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Synthesis, biological activity and structure–activity relationships of new benzoic acid-based protein tyrosine phosphatase inhibitors endowed with insulinomimetic effects in mouse C2C12 skeletal muscle cells

Rosaria Ottanà [a], Rosanna Maccari [a,*], Jérémie Mortier [b,c], Anna Caselli [d], Simona Amuso [a], Guido Camici [d,e], Archimede Rotondo [f], Gerhard Wolber [b], Paolo Paoli [d]

[a] Dipartimento di Scienze del Farmaco e dei Prodotti per la Salute, University of Messina, Polo Universitario dell'Annunziata, Viale SS. Annunziata, 98168 Messina, Italy
[b] Institute of Pharmacy, Department Pharmaceutical & Medicinal Chemistry, Freie Universität Berlin, 14195 Berlin, Germany
[c] Institute of Chemistry and Biochemistry, Organic Chemistry, Freie University Berlin, 14195 Berlin, Germany
[d] Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Sezione di Scienze Biochimiche, University of Firenze, Viale Morgagni 50, 50134 Firenze, Italy
[e] Center for Research, Transfer and High Education DENOthe, Viale Pieraccini 6, 50139 Firenze, Italy
[f] Dipartimento di Scienze Chimiche, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy

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ABSTRACT

Insulin resistance is a complex altered metabolic condition characterized by impaired insulin signaling and implicated in the pathogenesis of serious human diseases, such as diabetes, obesity, neurodegenerative pathologies. In pursuing our aim to identify new agents able to improve cellular insulin sensitivity, we have synthesized new 4-[(5-arylidene-4-oxo-2-phenylimino/oxothiazolidin-3-yl)methyl] benzoic acids (**5**, **8**) and evaluated their inhibitory activity towards human protein tyrosine phosphatases PTP1B, LMW-PTP and TCPTP, enzymes which are involved in the development of insulin resistance. Compounds **5** and **8** showed from moderate to significant selectivity toward PTP1B over both the highly homologous TCPTP and the two isoforms of human LMW-PTP. In addition, most of the tested compounds selectively inhibited LMW-PTP IF1 over the isoform IF2. Docking studies into the active sites of PTP1B and LMW-PTP aided the rationalization of the observed PTP inhibitory profile. Moreover, most tested compounds were capable to induce the insulin metabolic pathway in mouse C2C12 skeletal muscle cells by remarkably stimulating both IRβ phosphorylation and 2-deoxyglucose cellular uptake.

1. Introduction

Diabetes mellitus is a serious metabolic disease, which currently affects more than 371 million people worldwide, with a global prevalence of 8.3%. The number of people with diabetes is rapidly growing in every country, correspondingly with the current obesity epidemic, and is predicted to rise to almost 10% of total world population by 2030. More than 90% of diabetic patients suffer from type 2 diabetes (T2DM) [1]. The onset of T2DM is mainly linked to the development of insulin resistance, an altered metabolic condition in which the response of target tissues (such as fat tissue, liver and skeletal muscle) to the hormone is significantly impaired. Attenuated insulin signaling results in numerous metabolic and cellular alterations, including diminished cellular glucose uptake, increased liver gluconeogenesis, hyperglycemia and hyperlipidemia. Increased levels of blood glucose and lipids can induce further deterioration of both insulin action and secretion as well as trigger sequences of tissue and vascular dysfunctions that lead to chronic diabetic complications and increased cardiovascular risk [2,3].

The multiple metabolic and tissue abnormalities induced by insulin resistance can contribute to the development of metabolic syndrome, a cluster of correlated comorbid disorders that, beside

Abbreviations: 2-DOG, 2-deoxyglucose; IR, insulin receptor; LMW-PTP, low molecular weight protein tyrosine phosphatase; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PTP, protein tyrosine phosphatase; STAT, signal transducer and activator of transcription; TCPTP, T cell protein tyrosine phosphatase; T2DM, type 2 diabetes mellitus.

* Corresponding author. Tel.: +39 090 6766406.
E-mail address: rmaccari@unime.it (R. Maccari).

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T2DM or impaired glucose tolerance, include obesity, dyslipidemia, hypertension, increased levels of inflammatory mediators, endothelial dysfunction, impaired thrombolysis and increased risk of cerebrovascular and cardiovascular diseases [2,4].

Obesity can contribute to further attenuate the sensitivity of target tissues to insulin. In fact, a number of cytokines produced by adipose tissue (adipokines), such as tumor necrosis factor-α (TNFα) and leptin, can influence the response to the hormone. TNFα impairs several components of the insulin signal pathway, thus causing reduced glucose uptake and insulin resistance of target tissues [4,5]. Leptin activates specific receptors in hypothalamus, thus reducing food intake and increasing energy expenditure, and also exerts direct effects on cellular insulin sensitivity. States of leptin deficiency or resistance, which are associated to obesity, lead to lipid overaccumulation in nonadipose tissues, such as liver and muscle, thus attenuating insulin signaling and contributing to the development of insulin resistance [6,7].

Recent compelling evidence supports the pivotal roles of insulin in neuronal survival, synaptic plasticity and memory processing and highlights a direct implication of insulin resistance also in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease (AD), particularly in agedness [8–10]. AD has even been described as "type 3 diabetes" and it has been suggested that it could be considered mainly as a metabolic disease characterized by a progressive loss of the brain capacity to respond to insulin and insulin-like growth factor stimulation that results in cognitive impairment [11]. Peripheral insulin resistance contributes to the development of brain insulin resistance by inducing the production of toxic lipids that are able to promote neurodegeneration [11]. Thus, it has been suggested that the soaring incidence of diseases characterized by peripheral insulin resistance, such as obesity, T2DM and metabolic syndrome, might represent an important factor contributing to the current AD epidemic.

Considering that insulin resistance is one of the most prevalent metabolic disorders worldwide and is crucially implicated in the pathogenesis of common serious chronic diseases, there is an urgent need to discover new drugs able to treat this pathological condition.

The actions of insulin are mediated by multiple signal pathways triggered by the insulin receptor (IR) tyrosine kinase which acts on different intracellular substrates [12]. In this context, the pathophysiological events that underlie the development of insulin resistance are not fully understood and, consequently, fighting this condition may be a challenging task.

However, several alterations in post-receptor events of insulin cascade that lead to attenuated transduction of the hormone signal have been identified as major causes of insulin resistance in target tissues [3,13]. Out of these molecular mechanisms, the increased activity and/or overexpression of the cytosolic protein tyrosine phosphatase 1B (PTP1B) have been recognized to be key components in the development of insulin resistance, as established by numerous studies in both animal models and humans [14–17].

PTP1B acts as a crucial negative regulator of both insulin and leptin signal transduction. It controls the response of target tissues to insulin by inactivating IR through the dephosphorylation of specific phosphotyrosine (pTyr) residues on the receptor β subunits as well as by dephosphorylating insulin substrate proteins (IRS), which are downstream components of the insulin signal cascade [12]. Moreover, PTP1B negatively regulates leptin signaling by dephosphorylating and inactivating the Janus 2 kinase that is associated with leptin receptor, thus preventing the activation of downstream molecule STAT3 (signal transducer and activator of transcription 3) and critically modifying the action of leptin in the regulation of food intake and body weight [6,18].

Dysfunctions in PTP1B activity or expression can be induced by several factors, such as excessive release of free fatty acids, TNFα and pro-inflammatory cytokines. It has been demonstrated that PTP1B overexpression may lead to increased gene expression of the sterol regulatory element-binding protein-1 (SREBP-1), a transcriptional factor which plays a pivotal role in increasing fatty acid synthesis and triglyceride accumulation in liver. Through this mechanism, PTP1B mediates hepatic lipogenesis and postprandial hypertriglyceridemia, which are thought to be important factors in the pathogenesis of insulin resistance and metabolic syndrome [19].

Distinct studies demonstrated that, in mice, PTP1B knock-down enhances insulin sensitivity and resistance to diet-induced obesity, without causing abnormalities in growth or other vital functions [14,15]. Muscle-specific or liver-specific deletion of PTP1B led to improved glucose homeostasis and enhanced systemic insulin sensitivity in diet-induced insulin resistant animals, demonstrating that this phosphatase plays a crucial tissue-specific role in the control of insulin signaling, mainly in skeletal muscle and liver but not in adipose tissue [15,20–22]. PTP1B deficiency in both myocyte cultures and in mice confers protection against TNFα-induced insulin resistance [23].

Additionally, both systemic and neuron-specific PTP1B knockout mice are lean and hypersensitive to leptin, whereas specific PTP1B deletion in muscle or liver did not affect body weight, indicating that PTP1B regulates body weight and adiposity mainly through the central control of leptin signaling in hypothalamus [6,18,24]. In insulin resistant animals, elevated levels of PTP1B were also found in other specific brain regions, such as cerebral cortex and hippocampus, suggesting a possible general role of the enzyme in the impairment of brain insulin signaling [25].

The correlation of increased PTP1B expression with insulin resistance has also been demonstrated in humans [16]. In clinical trials, the administration of PTP1B antisense oligonucleotides (ASO) increased insulin sensitivity without inducing hypoglycemia [26].

Therefore, PTP1B is a promising validated target for the management of insulin resistance and, on this basis, the development of PTP1B inhibitors is considered a significant advancement in the search for new agents to treat T2DM, obesity and related pathologies.

Unfortunately, the search for potent, sufficiently selective and orally available PTP1B inhibitors has encountered difficulties, mainly due to the polar nature and high degree of homology of PTP catalytic sites. Despite the wealth of compounds so far investigated, only few inhibitors have been evaluated as drug candidates in clinical trials [27–30]. Currently, trodusquemine (Fig. 1), a selective peripheral and central PTP1B inhibitor, has reached phase 2 clinical trials as a promising antiobesity and antidiabetic agent [31]. However, in the design of active-site directed inhibitors of PTPs, targeting both catalytic site and unique loops or subpockets adjacent to the active site is a useful approach to achieve high-affinity binding and, consequently, effective and possibly selective inhibition [27,32–34].

In the structure of PTP1B, the catalytic domain is situated at the bottom of a deep crevice which is surrounded by both the flexible WPD loop, containing the catalytic residue Asp181, and the YRD loop, including Tyr46 which is critical for pTyr recognition. The phosphate-binding loop (P-loop) contains the signature motif CX_5R (S/T) that is conserved in all members of the PTP family and, in fact, includes the cysteine and arginine residues that are essential for the catalytic dephosphorylation mechanism common to all PTPs. In PTP1B, a secondary noncatalytic arylphosphate-binding site adjacent to the active site is also present, which is lined by poorly conserved residues, such as Arg24, Ala27, Phe52, Arg254, Met258, Gly259. This secondary pocket, which is lacking in several other