Advances in the studies of roles of Rho/Rho-kinase in diseases and the development of its inhibitors

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Advances in the studies of roles of Rho/Rho-kinase in diseases and the development of its inhibitors

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ABSTRACT

RhoA/Rho-kinase pathway plays a pivotal role in numerous fundamental cellular functions including contraction, motility, proliferation, differentiation and apoptosis. The pathway is also involved in the development of many diseases such as vasospasm, pulmonary hypertension, cancer and central nervous systems (CNS) disorders. The inhibitors of Rho kinase have been extensively studied since the Rho/Rho-kinase pathway was verified as a target for a number of diseases. Herein, we reviewed the advances in the studies of the roles of Rho/Rho-kinase in diseases and the development of Rho-kinase inhibitors in recent five years.

1. Introduction

Rho is a subfamily member of small molecular GTPases superfamily related to Ras. Ras comprises 20 members and are widely expressed in mammals, including RhoA, Rac1, and Cdc42. As the best-characterized Ras protein, RhoA acts as a molecular switch which cycles between an inactive GDP-bound and an active GTP-bound conformation, interacting with downstream targets to trigger a series of cellular responses. The activity of RhoA is regulated by many regulatory proteins, for example, guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and GDP dissociation inhibitors (GDIs). GEFs facilitate Rho to release GDP and bind with GTP subsequently while GAPs stimulate the Rho activity which hydrolyzes GTP into GDP. GDIs may suppress the transformation between Rho-GDP and Rho-GTP forms. The three proteins interact mutually and regulate the conversion of two forms of Rho (Fig. 1) [1–4].

'Unconventional' regulatory modes also exist in the control of Rho GTPase activities and cellular functions. Firstly, at pre- and post-transcriptional levels, the expression of Rho GTPases can be regulated by epigenetic modifications of their microRNAs and chromosomal DNAs. Then, palmitylation, prenylation and nuclear localization signals (NLSs) of a subset of Rho GTPases can make them target the proper intracellular compartments to perform cell functions. After that, post-translational covalent modifications, such as phosphorylation, transglutamination, AMPylation and SUMOylation, can mediate the activity of Rho GTPases. Finally, low level of active Rho GTPases at the protein level can

Abbreviations: ACS, acute coronary syndrome; AP-1, activator protein-1; AT1, angiotensin II type 1 receptor; CNS, central nervous system; CPI-17, PKC-potentiated inhibitory protein 17; CPK, creatine phosphokinase; CRD, C-terminal cysteine-rich domain; CRMP-2, collapsin response mediator protein 2; DMPK, myotonic dystrophy kinase; ERK, extracellular signal-regulated kinase; ERM, ezrin/radixin/moesin family; ET-1, endothelin-1; GAPs, GTPase-activating proteins; GDIs, GDP dissociation inhibitors; GEFs, guanine nucleotide exchange factors; HCC, hepatocellular carcinoma; 5-HT, 5-hydroxytryptamine; IP3, inositol 1,4,5-trisphosphate; LDH, lactate dehydrogenase; MCP-1, monocyte chemoattractant protein-1; MEK, mitogen-activated protein kinase; MES, maximal electroconvulsive shock; MLC, myosin light chain; MRCK, myotonic dystrophy kinase-related CDC42-binding kinase; MYPT-1, myosin phosphatase target subunit 1; NLSs, nuclear localization signals; OpN, optic nerve; PAH, pulmonary arterial hypertension; PAI-1, plasminogen activator inhibitor-1; PH, pleckstrin homology; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTZ, pentylenetetrazole; RBD, Rho-binding domain; ROCK, Rho-kinase; UPS, ubiquitin–proteasome system; VA, vasospastic angina; VCAM-1, vascular cell adhesion molecule-1; VSMC, vascular smooth muscle cell.

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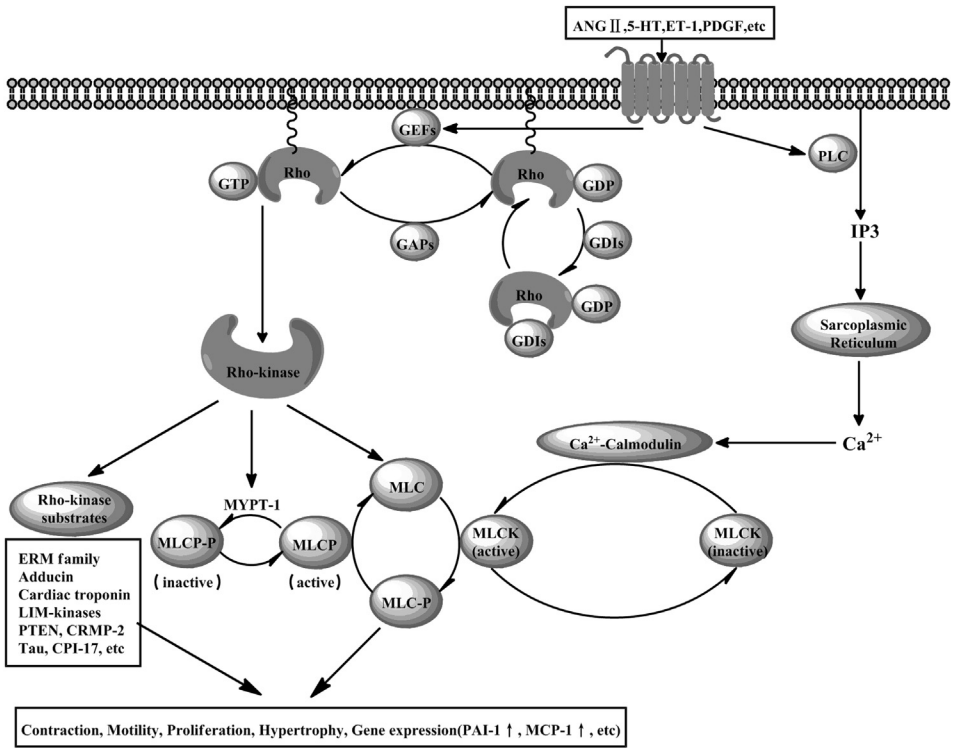


Fig. 1. The regulatory mechanisms of Rho GTPase and ROCK. GEFs, GAPs, and GDIs, the main regulatory components, control the cycling between GTP-bound and GTP-bound conformation. GEFs catalyze the exchange of bound GDP for GTP to activate RhoA, whereas GAPs are able to activate the hydrolysis of GTP and then to inactivate Rho GTPases, the active Rho species. Besides, RhoGDIs can combine with prenylated GDP-bound Rho proteins and allow Rho-GDP to translocate between the membranes and cytoplasm. Rho GTPases, small GTP-binding proteins can act as molecular switches and regulate many intracellular signaling pathways. RhoA cycles between an active GDP-bound and an inactive GTP-bound conformation, interacting with downstream targets, such as Rho-kinase. Besides, various substrates of Rho-kinase have been identified, including myosin phosphatase target subunit 1 (MYPT-1), myosin light chain (MLC), ezrin/radixin/moesin (ERM) family, adducin, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and LIM-kinases, etc (Modified from Liu, M., Trends Cell Biol., 2012 and Satoh, K. et al., Am. J. Physiol. Heart Circ. Physiol., 2011).

counterbalance Rho GTPase activation through the ubiquitin–proteasome system (UPS). Taken together, these regulatory mechanisms play important roles in the modulation of Rho GTPases in a variety of cellular processes including actin and microtubule dynamics, gene expression, cell cycle progression, cell adhesion, cell survival, membrane transport and polarity establishment [4]. To evoke more interests in the ROCK pathway, here we summarized the advances in the studies of the roles of Rho/Rho-kinase in diseases and the development of Rho-kinase inhibitors in recent five years.

2. Rho-kinase (ROCK)

2.1. The structures of ROCK

ROCK, belonging to serine/threonine kinases family, is a key downstream effector of RhoA. Till now, two isoforms of ROCK were confirmed, namely ROCK1 (also known as ROKβ and p160 ROCK) and ROCK2 (also known as ROKα and Rho-kinase). The two isoforms' functions are different. ROCK1 is mainly for circulating inflammatory cells and ROCK2 for vascular smooth muscle cells (VSMC) [1]. For distribution, ROCK1 is prominent in the lung, liver, spleen, kidney and testis, and ROCK2 is expressed preferentially in brain, muscle, and heart [2,5].

ROCK consists of an N-terminal kinase domain, a central coiled-coil-forming region containing a Rho-binding domain (RBD), and a pleckstrin homology (PH) motif with a C-terminal cysteine-rich domain (CRD) (Fig. 2). The genes of two ROCKs have been identified in mammalian systems. ROCK1 is located in chromosome 18 and encodes a 1354 amino acid protein, while ROCK2 is located in chromosome 2 and encodes a 1388 amino acids polypeptide. The two ROCKs share an overall homology of 65% in their amino acid sequences and 92% homology in their kinase domains. ROCKs are also homologous to other members of AGC kinase, such as myotonic dystrophy kinase-related CDC42-binding kinase (MRCK), citron kinase, and myotonic dystrophy kinase (DMPK) [3,6].

2.2. The regulation of ROCK

The ROCK C-terminus serves as a self automatic regulating inhibitor of the N-terminal kinase domain and modulates its function. The GTP-bound form of Rho, an activated form, interacts with the RBD of ROCK and subsequently activates ROCK by relieving repression of the N-terminal kinase domain by the RBD–PH-domain, finally resulting in the generation of an active 'open' kinase domain. Meanwhile, arachidonic acid binding to the PH domain or cleavage of the C-terminus by caspase-3 or granzyme B is able to induce the 'open' conformation. ROCK can also be activated by Rho through transphosphorylation of N-terminal or suppression of other small GTP-binding proteins such as Rad and Gem (Fig. 1). However, a structural analysis shows that phosphorylation at the activated loop and hydrophobic motif within the catalytic domain is not necessary in ROCK activation although necessary for the activation of other major AGC-family kinases [6].

It have been reported that dimerization is critical for ROCKs' function in cells and for its regulation by Rho or its inhibitors

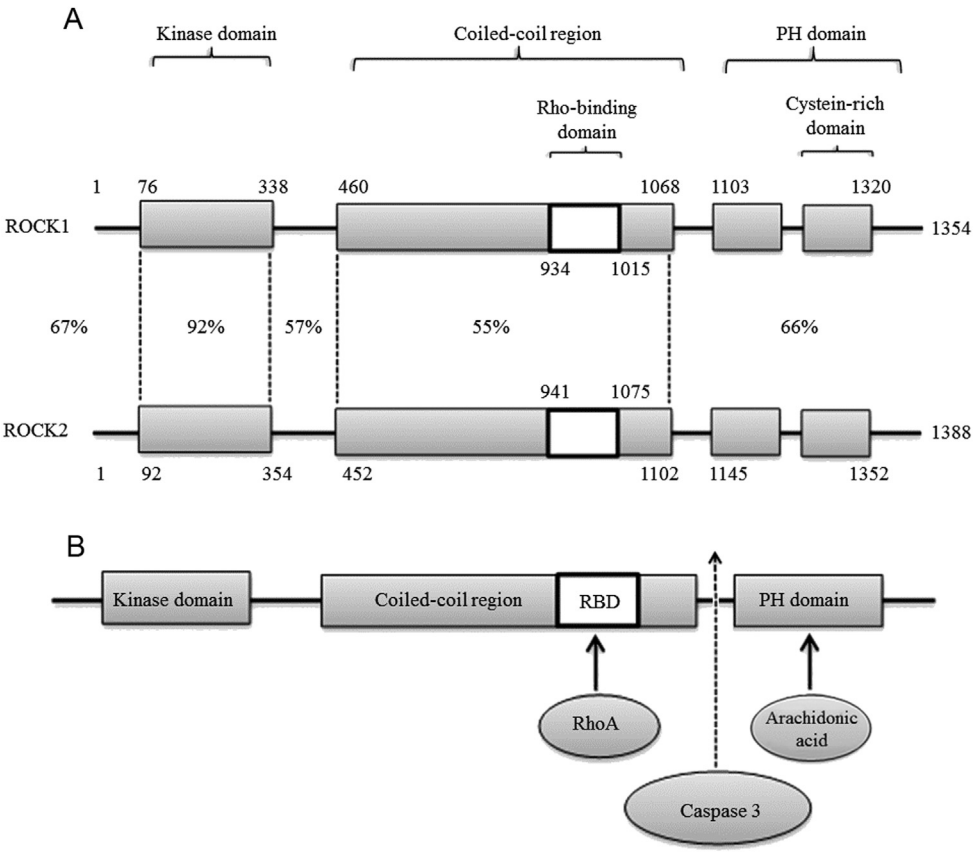


Fig. 2. ROCK structure and activation mechanism. A) Structural diagram of ROCK1 and ROCK2. Both isoforms consist of an N-terminal kinase domain, a coiled-coil-forming region which contains a RBD, and a C-terminal CRD sited in the PH domain. The two isoforms share overall 65% identity in amino acid sequence and 92% identity in their kinase domains (Modified from Zhou, Q. et al., Trends Pharmacol. Sci., 2011). B) Activation of ROCK. The open configuration of the active ROCK is regulated by binding of RhoA to the RBD, binding of arachidonic acid to the PH domain, and cleavage of C-terminal PH domain by caspase 3 (Modified from Zhou, Q. et al., Trends Pharmacol. Sci., 2011).

[7–10]. ROCKs can exist in an inactive state when the kinase domain interacts with a negative autoregulatory domain located near the C-terminus [8]. The crystal structures of the kinase domains of ROCK1 and ROCK2 demonstrated that the kinase regions crystallize as dimers [7,8]. For ROCK1, amino acids 6–415 dimerize primarily through the helical region N-terminal to the kinase domain, although parts of the kinase domain also contribute to dimer formation [8]. The ROCK2 structure includes additional amino acids C-terminal to the kinase domain, which contribute to dimerization by interacting with the N-terminal region [7]. Although ROCK1 and ROCK2 are highly homologous in their kinase domains, their N-terminal sequences are very different. For example, ROCK1 but not ROCK2 interacts with RhoE [7]. Moreover, two distinct regions of ROCK1 can mediate its dimerization, the N-terminal region and the kinase-proximal part of the central coiled-coil region. Dimerization via the N-terminus of ROCK1 is critical both for RhoE binding and for ROCK1 to induce actin reorganization in cells [9]. For ROCK2, Thr^{405} in hydrophobic motif in ROCK2 kinase domain is essential for kinase activity and dimerization through interaction with a single, conserved, residue in the N-terminal extension which containing Asp^{39} residue [11]. Both ROCK2 activity and dimerization were dependent upon the interaction between Thr (405) of the hydrophobic motif and Asp (39) of the N-terminal extension. A phosphomimetic residue at position 405 was inhibitory for ROCK2 activity and dimerization [11]. These findings have offered the support for development of small-molecule inhibitors designed to modulate ROCK activation by selectively disrupting the dimer formation.

2.3. The biological effects of ROCK

Phosphorylation of myosin light chain (MLC) is a key event in regulating VSMC contraction. MLC can be phosphorylated by Ca^{2+}-calmodulin-activated MLC kinase and dephosphorylated by MLC phosphatase. Agonists bind to G protein-coupled receptors, and induce contraction by increasing both cytosolic Ca^{2+} concentration and ROCK activity through mediating guanine nucleotide exchange factor. Numerous substrates of ROCK have been identified, including MLC, myosin-binding subunit or myosin phosphatase target subunit 1, ezrin/radixin/moesin family, adducin, phosphatase and tensin homolog on chromosome 10, and LIM-kinases. ROCK enhances MLC phosphorylation through the inhibition of myosin-binding subunit of myosin phosphatase and mediates agonists-induced VSMC contraction (Fig. 1) [1].

3. ROCK and diseases

Recent studies identified ROCK as an important target while it is observed to be involved in a variety of diseases [12]. The benefit of ROCK inhibition might extend to the treatment of cardiovascular diseases, nervous system disorders, cancer, diabetes and so on. Furthermore, they could potentially be used to treat nephropathy, heart diseases, obesity, ocular hypertension and glaucoma.

3.1. Cardiovascular diseases

The Rho/ROCK pathway has been demonstrated to play an important role in the pathogenesis of various cardiovascular

diseases. ROCK activity was increased in patients with acute coronary syndrome (ACS), particularly in those with myocardial infarction [13]. Inhibition of Rho/ROCKs might be an attractive therapeutic approach in the treatment of diseases, including coronary vasospasm, cerebral artery vasospasm, and systemic or pulmonary hypertension [14].

The vasodilatation effect caused by fasudil mesylate *in vivo* and *in vitro* has been proved [15]. Besides, Sang-Yong Yoo et al. [16] has demonstrated an association of ROCK2 gene polymorphisms with the risk of Vasospastic Angina (VA) and a protective effect of the haplotype G-T-C-T-G on the occurrence of VA. Their findings suggested that the ROCK2 gene might be involved in the pathogenesis of VA in the Korean race. Previous research results proved that long-term inhibition of Rho-kinase ameliorated pulmonary arterial hypertension (PAH) in animal models. Based on these, Hiroshi Fujita et al. [17] examined acute vasodilator effects of inhaled Fasudil as a more feasible form to be locally delivered for PAH. Later, aerosols of liposomal Fasudil were tested for prolonged pulmonary vasodilation in monocrotaline induced PAH rats [18].

3.2. Nervous system disorders

ROCK is involved in regulating neural cell migration, proliferation, survival, axon guidance, and regeneration [19]. It was found to be closely related to the pathogenesis of several nervous system disorders. Recent studies showed that ROCK inhibitors had potential therapeutic application for Alzheimer's disease [20,21], Parkinson's disease [22,23], stroke [24], epilepsy [25], chronic pain [26], autoimmune neuritis [27] and so on (more details are well summarized by M. Chen et al. [28]).

3.3. Cancer

Recent studies have demonstrated a more diverse range of the functions of ROCK. These findings indicated that ROCK inhibitors might be drug candidate to treat cancer by targeting stromal cells rather than tumor cells [29]. Hiromichi Nakabayashi et al. [30] showed that Fasudil suppressed glioma-induced angiogenesis by targeting Rho-ROCK and mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK/ERK) signal pathways, implying a potential of Fasudil to treat glioma. ROCK inhibitors showed a promising outlook on K-Ras-induced lung cancers and hematological malignancies [29] .In the studies of Xueying Yang et al. [31], Fasudil prevented proliferation and metastasis of NCI-H446 small cell lung cancer cells. Hany Eldawoody et al. [32] suggested that Fasudil attenuated induction of cerebral aneurysms in the rat model. Yuko Takeba et al. [33] showed that ROCK was expressed in hepatic tissues in hepatocellular carcinoma (HCC), and proved its involvements in cell survival in HCC cells using Fasudil.

3.4. Diabetes complications

Fasudil can also attenuate high glucose-induced monocyte adhesion to endothelial cells, ostensibly through limiting expression of endothelial vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1). These results suggested that the inhibition of Rho/ROCK signaling might have therapeutic potential in preventing diabetes associated vascular inflammation and atherogenesis [34]. Besides, the up-regulation of fibronectin induced by high glucose, which might lead to renal disease, was halted by ROCK inhibition. Studies also showed that bone marrow endothelial barrier dysfunction caused by diabetes was the effect of the activation of Rho/Rho-kinase pathway [35]. Fasudil prevented glomerular sclerosis, glomerular fibronectin up-regulation, and proteinuria in diabetic rats, with almost equipotent effectiveness to enalapril [36].

3.5. Other diseases

Some studies also showed that Fasudil has renoprotective effects through the suppression of inflammatory, apoptotic, and fibrogenic factors, offering a therapeutic approach to intervene cyclosporine-induced nephropathy [37]. Na Wang et al. [38] found that Fasudil significantly decreased plasma lactate dehydrogenase (LDH), dose dependently attenuated adriamycin-induced hemodynamic, histopathologic and ultra-structural changes in the left ventricle of the heart, likely by inhibiting RhoA/ROCK pathway. Robert D. Williams et al. [39] reported that AR-12286, a ROCK inhibitor in clinical research, provided statistically significant ocular hypotensive efficacy in patients who were diagnosed with ocular hypertension and glaucoma. More interestingly, ROCK1 was found to be involved in regulation of leptin action on energy balance, and ROCK1 deletion in arcuate nucleus resulted in increasing food intake and severe obesity [40], implying that ROCK might be a new target for the treatment of obesity.

4. Advances in the development of ROCK inhibitors

Various evidences have demonstrated that over-expression of ROCK is involved in the pathogenesis of numerous diseases and inhibition of ROCK shows beneficial effects in animal and human disease models. Consequently, an increasing amount of efforts have been made to the studies on the development of ROCK inhibitors. In 2007, Weigang Duan et al. [41] have reviewed the advances in the study of Rho kinase and its inhibitors. After that, many new potential Rho kinase inhibitors have been proved effective *in vivo* and *in vitro* experiments. Herein, we reviewed a series of new reported ROCK inhibitors according to their chemical classification.

4.1. Isoquinolines

Fasudil (also named HA1077) (**1**-**1**), a moderate inhibitor of ROCK, consists of the isoquinoline and the homopiperazine ring connected by a sulphonyl group. Fasudil, inhibiting ROCK via targeting ATP-dependent kinase domain, is the first and only clinically available ROCK inhibitor. It was initially approved for the treatment of cerebral vasospasm complicating intracranial hemorrhage. Fasudil has major vasodilatory activity and is currently undergoing clinical trials for the treatment of ischemic heart disease [14,15]. Although its IC_{50} value against ROCK2 (IC_{50} = 0.158 μM) is much lower than those for other kinases such as PKA and PKC, Fasudil still retains relative potent inhibition effects against these kinases [42]. Several analogs of Fasudil were developed in order to seek highly specific ROCK inhibitors. Masayuki Iwakubo et al. [43] showed that **1**-**2** displayed a higher inhibitory potency than its (S)-isomer in a cell-free kinase assay and in the cell migration assay ($IC_{50(ENZ)}$ = 25 nM and $IC_{50(MCP)}$ = 1 μM). The compound can be employed as a molecular probe to study the function of Rho kinase *in vivo*. **1**-**3** and **1**-**4** were found to be ROCK1 inhibitors by Peter Ray et al. [44]. Darja Lavogina et al. [45] showed that **ARC-1083** (**1**-**5**) possessed subnanomolar dissociation constant (Kd) towards several kinases including ROCK of the AGC-group, and **ARC-3002** (**1**-**6**) had high affinity towards ROCK2 (Kd = 20 pM), over 160-fold selectivity compared to that against PKAc (Fig. 3).

4.2. Pyridines

Y-27632 (**2**-**1**) is a non-specific inhibitor of both ROCK1 and ROCK2 by competing with ATP for binding to their catalytic sites. It

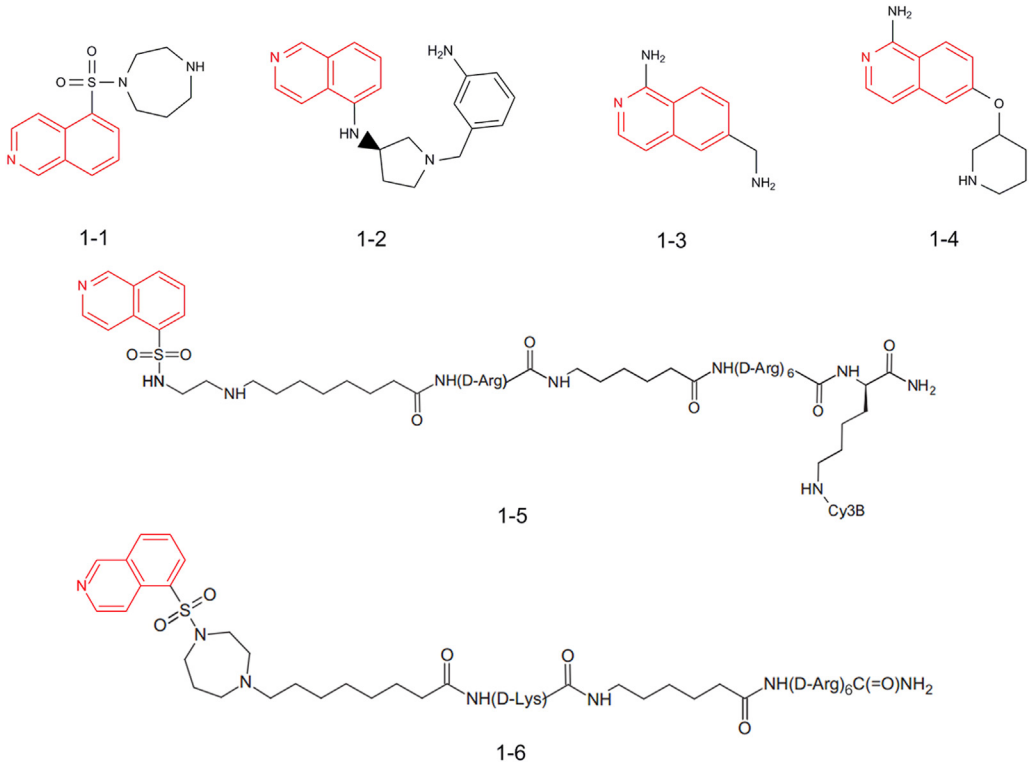


Fig. 3. Structures of ROCK inhibitors with isoquinoline ring.

inhibited smooth muscle contractility and normalized blood pressure in rat hypertensive models [14]. In the study of Koc-Kan Ho et al. [46], **2-2** was demonstrated to have *in vivo* activity in a spontaneously hypertensive rat model. Alan J. Henderson et al. [47] reported that **2-3** lowered intraocular pressure in *in vivo* studies. Rongshi Li et al. [48] discovered a novel series of ROCK inhibitors, in which **2-4** possessed more specificity against ROCK2 (IC_{50} = 100 nM) over ROCK1 (IC_{50} = 1690 nM) (Fig. 4).

4.3. Indazoles

Yangbo Feng et al. [49] have developed a series of 1H-indazol analogs of ROCK2 inhibitors, including SR-1459 (**3-1**) with IC_{50} 13 nM and **SR-899** (**3-2**) with IC_{50} 100 nM. Rongshi Li et al. [48] discovered that **3-3** was equipotent for ROCK1 (IC_{50} = 650 nM) and ROCK2 (IC_{50} = 670 nM). In the optimization of the initial dihydropyrimidinone **3-4**, Krista B. Goodman et al. [50] found that 6-fluoroindazole **3-5**, another potent ROCK1 inhibitor with 61% oral bioavailability, dramatically reduced mean arterial pressure in spontaneously hypertensive rats after oral administration. Clark A. Sehon et al. [51] developed a series of potent and selective dihydropyrimidine inhibitors of ROCK1 from **3-6**. Among them, **3-7** had promising oral bioavailability (49% in rat and 19% in monkey), good half-life (1.8 h in rat and 2.2 h in monkey), good selectivity over a panel of 31 kinases (>100 fold), and a dramatically improved P450 profile (>2.2 μM at all isozymes tested). Finally, oral administration of **3-7** gave a robust 25 mmHg reduction mean arterial pressure in spontaneously hypertensive rats at a single dose of 30 mg/kg (Fig. 5).

4.4. Pyrazoles

Yen Ting Chen et al. [52] synthesized a series of chroman-3-amides compounds (**4-1**) with remarkable inhibitory activity against ROCK2 and other pharmacological actions in the further study. Yan Yin et al. [53] discovered a series of benzothiazole derivatives as ROCK inhibitors, in which some compounds (**4-2**) possessed significantly biochemical and cellular potency. A series of tetrahydroisoquinoline-based amides as ROCK inhibitors starting from the initial **4-3** was developed by Xingang Fang et al. [54]. Among them, **4-4** had subnanomolar potency on ROCK2 and excellent cellular potency (IC_{50} = 51 nM). Sarwat Chowdhury et al. [55] showed that ROCK inhibitors **4-5** had high inhibitory potency on ROCK (IC_{50} = 1 nM) and showed good drug metabolism and

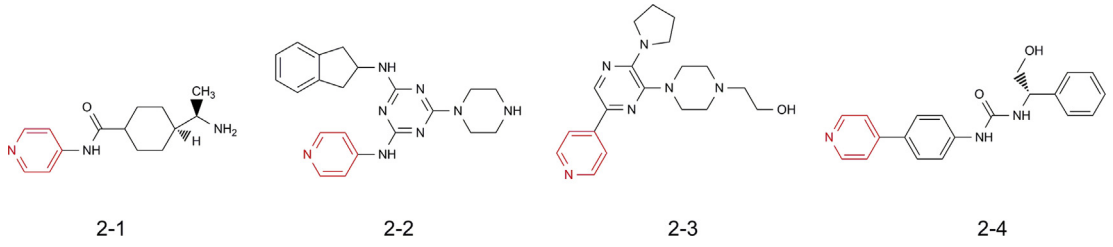


Fig. 4. Structures of ROCK inhibitors with pyridine ring.

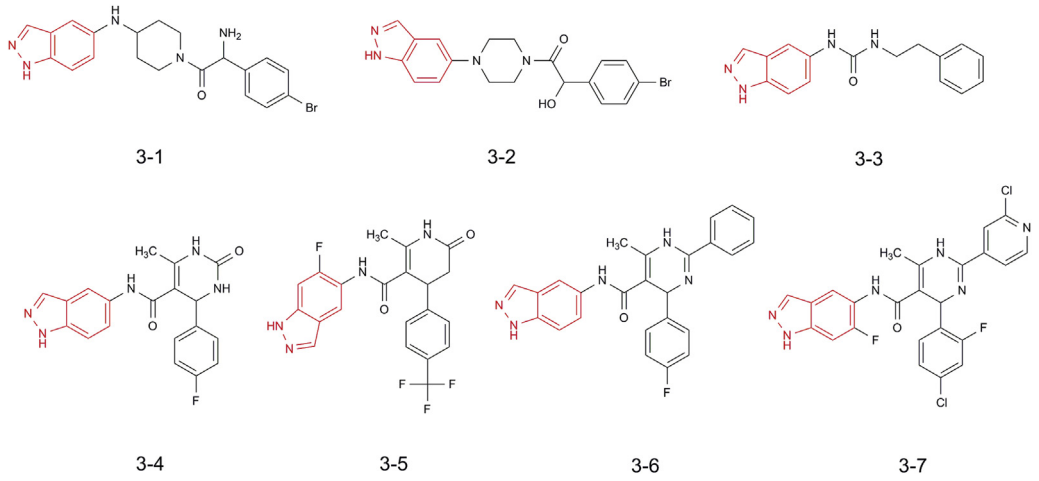


Fig. 5. Structures of ROCK inhibitors with indazole ring.

pharmacokinetic (DMPK) properties, making it a good candidate for further development. Xingang Fang et al. [56] described a series of quinazoline and 4-quinazolinone pyrazoles as potent ROCK inhibitors. Among them, **4-6** possessed good oral exposure levels favorable for systemic applications. E. Hampton Sessions et al. [57] found that **4-7** and **4-8** were good for nonsystemic local applications. Yan Yin et al. [58] developed a series of ROCK inhibitors among which **4-9** showed high inhibitory potency on ROCK2 ($IC_{50} < 1$ nM). After that, they [59] developed **4-10** and **4-11** which were found to be potent and selective inhibitors of ROCK2, both with IC_{50} 2 nM (Fig. 6).

4.5. Others

Robert A. Stavenger et al. [60] demonstrated that the aminofurazanyl-azabenzimidazole structure was a useful template

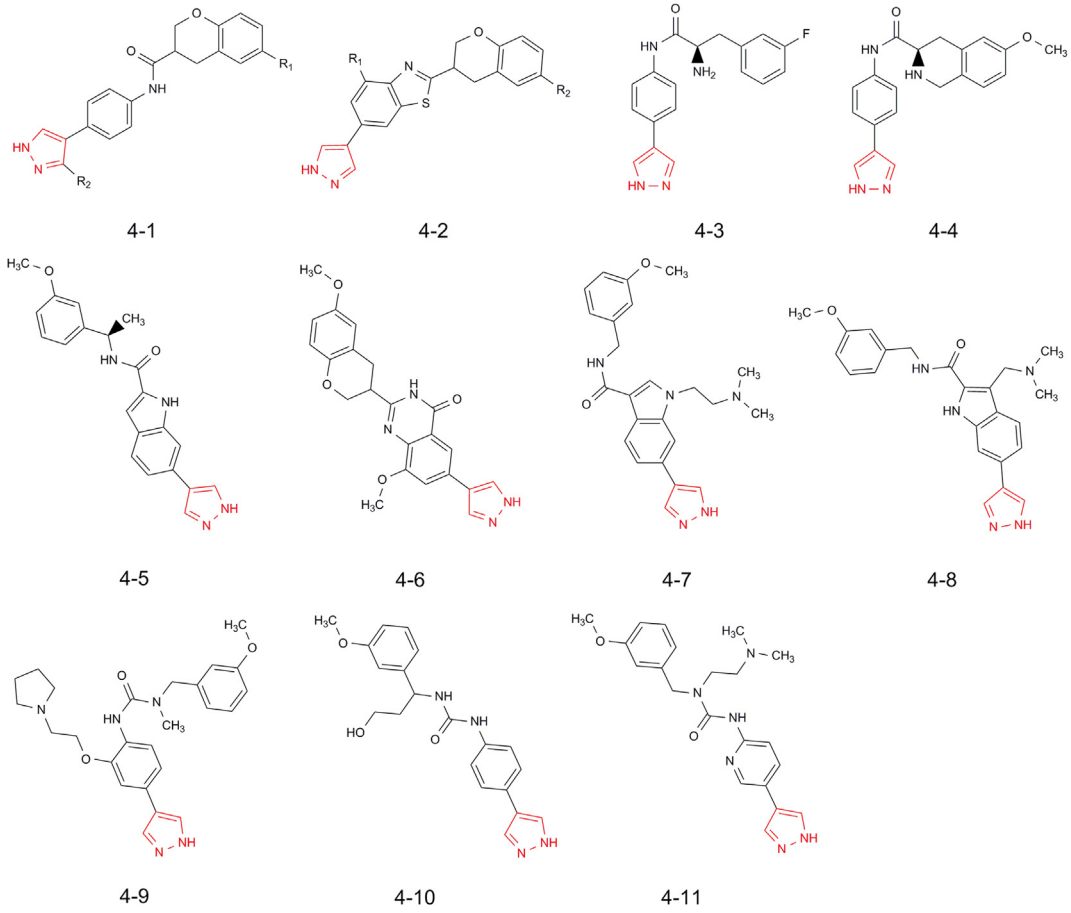


Fig. 6. Structures of ROCK inhibitors with pyrazole ring.

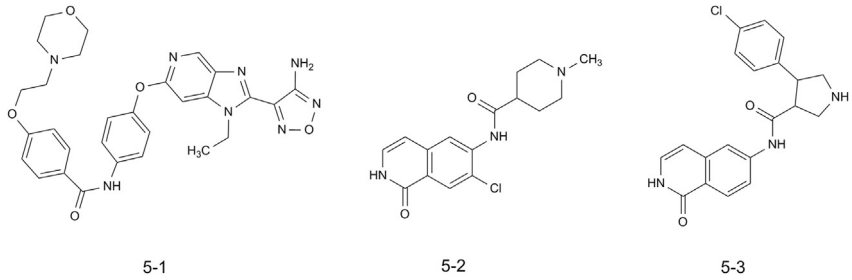


Fig. 7. Other structures of ROCK inhibitors.

for the development of ROCK1 inhibitors. In their study, **5-1** dramatically lowered blood pressure in a rat model of hypertension. Todd Bosanac et al. [61] described the discovery and SAR study of a series of beta-aryl substituted pyrrolidine 2*H*-isoquinolin-1-one inhibitors of ROCK derived from **5-2**, leading to the identification of pyrrolidine **5-3** with a 10-fold improvement in aortic ring (AR) potency over **5-2** (Fig. 7).

5. Prospect

Accumulative evidence has suggested that RhoA/Rho-kinase pathway is widely involved in the pathogenesis of a variety of diseases and that Rho-kinase inhibitors are useful for the treatment of those diseases. However, to date, Fasudil is the only ROCK inhibitor available for clinical use [14] possibly because of ROCK's widely distribution and various substrates, which may cause more untoward effects. A lot of reports also showed that Rho-GTPases could be a potential target for the treatment of some diseases, such as pulmonary vascular dysfunction [62] and Alzheimer's disease [63].

Further studies should aim to develop the selective Rho-kinase inhibitors such as ROCK1 inhibitor or ROCK2 inhibitor to reduce adverse effects, and also to improve the ability of penetrating blood–brain barrier for the treatment of CNS diseases. Moreover, since most of the diseases mentioned above are associated with multifactors, multi-targeted drugs with ROCK inhibitory property would be attractive candidates to treat these diseases.

As for the development of ROCK inhibitors, screening of plant extracts is an effective approach. In the study of Sumanta Kumar Goswami et al. [64], plant extracts with ROCK inhibitory activity were screened for the treatment of erectile dysfunction. Besides, high throughput screening assay is also a feasible way for the discovery of novel ROCK inhibitors, with which Kwang-Seok Oh et al. [12] found a novel scaffold with potent inhibitory activity and selectivity for ROCK.

In addition, it is worth to point out that the crystal and the co-crystal structures of ROCKs bound with inhibitors have been solved, paving a way for development of inhibitors by virtual screening, another attractive strategy for developing new drugs with higher efficiency over the traditional ways. Very recently, Hou T. et al. [65,66] found a series of compounds with good inhibitory activity against ROCK1 by virtual screening, indicating that we could find selective ROCK inhibitors using a computer aided drug design strategy. Till now, the co-crystal structures of ROCK bound with inhibitors are mainly for ROCK1. Fig. 8 is one example from PDB 3V8S, demonstrating the secondary structure of ROCK and the binding site of its inhibitors. Jacobs et al. determined the structures of ROCK1 bound to four different ATP-competitive small molecule inhibitors (Y-27632, fasudil, hydroxyfasudil, and H-1152P) [8]. Each of these compounds binds with reduced affinity to cAMP-dependent kinase (PKA), a highly homologous kinase. Subtle differences exist between the ROCK- and PKA-bound conformations of the inhibitors, suggesting that interactions with a single amino acid of the active site (Ala215 in ROCK and Thr183 in PKA) determine the relative selectivity of these compounds [8]. Yamaguchi et al. reported the crystal structure of the Rho-kinase catalytic domain in complex with a specific inhibitor Y-27632 [67]. Comparison with the structure of PKA bound to this inhibitor revealed a potential induced-fit binding mode that can be accommodated by the phosphate binding loop [67]. Young, E.R. et al. discovered phenylglycine substituted isoquinolones and beta-aryl substituted pyrrolidine 2*H*-isoquinolin-1-one inhibitors as potent ROCK1/ROCK2 inhibitors and obtained their co-crystal structures [61,68]. Using high concentration biochemical assays and fragment-based screening assisted by structure-guided design, Li R. et al. discovered a novel class of Rho-kinase inhibitors. The crystal structure of the compound **3**-**3**-ROCK1 complex revealed that compound **3**-**3** is a ROCK1 inhibitor that binds the hinge region in the ATP binding site [48]. There are also many other inhibitors with co-crystal structures of ROCK such as H-1152P, RKI1342, and RKI1447 [8,69,70]. All current co-crystal structures of ROCK1 and the inhibitors are summarized in Table 1. Beside of the ATP binding site, as described before, the dimerization

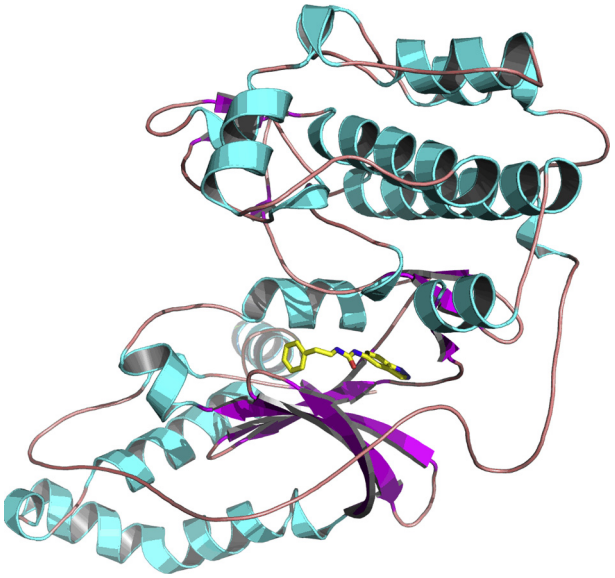
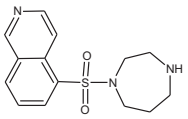
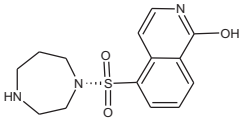
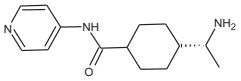
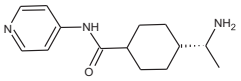
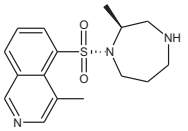
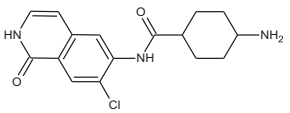
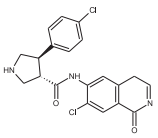
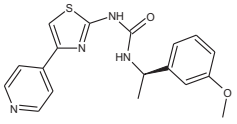
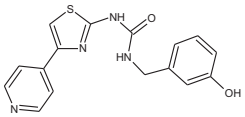
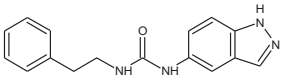


Fig. 8. The secondary crystal structure of ROCK1 bound by its inhibitor, compound **3**-**3**, an indazole derivative (PDB 3V8S). The crystal structure of the inhibitor-ROCK1 complex revealed that the indazole derivative bound the hinge region in the ATP binding site.

Table 1
Summary of Co-crystal structures of ROCK1 and its inhibitors.

PDB ID	Full name	Ligand ID	Ligand's chemical structure	Resolution (Å)
2ESM	Crystal structure of ROCK 1 bound to fasudil	M77		3.20
2ETK	Crystal structure of ROCK 1 bound to hydroxyfasudil	HFS		2.96
2ETR	Crystal structure of ROCK 1 bound to Y-27632	Y27		2.60
2H9V	Structural basis for induced-fit binding of Rho-kinase to the inhibitor Y27632	Y27		3.10
3D9V	Crystal structure of ROCK I bound to H-1152P, a di-methylated variant of fasudil	H52		3.30
3NCZ	X-ray co-structure of Rho-associated protein kinase (ROCK 1) with a potent 2*H*-isoquinolin-1-one inhibitor	3NC		3.00
3NDM	Crystal structure of Rho-associated protein kinase (ROCK 1) with a potent isoquinolone derivative	3ND		3.30
3TV7	Human Rho-associated protein kinase 1 (ROCK 1) in complex with RKI1342	07Q		2.75
3TWJ	Rho-associated protein kinase 1 (ROCK 1) in complex with RKI1447	07R		2.90
3V8S	Human Rho-associated protein kinase 1 (ROCK 1) in complex with indazole derivative (compound **18**)	0HD		2.29

Note: All crystal structures related information is retrieved from RCSB Protein Data Bank (http://www.rcsb.org/).

domains of ROCKs should be other attractive targets to be modulated for development of specific ROCK inhibitors [7–11,71].

In summary, given that ROCKs distribute in many tissues and also have multiple functions, more specific ROCK inhibitors are necessary to be developed. We hope novel and better ROCK inhibitor(s) will be developed for diseases in short future.

Conflict of interest

The authors state no conflict of interest.

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References

[1] K. Satoh, Y. Fukumoto, H. Shimokawa, Rho-kinase: important new therapeutic target in cardiovascular diseases, Am. J. Physiol. Heart Circ. Physiol. 301 (2011) H287–H296.
[2] H.B. Tan, Y.S. Zhong, Y. Cheng, X. Shen, Rho/ROCK pathway and neural regeneration: a potential therapeutic target for central nervous system and optic nerve damage, Int. J. Ophthalmol. 4 (2011) 652–657.
[3] B.K. Mueller, H. Mack, N. Teusch, Rho kinase, a promising drug target for neurological disorders, Nat. Rev. Drug Discov. 4 (2005) 387–398.
[4] M. Liu, F. Bi, X. Zhou, Y. Zheng, Rho GTPase regulation by miRNAs and covalent modifications, Trends Cell Biol. 22 (2012) 365–373.
[5] R. Shahin, S. Alqtaishat, M.O. Taha, Elaborate ligand-based modeling reveal new submicromolar Rho kinase inhibitors, J. Comput. Aided Mol. Des. 26 (2012) 249–266.
[6] Q. Zhou, C. Gensch, J.K. Liao, Rho-associated coiled-coil-forming kinases (ROCKs): potential targets for the treatment of atherosclerosis and vascular disease, Trends Pharmacol. Sci. 32 (2011) 167–173.
[7] H. Yamaguchi, M. Kasa, M. Amano, K. Kaibuchi, T. Hakoshima, Molecular mechanism for the regulation of rho-kinase by dimerization and its inhibition by fasudil, Structure 14 (2006) 589–600.
[8] M. Jacobs, K. Hayakawa, L. Swenson, S. Bellon, M. Fleming, P. Taslimi, J. Doran, The structure of dimeric ROCK I reveals the mechanism for ligand selectivity, J. Biol. Chem. 281 (2006) 260–268.
[9] R. Garg, K. Riento, N. Keep, J.D. Morris, A.J. Ridley, N-terminus-mediated dimerization of ROCK-I is required for RhoE binding and actin reorganization, Biochem. J. 411 (2008) 407–414.
[10] R. Dvorsky, L. Blumenstein, I.R. Vetter, M.R. Ahmadian, Structural insights into the interaction of ROCKI with the switch regions of RhoA, J. Biol. Chem. 279 (2004) 7098–7104.
[11] A.L. Couzens, V. Saridakis, M.P. Scheid, The hydrophobic motif of ROCK2 requires association with the N-terminal extension for kinase activity, Biochem. J. 419 (2009) 141–148.
[12] K.S. Oh, J. Mun, J.E. Cho, S. Lee, K.Y. Yi, C.J. Lim, J.S. Lee, W.J. Park, B.H. Lee, Discovery of novel scaffolds for Rho kinase 2 inhibitor through TRFRET-based high throughput screening assay, Comb. Chem. High Throughput Screen. 16 (2013) 37–46.
[13] M. Dong, J.K. Liao, B. Yan, R. Li, M. Zhang, C.M. Yu, A combination of increased Rho kinase activity and N-terminal pro-B-type natriuretic peptide predicts worse cardiovascular outcome in patients with acute coronary syndrome, Int. J. Cardiol. 167 (2013) 2813–2819.
[14] M. Dong, B.P. Yan, J.K. Liao, Y.Y. Lam, G.W. Yip, C.M. Yu, Rho-kinase inhibition: a novel therapeutic target for the treatment of cardiovascular diseases, Drug Discov. Today 15 (2010) 622–629.
[15] Q. Li, Y. Chen, L. Sun, G. Fu, L. Guo, Vasodilatation produced by fasudil mesylate in vivo and in vitro, Vascul. Pharmacol. 55 (2011) 121–126.
[16] S.Y. Yoo, J. Kim, S. Cheong, D.H. Shin, J. Jang, C. Lee, S.J. Tahk, J.H. Shin, S.Y. Choi, M.H. Yoon, Rho-associated kinase 2 polymorphism in patients with vasospastic angina, Korean Circ. J. 42 (2012) 406–413.
[17] H. Fujita, Y. Fukumoto, K. Saji, K. Sugimura, J. Demachi, J. Nawata, H. Shimokawa, Acute vasodilator effects of inhaled fasudil, a specific Rho-kinase inhibitor, in patients with pulmonary arterial hypertension, Heart Vessels 25 (2010) 144–149.
[18] V. Gupta, N. Gupta, I.H. Shaik, R. Mehvar, I.F. McMurtry, M. Oka, E. Nozik-Grayck, M. Komatsu, F. Ahsan, Liposomal fasudil, a rho-kinase inhibitor, for prolonged pulmonary preferential vasodilation in pulmonary arterial hypertension, J. Controlled Release 167 (2013) 189–199.
[19] D. Gonzalez-Forero, F. Montero, V. Garcia-Morales, G. Dominguez, L. Gomez-Perez, J.M. Garcia-Verdugo, B. Moreno-Lopez, Endogenous Rho-kinase signaling maintains synaptic strength by stabilizing the size of the readily releasable pool of synaptic vesicles, J. Neurosci. 32 (2012) 68–84.
[20] Y. Hou, L. Zhou, Q.D. Yang, X.P. Du, M. Li, M. Yuan, Z.W. Zhou, Changes in hippocampal synapses and learning-memory abilities in a streptozotocin-treated rat model and intervention by using fasudil hydrochloride, Neuroscience 200 (2012) 120–129.
[21] M. Raad, T. El Tal, R. Gul, S. Mondello, Z. Zhang, R.M. Boustany, J. Guingab, K.K. Wang, F. Kobeissy, Neuroproteomics approach and neurosystems biology analysis: ROCK inhibitors as promising therapeutic targets in neurodegeneration and neurotrauma, Electrophoresis 33 (2012) 3659–3668.
[22] B. Villar-Cheda, A. Dominguez-Meijide, B. Joglar, A.I. Rodriguez-Perez, M.J. Guerra, J.L. Labandeira-Garcia, Involvement of microglial RhoA/Rho-kinase pathway activation in the dopaminergic neuron death. Role of angiotensin via angiotensin type 1 receptors, Neurobiol. Dis. 47 (2012) 268–279.
[23] L. Tonges, T. Frank, L. Tatenhorst, K.A. Saal, J.C. Koch, E.M. Szego, M. Bahr, J.H. Weishaupt, P. Lingor, Inhibition of rho kinase enhances survival of dopaminergic neurons and attenuates axonal loss in a mouse model of Parkinson's disease, Brain 135 (2012) 3355–3370.
[24] A. Koumura, J. Hamanaka, K. Kawasaki, K. Tsuruma, M. Shimazawa, I. Hozumi, T. Inuzuka, H. Hara, Fasudil and ozagrel in combination show neuroprotective effects on cerebral infarction after murine middle cerebral artery occlusion, J. Pharmacol. Exp. Ther. 338 (2011) 337–344.
[25] S. Inan, K. Buyukafsar, Antiepileptic effects of two Rho-kinase inhibitors, Y-27632 and fasudil, in mice, Br. J. Pharmacol. 155 (2008) 44–51.
[26] E. Yoshimi, H. Yamamoto, Y. Furuichi, Y. Shimizu, N. Takeshita, Sustained analgesic effect of the Rho kinase inhibitor AS1892802 in rat models of chronic pain, J. Pharmacol. Sci. 114 (2010) 119–122.
[27] A.A. Pineda, M. Minohara, N. Kawamura, T. Matsushita, R. Yamasaki, X. Sun, H. Piao, H. Shimokawa, J. Kira, Preventive and therapeutic effects of the selective Rho-kinase inhibitor fasudil on experimental autoimmune neuritis, J. Neurol. Sci. 306 (2011) 115–120.
[28] M. Chen, A. Liu, Y. Ouyang, Y. Huang, X. Chao, R. Pi, Fasudil and its analogs: a new powerful weapon in the long war against central nervous system disorders? Expert Opin. Invest. Drugs 22 (2013) 537–550.
[29] N. Rath, M.F. Olson, Rho-associated kinases in tumorigenesis: re-considering ROCK inhibition for cancer therapy, EMBO Rep. 13 (2012) 900–908.
[30] H. Nakabayashi, K. Shimizu, HA1077, a Rho kinase inhibitor, suppresses glioma-induced angiogenesis by targeting the Rho-ROCK and the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) signal pathways, Cancer Sci. 102 (2011) 393–399.
[31] X. Yang, J. Di, Y. Zhang, S. Zhang, J. Lu, J. Liu, W. Shi, The Rho-kinase inhibitor inhibits proliferation and metastasis of small cell lung cancer, Biomed. Pharmacother. 66 (2012) 221–227.
[32] H. Eldawoody, H. Shimizu, N. Kimura, A. Saito, T. Nakayama, A. Takahashi, T. Tominaga, Fasudil, a Rho-kinase inhibitor, attenuates induction and progression of cerebral aneurysms: experimental study in rats using vascular corrosion casts, Neurosci. Lett. 470 (2010) 76–80.
[33] Y. Takeba, N. Matsumoto, M. Watanabe, S. Takenoshita-Nakaya, Y. Ohta, T. Kumai, M. Takagi, S. Koizumi, T. Asakura, T. Otsubo, The Rho kinase inhibitor fasudil is involved in p53-mediated apoptosis in human hepatocellular carcinoma cells, Cancer Chemother. Pharmacol. 69 (2012) 1545–1555.
[34] H. Li, W. Peng, W. Jian, Y. Li, Q. Li, W. Li, Y. Xu, ROCK inhibitor fasudil attenuated high glucose-induced MCP-1 and VCAM-1 expression and monocyte-endothelial cell adhesion, Cardiovasc. Diabetol. 11 (2012) 65.
[35] G. Mangialardi, R. Katare, A. Oikawa, M. Meloni, C. Reni, C. Emanueli, P. Madeddu, Diabetes causes bone marrow endothelial barrier dysfunction by activation of the RhoA-Rho-associated kinase signaling pathway, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 555–564.
[36] F. Peng, D. Wu, B. Gao, A.J. Ingram, B. Zhang, K. Chorneyko, R. McKenzie, J.C. Krepinsky, RhoA/Rho-kinase contribute to the pathogenesis of diabetic renal disease, Diabetes 57 (2008) 1683–1692.
[37] J.W. Park, C.H. Park, I.J. Kim, E.H. Bae, S.K. Ma, J.U. Lee, S.W. Kim, Rho kinase inhibition by fasudil attenuates cyclosporine-induced kidney injury, J. Pharmacol. Exp. Ther. 338 (2011) 271–279.
[38] N. Wang, P. Guan, J.P. Zhang, Y.Z. Chang, L.J. Gu, F.K. Hao, Z.H. Shi, F.Y. Wang, L. Chu, Preventive effects of fasudil on adriamycin-induced cardiomyopathy: possible involvement of inhibition of RhoA/ROCK pathway, Food Chem. Toxicol. 49 (2011) 2975–2982.
[39] R.D. Williams, G.D. Novack, T. van Haarlem, C. Kopczynski, A.R.P.A.S. Group, Ocular hypotensive effect of the Rho kinase inhibitor AR-12286 in patients with glaucoma and ocular hypertension, Am. J. Ophthalmol. 152 (2011) 834–841.
[40] H. Huang, D. Kong, K.H. Byun, C. Ye, S. Koda, D.H. Lee, B.C. Oh, S.W. Lee, B. Lee, J.M. Zabolotny, M.S. Kim, C. Bjorbaek, B.B. Lowell, Y.B. Kim, Rho-kinase regulates energy balance by targeting hypothalamic leptin receptor signaling, Nat. Neurosci. 15 (2012) 1391–1398.
[41] W.G. Duan, S.T. Yuan, H. Liao, M. Yan, L.Y. Zhang, Advances in the study of Rho kinase and its inhibitors, Yao Xue Xue Bao 42 (2007) 1013–1022.
[42] M. Tamura, H. Nakao, H. Yoshizaki, M. Shiratsuchi, H. Shigyo, H. Yamada, T. Ozawa, J. Totsuka, H. Hidaka, Development of specific Rho-kinase inhibitors and their clinical application, Biochim. Biophys. Acta 1754 (2005) 245–252.
[43] M. Iwakubo, A. Takami, Y. Okada, T. Kawata, Y. Tagami, M. Sato, T. Sugiyama, K. Fukushima, S. Taya, M. Amano, K. Kaibuchi, H. Iijima, Design and synthesis of rho kinase inhibitors (III), Bioorg. Med. Chem. 15 (2007) 1022–1033.
[44] P. Ray, J. Wright, J. Adam, J. Bennett, S. Boucharens, D. Black, A. Cook, A.R. Brown, O. Epemolu, D. Fletcher, A. Haunso, M. Huggett, P. Jones, S. Laats, A. Lyons, J. Mestres, J. de Man, R. Morphy, Z. Rankovic, B. Sherborne, L. Sherry, N. van Straten, P. Westwood, G.Z. Zaman, Fragment-based discovery of 6-substituted isoquinolin-1-amine based ROCK-I inhibitors, Bioorg. Med. Chem. Lett. 21 (2011) 97–101.
[45] D. Lavogina, K. Kalind, J. Bredihhina, M. Hurt, A. Vaasa, M. Kasari, E. Enkvist, G. Raidaru, A. Uri, Conjugates of 5-isoquinolinesulfonylamides and oligo-D-arginine possess high affinity and selectivity towards Rho kinase (ROCK), Bioorg. Med. Chem. Lett. 22 (2012) 3425–3430.
[46] K.K. Ho, J.R. Beasley, L. Belanger, D. Black, J.H. Chan, D. Dunn, B. Hu, A. Klon, S.G. Kultgen, M. Ohlmeyer, S.M. Parlato, P.C. Ray, Q. Pham, Y. Rong, A.L. Roughton, T.L. Walker, J. Wright, K. Xu, Y. Xu, L. Zhang, M. Webb, Triazine and pyrimidine based ROCK inhibitors with efficacy in spontaneous hypertensive rat model, Bioorg. Med. Chem. Lett. 19 (2009) 6027–6031.
[47] A.J. Henderson, M. Hadden, C. Guo, N. Douglas, H. Decornez, M.R. Hellberg, A. Rusinko, M. McLaughlin, N. Sharif, C. Drace, R. Patil, 2,3-Diaminopyrazines as Rho kinase inhibitors, Bioorg. Med. Chem. Lett. 20 (2010) 1137–1140.
[48] R. Li, M.P. Martin, Y. Liu, B. Wang, R.A. Patel, J.Y. Zhu, N. Sun, R. Pireddu, N.J. Lawrence, J. Li, E.B. Haura, S.S. Sung, W.C. Guida, E. Schonbrunn, S.M. Sebti, Fragment-based and structure-guided discovery and optimization of Rho kinase inhibitors, J. Med. Chem. 55 (2012) 2474–2478.

[49] Y. Feng, M.D. Cameron, B. Frackowiak, E. Griffin, L. Lin, C. Ruiz, T. Schröter, P. LoGrasso, Structure–activity relationships, and drug metabolism and pharmacokinetic properties for indazole piperazine and indazole piperidine inhibitors of ROCK-II, Bioorg. Med. Chem. Lett. 17 (2007) 2355–2360.
[50] H. Cui, Krista B. Goodman, Sarah E. Dowdell, Dimitri E. Gaitanopoulos, Robert L. Ivy, Clark A. Sehon, Robert A. Stavenger, Gren Z. Wang, Andrew Q. Viet, et al., Development of dihydropyridone indazole amides as selective rho-kinase inhibitors, J. Med. Chem. 50 (2007) 6–9.
[51] C.A. Sehon, G.Z. Wang, A.Q. Viet, K.B. Goodman, S.E. Dowdell, P.A. Elkins, S.F. Semus, C. Evans, L.J. Jolivette, R.B. Kirkpatrick, E. Dul, S.S. Khandekar, T. Yi, L.L. Wright, G.K. Smith, D.J. Behm, R. Bentley, C.P. Doe, E. Hu, D. Lee, Potent, selective and orally bioavailable dihydropyrimidine inhibitors of Rho kinase (ROCK1) as potential therapeutic agents for cardiovascular diseases, J. Med. Chem. 51 (2008) 6631–6634.
[52] Y.T. Chen, T.D. Bannister, A. Weiser, E. Griffin, L. Lin, C. Ruiz, M.D. Cameron, S. Schurer, D. Duckett, T. Schroter, P. LoGrasso, Y. Feng, Chroman-3-amides as potent Rho kinase inhibitors, Bioorg. Med. Chem. Lett. 18 (2008) 6406–6409.
[53] Y. Yin, L. Lin, C. Ruiz, M.D. Cameron, J. Pocas, W. Grant, T. Schroter, W. Chen, D. Duckett, S. Schurer, P. Lograsso, Y. Feng, Benzothiazoles as Rho-associated kinase (ROCK-II) inhibitors, Bioorg. Med. Chem. Lett. 19 (2009) 6686–6690.
[54] X. Fang, Y. Yin, Y.T. Chen, L. Yao, B. Wang, M.D. Cameron, L. Lin, S. Khan, C. Ruiz, T. Schroter, W. Grant, A. Weiser, J. Pocas, A. Pachori, S. Schurer, P. Lograsso, Y. Feng, Tetrahydroisoquinoline derivatives as highly selective and potent Rho kinase inhibitors, J. Med. Chem. 53 (2010) 5727–5737.
[55] S. Chowdhury, E.H. Sessions, J.R. Pocas, W. Grant, T. Schroter, L. Lin, C. Ruiz, M.D. Cameron, S. Schurer, P. LoGrasso, T.D. Bannister, Y. Feng, Discovery and optimization of indoles and 7-azaindoles as Rho kinase (ROCK) inhibitors (part-I), Bioorg. Med. Chem. Lett. 21 (2011) 7107–7112.
[56] X. Fang, Y.T. Chen, E.H. Sessions, S. Chowdhury, T. Vojkovsky, Y. Yin, J.R. Pocas, W. Grant, T. Schroter, L. Lin, C. Ruiz, M.D. Cameron, P. LoGrasso, T.D. Bannister, Y. Feng, Synthesis and biological evaluation of 4-quinazolinones as Rho kinase inhibitors, Bioorg. Med. Chem. Lett. 21 (2011) 1844–1848.
[57] E.H. Sessions, S. Chowdhury, Y. Yin, J.R. Pocas, W. Grant, T. Schroter, L. Lin, C. Ruiz, M.D. Cameron, P. LoGrasso, T.D. Bannister, Y. Feng, Discovery and optimization of indole and 7-azaindoles as Rho kinase (ROCK) inhibitors (part-II), Bioorg. Med. Chem. Lett. 21 (2011) 7113–7118.
[58] Y. Yin, M.D. Cameron, L. Lin, S. Khan, T. Schroter, W. Grant, J. Pocas, Y.T. Chen, S. Schurer, A. Pachori, P. LoGrasso, Y.B. Feng, Discovery of potent and selective urea-based ROCK inhibitors and their effects on intraocular pressure in rats, ACS Med. Chem. Lett. 1 (2010) 175–179.
[59] Y. Yin, L. Lin, C. Ruiz, S. Khan, M.D. Cameron, W. Grant, J. Pocas, N. Eid, H. Park, T. Schroter, P.V. Lograsso, Y. Feng, Synthesis and biological evaluation of urea derivatives as highly potent and selective rho kinase inhibitors, J. Med. Chem. 56 (2013) 3568–3581.
[60] R.A. Stavenger, H. Cui, S.E. Dowdell, R.G. Franz, D.E. Gaitanopoulos, K.B. Goodman, M.A. Hilfiker, R.L. Ivy, J.D. Leber, J.P. Marino Jr., H.J. Oh, A.Q. Viet, W. Xu, G. Ye, D. Zhang, Y. Zhao, L.J. Jolivette, M.S. Head, S.F. Semus, P.A. Elkins, R.B. Kirkpatrick, E. Dul, S.S. Khandekar, T. Yi, D.K. Jung, L.L. Wright, G.K. Smith, D.J. Behm, C.P. Doe, R. Bentley, Z.X. Chen, E. Hu, D. Lee, Discovery of aminofurazan-azabenzimidazoles as inhibitors of Rho-kinase with high kinase selectivity and antihypertensive activity, J. Med. Chem. 50 (2007) 2–5.
[61] T. Bosanac, E.R. Hickey, J. Ginn, M. Kashem, S. Kerr, S. Kugler, X. Li, A. Olague, S. Schlyer, E.R. Young, Substituted 2*H*-isoquinolin-1-ones as potent Rho-kinase inhibitors: part 3, aryl substituted pyrrolidines, Bioorg. Med. Chem. Lett. 20 (2010) 3746–3749.
[62] E.M. Storck, B. Wojciak-Stothard, Rho GTPases in pulmonary vascular dysfunction, Vascul. Pharmacol. 58 (2013) 202–210.
[63] M. Musilli, V. Nicolia, S. Borrelli, S. Scarpa, G. Diana, Behavioral effects of Rho GTPase modulation in a model of Alzheimer's disease, Behav. Brain Res. 237C (2012) 223–229.
[64] S.K. Goswami, M.K. Pandre, R. Jamwal, S. Dethe, A. Agarwal, M.N. Inamdar, Screening for Rho-kinase 2 inhibitory potential of Indian medicinal plants used in management of erectile dysfunction, J. Ethnopharmacol. 144 (2012) 483–489.
[65] M. Shen, H. Yu, Y. Li, P. Li, P. Pan, S. Zhou, L. Zhang, S. Li, S.M. Lee, T. Hou, Discovery of Rho-kinase inhibitors by docking-based virtual screening, Mol. Biosyst. 9 (2013) 1511–1521.
[66] M. Shen, S. Zhou, Y. Li, P. Pan, L. Zhang, T. Hou, Discovery and optimization of triazine derivatives as ROCK1 inhibitors: molecular docking, molecular dynamics simulations and free energy calculations, Mol. Biosyst. 9 (2013) 361–374.
[67] H. Yamaguchi, Y. Miwa, M. Kasa, K. Kitano, M. Amano, K. Kaibuchi, T. Hakoshima, Structural basis for induced-fit binding of Rho-kinase to the inhibitor Y-27632, J. Biochem. 140 (2006) 305–311.
[68] J.D. Ginn, T. Bosanac, R. Chen, C. Cywin, E. Hickey, M. Kashem, S. Kerr, S. Kugler, X. Li, A. Prokopowicz 3rd, S. Schlyer, J.D. Smith, M.R. Turner, F. Wu, E.R. Young, Substituted 2*H*-isoquinolin-1-ones as potent Rho-kinase inhibitors: part 2, optimization for blood pressure reduction in spontaneously hypertensive rats, Bioorg. Med. Chem. Lett. 20 (2010) 5153–5156.
[69] R.A. Patel, K.D. Forinash, R. Pireddu, Y. Sun, N. Sun, M.P. Martin, E. Schonbrunn, N.J. Lawrence, S.M. Sebti, RKI-1447 is a potent inhibitor of the Rho-associated ROCK kinases with anti-invasive and antitumor activities in breast cancer, Cancer Res. 72 (2012) 5025–5034.
[70] R. Pireddu, K.D. Forinash, N.N. Sun, M.P. Martin, S.S. Sung, B. Alexander, J.Y. Zhu, W.C. Guida, E. Schonbrunn, S.M. Sebti, N.J. Lawrence, Pyridylthiazole-based ureas as inhibitors of Rho associated protein kinases (ROCK1 and 2), MedChemComm 3 (2012) 699–709.
[71] J.D. Doran, X. Liu, P. Taslimi, A. Saadat, T. Fox, New insights into the structure-function relationships of Rho-associated kinase: a thermodynamic and hydrodynamic study of the dimer-to-monomer transition and its kinetic implications, Biochem. J. 384 (2004) 255–262.