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# Substituted 2*H*-isoquinolin-1-ones as potent Rho-kinase inhibitors: Part 2, optimization for blood pressure reduction in spontaneously hypertensive rats

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ABSTRACT

Phenylglycine substituted isoquinolones **1** and **2** have previously been described as potent dual ROCK1/ROCK2 inhibitors. Here we describe the further SAR of this series to improve metabolic stability and rat oral exposure. Piperidine analog **20** which demonstrates sustained blood pressure normalization in an SHR blood pressure reduction model was identified through this effort.

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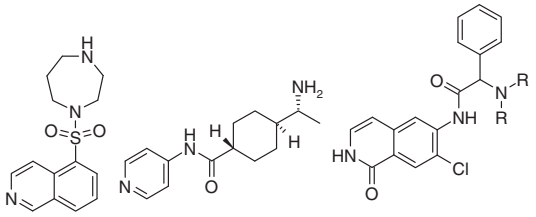
The serine–threonine Rho kinases, ROCK1 and ROCK2, have been repeatedly reviewed as desirable points of therapeutic intervention for many disease indications.[1] With respect to cardiovascular disease they are known to prolong smooth muscle contraction through an inactivating phosphorylation of myosin light chain kinase,[2] inhibit endothelium-mediated smooth muscle relaxation through reducing bioavailability of NO,[3] play an essential role in proinflammatory chemotactic cell migration,[4] and in some contexts may be profibrotic.[5] For these reasons, we became interested in the inhibition of Rho kinase for treatment of hypertension with the potential for independent end organ protection benefits.

To date Fasudil and Y-27632 have served as the primary chemical tools for studying the role of Rho kinases in many biological contexts (Fig. 1).[6] Although these compounds elicit only modest lowering of blood pressure, recent reports in the literature demonstrate that profound effects can be achieved in the spontaneous hypertensive rat (SHR) model with more potent orally bioavailable Rho-kinase inhibitors.[7] In the proceeding article, we described our lead identification efforts resulting in the discovery of 2*H*-isoquinolin-1-ones, exemplified by compound **1**.[8] This series provided a very attractive starting point with a desirable level of potency but had poor oral bioavailability consistent with poor in vitro microsomal stability (Table 1). Here we report on our further optimization of this series of dual ROCK1/ROCK2 inhibitors which now provide sustained normalization of blood pressure in the SHR.[9]

We found that the N-dealkylated analog **2** maintained molecular potency and improved microsomal stability. However, this compound also displayed a dramatic loss in potency in an aortic ring relaxation assay.[10] We hypothesized that this shift in tissue potency was attributable to decreased permeability due to decreased lipophilicity, increased H-bond donor count or both.[11] With these considerations in mind it was decided to further investigate the amide side chain SAR in the hopes of finding an appropriate balance of lipophilicity driven permeability and metabolic stability.

Docking of compound **2** into a ROCK1 crystal structure (Fig. 2) highlights key interactions of the isoquinolinone series with the ATP-binding site. In the hinge region two essential H-bonds are



**Fasudil** **Y-27632** **1 (R=Me) ROCK 2 $IC_{50}$ = 57 nM**
**Aortic Ring $EC_{50}$ = 90 nM**
**2 (R=H) ROCK 2 $IC_{50}$ = 26 nM**
**Aortic Ring $EC_{50}$ = 2400 nM**

**Figure 1.** Structure of Fasudil, Y-27632, and isoquinolones **1** and **2**.

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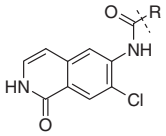
**Table 1**
α-Amino acid substituted isoquinolone analogs



| Compd | R | ROCK2 $IC_{50}$[a] (nM) | Aortic ring $EC_{50}$[b] (nM) | HLM $T_{1/2}$[c] (min) | *c*Log *P* |
|---|---|---|---|---|---|
| **1** | *N*,*N*-Dimethyl Phg | 19 | 69 | 15 | 1.80 |
| **2** | Phg | 59 | 2400 | 120 | 0.72 |
| **5** | Gly | 120 | 2500 | nt | −0.74 |
| **6** | L-Val | 12 | 500 | 89 | 0.50 |
| **7** | D-Val | 240 | nt | nt | |
| **8** | L-Leu | 340 | nt | nt | 1.03 |
| **9** | D-Leu | 19 | 700 | 16 | |
| **10** | L-Neopentylglycine | 845 | nt | nt | 1.43 |
| **11** | D-Neopentylglycine | 110 | nt | nt | |
| **12** | L-Chg | 20 | 180 | 34 | 1.69 |
| **13** | D-Chg | 400 | nt | nt | |
| **14** | *N*-Isopropyl L-Chg | 25 | 190 | 17 | 2.87 |

nt = not tested.
[a] Cambrex PKLight ATP detection reagent using luciferin–luciferase to quantify residual ATP. Values are means of at least three duplicate experiments.
[b] Relaxation of phenylephrine stimulated isolated rat aortic rings. Values are means of at least three experiments.
[c] Compounds were incubated with Human liver microsomes at a concentration of 1 mg protein/ml.

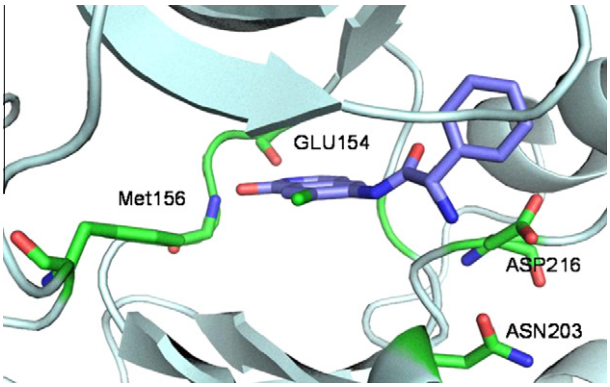
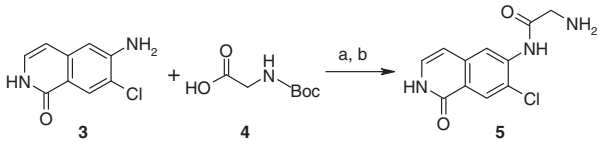



**Figure 2.** Docking of **2** into ROCK 1 homology model.

formed with the backbone NH of Met156 and the backbone carbonyl of Glu154. The phenyl side chain is oriented into a hydrophobic groove defined by the glycine-rich loop with the basic amine positioned to interact with polar residues in the phosphate binding region. Using this model as a guide we first desired to examine closely related α-amino acid analogs that maintained these interactions. This also provided the opportunity to assess the influence of the amino acid chiral center on potency, which was not possible with the readily epimerized phenylglycine derivatives.[12]

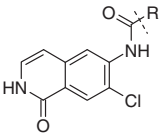
This effort was initially frustrated by the lack of reactivity of the 7-chloro-6-aminoisoquinolone (**3**) under standard amide coupling conditions (EDC, HATU, CDI, acid fluorides, etc.). Acid chlorides could effect the desired bond formation but generally resulted in low yields. A more convenient protocol for the formation of the desired amides was found utilizing a phosphorous oxychloride-mediated amide bond forming reaction.[13] These conditions allowed for rapid formation of the desired amides in good isolated yields without the loss of enantiomeric purity. The reaction conditions are compatible with most amine protecting groups, although Fmoc-protected α-amino acids were generally superior to Boc- or Cbz-protected α-amino acids. Tertiary amines were well tolerated under the reaction conditions. However, unprotected hydroxyl groups or amines resulted in only recovered starting materials. Shown in Scheme 1 is the synthesis of the glycine analog **5** from the Boc-protected amino acid. Other analogs were made in a similar fashion from commercially available materials.

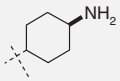
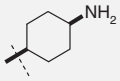
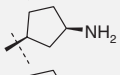
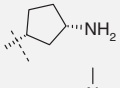
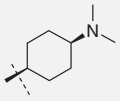
Compounds were first profiled for their inhibition of ROCK 2 using a luciferase based ATP detection assay.[8] Compounds that displayed sufficient activity against ROCK 2 were further examined for relaxation of isolated rat aortic rings following pre-constriction




**Scheme 1.** Synthesis of isoquinolones **5**. Reagents and conditions: (a) $POCl_3$, pyridine, 0 °C, 30 min, 50%; (b) HCl, DCM, rt, overnight, 50%.

**Table 2**
Cyclized amino acid isoquinolone analogs



| Compd | R | ROCK2 $IC_{50}$[a] (nM) | Aortic ring $EC_{50}$[b] (nM) | HLM $T_{1/2}$[c] (min) |
|---|---|---|---|---|
| **15** |  | 130 | 1120 | 17 |
| **16** |  | 35 | nt | 16 |
| **17** |  | 180 | nt | 49 |
| **18** |  | 67 | nt | >300 |
| **19** |  | 28 | 220 | 224 |
| **20** |  | 31 | 250 | >300 |
| **21** |  | 330 | nt | nt |
| **22** |  | 15 | 180 | 70 |
| **23** |  | 310 | nt | nt |
| **24** |  | 7.3 | 190 | 139 |
| **25** |  | 1000 | nt | nt |

nt = not tested.
[a] Cambrex PKLight ATP detection reagent using luciferin–luciferase to quantify residual ATP. Values are means of at least three duplicate experiments.
[b] Relaxation of phenylephrine stimulated isolated rat aortic rings. Values are means of at least three experiments.
[c] Compounds were incubated with human liver microsomes at a concentration of 1 mg protein/ml.

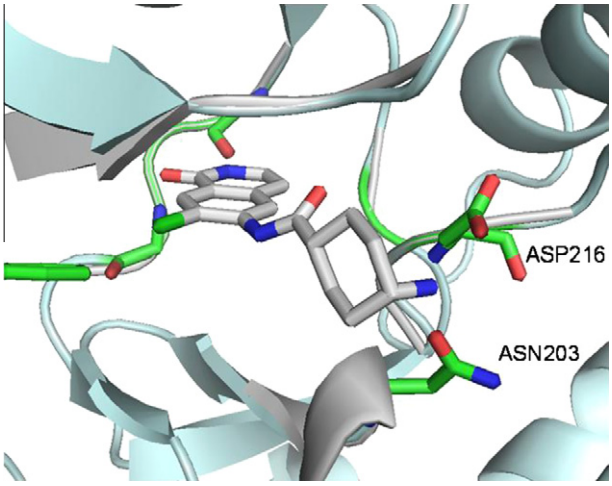



**Figure 3.** X-ray co-crystal structure of **22** bound in ROCK1.

**Table 3**
RLM, Caco2, and PK parameters for **19**, **20**, and **22**

| Compd | RLM $T_{1/2}$ (min) | Caco2 ab/ba | Cl (ml/min/kg) | AUC (ng h/ml) | | %F |
|---|---|---|---|---|---|---|
| | | | | iv[a] | po[b] | |
| **19** | nt | nt | 53 | 161 | 141 | 4.4 |
| **20** | 151 | 14/12 | 65 | 127 | 1779 | 70 |
| **22** | >300 | 0.35/2.53 | 60 | 119 | 338 | 20 |

Compounds were dosed at 0.5 mg/kg iv and 10 mg/kg po; nt = not tested.
[a] Mean value of plasma concentration from three rats.
[b] Plasma concentration from a single rat.

with phenylephrine. The results of the amino acid analogs are shown in Table 1. A lack of the hydrophobic G-loop interaction by deleting the phenyl side chain (glycine analog **5)** resulted in a sixfold loss in molecular potency compared to **1**. Reintroducing this interaction with a variety of lipophilic side chains restored potency. With these configurationally stable analogs significant chiral preferences were observed which were dependent on the nature of the individual amino acid side chain. For derivatives, in which branching occurs alpha to the chiral center, the (*S*) isomers derived from the natural L-amino acids were strongly preferred (**6** and **12**). Interestingly, for derivatives, which are branched in the beta-position, the (*R*) isomers are more potent (**9** and **11**). Molecular modeling of these compounds suggested a potential source for this side chain dependence is steric incompatibility between the (*S*) enantiomers of the beta-branched analogs (**8** and **10**) and residues in the glycine-rich loop. In these cases, the (*R*) enantiomers (**9** and **11**) are predicted to extend the side chain deeper into the phosphate binding region while maintaining the basic amine interaction with the ASP216.

Encouragingly, the more lipophilic cyclohexylglycine (Chg) analog (**12**) demonstrated aortic ring potency similar to the N,N-disubstituted phenylglycine derivate (**1**) without requiring substitution on the terminal amine. Comparison of the *c*Log *P* values for **12** and **1** supports the hypothesis that this enhanced tissue potency is due to a lipophilicity driven increase in permeability. N-substitution of the cyclohexylglycine analog (**14**) did not result in further improvements in the aortic ring potency suggesting that a maximal effect on permeability had already been achieved.

Unfortunately, the cyclohexyl glycine derivative **14** did not improve metabolic stability, suggesting a switch in the metabolic site from N-dealkylation to oxidation within the more lipophilic side chain. We felt that this could limit the effectiveness of our initial strategy and therefore focused our SAR efforts on simpler and potentially more metabolically robust structures. In particular, we pursued targets which might provide an opportunity to further improve potency through optimization of the basic amine interaction. The proline derivatives **15** and **16** were reasonably potent but did not provide improvements in microsomal stability (Table 2). However, the regioisomeric pyrrolidine derivative **18** demonstrated a significant improvement in microsomal stability and led to compounds such as **19** and **20** which successfully combine desirable molecular and tissue potency as well as excellent microsomal stability. We further profiled compound **20** to evaluate the effect this optimization had on the kinase selectivity seen in the original lead series. Compound **20** was tested against a panel of 50 kinases at 10 μM with >80% inhibition only observed for PRKG1, PRKCl2, MSK2, p70S6 K, PKB alpha, and PKA.[14]

Of particular interest were the differences in potency observed with stereoisomeric pairs **21**/**22**, and **23**/**24** indicating a highly

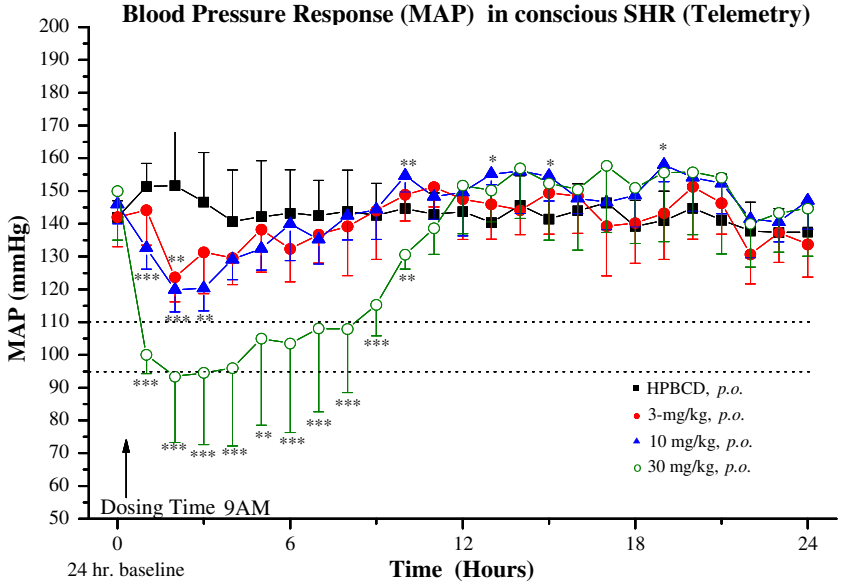



**Figure 4.** Effect of **20** on the mean arterial pressure (MAP) in conscious SHR. Compound was administered as a single dose oral gavage. Reported results represent the mean arterial pressure for three treated animals.

specific, exploitable ligand interaction. An X-ray co-crystal structure of **22** bound to ROCK1 (Fig. 3) revealed two hydrogen bonds between the terminal amine and the sidechains of ASP216 (2.9 Å) and ASN203 (3.3 Å) located in the phosphate binding region.[15]

That the increased molecular potency observed with both **22** and **24** did not translate to improved activity in the aortic ring assay is likely due to the poor permeability of the primary amines. Caco2 experiments show the permeability of the primary amine **22** is markedly reduced as compared to the tertiary amine **20** (Table 3). Attempts to correct this by alkylation were not successful. The large drop off in potency observed with **25** suggests that the exocyclic amine resides too close to the protein to allow for further substitution, in full agreement with the X-ray co-structure of **22** with ROCK1.

Compound **20** was selected for further evaluation in the SHR blood pressure reduction model based upon its rat PK profile and demonstrated a dose responsive effect at 10 and 30 mg/kg (Fig. 4).[16] Moreover, a single oral dose of 30 mg/kg successfully normalized blood pressure for 8 h and maintained a statistically significant reduction for up to 11 h.

*Conclusion*: In conclusion, the early lead optimization of isoquinolone based dual ROCK1/ROCK2 inhibitors has resulted in compounds with good tissue potency in conjunction with improved microsomal stability. This has lead us to identify compound **20**, which induces blood pressure normalization in an acute oral dosing SHR model.[17]

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14. $IC_{50}$ were determined for PRKG1 (230 nM), PRKCL2 (500 nM), p70S6 K (180 nM), and MSK2 (480 nM).
15. PDB ID: 3NCZ.
16. Rat PK data was obtained from an IV cassette study ($n = 3$, 0.2 mg/kg) followed by an oral leg ($n = 1$, 10 mg/kg) in male Sprague-Dawley rats.
17. A recent report from researchers at GSK demonstrated that upon chronic dosing of a Rho-kinase inhibitor the bp lowering effects diminished over time. The efficacy of compound **20** has not been examined in similar multi-dose studies. Goodman, K.; 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.; Jung, D.; Wright, L.; Smith, G.; Behm, D.; Bentley, R.; Doe, C.; Hu, E.; Lee, D. 238th ACS National Meeting, Washington, DC, Aug 16, 2009; paper MEDI 252.