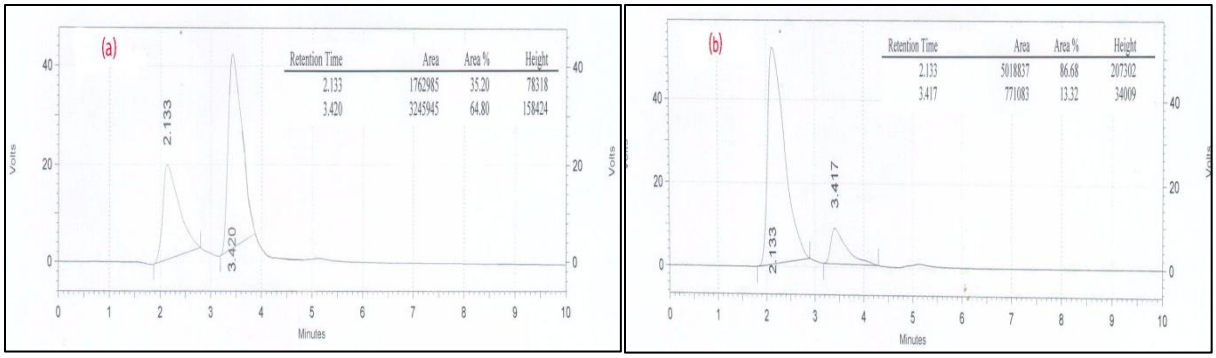


Figure 19. Chromatograms showing the intra-conversion of dopamine ProD 1 at 0.1N HCl (a) after one hour, (b) after 120 hours.

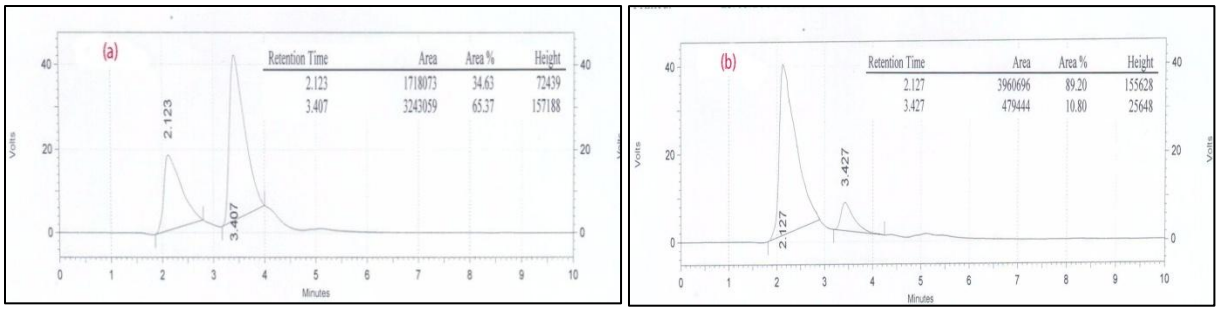


Figure 20. Chromatograms showing the intra-conversion of dopamine ProD 1 at pH 2.2 (a) after one hour and (b) after 144 hours.

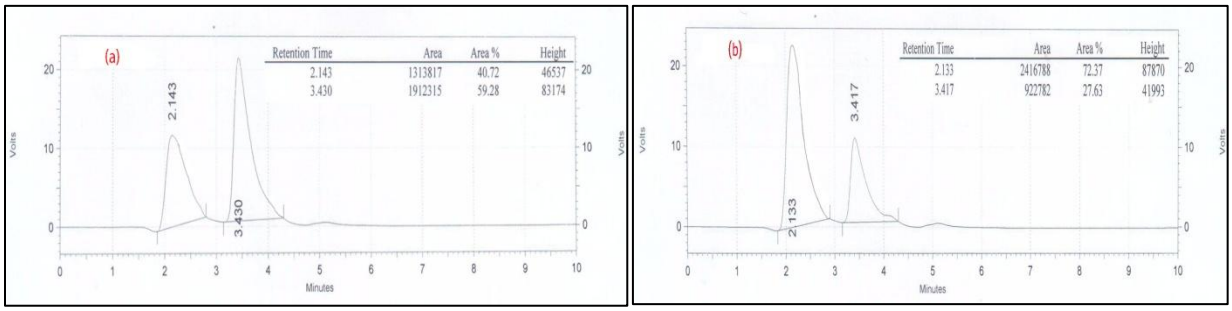


Figure 21. Chromatograms showing the intra-conversion of dopamine ProD 1 at pH 5.5 (a) after 48 hour, (b) after 144 hours.

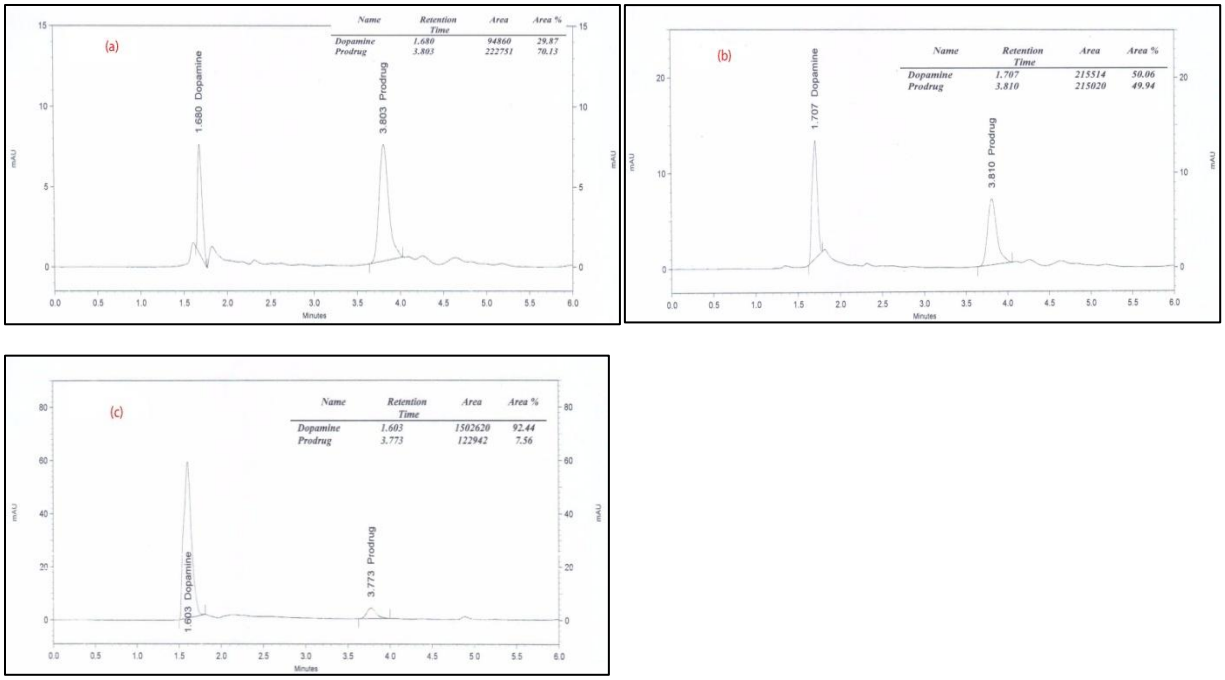


Figure 22. Chromatograms showing the intra-conversion of dopamine ProD 1 at pH 7.4 (a) after 24 hours, (b) after 48 hours and (c) after 216 hours.

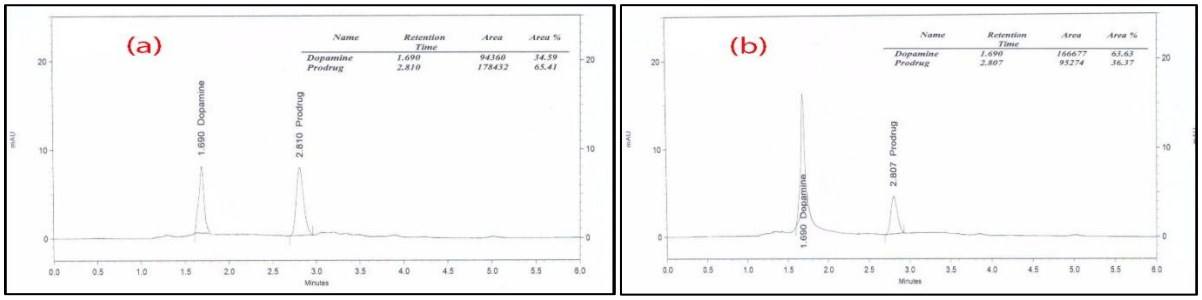


Figure 23. Chromatograms showing the intra-conversion of dopamine ProD 2 at 0.1N HCl (a) after 4 hours and (b) after 72 hours.

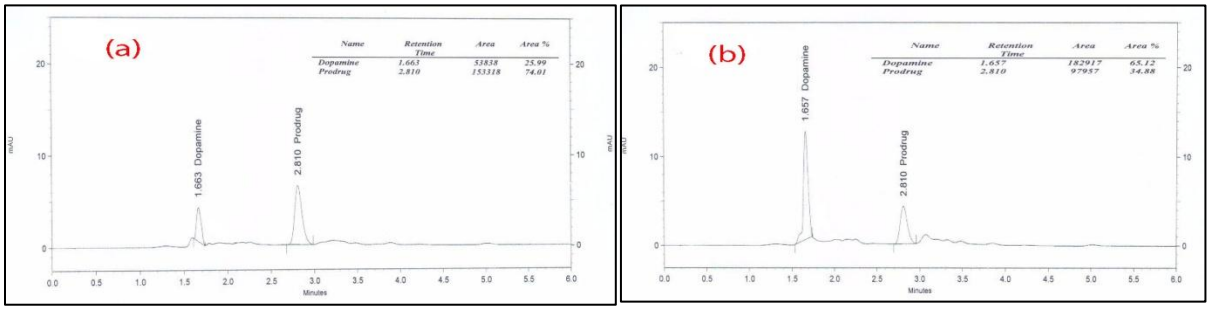


Figure 24. Chromatograms showing the intra-conversion of dopamine ProD 2 at pH 2.2 (a) after 4 hours and (b) after 72 hours.

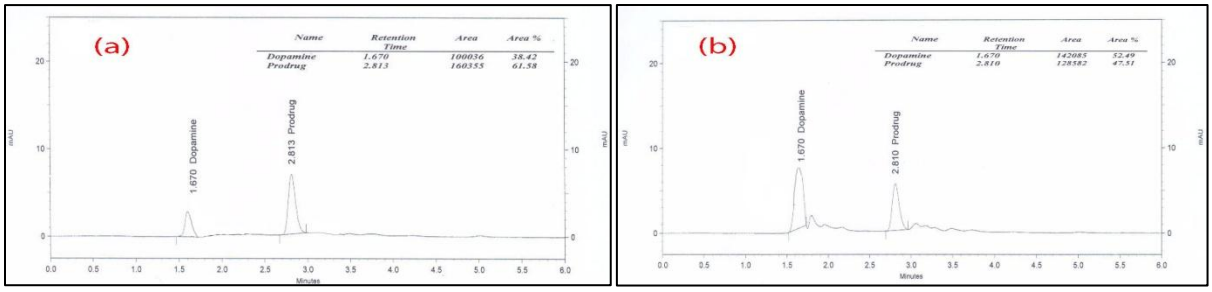


Figure 25. Chromatograms showing the intra-conversion of dopamine ProD 2 at pH 5.5 (a) after 12 hours and (b) after 48 hours.

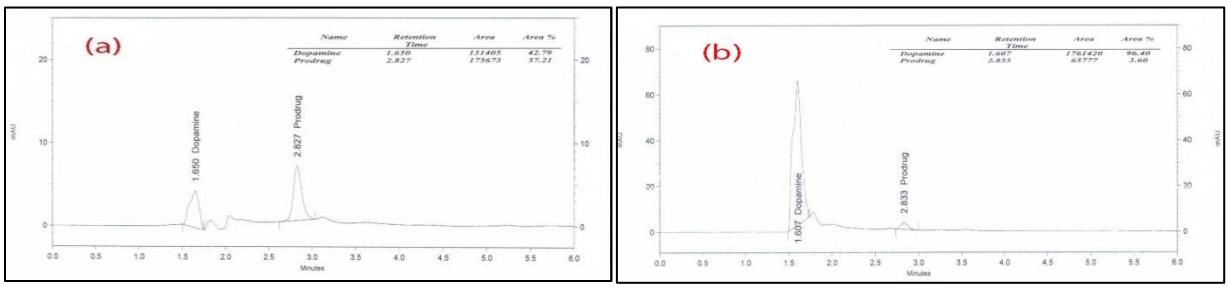
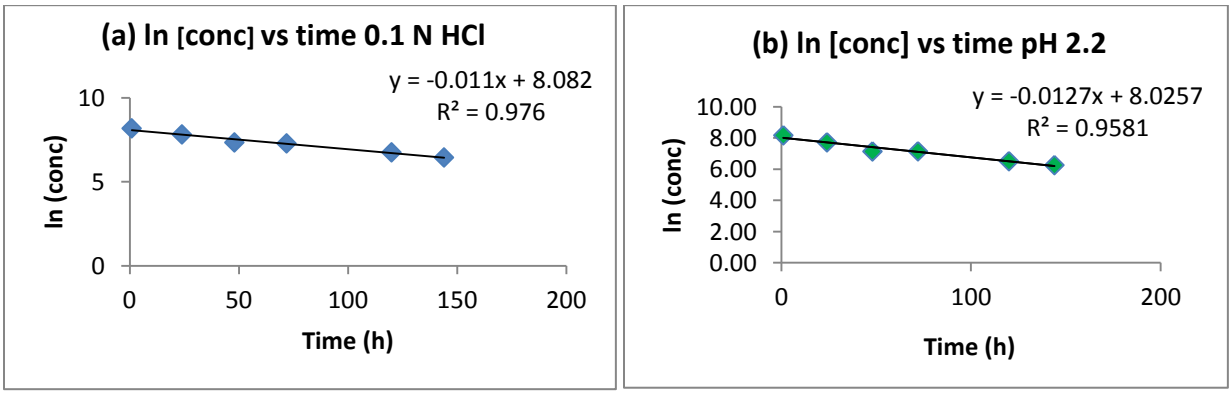


Figure 26. Chromatograms showing the intra-conversion of dopamine ProD 2 at pH 7.4 (a) at the start of reaction and (b) after 216 hours.



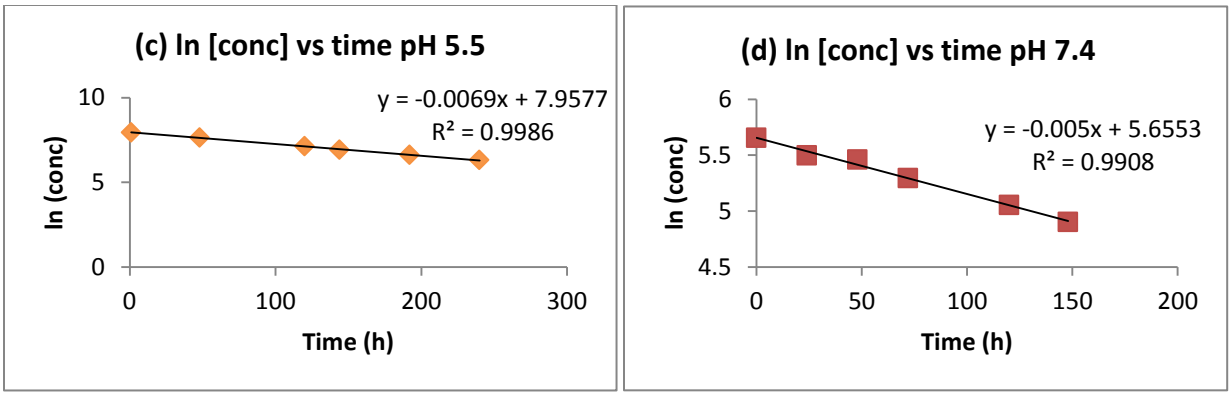


Figure 27. First order hydrolysis plot for dopamine ProD 1 in (a) 0.1N HCL, (b) buffer pH 2.2, (c) buffer pH 5.5 and (d) buffer pH 7.4.

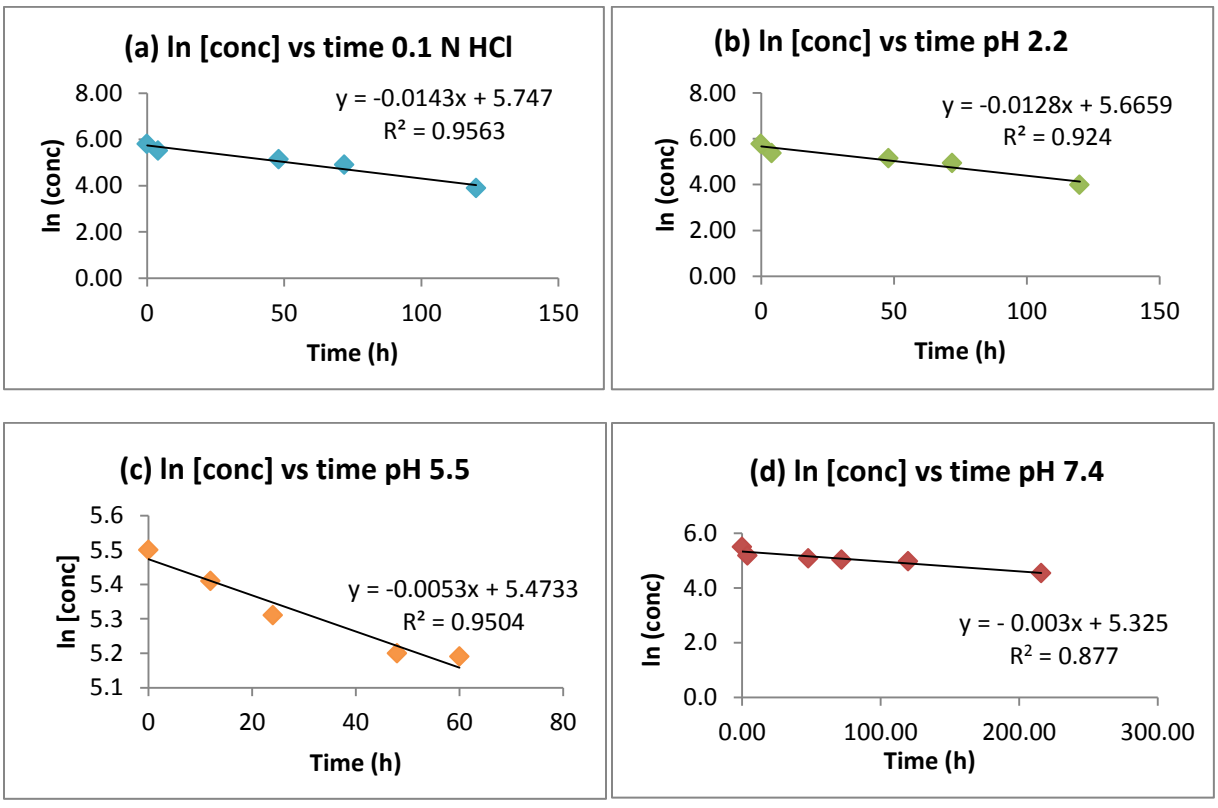


Figure 28. First order hydrolysis plot for dopamine ProD 2 in (a) 0.1N HCL, (b) buffer pH 2.2, (c) buffer pH 5.5 and (d) buffer pH 7.4.

4.4 Prediction of drug-likeness and *in silico* ADMET studies

A. Matching with Lipinski's rule of five:

The medicinal chemist Christopher Lipinski and his colleagues analyzed the physicochemical properties of more than 2,000 drugs and candidate drugs in clinical trials, and concluded that 90% of orally active drugs that have achieved phase II clinical status were associated with four simple physicochemical parameter ranges: molecular weight < 500, log P < 5, H-bond donors < 5 and H-bond acceptors < 10). Therefore, if a compound matches these physicochemical parameters, it is more likely to be membrane permeable and easy to be absorbed through membranes and possess the properties of drug like molecules [171].

In an attempt to improve the predictions of druglikeness, the rules have been extended to include, for example the following criteria: partition coefficient (log P) range −0.4 to +5.6, molar refractivity range 40 to 130, molecular weight range 180 to 500, total number of atoms range 20 to 70 and polar surface area not greater than 140 $Å^2$ [172].

The results of physicochemical properties revealed that the synthesized dopamine prodrugs were within the acceptable limit and comply with Lipinski's rule of five.

B. Determination of molecular lipophilicity (log P) and aqueous solubility (log S) of dopamine prodrugs

The log P and log S coefficients are well-known as the principal parameters for the estimation of lipophilicity and solubility of drugs and these two parameters significantly affect the pharmacokinetic properties of the drugs [173].

1. Aqueous Solubility (log S)

The aqueous solubility of a compound largely affects its absorption and distribution characteristics. Typically, a low solubility goes along with a poor absorption and therefore the general aim is to avoid poorly soluble compounds. Log S value is a unit stripped logarithm (base 10) of the solubility measured in mol/liter. Aqueous solubility is an important requirement for a CNS drug, as demonstrated by Alelyunas, who determined the solubility of 98 marketed CNS drugs in buffer pH 7.4. Over 85% of the drugs tested had high aqueous solubility (>100 uM) [174]. Another study showed that more than80% of the drugs on the market have logS values greater than -4 and less than 0 [173]. Both dopamine prodrugs were within this limit.

2. Molecular lipophilicity (log P)

Lipophilicity is a main physicochemical determinant influencing the bioavailability, permeability and the toxicity of drugs. Thus the 1-octanol/water partition coefficient is the ratio of the unionized compound concentration in 1-octanol to its concentration in water. Hence this coefficient is a measure of differential solubility of the compound between these two solvents. Usually one of the chosen solvents is water, while the second one is hydrophobic, such as 1-octanol. Both the partition and distribution coefficient are measures of how hydrophilic ("water loving") or hydrophobic (" water fearing") a chemical substance is. Moderately lipophilic drugs cross the blood brain barrier (BBB) by passive diffusion [173]. For several classes of CNS active substances, Hansch and Leo found that BBB penetration is optimal when the Log P values are in the range of 1.5-2.7 [175]. Results revealed that log P values for the synthesized dopamine prodrugs were found to be within the acceptable limit.

C. Prediction of oral bioavailability

Oral bioavailability measurements in rats for over 1100 drug candidates wereconducted at SmithKline Beecham Pharmaceuticals (now GlaxoSmithKline) to analyze the relative importance of molecular properties considered to influence the oral bioavailability of drug candidates. Their observations suggest that compounds which meet only the two criteria of (1) 10 or fewer rotatable bonds and (2) polar surface area (PSA) equal to or less than 140 $Å^2$ (or 12 or fewer H-bond donors and acceptors) will have a high probability of good oral bioavailability in the rat [176]. The results of molecular properties found that the two dopamine prodrugs have met with these two criteria and the predicted oral bioavailability was approximately 30% for both of them which is more than the marketed drugs bioavailability [177].

D. Prediction of log D

In addition, recently van de Waterbeemd et al. have showed that, of a set of 125 drugs, all those showing CNS activity could be found within the ranges of $-1 \leq \log D \text{ (pH } 7.5) \leq 4$ [178]. Results demonstrated that dopamine **ProD 1** was within this range, while dopamine **ProD 2** wasn't.

E. BBB permeability prediction and *in silico* log BB studies

A common measure of the degree of BBB penetration is the ratio of the steady-state concentrations of the drug molecule in the brain and in the blood, usually expressed as log(Cbrain/Cblood) or, more simply, log BB. Experimental values of log BB published to date cover the range about -2.00 to +1.00. Within this range, compounds with log BB > 0.3 cross the BBB readily, while compounds with log BB < -1.0 are poorly distributed to the brain [179].

BBB permeability software predicts that dopamine **ProD 2** has the ability to cross BBB, while dopamine **ProD 1**is not capable to permeate through BBB.

Table 3: Molecular properties of dopamine **ProD 1** and **ProD 2.**

	Molecular weight	No. of Hydrogen Bond Donors	No. of Hydrogen Bond Acceptors	TPSA	No. of Rotatable Bonds	Molar Refractivity	log P	log S	Total number of atoms	log D at pH 7.4
Drug-like properties	**< 500 [171]**	**< 5 [171]**	**< 10 [171]**	**<140 Å^2[176]**	**≤10 [176]**	**40 to 130 [172]**	**1.5-2.7 [175]**	**-4< log S < 0 [173]**	**20 to 70 [172]**	**-1 ≤ log D (pH 7.5) ≤ 4 [177].**
Dopamine ProD 1	**321**	**4**	**6**	**106.86**	**5**	**84.16 cm^3**	**2.39**	**-3.94**	**46**	**-0.39**
Dopamine ProD 2	**307**	**4**	**6**	**106.86**	**5**	**97.46 cm^3**	**1.59**	**-3.39**	**43**	**-1.19**

F. *In silico* ADMET prediction

In silico pharmacokinetic properties and toxicity were predicted for the two dopamine prodrugs, and the results are shown in Table 5. The results of the pharmacokinetic and toxicity predictions revealed that no prodrug had a high risk of toxicity, and all the prodrugs showed good pharmacokinetic properties.

Table 4: ADMET (absorption, distribution, metabolism, excretion and toxicity) prediction of dopamine **ProD 1**

Absorption	Blood-Brain Barrier	BBB-
	Human Intestinal Absorption	HIA+
	Caco-2 Permeability	Caco2-
	Oral bioavailability	Approximately 30 %
	Passive transport	Good(More than 70%) passive absorption across intestinal barrier
	Active transport	Not transported
	Plasma protein binding (%PPB)	87.47%
	Log BB	-0.47
Metabolism	CYP450 2C9 Substrate	Non-substrate
	CYP450 2D6 Substrate	Non-substrate
	CYP450 3A4 Substrate	Substrate
	CYP450 1A2 Inhibitor	Non-inhibitor
	CYP450 2C9 Inhibitor	Non-inhibitor
	CYP450 2D6 Inhibitor	Non-inhibitor
	CYP450 2C19 Inhibitor	Non-inhibitor
	CYP450 3A4 Inhibitor	Non-inhibitor
	CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity
Toxicity	Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor Non-inhibitor
	AMES Toxicity	Non AMES toxic
	Carcinogens	Non-carcinogens
	Fish Toxicity	High FHMT
	Tetrahymena Pyriformis Toxicity	High TPT
	Honey Bee Toxicity	Low HBT
	Biodegradation	Not ready biodegradable
	Acute Oral Toxicity	III
	Carcinogenicity (Three-class)	Non-required
	Genotoxicity Hazards	No hazards fragment have been found
	Acute toxicity (LD50, mg/kg)	Species/Administration route: LD50 (mg/kg) Mouse/Intraperitoneal: 120 Mouse/Oral: 1500 Mouse/Intravenous: 490 Mouse/Subcutaneous: 1000 Rat/Intraperitoneal: 280 Rat/Oral: 3800
	Endocrine disruption	No binding to estrogen receptor alpha
	MRDD (Max. recommended daily dose)	1.15 mg/kg/day
	Toxicity effect (Information on whether predicted rodent acute toxicity values provide any indication that the compound can be unsafe at higher dosage)	Gastrointestinal: Safe Lungs: Safe Cardiovascular: Safe Liver: Could be unsafe Blood: Safe Kidny: Safe Predicted oral LD50 >1000 mg/kg Predicted intravenous LD50 >200 mg/kg

Table 5:ADMET (absorption, distribution, metabolism, excretion and toxicity) prediction of dopamine **ProD 2**

Absorption	Blood-Brain Barrier	BBB+
	Human Intestinal Absorption	HIA+
	Caco-2 Permeability	Caco2-
	Oral bioavailability	Approximately 30 %
	Passive transport	Moderate (between 40% and 70%) passive absorption across intestinal barrier
	Active transport	Not transported
	Plasma protein binding (%PPB)	76.22%
	Log BB	-0.43
Metabolism	CYP450 2C9 Substrate	Non-substrate
	CYP450 2D6 Substrate	Non-substrate
	CYP450 3A4 Substrate	Non-substrate
	CYP450 1A2 Inhibitor	Non-inhibitor
	CYP450 2C9 Inhibitor	Non-inhibitor
	CYP450 2D6 Inhibitor	Non-inhibitor
	CYP450 2C19 Inhibitor	Non-inhibitor
	CYP450 3A4 Inhibitor	Non-inhibitor
	CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity
Toxicity	Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor
		Inhibitor
	AMES Toxicity	Non AMES toxic
	Carcinogens	Non-carcinogens
	Fish Toxicity	High FHMT
	Tetrahymena Pyriformis Toxicity	High TPT
	Honey Bee Toxicity	Low HBT
	Biodegradation	Ready biodegradable
	Acute Oral Toxicity	III
	Carcinogenicity (Three-class)	Non-required
	Genotoxicity Hazards	No hazards fragment have been found
	Acute toxicity (LD50, mg/kg)	Species/Administration route: LD50 (mg/kg) Mouse/Intraperitoneal: 110 Mouse/Oral: 1600 Mouse/Intravenous: 590 Mouse/Subcutaneous: 1100 Rat/Intraperitoneal: 290 Rat/Oral: 3000
	Endocrine disruption	No binding to estrogen receptor alpha
	MRDD (Max. recommended daily dose)	1.13 mg/kg/day
	Toxicity effect (Information on whether predicted rodent acute toxicity values provide any indication that the compound can be unsafe at higher dosage)	Gastrointestinal: Safe Lungs: Safe Cardiovascular: Safe Liver: Could be unsafe Blood: Safe Kidny: Safe Predicted oral LD50 >1000 mg/kg Predicted intravenous LD50 >200 mg/kg

Conclusions and Future directions

Chapter Five

Conclusions and Future directions

5.1. Conclusions

Parkinson's disease (PD) is a progressive, neurodegenerative disorder which involves the loss of dopaminergic neurons of the substantia nigra pars compacta. Current therapy is essentially symptomatic, and L-Dopa (LD), the direct precursor of dopamine(DA), is the treatment of choice in more advanced stages of the disease. Substitution therapy with LD is, however, associated with a number of acute problems. The peripheral conversion of LD by amino acid decarboxylase (AADC) to DA is responsible for the typical gastrointestinal (nausea, emesis) and cardiovascular (arrhythmia, hypotension) side effects. To minimize the conversion to DA outside the central nervous system (CNS), LD is usually given in combination with peripheral inhibitors of AADC (carbidopa or benserazide). In spite of that, other central nervous side effects such as dyskinesia, on-off phenomenon and end-of-dose deterioration still remain.

In order to improve the bioavailability, the prodrug approach appeared to be the most promising approach. Therefore, there was a need to synthesize new prodrugs for the treatment of Parkinson's disease having higher bioavailability than the current medications and have the potential to release DA in a sustained manner.

Based on the DFT calculations on proton transfer mechanism of Kemp‘s acid amides, two dopamine derivatives were designed, synthesized and characterized. These prodrugs have a carboxylic group as a hydrophilic moiety and the rest of the prodrug as a lipophilic moiety, where the combination of both moieties secures a moderate HLB. Furthermore, at physiological pH in the blood circulation the expected predominant form of dopamine is the ionized form while its prodrugs will exist in the ionic and free acid forms. Therefore, dopamine ProD 1 and dopamine ProD 2 may have a higher bioavailability than dopamine due to improved absorption. Also, the synthesized prodrugs can be used in many dosage forms (e.g. enteric coated tablets) because they are soluble in organic and aqueous media due to the ability of the carboxylic group to be converted to the corresponding carboxylate anion in physiological environments of pH 5.0-7.4 (intestine and blood circulation). The in vitro intra-conversion of these prodrugs to their parent drug, dopamine, revealed that the $t_{1/2}$ was largely affected by the pH of the medium. For dopamine **ProD 1** the experimental $t_{1/2}$ values in 0.1N HCl, buffer pH 2, buffer pH 5.5 and buffer pH 7.4 were 60.3hours, 54.66 hours, 99.93 hours and 138.13 hours, respectively. Dopamine **ProD 2** was readily converted in 0.1N HCl and pH 2, pH 5.5 and pH 7.4 with half -life time (t½) of 48 hours, 54.22 hours, 131.98 hours and 193.42 hours, respectively. In silico prediction of the

pharmacokinetic and toxicity revealed that no prodrug has a high risk of toxicity, and all the prodrugs showed good pharmacokinetic properties. Moreover, all prodrugs complied with Lipinski's rule of five.

5.2. Future directions

Our future directions are to evaluate if our newly synthesized prodrugs haveanti-Parkinson activity. In addition, *in vivo* pharmacokinetic studies will be launched in order to determine the bioavailability and the duration of action of the tested prodrugs.

References

[1] Goetz, C. G. (2011). The history of Parkinson's disease: early clinical descriptions and neurological therapies. *Cold Spring Harbor perspectives in medicine*, *1*(1), a008862.

[2] Parkinson, J. (2002). An essay on the shaking palsy. *The Journal of neuropsychiatry and clinical neurosciences*, *14*(2), 223-236.

[3] Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in neurosciences*, *12*(10), 366-375.

[4] Mandir, A. S., & Vaughan, C. (2000). Pathophysiology of Parkinson's disease.*International Review of Psychiatry*, *12*(4), 270-280.

[5] Samii, A., Nutt, J. G., Ransom, B. R. (2004). Parkinson's disease. Lancet, 363, 1783-1793.

[6] Chaudhuri, K. R., Healy, D. G., & Schapira, A. H. (2006). Non-motor symptoms of Parkinson's disease: diagnosis and management. *The Lancet Neurology*,*5*(3), 235-245.

[7] Konerth, M., & Childers, J. (2013). Exercise: a possible adjunct therapy to alleviate early Parkinson disease. *Journal of the American Academy of Physician Assistants*, *26*(4), 30-33.

[8] Olanow, C. W., & Koller, W. C. (1998). An algorithm (decision tree) for the management of Parkinson's disease Treatment guidelines. *Neurology*, *50*(3 Suppl 3), S1-S1.

[9] Obeso, J. A., Olanow, C. W., & Nutt, J. G. (2000). Levodopa motor complications in Parkinson's disease. *Trends in neurosciences*, *23*, S2-S7.

[10] Hermanns, M. (2011). Weathering the storm: living with Parkinson's disease.*Journal of Christian Nursing*, *28*(2), 76-82.

[11] Valente, A. X., das Neves, R. P., & Oliveira, P. J. (2012). Epigenetic engineering to reverse the Parkinson's expression state. *Parkinsonism & related disorders*, *18*(6), 717-721.

[12] Murphy, S. L., Xu, J., Kochanek, K. D., & Bastian, B. A. (2016). Deaths: Final Data for 2013. *National vital statistics reports: from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System*, *64*(2), 1-119.

[13] McInerney-Leo, A., Gwinn-Hardy, K., & Nussbaum, R. L. (2004). Prevalence of Parkinson's disease in populations of African ancestry: a review. *Journal of the National Medical Association*, *96*(7), 974.

[14] Van Den Eeden, S. K., Tanner, C. M., Bernstein, A. L., Fross, R. D., Leimpeter, A., Bloch, D. A., & Nelson, L. M. (2003). Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. *American journal of epidemiology*, *157*(11), 1015-1022.

[15] Muthane, U. B., Swamy, H. S., Satishchandra, P., Subhash, M. N., Rao, S., & Subbakrishna, D. (1994). Early onset Parkinson's disease: are juvenile-and young-onset different?. *Movement disorders*, *9*(5), 539-544.

[16] Grosset, D., Fernandez, H., Grosset, K., & Okun, M. (2009). *Parkinson's Disease: Clinican's Desk Reference*. CRC Press.

[17] Koller, W., Vetere-Overfield, B., Gray, C., Alexander, C., Chin, T., Dolezal, J., ... & Tanner, C. (1990). Environmental risk factors in Parkinson's disease.*Neurology*, *40*(8), 1218-1218.

[18] Hernán, M. A., Takkouche, B., Caamaño-Isorna, F., & Gestal-Otero, J. J. (2002). A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Annals of neurology*, *52*(3), 276-284.

[19] Jackson-Lewis, V., Jakowec, M., Burke, R. E., & Przedborski, S. (1995). Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine. *Neurodegeneration*,*4*(3), 257-269.

[20] Davie, C. A. (2008). A review of Parkinson's disease. *British medical bulletin*,*86*(1), 109-127.

[21] Lesage, S., & Brice, A. (2009). Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Human molecular genetics*, *18*(R1), R48-R59.

[22] Obeso, J. A., Rodríguez-Oroz, M. C., Benitez-Temino, B., Blesa, F. J., Guridi, J., Marin, C., & Rodriguez, M. (2008). Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease. *Movement Disorders*,*23*(S3), S548-S559.

[23] Schulz-Schaeffer, W. J. (2010). The synaptic pathology of α-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. *Acta neuropathologica*, *120*(2), 131-143.

[24] Obeso, J. A., Rodriguez-Oroz, M. C., Goetz, C. G., Marin, C., Kordower, J. H., Rodriguez, M., ... & Halliday, G. (2010). Missing pieces in the Parkinson's disease puzzle. *Nature medicine*, *16*(6), 653-661.

[25] Brocks, D. R. (1999). Anticholinergic drugs used in Parkinson's disease: an overlooked class of drugs from a pharmacokinetic perspective. *J Pharm Pharm Sci*, *2*(2), 39-46.

[26] Di Stefano, A., Sozio, P., Serafina Cerasa, L., & Iannitelli, A. (2011). L-Dopa prodrugs: an overview of trends for improving parkinson's disease treatment.*Current pharmaceutical design*, *17*(32), 3482-3493.

[27] Clarke, C. E., & Guttman, M. (2002). Dopamine agonist monotherapy in Parkinson's disease. *The Lancet*, *360*(9347), 1767-1769

[28] Montastruc, J. L., Rascol, O., & Senard, J. M. (1999). Treatment of Parkinson's disease should begin with a dopamine agonist. *Movement disorders*, *14*(5), 725-730.

[29] Ahlskog, J. E. (1994). Treatment of Parkinson's disease. From theory to practice. *Postgraduate medicine*, *95*(5), 52-4.

[30] Olanow, C. W. (1992). A rationale for dopamine agonists as primary therapy for Parkinson's disease. *Canadian Journal of Neurological Sciences/Journal Canadien des Sciences Neurologiques*, *19*(S1), 108-112.

[31] Barone, P. (2003). Clinical strategies to prevent and delay motor complications.*Neurology*, *61*(6 suppl 3), S12-S16.

[32] Jenner, P. (2002). Pharmacology of dopamine agonists in the treatment of Parkinson's disease. *Neurology*, *58*(suppl 1), S1-S8.

[33] Youdim, M. B., & Riederer, P. F. (2004). A review of the mechanisms and role of monoamine oxidase inhibitors in Parkinson's disease. *Neurology*, *63*(7 suppl 2), S32-S35

[34] Jenner, P. (2004). Preclinical evidence for neuroprotection with monoamine oxidase-B inhibitors in Parkinson's disease. *Neurology*, *63*(7 suppl 2), S13-S22.

[35] Schapira, A. H. (2011). Monoamine oxidase B inhibitors for the treatment of Parkinson's disease. *CNS drugs*, *25*(12), 1061-1071.

[36] Kaakkola, S. (2000). Clinical pharmacology, therapeutic use and potential of COMT inhibitors in Parkinson's disease. *Drugs*, *59*(6), 1233-1250.

[37] Crosby, N. J., Deane, K., & Clarke, C. E. (2003). Amantadine in Parkinson's disease. *The Cochrane Library*

[38] Bailey, E. V., & Stone, T. W. (1975). The mechanism of action of amantadine in Parkinsonism: a review. *Archives internationales de pharmacodynamie et de therapie*, *216*(2), 246-262.

[39] Metman, L. V., Del Dotto, P., Van Den Munckhof, P., Fang, J., Mouradian, M. M., & Chase, T. N. (1998). Amantadine as treatment for dyskinesias and motor fluctuations in Parkinson's disease. *Neurology*, *50*(5), 1323-1326.

[40] Iversen, L. L. (2010). *Dopamine handbook*. Oxford University Press, USA.

[41] Daubner, S. C., Le, T., & Wang, S. (2011). Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of biochemistry and biophysics*, *508*(1), 1-12.

[42] Shastry, B. S. (2001). Parkinson disease: etiology, pathogenesis and future of gene therapy. *Neuroscience research*, *41*(1), 5-12.

[43] Jana, S., Mandlekar, S., & Marathe, P. (2010). Prodrug design to improve pharmacokinetic and drug delivery properties: challenges to the discovery scientists. *Current medicinal chemistry*, *17*(32), 3874-3908.

[44] Gupta, S. K. (2004). *Drug screening methods*. Jaypee Brothers.

[45] Albert, A. (1958). Chemical aspects of selective toxicity. *Nature*, *182*(4633), 421.

[46] Higuchi, T., & Stella, V. J. (1975). *Pro-drugs as novel drug delivery systems*. The Society.

[47] Rautio, J., Kumpulainen, H., Heimbach, T., Oliyai, R., Oh, D., Järvinen, T., & Savolainen, J. (2008). Prodrugs: design and clinical applications. *Nature Reviews Drug Discovery*, *7*(3), 255-270.

[48] Stella, V. J. (2010). Prodrugs: Some thoughts and current issues. *Journal of pharmaceutical sciences*, *99*(12), 4755-4765.

[49] Rautio, J. (2010). Prodrug strategies in drug design. *Prodrugs and targeted delivery: towards better ADME properties*, 1-30.

[50] Ettmayer, P., Amidon, G. L., Clement, B., & Testa, B. (2004). Lessons learned from marketed and investigational prodrugs. *Journal of medicinal chemistry*,*47*(10), 2393-2404.

[51] Liederer, B. M., & Borchardt, R. T. (2006). Enzymes involved in the bioconversion of ester-based prodrugs. *Journal of pharmaceutical sciences*,*95*(6), 1177-1195.

[52] Karaman, R. (2013). Computationally designed enzyme models to replace natural enzymes in prodrug approaches. *Drug Designing: Open Access*, *2013*.

[53] Belpaire, F. M., & Bogaert, M. G. (1996). Cytochrome P450: genetic polymorphism and drug interactions. *Acta Clinica Belgica*, *51*(4), 254-260.

[54] Moser, V. C., Chanda, S. M., Mortensen, S. R., & Padilla, S. (1998). Age-and gender-related differences in sensitivity to chlorpyrifos in the rat reflect developmental profiles of esterase activities. *Toxicological sciences*, *46*(2), 211-222.

[55] Draganov, D. I., & La Du, B. N. (2004). Pharmacogenetics of paraoxonases: a brief review. *Naunyn-Schmiedeberg's archives of pharmacology*, *369*(1), 78-88.

[56] Ngawhirunpat, T., Kawakami, N., Hatanaka, T., Kawakami, J., & Adachi, I. (2003). Age dependency of esterase activity in rat and human keratinocytes.*Biological and Pharmaceutical Bulletin*, *26*(9), 1311-1314.

[57] Karaman, R., & Pascal, R. (2010). A computational analysis of intramolecularity in proton transfer reactions. *Organic and biomolecular chemistry*, *8*(22), 5174-5178.

[58] Karaman, R. (2010). The efficiency of proton transfer in Kirby's enzyme model, a computational approach. *Tetrahedron Letters*, *51*(16), 2130-2135.

[59] Karaman, R. (2011). Analyzing the efficiency of proton transfer to carbon in Kirby's enzyme model—a computational approach. *Tetrahedron Letters*, *52*(6), 699-704.

[60] Karaman, R. (2011). Analyzing the efficiency in intramolecular amide hydrolysis of Kirby's N-alkylmaleamic acids–A computational approach. *Computational and Theoretical Chemistry*, *974*(1), 133-142.

[61] Karaman, R. Prodrugs (2014)-Current and Future Drug Development Strategy. *Drug discovery*, *1*, 11.

[62] Hörter, D., & Dressman, J. B. (2001). Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Advanced drug delivery reviews*, *46*(1), 75-87.

[63] Stegemann, S., Leveiller, F., Franchi, D., De Jong, H., & Lindén, H. (2007). When poor solubility becomes an issue: from early stage to proof of concept.*European journal of pharmaceutical sciences*, *31*(5), 249-261.

[64] Stella, V. J., & Nti-Addae, K. W. (2007). Prodrug strategies to overcome poor water solubility. *Advanced drug delivery reviews*, *59*(7), 677-694.

[65] Fleisher, D., Bong, R., & Stewart, B. H. (1996). Improved oral drug delivery: solubility limitations overcome by the use of prodrugs. *Advanced drug delivery reviews*, *19*(2), 115-130.

[66] Müller, C. E. (2009). Prodrug approaches for enhancing the bioavailability of drugs with low solubility. *Chemistry & Biodiversity*, *6*(11), 2071-2083.

[67] Huttunen, K. M., Raunio, H., & Rautio, J. (2011). Prodrugs—from serendipity to rational design. *Pharmacological reviews*, *63*(3), 750-771.

[68] Chan, O. H., & Stewart, B. H. (1996). Physicochemical and drug-delivery considerations for oral drug bioavailability. *Drug Discovery Today*, *1*(11), 461-473.

[69] Kadam, V. B., Dhanawade, K. B., Salunkhe, V. A., & Ubale, A. T. (2014). Prodrugs: A New Approach to Drug Design & its Applications.

[70] Liederer, B. M., & Borchardt, R. T. (2006). Enzymes involved in the bioconversion of ester-based prodrugs. *Journal of pharmaceutical sciences*,*95*(6), 1177-1195.

[71] Taylor, M. D. (1996). Improved passive oral drug delivery via prodrugs.*Advanced drug delivery reviews*, *19*(2), 131-148.

[72] Di, L., & Kerns, E. H. (2015). *Drug-like properties: concepts, structure design and methods from ADME to toxicity optimization*. Academic Press.

[73] Kwan, K. C. (1997). Oral bioavailability and first-pass effects. *Drug metabolism and disposition*, *25*(12), 1329-1336.

[74] Singh, B., & Ahuja, N. (2002). Development of controlled-release buccoadhesive hydrophilic matrices of diltiazem hydrochloride: optimization of bioadhesion, dissolution, and diffusion parameters. *Drug development and industrial pharmacy*, *28*(4), 431-442.

[75] Chu, W. W. (1987). *PROD RUG STRATEGIES FOR BYPASSING THE FIRST· PASS METABOLISM OF PROPRANOLOL* (Doctoral dissertation, The University of Utah).

[76] Karaman, R. (2014). Prodrugs for Masking the Bitter Taste of Drugs.*Application of Nanotechnology in Drug Delivery*, 399-445.

[77] Pardridge, W. M. (2007). Blood–brain barrier delivery. *Drug discovery today*,*12*(1), 54-61.

[78] Brightman, M. W., & Reese, T. S. (1969). Junctions between intimately apposed cell membranes in the vertebrate brain. *The Journal of cell biology*,*40*(3), 648-677.

[79] Abbott, N. J., Patabendige, A. A., Dolman, D. E., Yusof, S. R., & Begley, D. J. (2010). Structure and function of the blood–brain barrier. *Neurobiology of disease*, *37*(1), 13-25.

[80] Rautio, J., Laine, K., Gynther, M., & Savolainen, J. (2008). Prodrug approaches for CNS delivery. *The AAPS journal*, *10*(1), 92-102.

[81] Oprea, T. I., & Gottfries, J. (1999). Toward minimalistic modeling of oral drug absorption. *Journal of Molecular Graphics and Modelling*, *17*(5), 261-274.

[82] Fischer, H., Gottschlich, R., & Seelig, A. (1998). Blood-brain barrier permeation: molecular parameters governing passive diffusion. *The Journal of membrane biology*, *165*(3), 201-211.

[83] Begley, D. J., & Brightman, M. W. (2003). Structural and functional aspects of the blood-brain barrier. In *Peptide transport and delivery into the central nervous system* (pp. 39-78). Birkhäuser Basel.

[84] Pardridge, W. M. (2001). *Brain drug targeting: the future of brain drug development*. Cambridge University Press.

[85] Abbott, N. J., Rönnbäck, L., & Hansson, E. (2006). Astrocyte–endothelial interactions at the blood–brain barrier. *Nature Reviews Neuroscience*, *7*(1), 41-53.

[86] Malakoutikhah, M., Teixidó, M., & Giralt, E. (2011). Shuttle-Mediated Drug Delivery to the Brain. *Angewandte Chemie International Edition*, *50*(35), 7998-8014.

[87] Denora, N., Trapani, A., Laquintana, V., Lopedota, A., & Trapani, G. (2009). Recent advances in medicinal chemistry and pharmaceutical technology-strategies for drug delivery to the brain. *Current topics in medicinal chemistry*,*9*(2), 182-196.

[88] Sozio, P., Cerasa, L. S., Abbadessa, A., & Di Stefano, A. (2012). Designing prodrugs for the treatment of Parkinson's disease. *Expert opinion on drug discovery*, *7*(5), 385-406.

[89] Oldendorf, W. H., Hyman, S., Braun, L., & Oldendorf, S. Z. (1972). Blood-brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. *Science*, *178*(4064), 984-986.

[90] Bodor, N., & Buchwald, P. (1999). Recent advances in the brain targeting of neuropharmaceuticals by chemical delivery systems. *Advanced drug delivery reviews*, *36*(2), 229-254.

[91] Bodor, N., & Buchwald, P. (2002). Barriers to remember: brain-targeting chemical delivery systems and Alzheimer's disease. *Drug discovery today*,*7*(14), 766-774.

[92] Anderson, B. D. (1996). Prodrugs for improved CNS delivery. *Advanced drug delivery reviews*, *19*(2), 171-202.

[93] Ohtsuki, S., & Terasaki, T. (2007). Contribution of carrier-mediated transport systems to the blood–brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development.*Pharmaceutical research*, *24*(9), 1745-1758.

[94] Tamai, I., & Tsuji, A. (2000). Transporter-mediated permeation of drugs across the blood–brain barrier. *Journal of pharmaceutical sciences*, *89*(11), 1371-1388.

[95] Lotharius, J., & Brundin, P. (2002). Pathogenesis of Parkinson's disease: dopamine, vesicles and α-synuclein. *Nature Reviews Neuroscience*, *3*(12), 932-942.

[96] Karaman, R. (2011). Computational-Aided Design for Dopamine Prodrugs Based on Novel Chemical Approach. *Chemical biology & drug design*, *78*(5), 853-863.

[97] Wade, L. A., & Katzman, R. (1975). Synthetic amino acids and the nature of L-DOPA transport at the blood-brain barrier. *Journal of neurochemistry*, *25*(6), 837-842.

[98] Aminoff, M. (2004). Pharmacologic Management of Parkinsonism & Other Movement Katzung Pharmacology Katzung, Basic and Clinical Pharmacology, 483-500.

[99] Howland, R. D., Mycek, M. J., Harvey, R. A., & Champe, P. C. (2006).*Lippincott's illustrated reviews: Pharmacology* (pp. 159-171). Philadelphia: Lippincott Williams & Wilkins.

[100] Parkinson Study Group. (2004). Levodopa and the progression of Parkinson's disease. *N Engl J Med*, *2004*(351), 2498-2508.

[101] Lang, A. E., & Lozano, A. M. (1998). Parkinson's disease. *New England Journal of Medicine*, *339*(15), 1044-1053.

[102] Standaert, D. G., & Young, A. B. (1996). Treatment of central nervous system degenerative disorders. *The pharmacological basis of therapeutics. IXth ed. New York: McGraw-Hill*, 503-519.

[103] LeWitt, P. A. (2008). Levodopa for the treatment of Parkinson's disease. *New England Journal of Medicine*, *359*(23), 2468-2476.

[104] Nutt, J. G., Woodward, W. R., Beckner, R. M., Stone, C. K., Berggren, K., Carter, J. H., ... & Gordin, A. (1994). Effect of peripheral catechol-O-methyltransferase inhibition on the pharmacokinetics and pharmacodynamics of levodopa in parkinsonian patients. *Neurology*, *44*(5), 913-913.

[105] Nutt, J. G. (1999). Effect of COMT inhibition on the pharmacokinetics and pharmacodynamics of levodopa in parkinsonian patients. *Neurology*, *55*(11 Suppl 4), S33-7.

[106] Kurlan, R., Rothfield, K. P., Woodward, W. R., Nutt, J. G., Miller, C., Lichter, D., & Shoulson, I. (1988). Erratic gastric emptying of levodopa may cause "random" fluctuations of parkinsonian mobility. *Neurology*, *38*(3), 419-419.

[107] Djaldetti, R., Baron, J., Ziv, I., & Melamed, E. (1996). Gastric emptying in Parkinson's disease Patients with and without response fluctuations.*Neurology*, *46*(4), 1051-1054.

[108] Palma, P. N., Bonifácio, M. J., Almeida, L., & Soares-da-Silva, P. (2007). 10 Restoring Dopamine Levels. *Protein Misfolding in Neurodegenerative Diseases: Mechanisms and Therapeutic Strategies*, 415.

[109] Gordin, A. (1997). COMT inhibitors in the treatment of Parkinson's disease.*Pharmacology and Toxicology-Supplements*, *81*(1), S13.

[110] Olanow, C. W. (2004). The scientific basis for the current treatment of Parkinson's disease. *Annu. Rev. Med.*, *55*, 41-60.

[111] Olanow, C. W., Obeso, J. A., & Stocchi, F. (2006). Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. *The Lancet Neurology*, *5*(8), 677-687.

[112] Di Stefano, A., Sozio, P., Serafina Cerasa, L., & Iannitelli, A. (2011). L-Dopa prodrugs: an overview of trends for improving parkinson's disease treatment.*Current pharmaceutical design*, *17*(32), 3482-3493.

[113] Di Stefano, A., Sozio, P., & Cerasa, L. S. (2008). Antiparkinson prodrugs.*Molecules*, *13*(1), 46-68.

[114] Chemuturi, N. V., & Donovan, M. D. (2007). Role of organic cation transporters in dopamine uptake across olfactory and nasal respiratory tissues. *Molecular pharmaceutics*, *4*(6), 936-942.

[115] Borgman, R. J., McPhillips, J. J., Stitzel, R. E., & Goodman, I. J. (1973). Synthesis and pharmacology of centrally acting dopamine derivatives and analogs in relation to Parkinson's disease. *Journal of medicinal chemistry*,*16*(6), 630-633.

[116] Bodor, N., Farag, H. H., & Brewster, M. E. (1981). Site-specific, sustained release of drugs to the brain. *Science*, *214*(4527), 1370-1372.

[117] Prokai, L., Prokai-Tatrai, K., & Bodor, N. (2000). Targeting drugs to the brain by redox chemical delivery systems. *Medicinal research reviews*, *20*(5), 367-416.

[118] Carelli, V., Liberatore, F., Scipione, L., Impicciatore, M., Barocelli, E., Cardellini, M., & Giorgioni, G. (1996). New systems for the specific delivery and sustained release of dopamine to the brain. *Journal of controlled release*,*42*(3), 209-216.

[119] Giannola, L., De Caro, V., Giandalia, G., Siragusa, M. G., & Lamartina, L. I. L. I. A. N. A. (2008). Synthesis and in vitro studies on a potential dopamine prodrug. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *63*(10), 704-710.

[120] More, S. S., & Vince, R. (2008). Design, synthesis and biological evaluation of glutathione peptidomimetics as components of anti-Parkinson prodrugs.*Journal of medicinal chemistry*, *51*(15), 4581-4588.

[121] Li, Y., Zhou, Y., Qi, B., Gong, T., Sun, X., Fu, Y., & Zhang, Z. (2014). Brain-specific delivery of dopamine mediated by N, N-dimethyl amino group for the treatment of Parkinson's disease. *Molecular pharmaceutics*, *11*(9), 3174-3185.

[122] Fernández, C., Nieto, O., Rivas, E., Montenegro, G., Fontenla, J. A., & Fernández-Mayoralas, A. (2000). Synthesis and biological studies of glycosyl dopamine derivatives as potential antiparkinsonian agents. *Carbohydrate research*, *327*(4), 353-365.

[123] Fernández, C., Nieto, O., Fontenla, J. A., Rivas, E., de Ceballos, M. L., & Fernández-Mayoralas, A. (2003). Synthesis of glycosyl derivatives as dopamine prodrugs: interaction with glucose carrier GLUT-1. *Organic & biomolecular chemistry*, *1*(5), 767-771.

[124] Bonina, F., Puglia, C., Rimoli, M. G., Melisi, D., Boatto, G., Nieddu, M., ... & Caprariis, P. D. (2003). Glycosyl derivatives of dopamine and L-dopa as anti-Parkinson prodrugs: synthesis, pharmacological activity and in vitro stability studies. *Journal of drug targeting*, *11*(1), 25-36.

[125] Ruocco, L. A., Viggiano, D., Viggiano, A., Abignente, E., Rimoli, M. G., Melisi, D., ... & Carnevale, U. G. (2008). Galactosylated dopamine enters into the brain, blocks the mesocorticolimbic system and modulates activity and scanning time in Naples high excitability rats. *Neuroscience*, *152*(1), 234-244.

[126] Karaman, R. (2015). From Conventional Prodrugs to Prodrugs Designed By Molecular Orbital Methods. *Frontiers in Computational Chemistry*, *2*, 187-249.

[127] Karaman, R. (2014). Prodrugs Design Based On Inter-And Intramolecular Processes. *Nova Science Publishers, Inc. NY, USA*, 1-76.

[128] Ala'Abu-Jaish, S. J., & Karaman, R. (2014) Prodrug Overview. *PRODRUGS DESIGN*,*1*, 77.

[129] Fattash, B., & Karaman, R. (2014). Chemical Approaches Used In Prodrugs Design. *Nova Science Publishers, Inc. NY, USA*, 103-138.

[130] Karaman, R. (2014). Using predrugs to optimize drug candidates. *Expert opinion on drug discovery*, *9*(12), 1405-1419.

[131] Karaman, R. (2008). Analysis of Menger's 'spatiotemporal hypothesis'.*Tetrahedron Letters*, *49*(41), 5998-6002.

[132] Karaman, R. (2009). Cleavage of Menger's aliphatic amide: a model for peptidase enzyme solely explained by proximity orientation in intramolecular proton transfer. *Journal of Molecular Structure: THEOCHEM*, *910*(1), 27-33.

[133] Karaman, R. (2010). A general equation correlating intramolecular rates with 'attack'parameters: distance and angle. *Tetrahedron Letters*, *51*(39), 5185-5190.

[134] Karaman, R. (2009). A new mathematical equation relating activation energy to bond angle and distance: a key for understanding the role of acceleration in lactonization of the trimethyl lock system. *Bioorganic chemistry*, *37*(1), 11-25.

[135] Karaman, R. (2009). Reevaluation of Bruice's proximity orientation. *Tetrahedron Letters*, *50*(4), 452-456.

[136] Karaman, R. (2009). The gem-disubstituent effect—a computational study that exposes the relevance of existing theoretical models. *Tetrahedron Letters*,*50*(44), 6083-6087.

[137] Karaman, R. (2009). Analyzing Kirby's amine olefin—a model for amino acid ammonia lyases. *Tetrahedron Letters*, *50*(52), 7304-7309.

[138] Karaman, R. (2009). The effective molarity (EM) puzzle in proton transfer reactions. *Bioorganic chemistry*, *37*(4), 106-110.

[139] Karaman, R. (2010). Effects of substitution on the effective molarity (EM) for five membered ring-closure reactions–A computational approach. *Journal of Molecular Structure: Theochem*, *939*(1), 69-74.

[140] Karaman, R. (2010). The effective molarity (EM) puzzle in intramolecular ring-closing reactions. *Journal of Molecular Structure: Theochem*, *940*(1), 70-75.

[141] Menger, F. M., & Karaman, R. (2010). A singularity model for chemical reactivity. *Chemistry–A European Journal*, *16*(5), 1420-1427.

[142] Karaman, R. (2010). The effective molarity (EM)–a computational approach.*Bioorganic chemistry*, *38*(4), 165-172.

[143] Karaman, R., Blasko, A., Almarsson, O., Arasasingham, R., & Bruice, T. C. (1992). Symmetrical and unsymmetrical quadruply aza-bridged, closely interspaced, cofacial bis (5, 10, 15, 20-tetraphenylporphyrin) s. 2. Synthesis, characterization, and conformational effects of solvents. *Journal of the American Chemical Society*, *114*(12), 4889-4898.

[144] Karaman, R. (2010). Proximity vs. strain in intramolecular ring-closing reactions. *Molecular Physics*, *108*(13), 1723-1730.

[145] Karaman, R. (2011). The role of proximity orientation in intramolecular proton transfer reactions. *Computational and Theoretical Chemistry*, *966*(1), 311-321.

[146] Karaman, R., & Bruice, T. C. (1991). Synthesis and characterization of the first water-soluble closely interspaced cofacial porphyrin dimer. *The Journal of Organic Chemistry*, *56*(11), 3470-3472.

[147] Karaman, R. (2011). Analyzing Kemp's amide cleavage: A model for amidase enzymes. *Computational and Theoretical Chemistry*, *963*(2), 427-434.

[148] Karaman, R., Ghareeb, H., Dajani, K. K., Scrano, L., Hallak, H., Abu-Lafi, S., ... & Bufo, S. A. (2013). Design, synthesis and in vitro kinetic study of tranexamic acid prodrugs for the treatment of bleeding conditions. *Journal of computer-aided molecular design*, *27*(7), 615-635.

[149] Karaman, R., Dajani, K. K., Qtait, A., & Khamis, M. (2012). Prodrugs of Acyclovir–A Computational Approach. *Chemical biology & drug design*, *79*(5), 819-834.

[150] Karaman, R., Dajani, K., & Hallak, H. (2012). Computer-assisted design for atenolol prodrugs for the use in aqueous formulations. *Journal of molecular modeling*, *18*(4), 1523-1540.

[151] Karaman, R. (2013). Prodrugs for masking bitter taste of antibacterial drugs—a computational approach. *Journal of molecular modeling*, *19*(6), 2399-2412.

[152] Karaman, R., Dokmak, G., Bader, M., Hallak, H., Khamis, M., Scrano, L., & Bufo, S. A. (2013). Prodrugs of fumarate esters for the treatment of psoriasis and multiple sclerosis—a computational approach. *Journal of molecular modeling*, *19*(1), 439-452.

[153] Karaman, R. (2010). Prodrugs of aza nucleosides based on proton transfer reaction. *Journal of computer-aided molecular design*, *24*(12), 961-970.

[154] Karaman, R., & Hallak, H. (2010). Computer-Assisted Design of Pro-drugs for Antimalarial Atovaquone. *Chemical biology & drug design*, *76*(4), 350-360.

[155] Karaman, R. (2013). Antimalarial Atovaquone Prodrugs Based on Enzyme Models-Molecular Orbital Calculations Approach. *Antimalarial Drug Research and Development, Banet, A C. & Brasier, P. Ed*, 1-67.

[156] Karaman, R., Fattash, B., Mecca, G., & Bader, M. (2014). Computationally designed atovaquone prodrugs based on Bruice's enzyme model. *Current computer-aided drug design*, *10*(1), 15-27.

[157] Karaman, R., Amly, W., Scrano, L., Mecca, G., & Bufo, S. A. (2013). Computationally designed prodrugs of statins based on Kirby's enzyme model. *Journal of molecular modeling*, *19*(9), 3969-3982.

[158] Karaman, R., Karaman, D., & Zeiadeh, I. (2013). Computationally-designed phenylephrine prodrugs–a model for enhancing bioavailability. *Molecular Physics*, *111*(21), 3249-3264.

[159] Almarsson, O., Karaman, R., & Bruice, T. C. (1992). Kinetic importance of conformations of nicotinamide adenine dinucleotide in the reactions of dehydrogenase enzymes. *Journal of the American Chemical Society*, *114*(22), 8702-8704.

[160] Jeon, S., Almarsson, O., Karaman, R., Blasko, A., & Bruice, T. C. (1993). Symmetrical and unsymmetrical quadruply aza-bridged closely interspaced cofacial bis (5, 10, 15, 20-tetraphenylporphyrins). 4. Structure and conformational effects on electrochemistry and the catalysis of electrochemical reduction of dioxygen by doubly, triply, and quadruply N, N-dimethylene sulfonamide bridged dimer bis (cobalt tetraphenylporphyrins). *Inorganic Chemistry*, *32*(11), 2562-2569.

[161] Horani, W., Thawabteh, A., Scrano, L., Bufo, S. A., Mecca, G., & Karaman, R. (2015). Anti-cancer Prodrugs-Three Decades of Design. *World Journal of Pharmacy & Pharmaceutical Sciences World Journal of Pharmacy & Pharmaceutical Sciences*, *4*(7), 1751-1779.

[162] Karaman, R. (2015). Design of prodrugs to replace commonly used drugs having bitter sensation. *World Journal of Pharmaceutical Research*, *4*(2), 49-58.

[163] Hejaz, H., Karaman, R., & Khamis, M. (2012). Computer-assisted design for paracetamol masking bitter taste prodrugs. *Journal of molecular modeling*,*18*(1), 103-114.

[164] Abu-Jaish, A., Mecca, G., Jumaa, S., Thawabteh, A., & Karaman, R. (2015). Mefenamic acid Prodrugs and Codrugs-Two Decades of Development. *World Journal of Pharmaceutical Research*, *4*(6), 2408-2429.

[165] Karaman, R. (2015). Computationally Designed Prodrugs Based on Enzyme Models‖ Aperito Journal of Drug Designing and Pharmacol 2015, 2: 111.

[166] Karaman, R., Jumaa, S., Awwadallah, H., Salah, S., Khawaja, Y., & Karaman, D. (2016). Intramolecular Processes and Their Applications in Prodrugs Approaches-Experimental and Computational Studies. *Current Organic Chemistry*, *20*(3), 289-315.

[167] Zhao, H. X., Mu, H., Bai, Y. H., Yu, H., & Hu, Y. M. (2011). A rapid method for the determination of dopamine in porcine muscle by pre-column derivatization and HPLC with fluorescence detection. *Journal of Pharmaceutical Analysis*, *1*(3), 208-212.

[168] Johnson, D. E., & Wolfgang, G. H. (2000). Predicting human safety: screening and computational approaches. *Drug Discovery Today*, *5*(10), 445-454

[169] ACD/lab percepta predictors, version 15.01, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2015.

[170] Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., ... & Tang, Y. (2012). admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *Journal of chemical information and modeling*, *52*(11), 3099-3105.

[171] Lipinski, C. A. (2004). Lead-and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337-341.

[172] Ghose, A. K., Viswanadhan, V. N., & Wendoloski, J. J. (1999). A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *Journal of combinatorial chemistry*, *1*(1), 55-68.

[173] Prakash, V., & Gupta, S. Determination of log P and log S value of CNS active drugs.

[174] Alelyunas, Y. W., Empfield, J. R., McCarthy, D., Spreen, R. C., Bui, K., Pelosi-Kilby, L., & Shen, C. (2010). Experimental solubility profiling of marketed CNS drugs, exploring solubility limit of CNS discovery candidate. *Bioorganic & medicinal chemistry letters*, *20*(24), 7312-7316.

[175] Hansch, C., & Leo, A. (1979). *Substituent constants for correlation analysis in chemistry and biology*. Wiley.

[176] Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of medicinal chemistry*, *45*(12), 2615-2623.

[177] Zheng, C., & Wang, Y. (2014). Prediction of Oral Bioavailability: Challenges and Strategies. *Journal of Bioequivalence & Bioavailability*, *2014*.

[178] Clark, D. E. (1999). Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 2. Prediction of blood–brain barrier penetration. *Journal of pharmaceutical sciences*, *88*(8), 815-821.

[179] Abraham, M. H., Takács-Novák, K., & Mitchell, R. C. (1997). On the partition of ampholytes: application to blood–brain distribution. *Journal of pharmaceutical sciences*, *86*(3), 310-

تصنيع ودراسة المواصفات والقوى المحركة المخبرية للأدوية المساعدة للدوبامين

إعداد: يحيى فؤاد رشيد خواجا

إشراف: بروفيسور رفيق قرمان

ملخص :

من المعروف أن مرضى الباركنسون (Parkinson) يعانون من نقص في مادة الدوبامين (dopamine) في مناطق معينه في الدماغ، لذلك كانت المحاولات لتعويض هذا النقص من الدوبامين. الدوبامين وحده لا يمر من الحاجز الدموي الدماغي لكن طليعه الليفودوبا (Levodopa) استطاع العبور إلى الجهاز العصبي المركزي (CNS) ليتم تحويله الى الدوبامين في الدماغ. عند اعطاء الليفودوبا (LD) عن طريق الفم كان التوافر الحيوي له أقل من 10% مع أقل من 1 % من الجرعة تخترق الدماغ. جرعات كبيرة من الليفودوبا مطلوبة، لأن الكثير منه يتم تحويله إلى الدوبامين خارج الدماغ مما يؤدي إلى الآثار الجانبية التي تشمل الغثيان، التقيؤ، عدم انتظام ضربات القلب وانخفاض ضغط الدم. للحد من التحويل إلى الدوبامين خارج الجهاز العصبي المركزي(CNS) عادة ما يعطى الليفودوبا مع مثبط الإنزيم المسئول عن نزع مجموعة الكربوكسيل (carboxyl group) من الدوبامين مثل (Carbidopa, benserazide) (كاربيدوبا و بنسيرازايد). على الرغم من ذلك، آثار عصبية مركزية جانبية أخرى كخلل في الحركة، وتدهور الحالة عند نهاية الجرعة لا تزال قائمة.

بناء على حسابات DFT تم تصنيع طلائع (Prodrugs) جديدة للدوبامين لعلاج مرضى الباركنسون حيث كانت هذه الطلائع لديها مجموعة الكربوكسيل المحبة للماء ومجموعة الهيدروكربون المحبة للدهون حيث أن الجمع بين كلتا المجموعتين يضمن توازن في قيمة HLB. من المتوقع أن يكون لهذه الطلائع (Prodrugs) توافر حيوي أعلى من الدواء الأم بسبب تحسن امتصاص طلائع الدوبامين المحتمل. علاوة على ذلك، يعتقد بان هذه الطلائع لها فعالية أكثر من الليفودوبا، لأن هذا الأخير يخضع لنزع الكربوكسيل في المحيط الخارجي قبل الوصول إلى حاجز الدماغ الدموي. بالإضافة إلى ذلك، طلائع الدوبامين المصنعة يمكن استخدامها في أشكال صيدلانية مختلفة

(بالوريد، تحت الجلد، أقراص،وغيرها) بسبب الذوبان المحتمل في الوسطين العضوي والمائي. بعد دراسة تحويل هذه الأدوية إلى الدواء الأصلي وجد أن $t_{1/2}$ تتأثر إلى حد كبير بدرجة حموضة الوسط. ل Dopamine **ProD 1** كانت النتائج التجريبية لل $t_{1/2}$ في حمض الهيدروكلوريد 0.1N HCl ، درجة حموضة 2.2 و درجة حموضة 5.5 ودرجة حموضه 7.4 كالتالي 60.3 ساعة، 54.66 ساعة، 99.93 ساعة و138.13 ساعات. في المقابل، كانت قيم $t_{1/2}$ التجريبية ل Dopamine **ProD 2** في حمض الهيدروكلوريد (0.1N HCl)، درجة حموضة 2.2 و درجة حموضة 5.5 ودرجة حموضة 7.4 كالتالي 48.34 ساعة، 54.22 ساعة، 131.98 ساعة و193.42 ساعة على التوالي. وأخيرا تم التنبؤ بالخصائص الفيزيوكيميائية ودرجة السمية لهذه الطلائع بالإضافة إلى التوافر الحيوي ومعرفة إذا كانت هذه الطلائع لها القدرة على المرور من الحاجز الدموي الدماغي.

Yahya's publications throughout my years of study and through the process of researching

1. **Khawaja Y**, Karaman R. A Novel Mathematical Equation For Calculating The Number of ATP Molecules Generated From Sugars In Cells. World Journal of Pharmaceutical Research., **2015**; 4(4): 303-312.

2. **Khawaja Y**, Karaman R. A New Equation For Calculating The Number of ATP Molecules Generated From Fatty Acids. World Journal of Pharmaceutical Research., **2015**; 6.

3. **Khawaja, Y**., & Karaman, R. Osteoporosis Drugs. *COMMONLY USED DRUGS-USES, SIDE EFFECTS, BIOAVAILABILITY AND APPROACHES TO IMPROVE IT*, 219.

4. Karaman R, Jumaa S, Awwadallah H, **Khawaja Y**. Intramolecular Processes and Their Applications for a Design and Synthesis of Prodrugs. Current Organic Chemistry, **2015**; 7.

5. Synthesis, Characterization, *In vitro* kinetic study and *In silico* ADMET prediction of Dopamine prodrugs (To be submitted).

6. Dopamine and Levodopa Prodrugs for the Treatment of Parkinson's Disease (To be submitted)