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ORIGINAL RESEARCH

Design, synthesis and biological evaluation of novel arylidine-malononitrile derivatives as non-carboxylic inhibitors of protein tyrosine phosphatase 1B

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Abstract In this study, we describe the design, synthesis, biological evaluation and molecular modelling studies of novel non-carboxylic arylidine malononitrile-based molecules as Protein Tyrosine Phosphatase 1B (PTP1B) inhibitors. The synthesized derivatives were evaluated in vitro for glucose reuptake using L6 muscle cell lines and enzymatic assay against PTP1B. The biological activity results showed that the 2-methoxy substituted (**14b**) compound exhibited significant activity in both the assays. The unsubstituted compound (**14a**) also possessed comparable activity on glucose reuptake in L6 muscle cell lines and better inhibitory activity on PTP1B enzyme assays. Docking analysis was performed on the most potent compound of the series to understand the nature of interactions governing the binding of the designed molecule with the PTP1B enzyme.

Keywords PTP1B · Diabetes · Arylidine malononitrile · Oxadiazole · Non-carboxylic acid inhibitors · Docking study

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Introduction

Diabetes mellitus is a major metabolic syndrome affecting human health in developing and developed nations. There are an estimated 285 million adults with diabetes in 2010 and this number will continue to increase globally to affect 300 million people by the year 2025 due to an ageing population, growth of population size, urbanization and high prevalences of obesity and sedentary lifestyle (Zhang *et al*., 2010; Simpson *et al*., 2003; Shaw *et al*., 2010; Dixit *et al*., 2007). Diabetes leads to both premature death and complications such as blindness, amputations, renal disease and cardiovascular diseases (Zhang *et al*., 2010; Egede and Ellis, 2010; Nicholas, 2003).

Protein tyrosine phosphatase 1B (PTP1B) has been the focus of antidiabetic drug discovery efforts owing to evidence of its role in downregulating insulin transduction mediated by receptor tyrosine kinases such as insulin receptor and epidermal growth factor receptor (Johnson *et al*., 2002; Cheon *et al*., 2004). Inhibitors of this enzyme are predicted to enhance insulin-stimulated glucose transport and have potential for the treatment of type II diabetes. Thus, there is a great interest in PTPs as molecular targets for the development of novel therapeutic agents (Cheon *et al*., 2004).

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In last few years, large numbers of potent and selective PTP1B inhibitors have been reported in the literature (Pei *et al*., 2003; Xin *et al*., 2003; Zhao *et al*., 2004; Cho *et al*., 2006; Wan *et al*., 2006). Most of the inhibitors reported incorporate a charged pTyr mimetic group such as phosphonates, carboxylic acids and sulphamic acids since the active site of enzyme accommodates pTyr, which contains two negative charges at physiological pH. Consequently, the molecules are generally not drug-like and they possess limited cell membrane permeability and bioavailability. Hence, small molecule PTP1B inhibitors devoid of any charged moieties and with good enzyme binding affinity is highly desirable.

Numerous reports on development of non-carboxylic PTP1B inhibitors have emerged recently in literature. Scientists have used many parent structures for novel molecules development, among them, thiazolidinedione derivatives (**1**) (Bhattarai *et al*., 2009), barbituric acid derivatives (**2**) (Kafle *et al*., 2011), isothiazolidinone inhibitors (**3**) (Douty *et al*., 2008), chromones derivatives (**4**) (Forghieri *et al*., 2009) and pyridazine analogues (**5**) (Liljebris *et al*., 2002) possessed significant activity (Fig. 1). As part of our efforts to discover novel non-carboxylic acid PTP1B inhibitors, we identified arylidine malononitrile as a suitable phosphate mimic through computer-aided drug design protocol involving docking analysis. The arylidene malononitrile moiety exhibits favourable interactions with residues flanking the pTyr in the active site of PTP1B and is also well suited for further chemical derivatization. To the best of our knowledge, inhibitory activity of this class of compounds against phosphatases has not been reported in the literature. The most potent compounds were also evaluated using zebrafish embryos for general toxicity related effects at 10 μM concentration. Developing embryos of the zebrafish (*Danio rerio*) are excellent animal models for studying specific developmental effects of small molecules, thus allowing effective in vivo evaluations of potential drugs before embarking on highly expensive studies on rodents and humans.

Results and discussion

Substituted 2-{4-[2-(5-phenyl-1,3,4-oxadiazol-2-ylthio) ethoxy] benzylidene} malononitrile-based derivatives (**14a–h**) were synthesized by known synthetic approaches in several stages, which included reactions of esterification, cyclization and condensation (Scheme 1). Substituted oxadiazoles (**10a–h**) were synthesized in three steps according to reported procedure (Zarghi *et al*., 2008) from the appropriate benzoic acids (**7a–h**). The linker moiety (**12**) was synthesized using para-hydroxybenzaldehyde (**11**) and 1,2 di-bromoethane by reported procedure (Kumar *et al*., 2007) and purified by column chromatography. The final step of synthesis included a simple Knoevenagel condensation of substituted aldehydes with malononitrile (Please refer Supplementary information for detailed synthetic procedure).

The effect of synthesized compounds **14a–h** on protein tyrosine phosphatase inhibition was studied using colorimetric, non-radioactive PTP1B tyrosine phosphatase drug discovery kit-BML-AK 822 from Enzo Life Sciences, USA (Joshi *et al*., 2012a, b). The synthesized compounds **14a–h** were also evaluated for glucose reuptake using L-6 muscle cell lines (Table 1). The percentage glucose reuptake results showed that the compound with 2-methoxy substitution in the aromatic ring (**14b**) possessed significant activity than any other compounds in the series. Introduction of nitro group (**14 h**) or electronegative fluoro group (**14f**) in the aromatic ring resulted in decreased glucose reuptake activity. Further, substitution in the meta position on the aromatic ring (**14c** and **14h**) appears to be unfavourable to glucose reuptake activity shown by the title compounds. The unsubstituted derivative (**14a**) shows significant activity than meta- and para-substituted compounds (**14c, 14e–h**) but these compounds (**14c, 14e–h**) exhibit comparable activity with the reference compound (rosiglitazone). Overall, the SAR study suggests that substitution in ortho position confers greater glucose uptake activity.

The PTP1B inhibitory activity results show that the unsubstituted compound (**14a**) possessed significant activity than other compounds in the series (**14b–h**). Together with the good glucose reuptake activity, the compound **14b** also possessed better PTP1B inhibitory activity than other compounds in the series (**14c–h**). The 2-chloro-substituted compounds (**14d**) exhibited the least activity in the series. In contrast to the glucose reuptake activity, the compounds substituted on the 3-position of the benzene ring (**14c** and **14h**) exhibited significant PTP1B inhibitory potency.

The biological activity results showed that the substitution on the benzene ring connected directly to the oxadiazole ring has little effect on enhancing PTP1B inhibition but elicits greater effect in glucose reuptake activity. It is confirmed from the activity results from both assays that the unsubstituted compound **(14a)** has remarkable activity. Even though the meta-substitution (**14c** and **14h**) on the benzene ring is detrimental for the glucose reuptake activity it is favoured for the PTP1B inhibition. Both electron releasing substituents such as methyl and methoxy and electron withdrawing substituents in the molecules exhibit enhanced PTP1B inhibitory activity, while electronegative halogen groups (**14d–f**) were found to be unfavourable for the activity. Moreover, the lack of correlation between the glucose uptake assay results and PTP1B inhibition assay result highlights that multiple mechanisms may be involved in the antidiabetic potency showed by the studied compounds. Nevertheless, the

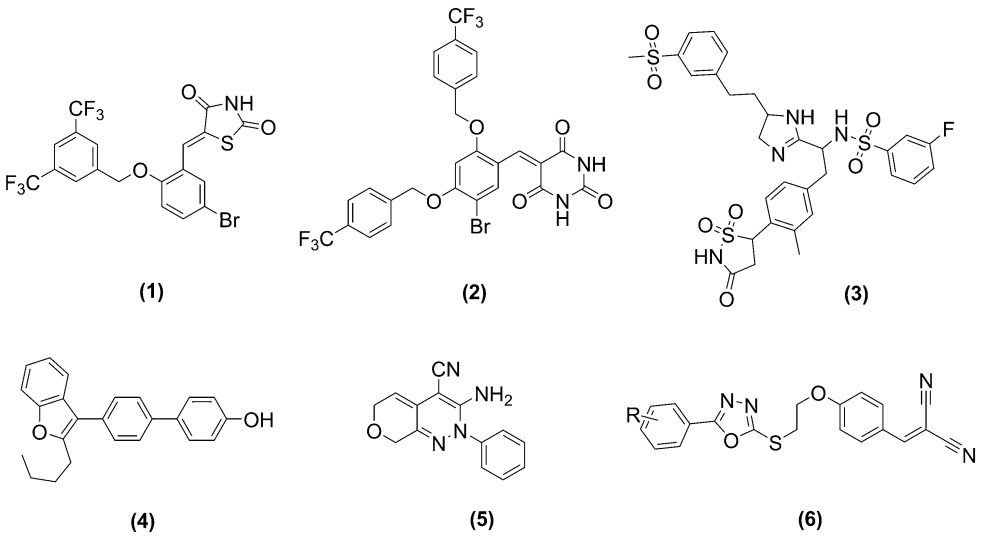
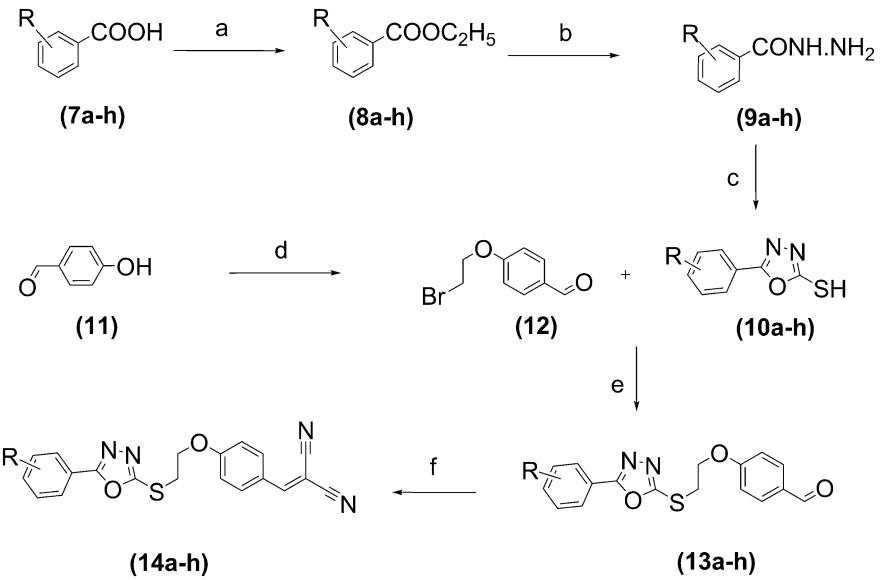


Fig. 1 Structure of potent PTP1B inhibitors and compounds synthesized in the study



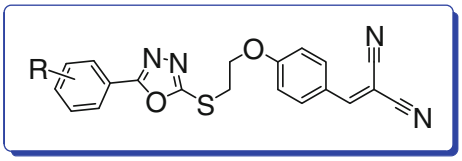
Scheme 1 Reagent and conditions: *a* EtOH, reflux, 8 h; *b* $NH_2NH_2.H_2O$, reflux, 6 h; *c* CS_2, Ethanol, reflux, 5 h; *d* 1,2-di-bromoethane, K_2CO_3, acetone, 70 °C, 20 h; *e* K_2CO_3, acetone, 70 °C, 2–4 h; *f* malononitrile, methanol, R.T., 1–2 h

PTP1B inhibition showed by the compounds and the novelty of the structure in comparison to the previously reported PTP1B inhibitors warrant further in silico studies to understand their binding mode to PTP1B enzyme.

Related to the foregoing, molecular docking studies were performed in order to gain further insight into the binding mode of the compounds into PTP1B enzyme. The most potent compounds of the series were docked into the active site of the enzyme using using Maestro, version 9.2 implemented from Schrodinger software suite (Maestro, 2011). The protein for docking study was obtained from Protein Data Bank (PDB ID: 1Q1M), was prepared by removing solvent, adding hydrogens and minimization in the presence of bound ligand using protein preparation wizard. Grids for molecular docking were generated with bound co-crystallized ligand. The compounds were docked using Glide in extra-precision (XP) mode, with up to three poses saved per molecule.

The docking study has predicted good binding of the compounds with the catalytic binding site of PTP1B. The Docking orientation and interactions of molecule **14a** with catalytic site residues of PTP1B are shown in Figs. 2 and 3. As expected, the arylidine-malononitrile moiety extends towards the arginine binding region (SITE A) and the nitrile group interacts with Arg221 residue in the active site through hydrogen bonds (Fig. 3). Further, the phenyl ring of the arylidine malononitrile shows hydrophobic interaction with phenyl ring of Tyr46. In addition to the PTP1B catalytic site, Puius *et al*., (1997) has reported an additional aryl phosphate-binding site in PTP1B. This second aryl phosphate-binding site is made up of residues such as

Table 1 In vitro glucose uptake and PTP1B enzyme inhibitory activity for compounds **14a–h**



Compound	*R*	% PTP1B inhibition (at 10 μM)	% glucose uptake in L6 muscle cell line (at 50 μM)
14a	H	54.6	25.3
14b	2-MeO	50.0	38.5
14c	3-MeO	39.0	13.5
14d	2-Cl	23.4	26.6
14e	4-Cl	34.3	24.2
14f	4-F	30.4	13.3
14g	4-CH_3	44.5	18.8
14h	3-NO_2	41.4	11.8
Metformin	–	–	30.2*
Rosiglitazone	–	–	25.2**

* At 500 μM

** At 100 μM

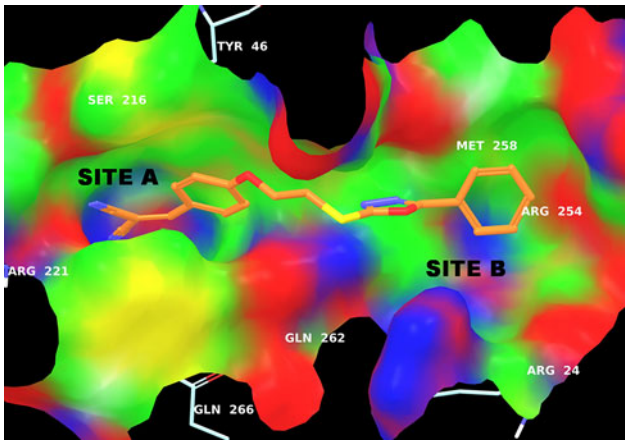


Fig. 2 Docking orientation of **14a** in catalytic pocket (Site A & B) of PTP1B

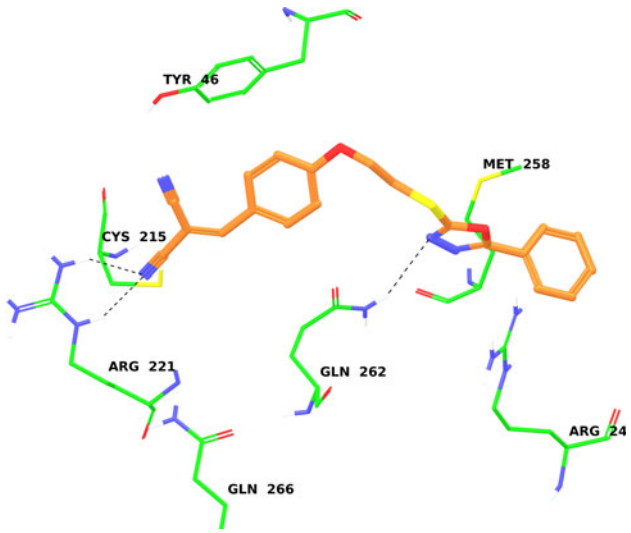


Fig. 3 Binding pose and interactions of **14a** with catalytic site residues of PTP1B

Arg24, Arg254, Met258, Phe52 and Gln262. The nitrogen atom (N3) of 1,3,4-oxadiazole ring is interacting with Gln262 of additional phosphate-binding site by hydrogen bonding as well as few hydrophobic interactions between substituted phenyl ring and hydrophobic residues of this additional phosphate-binding site are also observed. Our special interest was potential interactions between inhibitor functionality with residues in this additional aryl phosphate-binding site, as it has been proven to contribute towards PTP1B selectivity. By design, it was expected that the phenyl oxadiazole moiety would be accommodated in the additional aryl phosphate-binding site (SITE B) and this orientation would be helped by flexible diethylene linker. The docking studies show that this orientation can exist where the phenyl ring attached oxadiazole is flanked by hydrophobic residues such as Phe52 and Met258.

The most active compounds **14a** and **14b** were screened for general toxicity studies using zebrafish embryos. The embryos were grown in the presence of the compounds for 24, 48 and 72 h. Control embryos were incubated in 0.1 % DMSO. Finally, embryos were observed using visible light microscopy at regular intervals after compound treatment to document general toxicity related effects such as

developmental delays, deformations, oedema and death. The results indicate that none of the tested compounds were found to be having significant toxic effect on zebrafish embryos (please refer supplementary information for detailed experimental procedure).

Conclusion

In conclusion, this work reports novel arylidine-malononitrile derivatives as non-carboxylic inhibitors of protein tyrosine phosphatase 1B. Compound **14a**, the unsubstituted derivative showed maximum PTP1B inhibition whereas the compound **14b**, the 2-methoxy substituted derivative exhibited maximal efficacy in glucose reuptake assay. Molecular docking studies of the compound **14a** showed that arylidine-malononitrile group in the compounds interacts with the PTP1B catalytic site and the phenyl oxadiazole moiety in the compounds is accommodated in the additional aryl phosphate-binding site. Overall, the present research work has clearly established the studied arylidene malononitrile derivatives as potential non-carboxylic protein tyrosine phosphatase 1B inhibitors. Further refinement of analogues is ongoing and will be reported in due course.

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