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Triazine and pyrimidine based ROCK inhibitors with efficacy in spontaneous hypertensive rat model

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ABSTRACT

The profile of a series of triazine and pyrimidine based ROCK inhibitors is described. An initial binding mode was established based on a homology model and the proposed interactions are consistent with the observed SAR. Compounds from the series are potent in a cell migration assay and possess a favorable kinase selectivity. In vivo activity was demonstrated for compound **1A** in a spontaneous hypertensive rat model.

Rho-kinase (ROCKI and ROCKII), a downstream effector of RhoA G protein, is one of the key mediators in regulating vascular smooth muscle tone.[1,2] Activation of ROCK by pathological agents, such as angiotensin II, endothelin-1, 5-hydroxytryptamine and oxyhemoglobin, leads to inhibition of myosin light chain phosphatase, and in turn increases the vascular tone.[1] Fasudil, a ROCK inhibitor and a dilator for injured cerebral arteries, has been used clinically in Japan since 1995 for the treatment of cerebral vasospasm.[3] Since then, pharmaceutical companies have been investigating small molecule ROCK inhibitors as potential treatments of hypertension and related cardiovascular disease.[4]

ROCK also plays an important role in cellular functions such as cell motility and cell migration.[5] Inhibition of ROCK may stabilize lesions by reducing monocyte infiltration directly by inhibiting the motility of monocytes, and indirectly by preventing the increase in endothelial cell permeability. Therefore, the modulation of Rho-kinase activity could be a useful treatment for atherosclerosis.[6]

A high-throughput screening campaign was initiated to identify ROCK inhibitors. An IMAP®[7] based ROCKI assay was used to screen Pharmacopeia libraries (5 million compounds, 2005). One of the hit series represented by structure **1A** was identified and was subsequently demonstrated to possess excellent cell based activity in a Monocyte Chemotactic Protein-1 (MCP-1) induced THP-1 cell migration assay (Table 1). Since compound **1A** contains a triaminotriazine motif which is common to many kinase inhibitors, it was a priority to assess its general kinase selectivity. Thus, compound **1A** was tested against a panel of 24 kinase at a concentration of 2 μM. Compound **1A** shows an acceptable selectivity profile (>25-fold) against the kinase panel.[8]

This Letter describes the structural–activity relationships around the compound and a general binding mode is proposed for the series based on a homology model.

Triazine and pyrimidine analogs were readily accessible via the synthetic route outlined in Schemes 1 and 2. It is based on sequential addition of amines to cyanuric chloride or trichloropyrimidine. One key step involves the displacement of the chloride in a diaminochlorotriazine (**1D** to **1E**) with aminopyridines. Although a direct displacement with 4-aminopyridine under strong heating condition provided sufficient quantity for the initial analoging, similar conditions used to attach 3-aminopyridine and 2-aminopyridine to the chlorotriazine were met with low yield and impure products. Subsequently, a palladium mediated

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† In 2008, Pharmacopeia, Inc was acquired by Ligand Pharmaceuticals, Inc.

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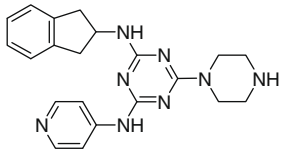
Table 1
Profile of hit **1A** identified in a ROCKI screening campaign



1A

Assays/parameters	IC_{50}[a] (nM)
ROCKI (IMAP)	6 (±2)
THP-1 (MCP-1)	30 (±1)
Selectivity (24 kinases)	<50% inhibition at 2 μM
MW	405
A log P	3.71

[a] Values are means of two or more measurements, standard deviation is given in parentheses (na = not active).

coupling procedure was used to prepare the 3-aminopyridyl (**4A**) and 2-aminopyridyl (**4B**) analogs.

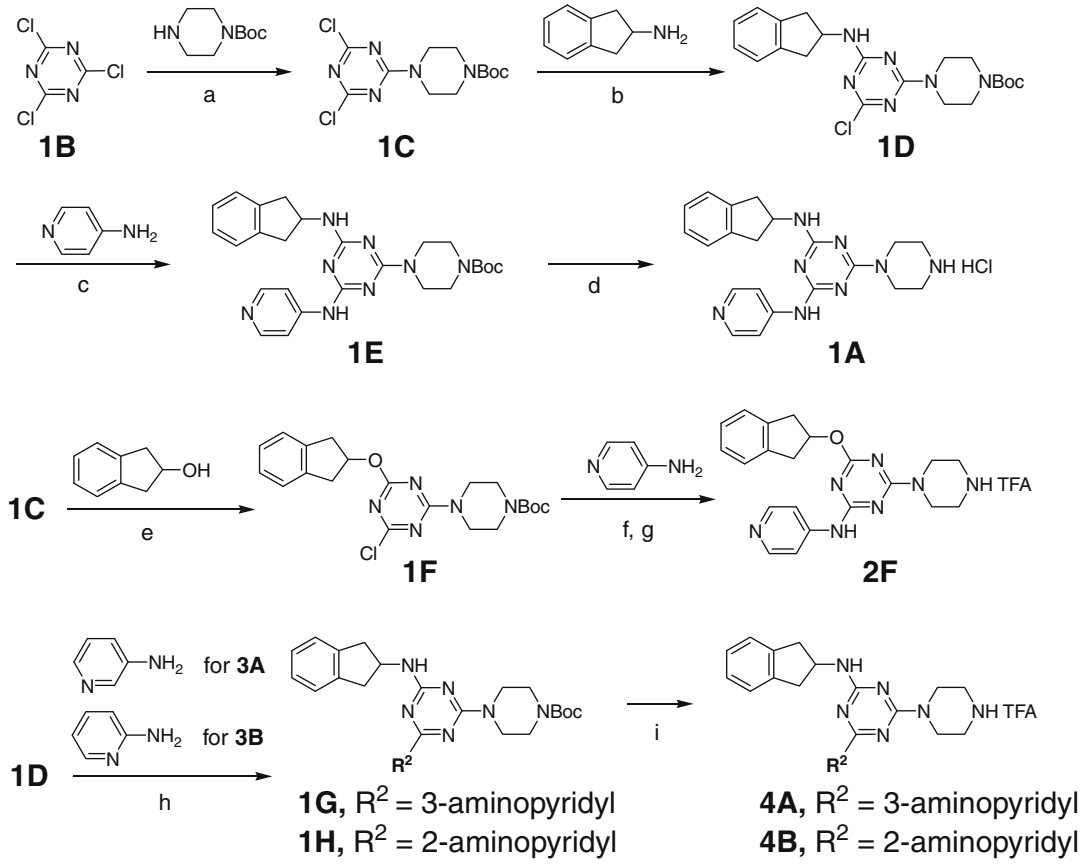
The SAR was first established for the 2-aminoindane moiety (**R**[1], Table 2). Weaker potency was observed for the cyclopentylamino analog (**3A**) and the data suggested that an aromatic group is required for potency. The orientation of the aromatic moiety for optimal interaction was explored by two ring opening analogs (**3B** and **3C**), a 2-aminotetrahydronaphthalene analog (**3D**) and a 1-aminoindane analog (**3E**). The superior potency observed in **1A** in comparison to these compounds showed that the fused ring in the 2-aminoindane imposed a favorable geometry for ligand enzyme interaction. The exocyclic NH in this domain does not appear to be involved in any binding interaction since an ether analog (**3F**) is equal potent to the N-linked **1A**.

The 3-pyridyl (**4A**) and 2-pyridyl (**4B**) isomers were prepared in order to establish the optimal position of the pyridyl nitrogen in the **R**[2] domain (Table 3). Significant decrease in potency in both cases suggested that the endocyclic pyridyl nitrogen may involve in a hydrogen bonding interaction. The hypothesis is further strengthened by the fact that an aniline analog (**4C**) has inferior potency.

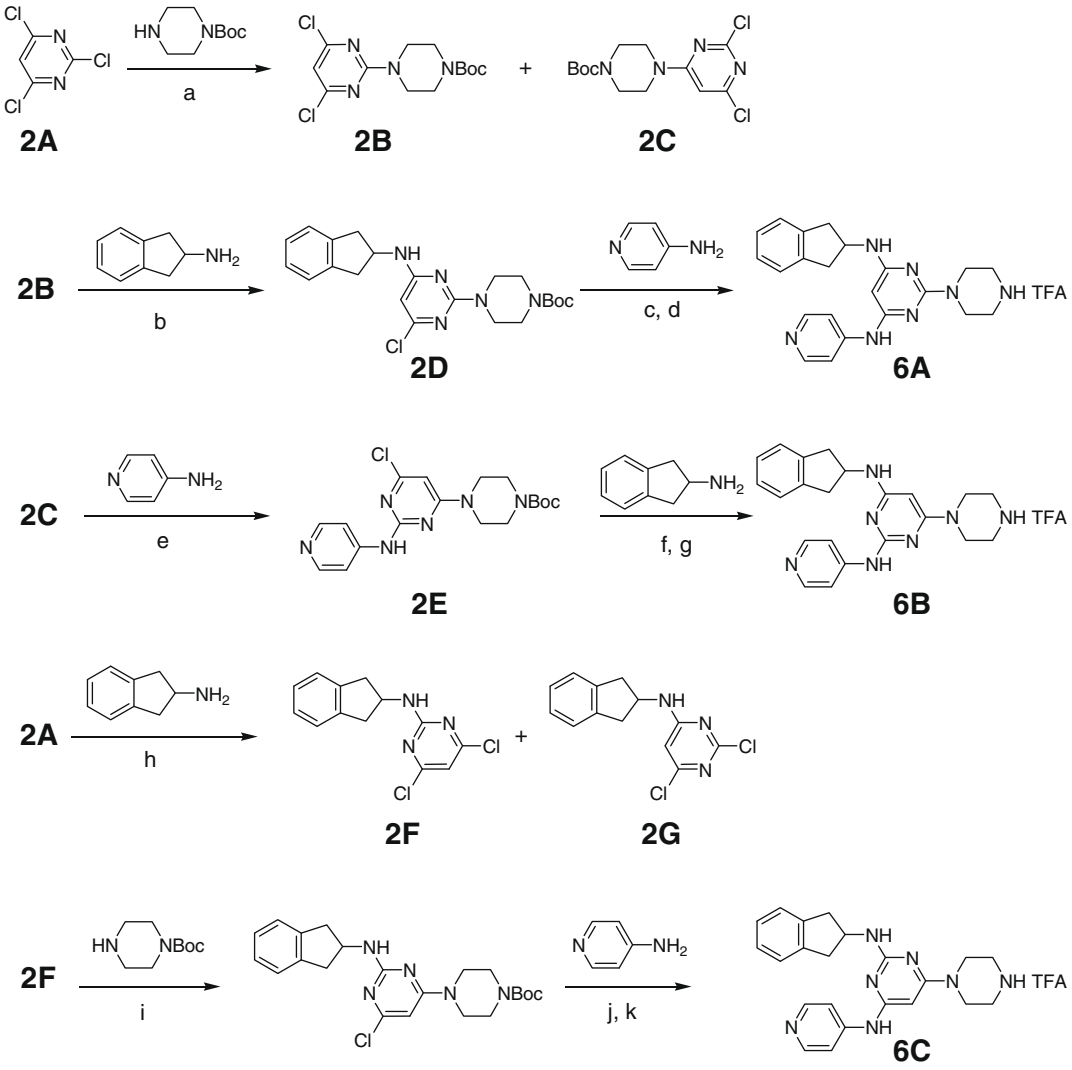
Next, the piperidine domain (R^3, Table 4) was evaluated. The basic nitrogen distal from the triazine is required for potency. Deletion (**5A**) or methylation (**5B**) of the distal nitrogen yielded weaker compounds (R^3, Table 4). Cyclic amines (**1A** and **5C**) are preferred over the linear aliphatic amine (**5D**). Four methylpipridine analogs were prepared (**5E** to **5H**) in order to elucidate the preferred position and chirality for the methyl substitution. Among the four isomers, compound **5E** shows superior potency in both the IMAP assay and cell migration assays.

We next turned our attention to the triazine core. The core in **1A** was replaced by the three possible pyrimidine isomers (Table 5). Stepwise replacement of a nitrogen in the core with a CH established that the nitrogen between the 4-aminopyridine and piperizine moieties (position **Z** in general structure **6**) is required for potency. Thus, compound **6C**, in which the Z group is a CH, shows significantly weaker potency in comparison to **6A** and **6B**. We speculated that the nitrogen may involve in a specific interaction with the enzyme.

In the absence of a ROCK crystal structure (year 2005),[9] a homology model of ROCKI was built with MOE (Chemical Computing



Scheme 1. Synthesis of triazine based ROCK inhibitors. Reagents and conditions: (a) $NaHCO_3$, acetone, H_2O, 0–5 °C; (b) CH_2Cl_2, NEt_3, rt; (c) K_2CO_3, THF, 140 °C, sealed-tube; (d) HCl in dioxane; (e) NaOH, acetone, H_2O; (f) K_2CO_3, DMSO, 80 °C; (g) TFA, CH_2Cl_2; (h) $Pd(OAc)_2$, BINAP, Cs_2CO_3, dioxane, microwave target temperature 160 °C; (i) TFA, CH_2Cl_2.



Scheme 2. Synthesis of pyrimidine based ROCK inhibitors. Reagents and conditions: (a) DIEA, CH_2Cl_2, −78 °C, then separate by column chromatography; (b) K_2CO_3, CH_3CN, reflux; (c) $Pd(OAc)_2$, BINAP, Cs_2CO_3, dioxane, microwave target temperature 160 °C; (d) TFA, CH_2Cl_2; (e) K_2CO_3, CH_3CN, reflux; (f) K_2CO_3, THF, reflux; (g) TFA, CH_2Cl_2; (h) CH_2Cl_2, −78 °C, then separate by column chromatography; (i) K_2CO_3, CH_3CN, reflux; (j) $Pd(OAc)_2$, BINAP, Cs_2CO_3, dioxane, microwave target temperature 160 °C; (k) TFA, CH_2Cl_2.

Group, Montreal, Quebec, Canada, version 2006.08) using a crystal structure of PKA (1Q8T) as a template.[10] Docking of **1A** was performed with Glide in standard precision (SP) mode.[11] Key interactions between the ligand and the enzyme are shown in Figure 1 and are consistent with the observed SAR.

In the proposed binding model for 1, the 2-aminoindane moiety interacts with ROCKI via hydrophobic contact with the aromatic ring of Phe-368. The nitrogen has no observed role in forming specific hydrogen bonding interactions, a conclusion that is supported by the observed equipotency of compound **3F** with **1A** (Table 2).

A key intermolecular hydrogen bond is observed between the pyridyl nitrogen and the backbone NH of Met-156 in the hinge domain. The model suggests that the geometric orientation of this contact is critical for binding to ROCKI, and this is borne out in the SAR by substantial losses in potency when the location of the nitrogen on the ring is altered (**4A** and **4B**) or is removed altogether (**4C**).

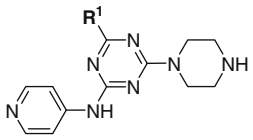
The model for the binding mode of **1A** places the basic nitrogen of the piperidine ring in position to form a hydrogen bond with one of the side chain oxygen atoms of Asp-216. Removal of this hydrogen bonding interaction yielded substantially weaker compounds (**5A** and **5B**).

The triazine nitrogen between the 4-aminopyridine and piperizine moieties (**6C**) does not appear to have a specific role in the proposed binding mode. The loss of potency of replacing this nitrogen can be rationalized in one of two ways. Either there is enough conformational flexibility in the Lys-105 side chain to accommodate a direct interaction between the side chain nitrogen with the core nitrogen of **6C** (distance 5.6 Å), or a water-mediated hydrogen bond is necessary for interactions with hydrogen bond acceptors/donors as represented by Lys-105. The other two nitrogens in the triazine core have no role in the proposed binding model, which is consistent with the observation that their removal has little effect on their IMAP® potency (**6A** and **6B**).

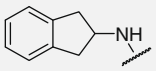
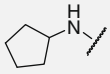
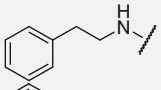
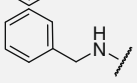
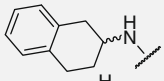
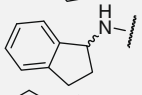
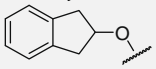
A representative example from the series (**1A**) was tested in a spontaneous hypertensive rat model. Compound was dosed via sc in three doses (1, 3 and 10 mg/kg).[14] A significant decrease in mean arterial blood pressure (MAP) was observed at 3 and 10 mg/kg with an apparent dose–response (Fig. 2).

In conclusion, triazine and pyrimidine based ROCK inhibitors were identified. An initial binding mode was established based on a homology model and the proposed interactions are consistence with the observed SAR. Compounds from the series are

Table 2
Modifications of **R[1]**

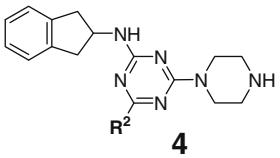


3

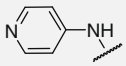
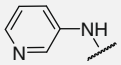
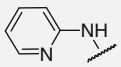
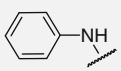
Compds	**R[1]**	ROCKI (IMAP) IC_{50}[a] (nM)	THP-1 (MCP-1) IC_{50}[a] (nM)
1A		6 (±2)	30 (±1)
3A		890 (±315)	nd
3B		326 (±3)	nd
3C		400 (±6)	nd
3D		23 (±1)	nd
3E		185 (±93)	nd
3F		8 (±2)	57 (±30)

[a] Values are means of two or more measurements, standard deviation is given in parentheses (nd = not determined).

Table 3
Modifications of **R[2]**



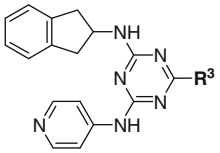
4

Compds	**R[2]**	ROCKI (IMAP) IC_{50}[a] (nM)	THP-1 (MCP-1) IC_{50}[a] (nM)
1		6 (±2)	30 (±1)
4A		8400 (±200)	nd
4B		1000 (±100)	nd
4C		7400 (±900)	nd

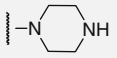
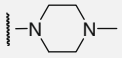
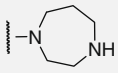
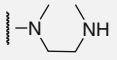
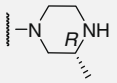
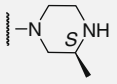
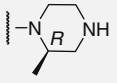
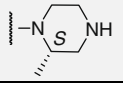
[a] Values are means of two or more measurements, standard deviation is given in parentheses (nd = not determined).

potent in a cell migration assay and possess a favorable kinase selective. In vivo activity was demonstrated in the spontaneous hypertensive rat model.

Table 4
Modifications of R^3

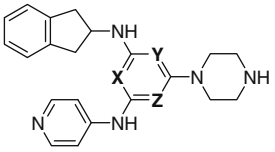


5

Compds	**R^3**	ROCKI (IMAP) IC_{50}[a] (nM)	THP-1 (MCP-1) IC_{50}[a] (nM)
1A		6 (±2)	30 (±1)
5A		8300 (±4900)	nd
5B		305 (±14)	nd
5C		11 (±1)	17 (±10)
5D		81 (±2)	nd
5E		8 (±2)	26 (±13)
5F		135 (±57)	nd
5G		118 (±6)	nd
5H		87 (±7)	nd

[a] Values are means of two or more measurements, standard deviation is given in parentheses (nd = not determined).

Table 5
Replacement of triazine with pyrimidines



6

Compds	**X**	**Y**	**Z**	ROCKI (IMAP) IC_{50}[a] (nM)	THP-1 (MCP-1) IC_{50}[a] (nM)
1A	N	N	N	6 (±2)	30 (±1)
6A	CH	N	N	2 (±1)	12 (±2)
6B	N	CH	N	14 (±4)	154 (±53)
6C	N	N	CH	1200 (±120)	nd

[a] Values are means of two or more measurements, standard deviation is given in parentheses (nd = not determined).

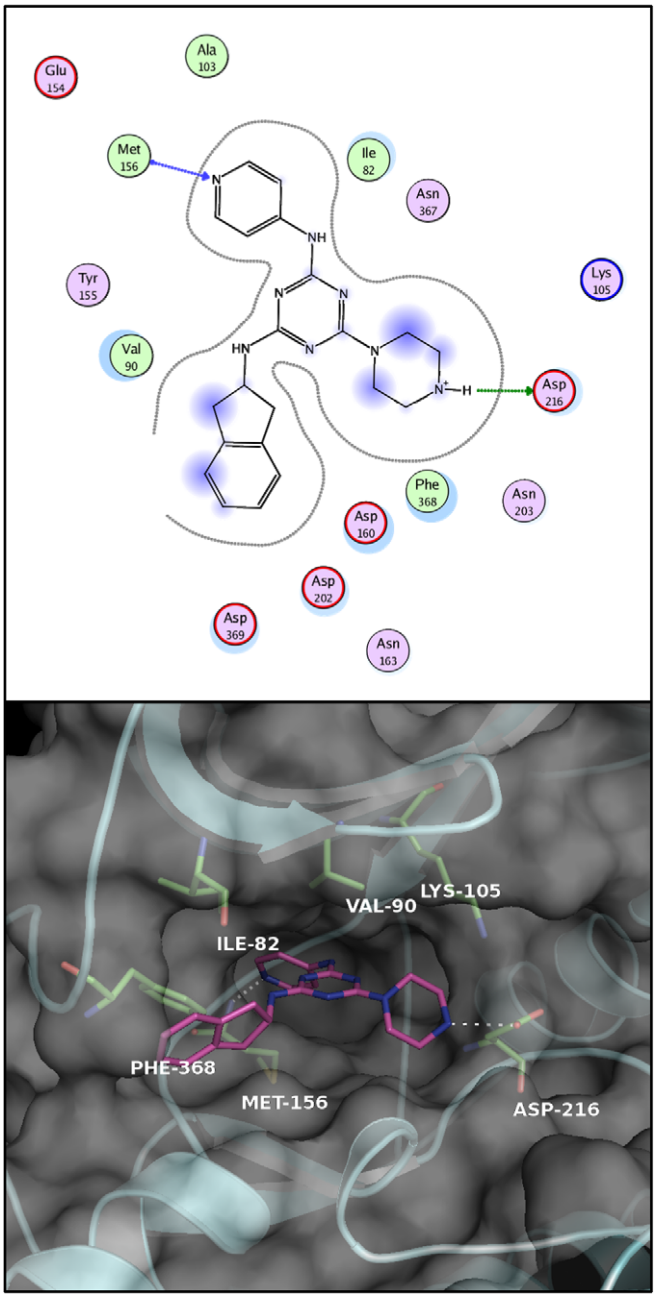


Figure 1. Interaction of 1 with the ROCK1 homology model. The top panel was generated with MOE.[12] The bottom panel was generated with PyMOL.[13] The distal nitrogen of the piperzine is in quaternary form. One of the hydrogen was deleted for clarity reason.

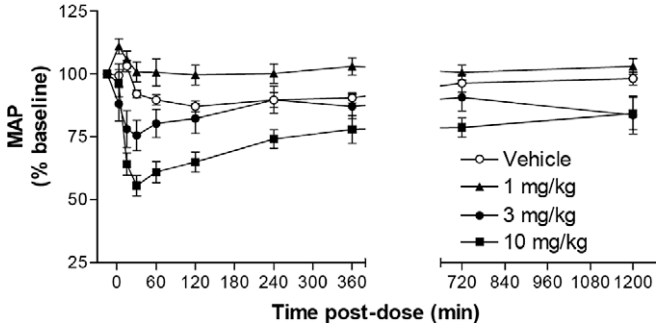


Figure 2. Effect of compound **1A** in mean arterial blood pressure in a spontaneous hypertensive rat model (1, 3 and 10 mg/kg sc).

References and notes

1. Budzyn, K.; Marley, P. D.; Sobey, C. G. *Trends Pharmacol. Sci.* **2006**, *27*, 97.
2. Lee, D. L.; Webb, R. C.; Jin, L. *Hypertension* **2004**, *44*, 796.
3. Mueller, B. K.; Mack, H.; Teusch, N. *Nat. Rev. Drug Disc.* **2005**, *4*, 387.
4. Hu, E.; Lee, D. *Exp. Opin. Ther. Targets* **2005**, *9*, 4; Stavenger, R. A.; Cui, H.; Dowdell, S. E.; Franz, R. G.; Gaitanopoulos, D. E.; Goodman, K. B.; Hilfiker, M. A.; Ivy, R. L.; Leber, J. D.; Marino, J. P., Jr.; Oh, H.-J.; Viet, A. Q.; Xu, W.; Ye, G.; Zhang, D.; Zhao, Y.; Jolivette, L. J.; Head, M. S.; Semus, S. F.; Elkins, P. A.; Kirkpatrick, R. B.; Dul, E.; Khandekar, S. S.; Yi, T.; Jung, D. K.; Wright, L. L.; Smith, G. K.; Behm, D. J.; Doe, C. P.; Bentley, R.; Chen, Z. X.; Hu, E.; Lee, D. *J. Med. Chem.* **2007**, *50*, 2; Goodman, K. B.; Cui, H.; Dowdell, S. E.; Gaitanopoulos, D. E.; Ivy, R. L.; Sehon, C. A.; Stavenger, R. A.; Wang, G. Z.; Viet, A. Q.; Xu, W.; Ye, G.; Semus, S. F.; Evans, C.; Fries, H. E.; Jolivette, L. J.; Kirkpatrick, R. B.; Dul, E.; Khandekar, S. S.; Yi, T.; Jung, D. K.; Wright, L. L.; Smith, G. K.; Behm, D. J.; Bentley, R.; Doe, C. P.; Hu, E.; Lee, D. *J. Med. Chem.* **2007**, *50*, 6; Schirok, H.; Kast, R.; Figueroa-Pérez, S.; Bennabi, S.; Gnoth, M. J.; Feurer, A.; Heckroth, H.; Thutewohl, M.; Paulsen, H.; Knorr, A.; Hütter, J.; Lobell, M.; Münter, K.; Geiß, V.; Ehmke, H.; Lang, D.; Radtke, M.; Mittendorf, J.; Stasch, J.-P. *ChemMedChem* **2008**, *12*, 1893.
5. Saito, H.; Minamiya, Y.; Saito, S.; Ogawa, J. *J. Leukocyte Biol.* **2002**, *72*, 829.
6. Shimokawa, H.; Morishige, K.; Miyata, K.; Kandabashi, T.; Eto, Y.; Ikegaki, I.; Asano, T.; Kaibuchi, K.; Takeshita, A. *Cardiovasc. Res.* **2001**, *51*, 169; Morishige, K.; Shimokawa, H.; Eto, Y.; Kandabashi, T.; Miyata, K.; Matsumoto, Y.; Hoshijima, M.; Kaibuchi, K.; Takeshita, A. *Arterioscler. Thromb. Vasc. Biol.* **2001**, *21*, 548.
7. Registered trademark of Molecular Devices Sportsman, J. R.; Gaudet, E. A.; Boge, A. *Assay Drug Dev. Technol.* **2004**, *2*, 205.
8. The following kinases were screened by Upstate (Millipore, MA, USA) at 2 μM concentration: EGFR, IR, LKB1, MEK1, PDGFRβ, TrkB, AuroraA, CaMKIV, CDK2, CHK1, CK1δ, CK2, cRAF, CSK, cSRC, GSK3β, LCK, MAPK2, MAPKA, Met, NEK2, PAK2, Rsk2 and Zap-70.
9. The modeling study was conducted in 2005. A ROCK crystal structure was published in 2006 Jacobs, M.; Hayakawa, K.; Swenson, L.; Bellon, S.; Fleming, M.; Taslimi, P.; Doran, J. *J. Biol. Chem.* **2006**, *281*, 260.
10. Breitenlechner, C.; Gassel, M.; Hidaka, H.; Kinzel, V.; Huber, R.; Engh, R. A.; Bossemeyer. *Structure* **2003**, *11*, 1595.
11. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739; Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. *J. Med. Chem.* **2004**, *47*, 1750.
12. Clark, A. M.; Labute, P.; Santavy, M. *J. Chem. Inf. Model.* **2006**, *46*, 1107.
13. DeLano, W. L. DeLano Scientific: Palo Alto, CA, USA, 2002, http://www.pymol.org.
14. Y-27632, a known ROCK inhibitor, was used as positive control in the spontaneous hypertensive rat model. Y-27632 was dosed orally at 30 mg/kg and significant decrease in MAP was observed. The study was conducted at Aptuit (CT, USA).