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Novel ROCK inhibitors for the treatment of pulmonary arterial hypertension

Duncan Shaw [a,*], Greg Hollingworth [a], Nicolas Soldermann [b], Elizabeth Sprague [c], Walter Schuler [b], Eric Vangrevelinghe [b], Nicholas Duggan [a], Matthew Thomas [a], Takatoshi Kosaka [a], Nigel Waters [a], Maurice J. van Eis [b,*]

[a] Novartis Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex RH12 5AB, United Kingdom
[b] Novartis Institutes for Biomedical Research, Forum 1 Novartis Campus, 4056 Basel, Switzerland
[c] Novartis Institutes for Biomedical Research, Cambridge, MA 02139, USA

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ABSTRACT

A novel class of selective inhibitors of ROCK1 and ROCK2 has been identified by structural based drug design. PK/PD experiments using a set of highly selective Rho kinase inhibitors suggest that systemic Rho kinase inhibition is linked to a reversible reduction in lymphocyte counts. These results led to the consideration of topical delivery of these molecules, and to the identification of a lead molecule **7** which shows promising PK and PD in a murine model of pulmonary hypertension after intra-tracheal dosing.

Pulmonary arterial hypertension (PAH) is a rare chronic progressive condition with a complex pathogenesis. There remains a significant unmet medical need for disease modifying treatments. The significant symptomatic relief obtained with the current standard of care has not greatly improved the prognosis, and life expectancy after diagnosis still averages around seven years.[1] PAH is characterised by a high degree of remodelling of the pulmonary arterioles and formation of plexiform lesions, the consequent restriction of blood flow to the lungs leads to an increase in blood pressure in the pulmonary artery (>25 mmHg).

Inhibition of the PKC family (AGC) serine threonine kinases Rho kinase 1 and 2 has been postulated as a potential therapy for PAH. The RhoA/Rho kinase pathway has been shown to play a prominent role in smooth muscle cell contraction as a result of activation by various vasoactive substances including angiotensin II and serotonin, which have been shown to have a role in the pathogenesis of PAH.[2] Furthermore the RhoA/Rho kinase pathway is associated with both cell migration, and proliferation by its activation of myosin light chain phosphorylation. The pathway has been shown to be abnormally activated in animal models of PAH[3,4] and in the disease itself.[5] Rho kinase has been demonstrated to mediate both vasoconstriction[6] and remodelling[7] in iPAH models. Inhibitors of Rho kinase 1 and 2 (ROCK1,2) are postulated to have dual therapeutic benefit in PAH by leading to pulmonary vasodilation and also inhibiting the remodelling of the pulmonary arterioles. Clinical efficacy has recently been shown with Fasudil in PAH[8–10] although it is unclear whether the activity observed is due to inhibition of Rho kinase.

Given the involvement of Rho kinase in a large number of signaling pathways, we reasoned that it would be desirable to avoid systemic Rho kinase inhibition in the treatment of PAH in order to minimize undesired side-effects. In order to evaluate the effects related to systemic Rho kinase inhibition (in addition to the well described effect of Rho kinase in the regulation of systemic blood pressure) a set of known, highly selective Rho kinase inhibitors[11–13] were tested in a high-dose PK experiment in rats. Compounds were profiled against a panel of kinases including ROCK1,2[14] as well as a Rho kinase-dependent functional cellular assay (MCP-1 driven human monocyte migration[15]) to confirm inhibitory activity of ROCK, kinase selectivity and cellular activity (see, Table 1). A single high-dose (30 mg/kg) of the compounds was orally applied to Lewis rats and blood samples were collected at 1, 2, 4, 8 and 24 h. All compounds were well tolerated, however, analysis of the blood samples surprisingly revealed a strong

* Corresponding authors. Tel.: +1 617 871 7739, +41 61 324 8293.
E-mail addresses: duncan.shaw@novartis.com (D. Shaw), maurice.van_eis@novartis.com (M.J. van Eis).

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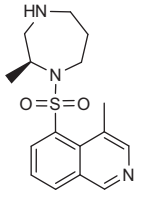
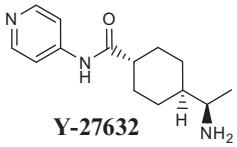
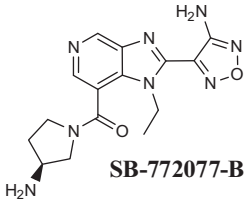
Table 1
Enzymatic, cellular and in vivo activity of selective ROCK inhibitors

Compound	ROCK1,2 IC_{50}[a] (nM)	Kinase selectivity <50 fold[b]	Monocyte migration IC_{50}[c] (nM)	Reduction of lymphocyte counts[d,e] (%)
 **H-1152P**	5, 5	0/77	74	65
 **Y-27632**	93, 89	3/84 PKCθ PKN1 PKN2	483	53
 **SB-772077-B**	14, 7	1/23 PKA	59	84

[a] See Ref. 14.
[b] Number of kinases inhibited with less than 50-fold selectivity over ROCK1 versus total number of kinases tested.
[c] See Ref. 15.
[d] Maximum reduction after a single 30 mg/kg po dose to Lewis rats.
[e] Average value, $n = 2$.

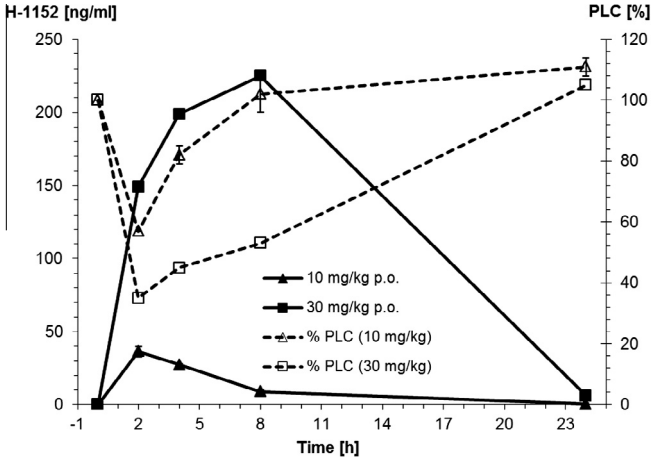
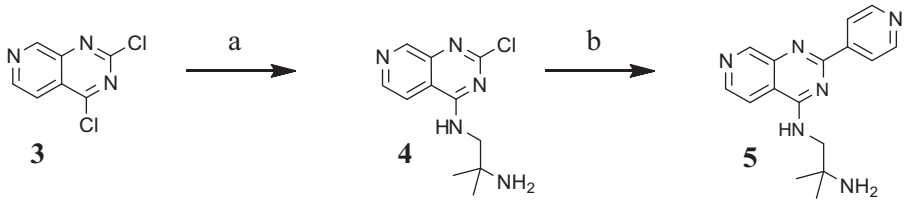


Figure 1. PK-PD for **H-1152P**. Single 30 mg/kg po dose to Lewis rats; average values; $n = 2$; PLC = peripheral lymphocyte counts.

reduction in peripheral lymphocyte counts (B- and T-cells). The reduction was shown to be transient and an excellent PK/PD relationship was observed (shown for **H-1152P** in Fig. 1). The in vivo effect is similar to what is observed with S1P1 (sphingosine-1-phosphate receptor 1) antagonist such as Gilenya®. Although a role of Rho kinase in the S1P signaling pathway has been described[16] this is to our best knowledge the first time that it has been shown that Rho kinase has an effect on lymphocyte trafficking in vivo. The exact underlying mechanism remains to be elucidated. Since the transient reduction in peripheral lymphocyte counts could be demonstrated with potent and highly selective Rho kinase inhibitors belonging to diverse chemical scaffolds, we feel that this effect can be convincingly tied to Rho kinase inhibition. This strengthened our conviction that it is highly desirable to minimize systemic Rho kinase inhibition.

The starting point for our optimization program (compound **1**) had originally been identified as a protein kinase C (PKC) inhibitor using a high-throughput docking (HTD) approach.[17] Screening of **1** against a panel of kinases revealed that the compound inhibits a set of structurally closely related AGC kinases including ROCK1,2 and PKN1,2 but is otherwise remarkably selective. In line with



Scheme 1. Reagents and conditions: (a) 2-methylpropane-1,2-diamine, NEt_3, THF, 0 °C, 2 h, 70%; (b) pyridin-4-ylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH, 100 °C, 16 h, 34%.