Development of new Rac inhibitors to study the role of Rac protein in cardiac hypertrophy

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The low-molecular weight or small GTPase protein Rac belongs to the Rho/Rac/cdc42 subfamily. The activation of Rho proteins depends on the release of GDP and the binding with GTP. This cycling is finely regulated by three groups of proteins: the guanine nucleotide exchange factors (GEFs) as activators; the GTPase activating proteins (GAPs) and GDP dissociation inhibitors (GDIs) as negative regulators. When bound to GTP, Rho GTPases interact with their downstream effectors, which include protein kinases, regulators of actin polymerization, and other proteins with adaptor functions. The selective interaction of the different Rho GTPases with a variety of effectors determines the final outcome of their activation.
Rac is a protein involved in numerous cellular events in various cell types as cardiomyocytes, smooth muscle cells, monocytes, endothelial cells and platelets and therefore is also involved in numerous pathologies such as atherosclerosis, cardiac hypertrophy and tumors.
Several data indicate that Rac1 is involved in many events of cardiovascular diseases. In particular Rac1, through the regulation of NADPH oxidase, plays a critical role in the development of cardiac hypertrophy in mice C57/BL6 in response to Angiotensin II. In fact, treatment with Angiotensin II activates NADPH oxidase, increases O2 production, induces cardiac fetal gene expression, and increases left ventricular mass. These findings indicate that cardiomyocyte Rac1 plays a pivotal role in the development of cardiac hypertrophy in response to Ang II.

Starting from our previous identification of Rac inhibitors [1], through a computational approach, 57 chemical entities, with the 3-aryl-1H-pyrazole-5-carboxamide nucleus, were identified and their Rac inhibitory efficacy evaluated by G-LISA assay. Twenty-three compounds were found to reduce Rac-GTP levels in cultured cells by more than 25%. Compounds **4**, **5**, **6**, **11** and **21** resulted the most potent without interfering with RhoA protein activity, with compound **11** that was less Rac-selective. The IC50s for Rac inhibition of compounds **3**, **4**, **5** and **21** were between 4.4 and 29.1 μM. The treatment with compound **4** reduced the Rac1-GTP levels induced by the expression of Tiam1, TrioN, or Vav2 in a human smooth muscle cell line.

Compound **4** inhibited cell migration in response to PDGF-BB in a concentration dependent manner with an IC50 value of 5.8 μM. The incubation of human monocytic cell line THP-1 with compound **4** (10 μM) completely abrogated their adhesion to cultured human umbilical endothelial cells (HUVEC) indicating a potent anti-inflammatory activity[2]. A preliminary pharmacodynamic study in C57BL/6 mice was performed by administering compound **4** at 50 and 100 mg/kg i.p. At these doses, a 35% (50 mg/kg) and 70% (100 mg/kg) reduction of Rac1-GTP levels were observed in heart homogenates.
In conclusion, in the present study we identified a new selective Rac inhibitor capable to interfere with Rac-mediated cellular events such as cell migration and monocyte-endothelial cell adhesion. Moreover this compound reduces Rac-GTP levels in heart of C57/BL6 mice, supporting a future *in vivo* studies on experimental models of cardiac hypertrophy.
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