VIRTUAL SCREENING OF PROTEIN TYROSINE PHOSPHATASE 1B IN SEARCH FOR NATURAL PRODUCTS WITH POTENTIAL ACTIVITY AGAINTS DIABETES MELLITUS TYPE 2

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by

HO YUENG HSING

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This Master research project had taught me about the importance of time management, learn from experts, the skill to bridge theory on books with practical hands-on and most importantly stay positive even when something went wrong. It is a truly remarkable experiment that no one can teach you if you never experienced it firsthand. I enjoyed Master project and I believed that it will only bring sweet remembrance throughout my life. Herby, I claim all the work in this thesis are done by me and willing to take the responsibility for it.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree Celcius
µL	Micro liter
µM	Micro molar
3D	Three Dimensional
Å	Angstrom
Absorbance Unit	AU
ACC	Coenzyme A Carboxylase
ACO	Acyl-coenzyme A oxidase
Akt	Protein kinase
ASCVD	Atherosclerotic Cardiovascular Disease
CPT-1	Carnitine Palmitoyl Transferase 1
CVDs	Cardiovascular Diseases
DKA	Diabetic ketoacidosis
DMSO	Dimethyle Sulfoxide
DOF	Degree of Freedom
EP	Evolutionary Programming
FAA	Fatty Acid Synthase
FEB	Free Energy of Binding
g	Gram
GA	A hybrid genetic algorithm
GLUT4	Glucose transporter translocation
GOLD	Genetic Optimisation for Ligand Docking
GSK-3	Glycogen synthase kinase
HBA	Hydrogen Bond Acceptor
i.p.	Intraperitoneally
i.v.	Intravenously
IC_{50}	Half maximal inhibitory concentration
IFG	Impaired Fasting Glycaemia
IGT	Impaired Glucose Tolerance
IPHARM	Malaysian Institute of Pharmaceuticals and Nutraceuticals
IRS	Insulin receptor substrate
IUPAC	International Union of Pure and Applied Chemistry
kcal/mol	Kilo calories per mole
kg	Kilogram
LGA	Lammarckian Generic Algorithm
LS	Local search method
MMFF	Molecular Mechanics Force Field
mmol/L	Mili mole per Liter
NADI	Natural Products Discovery System
Nmol	Nano mole
NCDs	Non-communicable Diseases
NegIoniz	Negative Ionisable
ng/µL	Nano gram per micro liter
NHMS	National Health and Morbidity Survey
nmol	Nano mole
NMR	Nuclear Magnetic Resonance
OAI	6-(oxalyl-amino)-1h-indole-5-carboxylic acid
OBA	2-(oxalylamino)-bezoic acid
Ob-R1	Leptin receptor

outlev	Level of Output
parts per million	ppm
PDB	Protein Data Bank
PDK1	PI3K-dependend kinase 1
PI3K	Phosphatidylinositol 3-kinase
PO_4^{2-}	Phosphate ions
PTP1B	Protein Tyrosine Phosphatase 1B
RAM	Random Access Memory
RMS Distance	Root Mean Square Distance
RMSD	Root Mean Square Deviation
rmstol	Root Mean Square Tolerance
SA	Monte Carlo Simulating Annealing
SW or pSW	Pure local search
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
UKPDS	United Kingdom Prospective Diabetes study
WHO	World Health Organization

PENYARINGAN MAYA PROTEIN TIROSIN FOSFATASE 1B DALAM PENCARIAN HASILAN SEMULA JADI YANG MEMPUNYAI AKTIVITI BERPOTENSI TERHADAP DIABETES MELITUS JENIS 2

ABSTRAK

Populasi pesakit Diabetes Melitus Jenis 2 telah mencecah 422 juta orang daripada keseluruhan populasi dunia pada tahun 2014 dan dijangka akan mencapai kedudukan ketujuh sebagai penyebab utama kematian di tahun 2030 menurut Organisasi Kesihatan Dunia (WHO). Kajian telah menunjukkan 90-95% daripada penyakit diabetes terdiri daripada Diabetes Melitus Jenis 2 (T2DM). Berdasarkan peratusan tersebut, Diabetes Melitus telah merangkumi 6% daripada populasi orang dewasa di dalam kalangan masyarakat barat. Di Malaysia, penularan penyakit T2DM telah meningkat dua kali ganda dari 8.3% (1996) kepada 17.5% (2015) dalam tempoh sepuluh tahun. Kini, 1 daripada 5 rakyat Malaysia merupakan pengidap diabetik di mana 45% (1 juta orang) daripada populasi obesiti terdiri daripada kanak-kanak. Kajian ini melibatkan penyaringan maya ke atas sebatian dari pangkalan data Natural Product Discovert (NADI) terhadap protein tirosin fosfatase 1B (PTP1B) dengan menggunakan dua kaedah *in silico* untuk mengenalpasti sifat anti-diabetik daripada sebatian tersebut. PTP1B adalah penyelia negatif laluan isyarat insulin dan leptin. Bukti kajian menunjukkan bahawa perencat PTB1B mungkin sasaran dadah yang menyakinkan untuk pesakit T2DM dan obes-berisiko. Sebanyak 4000 sebatian telah melalui penyaringan maya dengan AutoDock 4.2 dan 1933 ligan telah diterjemahkan ke dalam model pharmacophore HYPO1menggunakan Discovery Studio 2.5. Sebanyak 18 tumbuhan telah dipilih selepas ikatan tenaga terendah (FEB), nilai kesesuaian (Fit Value), pembentukan ligan-protein, interaksi ligan-protein, populasi sebatian yang berkaitan, dan ketersediaan komersial ligan paling aktif daripada pemeriksaan kedua-dua penyaringan maya tersebut. 20 ekstrak daripada tumbuhan yang di pilih kemudiannya dikenakan ujian *in vitro* enzimatik. *Pandanus amaryllifolius* (daun) menunjukkan peratusan perencat yang tertinggi, iaitu sebanyak 94.38%, dan diikuti oleh *Vitex negundo* (daun) (89.03%), *Piper nigrum* (buah)

(81.39%), *Cymbopogon nardus* (daun) (79.78%), *Artocarpus champeden* (kulit) (66.90%), *Cymbopogon nardus* (daun) (66.02%) dan *Manilkara zapota* (buah) (62.06%). Kajian pada masa hadapan boleh ditumpukan untuk mengenalpasti unsur sebatian secara terperinci, "scaffold-hopping" terhadap ligan yang aktif, dan modifikasi kimia ke atas struktur sebatian perencat; untuk menemui perencat PTP1B yang lebih berkesan dan khusus untuk pesakit T2DM.

VIRTUAL SCREENING OF PROTEIN TYROSINE PHOSPHATASE 1B IN SEARCH FOR NATURAL PRODUCTS WITH POTENTIAL ACTIVITY AGAINST DIABETES MELLITUS TYPE 2

ABSTRACT

The world population of diabetes mellitus patients had reached 422 million in the year 2014 and projected to be the seventh leading cause of death in the year 2030 by World Health Organization. Type 2 Diabetes Mellitus (T2DM) was responsible for 90-95% of all diabetes and it had affected approximate 6% of the adult population in the western society. In Malaysia, the prevalence of T2DM had doubled from 8.3% (1996) to 17.5% (2015) in duration of ten years. Now, 1 out of 5 Malaysian are diabetic and with over 45% of overweight and obese population with 1 million obese children. This study involved virtual screening of the Natural Product Discovery (NADI) database compounds against protein tyrosine phosphatase 1B (PTP1B) using two *in silico* methods, molecular docking (structure-based virtual screening) and pharmacophore mapping (ligand-based virtual screening), in order to predict their anti-diabetic properties. PTP1B is a negative regulator of both insulin and leptin signalling pathways. Evidence had shown that PTP1B inhibitors might be a convincing drug target for T2DM patients and at-risk obese patients. 4000 compounds from NADI database were virtually screened using AutoDock 4.2 while 1933 ligands were mapped to pharmacophore model HYPO1 using Discovery Studio 2.5. 18 plants were selected after cross examine the lowest binding energy (FEB), fit value, ligand-protein conformation, ligand-protein interactions, the population of the related compound and commercial availability in the virtually active top hits from two virtual screening results. 20 extracts from 18 selected plants were then subjected to *in vitro* enzymatic assay. *Pandanus amaryllifolius* (leaves) showed highest inhibitory at 94.38% followed by *Vitex negundo* (leaves) (89.03%), *Piper nigrum* (fruits) (81.39%), *Cymbopogon nardus* (leaves) (79.78%), *Cymbopogon nardus* (peel) (66.90%), *Cymbopogon nardus* (leaves) (66.02%) and *Manilkara zapota* (fruits) (62.06%). Future studies focusing on compounds elucidation,

scaffold-hopping of virtually active ligands, and chemical modification of the structure of inhibiting compounds could be carried out in order to discover a more potent and specific PTP1B inhibitor for drug design in the prospect of T2DM.

CHAPTER ONE

INTRODUCTION

1.1 Statement of Problem

Diabetes mellitus is one of the most common chronic, non-communicable, diseases around the world (Whiting *et al.*, 2011). The ever-changing lifestyle with reduced physical activity and increased obesity along the economic development and urbanization had significantly increased the prevalence of diabetes mellitus patients globally. The number of people with diabetes had risen tremendously from 108 million in the year 1980 to 422 million in the year 2014 (WHO, 2016). It is the major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation, and The World Health Organization (WHO) is projecting diabetes to be the seventh leading cause of death in the year 2030 (WHO, 2016). The studies all over the world showed that the prevalence of Type 2 Diabetes Mellitus (T2DM) is escalating at phenomenal scale and most likely heading towards epidemical level (Zaini, 2000).

Diabetes prevalence has been rising more rapidly in middle and low-income countries (WHO, 2016) and the projected rise (from the year 1995 to the year 2025) in developed countries is 42% whereas developing countries will witness an escalation of 170% (King *et al.*, 1998). In Malaysia, the prevalence of diabetes mellitus (18 years old and above) had risen from 8.3% in the year 1996 to 17.5% in the year 2015 (Malaysia Institute For Public Health, 2015). It is estimated that 3.5 million Malaysian are diabetic or, in another word, 1 out of 5 Malaysian is living with this chronic disease. The situation in Malaysia became more agonised as the overweight (BMI between 25 kg m^{-2} and 30 kg m^{-2}) and obese (BMI above 30 kg m^{-2}) population had risen from 16.6% and 4.4%, respectively, in the year 1996 to 30% and 17.7%, respectively, in the year 2015. At the same time, Malaysia has 1 million obese children (below 18 years old) which represent 11.9% of the total underage population (Malaysia Institute For Public Health, 2015).

Although varies diabetes treatments are available in the market but most of these treatments are not only costly but also causing side effects, such as nausea, migraine and fatigue. Weight gain is one of the most common side effects from T2DM treatments due to the close relationship between insulin and lipase pathway. Obesity is the state of increased body weight (especially adipose tissues) of a certain level to cause advert health issue (Spiegelman & Flier, 2001). This is a vicious circle for T2DM patients as weight gain is a commonly recognised contributing factor for insulin resistance, impaired glucose tolerance (IGT) and T2DM. New therapies through different pathways and provoke less side effects are necessary.

In the recent decades, protein tyrosine phosphatase 1B (PTP1B) had emerged as a new potential target in the road of finding a cure for T2DM. T2DM is strongly associated with obesity and insulin resistance act as a common link between them. The inhibition of PTP1B might enhance the action of insulin and give advantage in T2DM treatment (Zhang & Lee, 2003). With all the evidence published throughout the decades, PTP1B seems to be a convincing candidate at all genetic, molecular, biochemical and physiological level for drug design in order to treat T2DM and at-risk obese patients (Johnson *et al.*, 2002).

1.2 Objectives and Scopes of Study

This study involved screening of the Malaysian Plants' Natural Products from Natural Products Discovery (NADI) database using two different *in silico* methods in order to predict their antidiabetic properties. First, virtual screening was conducted using both structure – based molecular docking and ligand-based pharmacophore mapping. The compounds in the database were subjected to PTP1B, an enzyme that played important role in negative regulation of insulin pathway, associating with metabolic syndrome and type 2 diabetes mellitus. A few host plants for the top ranked compounds from the virtual screening were then preceded to bio-assay guided extraction. These extracts were evaluated using enzymatic bio-assay.

The final validated antidiabetic properties were then cross referenced with literature and ethno-medical knowledge in the aim to find a competitive PTP1B inhibitor which acted as a starting point for optimisation in the drug design.

Specifically, the objectives of this study are:

1. To screen for Protein Tyrosine Phosphatase 1B (PTP1B) inhibitors using Natural Products Discovery (NADI) database implicated in diabetes mellitus type 2.
2. To validate *in silico* predictions using suitable *in vitro* assay.
3. To correlate these predictions and confirmation of the antidiabetic properties with ethno-medical uses.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes is a heterogeneous group of disorders occurred either when the pancreas do not produce enough insulin or the body cannot effectively use the insulin it produces (WHO, 2016). This disease is now a major public health problem listed on the four priority non-communicable diseases (NCDs) along side with cardiovascular diseases, cancer and chronic lung diseases. Historically, the word diabetes which means "to pass through" in Greek was first used by Apollonius of Memphis in 230 BC. The modern classification of diabetes mellitus and the tests used for its diagnosis were created by the National Diabetes Data Group of the USA and the second World Health Organization (WHO) Expert Committee on Diabetes Mellitus in the year 1979 and 1980 (Alberti & Zimmet, 1998).

Diabetes is categorised into four clinical classifications: Type 1 diabetes mellitus (T1DM) (previously known as insulin-dependent diabetes), Type 2 diabetes mellitus (T2DM) (previously known as non-insulin-dependent diabetes), gestational diabetes and diabetes due to other causes such as, genetic defects or medication by the American Diabetes Association (ADA) (Clark *et al.*, 2012). Other than the four clinical classifications, WHO had also recognised impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) as the intermediate conditions before diabetes. As the conditions are not inevitable, those with these transition conditions are taken in to account for undiagnosed or potential diabetes patient (especially T2DM). The diagnosis criteria for diabetes were illustrated in Table 2.1.

Table 2.1 Diagnosis criteria for diabetes using oral glucose tolerance test.

Fasting plasma glucose, mmol/L	< 6.1	Normal
	6.1 – 6.9	Impaired fasting glycaemia (IFG)
	≥ 7.0	Diabetes
2-hours plasma glucose, mmol/L	< 7.8	Normal
	7.8 – 11.0	Impaired glucose tolerance (IGT)
	≥ 11.0	Diabetes

T1DM is mainly caused by insulin deficiency in the body. Patients with T1DM needed daily insulin injection to help regulate the blood glucose level. Unfortunately, T1DM cannot be prevented with the current understanding and knowledge. The cause remained unknown. T1DM patients will not survive without regular insulin administration. On the other hand, T2DM are the result of insulin inefficacy, in term of both, action and excretion (Nolan, 2002). It is a chronic and progressive metabolic disorder characterised by hyperglycaemia.

The blood glucose level is regulated by insulin and glucagon hormones. Insulin is primarily excreted from β-cell of the Islet of Langerhans in pancreas while glucagon is expressed from the α-cell of the pancreas. Insulin inhibits glycogenolysis, gluconeogenesis, lipolysis, proteolysis and ketogenesis but at the same time promote glucose uptake in muscle and adipose tissues. On the other hand, glucagon acts as an antagonist of insulin in the liver increasing the blood glucose level (Clark *et al.*, 2012).

A diabetic individual may express symptoms such as excessive urination (polyuria) and great thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue. These symptoms are remarkably more severe in T1DM patients while sometimes recorded as absent by T2DM patients.

2.1.1 Type 2 Diabetes Mellitus in the world

As reported by WHO (WHO, 2016), there are estimated 422 million diabetic adults in the year 2014. More than 6% of the adult population from the Western society is afflicted by diabetes and T2DM responsible for 90-95% of the total prevalence (Moller, 2001). The global prevalence of diabetes nearly doubled from 4.7% of 1980 to 8.5% of 2014. International Diabetes Foundation (IDF) projected that the global diabetes prevalence will reach 552 million by 2030 (Whiting *et al.*, 2011) while WHO projected it as the seventh leading cause of death in the same year. The diabetes prevalence in middle and low-income

countries are rising more rapidly compared to high-income countries. IDF predicted the African region to have the largest proportional increase in diabetic adult number by 2030 followed by the Middle East and North Africa region. However, the Western Pacific region will continue to have the largest number of diabetic adults primarily due to the large diabetic population in China.

In 2012, an estimated of 1.5 million deaths worldwide are directly due to diabetes. It is the eighth leading cause of death among all gender and fifth leading cause of death among women (WHO, 2016). The numbers increases to 1.6 million in the year 2015 (WHO, 2017). Diabetes mellitus is also well known for its development of complications if the patient didn't manage the blood glucose level well. Diabetic ketoacidosis (DKA) (T1DM and T2DM) and hyperosmolar coma are common complications triggered by constant high blood glucose level. Prolonged diabetes will damage the heart, blood vessels, eyes (retinopathy), kidneys (one of the leading cause of kidney failure), nerves and increase the risk of stroke. Serious neuropathy in limbs might lead to amputation. Statistically, atherosclerotic cardiovascular disease (ASCVD) had been reported account for 80% of diabetic mortality and more than 75% of all hospitalisations for diabetic complications (Moller, 2001). The total deaths related to high blood glucose level is approximately 3.7 million in the year 2012 including 2.7 million deaths from diabetic related complications (Figure 2.1). Of these, Middle and low-income countries contributed the highest death proportion in all age group and significantly increased after age of 50 (Figure 2.1).

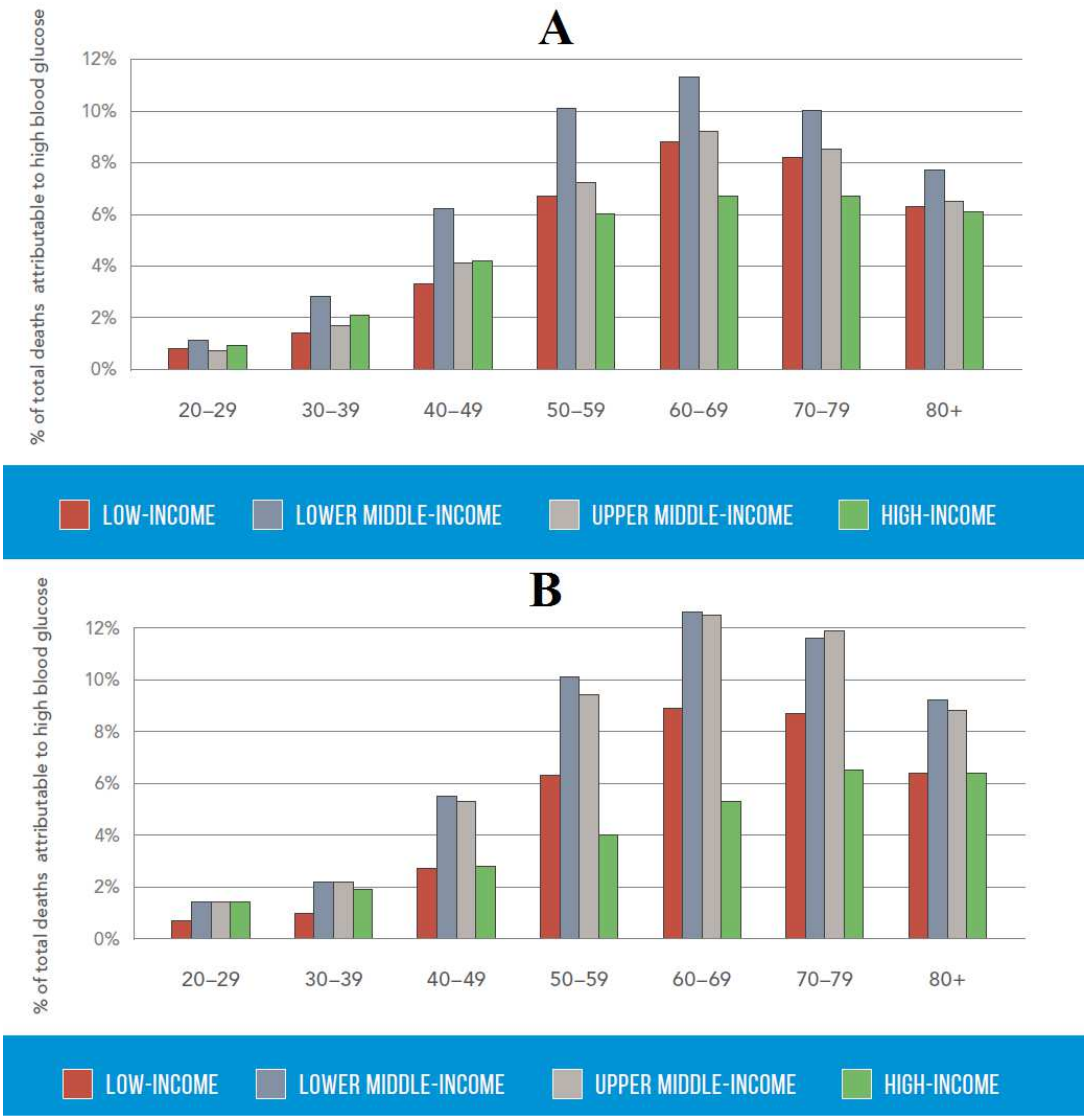

Figure 2.1 Percentage of all cause deaths attributed to high blood glucose, by age and country by income group in 2012 (categorised by the Wolrd Bank, 2012). Graph A for male and graph B for female, respectively (WHO, 2016).

2.1.2 Type 2 Diabetes Mellitus in Malaysia

The MalaysiaNational Health and Morbidity Survey (NHMS) is a nationwide survey initiated in the year 1986 which aims to monitor the health and nutritional level of Malaysian. The interval of the NHMS had shortened into every 4 years instead of 10 since the year 2011. In the year 2015, diabetes mellitus prevalence in Malaysia had reached 17.5% (approximately 3.5 million) of the population (Table 2.2) (Institute For Public Health, 2015; Letchuman *et al.*, 2010). The figure had doubled from 8.3% (1996) to 17.5% (2015) in the duration of ten years. The situation in Malaysia has became more agonised as the overweight and obese Malaysian had risen from 16.6% and 4.4%, respectively, in the year 1996 to 30% and 17.7%,respectively, in the year 2015 (Table 2.3) (Institute For Public Health, 2015; Letchuman *et al.*, 2010). At the same time, Malaysia has 1 million obese children (below 18 years old) which represent 11.9% of the total underage population (Institute For Public Health, 2015). It is generally agreed that ethnicity, family history of diabetes, overweight and obesity, physical inactivity and smoking are risk factors for T2DM (WHO, 2016). With over 45% of total obesity and pre-obese population and 22.8% of the smoking community, T2DM had already risen as one of the major public health problems in Malaysia. Fortunately, with the increase of awareness, risk factor physical inactivity had decreased from 88.4% (1996) to 33.5% (2015) (Table 2.3) (Institute For Public Health, 2015; Letchuman *et al.*, 2010).

Table 2.2 Prevalence of diabetes in Malaysia from Malaysia National Health Morbidity Surveys (Institute For Public Health, 2015; Letchuman *et al.*, 2010).

	NHMS II (1996)	**NHMS III (2006)**	**NHMS 2011**		**NHMS 2015**	
Age group	≥30 years	≥18 years	≥18 years	Est. Population	≥18 years	Est. Population
Prevalence	***8.3%***	***11.6%***	***15.2%***	***2,622,284***	***17.5%***	***3,500,000***
Known diabetes	6.5%	7.0%	7.2%	1,247,366	8.3%	N/A
Undiagnosed	1.8%	4.5%	8.0%	1,374,918	17.2%	N/A
Impaired Glucose Tolerance * / Impaired Fasting Glucose **	4.3% *	4.2% **	4.9% **	841,477	N/A	N/A

Table 2.3 Prevalence of nutritional status and dietary practise of Malaysian from Malaysia National Health Morbidity Surveys (Institute For Public Health, 2015; Letchuman *et al.*, 2010).

	NHMS II (1996)	**NHMS III (2006)**	**NHMS 2011**	**NHMS 2015**
Age group	≥18 years	≥18 years	≥18 years	≥18 years
Smoking	24.8%	21.5%	23.1%	22.8%
Fruits & Vegetable < 5 servings/day	N.A.	N.A.	92.5%	94.0%
Physically Inactive	88.4%	43.7%	35.2%	33.5%
Overweight (BMI ≥25 kg/m^2 & < 30 kg/m^2)	***16.6%***	***29.1%***	***29.4%***	***30.0%***
Obesity (BMI ≥ 30 kg/m^2)	***4.4%***	***14.0%***	***15.1%***	***17.7%***

2.1.3 Relationship between insulin signalling pathway and Type 2 Diabetes Mellitus

Pathophysiology of a disease determined its treatment. There are three events occurred in a coordinated manner after the ingestion of glucose (DeFronzo, 1999). First, the secretion of insulin. Secondly, the suppression of endogenous (primarily hepatic) glucose production by insulin. Then, insulin will trigger the glucose uptake by peripheral tissue, mainly muscles. Insulin is the most potent known anabolic hormone which is in charge of the synthesis promotion and storage of carbohydrates, lipids and proteins while inhibiting their degradation and release in circulation (Figure 2.2) (Saltiel & Kahn, 2001). Pancreatic cells, the primary insulin secretion cell, are capable of moderate insulin action and secretion accordingly. IGT or IFG will develop whenever pancreatic β-cell could not function properly for a prolonged duration.

Insulin resistance, a condition of insulin inefficacy, was first reported by Himsworth in 1936 based on his observation of decreased insulin action in obese patients (Himsworth, 2013). It is the hallmark of T2DM which indicates attenuated insulin effect to promote glucose transport and metabolism in peripheral tissues, such as liver, adipose and skeletal muscle (Zhang & Lee, 2003). Insulin action started when it binds to the insulin receptor (IR) and the conformation change will trigger autophosphorylation of several downstream protein substrates namely insulin receptor substrate (IRS) 1 to 4 and a few adapter proteins including Gab1 and Shc (Figure 2.3). The production of IRSs and adapter proteins triggered some important kinases in a very specific order. Phosphatidylinositol 3-kinase (PI3K) activated PI3K-dependent kinase 1 (PDK1) then protein kinase Akt. Akt is essential for glucose transporter translocation (GLUT4) in transporting glucose into the cell and glycogen synthase kinase (GSK-3) in glycogen synthesis (Saltiel & Kahn, 2001; Zhang & Lee, 2003).

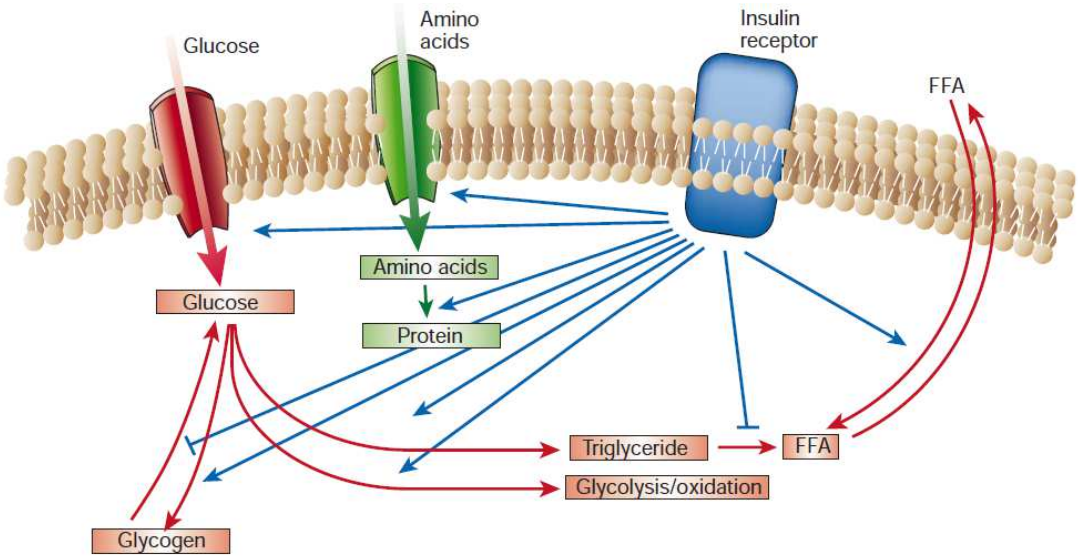

Figure 2.2 Insulin stimulates the glucose, amino acids and fatty acids uptake, stop their degradation and promote enzyme expression or activity that synthesis glycogen, lipid and protein (modified from Saltiel & Kahn, 2001).

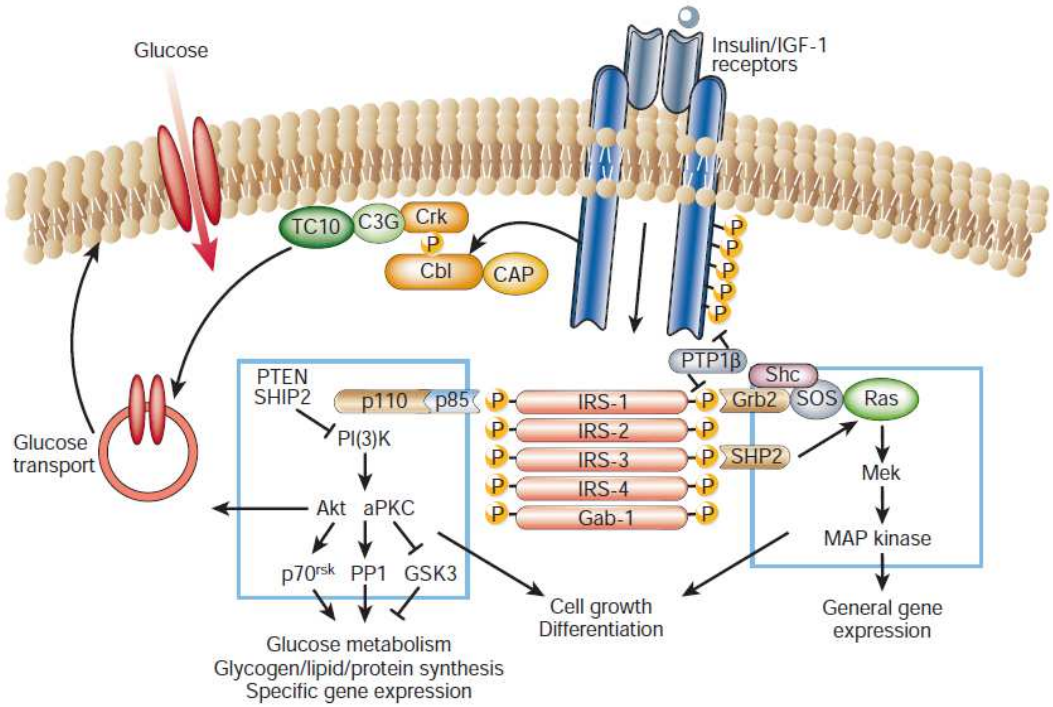

Figure 2.3 Signal transduction of in insulin action (modified from Saltiel & Kahn, 2001).

Insulin resistance in both T2DM and obesity involved defection in multiple levels including decreases in receptor concentration and kinase activity, the concentration and phosphorylation of IRS-1 to 4, PI3K activity, GLUT4 and the activity of intracellular enzymes (Pessin & Saltiel, 2000). On the other hand, the defects in genetic or acquired factors, even though rare, can cause the most severe form of insulin resistance (Taylor Simeon & Arioglu, 1998). Table 2.4 and Table 2.5 summarised some important research observations involved *in vivo* targeted deletion of insulin signalling (homologous recombination) components and role of specific tissues in diabetes respectively (Saltiel & Kahn, 2001). Insulin sensitivity in PTP1B and SHIP2 knockout mice increased and GLUT4 role in insulin resistance was once again being confirmed (Table 2.4 & Table 2.5).

Table 2.4 Mice phenotypes with single-gene knockouts in signalling pathways (Saltiel & Kahn, 2001).

Gene	Phenotype
Insulin receptor	Normal intrauterine growth; die of diabetec ketoacidosis at 3-7 days.
IGF1 receptor	Intrauterine and postnatal growth retardation; normal glucose homeostasis
IRS1	Insulin resistance/ impaired glucose tolerane; IGF resistance/growth retardation
IRS2	Insulin resistance/ decreased β-cell development; T2DM
IRS3	Normal growth/ normal glucose tolerance
IRS4	Normal growth/ normal glucose tolerance
Akt	Insulin resistance in liver and muscle
GLUT4	Cardiac hypertrophy/ failure; normal glucose tolerance
P85α	Increased insulin sensitivity; hypoglycaemia
PTP1B	Increased insulin sensitivity; resistance to diet-induced obesity
SHIP2	Increased insulin sensitivity

Table 2.5 Defect s in tissues-specific knockout mice (Saltiel & Kahn, 2001).

Gene	Tissue	Phenotype
Insulin receptor	Skeletal muscle	Normal glucose tolerance; increased fat mass; increased triglycerides and FFAs
	Liver	Impaired glucose tolerance; hyperinsulinaemia and reduced insulin clearance; decreased hepatic function
	β-cell	Loss of glucose-stimulated insulin secretion; progressively impaired glucose tolerance; decreased β-cell growth in adults
	Brain	Increased appetite; increased fat and leptin; insulin resistance; hypothalamic hypogonadism
GLUT4	Skeletal muscle	Reduced basal, insulin and contraction-stimulation glucose transport; severe insulin resistance; glucose intolerance
	Fat	Impaired glucose tolerance; hyperinsulinaemia; secondary insulin resistance in muscle and liver
Glukokinase	β-cell	Die within a few days of birth with severe diabetes
	Liver	Mild hyperglycaemia; pronounced defects in glycogen synthesis and glucose turnover; impaired glucose-stimulated inculin secretion

2.1.4 Therapeutic approaches in Type 2 Diabetes Mellitus

In the old days, T2DM was believed could not be cured and hence the patients were advised to manage their blood glucose level by oral administration drugs or insulin injection (pharmacological agents), diet and lifestyle adjustment in order to delay the development of severe insulin resistance or complications for prolonged high blood glucose level (Moller, 2001; Olokoba *et al.*, 2012). Current therapeutic approaches are mainly developed with the absence of defined molecular target but targeting classic action sites such as liver and pancreas (Table 2.6) (Moller, 2001) with a four fundamental pharmacological approaches including promoting insulin secretion, enhancing insulin efficiency (action), reducing excessive hepatic glucose production and inhibiting glucose absorption from the gut (Doherty, 2005). Weight gain is one of the common side effects from the current agents which lead to a vicious cycle for T2DM patients as weight gain is a commonly recognised contributing factor for insulin resistance, IGT, IFG and T2DM.

According to the United Kingdom Prospective Diabetes Study (UKPDS), T2DM can initially treat by mono-therapy but eventually required additional oral agent. In many cases, insulin therapy will be needed to achieve targeted blood glucose level (DeFronzo, 1999). Current treatments (Table 2.6) include injectable insulin and oral hypoglycemic agents such as metformin, sulfonylureas, the thiazolidinediones, and α-glucosidase inhibitors. However, no combination of these therapies is completely successful in ameliorating type II diabetes for many patients, and more efficacious agents are needed. A major goal of new therapies for T2DM is to potentiate the action of insulin (Szczepankiewicz *et al.*, 2003).

Three striking characteristics of actions of drugs indicate very strongly that they are concentrated by cells in small, specific areas known as the receptor. The three characters are as following (Albert, 2013; Cushny, 1903):

1. Drugs can retain its potency up to nanomolar level.
2. Drugs having very high specificity, for example, D- and L- isomers of a substance can have different pharmacological actions.
3. Drugs having very high biological specificity, for example, adrenaline works on cardiac muscle but its effect is very little on striatal muscle.

Other than the pharmacological agents listed in Table 2.6, some new drugs were introduced in the recent years including incretin-based therapies (exenatide-imitative and Liraglutide), dipeptidyl peptidase-IV inhibitors, new insulin analogues (lispro, aspart, analog and glargine), inhaling insulin drug and bromocriptine (Olokoba *et al.*, 2012). With the advance in biochemistry knowledge and increasing understanding in the diabetes pathophysiology, a few negative regulators of insulin signalling pathway, including PTP1B had been implicated as new novel drug targets (Table 2.7) (Moller, 2001).

Table 2.6 Current therapeutic agents for T2DM.

Drug class	Molecular target	Site of action	Adverse events
Insulin	Insulin receptor	Liver, muscle, fat	Hypoglycaemia; weight gain
Sulphonylureas (glibenclamide, nateglinide, repaglinide)	SU receptor/ K^+ ATP channel	Pancreas β-cell	Hypoglycaemia
Metformin-biguanides	Unknown	Liver, muscle	Gastrointestinal disturbances; lactic acidosis
Acarbose	α-glucosidase	Intestine	Gastrointestinal disturbances
Pioglitazone, rosiglitazone (thiazolidinediones)	PPARγ	Fat, muscle, liver	Weight gain; oedema; anaemia

Table 2.7 Potential drug targets in the insulin signalling pathway.

Target	Validation	Potential mechanism
Insulin receptor	Insulin, small molecule activators/ potentiators	Apparent direct activation of the receptor
PTP1B	Efficacy of vanadium compounds; PTP1B knockout mice (insulin sensitivity increased and resist to obesity); efficacy of PTP1B antisense oligonucleotide	Mediates dephosphorylation of insulin receptor
SHP2	SHP2 knockout mice (insulin sensitivity increased)	Dephosphorylation of phosphionositides
GSK3	Efficacy of GSK3 inhibitors in rodent models	Phosphorylation of glycogen synthase eading to inhibition of glycogen synthesis; potential negative regulator of other insulin signalling events
IkB kinase	Efficacy of high-dose salicylate (inhibits IkB kinase); IkB kinase knockout mice (insulin sensitivity increased)	Serine-threonine phosphorylation of insulin signalling intermediates
PKCθ	Activated in muscle in association with fatty acid induced insulin resistance	Negative regulation of insulin signalling; potential serine-threonine phosphorylation of IRS proteins

2.2 Protein tyrosine phosphatase 1B, PTP1B

Protein phosphorylation is reversible and controlled by the protein kinase (phosphorylate) and phosphatase (dephosphorylate), respectively, where these enzyme having opposing action mechanism. The phosphorylation of the tyrosine residues is essential to many signal transduction pathways triggered by hormones, mitogens and oncogenes that lead to processes such as cell growth, proliferation and differentiate (Fantl *et al.*, 1993; Johnson *et al.*, 2002). The role of protein tyrosine kinase (PTKs) are well documented since the 1980s and the role of protein tyrosine phosphatase (PTPs) was widely researched in 1990s (Charbonneau & Tonks, 1992; N. K. Tonks, 1993; Walton & Dixon, 1993).

PTPs family is large where there are more than 40 members recorded by 1994 (Barford *et al.*, 1994) and the number had doubled to more than 100 in 2017. All the members of PTPs family are highly conserved at a 11 residues motif (I/V)HCXAGXXR(S/T)G where cysteine and arginine residues (Figure 2.4) act as the catalytic site of the enzyme. It is worth to mention that PTPs sequence shared no similarity to serine or threonine, acid and alkaline phosphatase (Charbonneau & Tonks, 1992). The diversity of the structure within the PTPs family is mainly occurs at non-catalytic sequences attached to theNH_2- or COOH- termini of the catalytic domain.

PTP1B is the first PTP being isolated in homogeneous form and served as a model to illustrate several of the properties of PTPs. It was purified from human placenta as a 321 amino acids protein (Charbonneau *et al.*, 1989; N K Tonks *et al.*, 1988). The cDNA indicated PTP1B is a 435 amino acids protein but the catalytic conserved motif occurred at residues 30 to 278. The COOH- terminal 35 residues of PTP1B targetted to the cytoplasmic face of the endoplasmic reticulum whereas preceding 122 residues are predominantly hydrophilic and contain sites for serine phosphorylation. Overexpression of PTP1B retarded the action of oncogenic PTKs such as Src (Woodford-Thomas *et al.*, 1992) and Neu (Brown-Shimer *et al.*, 1992). It is also linked to insulin signalling pathway (Kenner *et al.*, 1993).

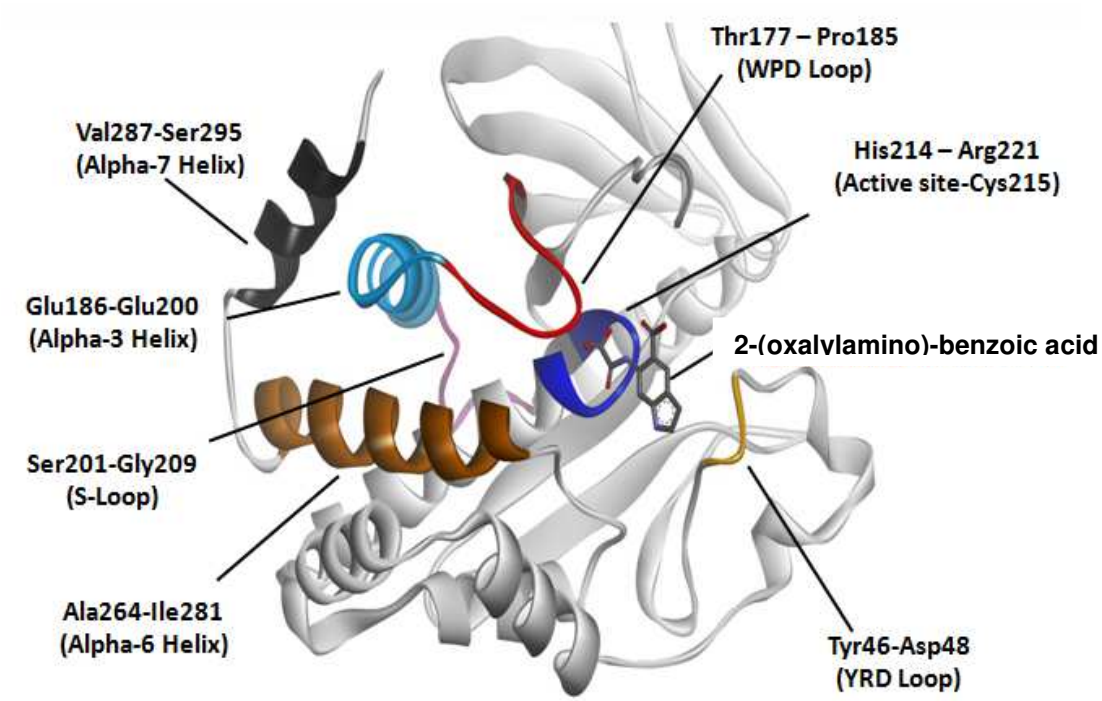

Figure 2.4 Important residues of PTP1B

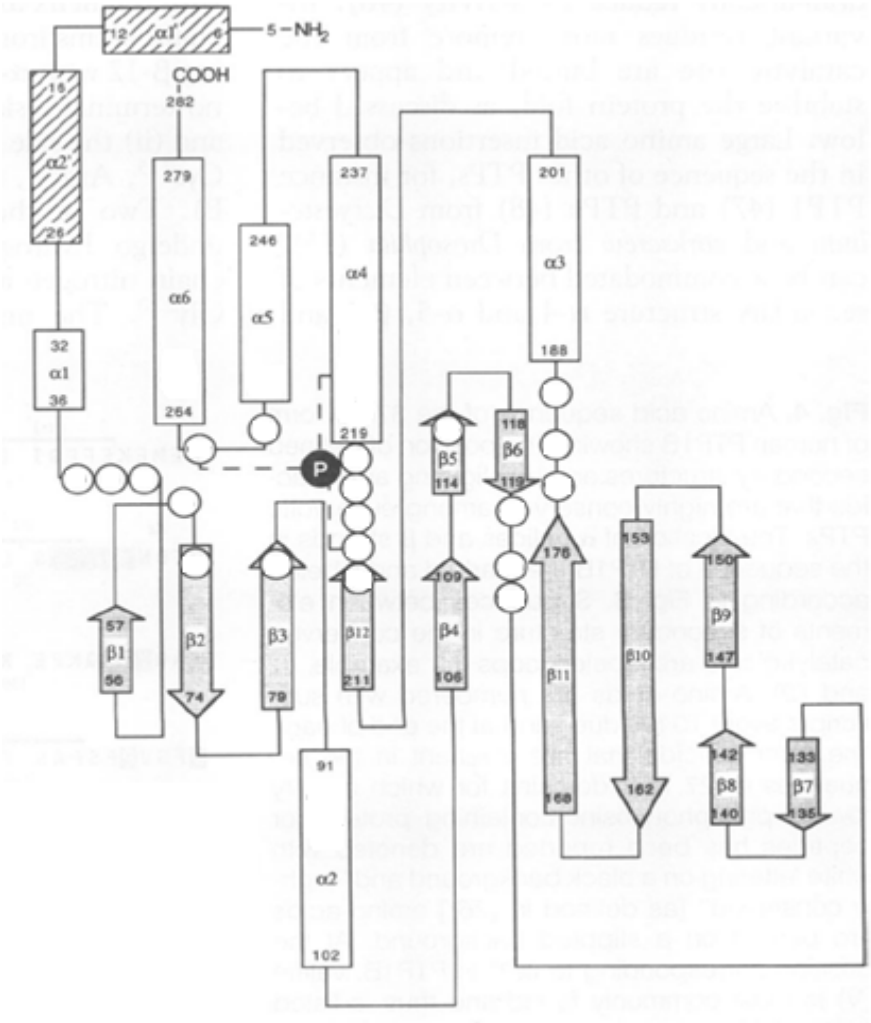

Figure 2.5 Topology diagram of PTP1B secondary structural elements.

2.2.1 PTP1B Protein Crystal Structure in Protein Data Bank

There are 124 PTP1B protein crystal structures of *Homo sapiens* and 2 structures from *Oryctolagus cuniculus* originated from more than 60 published journals (Table 2.8) are available in the Protein Data Bank (www.rcsb.org/pdb). The objectives of these published journals were varied from PTP1B inhibitor discoveries (Asante-Appiah *et al.*, 2006; Douty *et al.*, 2008; Groves *et al.*, 1998; Liu *et al.*, 2008; Zhao *et al.*, 2004), chemical modification of PTP1B inhibitors oral drug design (Han *et al.*, 2008; Iversen *et al.*, 2000; Iversen *et al.*, Scapin *et al.*, 2003; Wan *et al.*, 2007), insulin or insulin receptor related augmentation (Bleasdale *et al.*, 2001; Salmeen *et al.*, 2000), PTP1B protein structure understanding (Peters *et al.*, 2000; Scapin *et al.*, 2001; Wilson *et al.*, 2007) and many more. These vigorous researches around the world displayed the importance and potential of PTP1B as a drug target.

Table 2.8 PTP1B protein crystal structures in Protein Data Bank.

PDB ID. (Resolution)	PDB Title	Published Journals	References
2NT7(2.1Å) 2NTA(2.1Å)	Crystal structure of PTP1B-inhibitor complex	Probing acid replacements of thiophene PTP1B inhibitors	(Wan *et al.*, 2007)
1XBO(2.5Å)	PTP1B comf1plexed with Isoxazole Carboxylic Acid	Isoxazole carboxylic acids as protein tyrosine phosphatase 1B (PTP1B) inhibitors.	(Zhao *et al.*, 2004)
3CWE(1.6Å)	PTP1B in complex with a phosphonic acid inhibitor	Discovery of [(3-bromo-7-cyano-2-naphthyl)(difluoro)methyl]phosphonic acid, a potent and orally active small molecule PTP1B inhibitor	(Han *et al.*, 2008)
1BZC(2.35Å) 1BZH(2.1Å) 1BZJ(2.25Å)	Cyclic peptide inhibitor of human PTP1B	Structural basis for inhibition of the protein tyrosine phosphatase 1B by phosphotyrosine peptide mimetics	(Groves *et al.*, 1998)
1LQF(2.5Å)	Structure of PTP1b in Complex with a Peptidic Bisphosphonate Inhibitor	The structure of PTP-1B in complex with a peptide inhibitor reveals an alternative binding mode for bisphosphonates	(Asante-Appiah *et al.*, 2002)
1NWL(2.4Å) 1NWE(3,1Å)	Crystal structure of the PTP1B complexed with SP7343-SP7964, a ptyr mimetic	Discovery of a New Phosphotyrosine Mimetic for PTP1B Using Breakaway Tethering	(Erlanson *et al.*, 2003)
3EAX(1.9Å) 3EB1(2.4Å)	Crystal structure PTP1B complex with small molecule compound LZP-6	Targeting inactive enzyme conformation: aryl diketoacid derivatives as a new class of PTP1B inhibitors.	(Liu *et al.*, 2008)
1L8G(2.5Å)	Crystal structure of PTP1B complexed with 7-(1,1-Dioxo-1H-benzo[d]isothiazol-3-yloxymethyl)-2-(oxalyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid	Steric hindrance as a basis for structure-based design of selective inhibitors of protein-tyrosine phosphatases	(Iversen *et al.*, 2001)
2VEU(2.4Å) 2VEV(1.8Å) 2VEW(2.0Å) 2VEX(2.2Å) 2VEY(2.2Å)	Crystal Structure of Protein Tyrosine Phosphatase 1b In Complex with an Isothiazolidinone-Containing Inhibitor	Isothiazolidinone Inhibitors of Ptp1B Containing Imidazoles and Imidazolines	(Douty *et al.*, 2008)
1G7G(2.2Å) 1G7F(1.8Å)	Human ptp1b catalytic domain complexes with pnu179326	Small molecule peptidomimetics containing a novel phosphotyrosine bioisostere inhibit protein tyrosine phosphatase 1B and augment insulin action.	(Bleasdale *et al.*, 2001)
3EU0(2.7Å)	Crystal structure of the S-nitrosylated Cys215 of PTP1B	Cysteine S-Nitrosylation Protects Protein-tyrosine Phosphatase 1B against Oxidation-induced Permanent Inactivation	(Chen *et al.*, 2008)

Table 2.8 PTP1B protein crystal structures in Protein Data Bank. (Cont.)

PDB ID. (Resolution)	PDB Title	Published Journals	References
1Q6J(2.2Å) 1Q6M (2.2Å) 1Q6N(2.1Å) 1Q6P(2.3Å) 1Q6S(2.2Å) 1Q6T(2.3Å)	The structure of phosphotyrosine phosphatase 1b in complex with compound 2	The Structural Basis for the Selectivity of Benzotriazole Inhibitors of Ptp1B	(Scapin *et al.*, 2003)
1GFY(2.13Å)	Residue 259 is a key determinant of substrate specificity of protein-tyrosine phosphatase 1b and alpha	Residue 259 is a key determinant of substrate specificity of protein-tyrosine phosphatases 1B and alpha.	(Peters *et al.*, 2000)
1C83(1.8Å) 1C84(2.35Å) 1C85(2.72Å) 1ECV(1.95Å)	Crystal structure of protein tyrosine phosphatase 1b complexed with 6-(oxalyl-amino)-1h-indole-5-carboxylic acid	2-(oxalylamino)-benzoic acid is a general, competitive inhibitor of protein-tyrosine phosphatases	(Andersen *et al.*, 2000).
1C86(2.3Å) 1C87(2.1Å) 1C88(1.8Å)	Crystal structure of protein tyrosine phosphatase 1b (r47v,d48n) complexed with 2-(oxalyl-amino-4,7-dihydro-5h-thieno[2,3-c]pyran-3-carboxylic acid	Structure-based design of a low molecular weight, nonphosphorus, nonpeptide, and highly selective inhibitor of protein-tyrosine phosphatase 1B.	(Iversen *et al.*, 2000)
2QBP(2.5Å) 2QBQ(2.1Å) 2QBR(2.3Å) 2QBS(2.1Å)	Crystal structure of ptp1b-inhibitor complex	Structure-based optimization of protein tyrosine phosphatase 1B inhibitors: from the active site to the second phosphotyrosine binding site.	(Wilson *et al.*, 2007)
1EEN(1.9Å), 1EEO(1.8Å)	Crystal structure of protein tyrosine phosphatase 1b complexed with acetyl-d-a-d-bpa-ptyr-l-i-p-q-q-g	Structural basis of plasticity in protein tyrosine phosphatase 1B substrate recognition.	(M. Sarmiento *et al.*, 2000)
1PXH(2.15Å)	Crystal structure of protein tyrosine phosphatase 1B with potent and selective bidentate inhibitor compound 2	Crystal structure of PTP1B complexed with a potent and selective bidentate inhibitor.	(Sun *et al.*, 2003)
2H4G(2.5Å) 2H4K(2.3Å) 2HB1(2.0Å)	Crystal structure of PTP1B with monocyclic thiophene inhibitor	Monocyclic thiophenes as protein tyrosine phosphatase 1B inhibitors: Capturing interactions with Asp48.	(Wan *et al.*, 2006)
1OEO(2.15Å) 1OEM(1.8Å)	Ptp1b with the catalytic cysteine oxidized to sulfonic acid	Redox Regulation of Protein Tyrosine Phosphatase Involves a Sulfenyl-Amide Intermediate	(Salmeen *et al.*, 2003)

Table 2.8 PTP1B protein crystal structures in Protein Data Bank. (Cont.)

PDB ID. (Resolution)	PDB Title	Published Journals	References
2AZR(2.0Å) 2B07(2.95Å)	Crystal structure of PTP1B with Bicyclic Thiophene inhibitor	Bicyclic and tricyclic thiophenes as protein tyrosine phosphatase 1B inhibitors.	(Moretto *et al.*, 2006)
3D9C(2.3Å)	Crystal Structure PTP1B complex with aryl Seleninic acid	Seleninate in place of phosphate: irreversible inhibition of protein tyrosine phosphatases.	(Abdo *et al.*, 2008)
2F6F(2.0Å)	The structure of the S295F mutant of human PTP1B	Residues distant from the active site influence protein-tyrosine phosphatase 1B inhibitor binding.	(Montalibet *et al.*, 2006)
1PTY(1.85Å) 1AAX(1.9Å)	Crystal structure of protein tyrosine phosphatase 1b complexed with two phosphotyrosine molecules	Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: a paradigm for inhibitor design.	(Puius *et al.*, 1997)
2F6T(1.7Å) 2F6V(1.7Å) 2F6W(2.2Å) 2F6Y(2.15Å) 2F6Z(1.7Å) 2F70(2.12Å) 2F71(1.55Å)	Protein tyrosine phosphatase 1B with sulfamic acid inhibitors	1,2,3,4-Tetrahydroisoquinolinyl sulfamic acids as phosphatase PTP1B inhibitors	(Klopfenstein *et al.*, 2006)
1G1G(2.2Å) 1G1H(2.4Å) 1G1F(2.0Å)	Crystal structure of protein tyrosine phosphatase 1b complexed with a mono-phosphorylated peptide (etdy(ptr)rkggkgll) from the insulin receptor kinase	Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B.	(Salmeen *et al.*, 2000)
1Q1M(2.6Å)	A Highly Efficient Approach to a Selective and Cell Active PTP1B inhibitors	Fragment screening and assembly: a highly efficient approach to a selective and cell active protein tyrosine phosphatase 1B inhibitor.	(Liu, Xin, Pei, *et al.*, 2003)
1JF7(2.2Å)	Human ptp1b catalytic domain complexed with pnu177836	Synthesis and biological activity of a novel class of small molecular weight peptidomimetic competitive inhibitors of protein tyrosine phosphatase 1B.	(Larsen *et al.*, 2002)
1ONY(2.15Å) 1ONZ(2.4Å)	Oxalyl-Aryl-Amino Benzoic Acid inhibitors of PTP1B, compound 17	Discovery and Structure-Activity Relationship of Oxalylarylaminobenzoic Acids as Inhibitors of Protein Tyrosine Phosphatase 1B	(Liu, Szczepankiewicz, *et al.*, 2003)

Table 2.8 PTP1B protein crystal structures in Protein Data Bank. (Cont.)

PDB ID. (Resolution)	PDB Title	Published Journals	References
2B4S(2.3Å)	Crystal structure of a complex between PTP1B and the insulin receptor tyrosine kinase	Crystal Structure of a Complex between Protein Tyrosine Phosphatase 1B and the Insulin Receptor Tyrosine Kinase.	(Li *et al.*, 2005)
1I57(2.1Å)	Crystal structure of apo human ptp1b (c215s) mutant	The structure of apo protein-tyrosine phosphatase 1B C215S mutant: more than just an S --> O change.	(Scapin *et al.*, 2001)
1PH0(2.2Å)	Non-carboxylic Acid-Containing Inhibitor of PTP1B Targeting the Second Phosphotyrosine Site	Selective Protein Tyrosine Phosphatase 1B Inhibitors: Targeting the Second Phosphotyrosine Binding Site with Non-Carboxylic Acid-Containing Ligands.	(Liu, Xin, Liang, *et al.*, 2003)
1WAX(2.2Å)	Protein tyrosine phosphatase 1b with active site inhibitor	Fragment-based lead discovery using x-ray crystallography	(Hartshorn *et al.*, 2005)
2FJN(2.2Å) 2FJM(2.1Å)	The structure of phosphotyrosine phosphatase 1B in complex with compound 2	Conformation-assisted inhibition of protein-tyrosine phosphatase-1B elicits inhibitor selectivity over T-cell protein-tyrosine phosphatase.	(Asante-Appiah *et al.*, 2006)
2SHP(2.0Å)	Tyrosine phosphatase shp-2	Crystal structure of the tyrosine phosphatase SHP-2.	(Hof *et al.*, 1998)
1A5Y(2.5Å)	Protein tyrosine phosphatase 1b cysteinyl-phosphate intermediate	Visualization of the cysteinyl-phosphate intermediate of a protein-tyrosine phosphatase by x-ray crystallography.	(Pannifer *et al.*, 1998)
2PA5(1.6Å)	Crystal structure of human protein tyrosine phosphatase PTPN9	Large-scale structural analysis of the classical human protein tyrosine phosphatome.	(Barr *et al.*, 2009)
1NZ7(2.4Å)	Potent, selective inhibitors of PTP1B using a second phosphotyrosine binding site, complexed with compound 19	Potent, selective inhibitors of protein tyrosine phosphatase 1B.	(Xin *et al.*, 2003)
1NL9(2.4Å) 1NNY(2.4Å) 1NO6(2.4Å)	Potent, Selective Protein Tyrosine Phosphatase 1B Inhibitor Compound 12 Using a Linked-Fragment Strategy	Discovery of a potent, selective protein tyrosine phosphatase 1B inhibitor using a linked-fragment strategy.	(Szczepankiewicz *et al.*, 2003)

2.2.2 PTP1B in diabetes and obesity

Sedentary lifestyle and unbalanced diet with high fat intake have created a large overweight and obese population in the developing and developed countries. More than 30% of the adult population in the United State of America are considered obese. Obesity is defined as a medical state where the body weight (especially adipose tissue) reached a certain level where caused advert health problem to the person. The most popular among all measures is the waist/hip circumference ratio (WHR) and Body Mass Index (BMI) (Kopelman *et al.*, 2009). In the case of BMI, according to WHO, any reading between BMI 25 to 30 is considered as overweight while readings above BMI of 30 will be categorised as obese. Since the ground breaking work of Vague in the year 1947 (Vague, 1956, 1996), it is slowly but widely accepted that different body morphology or types of fat distribution are independently related to the health risk associated with obesity. Vague was also the first to observe a constellation of the risk factor for T2DM, dyslipidemia and atherosclerosis in obese patient (Farooq *et al.*, 2015).

T2DM is strongly associated with obesity and insulin resistance acts as a common link between them. Another common factor between T2DM and obesity is the presence of PTP1B enzyme within the insulin and leptin signalling pathways which regulated blood glucose level and fatty acid homeostasis respectively. PTP1B acts as a negative regulator in both insulin (Figure 2.6) and leptin (Figure 2.7) signalling pathways. The inhibition of PTP1B might enhance the action of insulin and in turn advantageous to T2DM treatment (Zhang & Lee, 2003) as it avoids one of the most common side effect in T2DM treatment, that is the weight gain.