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PDI inhibitors

Scientific hypothesis

Protein disulphide isomerase (PDI) family is a group of enzymes regulating endoplasmic reticulum protein folding with PDIA1 and PDIA3 representing the major isoforms. In addition to well-known role of PDI as molecular chaperones, their cell-surface disulfide exchange activity controls thrombosis, cancer cell adhesion, metastasis and vascular function. Thus, protein disulphide isomerase seems to be an interesting therapeutic target to be exploited to afford anti-thrombotic, anti-metastatic and vasoprotective action. To the best of our knowledge there is no specific PDI inhibitor that has been approved, though flavonoid-based PDI-inhibition is currently in clinical trial to treat cancer associated thrombosis.

Therapeutic area

Suppression of intracellular PDI inhibits platelet activation and thrombus formation. Inhibition of extracellular PDI regulates cancer cell invasion and migration. Finally, inhibition of PDI has vasoprotective effects. Moreover, there is an evidence suggesting that inhibition of PDI display an anti-viral effects.

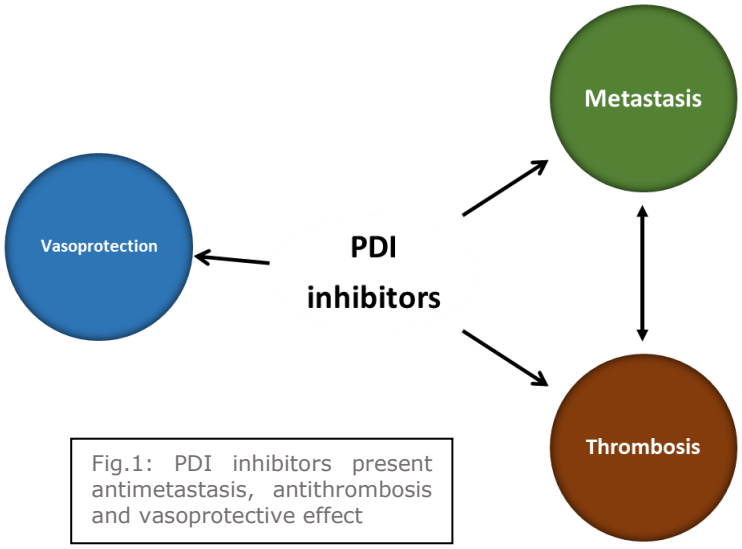

Fig.1: PDI inhibitors present antimetastasis, antithrombosis and vasoprotective effect

Accordingly, inhibition of PDI affords anti-metastatic, anti-thrombotic and vasoprotective effects. Our results supporting this profile of pharmacological activities of PDI inhibition are presented in Table 2.

Lead Compounds

Compounds C-3389 and C-3399, the selective PDIA1 inhibitor and non-selective PDIA1/PDIA3 inhibitor, have been chosen from the library of 82 rationally designed and tested derivatives. These compounds or their congeners display a remarkable pharmacological activities in *in vitro* and *in vivo* assays. **Importantly, both of these compounds are not toxic in acute toxicity assay *in vivo***. Summary of results with PDI inhibitors based on *in vitro* and *in vivo* experiments, are presented in Table 1.

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Table 1: Profile of C-3389 and C-3399, the selective PDIA1 inhibitor and non-selective PDIA1/PDIA3 inhibitor.

	Measured parameter	C-3399	C-3389
***In vitro* data**	**Inhibition of PDIA1 (EC 50, µM)**	**0.12**	**0.03**
	Inhibition of PDIA3 (EC 50, µM)	**4.0**	**23.3**
	Anti-aggregatory effect *in vitro* (EC 50, µM):	20±10	-
	Anti-adhesive effect *in vitro* (adhering cancer cells to fibronectin in comparison to control group)	Compound conc.: 30µM MDA-MB-231 – 62% MCF-7 – 41%	Compound conc.: 30µM MDA-MB-231 – 70% MCF-7 – 64%
	In vitro antiproliferative effect towards panel of cancer cells (48-hour exposition) (Cytotoxicity, IC50, µM):	HT-1080: 7.9±0.7, CaCo-2: 55±7, MDA-MB-231: 17±1, MCF-7: 41±6	HT-1080: 36±3, CaCo-2: 49±2, MDA-MB-231: 32±3, MCF-7: 47±6
***In vivo* data**	Pharmacokinetics: intraperitoneal (i.p.) administration to mice (n=4, dose: 30mg/kg b.w) (*metabolite parameters)	C_{max} *= 50±11 µM at t_{max} *= 15min, MRTlast *= 27.23±1.83 min	C_{max} = 17.97±2.91 µM at t_{max} = 15min MRTlast= 25.71±0.89min
	Acute toxicity: (Globally Harmonized System of Classification and Labelling of Chemicals)	category 5/unclassified	category 5/unclassified

Patent protection

Project is patent pending

Priority date – 2020.01; PCT/PL2020/050004 and PCT/PL2020/050005

Unique Selling Point

PDIA3 and PDIA1 irreversible inhibition by the compound C-3399 is mediated by its metabolite. After administration, it is metabolised immediately to labile active

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metabolite, inhibiting overactivated PDI. Due to irreversible character of inhibition, therapeutic effect lasts long, despite the rapid degradation of the compound to the final non-active compound. This pro-drug approach allowed to obtain all therapeutic advantages of the inhibition of extracellular PDI, simultaneously omitting high toxicity connected with the inhibition of intracellular PDI. Compound C-3399 and its congeners, were designed for the effective blockade of platelets aggregation and antithrombotic effects making this compounds the unique anti-thrombotic agent.

Selective PDIA1 inhibition by compound C-3389 endowed with low nanomolar EC50, was designed to block cancer cells adhesion to endothelium, and displays also ancillary vasoprotective effects and anti-thrombotic effects, that makes discovered compound a unique molecule for anti-cancer/anti-metastatic treatment.

Molecular target

Sulphonamides of aziridine-2-carboxylic acid derivatives were developed as selective PDIA1 or non-selective PDIA1 and PDIA3 inhibitor targeted to both cysteines of the catalytic centre of PDIA1 (*C53 and C56)*. They inhibits PDI by covalent binding and the alkylation of cysteines of the catalytic centre of PDIA1.

Formulation for *in vivo* assays

Final compounds are obtained in solid state as water-insoluble substances of high LC-MS purity. For the needs of *in vivo* experiments, investigated compounds were dissolved in PEG-6.

Synthesis

The synthesis pathway was developed and successfully performed on non-industrial scale. Details of that process were included in patent application. In general, solution of appropriate aziridine derivative is treated with designated aromatic or heteroaromatic sulphonic acid chloride.

References available on request

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Additional data supporting unique therapeutic efficacy of PDI inhibitors

Table 2: *S*upporting data from in vivo studies

Metastasis	**The anticancer activity of selected PDIA1 inhibitors *in vivo* (the murine model of Lewis Lung Carcinoma (LLC))**	
Measured parameter	**C-3281**	**C-3329**
PDIA1 IC50	0.14 µM	1.1 µM
Tumour growth inhibition	65-71%	58%

Thrombosis	**Anti-thrombotic effects of PDIA1 inhibitors *in vivo* (rat model of arterial thrombosis)**	
Measured parameter	**C-3257**	**Rutin (reference PDIA1 inhibitor)**
PDIA1 / PDIA3 IC50	0.08 / 48.5 µM	4.0 / >200 µM
Thrombus weight (compared to vehicle)	75.2%	66.98%

Vasoprotection	**Vasoprotective effect of PDI inhibitor *in vivo*: (Ang II-induced hypertension in mice)**	
Measured parameter	**Hypertensive mice (control group)**	**Hypertensive mice treated with Bepristat 2a (reference PDIA1 inhibitor)**
In vivo vascular function based on Ach-induced vasodilatation [% changes]	vasoconstriction by 9.2 %	vasodilation by 1.5 %
In vivo vascular stiffness based on pulse wave velocity (PWV) measurements [mm/ms]	stiff vessels (PWV=4.9)	reduced stiffness PWV=3.5

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