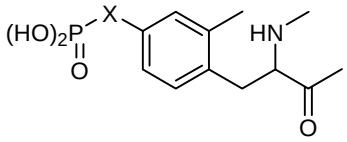
Review of Literature

1. REVIEW OF LITERATURE

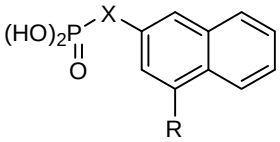
The discovery of novel Protein Tyrosine Phosphatase 1B (PTP1B) inhibitor pharmacophore has been a difficult task for both biologists and medicinal chemists. Data from large High throughput screening (HTS) collections has uncovered only a handful of novel competitive inhibitors. Sensitivity of PTP1B towards extremely hydrophobic compounds and oxidation of catalytic Cys 215 residue has produced many false positive results[13]. Although pTyr by itself binds weakly to PTP1B, the recognition pocket represents the dominant driving force for peptide binding since pTyr contributes about to 53% of peptide solvent-accessible area[4]. Several pTyr mimetics based on natural phosphopeptide substrates and available PTP1B crystal structure were designed and targeted for development of PTP1B inhibitors. Based on structure based drug design (SBDD) led to the identification of non-hydrolysable phosphonate and carboxylic acid pTyr mimetics.

2.1 Nonhydrolyzable phosphorous containing pTyr mimetics

Initial work in this area showed the replacement of the pTyr residue with the pTyr mimetic phosphonomethylphenylalanine (Pmp, **1**)[14,15]. Subsequent studies have shown that adding fluorine to the **1** methylene bridge, giving difluorophosphonomethylphenylalanine (F_2Pmp, **2**)[16], resulted in 1000-fold enhancement in PTP1B inhibitory potency[17].



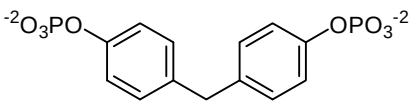
1 Pmp; X = CH_2, **2** F_2Pmp ; X = CF3



3 X = CF_3; R = H, **4** X = CF_3; R = OH

Structure activity studies on a series of arylphosphonates indicated that substituted naphthyldifluorophosphonates having more extended naphthyl ring system show good inhibitory potency[18]. A 4-hydroxy analogue **4**[19] was rationally designed and synthesized by examining the binding interaction of X-ray structure of 2-difluoromethylnaphthylphosphonic acid **3** with PTP1B active site, which showed two fold enhanced binding affinity (Ki = 94 μM) compared to parent **3** (Ki = 179 μM)[20]. The

identification of a second binding site in PTP1B by Puius et al., by solving the X-ray crystal structure of an active site Cys215 to Ser mutant PTP1B complexed with bis-(para-phosphophenyl)methane (BPPM, **5**)[21] suggested a new strategy for inhibitor design, where appropriate compounds may be made to simultaneously occupy both the binding sites to gain much higher affinity and selectivity. To prove this hypothesis and gain further insights into the structural basis of inhibitor binding, Zongchao Jia et al., have determined the crystal structure of PTP1B complexed with two non-peptidyl inhibitors, **6** and **7** to reveal that it did not bind both the active site and the adjacent noncatalytic site, as expected. The second or distal phosphonate group instead, extended into the solvent to make water mediated interaction with Arg47. Intended to interact with the PTP1B Arg47 further redesign, synthesis and biological evaluation of **3** using molecular dynamics calculation peptide based inhibitor was made, which result in more potent analogue **8** (Ki = 2 μM), and showed higher affinity than parent **3** (Ki = 179 μM).



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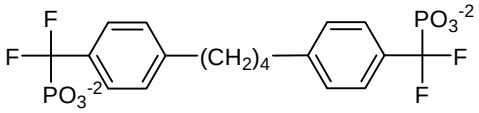

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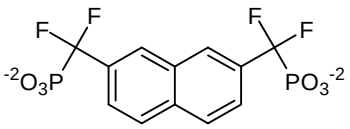

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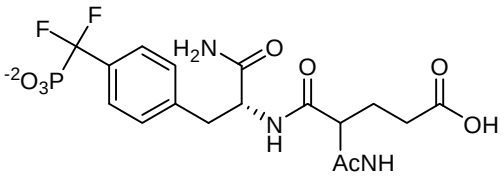
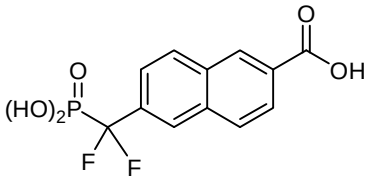


8; N-Ac-Glu-F_2Pmp-amide

Surprisingly, although the 2-carboxyl group of **9** lacked extension sufficient to reach the Arg47 residue, it exhibited eight fold higher binding affinity (Ki=22 μM) than the corresponding analogue **3**, which lacks a 2-carboxyl group[22]. X-ray crystallographic analyasis of **9** revealed that enhanced binding was achieved by interaction of its 2-carboxyl group with the protein backbone indirectly through a bridging water molecule[23]. Using molecular modeling, the 2-carboxyl group of **9** was replaced with sulfonamido group which showed enhanced binding affinity and potency **10** (IC_{50}=0.35 μM)[24]. In an effort to develop more potent PTP1B inhibitors

containing α,α-difluoromethylenephosphonic acid (DFMP) a series of phenyl derivatives bearing a single DFMP group were designed and synthesized but were not superior to the parent compound α,α-difluorobenzylphosphonic acid **11**, with the meta-phenyl susbstituted showing seventeen fold decrease in IC_{50} (IC_{50} = 35 μM) relative to parent **11** (IC_{50} = 610 μM). However, compound containing 2 DFMP groups showed very potent competitive inhibitory activity with IC_{50} of 4.4 μM, and Ki of 1.5 μM **6**[25].



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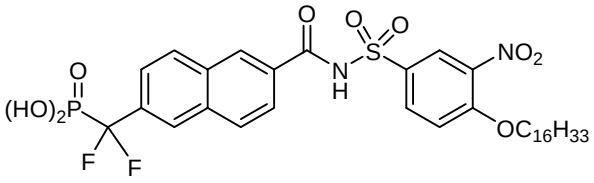

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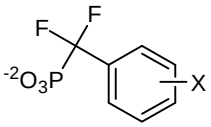
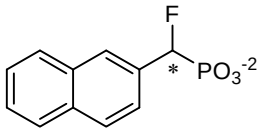
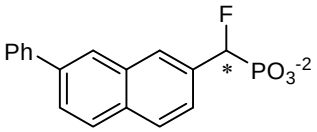


11; α,α-difluoromethylenephosphonic acid

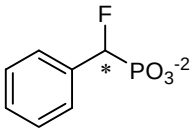
To give a possible explanation for the role of fluorine and why the difluoro derivatives show 1000 times more inhibitory activity than non fluorinated inhibitors, Christopher and co-workers reported the synthesis of enatiomerically pure α-monofluoroalkylphosphonic acid using (-)-ephedrine as the chiral auxiliary. Inhibition studies with **12-17** and PTP1B revealed that the *R*-enantiomers were 9.5-fold more potent inhibitors than the corresponding *S*-enantiomers, but 9.5-fold less potent than their α,α-difluoro analogues explaning that the pro-*S* fluorine has some role in enhancing the affinity of difluoro inhibitors **11**[26] and supports the argument[25,27] that fluorine does not enhance binding by reducing the pKa of the phosphonic acid moiety.



12; (*R*)
13; (*S*)

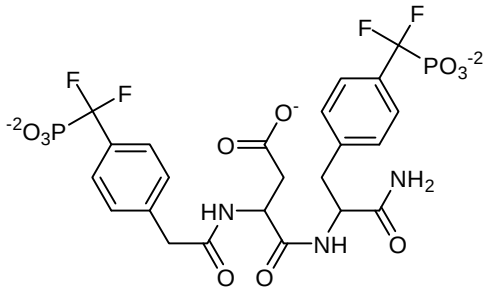


14; (*R*)
15; (*S*)



16; (*R*)
17; (*S*)

Thorough detailed kinetic analyses of PTP1B mutants targeted to amino acid residues implicated by the H/D exchange experiments led to the identification of twelve PTP1B residues (Lys36, Lys41, Tyr46, Arg47, Asp48, Val49, Ser50, Lys116, Phe182, Arg254, Arg257, and Gln262)[28] that are important for compound **18** recognition which showed 10-fold selectivity over its closest structural homologue, TCPTP. Residue Tyr46, Val49, Phe182, Arg254, Arg257and Gln262 contribute primarily to the potency of compound **18**, while other residue Lys36, Lys41, Arg47, Asp48, Ser50 and Lys116 are important for both potency and selectivity of compound **18**. Consequently, there has been considerable interest in the development of less highly charged pTyr mimics[29,30].



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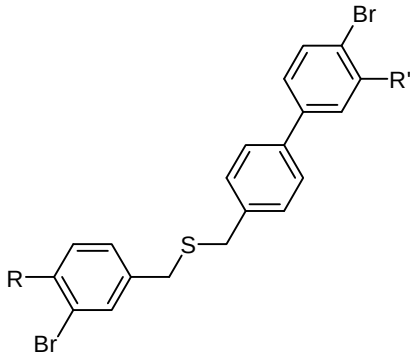
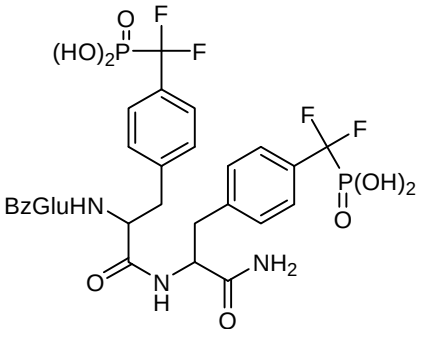


19-23

19; R= $CF_2PO_3^{-2}$, R'= SO_2NH_2
20; R= $CF_2SO_3^{-1}$, R'= SO_2NH_2
21; R= $CH_2SO_3^{-1}$, R'= SO_2NH_2
22; R= $CF_2PO_4^{-2}$, R'= $CF_2SO_2NH_2$
23; R= $CF_2SO_3^{-1}$, R'= $CF_2SO_2NH_2$

The difluorophosphonic acid (DFMS) group bearing peptidyl and non-peptidyl inhibitors was found to be the most potent, non-hydrolyzable and monoanionic pTyr mimetic. Although the DFMS group bearing small molecule scaffolds was only 3- to 8-fold poor inhibitors than the DFMP group bearing compounds which suggest that DMFS group may prove to be a very useful monoanionic phosphate mimetic for preparing small molecule inhibitors of PTP1B[31]. The effective synthesis of **19**[32], with a DFMS analogue **20** and non-fluorinated methylenesulfonyl analogue **21** and synthesis of other two derivative **22** and **23**, in which the sulphonamide moiety in compound **19**

and **20** is replaced with a difluoromethylene sulphonamide group showed that none of the new compounds (**20-23**) were as potent as the parent compound **19** (IC_{50}=0.006 μM). Compound **20** (IC_{50}=13 μM) is 1000-fold a less potent inhibitor than **19** while its non-fluorinated analogue **21** (IC_{50}=19 μM) has almost the same potency. Compound **22** (IC_{50}=0.03 μM) exhibited 5-fold increase in IC_{50} compared to compound **19**. Compound **23** (IC_{50}=6.0 μM) exhibited a slight decrease in IC_{50} compared to the sulfonate **20**[33].



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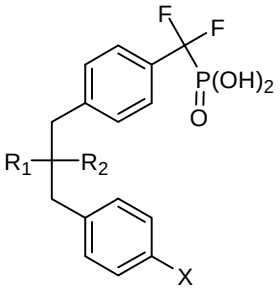


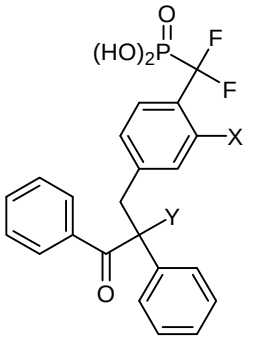
25,26

25; R_1=CO_2Bn, R_2=CO_2Bn, X=$CF_2PO_3H_2$
26; R_1=CO_2Bn, R_2=CO_2Bn, X=CF_2COOH

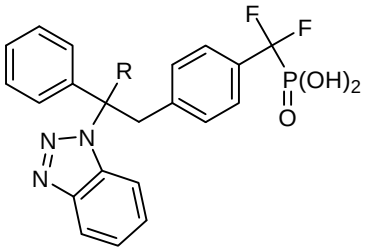
Malonate ester containing phosphate base inhibitors designed based on SAR and structural information from enzyme-peptide **24** complex, produced potent, selective (except TCPTP) and reversible PTP1B bisphosphonate inhibitor **25** (FDP Assay; IC_{50}=0.06 μM, Sf9 cell based assay; IC_{50}=0.54 μM) and monophosphonate inhibitor **26** (FDP Assay; IC_{50}=0.04 μM, Sf9 cell based assay; IC_{50}=1.90 μM). Due to the instability of malonate ester to blood esterases, a deoxybenzoin containing inhibitor was designed **27** (FDP Assay; IC_{50}=0.06 μM, Sf9 cell based assay; IC_{50}=0.58 μM), while the ortho bromo analogue **28** (FDP Assay; IC_{50}=0.12 μM, Sf9 cell based assay; IC_{50}=1.22 μM) was found to be orally bioavailable (*F*=13%) in rats with C_{max} of 35 μM[34].

Based on sequence alignment of the highly-homologous PTP1B and TCPTP, it was observed that only two amino acid residues differ in the two enzymes in the first shell of the active site located at the periphery of the secondary binding site. Phenylalanine 52 (Phe52) and alanine 27 (Ala27) in PTP1B are replaced by tyrosine and serine respectively in TCPTP. From these observations the tetrasubstituted benzotriazole compounds were designed and synthesized. Analogue **29** was found to be the most

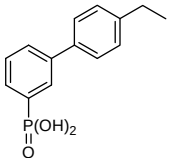
potent PTP1B inhibitor (IC_{50}=0.003μM) but showed no selectivity over TCPTP. However, disubstituting the methyl by making methoxyisobutylquinoline analogue **30a** (PTP1B, IC_{50}=0.005 μM; TCPTP, IC_{50}=0.036 μM) and **30b** (PTP1B, IC_{50}=0.007 μM; TCPTP, IC_{50}=0.041 μM) out of four diasteromers of **30** showed moderate selectivity over TCPTP[35].



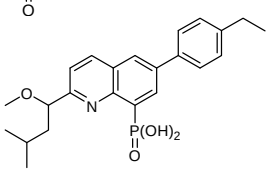
27; X=H, Y=*p*-$CF_2PO_3H_2$benzyl, **28;** X=Br, Y=H



29 ; R =

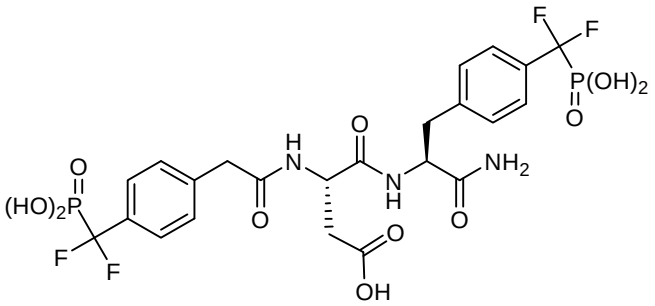


30, 30a, 30b ; R =

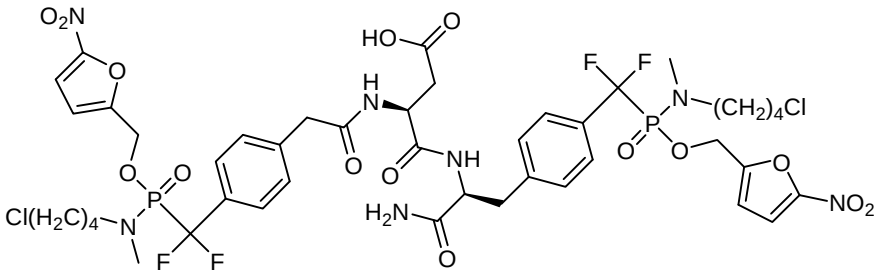


29-30b

Since a number of organophosphonate-based prodrug have advanced to the clinic and beyond[36], prodrug approach to improve the *in vivo* efficacy of the potent and selective compound **31a** (Ki = 2.4 nM, tenfold selectivity over TCPTP) originally discovered by Zhang et al[37] was attempted by Borch et al. The efficacy of the prodrug **31b** was determined on insulin signalling in human hepatoma HepG2 cells. Prodrug **31b** increases the insulin induced activation of both IRβ and its downstream target ERK1/2 at 20 nM which enhanced the phosphorylation level of IRβ and ERK1/2 by 1.9 and 1.3 fold respectively and at 100 nM showed 2.1 and 1.6 fold increase in phosphorylation level of IRβ and ERK1/2 respectively and showed conversion half-life ($t_{1/2}$) of 44 minute[38].



31a

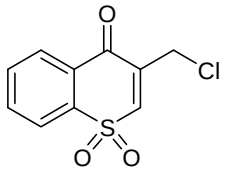


31b

2.2 Carboxylic acid pTyr mimetics

Most of the PTP1B inhibitors which have been previously described are peptide-based, containing negatively charged phosphonic acid or sulphate derivatives. These compounds are inefficient in crossing cell membranes and are unstable *in vivo*. Thus, there was a need to identify small organic compounds which exhibit potent and selective inhibitory profiles against PTP1B. The crystal structure of *Yersinia* PTP complexed with the sulphate indicated that it binds to the active site Cys in the phosphotyrosine binding pocket[39]. In addition it was reported that trisulfotyrosyl peptide, corresponding to the major autophosphorylation site of the insulin receptor can inhibit PTP1B[40,41]. Casein sulfotyrosyl peptide of sequence NANEEE(sY)SIGSA, **1** corresponding to high affinity phosphotyrosyl peptide substrate for PTP1B (Km ~ 0.2 μM)[42] was synthesized and examined for inhibition potential for PTP1B and CD45 while the kinetic analysis of both CD45 and PTP1B showed Ki of 15 μM and 3 μM respectively. The minimum sequence required for the inhibition of PTP1B by sulfotyrosyl peptide, **1** was determined by systematically deleting the N-terminal and C-terminal of the peptide, which showed that the three glutamic acids (-1 to -3 residues) immediately N-terminal to the sulfotyrosyl residue are essential for high-

affinity binding to PTP1B, while the modification of C-terminal shows modest change in potency[43]. Seung and co-workers reported the first selective and irreversible PTP1B inactivation by sulfone analogue of napththoquinone **2** which showed the dissociation constant Ki to be 3.5 μM and inactivation rate constant K_{inact} to be 2.2 x 10^{-2} sec^{-1} without touching upon the interaction studies[44]. Dephostatin **3,** as the first naturally occurring selective PTPase inhibitor[45,46] was further rationally designed based on the crystal structure of C215S PTP1B-*O*-Phosphotyrosine complex. The most favourable interaction between PTP1B and Me-3,4-desphostatin **4** (IC_{50} = 0.52 μg/mL) derivatives was obtained by calculation using the CVFF force field parameter. Out of the synthesized derivatives the pentyl derivative **5** (IC_{50} = 0.30 μg/mL) showed potent inhibitory activity but due to the weak mutagenic activity of nitrosamine moiety, inhibitors without nitrosamine moiety were designed.



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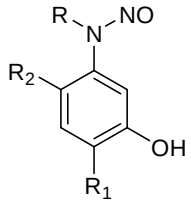
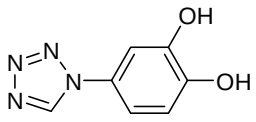


3-5

	R	R_1	R_2
3	CH_3	H	OH
4	CH_3	OH	H
5	C_5H_{11}	OH	H

The tetrazole derivative **6** (IC_{50} = 47 μg/mL) showed no activity possible because of geometrical mismatch, while the methoxime compound **7 (**IC_{50} = 2.9 μg/mL) shows five times higher IC_{50} than that of CH_3-3,4-dephostatin, **4**[47].



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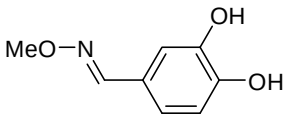
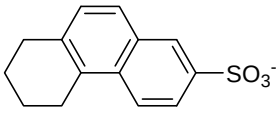


7

Mauro Sarmiento and co-workers identified 25 small molecule hits (17 based on distance screen and 8 on force field screen) by carrying out a structure-based, computer-assisted search of a chemical database using the DOCK methodology. Out of

25 hits 7 exhibited measurable inhibition of PTP1B and displayed significant selectivity against PTPα, LAR and dual specific phosphatase VHR. Kinetic characterization of compounds **8-12** showed competitive inhibition, while compound **13** and **14** showed mixed inhibition. Structural basis for PTP1B inhibition by compounds **8-14** suggested that, planar ring system is preferred, a great deal of plasticity exist in the active site which can accommodate extended aromatic system and additional binding regions proximal to the active site are important in development of PTP1B inhibitor[48].



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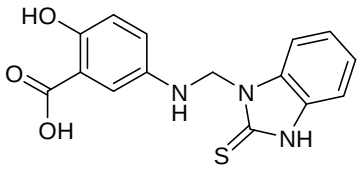

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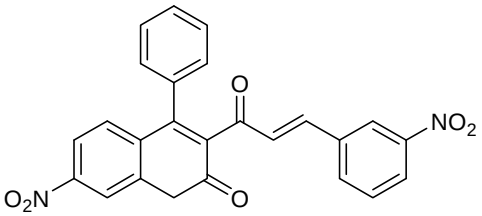

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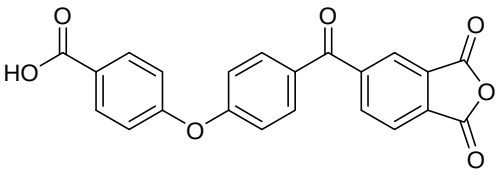

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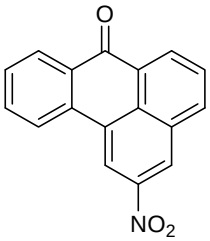

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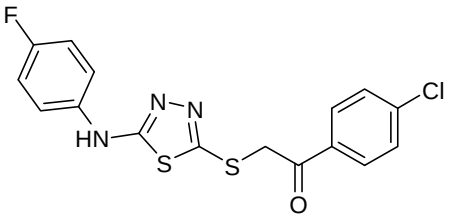
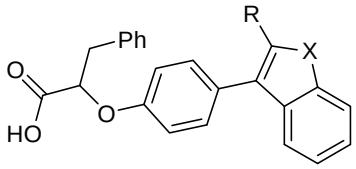


14

Two novel series of benzofuran/benzothiophene biphenyl oxo-acetic acids **15,** and sulfonyl-salicylic acids **16,** as potent inhibitors of PTP1B with good oral antihyperglycemic activity was developed by detailed systematic structure-activity relationship (SAR) studies. Resolution of crystal complexes has suggested that in the oxo-acetic acid series, hydrophobic substitutents at position-2 of the benzofuran/benzothiophene biphenyl framework interacted with Phe182 of the catalytic site and were very critical to the intrinsic activity of the molecule. Similar

ortho aromatic substitutions on the salicylic acid-type inhibitors had no effect, primarily due to the different orientation of these inhibitors in the catalytic site. Compound **17** (IC_{50} = 0.32 μM) was one of the most active compounds *in vivo*, normalizing plasma glucose levels at 25 mg/kg dose (po) and 1 mg/kg dose (ip). Compound **17** was also selective against several other PTPases (no data provided for TCPTP)[49]. 2-(oxalyl-amino)-benzoic acid (OBA), **18** (Ki = 23 μM) was found to be a general inhibitor of PTPs[50], substituting the phenyl ring in OBA by thiophene, resulted in compounds with little difference in potency, **19** (Ki = 58 μM). Consequently, compound **20** has showed about eight fold more potency against PTP1B than compound **19**.



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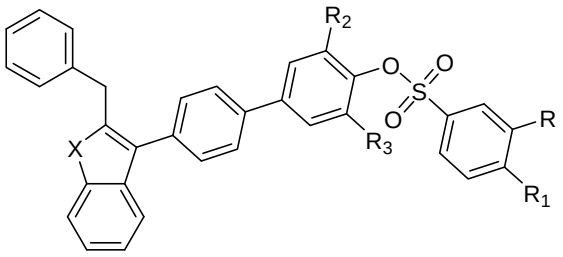

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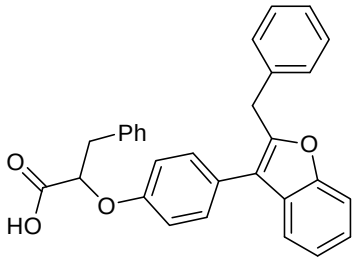

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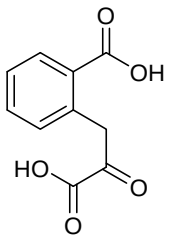

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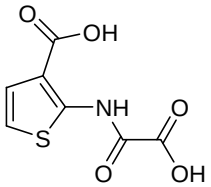

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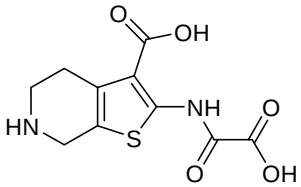
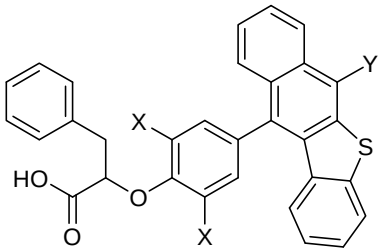


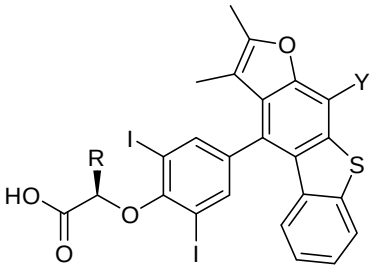
20

A considerable selectivity was achieved over many other PTPS by introducing the basic nitrogen in the saturated ring in compound **20** which is sufficiently close to Asp48 to allow the formation of salt bridge and in addition because of repulsive forces between the positive ligand charge and the asparagine side chain in many other PTPs[51] PTP1B inhibition and oral antidiabetic activity of a series of 11-aryl

benzo[*b*]naphtha[2,3-*d*]thiophene **21** was reported by Worbel *et al*. In an effort to define the role and improve upon the properties of the tetracyclic ring portion of **21** (IC_{50} = 61 nM) and **22** (IC_{50} = 179 nM) 4-aryl-1-oxo-9-thiacyclopenta[*b*]fluorenes **23** (IC_{50} = 284 nM) and **24** (IC_{50} = 74 nM) were designed and synthesized[52]. A detailed systematic structure-activity relationship (SAR) study of azolidinediones 25 as PTPase inhibitors was studied by Michael and co-workers. Several of the developed inhibitors showed good *in vivo* and *in vitro* activity; from SAR study it was found that the elongated spacer between the azolidinedione moiety and the central aromatic portion of the molecule was found to be very important to the inhibitory activity.

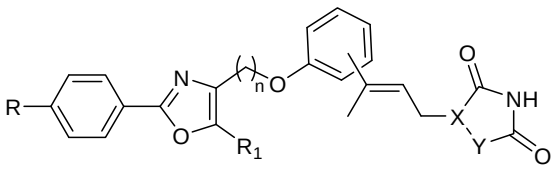


21; X=Br, Y=Br, **22**; X =I, Y =H



23; R=CH_3, **24**; R=CH_2Ph

A large hydrophobic group in the vicinity of this aromatic central region affords potency. Substitutions at position-5 of the oxazole moiety with a hydrophobic substituent produce very good inhibitors, **26** and **27**. But the *in vitro* enhanced activity of several compounds does not translate to higher *in vivo* potency probably because of the increased lipophilic properties of these compounds[53].



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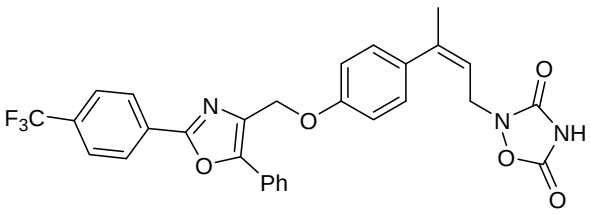
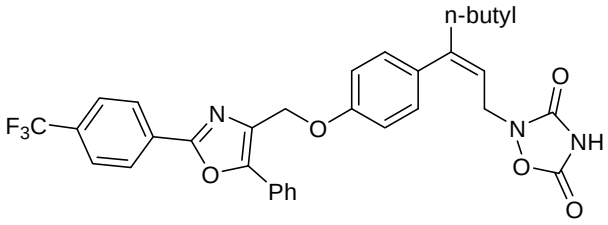


26

An analog program was launched to attenuate the peptidic character of tripeptide Ac-Asp-Tyr-(SO_3H)-Nle-NH_2 (Ki = 5μM)[54] and to enhance its potency. The most significant analog arisen from this work was **28**, which possesses submicromolar affinity for PTP1B (Ki = 0.22μM) without significantly inhibiting LAR or SHP-2 at concentration up to 100 μM and its triester prodrug **29** enhances insulin signalling in

two different cell lines but none of the compounds exhibited significant selectivity between PTP1B and TCPTP[55]. Because cell permeability is a key issue for these compounds a series of analogs of **30** (Ki = 6.5 μM)[56] and **31** (Ki = 2.0 μM)[55], wherein the carboxylic acids have been replaced with various groups with the primary aim of increasing permeability and potency were synthesized.



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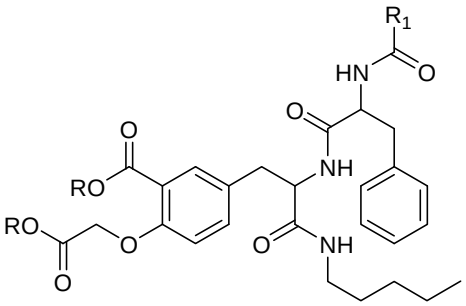
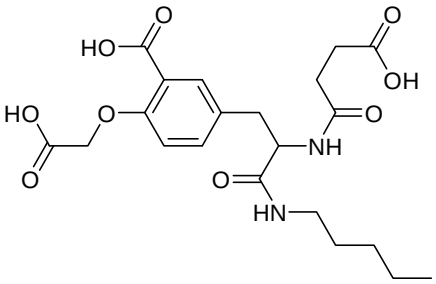


28; R=H, R_1=CH_2CH_2COOH
29; R=CH_3, R_1=$CH_2CH_2COOCH_3$



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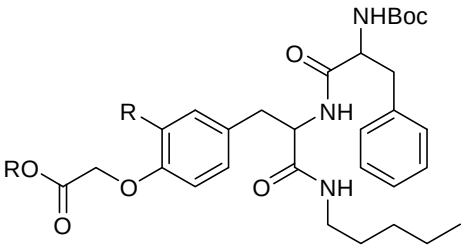
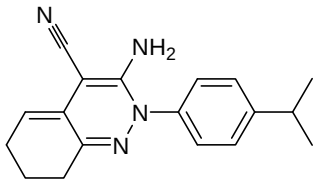


31; R=COOH, **32;** R= 

The ortho tetrazole analog **32** (Ki = 2.0 μM) was found to be equipotent to the dicarboxylic acid derivative **31** as a PTP1B inhibitor, which also displayed increased permeability into Caco-2 cells and exhibited modest enhancement of insulin-stimulated 2-deoxyglucose uptake by L6 myocytes[57]. First time reversible non-competitive pyridazine analog as inhibitors of PTP1B were reported by Charlotta and co-workers and indicated that there may exist another site in the enzyme. Some of the synthesized compounds demonstrated high selectivity, compound **32** (IC_{50} = 5.6μM) was twenty fold more selective for PTP1B against both LAR and TCPTP. Furthermore, due to the small molecular weight and non-polar properties of these compounds, they showed good cellular activity[58]. The SAR of α-bromoacetophenone derivatives as

PTP1B and SHP-1 was developed. Bromides were much more potent than the corresponding chlorides, and the phenyl ring is markedly tolerant to modification.



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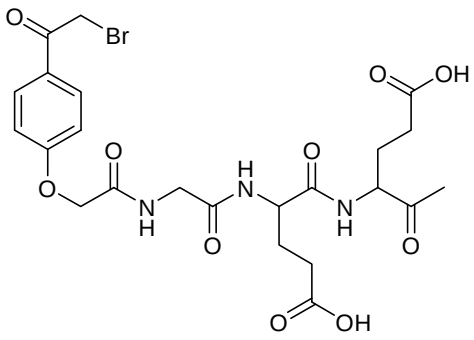

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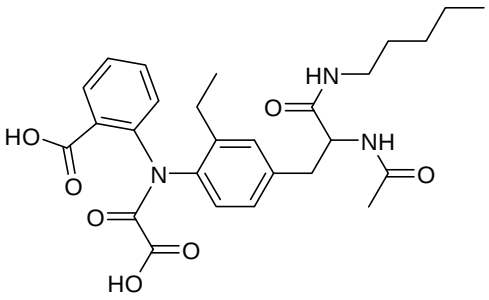

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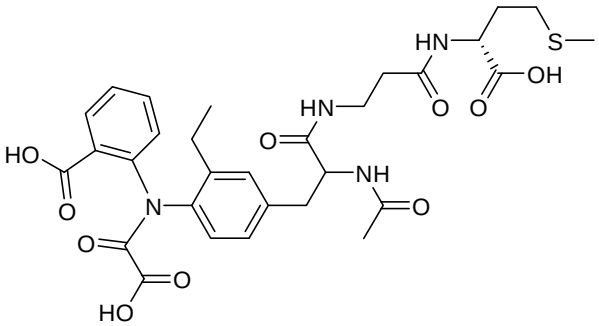
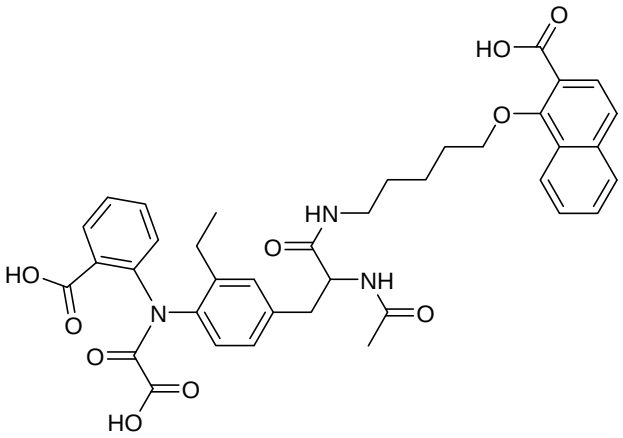


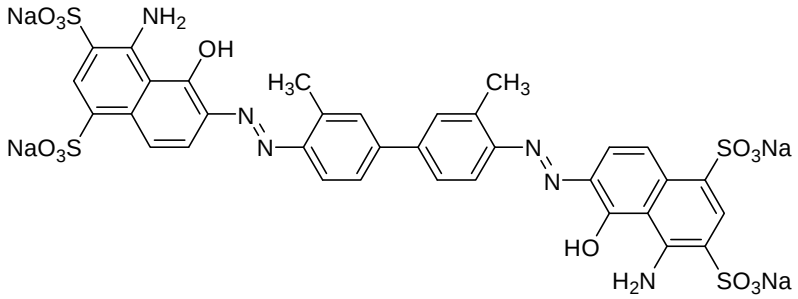
35

Derivatization of phenyl ring with tripeptide Gly-Glu-Glu **33** (Ki = 2.8μM) results in potent and selective inhibition[59]. An oxalyl-aryl-amino benzoic acid was discovered by NMR-based fragment screening as a novel PTP1B inhibitor and subsequent structure-based drug design approach facilitated the discovery of relatively potent inhibitors of PTP1B. The X-ray crystal structure of **34** revealed that two hydrogen bonding interactions with Asp48 of PTP1B that are critical for the enhanced affinity. Utilizing a solution phase parallel- synthesis approach, a highly potent PTP1B inhibitor **35** (Ki = 76 nM) with moderate selectivity (5-fold) over TCPTP through interacting with a second phosphotyrosine binding site (site 2) was identified[60]. Similarly, based on NMR based linked-fragment strategy and starting with low affinity leads, an inhibitor series was constructed to rationally improve both potency and selectivity. Compound **36** was the most potent PTP1B (Ki = 22 nM) inhibitor and displays excellent selectivity against LAR (Ki = 1.3 μM), SHP-2 (Ki = 2.49 μM), CD-45 (Ki = 53.6 μM) and calcineurin (Ki > 300 μM) and modest selectivity against TCPTP (Ki = 49 nM)[61].



36

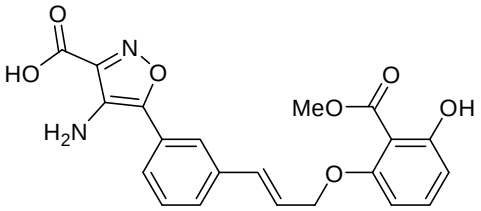
It was found that dye-related compounds suramin and derivatives of suramin **36-38** (IC_{50} < 5 μM) are potent inhibitors of protein tyrosine phosphatases (PTPases)[62,63,64]. Suja Shrestha et al have examined 13 arbitrarily selected dyes against human PTP1B, membrane proximal catalytic domain of human LAR (LAR-D1) and YPTP1 from Saccharomyces cerevisiae[65]. Initial enzyme assay proved that all the dyes tested behave as inhibitors of the PTPases with different potencies. Evans Blue **39** was the most potent inhibitor of PTP1B and YPTP1 with IC_{50} of 1.3 μM and 1.2 μM, respectively[66].



39

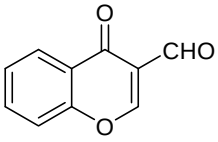
Guided by X-ray crystallography, introduction of an amino group to the only chemically modifiable position 4 of the isoxazole ring provided a more active inhibitor **40** with good selectivity over TCPTP (Ki 2.1 μM, PTP1B; Ki > 30 μM, TCPTP) and showed good cell permeability in COS7 cells[67]. Many quinones are catalytic inactivators of protein tyrosine phosphatases under aerobic conditions, through the specific oxidation of their catalytic cysteine. Polyaromatic quinones, such as the environmental pollutants 9,10-phenanthrenediones, elicit a wide range of responses

including growth inhibition, immune suppression, and glucose normalization in diabetic models[68].



40

3-formylchromone (4-oxo-4H-1-benzopyran-3-carboxaldehyde, **41**) and its derivatives behave as inhibitors of PTPases[64] to take the advantage that 3-formylchromone moiety can serve as a neutral pharmacophore for PTPase inhibitor design, intensive derivatization of 3-formylchromone were performed and evaluation of the inhibitory potency of the derivatives indicated that **42** and **43** were PTP1B-selective with IC_{50} values 1.1 ± 0.3 μM and 1.0 ± 0.2 μM[69].



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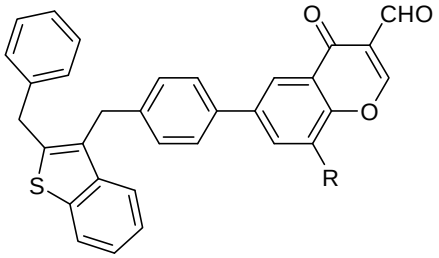
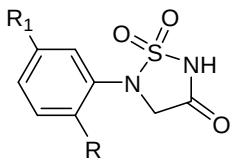
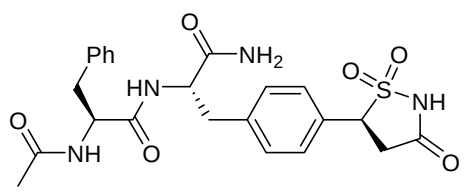


42; R=H, **43;** R= Ph

Incorporating the 1,2,5-thiadiazolidin-3-one-1,1-dioxide template and using structure-based design, a new class of inhibitors of protein tyrosine phosphatase-1B (PTP1B) has been identified and synthesized. Following this approach it was determined that the 5-position of the aromatic ring was the preferred point for substitution. Subsequently, compounds **44** and **45** were synthesised (IC_{50} = 2.47 μM and 132 μM)[70].

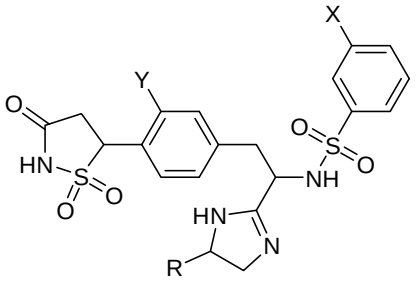


44; R=OMe, R_1=Ph, **45**; R=H, R_1=Ph



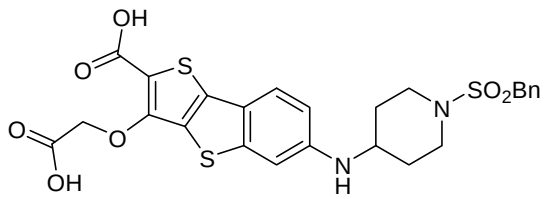
46

Through meticulous analysis and overlay of structural information available in the PDB, a novel dipeptide containing the (S)-IZD pTyr mimetic was synthesized which demonstrated competitive, reversible, and potent inhibition of PTP1B (**46**, IC_{50} = 190 nM and Ki = 180 nM). An X-ray crystal structure of PTP1B complexed with **46** showed that the ligand bound precisely as designed in silico. Furthermore, the (S)-IZD pTyr mimetic was found to be a 10-fold more potent inhibitor of PTP1B than an analogous peptidic compound bearing a DFMP[71,72,73]. Structure based modification of isothiazolidinone (IZD) containing imidazoles and imidazolines inhibitors to interact with the B site of PTP1B led to the development of an inhibitor **47A** (IC_{50} = 22 nM) in the imidazoline series and **47B** (IC_{50} = 32 nM) in the imidazole series[74].



	X	R	Y
47A	F	(*R*)$(CH_2)_4O$(2-CO_2H, 3-OH-Ph)	Me
47B	F	$(CH_2)_4O$(2-CO_2H, 3-OH-Ph)	Me

A novel pyridothiophene inhibitor of PTP1B was discovered by rational screening of phosphotyrosine mimics. The potency of this lead compound has been improved significantly by medicinal chemistry guided by X-ray crystallography and molecular modeling **48** [Ki = 0.37 μM] Excellent consistency has been observed between structure–activity relationships and structural information from PTP1B-inhibitor complexes[75].



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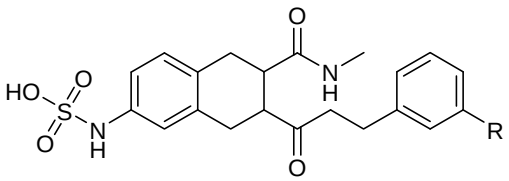
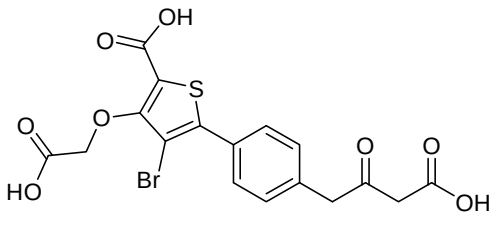


49; R = SO_3H, **50**; R = CO_2H

High-throughput screening of the P&GP corporate repository against several protein tyrosine phosphatases identified the sulfamic acid moiety as potential phosphotyrosine mimetic. Incorporation of the sulfamic acid onto a 1,2,3,4-tetrahydroisoquinoline scaffold provided a promising starting point for PTP1B inhibitor design. Analog **49** was screened against a panel of 14 PTPases but shows only 2-fold selectivity over the closely homologous TC-PTP and 3-carboxylate analog **50** which provided a 16-fold increase in potency[76]. A key hydrogen bond with Asp48 was proven to be pivotal in achieving better inhibition against PTP1B. A series of monocyclic thiophenes was discovered to be competitive and reversible PTP1B inhibitors with good potency and selectivity against other PTPases with the exception of TCPTP **51** (Ki = 0.14μM). This key interaction was confirmed by X-ray co-crystal structures[77]. Replacement of the C2 carboxylic acid of diacid thiophene with another ionizable functional group afforded a reversible and competitive inhibitor. Use of a tetrazole ring or 1,2,5-thiadiazolidine-3-one-1,1-dioxide **52**, **53** as a carboxylate mimetic led to the discovery of two unique starting series that showed improved permeability (PAMPA) and potency of the order of 300 nM[78]. Structure-based optimization of thiophene derivatives from the active site to the second phosphotyrosine binding site to improve potency and selectivity in an efficient manner gave a more than 300,000-fold improvement in potency and examination of PK data led to the discovery of an active uptake mechanism of this class of compounds **54** (Ki = 0.004 μM) into hepatocytes[79].



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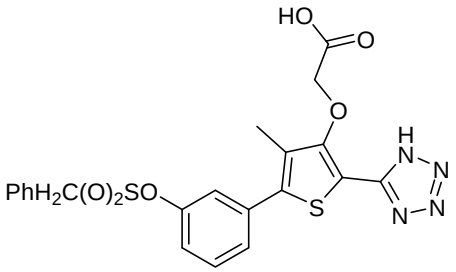

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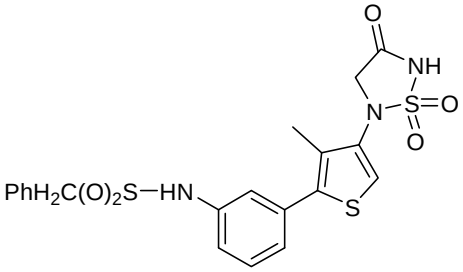

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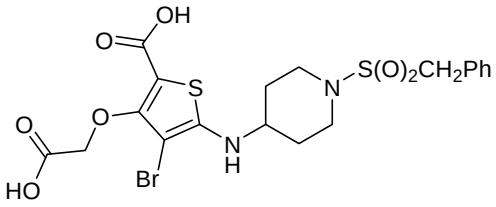
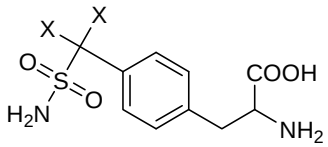


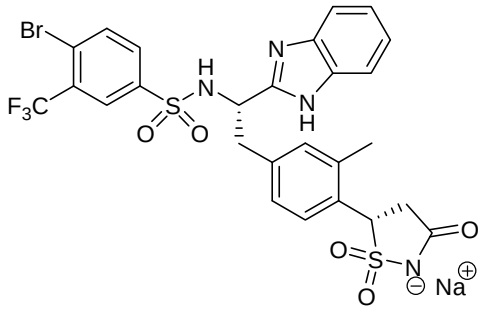
54

Enantioselective synthesis of protected L-4-[sulfonamido(methyl)]phenylalanine **55** and L-4-[sulfonamido(difluoromethyl)]phenylalanine **56** by Bryan Hill *et al* and incorporation of these species into a hexapeptide that has been widely used for assessing pTyr mimics as well as the tripeptide, FmocGlu(OBn)-X-LNH2, has been used by others for evaluating pTry mimics. Although the tripeptides bearing these mimics were relatively good inhibitors results suggest that the phosphate mimicking portion of the tripeptides may not be contributing significantly to their potency and hexapeptide platform may limit the depth of insertion and freedom of the pTyr mimic within the catalytic pocket[80]



55; X=H, **56**; X=F

Synthesis of benzimidazole sulfonamide containing heterocyclic (S)-isothiazolidinone phosphotyrosine mimetic **57 and 58**[81,82], cyclopenta[d][1,2]-oxazine derivatives **59**[83] and derivatives of ursolic acid **60**[84] showed good inhibitory activity with IC_{50} = 18 nM and 270 nM, 0.81 μM and 0.34±0.04 μM respectively against PTP1B.



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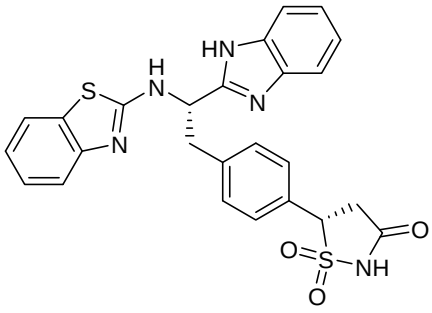

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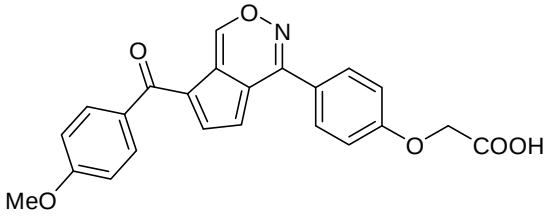

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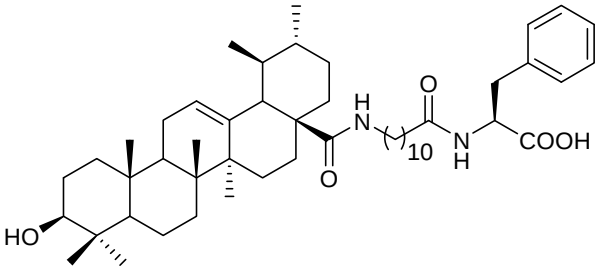
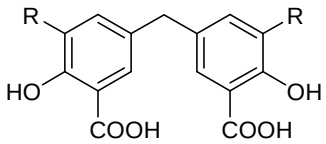
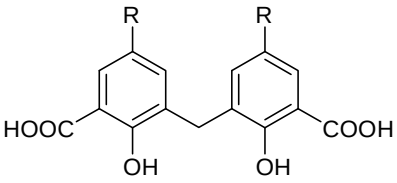


60

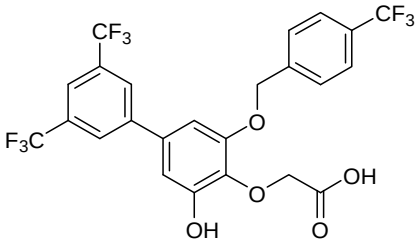
Suja Shrestha et al reported synthesis of methylenedisalicylic acid derivatives and their inhibitory activities against protein tyrosine phosphatases (PTPases). Two of the compounds, **61** and **62**, showed Ki values of 9.4 and 6.3 μM against PTP1B, and compound **63** shows 14-fold selectivity over TCPTP[85,86]. Synthesis of 2-O-carboxymethylpyrogallol derivative **64** shows seven-fold selectivity over TCPTP with Ki value of 1.1 μM against PTP1B[87].



61; R = Ph, **62**; R = Bz

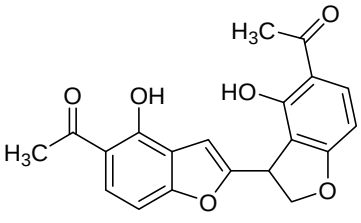


63; R = Ph



64

A series of hydroxy benzofuran methyl ketones and their naturally mimicking dimers and linear and angular furanochalcones and flavones have been evaluated against PTP-1B. Benzofuran dimmers **65**, **66** showed good inhibitory activity (IC_{50}: 58.8 and 56.3 μM) against PTP-1B compared to their monomers. The linear furanoflavonoids **67**, **68** showed better inhibition (67.5%, 75.6%) against PTP-1B[88].



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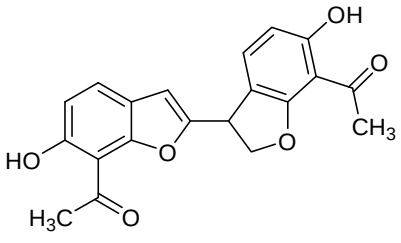

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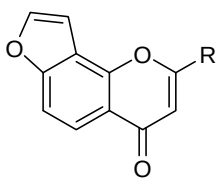
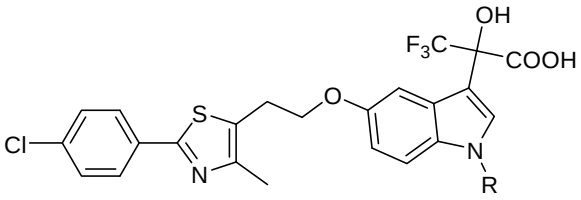
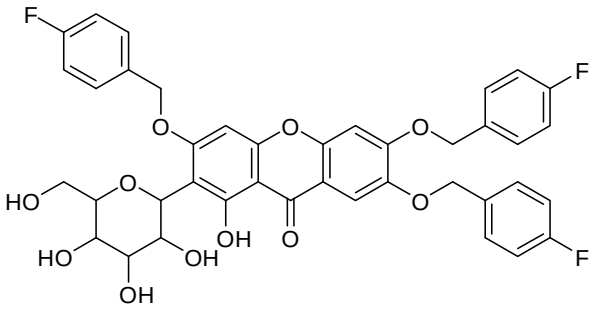


67; R = Phenyl, **68;** R = 4-OMe-C_6H_4



69; R = MOM or SEM or Bn

A new class of 2-Aryl-3,3,3-trifluoro-2-hydroxypropionic acid based inhibitors of PTP1B has been identified by structure-based design. Compounds with 2-(indol-3-yl)- and 2-phenyl-3,3,3-trifluoro-2-hydroxypropionic acid **69** core units targeted at the enzyme's primary site and a hydrophobic chlorophenylthiazole extension in its 2º site exhibit 3–60 μM IC_{50} for PTP1B inhibition in an Sf9 cell-based assay[89]. Synthesis of mangiferin derivates **70**[90] and stilbene derivatives based on lithospermic acid B **71**[91] were found to be good inhibitors of PTP1B, similarly acrolein **72** shows potent time-dependent inactivation of the enzyme PTP1B (k_{inact} = 0.02±0.005 S^{-1} and KI = 2.3 ± 0.6 × 10^{-4} M)[92].



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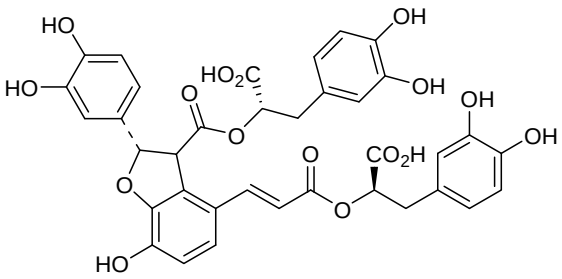
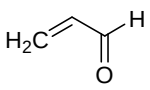


71

Matthew Stuible et al reported synthesis of uncharged thioxothiazolidinone derivatives **73**, an emerging therapeutic target, that provided a starting point for the development of specific inhibitors of PTPs[93].



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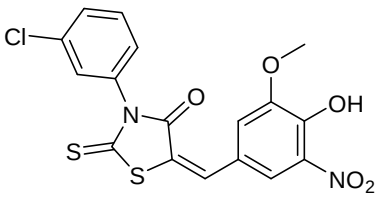
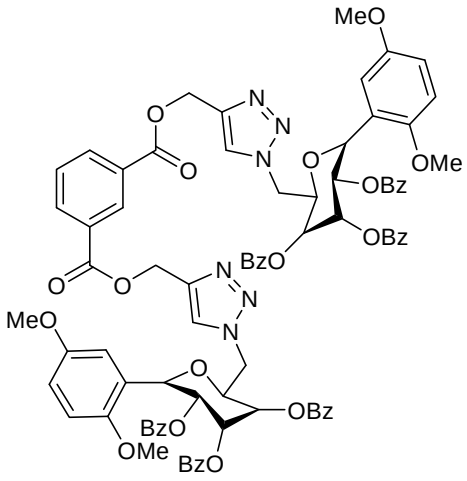


73

Synthesis of dimeric acetylated and benzoylated β-C-D-glucosyl and β -C-D-galactosyl 1,4-dimethoxy benzenes or naphthalenes by click chemistry and were further transformed into the corresponding β-C-D-glycosyl-1,4-quinone derivatives by CAN oxidation. The *in vitro* inhibition test showed that dimeric benzoylated β-C-D-glycosyl 1,4-dimethoxybenzenes **74** or 1,4-benzoquinones **75** were good inhibitors of PTP1B (IC_{50} = 0.62–0.88 μM), with no significant difference between gluco and galacto derivatives[94]. Similarly synthesis of sugar-based β-C-Glycosiduronic acid quinones and β-C-glycosyl compounds showed IC_{50} values of 0.77–5.27 μM against PTP1B, with compounds bearing an acidic function being the most potent **76**[95].



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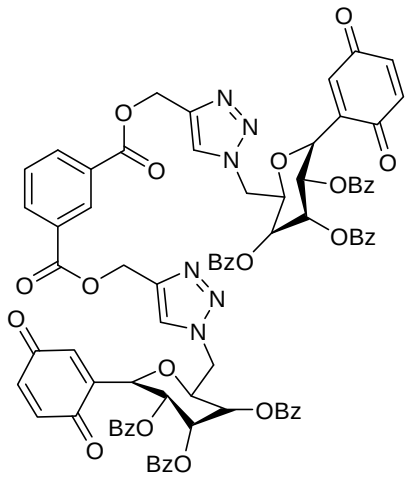

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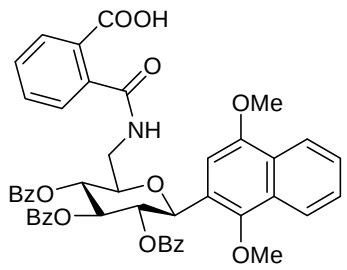

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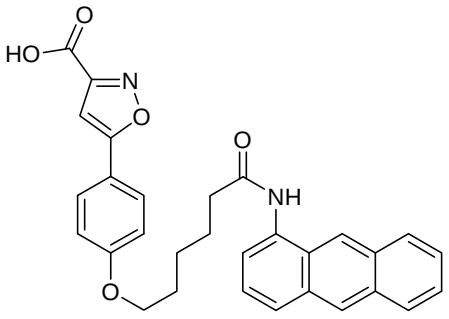
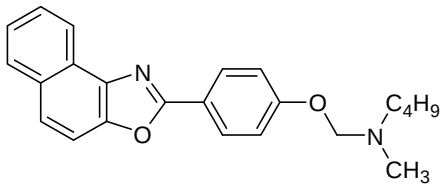


77

Rajavel Srinivasan et al reported the solid phase synthesis of 70-member combinatorial library of analogues of a known PTP1B inhibitor, which upon direct *in situ* screening revealed a potent inhibitor **77** (Ki = 7.0 μM) against PTP1B[96]. Synthesis

and evaluation of *in vitro* PTP-1B inhibitory activity and *in vivo* anti diabetic activity of 2-aryl-naphtho[1,2-*d*]oxazole derivatives **78**[12] (IC_{50} = 2.50 μM) and 3,5-diarylisoxazole derivatives **79** (IC_{50} = 1.50 μM) showed promising activity[97,98].



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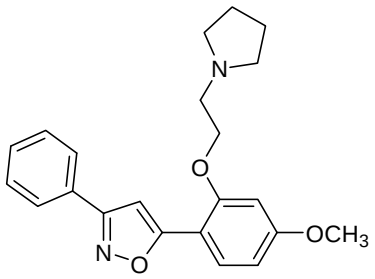
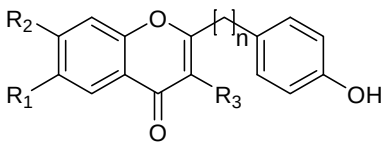


79

Derivatives of chromones **80** [99] and isochroman carboxylic acid **81**[100] was found to be good inhibitor of PTP1B and low moleculae weight PTPs. Synthesis of maslinic acid derivatives **82** (IC_{50} = 0.61 μM) showed good inhibitory as well as selectivity data over TCPTP [101]. Various functionalized mono- and diarylanthranilo-1,3-dinitriles were synthesized and evaluated for their *in vitro* antihyperglycemic activity against PTP-1B, glucose-6-phosphatase, glycogen phosphorylase and α-glucosidase enzymes. Among various screened compounds, 5,6-diaryl substituted anthranilo-1,3-dinitriles **83-85** showed good inhibitory activity against PTP-1B with IC_{50} values of 58– 72 μM[102].



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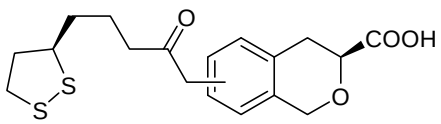

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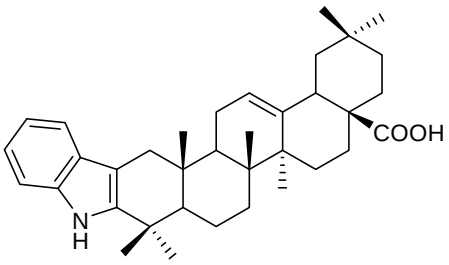

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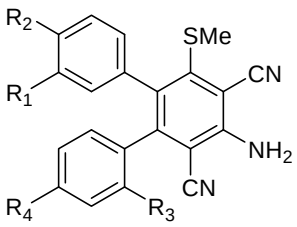
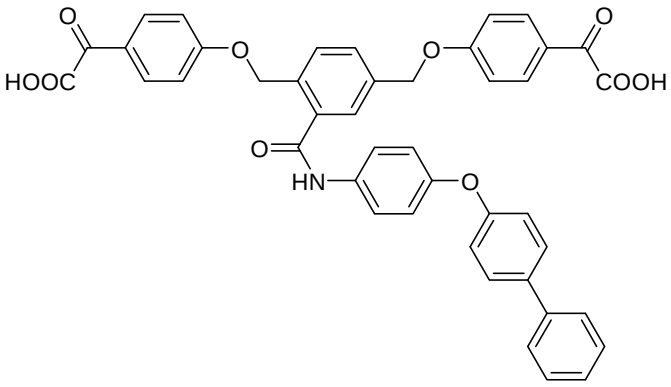


83-85

	R_1	R_2	R_3	R_4
83	H	H	H	H
84	H	H	H	OMe
85	H	OMe	H	OMe

A focused library of bidentate α-ketoacid-based inhibitors has been screened against several PTPs, out of which compound **86** was identified as a potent inhibitor (IC_{50}=220nM) of PTP1B with 30-fold selectivity over TCPTP[103].

As a direct result of chimerical *de novo* design, the synthesis of vanadium coordination compound **87** shows blood glucose lowering effects in rats and among vanadium compounds it as a mild toxicity agent when compared with literature data[104]. Synthesis and characterization of oxovanadium glutamate complex, $Na_2[V(IV)O(Glu)_2(CH_3OH)]H_2O$ ($1.H_2O$) **88** and mixed-ligand oxovanadium(IV) complexes showed potent inhibition against PTP1B with IC_{50} in the 0.21–0.37 μM and 41–75 nM respectively with moderate selectivity over TCPTP[105,106].



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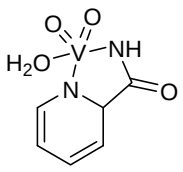

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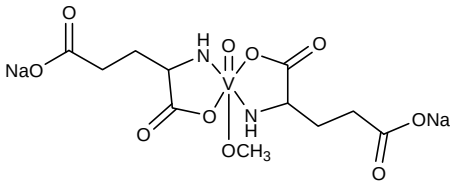
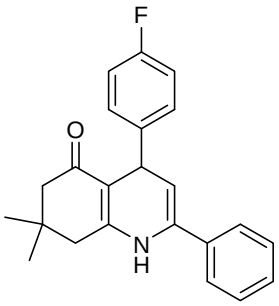


88

Synthesis of a series of 2,4-disubstituted polyhydroquinoline **89**[107], thiophene derivatives **90**[108] and substituted phenoxy-3-piperazin-1-yl-propan-2-ols **91**[109] shows moderate to good inhibitory activity against PTP1B.



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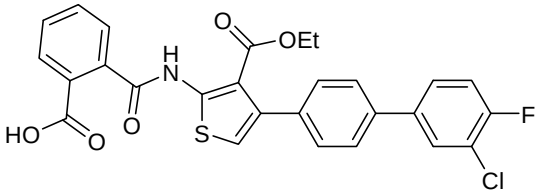

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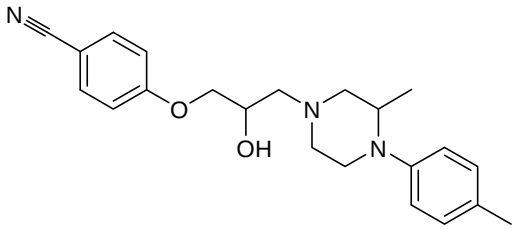

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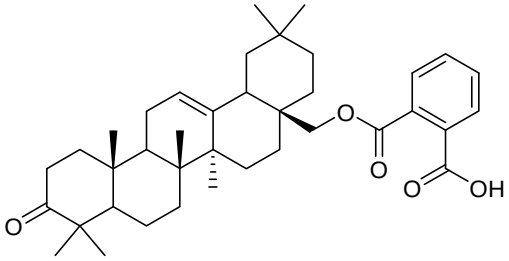
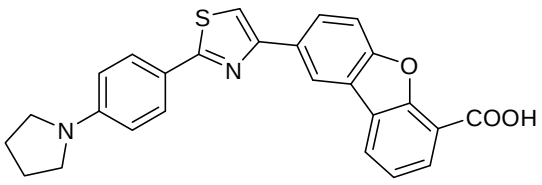


92

Synthesis and evaluation of PTP1B inhibitory activity of derivatives **92** [IC_{50} =3.12 μM] of oleanolic acid demonstrated that the integrity of the A ring and 12-ene moiety is important in the retention of PTP1B enzyme inhibitory activities. In addition, hydrophilic and acidic groups as well as the distance between the oleanene and acid moieties are associated with PTP1B inhibitory activities[110]. With the strategy of occupying both the catalytic site and the nearby, less homologous, non-catalytic phosphotyrosyl binding site, a series of dibenzo[b,d]furan mono-carboxylic acid derivatives **93** [IC_{50} = 82±0.43 nM] were synthesized and on examination revealed the residues important for achieving PTP1B specificity over TCPTP[111].



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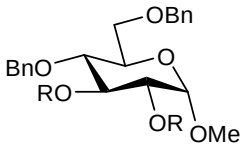

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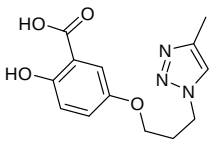
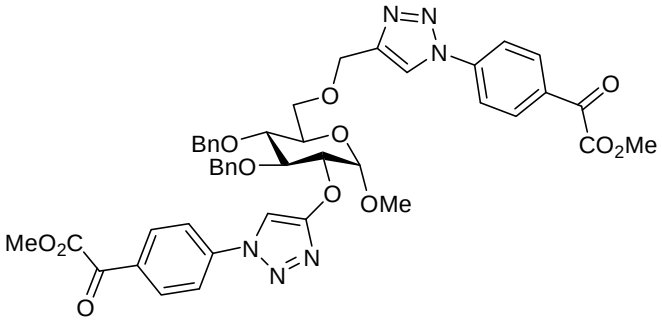


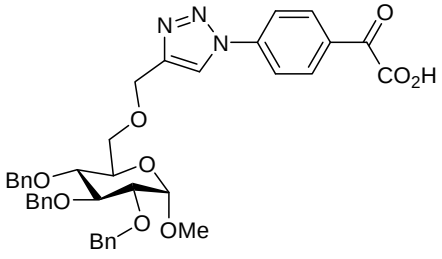


95

PTP1B Inhibitors via click chemistry were reported by Yan-Hui Tang et al and Zhuo Song et al. Synthesis of salicylate-based bidentate PTP1B inhibitors with monosaccharide as a central scaffold and *in vitro* evaluation of PTP1B inhibitory activity led to an identification of the 2,3-disubstituted salicylic 1-methoxy-O-glucoside **94** [IC_{50} =7.7 μM] as the structurally privileged inhibitor[112]. Similarly bidentate 1-O-methyl-α-D-pyranoglucosides bearing two triazolyl α-ketoester groups on the 2,6- or 3,4-positions of sugar scaffold **95** [IC_{50} =1.5 μM] were efficiently synthesized via Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition. These newly featured sugar derivatives displayed favorable inhibitory activity on PTP1B[113]. Glycosylated acetophenone, benzoic acid, and α-ketocarboxylic acid derivatives were efficiently synthesized via Cu(I)-catalyzed 1,3-dipolar cycloaddition. The glycosyl α-ketocarboxylic acids **96** [IC_{50} =3.2 μM] were identified as promising sugar-based PTP1B with several fold selectivities over a panel of homologous PTPs[114]. Xiao-Peng He reported the microwave-assisted construction of triazole-linked amino acid–glucoside conjugates as novel PTP1B inhibitors. Compounds bearing one or two phenylalanine or tyrosine derivatives on the 6-, 2,3-, 2,6-, 3,4- and 4,6-positions of the glucosyl scaffolds were efficiently constructed via the microwave-assisted Cu(I)-catalyzed azide–alkyne cycloaddition. Docking study suggested that 4,6-substituted glucosyl acid **97** [10.8±0.8 μM (Ki =14.4 μM)] possibly performed as a bidentate inhibitor[115].

Evaluation of (±)-3-(2-(2-fluorobenzyloxy)naphthalen-6-yl)-2-aminopropanoic acid derivatives and Pyrrolo[2,3-c]azepine derivatives as novel PTP1B inhibitors

demonstrated compound **98** [IC_{50} =1.25±0.24 μM)] and **99** [IC_{50} =13.92 μM] as good to moderate inhibitors[116,117]



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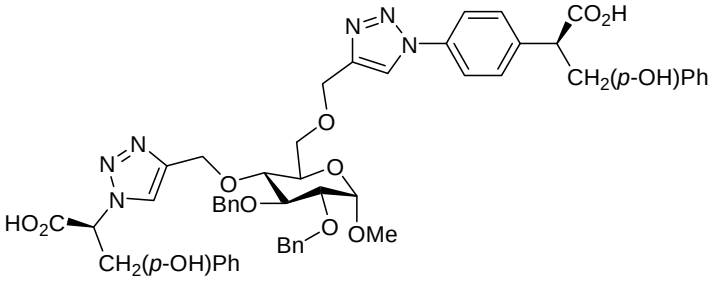

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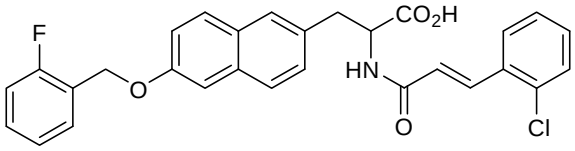

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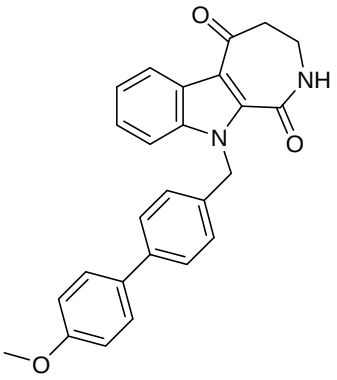
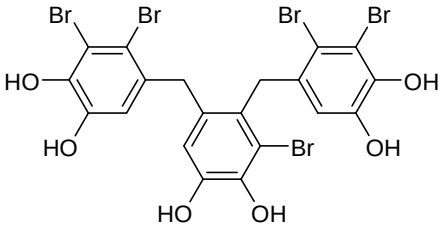


99

3-Bromo-4,5-bis(2,3-dibromo-4,5-dihydroxybenzyl)-1,2-benzenediol (BDB) **100**, is a bromo phenol purified from the marine red alga Rhodomela confervoides and exhibits potent protein tyrosine phosphatase 1B (PTP1B) inhibition (IC_{50} = 1.7 μmol/L). In an effort to improve the PTP1B inhibitory activity, a series of derivatives were designed, synthesized, and evaluated *in vitro*. The preliminary structure-activity relationship indicated that the tricyclic scaffold and multi-bromine atoms (four to five) attached to the aryl rings are important for PTP1B inhibition. Among these, compound **101** exhibited remarkable inhibitory activities against PTP1B with an IC_{50} of 0.89 μmol/L, approximately two fold more potent than **100**[118].



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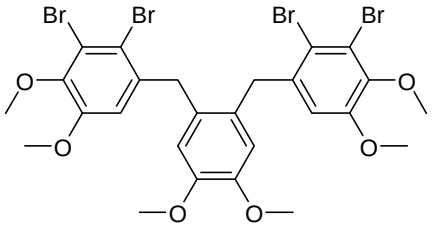
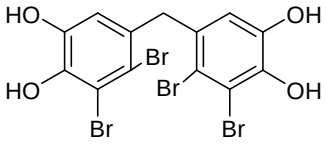


101

Another series of bromophenol derivatives were synthesized based on compound **102** (IC_{50} = 2.42 μmol/L) isolated from red algae Rhodomela confervoides and evaluated as PTP1B inhibitor *in vitro* and *in vivo*. All synthesized compounds displayed weak to good PTP1B inhibition. Among them, highly brominated compd. **103** exhibited promising inhibitory activity against PTP1B with IC_{50} 0.68 μmol/L, with high selectivity against other PTPs (TCPTP, LAR, SHP-1 and SHP-2) and inspiring *in vivo* antidiabetic activity[119].



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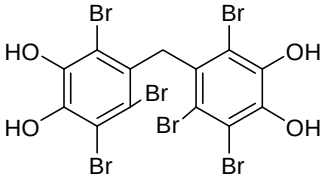
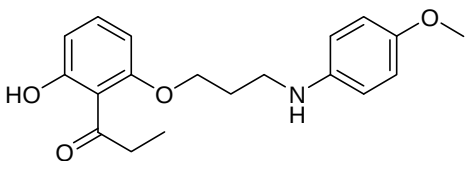
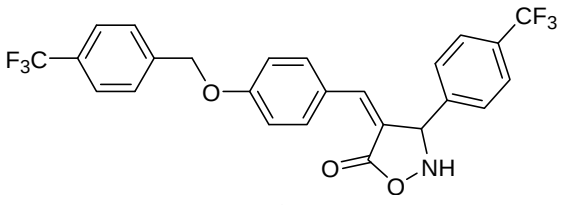


103

Derivatives of propiophenone were synthesized and evaluated for their in vivo antihyperglycemic activities in sucrose loaded model (SLM), sucrose challenged streptozotocin (STZ-S) induced diabetic rat model and C57BL/KsJ db/db diabetic mice model. Compound **104** shows potent antihyperglycemics and lipid lowering. Possible mechanism of action for the propiophenone derivatives was established by the evaluation in various in vitro models and found inhibitor of PTP-1B[120].

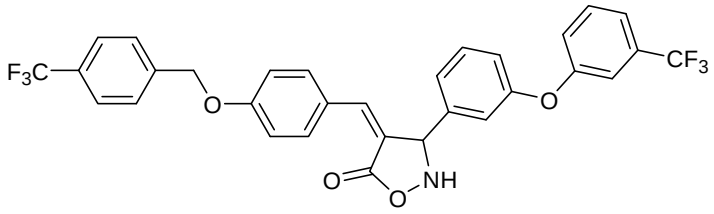


104



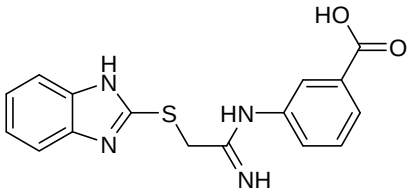
105

Synthesis of an isoxazol-5(4H)-one derivative **105** inhibited PTP1B with an IC_{50} value of 2.3 μM and suppressed weight gain in mice upon feeding a high fat diet (HFD). Further structural variation was made on the isoxazol-5(4H)-one moiety to improve the inhibitory potency of the compound **105** and compound **106** was found 3.3-fold more potent[121].

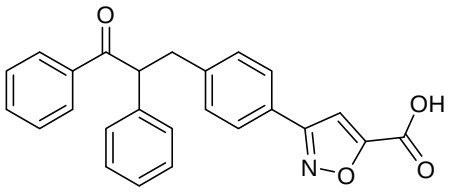


106

Identification and synthesis of novel **107** lead molecules through HTS screening of Zinc database against catalytic domain of PTP1B employing docking algorithm Glide. *in vitro* cellular glucose uptake assay and animal models of hyperglycemia shows promising inhibition of PTP1B enzyme at 10 μm assay[122].

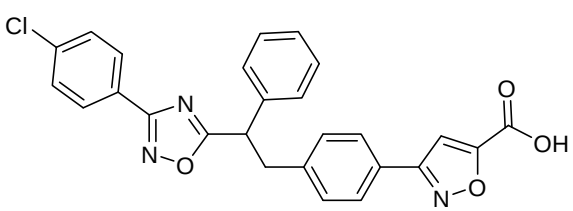


107

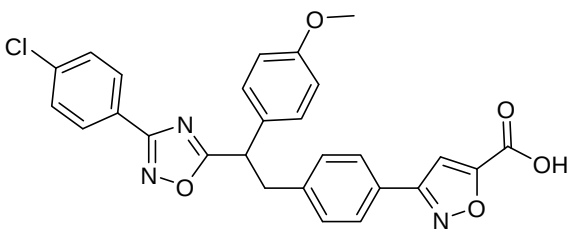


108

Synthesis and SAR optimization of a series of hydrophobic tail containing novel heterocyclic carboxylic acid based inhibitors resulted in identification of several potent, selective (over the highly homologous T-cell protein tyrosine phosphatase, TCPTP) and metabolically stable PTP1B inhibitors. Compound **108-110** showed favorable cell permeability and pharmacokinetic properties in mouse with moderate to very good oral (%F=13-70) bio-availability[123].

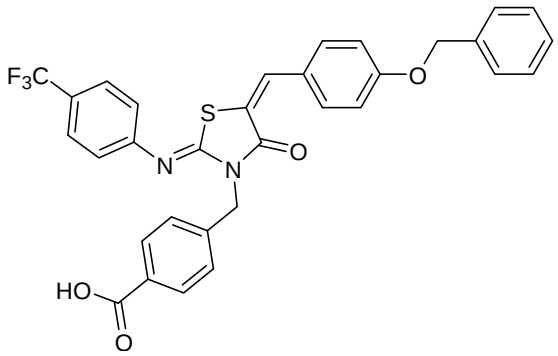


109

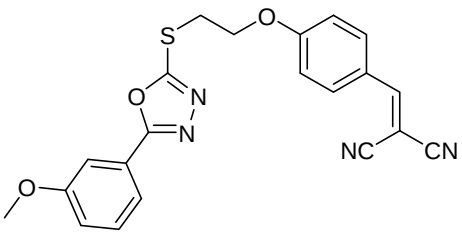


110

Identification of 4-[(5-arylidene-2-arylimino-4-oxo-3-thiazolidinyl)methyl] benzoic acids as a potent inhibitors of PTP1B and LMW-PTP showed selectivity towards PTP1B over the closely related TC-PTP. These compounds were found to activate the insulin-mediated signaling on mouse C2C12 skeletal muscle cells by increasing the phosphorylation levels of the insulin receptor and promoting cellular 2-deoxyglucose uptake. Compound **111** found the best *in vitro* inhibitor of PTP1B and the isoform IF1 of LMW-PTP, provided the highest activation level of the insulin receptor and was found to be endowed with an excellent insulinomimetic effect on the selected cells[124].



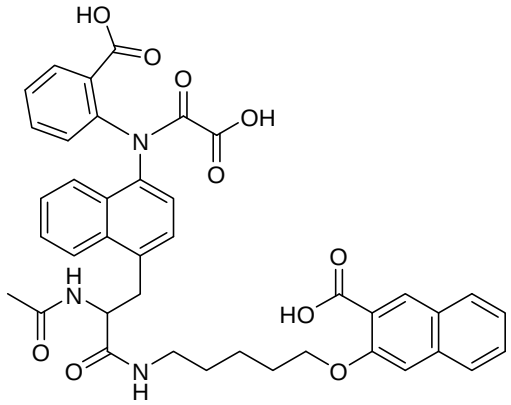
111



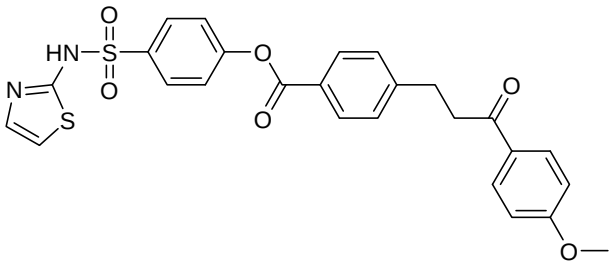
112

Synthesis, biological evaluation and molecular modeling studies of novel non-carboxylic arylidine malononitrile-based molecules were evaluated *in vitro* for glucose reuptake using L6 muscle cell lines and PTP1B. The biological activity results showed that the 2-methoxy substituted **112** (14b) compound exhibited significant activity in both the assays[125].

Based on previously reported allosteric inhibitor **113** of PTP1B, design and synthesis of novel sulfathiazole were evaluated against human recombinant PTP1B. The most active compd. **114** showed IC_{50} value of 3.2 μM and found non-competitive inhibitor of PTP1B. Furthermore, compound **114** demonstrated excellent selectivity to PTP1B over other PTPs. It also displayed in vivo insulin sensitizing effect in the insulin resistant mice[126].

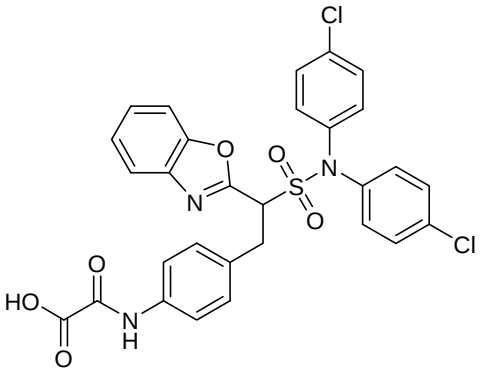


113

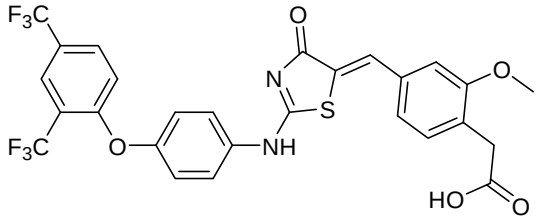


114

A series of oxamic acid containing benzoxazole compounds were synthesized and screened for the PTP1B inhibition. Compound **115** showed (Ki) of 6.7 μM [127].



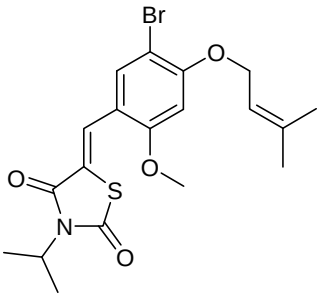
115



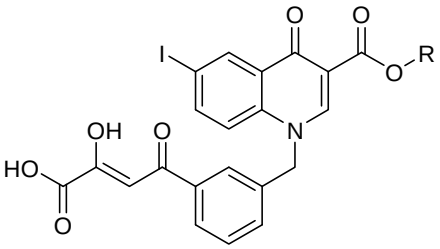
116

In series of thiazolidinone derivatives most of the synthesized compounds behaved as inhibitors of PTP1B. Compound **116** observed with the lowest IC_{50} of 1.4 μM and it showed 4-34 fold selectivity over other phosphatases[128].

Based on previous studies synthesis of 5-(substituted benzylidene)thiazolidine-2,4-dione derivatives were evaluated as competitive inhibitors of protein tyrosine phosphatase 1B (PTP1B). Compound **117**, the most potent among the series, had an IC_{50} of 4.6 μM. Docking studies shows high affinity to PTP1B residues (Gly220, Ala217, Arg221, Asp181, Ser216, Cys215, Phe182, Gln262 and Ile219) in the active sites, indicating that it may stabilize the open form and generate tighter binding to the catalytic sites of PTP1B[129].

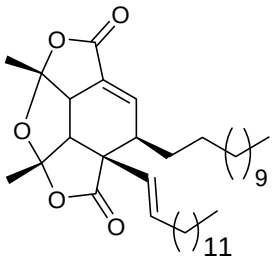


117

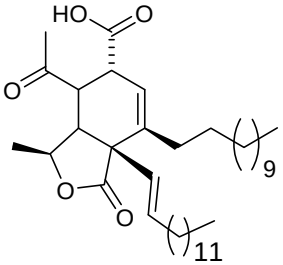


118-119
118; R = H
119; R = OEt

Substitution of hydrophobic groups at C-6, N-1 and C-3 positions of the quinolone motif afforded potent PTP1B inhibitors with low micromolar IC_{50} values. These 4-quinolone-3-carboxylate based PTP1B inhibitors displayed a 2-10-fold selectivity over a panel of PTP's. Furthermore, the bidentate inhibitors of 4-quinolone-3-carboxylic acids conjugated with aryl diketoacid or salicylic acid were cell permeable and enhanced insulin signaling in CHO/hIR cells. The kinetic studies and molecular modeling suggest that the 4-quinolone-3-carboxylates **118** and **119** act as competitive inhibitors by binding to the PTP1B active site in the WPD loop with closed conformation[130].



120

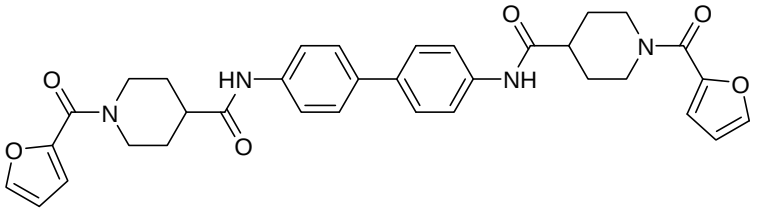


121

A series of structurally related analogs of the natural product paracaseolide A **120** were synthesized and identified as potent PTP1B inhibitors. Among these analogs, compound **121** II in particular showed improved PTP1B enzyme inhibitory activity, high selectivity for PTP1B over TC-PTP, and improved cellular effects[131].

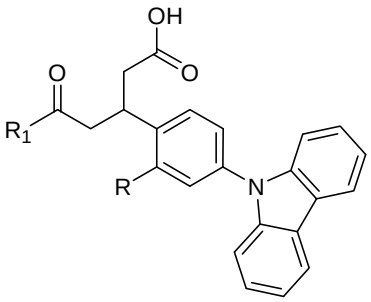
A series of bis-aromatic amides was designed, synthesized, and evaluated as a new class of inhibitors with IC_{50} values in the micromolar range against protein tyrosine phosphatase 1B (PTP1B). Among them, compound 122 displayed an IC50 value of 2.34

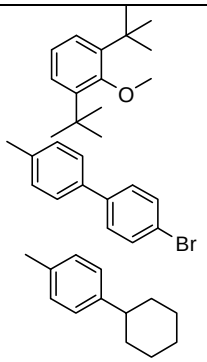
± 0.08 μM with 5-fold preference over TCPTP. More importantly, the treatment of CHO/HIR cells with compound **122** resulted in increased phosphorylation of insulin receptor (IR), which suggested extensive cellular activity of compound **122**. These results provided novel lead compounds for the design of inhibitors of PTP1B as well as other PTPs[132].



122

Previously identified 3-phenylpropanoic acid-based PTP1B inhibitors were synthesized, and three of them, **123**, **124** and **125** showed IC_{50} values at sub-micromolar level. Further in vivo evaluation indicated the sodium salt of **123** not only exhibited significant insulin-sensitizing and hypoglycemia effects, but also decreased the serum levels of triglyceride and total cholesterol in high-fat-diet-induced insulin resistance model mice. Preliminary in vivo pharmacokinetic studies on the sodium salt of **123** revealed its pharmacokinetic profile after oral administration in rats. These results provide proof-of-concept for the dual effects of PTP1B inhibitors on both glucose and lipid metabolism[133].



	R	R_1
123	H	
124	H	
125	F	

123-125