Insight into the protein tyrosine phosphatase 1B (PTP1B) inhibitory activity of pyrazoles

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Introduction

Diabetes *mellitus* (DM) is a long-term chronic condition characterized by hyperglycemia, being one of the biggest global health emergencies of the 21st century. Type 2 DM is the most common form of DM, representing 90-95% of patients with DM. Insulin resistance is the earliest detectable abnormality and the characteristic feature in individuals with type 2 DM. Protein tyrosine phosphatase 1B (PTP1B) has been emerging as a promising drug target for the management of type 2 DM. Different causes disrupt a complex network of signaling cascades where insulin is involved, impairing its play role on glucose homeostasis. The insulin binding to the α subunit of its extracellular receptor (IR) (Fig. 1) unleashes the phosphorylation of the tyrosine residues of the β subunits, subsequent protein–protein interactions and the phosphorylation of insulin receptor substrates (IRS). Then, IRS proteins serve as docking for other signaling molecules, to onset protein kinase B (PKB) (also known as Akt) activation and translocation of glucose transporter 4 (GLUT4) storage vesicles to the plasma membrane for glucose uptake. PTP1B, as a negative regulator of insulin signaling pathway, acts by dephosphorylation of IR and IRS, leading to the attenuation of the insulin signal. PTP1B inhibitors amplify the level of phosphorylation of the IR and its substrates, rising the translocation of glucose transporters and glucose uptake in insulin-dependent cells, improving insulin sensitivity.

Main goal: Compare the inhibitory effect of a panel of new structurally related pyrazoles (Table 1) against human PTP1B activity, using a microanalysis screening system, and determine the mechanism of inhibition of the most active compounds, graphically by Lineweaver-Burk plots and by non-linear least squares regression using the Solver™ supplement of Excel Microsoft Office™.

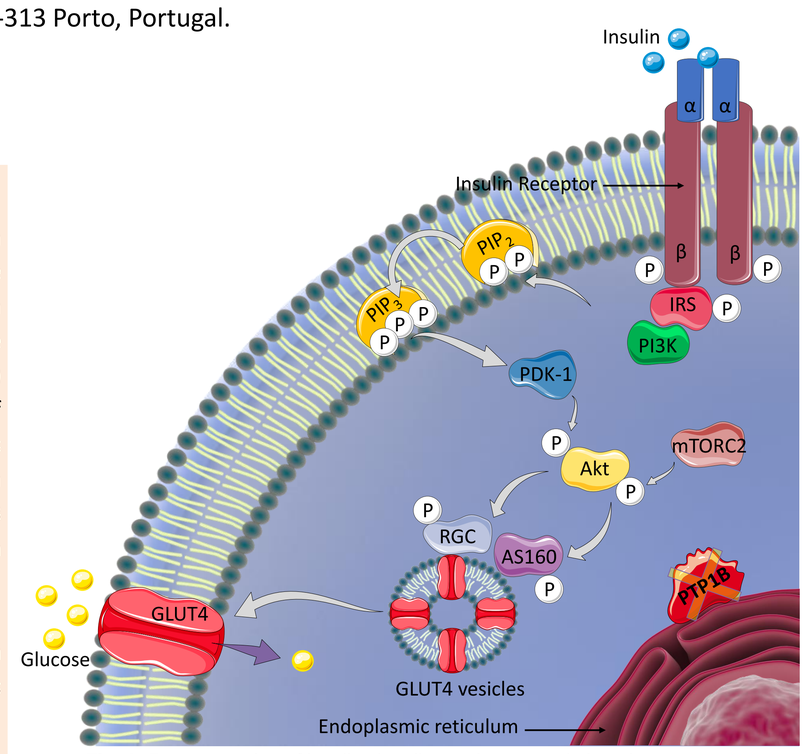


Fig. 1: Representation of insulin signaling pathway regulating glucose transport.

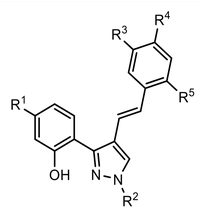
Methods

***In vitro* inhibitory assay for PTP1B activity**

***p*NPP**: *p*-nitrophenyl phosphate

Results

Table 1: Structures and *in vitro* PTP1B inhibition, expressed as % inhibition (at the highest tested concentration indicated in superscript) and IC_{50} values (mean ± SEM) of the tested pyrazoles.



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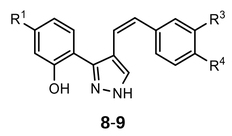
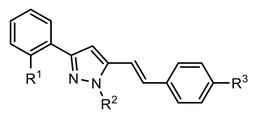


8-9

4-Styrylpyrazoles (1-9)

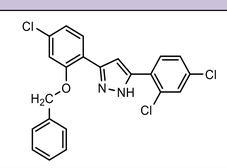
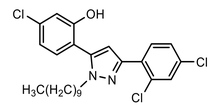
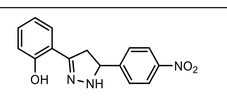
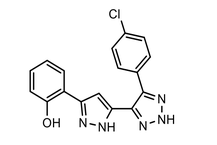
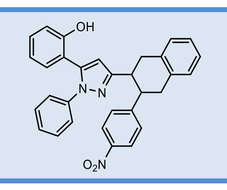
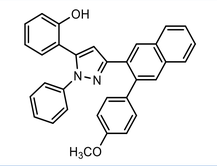
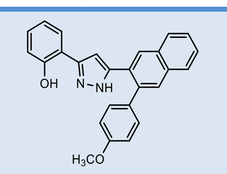
Compounds	R^1	R^2	R^3	R^4	R^5	PTP1B inhibitory activity
1	-	H	-	-	-	<30% 100 µM
2	-	H	-	Cl	-	≈100 µM (a)
3	-	H	-	OCH_3	-	<30% 100 µM
4	-	H	-	-	CF_3	35±2% 50 µM
5	-	$(CH_2)_9CH_3$	-	-	-	<30% 50 µM
6	-	$(CH_2)_{11}CH_3$	-	-	-	<30% 50 µM
7	-	$(CH_2)_{11}CH_3$	-	Cl	-	<30% 50 µM
8	-	-	NO_2	-	-	<30% 50 µM
9	OCH_3	-	-	NO_2	-	<30% 50 µM



5-Styrylpyrazoles (10-15)

Compounds	R^1	R^2	R^3	PTP1B inhibitory activity
10	OH	H	-	<30% 100 µM
11	OH	H	Cl	41±6% 50 µM
12	OH	-Ph	OCH_3	46±6% 50 µM
13	OH	-Ts	OCH_3	<30% 25 µM
14	-O-Ts	-Ts	-	<30% 25 µM
15	-O-Ts	-Ts	OCH_3	<30% 25 µM

Miscellaneous (16-22)

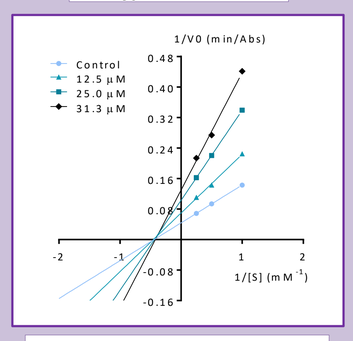
Compounds	Structure	PTP1B inhibitory activity
16		<30% 50 µM
17		<30% 50 µM
18		<30% 50 µM
19		<30% 50 µM
20		27±3 µM (b)
21		40±4 µM (c)
22		36±3 µM (b,c)

The IC_{50} with different lowercase superscript letters are statistically different from each other ($p<0.05$).

Most active pyrazoles

Pyrazole 20

IC_{50} = 27±3 µM

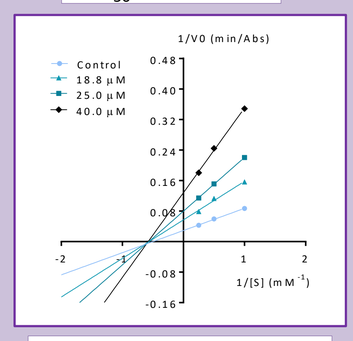


Non-competitive inhibition

Fig. 2: Kinetic analysis of PTP1B inhibition by pyrazole 20.

Pyrazole 22

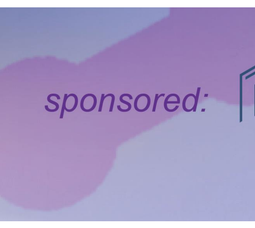
IC_{50} = 36±3 µM



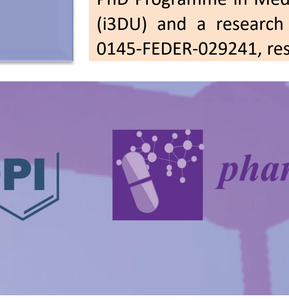
Non-competitive inhibition

Fig. 3: Kinetic analysis of PTP1B inhibition by pyrazole 22.

Positive control: Ursolic acid



IC_{50} = 7.4±0.4 µM



Non-competitive inhibition

Fig. 4: Kinetic analysis of PTP1B inhibition by the positive control, ursolic acid.

Table 2: Type of inhibition (using Solver™ supplement) of pyrazoles **20** and **22**, and the positive control, **ursolic acid**, and the respective kinetic constant values: V_{max}, K_m, K_{ic} and K_{iu} (mean ± SEM).

Compound	Type of inhibition	V_{max} (µM/min)	K_m (µM)	K_{ic} (µM)	K_{iu} (µM)
Pyrazole 20	Non-competitive	22.7±0.2	2208±38	18.0±0.2	18.0±0.2
Pyrazole 22		38.7±0.5	2435±71	16.2±0.3	16.2±0.3
Ursolic acid (positive control)		38.7±1.7	2653±200	5.5±0.2	5.5±0.2

Conclusions:

- ✓ It was possible to conclude that the type of substituents in the pyrazole moiety influence the inhibitory activity of the compounds.
- ✓ Pyrazole 20 and pyrazole 22 were the most promising compounds. The mechanism of inhibition was assessed using Lineweaver–Burk plots and non-linear least squares regression, since linear transformations are less exact. Both methods showed a non-competitive inhibition mechanism.
- ✓ The presence of additional benzene rings seems to benefit the inhibitory activity against PTP1B in the pyrazole scaffold.
- ✓ This study provides significant key considerations about the pyrazoles' scaffold and their antidiabetic effects as PTP1B inhibitors.

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