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Substituted 2*H*-isoquinolin-1-ones as potent Rho-kinase inhibitors: Part 3, aryl substituted pyrrolidines

Todd Bosanac [a,*], Eugene R. Hickey [a], John Ginn [a], Mohammed Kashem [a], Steven Kerr [b], Stanley Kugler [a], Xiang Li [a], Alan Olague [a], Sabine Schlyer [a], Erick R. R. Young [a]

[a] Department of Medicinal Chemistry, Boehringer-Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877-0368, United States
[b] Department of Cardiometabolic Diseases, Boehringer-Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877-0368, United States

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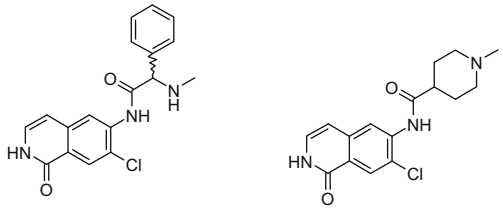
ABSTRACT

The discovery and SAR of a series of β-aryl substituted pyrrolidine 2*H*-isoquinolin-1-one inhibitors of Rho-kinase (ROCK) derived from **2** is herein described. SAR studies have shown that aryl groups in the β-position are optimal for potency. Our efforts focused on improving the ROCK potency of this isoquinolone class of inhibitors which led to the identification of pyrrolidine **32** which demonstrated a 10-fold improvement in aortic ring (AR) potency over **2**.

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 independent add on benefits for end organ protection.

Recently we described the discovery of a new class of 2*H*-isoquinolin-1-one based ROCK1/ROCK2 dual inhibitors[6] along with our early optimization efforts to identify analogs demonstrating sustained blood pressure lowering in the spontaneous hypertensive rat model.[7] A key finding for achieving oral exposure with this series was the flexibility to sacrifice the metabolically labile phenyl glycine side chain of **1** (Fig. 1). This was realized upon further optimizing potency through the basic amine-phosphate binding region interaction leading to the identification of **2**.

We had previously observed that the aryl group of the phenyl glycine residue contributes up to a 10-fold improvement in binding potency when compared to an unsubstituted analog (Table 1). Docking experiments with a ROCK1 homology model reveal the likely source of this binding interaction is extension of the aryl substituent from the α-carbon into a hydrophobic groove defined by the glycine rich loop vide infra. The goal of the work described herein was to reincorporate this binding interaction into piperidine analog **2** without compromising metabolic stability and oral bioavailability.



1
ROCK2 IC_{50} = 12 nM
AR EC_{50} = 170 nM
HLM = 15 min

2
ROCK2 IC_{50} = 31 nM
AR EC_{50} = 250 nM
HLM = >300 min

Figure 1.

Based upon binding overlays of **2** and **4** we reasoned that either the α or β ring carbon of piperidines or related pyrrolidines might provide the appropriate trajectory to access the G-loop hydrophobic pocket without disrupting the equally important amine interaction with residues in the phosphate binding region (Fig. 2).

Substituted analogs of **2** at the α- and β-carbons were prepared from their corresponding *N*-Boc protected acids **5** (Scheme 1).[8] The α-analogs were prepared via conversion to the acid chloride followed by coupling with isoquinolone **6** and removal of the protecting group. Alternatively, the β-analogs were synthesized using the

* Corresponding author.
E-mail address: todd.bosanac@boehringer-ingelheim.com (T. Bosanac).

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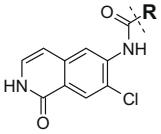
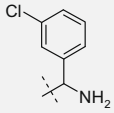
Table 1
Activity of 2*H*-isoquinolin-1-ones



Compd	R	ROCK2 IC_{50}[a] (nM)
3		120
4		15

[a] Cambrex PKLight ATP detection reagent using luciferin–luciferase to quantify residual ATP. Values are means of at least two duplicate experiments.

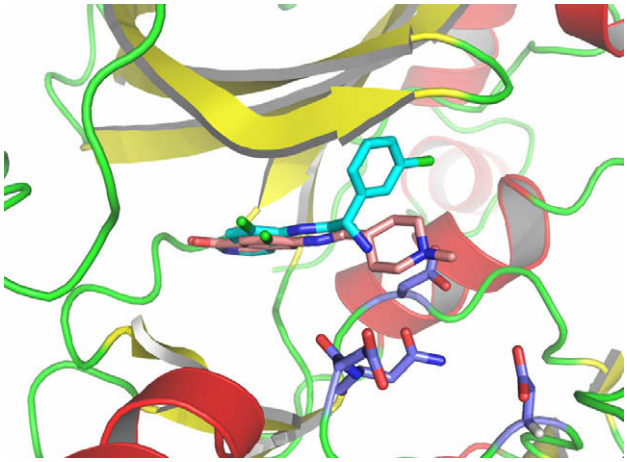
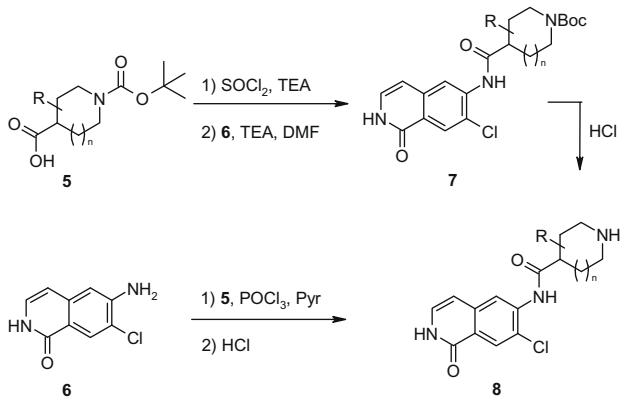


Figure 2. Overlay of inhibitor **2** (salmon) and **4** (blue) in a ROCK homology model.

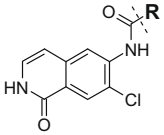


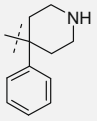
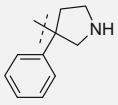
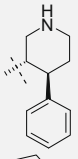
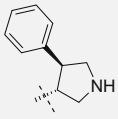
Scheme 1. Synthesis of isoquinolone inhibitors.

phosphorous oxychloride/pyridine conditions described previously to effect coupling.[7]

Our initial array of targets focused on identifying the optimal heterocycle and substitution pattern. Compounds were first profiled for their inhibition of ROCK 2 using a luciferase based ATP detection assay.[6] Compounds that displayed sufficient activity

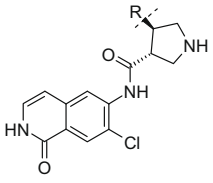
Table 2
SAR of substituted piperidines and pyrrolidines

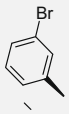
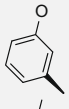
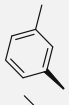
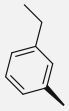


Compd	R	ROCK2[a] IC_{50} (nM)	AR EC_{50}[b] (nM)	HLM[c] (min)
9		28	220	224
10		67	nt	>300
11		47	550	71
12		230	nt	nt
13		240	nt	nt
14-*rac*		6.0	55	37
15-*rac*		6.0	89	130

[a] Cambrex PKLight ATP detection reagent using luciferin–luciferase to quantify residual ATP. Values are means of at least two 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 of protein/mL. nt = not tested.

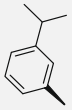
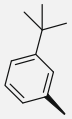
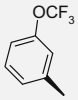
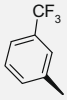
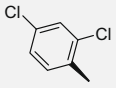
Table 3
SAR of substituted phenyl pyrrolidines



Compd	R	ROCK2 IC_{50}[a] (nM)	AR EC_{50}[b] (nM)	HLM[c] (min)
16-*rac*		2.0	63	43
17-*rac*		4.0	88	30
18-*rac*		5.0	nt	nt
19-*rac*		4.0	nt	nt

(*continued on next page*)

Table 3 (*continued*)

Compd	R	ROCK2 IC_{50}[a] (nM)	AR EC_{50}[b] (nM)	HLM[c] (min)
20-*rac*		6.0	nt	nt
21-*rac*		11	nt	nt
22-*rac*		7.0	nt	nt
23-*rac*		4.0	nt	nt
24-*rac*		8.0	nt	nt
25-rac		14	210	58
26-rac		8.0	150	49
27-rac		26	66	nt
28-*rac*		9.0	160	61
29-*rac*		4.0	140	238
30-*rac*		6.0	60	>300
31-(3*R*,4*S*)		11	40	nt
32-(3*S*,4*R*)		4.0	23	nt

[a] Cambrex PKLight ATP Detection Reagent using luciferin–luciferase to quantify residual ATP. Values are means of at least two 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 of protein/mL. nt = not tested.

against ROCK 2 were further examined for dilation of isolated rat aortic rings following pre-constriction with phenylephrine.[9] The initial heterocycle starting points demonstrated comparable ROCK potency to piperidine **2** with a preference for the *R*-enantiomers of both the piperidine **10** and pyrrolidine **11** as previously reported (Table 2).[7] α-Phenyl substitution of either the piperidine or pyrrolidine led to a loss in potency (compare **9**–**12** and **11**–**13**). However, both the racemic *trans* β-substituted piperidine **14** and pyrrolidine **15** improved molecular potency which also translated into improved aortic ring potency. Pyrrolidines were prioritized for further exploration with the discovery that **15** successfully maintained good microsomal stability and displayed higher oral bioavailability in rat (97% vs 13% compared to piperidine analog **14**).[10]

Substitution around the phenyl ring of **15** was further explored and both electron donating and withdrawing groups were found to be well tolerated with respect to molecular potency (Table 3). Initial exploration of substitution focused on the 3-position which demonstrated good aortic ring potency (3-Br and 3-OMe substituted analogs **16** and **17**), however poor microsomal stability was observed with these inhibitors. We reasoned that metabolism was occurring on the aryl ring and chose to investigate both replacement of the phenyl group with a pyridine, an effective strategy to alter metabolism,[11] and halogenation at the 4-position. The pyridyl compounds **25–27** did not improve the microsomal stability. Significant differences in the microsomal stability of the 4-halogenated derivatives were observed (compare **28**–**30**). In particular the 4-Cl compound **30** demonstrated both the best aortic ring potency and HLM stability and was resolved through chiral hplc to afford the discrete enantiomers **31** and **32**.[12] The absolute stereochemistry of **31** was determined by an x-ray co-crystal structure with ROCK1 (vide infra).

A modest separation in potency was seen with the (3*S*,4*R*) isomer **32** being more potent. The activity of both **31** and **32** could be rationalized with our ROCK1 homology model (Fig. 3) in which both compounds are capable of simultaneously participating in both the targeted G-loop and phosphate binding region interactions.

A co-crystal structure of inhibitor **31** bound to ROCK1 was solved at 3.2Å resolution (Fig. 4), which revealed a hydrogen bond between the pyrrolidine nitrogen and the carboxylate of ASP202, consistent with the docked structure of **31** in the ROCK homology model. The co-crystal structure also shows the 4-Cl-phenyl group is inserted right beneath the G-loop making hydrophobic contacts with Leu107 and Lys105. The G-loop is significantly shifted (about 1.8 Å) compared to the co-structure with inhibitor **22** described previously[7] in order to accommodate the phenyl group of **31**.

In conclusion, we have designed a series of β-aryl-pyrrolidine 2*H*-isoquinolin-1-one dual ROCK2 inhibitors which we believe access both the G-loop lipophilic interaction of the lead phenyl glycine series and the phosphate binding region polar interaction of the isonipocotic acid derivatives.[7] Execution of this strategy accomplished the goal of improving aortic ring potency over the previously reported **2**, 10-fold while maintaining excellent microsomal stability.

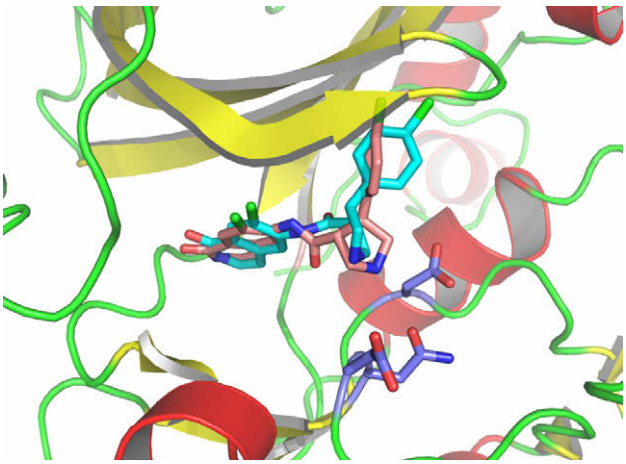


Figure 3. Overlay of inhibitor **31** (blue) and **32** (salmon) in a ROCK homology model.

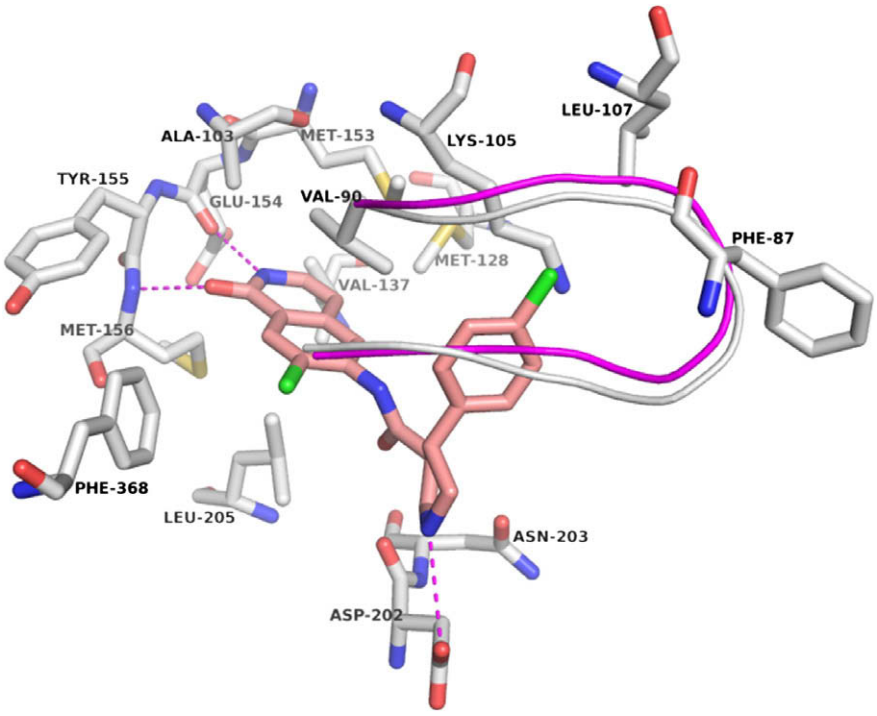


Figure 4. X-ray co-crystal structure of **31** bound in ROCK1. The section of the G-loop in the co-structure with inhibitor **22** from Ref. 7 is shown in magenta to highlight the movement of the G-loop caused by the phenyl ring of **31**.

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10. 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.
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12. Compound **32** was tested against the same panel of kinases in Ref. 7 (IC_{50} (nM); PRKG2 = 24, PRKCL2 = 14, PRKCE = 1000, CDC42 = 54).