



**ISO-STERIC REPLACEMENT OF THIAZOLIDIENE DIONE TO
IMIDAZOLINE DIONE (HYDANTOIN) AS A NOVEL SCAFFOLD:
SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF PTP1B
INHIBITORS.**

Ankit C. Borisa^{1*}, Hardik G. Bhatt¹, Dinesh Thakkar², Sneha Trivedi³.

¹Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University,
Ahmedabad 382 481. India.

²Department of Medicinal chemistry, A.R. College of Pharmacy & G. H. Patel Institute of
Pharmacy, Vallabh Vidyanagar, Anand - 388 120. India.

³Institute of Science, Shree P.M. Patel College of Paramedical Science & Technology,
Anand, 388 120, India.

Article Received on
11 Jan 2015,

Revised on 07 Feb 2015,
Accepted on 02 Mar 2015

***Correspondence for
Author**

Ankit C. Borisa

Department of
Pharmaceutical
Chemistry, Institute of
Pharmacy, Nirma
University, Ahmedabad,
382 481. India.

Ankit_borisa@outlook.com.

ABSTRACT

Diabetes affects 12% of the total population in world. So it is very important to treat this fatal disease. There are many marketed preparation is available to treat diabetes but not a single medicine have the ability to cure it. A thirst in research area is more and more increased now a day. Novel pathways and targets are identified in current time that may be kill this dragon named diabetes. Inhibition of a novel target PTP1B is chosen for attack on diabetes. PTP1B inhibition reported as negative regulator of insulin signaling pathway which increased the insulin sensitivity on insulin receptor. The numerous scaffold are reported and still so much research task going on for PTP1B inhibition. We choose Hydantoin scaffold as novel inhibition of PTP1B and design molecule that binds with this enzyme and inhibit it. A series of substituted 2-[4-[(2,5-dioxo imidazolidin-4-

ylidene) methyl] Aryloxy] -N-arylacetamide is decided to synthesized. The inhibition of PTP1B is *in vitro* tested over PTP1B enzymatic kit. Among all the tested compounds, two compound were found most potent activity 90.59% & 97.56 % inhibition respectively (N-(4-bromophenyl)-2-[4-[(2,5- dioxoimidazolidin-4-ylidene)methyl] phenoxy]acetamides) and (2-

[[2-[4-[(2,5- dioxoimidazolidin-4-ylidene)methyl] phenoxy] acetyl]amino]benzoic acid) with compared to standard drug suramin.

KEYWORDS: Diabetes, PTP1B, Hydantoin, imidazoline 2-4 dione.

1. INTRODUCTION

Hyperglycemia and dyslipidemia, simply called as diabetes, occurs due to defects in insulin secretion, insulin action, or both. Diabetes mellitus (DM) and diabetes insipidus (DI), almost same name but different conditions with unrelated mechanisms. DM is considered as resistance of insulin-sensitive tissues, such as muscle, liver, and fat, to insulin action, but the mechanism of the insulin resistance is unknown.^[1] The incidence of diabetes in the general population is 3 in 100,000 while DM is a common chronic disease and it is estimated that by 2030, 10% of world population will suffer from DM. The incidence of diabetes mellitus reached 210 million by the year 2013 and is estimated to reach 300 million by the year 2025. Several studies have been carried out covering different aspects of pharmacological interventions (new and old drugs) along with the effects of weight loss, diet and exercise.^[2-3]

Protein tyrosine phosphatase 1B (PTP1B) was discovered in 1988 by Fischer and co-workers which belongs to family of protein tyrosine phosphatase.^[4] This target had a very long development period which started from 19th century observations when vanadium and its derivatives were screened for its therapeutic utility in diabetes, followed by the biochemical discovery that vanadate is a potent nonselective inhibitor of phosphatases. PTP1B is universally expressed, well studied non-receptor PTP (NRPTP) anchored to the endoplasmic reticulum (ER) membrane.^[5] It is considered as negative regulator of insulin signaling by interact with the insulin receptor (IR) and removes tyrosine phosphates which induced by auto-phosphorylation in response to insulin binding. PTP1B is also able to dephosphorylate insulin receptor substrates (IRSs). PTP1B is an effective target for the treatment of both type 2 diabetes and obesity, however, targeting PTP1B is big challenge for drug discovery scientists because PTP1B consist of the highly conserved and positively charged active-site pocket.^[6] Much progress has been made in the development of potent and selective PTP1B inhibitors that engage both the active site and catalytic sites. Future efforts will probably transform the potent and selective PTP1B inhibitors into orally available drugs with desirable physicochemical properties and *in vivo* efficacies. PTP1B is involved in dephosphorylation of the activated IR and thus in termination of the insulin signal. Therefore, any changes in expression levels or activity of PTP1B relative to the IR could affect insulin signaling and

possibly play role in insulin resistance which is observed in DM. [7] There are a number of examples in which increased expression of PTP1B decreases the amount of IR tyrosine phosphorylation. Mice which lack PTP1B had enhanced sensitivity to insulin, with prolonged tyrosine phosphorylation of IR in muscle and liver. PTP1B was shown to bind and dephosphorylate JAK2, which is downstream of leptin receptor. [8-9] Thus, the resistance to diet-induced obesity observed in PTP1B mice is likely to be related with increased energy expenditure owing to enhanced leptin sensitivity. In addition, small-molecule inhibitors of PTP1B can work synergistically with insulin to increase insulin signalling and expand insulin-stimulated glucose uptake. Studies on the cellular role of the PTP1B have now clearly shown that it serves as a key negative regulator of the tyrosine phosphorylation cascade integral to the insulin signaling pathway. [10-11] Overall, these studies have covered the way for the commercial development of PTP1B inhibitors that may serve as a novel type of “insulin sensitizer” in the management of type 2 diabetes. Insulin resistance is present when insulin produces less than the expected of its actual response in various tissues. Insulin resistance is a fundamental pathogenic flaw leading to the metabolic syndrome which underlies the increased cardiovascular risk in the majority of overweight and obese patients and is also associated with reduced glucose tolerance or frank diabetes. This will focus on PTP1B. [12]

As a drug target, inhibition of PTP1B might be expected to enhance insulin action in diabetes as well as improving insulin resistance in patients with the metabolic syndrome. Only two compounds, Ertiprotafib (**1**) and Trodusquemine (**2**) shown in **Figure 1** have progressed to clinical trials. Ertiprotafib, however, cancelled in phase II due to side effects as well as low rate of *in vivo* efficacy. Trodusquemine is currently in a phase I clinical challenge with promising preclinical results in diet-induced obese (DIO) rats. A first-in-class drug yet to be launched with extensive research is under way to develop a potential blockbuster drug [13].

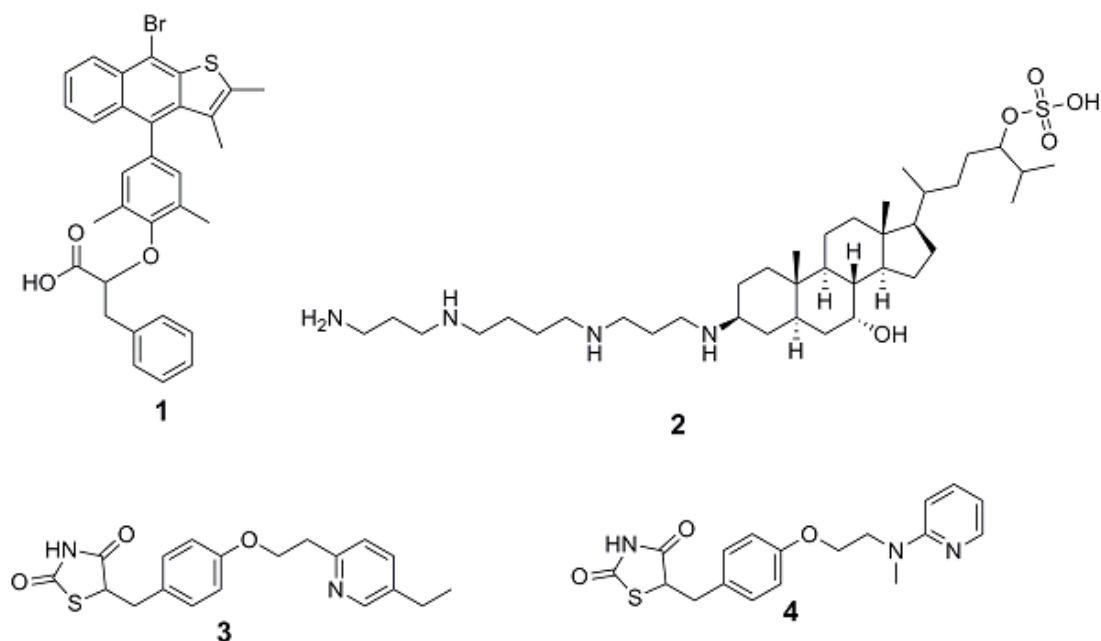


Figure 1. (1) Ertiprotafib, (2) Trodusquemine (3) Pioglitazone (4) Rosiglitazone

Iso-steric replacement: In the medication for diabetes many drugs are used to reduce glucose level. Insulin sensitizers are very important in this class. Thiazolidiene 2-4 dione derivatives are involved as anti-diabetic drugs like pioglitazone (3) and rosiglitazone (4). In our design part we designed on the basis of iso-steric replacement of thiazolidiene 2-4 dione with hydantoin derivatives as PTP1B inhibitors showed in **Figure 2**. This change also may reduce the side effect of this category. In research many scaffolds are reported but not hydantoin. Designed compounds and its requirements are shown in **Figure 3**.

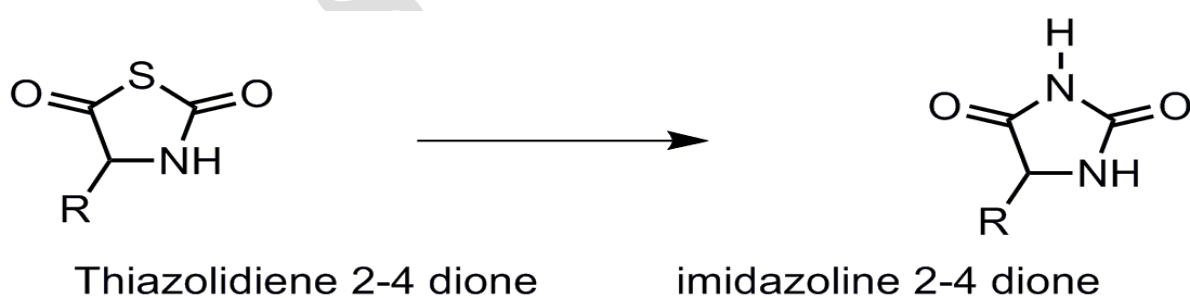


Figure 2. Iso-steric Replacement

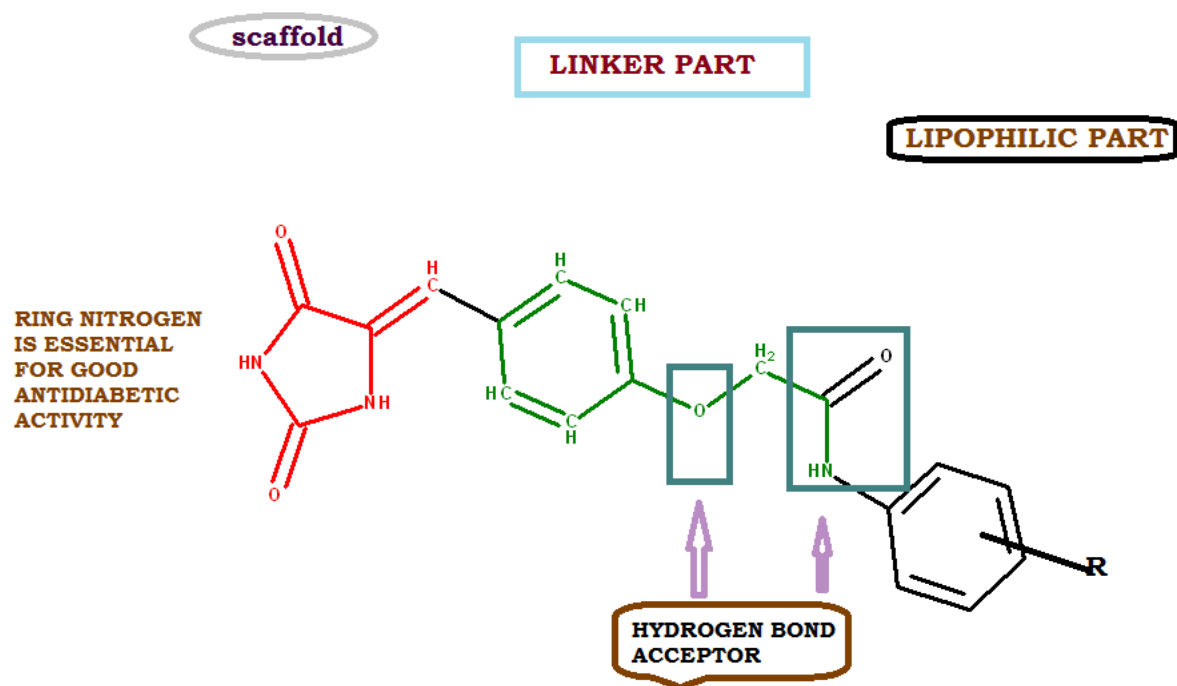


Figure: 3. Design of Hydantoin Derivatives

2. MATERIAL AND METHOD

2.1 Instrumentation

All the chemicals used for the synthesis of title compounds were procured from Sigma Aldrich, USA. S.D.Fine chem., Mumbai, India and Spectrochem, Delhi, India. The chemicals were used with further purification. The melting point of the synthesized compounds were determined in open capillary using VEEGO MELTING POINT APPARATUS model VMP-D and recorded in Celsius without correction. Thin layer chromatography was performed on microscopic slides (2 X 7.5cm) coated with Silica Gel-G with suitable solvent system. The R_f values were recorded accordingly. This method is also applied to determine the progress of the reaction and examine the purity of the end product. After the development of chromatogram, the spots were visualized by placing the plate in UV chamber or by exposure to iodine vapor. The Infrared spectra for the synthesized compounds were recorded in KBr discs on Perkin Elmer spectrum GX FT-IR spectrophotometer. ¹H NMR spectra of the synthesized compounds were taken using BRUKER Advance-II 400 MHz spectrometer using tetra methyl silane (TMS) as an internal standard. ¹H NMR spectra were recorded with DMSO as a solvent and the chemical shift data were expressed as delta values related to TMS. Mass spectra were recorded using (LCQ Fleet and TSQ quantum Access with surveyor plus HPLC system) spectrometer in methanol.

2.1.1 Procedure for synthesis of hydantoin: Imidazolidine-2,4- dione (1a)

To a stirred solution of glyoxal (0.6 mmol) in H₂O (10 ml), aq 85% H₃PO₄ (2.5 mmol) & urea (0.6 mmol) was added and the mixture was stirred at room temperature for 10 min. The end of the reaction occurred after 2 hrs and the hydantoins were obtained in 60% yield, m.p. 220-225°C, White solid powder, R_f = 0.3 (ethyl acetate: hexane, 6:4). IR (KBr cm⁻¹) 3302 (N-H), 1705 (C=O).

2.1.2 Procedure for synthesis of the 5-(4-hydroxy benzylidene) hydantoin: (2a)

In 250 ml R.B.F suspension of 4-Hydroxybenzaldehyde (4 mmol) and hydantoin (1.1 equiv.) in piperidine (5ml approx. as catalytic quantity) was heated at reflux for 6 hr. The reaction mixture was cooled, and water was added at about. Any traces of tarry material were removed by filtration. The filtrate was acidified with 6 N HCl at room temperature. The precipitates generated were then filtered off using a Buchner funnel and washed with cold water. They were then dried, and the 5-(4-hydroxy benzylidene) hydantoin products yields: 82% were obtained. m.p. >300°C, Pale yellow powder, R_f = 0.6 (Acetone: methanol, 3:1) IR data cm⁻¹: 1708 (C=O) carbonyl, 1650 (C=O) amide, 3344 (N-H), 1207 (C-O).

2.1.3 General Synthesis of 2-Chloro-N-phenylacetamide Chloracetylation (3a-3i).

Substituted anilines (0.01 M) were dissolved in 10 mL of glacial acetic acid in a clean dry conical flask. Then chloroacetylchloride (0.015 M) was added drop wise and the mixture was warmed on a water bath for 15-30 min with occasional swirling. Saturated solution of anhydrous sodium acetate was added to the warm solution to yield the precipitate on cooling in an ice bath. The product was filtered, washed well with water and purified by recrystallization from ethanol.

2.1.3.1 (3a) 2-Chloro-N-phenylacetamide, White solid, m.p:130-133°C, %yield=68.12%, TLC ethyl acetate: n-hexane (1:1) R_f value: 0.6, IR (KBr) cm⁻¹:750 (C-Cl), 1672 (C=O), 3267 (N-H).

2.1.3.2 (3b) 2-chloro N-(4-chlorophenyl)acetamide, White solid, m.p:163-166°C, %yield=89%, TLC ethyl acetate: n-hexane (1:1) R_f value: 0.75, IR (KBr) cm⁻¹: 3264 (NH-stretch), 1670 (C=O stretch), 1551(C-N stretch), 738(C-Cl stretch), 825 (Para-disubstituted benzene ring), 1070 (C-Cl).

2.1.3.3 (3c) 2-chloron-(4-bromophenyl)acetamides, White solid, m.p:175-179°C, %yield=72%, TLC ethyl acetate: n-hexane (1:1) R_f value: 0.77, IR (KBr) cm^{-1} : 3264 (NH-stretch), 1670 (C=O stretch), 1550(C-N stretch), 738(C-Cl stretch), 822(Para-disubstituted benzene ring), 1073 (C-Br stretch).

2.1.3.4 (3d) 2-chloro-N-(4-isopropylphenyl) acetamide, Brown solid, m.p:156-159°C, %yield=56%, TLC ethyl acetate: n-hexane (4:2) R_f value: 0.4, IR (KBr) cm^{-1} : 3273 (NH-stretch), 1674 (C=O stretch), 1512 (C-N stretch), 779 (C-Cl stretch), 835 (Para-disubstituted benzene ring).

2.1.3.5 (3e) 2-chloro-N-(2-isopropylphenyl)acetamide, Brown solid, m.p:145-150°C, %yield=60%, TLC ethyl acetate: n-hexane (4:2) R_f value: 0.4, IR (KBr) cm^{-1} : 3281 (NH-stretch), 1670 (C=O stretch), 1515 (C-N stretch), 775 (C-Cl stretch), 635 (Ortho-disubstituted benzene ring).

2.1.3.6 (3f) 2-chloro-N-(4-methoxy phenyl) acetamide, slightly blue solid, m.p:105-108°C, %yield=72%, TLC ethyl acetate: n-hexane (4:2) R_f value: 0.86, IR (KBr) cm^{-1} : 3281 (NH-stretch), 1670 (C=O stretch), 1515 (C-N stretch), 775 (C-Cl stretch), 835 (para-disubstituted benzene ring).

2.1.3.7 (3g) 2-[(2-chloro acetyl) amino] benzoic acid, White solid, m.p:185-187°C, %yield=72%, TLC ethyl acetate: n-hexane (4:2) R_f value: 0.8, IR (KBr) cm^{-1} : 3487 (O-H), 3267(NH-), 1670(C=O), 1545 (C-N), 784(C-Cl), 1725(C=O) of carbonyl carbon of acid.

2.1.3.8 (3h) N-benzyl-2-choro acetamides, White solid, m.p:89-91°C, %yield=85%, TLC ethyl acetate: n-hexane (4:2) R_f value: 0.3, IR (KBr) cm^{-1} : 3279 (NH-), 1649(C=O), 785(C-Cl).

2.1.3.9 (3i) 2-chloro-N-(p-acetophenyl) acetamide, Yellow solid, m.p:158-163°C, %yield=86%, TLC ethyl acetate: n-hexane (4:2) R_f value: 0.7, IR (KBr) cm^{-1} : 3325 (NH-), 1655(C=O), 1539 (C-N), 784 (C-Cl), 1707 (C=O).

2.1.4 General synthetic procedure of 2- [4- [(2,5-dioxo imidazolidin-4-ylidene) methyl] aryloxy] -N-aryl-acetamide derivatives (4a-4i)

In 250 ml R.B.F 5-(4-hydroxybenzylidene)-imidazoline 2-4dione (2a) (1.0 mol) along with 1.5 mol of potassium carbonate was taken in dimethyl formamide (DMF). To this solution

added solution of chloroacetylated product (3a-3i) (1.5 mol) in DMF. Reaction mixture was stirred at room temperature till the completion of reaction and monitored by TLC. After completion of reaction water was added to precipitate product. Solids collected and recrystallize using rectified spirit. All compounds are purified using column chromatography.

2.1.4.1 (4a) 2-[4-[(2,5-dioxoimidazolidin-4-ylidene)methyl]phenoxy]-N-phenylacetamide: Yield 53%, m.p. >300°C, TLC in ethylacetate:benzene(6:4) $R_f = 0.7$, IR (KBr) cm^{-1} : 3291 (N-H), 1708 (C=O), 1244(C-O), 1667(C=O), 1581(N-H), $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 10.39 (1H, -NH), 6.51-7.63(11H, aromatic protons and benzyldiene proton) 4.39 (2H, -CH₂-), 3.47(1H,N-H), m/z-100% at 336.27 (M-1).

2.1.4.2 (4b) N-benzyl-2-[4-[(2,5-dioxoimidazolidin-4-ylidene) methyl]phenoxy] acetamide: %yield =41%, m.p. >300°C, TLC in ethylacetate:benzene (5:2) $R_f = 0.62$, IR (KBr) cm^{-1} : 3276 (N-H), 1709 (C=O), 1248 (C-O), 1651 (C=O), 1604 (N-H), $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 10.73 (1H, -NH), 6.57-7.6 (11H, aromatic protons and benzyldiene proton), 7.6 (s, 1H, benzyldiene proton), 7.3 (m, 4H, aromatic protons), 4.37 (2H, -CH₂-), 4.37 (1H,N-H), 4.2(benzylic proton). m/z- 100% at 350.53 (M-1).

2.1.4.3 (4c) N-(4-chlorophenyl)-2-[4-[(2,5-dioxoimidazolidin-4-ylidene) methyl] phenoxy] acetamide: %yield=68%, m.p. >300°C, TLC in ethylacetate:benzene(1:4) $R_f = 0.8$, IR (KBr) cm^{-1} : 3328 (N-H), 1711 (C=O), 1249 (C-O), 1662 (C=O), 1604 (N-H), 825 (Para-disubstituted benzene ring), 1093 (C-Cl) aryl chloride. $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 10.45 (1H, -NH), 6.53-7.66 (9H, aromatic protons benzyldiene proton), 4.31(2H, -CH₂-), 4.27(1H, -NH). m/z- 100% at 370.3 (M-1) and 38% at 372.51 (M+2) chloride.

2.1.4.4 (4d) N-(4-bromo phenyl)-2-[4-[(2,5- dioxo imidazolidin-4- ylidene) methyl] phenoxy] acetamide: %yield=61%, m.p. >300°C, TLC in ethylacetate:benzene(1:4) $R_f = 0.7$, IR (KBr) cm^{-1} : 3317 (N-H), 1709 (C=O), 1246 (C-O), 1652 (C=O), 1602 (N-H), 821 (Para-disubstituted benzene ring), 1070 (C-Br) aryl bromide. $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 10.54 (1H, -NH), 6.54-7.7 (10H, aromatic protons and benzyldiene proton), 4.36 (2H, -CH₂-), 2.5 (1H, N-H). m/z- 100% at 414.69 (M-1) and 417.11 (M+2) bromide.

2.1.4.5 (4e) 2-[[2-[4-[(2,5-dioxoimidazolidin-4-ylidene) methyl] phenoxy] acetyl] amino] benzoic acid: %yield=76%, m.p. >300°C, TLC in ethylacetate:benzene(1:9) $R_f = 0.4$,

IR(KBr) cm^{-1} : 3350(N-H), 1709(C=O), 1223 (C-O), 1270 (C-O) acid, 1652 (C=O), 1604 (N-H), 653 (ortho-disubstituted benzene ring), 1751(C=O) acid. $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 11.0 (1H, O-H), 10.23 (1H, -NH), 6.23-7.7 (10H, aromatic protons and benzyldiene proton), 3.96(2H, -CH₂-), 2.4(1H, N-H). m/z - 100% at 381.98 (M+1).

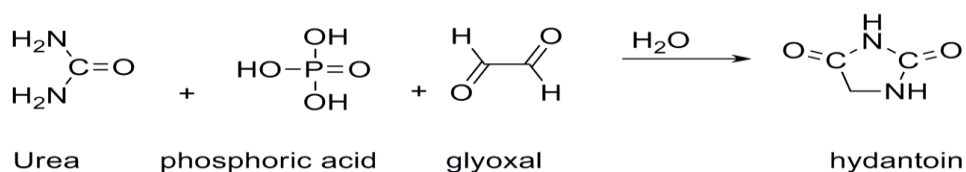
2.1.4.6 (4f) N-(4-acetylphenyl)-2-[4-[(2,5-dioxoimidazolidin-4-ylidene)methyl]phenoxy]acetamide: % yield=51%, m.p. >300°C, TLC in ethylacetate:benzene(1:9) $R_f = 0.7$, IR (KBr) cm^{-1} : 3328 (N-H), 1707 (C=O), 1264 (C-O), 1655 (C=O), 1602 (N-H), 828 (Para-disubstituted benzene ring), 1756 (C=O). $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 10.73 (1H, -NH), 7.9 (2H, benzyldiene proton), 7.5 (2H, aromatic protons), 6.8 (2H, aromatic protons), 4.34 (2H, -CH₂-), 3.5 (1H, -CH₃). m/z - 100% at 380.11 (M+1).

2.1.4.7 (4g) 2-[4-(2,5-dioxoimidazolidin-4-ylidene)methyl]phenoxy]-N-(4-isopropyl phenyl) acetamides: % yield=36%, m.p. >300°C, TLC in ethylacetate:benzene(4:1) $R_f = 0.8$, IR (KBr) cm^{-1} : 3345 (N-H), 1707 (C=O), 1270 (C-O), 1650 (C=O), 1604 (N-H), 824 (Para-disubstituted benzene ring). $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 11.03 (1H, -NH), 7.4 (2H, benzyldiene proton), 7.2 (2H, aromatic protons), 6.3(2H, aromatic protons), 4.64(2H, -CH₂-), 1.5(2H, alkyl -CH-), 1.1(1H, -CH-). m/z - 100% at 380.25 (M+1).

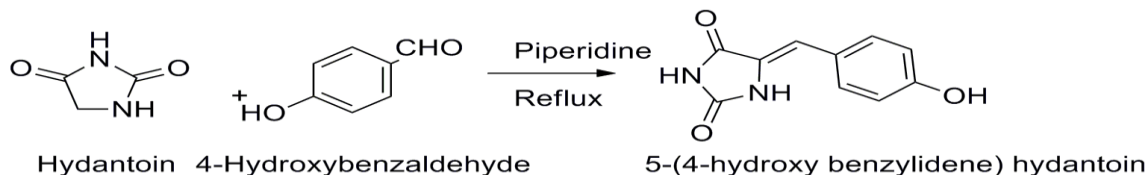
2.1.4.8 (4h) 2-[4-(2,5-dioxoimidazolidin-4-ylidene)methyl]phenoxy]-N-(2-isopropyl phenyl) acetamide: % yield=40%, m.p. >300°C, TLC in ethylacetate:benzene(4:1) $R_f = 0.7$, IR (KBr) cm^{-1} : 3351 (N-H), 1712 (C=O), 1275 (C-O), 1650 (C=O), 1608 (N-H), 631 (ortho-disubstituted benzene ring). $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 11.21 (1H, -NH), 7.6 (2H, benzyldiene proton), 7.4 (2H, aromatic protons), 6.8 (2H, aromatic protons), 4.4 (2H, -CH₂-), 1.25 (2H, alkyl -CH-), 1.21 (1H, -CH-). m/z - 100% at 380.61 (M+1).

2.1.4.9 (4i) 2-[4-[(2,5-dioxoimidazolidin-4-ylidene)methyl]phenoxy]-N-(4-methoxy phenyl) acetamide: % yield=68%, m.p. >300°C, TLC in ethylacetate:hexane(3:2) $R_f = 0.4$, IR (KBr) cm^{-1} : 3321 (N-H), 1727 (C=O), 1261 (C-O), 1621 (C=O), 1601 (N-H), 824 (Para-disubstituted benzene ring). $^1\text{H-NMR}$ (400MHz in DMSO value in ppm) δ ppm: 10.75 (1H, -NH), 7.62 (2H, benzyldiene proton), 7.20 (2H, aromatic protons), 6.21(2H, aromatic protons), 4.51(2H, -CH₂-), 2.5(3H, alkyl -CH₃). m/z - 100% at 366.51 (M-1).

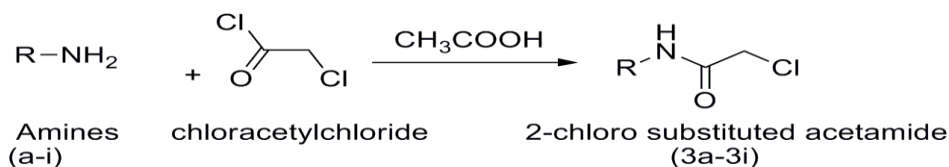
STEP-1: Synthesis of hydantoin ring



STEP-2:



STEP-3: synthesis of substitute amines (chloroacetylation)



STEP-4: Condensation reaction

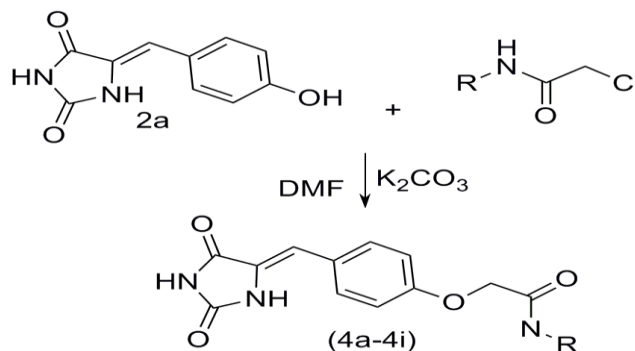


Figure: 4. Scheme for Synthesis of Hydantoin Derivatives

3. PTP1B ENZYMATIC ACTIVITY

3.1 Principle assay

The Calbiochem[®] PTP1B assay kit is a colorimetric, non-radioactive assay which was designed to measure PTP1B activity in purified preparations and for inhibitors screening. The kit comprised human recombinant PTP1B (Residues 1-322; M.W. 37,400) which was expressed in *E. coli*. The peptide substrate having phosphate that supplied with this kit contains sequence from the insulin β subunit domain that must be auto-phosphorylated to accomplish receptor kinase activation. This “activation loop” was the target of several protein tyrosine phosphatase regulators of insulin signaling, including particularly, PTP1B. The detection of free phosphate released is based on the classic malachite green assay. The PTP1B inhibitor Suramin is supplied as a control for inhibitor detection. An extremely low

reading generally indicates that the test sample is a potent inhibitor. The PTP1B inhibitory activity of our synthesized compounds was tested against PTP1B enzyme assay kit. All the reagents were reconstituted by procedure given in the protocol and standard phosphate curve was obtained by measuring the absorbance of concentration 0, 0.5, 1, 2, 3, 4, 5, 6, 7 nmoles of inorganic phosphates given in Table 1 Standard phosphate curve shown **Figure 5**.

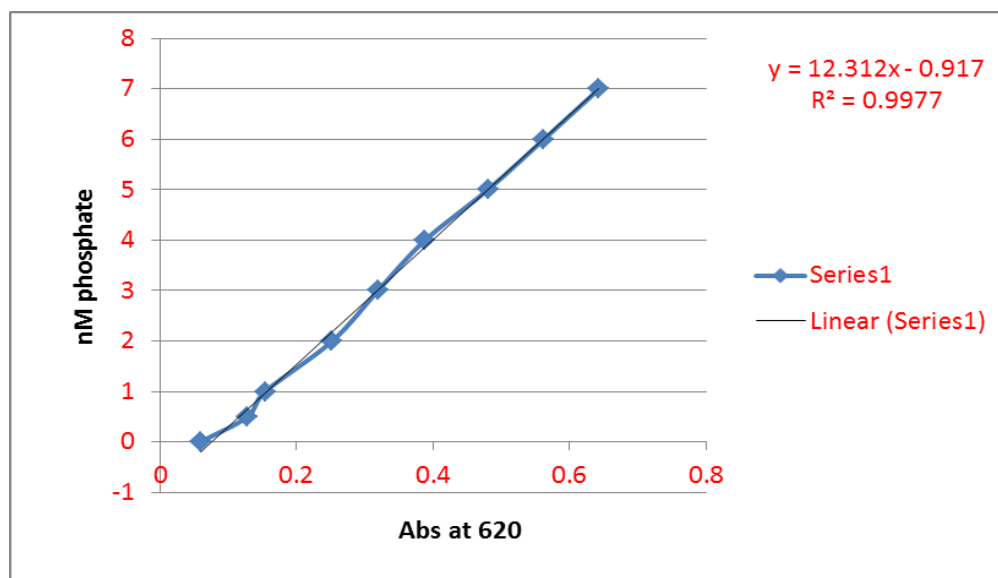


Figure: 5. Phosphate Standard Curve

The phosphopeptide sequence from the insulin receptor β subunit domain (IRs 5) was used as substrate. The test compound solutions were prepared in 1X Assay Buffer (provided in kit) at 10 times the desired final 10 μM concentration and warmed to 30°C. Stock solution of suramin (10 mM) was prepared by dissolving 10 mg of solid in 0.7 ml of 1X Assay Buffer. For a final concentration of 10 μM , 10X stock (100 μM) was prepared by mixing 10 μl of the 10 mM stock with 990 μl Assay Buffer. Then 35 μl of 1X Assay Buffer was added to each well and warmed 30°C. Subsequently 10 μl of test compound 10X stocks was added to appropriate wells and 10 μl of 1X Assay Buffer was added to control wells. Simultaneously 5 μl of the PTP1B enzyme dilution (0.4 nmol) was added to each well. After that reactions were initiated by addition of 50 μl of the warmed 2X PTP1B substrate (120 μM). Samples were incubated at 30°C, for desired length of 30 min. After incubating wells for desired duration, including the wells for standard curve, reactions were terminated by addition of 25 μl malachite green reagent provided in kit. Well plate was agitated or triturated gently to mix and kept for 20-30 min. for development of color. Absorbance was taken at 620 nm (A_{620}) on plate reader and data analysis was performed.

Table 1 Observation of Phosphate standard curve

Conc. of phosphate (nmol)	Absorbance at 620 nm
0	0.059
0.5	0.128
1	0.154
2	0.252
3	0.319
4	0.388
5	0.388
6	0.562
7	0.643

Table 2 *In-vitro* % inhibition of test samples against PTP1B enzyme

Compound Name/No.	Abs at t=0	nmol of phosphate t=0	Abs at t=30	nmol of phosphate t=30	% Inhibition at 10 μ M
Control	0.214	1.71	0.501	5.10	-
Suramin	0.134	0.73	0.161	0.90	95.54
4a	0.328	3.12	0.369	4.07	71.92
4b	0.482	5.01	0.538	5.70	79.51
4c	0.110	0.43	0.241	2.73	12.19
4d	0.325	3.08	0.317	3.49	90.59
4e	0.411	4.12	0.416	4.20	97.56
4f	0.243	2.07	0.299	3.29	65.50
4g	0.490	5.11	0.681	6.19	68.29
4h	0.680	7.45	0.771	8.11	80.48
4i	0.719	7.49	0.866	9.20	51.91

The results of the test compound assay were expressed as an amount of phosphate in nmoles.

The % Inhibition of test compounds was calculated by the equation given below,

$$\% \text{ Activity} = \frac{\text{compound (nmol PO}_4^{2-}) - \text{“time zero” (nmol PO}_4^{2-})}{[\text{Control (nmol PO}_4^{2-}) - \text{“time zero” (nmol PO}_4^{2-})]} \times 100 \%$$

$$\% \text{ Inhibition} = 100 - \% \text{ Activity}$$

All synthesized compounds are checked in this enzyme kit and its data given in Table.2

4. RESULT AND DISCUSSION

4.1 Chemistry

Hydantoin (1a) have been synthesized in water at room temperature from urea and glyoxal in presence of phosphoric acid. In step 2 synthesized hydantoin was condensed with 4-hydroxy benzaldehyde in presence of piperidine in catalytic quantity and reflux for 5-6 hrs that affords 5-(4-hydroxy benzylidene) hydantoin (2a). In third step chloracetylation, different substituted amine dissolved in glacial acetic acid followed by addition of chloroacetylchloride and heat

on water bath for half an hour. Crude chloroacetylated different products (3a-3i) were collected and dried. In final step of reaction 5-(4-hydroxy benzylidene) hydantoin (2a) and 2-chloro substituted acetamides (3a-3i), were condensed in presence of potassium carbonate in presence of DMF using magnetic stirrer for 4-5 hrs. The crude product was obtained after pouring in water and filter, dried, recrystallize using methanol.

4.2 PTP1B activity

In our test solution, compound 4d (N-(4-bromophenyl)-2-[4-[(2,5-dioxoimidazolidin-4-ylidene)methyl] phenoxy]acetamides) and 4e (2-[[2-[4-[(2,5-dioxoimidazolidin-4-ylidene)methyl] phenoxy] acetyl]amino]benzoic acid) show tremendous % inhibition of PTP1B at 90.59 and 97.56 % respectively. Other hand compound 4c N-(4-chlorophenyl)-2-[4-[(2,5-dioxo imidazolidin -4-ylidene)methyl] phenoxy]acetamides was found less potent having 12.7% inhibition. Compound 4a, 4b, 4f and 4g gave moderate %inhibition 71.92%, 79.51%, 65.50%, 68.29% and 80.48% respectively. Remaining compounds 4i show 51.91%, inhibition

5. CONCLUSION

From all these data we can say compound having acidic compound and electronegative atom greatly increase activity and effective binding over PTP1B enzyme and to inhibit it. Some of the other unsubstituted acetamide derivatives also show effective binding which makes strong hydrogen bonding complex with linker part of compounds. Compound having bulky group at para-position like isopropyl, methoxy, acetophenone also exhibit great activity on PTP1B inhibition.

REFERENCES

1. Jong, S, 2004. Diagnosis and Classification of Diabetes Mellitus. American Diabetes Association, Diabetes Care, 27.
2. Krishnaswami, C.V., Current trends in management of diabetes, TAMPA & IMA Chennai Central Felicitation Function on the Occasion of Doctors day, presentation at Chennai, 11 july-2010.
3. Modi, P. Diabetes Beyond insulin-review of new drugs for treatment of Diabetes mellitus. Curr Drug Discov Technol. 2007;4: 39-46.
4. Zhang, S., Zhang, Z., PTP1B as a drug target : recent developments in PTP1B inhibitor discovery. Drug Discovery Today. 2007; 7: 12-15.

5. Douty, B., Wayland, B., Ala, P.J., Bower, M.J., Pruitt, J., Bostrom, L., Wei, M., Klabe, R., Gonneville, L., Wynn, R., Burn, T.C., Liu, P.C., Combs, A.P., Yue, E.W., Isothiazolidinone inhibitors of PTP1B containing imidazoles and imidazolines. *Bioorg Med Chem Lett.* 2008; 18: 66-70.
6. Patel, D., Jain, M., Shah, S.R., Bahekar, R., Jadav, P., Joharapurkar, A., Dhanesha, N., Shaikh, M., Sairam, K.V., Kapadnis, P. Discovery of potent, selective and orally bioavailable triaryl-sulfonamide based PTP1B inhibitors. *Bioorg Med Chem Lett.* 2012; 22:1111-21.
7. Goldstein, B.J. Protein-tyrosine phosphatase 1B (PTP1B): a novel therapeutic target for type 2 diabetes mellitus, obesity and related states of insulin resistance. *Curr Drug Targets Immune Endocr Metabol Disord.* 2001; 275: 4283-87.
8. Hooft van Huijsduijnen, R., Bombrun, A., Swinnen, D. Selecting protein tyrosine phosphatases as drug targets. *Drug Discov Today.* 2002; 11: 730-37.
9. Bamane, V.R. and Chitre, S.T.. Molecular docking studies of quinazoline-2-hydrazide as novel PTP1B inhibitors as potential antihyperglycemic agents. *Der Pharma Chemica.* 2011; 3: 257-65.
10. Bhattarai, B.R., Kafle, B., Hwang, J.S., Ham, S.W., Lee, K.H., Park, H., Han, I.O., Cho, H. Novel thiazolidinedione derivatives with anti-obesity effects: dual action as PTP1B inhibitors and PPAR-gamma activators. *Bioorg Med Chem Lett.* 2010; 20: 6758-75.
11. Patil, V., Tilekar, K., Mehendale-Munj S., Mohan, R., Ramaa, C.S. Synthesis and primary cytotoxicity evaluation of new 5-benzylidene-2,4-thiazolidinedione derivatives. *Eur J Med Chem.* 2010; 45: 4539-48.
12. Wiesmann, C, *et al*, Protein Tyrosine Phosphatases and their Inhibitors. *Nat. Struct. Mol. Biol.*, 2004; 11(8): 730-37.
13. Xiang, D. Novel protein tyrosine phosphatase 1B inhibitors: interaction requirements for improved intracellular efficacy in type 2 diabetes mellitus and obesity control. *Biochem Biophys Res Commun.* 2011; 410:377-85.