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PTP1B INHIBITION AS A NOVEL THERAPEUTIC APPROACH IN THE MANAGEMENT OF TYPE-2 DIABETES: A SHORT REVIEW

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ABSTRACT

Diabetes mellitus (Type-1 DM and Type-2 DM) is the 8th leading cause of death worldwide. India is the worst affected country having more than 62 million population affected. Diabetes mellitus is considered as group of metabolic disorders characterized by high blood glucose level resulting in classic symptoms like polyurea, polydypsia, polyphagia, weight loss and several other life threatening complications. Both the type 1 and type 2 exhibits impaired insulin functions. Moreover, Type-2 DM (non insulin dependent DM) progresses to other complications if left untreated, indicates dysfunctional utilization of insulin by cells. Various researchers have suggested role of PTB1B in insulin desensitization since PTP1B is a negative regulator of the insulin signalling pathway and is considered as a promising potential therapeutic target, in particular for treatment of T2DM. PTP1B has been identified to possess a dual function in IR signalling i.e. to maintain the IR in a dephosphorylated state during biosynthesis to allow for proper processing and secondly, to dephosphorylate the insulin-activated receptor to attenuate insulin signaling and allow for recycling or degradation. Inhibitors are designed on the basis of enzyme-peptide complex or on the basis of transition state of enzyme-peptide complex.

This review encompasses above approaches employed for designing PTB1B inhibitors for the management of type 2 diabetes and additional benefits like obesity control and cancer.

Keywords: PTB1B, type 2 diabetes mellitus (T2DM), insulin signalling, PTB1B inhibitors, Drug design strategies

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long term complications include heart disease, kidney failure, and damage to the eyes. Diabetes is due to either the pancreas not producing enough insulin, or because cells of the body do not respond properly to the insulin that is produced. There are three types of Diabetes mellitus.

Type 1 Diabetes mellitus (T1DM): It results from the body's failure to produce insulin. This form was previously referred to as "Insulin Dependent Diabetes mellitus" (IDDM) or "juvenile diabetes".

Type 2 Diabetes mellitus (T2DM): It results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes also with an absolute insulin deficiency. This form was previously referred to as “Non insulin Dependent Diabetes mellitus”(NIDDM) or "adult-onset diabetes".

Gestational diabetes: It is the third main form and occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level.[1]

Complications of Type 2 Diabetes mellitus: It is a most common chronic disease with a ten year shorter life expectancy. Complications are risk of cardiovascular disease, ischemic heart disease and stroke, increase in lower limb amputations, and increased rates of hospitalizations. T2DM is the largest cause of nontraumatic blindness and kidney failure. It has also been associated with an increased risk of cognitive dysfunction, Alzheimer's disease and vascular dementia. Other complications are acanthosis nigricans, sexual dysfunction and frequent infections.[2]

EPIDEMIOLOGY OF DIABETES:

Global Epidemiology: Globally on 2010, an estimated 227 to 285 million people had diabetes, with T2DM making up about 90% of the cases. In 2011 it resulted in 1.4 million deaths worldwide making it the 8th leading cause of death. This is an increase from 1 million deaths in 2000. Its rate has increased, and by 2030, this number is estimated to almost double. The greatest increase in rates is expected to occur in Asia and Africa, where most people with diabetes will probably be found by 2030 (Figure 1).

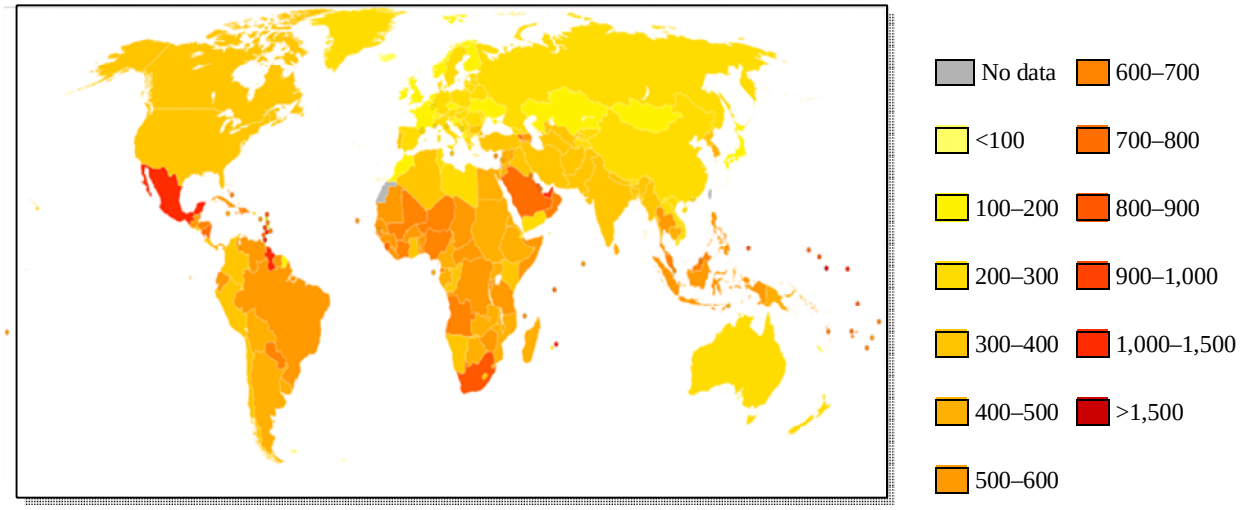


Fig.1. Geographical Distribution of Diabetes mellitus per 100000 inhabitants[3]

India Epidemiology

India has more diabetics than any other country in the world, according to the International Diabetes Foundation. The disease affects more than 62 million Indians, which is more than 7.1% of India's Adult Population. An estimate shows that nearly 1 million Indians die due to Diabetes every year. The average age on onset is 42.5 years, a study by the American Diabetes Association reports that India will see the greatest increase in DM by 2030.[4]

SYMPTOMS OF DIABETES MELLITUS: The classic symptoms of diabetes are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger) and weight loss. Other symptoms that are commonly present at diagnosis include a history of blurred vision, itchness, and peripheral neuropathy, recurrent vaginal infections and fatigue. Many people have no symptoms during the first few years and are diagnosed on routine testing. People with type 2 diabetes mellitus may rarely present with hyperosmolar hyperglycemic state (a condition of very high blood sugar associated with a decreased level of consciousness and low blood pressure).[4]

Table.1. Comparison between Type 1 and Type 2 Diabetes me llitus[4]

Type 1 DM	Type 2 DM
Symptoms start in childhood or young adulthood. People often seek medical help, because they are seriously ill from sudden symptoms of high blood sugar.	The person may not have symptoms before diagnosis. The disease is discovered in adulthood, but an increasing number of children are being diagnosed with the disease.
Episodes of low blood sugar level (hypoglycemia) are common.	There are no episodes of low blood sugar level, unless the person is taking insulin or certain diabetes medicines.
It cannot be prevented.	It can be prevented or delayed with a healthy lifestyle, including maintaining a healthy weight, eating sensibly, and exercising regularly.

INTRODUCTION TO PTP1B

PTP1B stands for Protein Tyrosine phosphatase 1B (Figure 2). It is also known as Tyrosine-protein phosphatase non-receptor type 1(PTPN1). It is an enzyme that is the founding member of the protein tyrosine phosphatase (PTP) family. In humans, it is encoded by the PTPN1 gene.

PTP1B is a negative regulator of the insulin signalling pathway and is considered as a promising potential therapeutic target, in particular for treatment of T2DM. It has also been implicated in the development of breast cancer and explored as a potential therapeutic target in that avenue as well. In addition to insulin sensitization, inhibition of PTP1B also has the potential to cause weight loss, which is a benefit because obesity is an important component of the type 2 diabetic pathology.[5] Protein tyrosine phosphatase 1B (PTP1B) is a prototype non receptor cytoplasmic PTPase enzyme that has been implicated in regulation of insulin signaling pathways. Studies on PTP1B knockout mice and PTP1B antisense treated mice suggested that inhibition of PTP1B would be an effective strategy for the treatment of type II diabetes and obesity.[6]

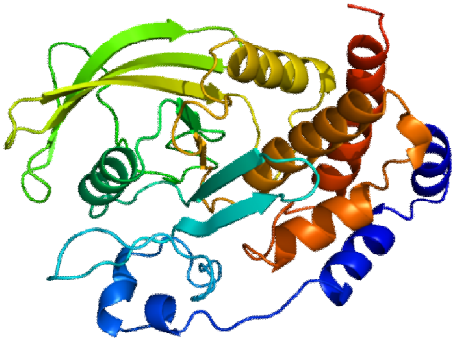


Fig.2. Crystal structure of PTP1B

The increased stress on the insulin secreting pancreatic β-cell from glucotoxicity, lipotoxicity, inflammatory cytokines and genetic sensitivities leads to their failure. The insulin resistance observed in T2DM patients results from the inability of the cells to propagate the insulin signal. The aberrant insulin signalling observed in T2DM patients is due to events downstream from the receptor. Inhibition of PTP1B alleviates the resistant state by removing negative pressure on the pathway. PTP1B is located in the 13q region of chromosome 20 that has been associated with diabetes and obesity. PTP1B coding region reveals a total of 35 single nucleotide polymorphisms (SNPs). A block of eight noncoding SNPs (rs941798, rs3787345, rs754118, rs2282147, rs718049, rs718050, rs3787348 and 1484insG in PTP1B are significant contributors to T2DM.[9]

ROLE OF PTP1B IN INSULIN SIGNALLING: Insulin signalling is turned off by dephosphorylation of the tyrosine residues on the activation loop of the IR abolishing its kinase activity. There have been several protein tyrosine phosphatases proposed to carry out this role namely PTPa, LAR, CD45, PTPe, SHP2, T-cell protein tyrosine phosphatase (TCPTP) and PTP1B. These all have abeen observed to control insulin signalling *in vitro* when overexpressed in cell lines but knockout mice studies showed no obvious effects on insulin signalling for any of them except PTP1B (Figure 3).Insulin binds α-chain of IR cause conformational change, activates kinase activity of β-chain and

autophosphorytion of activation loop. Phosphorylation of the insulin receptor substrates (IRS) is carried out. The phosphorylated IRS recruits phosphatidyl inositol 3-kinase (PI3K). PI3K is activated and converts PI3K to 3,4-bis and 3,4,5-trisphosphate. This stimulate the activity of phosphoinositide-dependent kinase 1 or 2 (PDK1/2) This activates Akt involved in translocation of GLUT4 and transport of Glucose.[5]

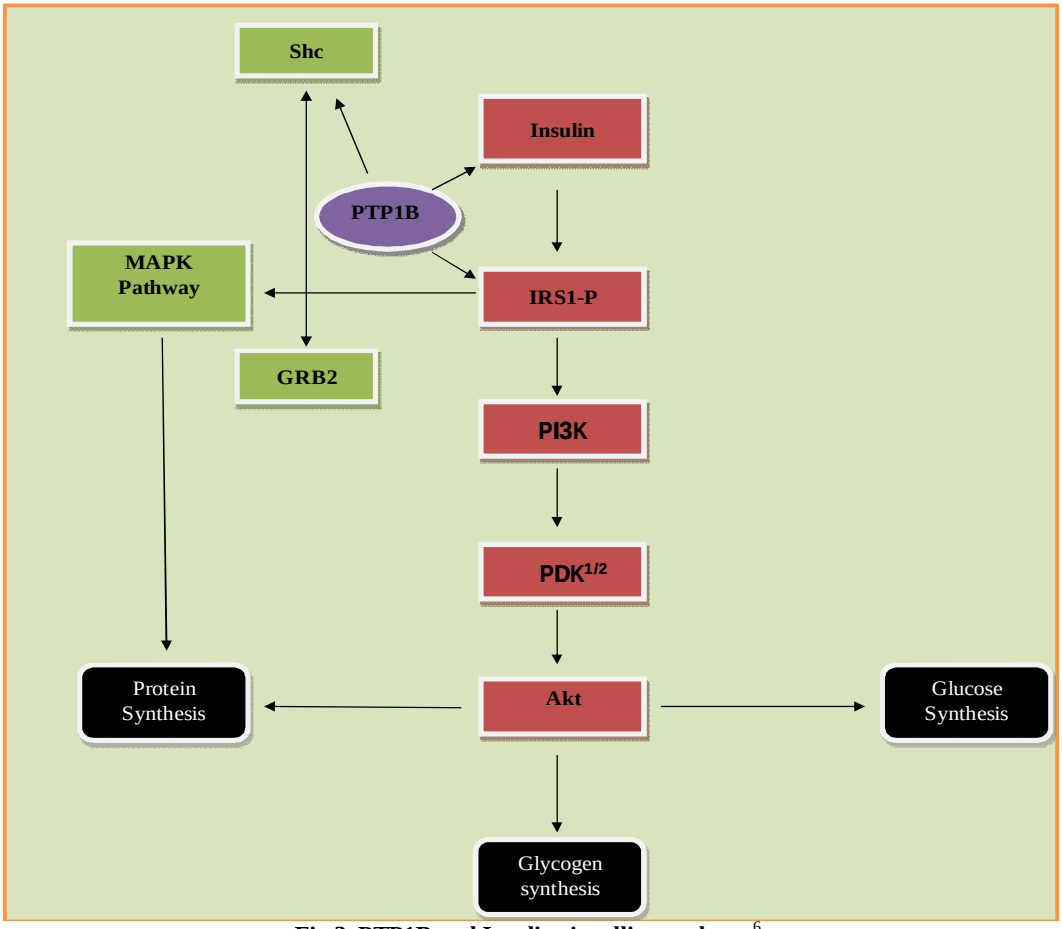


Fig.3. PTP1B and Insulin signalling pathway[6]

PTP1B is present in the endoplasmic reticulum (ER) and IR in the plasma membrane. BRET and FRET (bioluminescence and fluorescence resonance energy transfer, respectively) experiments have shown a direct interaction between PTP1B and the IR. Insulin stimulation causes a rapid increase of this interaction resulting from the internalization of the IR into an endosomic compartment which then interactes with PTP1B in the ER.[8]

There is also a significant level of a basal interaction (in the absence of insulin) between PTP1B and the IR suggested that PTP1B have an additional function to maintain the IR precursor in a dephosphorylated state as it trafficked through the ER during its biosynthesis.[10]

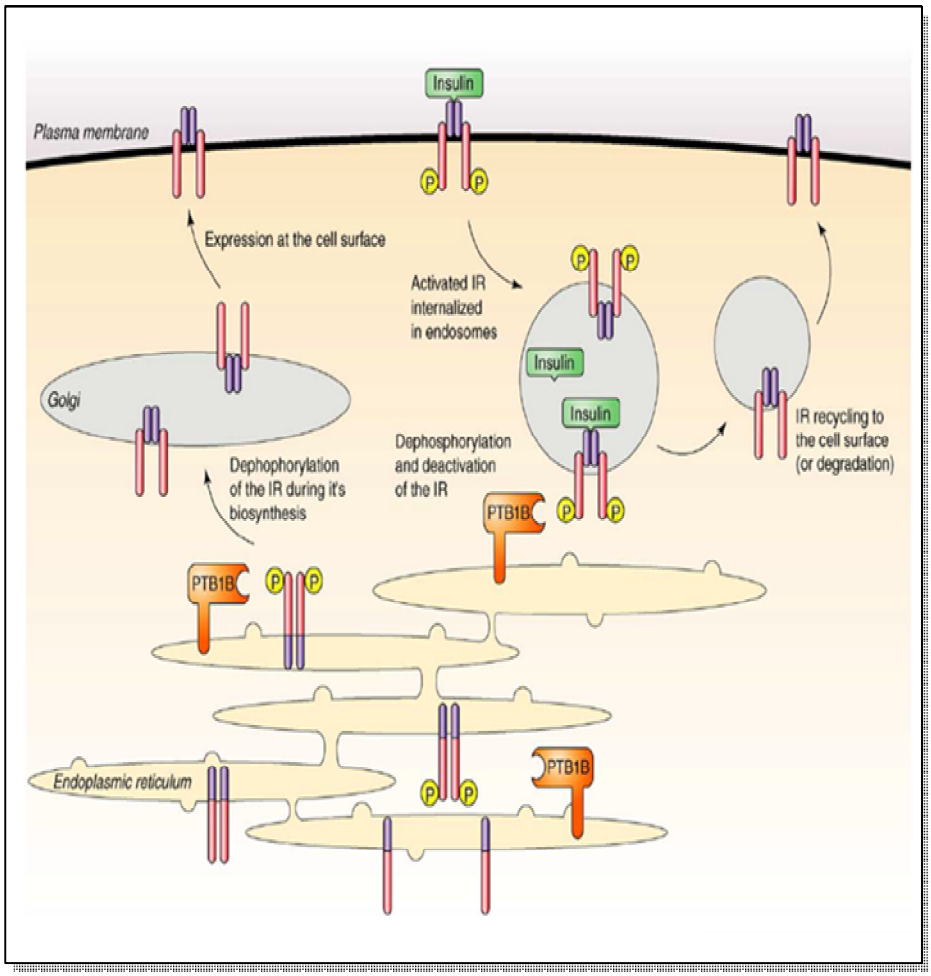


Fig.4. Cellular representation of Insulin signalling with PTP1B[6]

PTP1B have a dual function in IR signalling: (1) to maintain the IR in a dephosphorylated state during biosynthesis to allow for proper processing and (2) to dephosphorylate the insulin-activated receptor to attenuate insulin signalling and allow for recycling or degradation.[11]Among the current therapies for T2DM, thiazolidenediones address the problem of peripheral insulin resistance but have weight gain as a secondary side. Inhibition of PTP1B results in sensitization to insulin signalling and protection against diet induced obesity. This dual effect of PTP1B makes it an exciting target the treatment of T2DM by addressing key issues not filled by current treatment modalities.[12]

STRATEGY OF DRUG DESIGN

Based on transition state of enzyme-peptide complex: During the dephosphorylation of pTyr on insulin receptor, the phosphate group is situated such that it coordinates with main chain amide bonds and Arg-221 side chain of PTP1B. This orients the phosphorous so that it is susceptible to nucleophilic attack by the active site cysteine. Additional hydrophobic interactions between the aliphatic moiety of Arg-221 and Trp-179 as well as p-stacking interactions between

phenyl ring of pTyr and Phe-182 helps to stabilize the transition state.

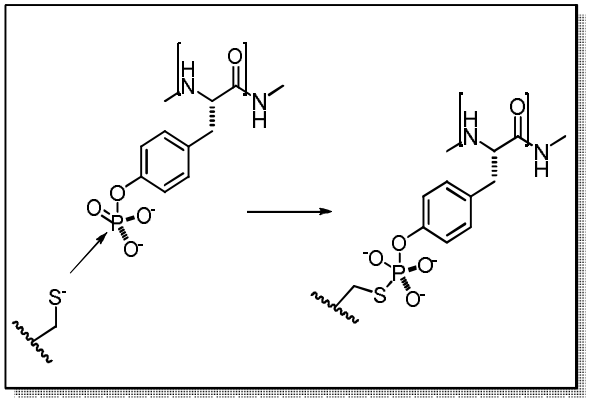


Fig.5. Nucleophilic attack of Cys-215 to form PTP1B-Cys-pTyr Transition state

Nucleophilic attack of Cys-215 thiol forms a trigonal bipyramidal PTP1B-Cys-phosphate transition state intermediate. Dephosphorylation of pTyr is assisted by protonation of the phenolic oxygen of pTyr by Asp-181. Collapse of the transition state results in the release of Tyr and the formation of a thiophosphate ester intermediate. Hydrolysis of the intermediate regenerates the active enzyme (Figure 5).[13]Many compounds are used nonhydrolizable phosphonates, sulfonates, carboxylic acids, and α-keto acids which serve as phosphate mimics and coordinate to Arg-221 in the active site. Small potential PTP1B inhibitors are designed based on either their potential as transition state mimics or their ability to interact with the active site Cys (Figure 6).[12]Potential small molecule transition state mimetics should posses a planar arrangement of oxygens around an electrophilic center similar to that of the transition state intermediate. Additionally, such mimetics that coan provide additional p-stacking interactions with Phe-182 can potentially be beneficial. Moniliformin and deltic acid are compounds that both have a planar arrangement of oxygens about an electrophilic ring similar to the arrangement of oxygens in the trigonal bipyramidal transition state complex.[14]

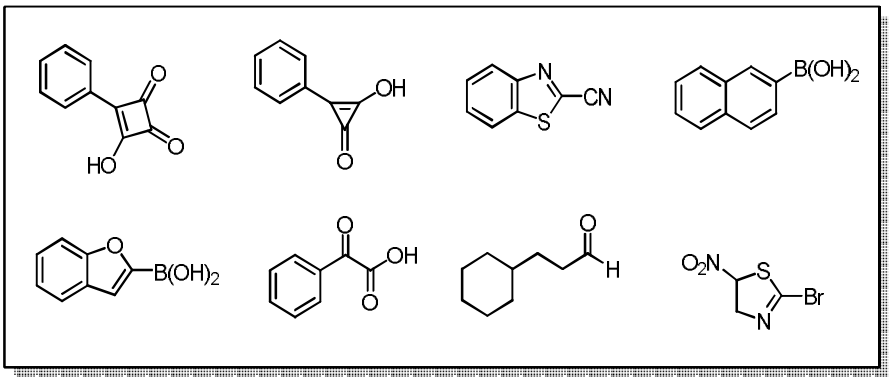


Fig.6. Transition state mimetics as PTP1B inhibitors

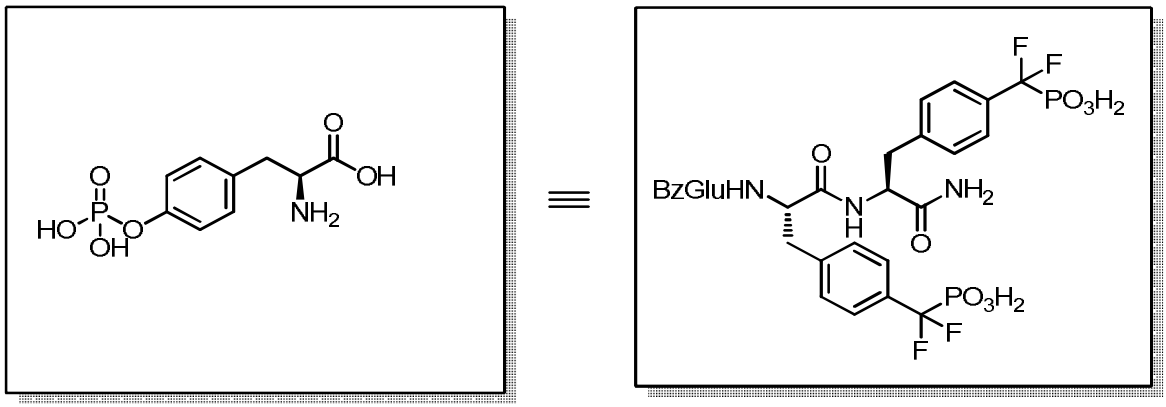
Based on enzyme-peptide complex: PTPs have a high affinity for phosphotyrosine. Difluorophosphonomethyl-phenylalanine (F_2PMP) is a mimetic of phosphotyrosine with similar binding affinity (Figure 7).

The X-ray crystal structure of peptide in complex with PTP1B shows that the C-terminal (F_2PMP) is located in the active site. It reduces 40% blood-glucose level analysed in oral glucose tolerance test. Thus, prolong insulin signalling. Thus the efforts can be made to design low molecular weight inhibitors based on the peptide SAR and structural information from the enzyme-peptide I complex (Figure 8).[17]

Synthesis of Dibenzyl malonate derivative: Malonate esters were chosen to replace the peptide backbone. Monophosphonate and Biphosphonates were identified to be the most potent inhibitor with an IC_{50} of 43%.[16]

Unfortunately, malonate esters are predicted unstable to blood esterases due to hydrolysis of the ester group resulting in the poor pharmacokinetics in rats. So, they are replaced by deoxybenzoin as a possible template to introduce the phosphonate (Figure 9).[18]

Synthesis of Deoxybenzoin derivative: Deoxybenzoin was deprotonated with sodium hydride or potassium tert-butoxide and alkylated with benzyl bromide giving the tetrapode. This series has provided potent inhibitors which are found to reduce blood glucose levels by 63% of IC_{50} (Figure 10) .[19-20]



Phosphotyrosine (pTyr)

Enzyme-peptide complex
(PTP1B complexed with F_2PMP)

Fig.7. Structural similarity between pTyr and PTP1B complexed with F_2PMP

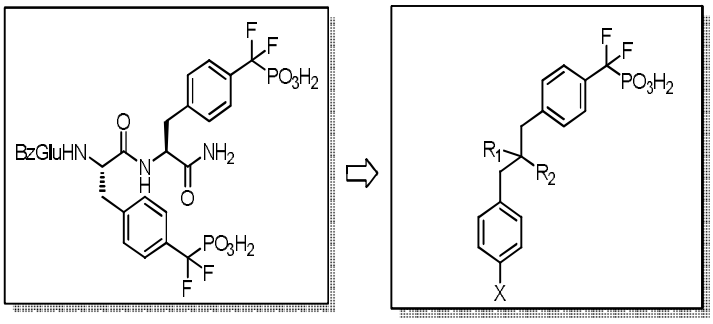
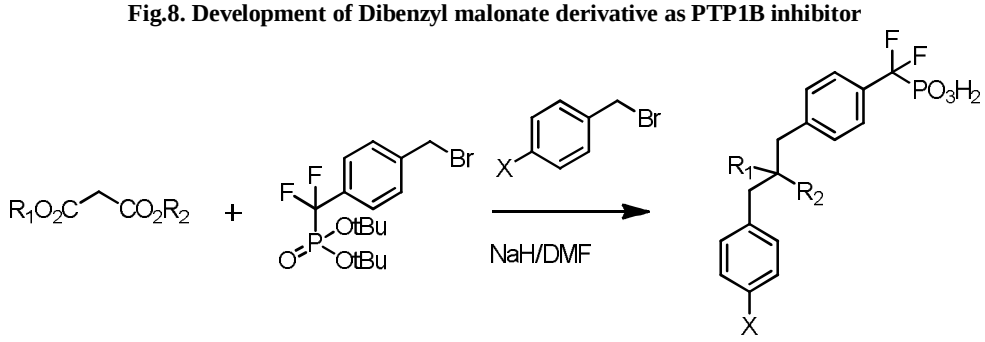


Fig.8. Development of Dibenzyl malonate derivative as PTP1B inhibitor



Scheme 1: Synthesis of Dibenzyl malonate derivative

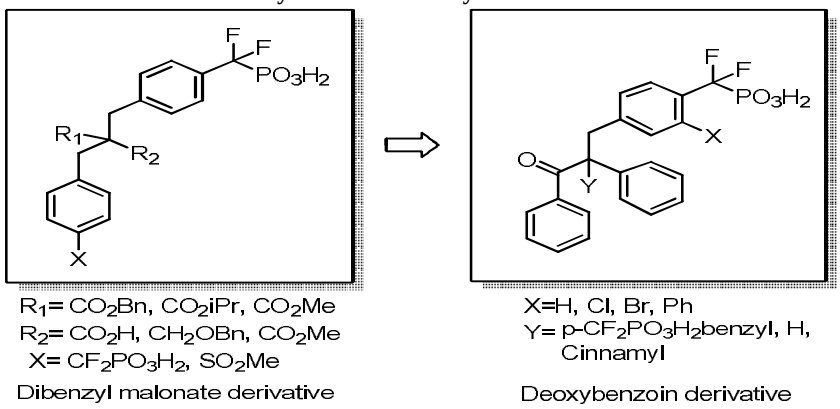
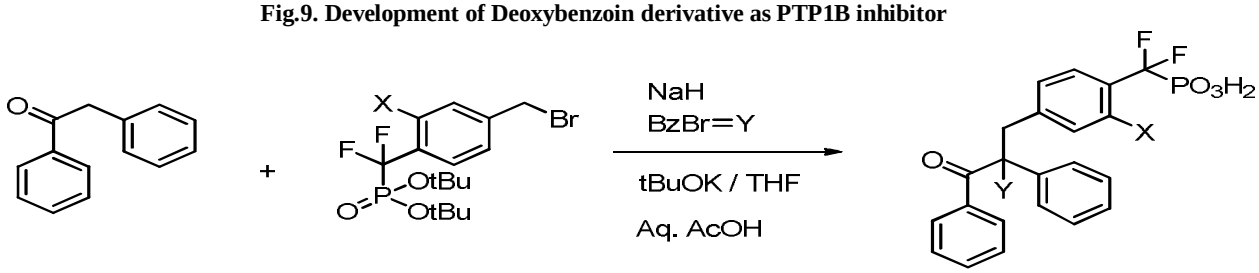


Fig.9. Development of Deoxybenzoin derivative as PTP1B inhibitor



Scheme 2: Synthesis of Deoxybenzoin derivative

Summary of designing PTP1B inhibitors based on enzyme-peptide complex

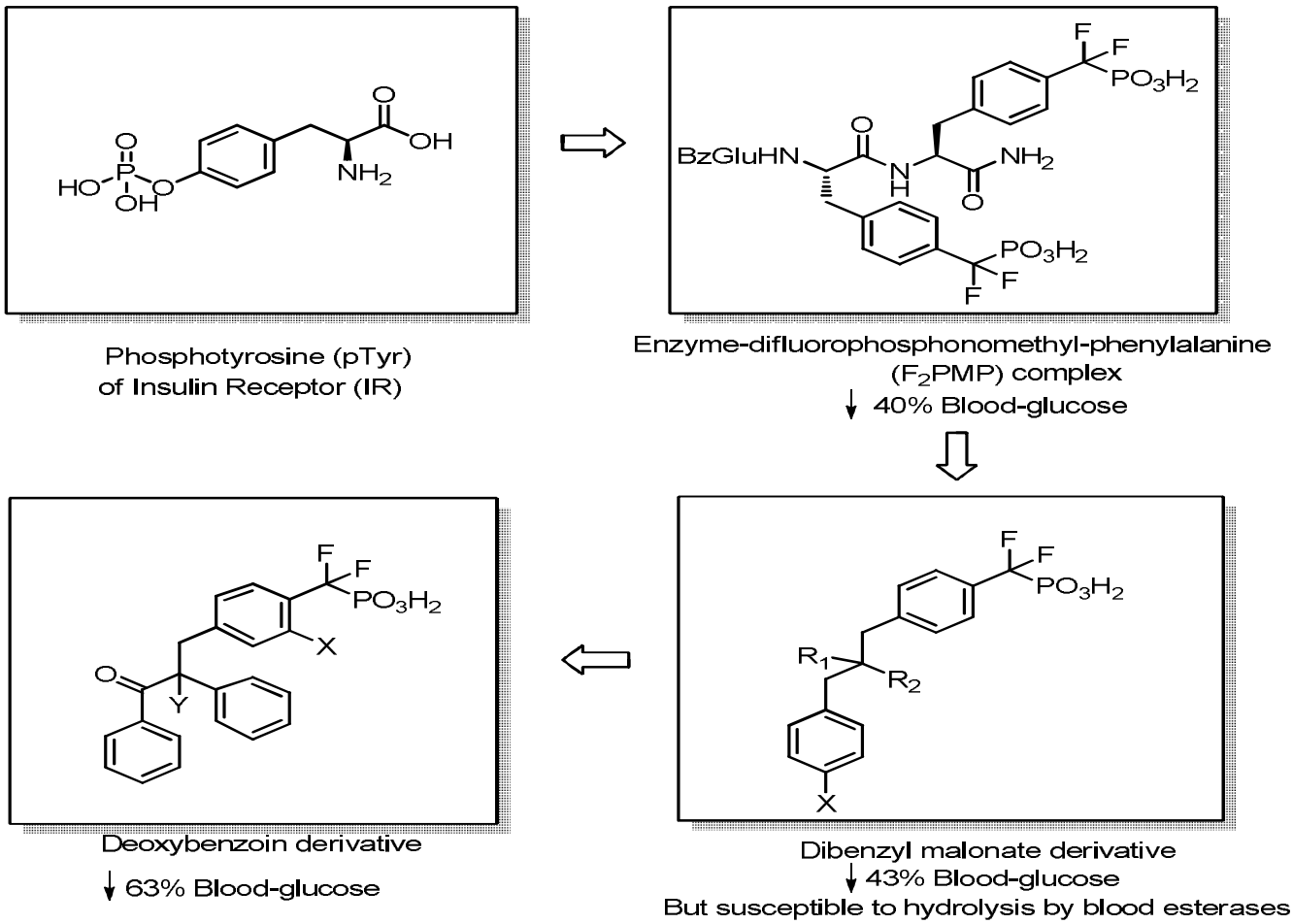


Fig.10. Overall approach in PTP1B inhibition based on enzyme-peptide complex

CONCLUSION: PTP1B play an important role in insulin signalling. Development of PTP1B inhibitors directed to the active site of the enzyme has been the major focus for most drug development programs. Thus, by targeting PTP1B, which is actively involved in the dephosphorylation of Insulin, drugs have been designed either based on transition state of enzyme-peptide complex eg. Dibenzyl malonate or Deoxybenzoin derivatives are used to overcome the threatening disease diabetes. Also the drugs are tested with additional therapeutic benefit like reduction of obesity and cancer. So, the compounds have been prepared having PTP1B inhibitory activity, reducing blood-glucose level, devoid of resistance which is most common in other antidiabetic drugs like Thiazolidenedione plus havng additional therapeutic benefits like treating obesity associated with diabetes and also cancer. Thus, PTP1B inhibition is considered as an important and novel therapeutic approach for the management of Diabetes.

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REFERENCE

[1] Tamrakar AK, Maurya CK and Rai AK, PTP1B inhibitors for type 2 diabetes treatment: a patent review (2011 - 2014).

[2] American Diabetes Association: Economic costs of diabetes in the U.S. in 2012, Diabetes Care 36 (4), 2013, 1033–46.

[3] Kleinfield NR, Modern Ways Open India's Doors to Diabetes, New York Times, Retrieved 8 June 2012.

[4] International Diabetes Foundation: Diabetes Atlas . Retrieved on 4th April 2014 http://www.idf.org/diabetesatlas.

[5] Fasanmade OA, Odeniyi IA, Ogbera AO, Diabetic ketoacidosis : diagnosis and management, African journal of medicine and medical sciences, 37(2), 2008, 99-105.

[6] Reddy MV, Ghadiyaram C, Panigrahi SK, Krishnamurthy NR, Hosahalli S, Chandrasekharappa AP, Manna D, Badiger SE, Dubey PK, Mangamoori LN, X-ray structure of PTP1B in complex with a new PTP1B inhibitor, Protein Pept Lett., 21(1), 2014, 90-3.

[7] deLorenzo C, Greco A, Fiorentino TV, Mannino GC and Hribal ML, Variants of insulin-signalling inhibitor genes in type 2 diabetes and related metabolic abnormalities, Int J Genomics, 2013, 376454.

[8] Luo DG, Liu T, Chen C, Li MY, Wang ZY, Chen RS, Wei GX, Wang XY, Data in support of fumosorinone, a novel PTP1B inhibitor, activates insulin signalling in insulin-resistance HepG2 cells and shows anti-diabetic effect in diabetic KKAy mice, Data Brief, 4, 2015, 159–161.

[9] Combs AP, Recent advances in the discovery of competitive protein tyrosine phosphatase 1B inhibitors for the treatment of diabetes, obesity, and cancer, J. Med. Chem., 53(6), 2010, 2333–44.

[10] Chen PJ, Cai SP, Huang C, Meng XM and Li J, Protein tyrosine phosphatase 1B (PTP1B): A key regulator and therapeutic target in liver diseases, Toxicology 337, 2015, 10-20.

[11] Saltiel AR, Kahn CR, Insulin signalling and the regulation of

glucose and lipid metabolism, Nature 414, 2001, 799–806.

[12] Alonso A, Sasin J, Bottini N, Friedberg I, et al, Protein tyrosine phosphatases in the human genome, Cell 117, 2004, 699–711.

[13] Ockey DA, Gadek TR, Discovery of novel PTP1B inhibitors, Bioorg. Med. Chem. Lett. 14, 2003, 389-390.

[14] Ahn JH, Discovery of novel PTP1B inhibitors, Bioorg. Med. Chem. Lett., 12, 2002, 1940-41.

[15] Sheng Z, Zhong YZ, PTP1B as a drug target: recent developments in PTP1B inhibitor discovery, Drug Discov Today, 2007, 373-75.

[16] Dufresne C, Roy P, Wang Z, Asante-Appiah E, Cromlish W et al, The development of potent non-peptidic PTP-1B inhibitors, Bioorg. Med. Chem. Lett., 14, 2004, 1039-1041.

[17] Larsen SD, The development of potent non-peptidic PTP-1B inhibitors, J. Med. Chem., 45, 2002, 596-98.

[18] Lau CK, Ramachandran C, Gauthier JY, Li CS, Therien M, Asante-Appiah et al, Structure based design of a series of potent and selective non peptidic PTP-1B inhibitors, Bioorg. Med. Chem Lett., 23;14(4), 2004, 1043-8.

[19] Maccari R, Ottana R, Identification of new inhibitors for low molecular weight protein tyrosine phosphatase isoform B, J. Med. Chem., 55, 2012, 2.

[20] Seiler CL, Richards KA, Jakubowski HV, McIntee EJ, Identification of new inhibitors for low molecular weight protein tyrosine phosphatase isoform B, Bioorg. Med. Chem. Lett., 23, 2013, 5912-5914.