Design, synthesis and biological evaluation of some tetrazole acetamide derivatives as novel non-carboxylic PTP1B inhibitors

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ABSTRACT

A series of ten \textit{N}-(3-(1H-tetrazole-5-yl)phenyl)acetamide derivatives (NM-07 to NM-16) designed from a lead molecule identified previously in our laboratory were synthesized and evaluated for protein tyrosine phosphatase 1B (PTP1B) inhibitory activity. Among the synthesized molecules, \textbf{NM-14}, a 5-Cl substituted benzothiazole analogue elicited significant PTP1B inhibition with an IC\textsubscript{50} of 1.88 µM against reference standard suramin (IC\textsubscript{50} ≥ 10 µM). Furthermore, this molecule also showed good \textit{in vivo} antidiabetic activity which was comparable to that of standard antidiabetic drugs metformin and glimepiride. Overall, the results of the study clearly reveal that the reported tetrazole derivatives especially \textbf{NM-14} are valuable prototypes for the development of novel non-carboxylic inhibitors of PTP1B with antidiabetic potential.

1. Introduction

Protein tyrosine phosphatase 1B (PTP1B), is an intracellular tyrosine phosphatase which modulates a number of cellular signal transduction pathways in insulin-responsive tissues such as fat, muscle or liver [1,2]. PTP1B functions as a negative modulator of insulin and leptin signalling pathways that regulates lipid and glucose metabolism in adipose tissues and skeletal tissues [3-6]. Hyperactivation of PTP1B is implicated in metabolic disorders such as type 2 diabetes mellitus (T2DM) and obesity [7-10]. Knockout studies in mice models have shown that the removal of PTP1B gene increased sensitivity towards insulin and also enhanced glucose tolerance without interfering normal growth or other essential functions [11]. Hence, PTP1B is considered to be a promising therapeutic target for the development of new drug molecules for the management of type 2 diabetes [12,13]. Consequently, there have been extensive reports from industry and academia on development small-molecule inhibitors of PTP1B that targeted the active site of the enzyme (Fig. 1) [12-15]. However, the clinical translation of these PTP1B inhibitors have not been successful owing to lack of cell permeability and bioavailability. This is primarily because the catalytic site of PTP1B is positively charged at normal body pH and competitive inhibitors of PTP1B requires incorporation of a negatively charged pTyr mimetic group in their molecular framework for inhibitory potency [16,17]. However, this negative charge reduces cell permeability and bioavailability which impedes clinical development [18]. Thus, small molecule PTP1B inhibitors with good enzyme binding affinity and devoid of any charged functional groups are highly desirable to combat the type II diabetes.

As a part of our continuing efforts expended towards development of ‘drug-like’ PTP1B inhibitors that lacks negatively charged pTyr mimetic, we have previously reported a series of \textit{N}-(3-(1H-tetrazole-5-yl) phenyl)acetamide derivatives as cell permeable and orally bioavailable PTP1B inhibitors. The aforementioned tetrazole derivatives were derived from a lead molecule ZINC02765569 by replacing the carboxylic phosphate 1B (PTP1B) inhibitory activity. Among the synthesized molecules, \textbf{NM-14}, a 5-Cl substituted benzothiazole analogue elicited significant PTP1B inhibition with an IC\textsubscript{50} of 1.88 µM against reference standard suramin (IC\textsubscript{50} ≥ 10 µM). Furthermore, this molecule also showed good \textit{in vivo} antidiabetic activity which was comparable to that of standard antidiabetic drugs metformin and glimepiride. Overall, the results of the study clearly reveal that the reported tetrazole derivatives especially \textbf{NM-14} are valuable prototypes for the development of novel non-carboxylic inhibitors of PTP1B with antidiabetic potential.
and also replaced the benzimidazole with 'biosisosteric' benzthiazole (NM-13 to NM-15) and benzoxazole ring (NM-16). The synthesis and in vitro evaluation of PTP1B inhibitory activity of the newly designed tetrazole analogues (NM-07 to NM-16) are described herein.

All the designed molecules were synthesized by a two-step reaction as given in scheme 1 [12]. The reaction started with chloroacetylation of 3-(1H-tetrazole-5-yl)aniline (7) under basic conditions in DMF to get an intermediate compound N-(3-(1H-tetrazole-5-yl)phenyl)-2-chloroacetamide (8) which was subsequently reacted with substituted benzimidazole, benzoxazoles and benzothiazole thiols under base catalysis to obtain the title molecules (NM-07 to NM-16). The chemical structures of all the synthesized molecules of the series were confirmed by spectral data.

All the compounds were assayed for PTP1B inhibitory activity according to the protocol provided with assay kit. Initially, all the molecules were tested in vitro for % inhibition of PTP1B at 30 μM and those showing less than 80% inhibition at this concentration were regarded as inactive while compounds displaying more than 80% inhibition were considered as active. These active molecules were further assessed at various concentrations to calculate the IC50 values using non-linear regression method through dose-response data. The results obtained from this assay are shown in Table 1.

The SAR analysis of PTP1B inhibition data of benzimidazole derivatives (NM-07 to NM-12) revealed that introduction of electron releasing group (NM-07, NM-09, NM-11) and electron withdrawing group (NM-07) in the benzo ring resulted in decreased PTP1B inhibition activity while substitution with halogen atoms (NM-10 & NM-12) caused a moderate improvement in PTP1B inhibitory activity compared to unsubstituted analogue NM-01 (≈33% inhibition). In case of benzothiazole derivatives (NM-13 to NM-15), 6-NO2 substituted compound NM-13 and 5-Cl substituted compound NM-14 in the benzo ring, showed potent PTP1B inhibition at less than 10 μM concentration. In fact, compound NM-14 is the most potent among the studied of N-(3-(1H-tetrazole-5-yl)phenyl)acetamide analogues which have shown an IC50 value of 1.88 μM. Replacement of benzothiazole of NM-14 with benzoxazole ring (NM-16) resulted in an almost two-fold reduction in PTP1B inhibition (IC50 = 6.04 μM).

Molecular docking study was carried out for the most active compound (NM-14) to understand its binding mode interactions with the catalytic binding site of enzyme (1Q1M as PDB ID) [22] by using the Glide docking algorithm [23]. The docking orientation of the NM-14 in the catalytic site of enzyme is shown by Fig. 3 where amino acid residue Glu262 interacted with the nitrogen atom of benzothiazole moiety and acetamido linker group respectively through hydrogen bonds. Another single hydrogen bonding interaction was observed between guanidine moiety of amino acid Arg221 and the nitrogen atom of the tetrazole nucleus at the catalytic site of the PTP1B enzyme.

The synthesized molecule NM-14 elicited potent in vitro inhibition
of PTP1B enzyme (IC$_{50}$ < 2 µM). Therefore, in vivo animal studies were performed to establish its antihyperglycaemic efficiency in animal models. Firstly, the compound was evaluated for its antidiabetic activity in oral glucose tolerance (OGTT) test using Sprague dawley rats (Refer supplemeny data). Initially, all the rats were loaded with oral glucose (2 g/kg) after 30 min, test compounds and standard drug, glimepiride (30 mg/kg and 0.1 mg/kg respectively) were administered orally. The level of serum glucose was measured at a different time period from 0, 15, 30, 60, 90, 120 and 180 min. (Fig. 4) The serum glucose level was found to be elevated in control groups after giving a glucose load. However, the animals treated with test compounds and standard drug showed a decrease in the glucose level after some time (Fig. 4). The variation observed in Tmax values indicates significantly different pharmacodynamic behaviour for glimepiride and test compounds respectively. The reduction in glucose level after treatment through the standard drug (glimepiride) and test compound (NM-14) was observed by 23.61% and 30.25% respectively. Overall, the outcome of OGTT suggested that NM-14 can reduce blood glucose more effectively than glimepiride.

The streptozotocin (STZ) induced type II diabetic rat model was also used to ascertain in vivo antidiabetic activity of test compound NM-14 (Refer supplementery data) which revealed that the ability for the reduction in glucose level of the diabetic animals by compound NM-14 (30 mg/kg) is almost same as metformin (300 mg/kg) which suggest that test compound produced an almost comparable antidiabetic effect to standard drug metformin (Fig. 5). Furthermore, less statistical difference was observed in blood glucose levels of normal rat and control rat beyond one month (28th day) of treatment which indicates the lack of hypoglycaemic side effect of the test compound during chronic treatment.

In conclusion, a series of N-(3-(1H-tetrazole-5-yl) phenyl)acetamide analogues were synthesized and evaluated for their PTP1B inhibitory activity. Preliminary SAR analysis reveals that halogen substituents on the benzo ring of benzimidazole and benzothiazole containing N-(3-(1H-tetrazole-5-yl) phenyl) acetamide analogues enhances PTP1B inhibition shown by these compounds. Compound NM-14, a 5-Cl substituted benzothiazole derivative showed most potent PTP1B inhibitory potency with an IC$_{50}$ value of 1.88 µM. Furthermore, NM-14 also showed comparable antidiabetic efficacy to that of standard anti-diabetic drugs in two in vivo models. The potent in vitro PTP1B inhibitory activity and in vivo antidiabetic efficiency clearly establish compound NM-14 as an important lead molecule for further exploration. To this end, studies are underway in our laboratory to optimize the structural features of NM-14 with an objective to improve its inhibitory efficacy or in vivo potency against PTP1B and the outcomes of the study will be published in due course.

Scheme 1. Synthesis of N-(3-(1H-tetrazole-5-yl) phenyl)acetamide analogues.
<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Structure</th>
<th>% Inhibitory activity (30μM)</th>
<th>IC₅₀ (μM)</th>
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</table>
Fig. 3. The interaction pattern of most active PTP1B inhibitor at PTP1B enzyme.

Fig. 4. Effects on blood glucose levels by NM-14 in OGTT.

Fig. 5. Effects on blood glucose levels by NM-14 in STZ induced diabetic rats.
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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103221.

References


