Bioorganic & Medicinal Chemistry Letters 19 (2009) 6686–6690

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Benzothiazoles as Rho-associated kinase (ROCK-II) inhibitors

Yan Yin [a], Li Lin [a], Claudia Ruiz [a], Michael D. Cameron [a], Jennifer Pocas [a], Wayne Grant [a], Thomas Schröter [a], Weimin Chen [a], Derek Duckett [a], Stephan Schürer [b], Philip LoGrasso [a], Yangbo Feng [a,*]

[a] Translational Research Institute and Department of Molecular Therapeutics, The Scripps Research Institute, Florida, 130 Scripps Way, #2A1, Jupiter, FL 33458, United States
[b] Department of Pharmacology and Center for Computational Science, University of Miami, Miami, FL 33136, United States

ARTICLE INFO

Article history:
Received 28 August 2009
Revised 28 September 2009
Accepted 30 September 2009
Available online 3 October 2009

Keywords:
ROCK
Rho
Benzothiazole
Chroman
Pyrazole
Glaucoma

ABSTRACT

A series of benzothiazole derivatives as ROCK inhibitors have been discovered. Compounds with good biochemical and cellular potency, and sufficient kinase selectivity have been identified.

Rho-associated kinases (ROCK) is a serine/threonine protein kinase from the AGC kinase family with two isoforms identified as ROCK-I (or $ROCK_{\beta}$) and ROCK-II (or $ROCK_{\alpha}$).[1] Upon activation by GTP-bound RhoA, ROCK phosphorylates many substrates such as myosin light chain (MLC) and controls a variety of cellular functions, including smooth muscle contraction, proliferation, stress-fiber formation, cell migration, cell adhesion, and gene expression.[2] Inhibition of the Rho/ROCK pathway has proven to be a promising strategy for several indications such as glaucoma,[3] asthma,[4] multiple sclerosis,[5] stroke,[6] cancer,[7] erectile dysfunction,[8] and hypertension.[2b,9]

Small molecule ROCK inhibitors have been reported by several groups.[10] Recently, our group also disclosed ROCK-II inhibitors from multiple structural classes (Fig. 1).[11] These include piperazine and aminopiperidine substituted indazoles (**1** and **2**),[11a,e] benzodioxan- and chroman-based amides (**3**),[11b,c] and benzimidazole and benzoxazole based compounds (**4** and **5**).[11d,e] Here, we report a new class of ROCK inhibitors. This class was based on a benzothiazole scaffold and was analogous to benzimidazole **4** and benzoxazole **5**. The benzothiazole ring is larger than the benzoxazole and benzimidazole rings and is expected to give different properties. Thus, the benzothiazole-chromane derivative **6a** was identified as a potent ROCK-II inhibitor (IC_{50} = 9 nM) with good stability in liver microsomes ($t_{1/2}$ = 47 min in human, 23 min in rat), and low inhibition against human CYP isoforms.[12] On the other hand, this compound had a moderate selectivity against PKA (IC_{50} = 156 nM, ~17-fold) and a low cellular potency (IC_{50} = 1500 nM) as assessed by myosin light chain bis-phosphorylation (ppMLC) assays.[13] Using **6a** as the lead compound, a series of SAR studies were performed to improve the pharmaceutical properties of benzothiazole based ROCK inhibitors.

The SAR of various nitrogen-containing five- or six-membered heterocycles as the hinge-binding moieties was first investigated (Table 1). Replacement of the aminopyrimidine by a pyridine (**6b**) slightly reduced ROCK-II potency while maintaining PKA selectivity (~25-fold). Substitution on the pyridine moiety (**6c**) further reduced the compound activity. Notably, application of a pyrazole group as the hinge-binding moiety led to compound **6d** which had similar ROCK inhibitory potency to **6a** in both enzyme and cell-based ppMLC assays, but with improved selectivity against PKA (30-fold). On the other hand, methyl substitution on the pyrazole ring (**6e**) reduced both the potency and the selectivity over PKA. It is interesting to compare these results to what we observed for the benzimidazole/benzoxazole series, where a 2-aminopyrimidine as the hinge-binding moiety was the optimal in terms of ROCK-II potency and selectivity against PKA.[11d] Therefore, the unsubstituted pyrazole moiety was used in all subsequent optimizations.

The effect of substitutions on the benzothiazole ring was studied (Table 2). Fluoro-substitution on the 5-position (**6f**) reduced both the potency (IC_{50} = 47 nM compared to 11 nM in **6d**) and the selectivity against PKA (11-fold compared to 30-fold in **6d**). However, fluoro-substitution on the 4-position led to compound

* Corresponding author.
E-mail address: yfeng@scripps.edu (Y. Feng).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.115

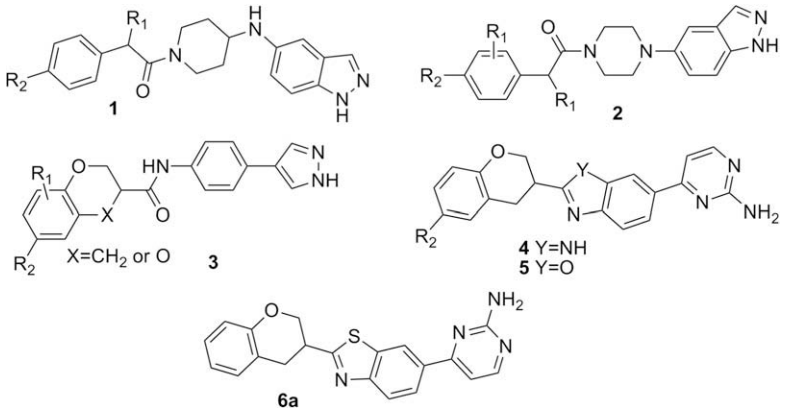
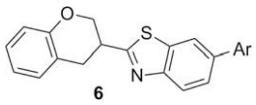


Figure 1. ROCK-II inhibitors.

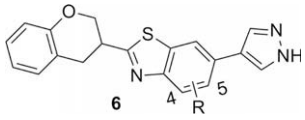
Table 1
SAR of hinge-binding moieties

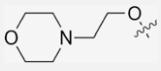
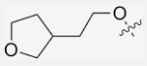
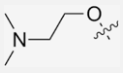
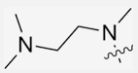


Compd	Ar	ROCK-II[a] (nM)	PKA[a] (nM)	ppMLC[a] (nM)
6a	2-Aminopyrimidine-4-yl	9	156	1500
6b	Pyridin-4-yl	42	1097	nd[b]
6c	2-Methylpyridin-4-yl	1200	7993	nd[b]
6d	1*H*-Pyrazol-4-yl	11	341	1550
6e	3-Methyl-1*H*-pyrazol-4-yl	25	404	nd[b]

[a] IC_{50} values are means of two or more experiments with standard deviation ⩽30%.
[b] Not determined.

Table 2
SAR of **6** on the benzothiazole ring



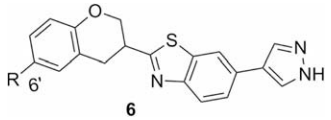
ID	R[a]	ROCK-II[b] (nM)	PKA[b] (nM)	ppMLC[b] (nM)
6f	5-F[c]	47	537	>2700
6g	F	14	644	>2700
6h	OCH_3	32	>5000	1241
6i		314	>5000	nd[d]
6j		500	>5000	nd[d]
6k		66	>5000	365
6l		51	702	365

[a] Substitution at 4-position.
[b] IC_{50} values are means of two or more experiments with standard deviation ⩽30%.
[c] Substitution at 5-position.
[d] Not determined.

6g which had similar potency against ROCK-II as compounds **6a** and **6d** and improved selectivity over PKA (43-fold). Introduction of a methoxy group on the 4-position (**6h**) significantly increased the PKA selectivity (⩾160-fold) although the ROCK-II inhibition was reduced slightly. Encouraged by the results of compound **6h**, other larger substitutions on this position were investigated. The 2-morpholinoethoxy and the 2-tetrahydrofuranylethoxy moieties (**6i** and **6j**, respectively) were not tolerated, probably due to the large rings in these two groups. On the other hand, the 2-dimethylaminoethyl substitutions (**6k** and **6l**) produced effective ROCK-II inhibitors.[14] Moreover, the alkoxy compound **6k** had good selectivity against PKA (⩾75-fold), while the tertiary amine linked compound **6l** had diminished selectivity (~12-fold). It is noteworthy that both **6k** and **6l** demonstrated better cell activities compared to **6d**.

SAR on the chroman ring was investigated to obtain more selective compounds which also had high cell potency (Table 3). Mapping different positions of the chroman ring with a methoxy group in closely related series[11b] demonstrated that a substitution at the 6-position (**6m**) provided the best compound. Thus, small functional groups such as electron-donating groups (**6m** and **6n**) and electron-withdrawing groups (**6o**, **6p**, and **6q**) were applied at the 6-position. These compounds showed slightly reduced ROCK-II potency but improved selectivity against PKA with the exception of **6o**. The best compounds came from 6-carboxamide substitutions containing a tertiary amine (**6r**, **6s**). These compounds had high ROCK-II potency (sub-nanomolar or single-digital nanomolar), high selectivity against PKA (83-fold and 847-fold for

Table 3
SAR of **6** on the chroman ring



ID	R	ROCK-II[a] (nM)	PKA[a] (nM)	ppMLC[a] (nM)
6m	OCH_3	45	>20,000	>2700
6n	CH_3	93	>20,000	nd[b]
6o	Cl	75	1775	nd[b]
6p	F	38	1929	1485
6q	$COOCH_3$	59	>5000	1485
6r	$CONH(CH_2)_2N(CH_3)_2$	7	582	20
6s SR6074	$CONH(CH_2)_3N(CH_3)_2$	0.9	765	<4

[a] IC_{50} values are means of two or more experiments with standard deviation ⩽50%.
[b] Not determined.

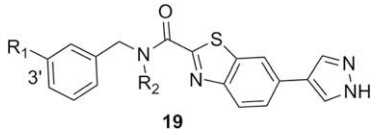
6r and **6s**, respectively), and excellent cell potency. The high cell activity obtained was probably due to the high aqueous solubility of **6r** and **6s**, in addition to their high potency against ROCK-II. Compounds **6r** and **6s** also exhibited favorable human microsomal stability with a half-life of 35 min and 34 min, respectively.

The next step in our optimizations of benzothiazole based ROCK inhibitors was to replace the chroman moiety with achiral groups. After screening several classes of substitutions at this position, benzyl carboxamides were discovered to give potent and selective ROCK-II inhibitors. As shown in Table 4, secondary benzyl amides **19a–c** showed good ROCK-II activity and selectivity over PKA. Among these three, compound **19b** was the best with an IC_{50} value of 18 nM for ROCK-II and 300-fold selectivity over PKA. Alkylation of the amide NH was applied to increase the cell potency and aqueous solubility. Thus, simple alkyl groups such as methyl (**19d**), ethyl (**19e**), and cyclopropyl (**19f**) were evaluated. Although ROCK-II inhibition decreased slightly, the cell potency was significantly improved due to the substitution by these alkyl groups. Substitution of the amide NH of **19b** with groups possessing heteroatoms gave even better inhibitors. Compound **19g**, with a methoxyethyl group, showed good inhibition of ROCK-II, and 2000-fold selectivity over PKA. The dimethylaminoethyl compound (**19h**, **SR6494**) showed excellent inhibitions in both enzyme and cellular assays (IC_{50} values of 0.4 nM and <6 nM, respectively) and good selectivity over PKA (>100-fold). Compound **19h** had also reasonable human microsomal stability ($t_{1/2}$ = 15 min). The pharmacokinetic properties of **19h** in rats (*Cl* = 50 ml/min/kg, *F* = 0%)[15] indicated that this compound would have minimum systemic exposure, possibly making it a good candidate for topical application such as in a glaucoma therapy.

To assess the selectivity of the benzothiazole based inhibitors against other kinases, compounds **6s**, **19e**, and **19h** were chosen for counter screening over several related or undesirable kinase targets such as MRCKα, JNK3, and p38 (p38 and PKA are undesirable targets).[11b–d] The results shown in Table 5 demonstrated that these compounds were quite selective except for **19h** which is a potent inhibitor of PKA. Screening against ROCK-I indicated that these benzothiazole compounds are pan-ROCK inhibitors. In the future, broader kinase counterscreening will be done on the key compounds that will be advanced to determine specificity beyond the limited number of kinases tested here.

The synthesis of various 2-chromanylbenzothiazoles **6** is outlined in Scheme 1. A S_N2 reaction of 4-bromo-2-fluoro-1-nitrobenzene (**7**) with (4-methoxyphenyl)-methane-thiol gave thiol **8**. In the presence of 5 equiv of tin(II) chloride dihydrate, nitrobenzene **8** was reduced to aniline **9**. Amide coupling reactions went smoothly by treating **9** with chroman-3-carbonyl chlorides (prepared by treating the corresponding acids with thionyl chloride in CH_2Cl_2) to produce **10**. Bromide **11** was then obtained through one pot, microwave-assisted deprotection and cyclization reactions. Finally, Suzuki coupling was applied to obtain products **6a**–**6f** and **6m–6s**.

Table 4
SAR of benzothiazole-2-carboxamides **19**



19

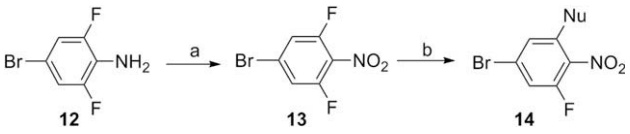
ID	R_1	R_2	ROCK-II[a] (nM)	PKA[a] (nM)	ppMLC[a] (nM)
19a	H	H	53	4644	nd[b]
19b	OCH_3	H	18	5291	>2700
19c	F	H	72	2675	nd[b]
19d	OCH_3	CH_3	59	>20,000	nd[b]
19e	OCH_3	C_2H_5	26	16,330	455
19f	OCH_3	Cyclopropyl	94	19,640	nd[b]
19g	OCH_3	Methoxyethyl	10	>20,000	871
19h SR6494	OCH_3	Dimethylamino ethyl	0.4	45	<6

[a] IC_{50} values are means of two or more experiments with standard deviation ⩽50%.
[b] Not determined.

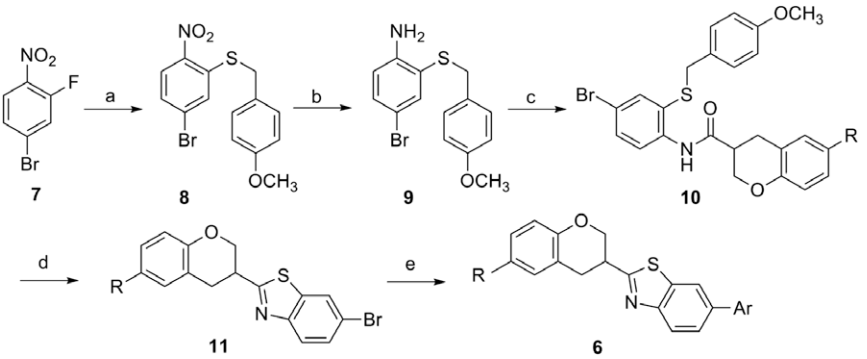
Table 5
Selectivity studies over other kinases, IC_{50} (nM)[a]

ID	ROCK-II	PKA	ROCK-I	MRCKα	JNK3	p38
6s	0.9	765	2	9552	5456	>20,000
19e	26	16,330	180	7861	>20,000	>20,000
19h	0.4	45	3.5	247	13,760	>20,000

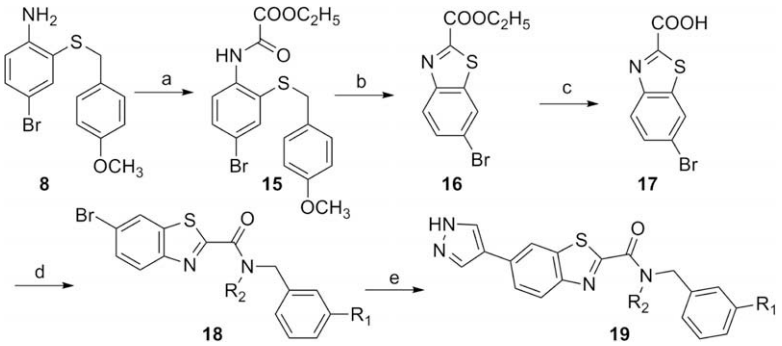
[a] Data are means of two or more measurements with standard deviation ⩽25%.



Scheme 2. Reagents and conditions: (a) $NaBO_3{\cdot}4H_2O$, CH_3COOH, 70 °C, 76%; (b) amine or alcohol nucleophilic reagents, inorganic bases, DMF, 0 °C to rt, 63–82%.



Scheme 1. Reagents and conditions: (a) (4-methoxyphenyl)methanethiol, K_2CO_3, DMF, rt, 92%; (b) $SnCl_2{\cdot}2H_2O$, EtOAc, rt, 86%; (c) chroman-3-carbonyl chlorides, pyridine, CH_2Cl_2, 0 °C; (d) TFA, microwave, 160 °C; (e) boronic acid, $Ph(PPh_3)_4$, K_2CO_3, dioxane, H_2O, 95 °C; or (i) bis-pinacolatodiboron, $PdCl_2(dppf)$, KOAc, dioxane, reflux; (ii) Ar–Br, $Ph(PPh_3)_4$, dioxane, K_2CO_3, H_2O, 95 °C, 60–80% in two steps.



Scheme 3. Reagents and conditions: (a) ethyl oxalyl chloride, pyridine, CH_2Cl_2, 0 °C, 76%; (b) TFA, microwave, 160 °C, 30 min; (c) NaOH, MeOH, H_2O, rt; (d) oxalyl chloride, DMF, CH_2Cl_2/amine, pyridine, CH_2Cl_2, 0 °C; (e) 1*H*-pyrazoleboronic acid pinacol ester, $Ph(PPh_3)_4$, K_2CO_3, dioxane, H_2O, 95 °C, 45–63% in four steps.

Benzothiazoles **6g–6l** were synthesized via intermediates **13** or **14** which were prepared as shown in Scheme 2. In the presence of excess sodium perborate tetrahydrate, 4-bromo-2,6-difluoroaniline (**12**) was oxidized to nitrobenzene **13**. Nucleophilic substitution of one of the fluorines afforded **14**. Compounds **6g–6l** were prepared using **14** and the chemistry described in Scheme 1.

Synthesis of compound **19** (Scheme 3) began with the acylation of aniline **8** with ethyl oxalyl chloride to give 2-oxoacetate **15**. Microwave-assisted thiol deprotection and cyclization in TFA produced benzothiazole-2-carboxylate **16**, which was hydrolyzed to acid **17**. Finally, amide formation with substituted or unsubstituted benzyl amines, followed by Suzuki–Miyaura coupling, gave compound **19**.

To help understand the binding motif of our benzothiazole based ROCK inhibitors, inhibitor **SR6494** was docked into the catalytic domain of a homology ROCK-II model by methods described previously.[11c] The enzyme–ligand complex with the lowest docking energy is shown in Figure 2. In this mode, the pyrazole group forms two hydrogen bonds with the enzyme: one between the backbone carbonyl group of Glu170 and the hydrogen atom of NH on the pyrazole ring, another between the backbone amino group of Met172 and another nitrogen atom of the pyrazole ring. A third hydrogen bond is formed in the phosphate binding site between the amine side chain of Lys121 and the carboxamide carbonyl group of **SR6494**. A fourth hydrogen bond is formed between the carbonyl group of Asp232 and the protonated tertiary amine of the dimethylaminoethyl moiety of the inhibitor. In addition to these hydrogen bonding interactions, another factor to the high potency of **SR6494** is the hydrophobic interaction between the benzyl amide phenyl ring and the hydrophobic pocket under the P-loop which is composed of the side chains of Phe103, Leu123 and Phe136.

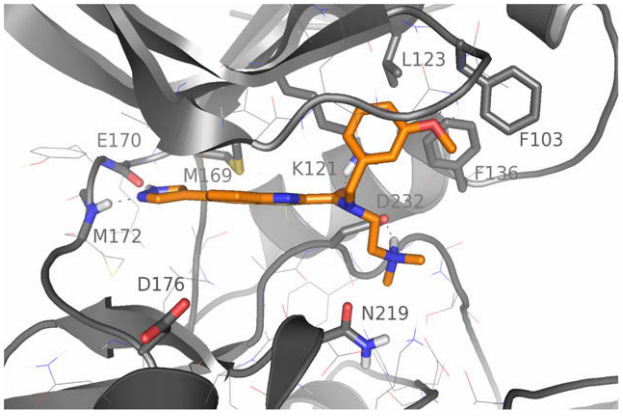


Figure 2. Model of **SR6494** docked in the catalytic domain of ROCK-II.

In conclusion, a series of potent and selective ROCK-II inhibitors based on the benzothiazole scaffold were developed. Compounds **6s** (**SR6074**) and **19h** (**SR6494**) were demonstrated to be highly potent and selective ROCK inhibitors. Future optimizations of these compounds will be mainly focused on issues such as physicochemical properties, toxicity, and selectivity against other undesirable protein targets (and a few selected inhibitors will be subjected to large panel counterscreens).

Acknowledgments

We thank Professor Patrick Griffin and Professor William Roush for their support. We also acknowledge the resources from the University of Miami Center for Computational Science (CCS publication #158).

References and notes

1. Nakagawa, O.; Fujisawa, K.; Ishizaki, T.; Saito, Y.; Nakao, K.; Narumiya, S. *FEBS Lett.* **1996**, *392*, 189.
2. (a) Leung, T.; Chen, X. Q.; Manser, E.; Lim, L. *Mol. Cell. Biol.* **1996**, *16*, 5313; (b) Uehata, M.; Ishizaki, T.; Satoh, H.; Ono, T.; Kawahara, T.; Morishita, T.; Tamakawa, H.; Yamagami, K.; Inui, J.; Maekawa, M.; Narumiya, S. *Nature* **1997**, *389*, 990; (c) Somlyo, A. P.; Somlyo, A. V. *J. Physiol.* **2000**, *522*, 177; (d) Riento, K.; Ridley, A. *J. Nat. Rev. Mol. Cell. Biol.* **2003**, *4*, 446.
3. (a) Tanihara, H.; Inatani, M.; Honjo, M.; Tokushige, H.; Azuma, J.; Araie, M. *Arch. Ophthalmol.* **2008**, *126*, 309; (b) Whitlock, N. A.; Harrison, B.; Mixon, T.; Yu, X.; Wilson, A.; Gerhardt, B.; Eberhart, D. E.; Abuin, A.; Rice, D. S. *J. Ocul. Pharmacol. Ther.* **2009**, *25*, 187; (c) Fukunage, T.; Ikesugi, K.; Nishio, M.; Sugimoto, M.; Sasoh, M.; Hidaka, H.; Uji, Y. *Curr. Eye Res.* **2009**, *34*, 42.
4. (a) Gosens, R.; Schaafsma, D.; Nelemans, S. A.; Halayko, A. J. *Mini-Rev. Med. Chem.* **2006**, *6*, 339; (b) Schaafsma, D.; Gosens, R.; Zaagsma, J.; Halayko, A. J.; Meurs, H. *Eur . J. Pharmacol.* **2008**, *585*, 398.
5. Sun, X.; Minohara, M.; Kikuchi, H.; Ishizu, T.; Tanaka, M.; Piao, H.; Osoegawa, M.; Ohyagi, Y.; Shimokawa, H.; Kira, J. I. *J. Neuoimmunol.* **2006**, *180*, 126.
6. (a) Rikitake, Y.; Kim, H. H.; Huang, Z.; Seto, M.; Yano, K.; Asano, T.; Moskowitz, M. A.; Liao, J. K. *Stroke* **2005**, *36*, 2251; (b) Shin, H. K.; Salomone, S.; Ayara, C. *Exp. Opin.* **2008**, 1547.
7. Ying, H.; Biroc, S.; Li, W. W.; Alicke, B.; Xuan, J. A.; Pagila, R.; Ohashi, Y.; Okada, T.; Kamata, Y.; Dinter, H. *Mol. Cancer Ther.* **2006**, *5*, 2158.
8. Park, K.; Kim, S. W.; Rhu, K. S.; Paick, J. S. *J. Sex Med.* **2006**, *3*, 996.
9. Shimokawa, H.; Takeshita, A. *Arterioscler. Throm. Vasc. Biol.* **2005**, *25*, 1767.
10. (a) Gingras, K.; Avedissian, H.; Thouin, E.; Boulanger, V.; Essagian, C.; McKerracher, L.; Lubell, W. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4931; (b) Takami, A.; Iwakubo, M.; Okada, Y.; Kawata, T.; Odai, H.; Takahashi, N.; Shindo, K.; Kimura, K.; Tagami, Y.; Miyake, M.; Fukushima, K.; Inagaki, M.; Amano, M.; Kaibuchi, K.; Iijima, H. *Bioorg. Med. Chem.* **2004**, *12*, 2115; (c) Iwakubo, M.; Takami, A.; Okada, Y.; Kawata, T.; Tagami, Y.; Ohashi, H.; Sato, M.; Sugiyama, T.; Fukushima, K.; Iijima, H. *Bioorg. Med. Chem.* **2007**, *15*, 350; (d) Iwakubo, M.; Takami, A.; Okada, Y.; Kawata, T.; Tagami, Y.; Ohashi, H.; Kawata, T.; Tagami, Y.; Sato, M.; Sugiyama, T.; Fukushima, K.; Taya, S.; Amano, M.; Kaibuchi, K.; Iijima, H. *Bioorg. Med. Chem.* **2007**, *15*, 1022; (e) Stavenger, R.; Cui, H.; Dowdell, S.; Franz, R.; Gaitanopoulos, D.; Goodman, K.; Hilfiker, M.; Ivy, R.; Leber, J.; Marino, J.; Oh, H.; Viet, A.; Xu, W.; Ye, G.; Zhang, D.; Zhao, Y.; Jolivette, L.; Head, M.; Semus, S.; Elkins, P.; Kirkpatrick, R.; Dul, E.; Khandekar, S.; Yi, T.; Jung, D.; Wright, L.; Smith, G.; Behm, D.; Doe, C.; Bentley, R.; Chen, Z.; Hu, E.; Lee, D. *J.*

Med. Chem. **2007**, *50*, 2; (f) Goodman, K.; Cui, H.; Dowdell, S.; Gaitanopoulos, D.; Ivy, R.; Sehon, C.; Stavenger, R.; Wang, G.; Viet, A.; Xu, W.; Ye, G.; Semus, S.; Evans, C.; Fries, H.; Jolivette, L.; Kirkpatrick, R.; Dul, E.; Khandekar, S.; Yi, T.; Jung, D.; Wright, L.; Smith, G.; Behm, D.; Bentley, R.; Doe, C.; Hu, E.; Lee, D. *J. Med. Chem.* **2007**, *50*, 6; (g) Sehon, C.; Wang, G.; Viet, A.; Goodman, K.; Dowdell, S.; Elkins, P.; Semus, S.; Evans, C.; Jolivette, L.; Kirkpatrick, R.; Dull, E.; Khandekar, S.; Yi, T.; Wright, L.; Smith, G.; Behm, D.; Bentley, R.; Doe, C.; Hu, E.; Lee, D. *J. Med. Chem.* **2008**, *51*, 6631; (h) 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.; Geiβ, V.; Ehmke, H.; Lang, D.; Radtke, M.; Mittendorf, J.; Stasch, J. P. *ChemMedChem* **2008**, *3*, 1893.

11. (a) Feng, Y.; Cameron, M.; Frackowiak, B.; Griffin, E.; Lin, L.; Ruiz, C.; Schröter, T.; LoGrasso, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2355; (b) Chen, Y. T.; Bannister, T.; Weiser, A.; Griffin, E.; Lin, L.; Ruiz, C.; Cameron, M.; Schürer, S.; Duckett, D.; Schröter, T.; LoGrasso, P.; Feng, Y. *Bioorg. Med. Chem. Lett.* **2008**, 18, 6406; (c) Feng, Y.; Yin, Y.; Weiser, A.; Griffin, E.; Cameron, M.; Lin, L.; Ruiz, C.; Schürer, S. C.; Inoue, T.; Rao, V.; Schröter, T.; LoGrasso, P. *J. Med. Chem.* **2008**, *51*, 6642; (d) Sessions, E. H.; Yin, Y.; Bannister, T.; Weiser, A.; Griffin, E.; Pocas, J.; Cameron, M.; Ruiz, C.; Lin, L.; Schürer, S. C.; Schröter, T.; LoGrasso, P.; Feng, Y. *Bioorg. Med. Chem. Lett.* **2008**, 18, 6390; (e) LoGrasso, P.; Feng, Y. *Curr. Top. Med. Chem.* **2009**, *9*, 704.
12. % Inhibition of CYP450 isoforms 1A2/2C9/2D6/3A4 at 10 μM was 2/10/28/-14.
13. Schröter, T.; Griffin, E.; Weiser, A.; Feng, Y.; LoGrasso, P. *Biochem. Biophys. Res. Commun.* **2008**, *374*, 356.
14. Although the corresponding compound of **6l** with a secondary amine (NH) linkage (instead of the tertiary amine linkage in **6l**) was not prepared and evaluated, compounds with this side chain (dimethylaminoethylamine-) were synthesized in closely related series and were discovered to have much reduced ROCK potency.
15. In vivo pharmacokinetic data were obtained in rats from three determinations with standard deviation $\leqslant$20%.