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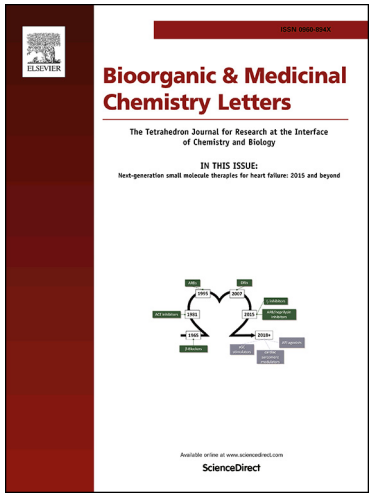

ROCK Inhibitors 2. Improving Potency, Selectivity and Solubility through the Application of Rationally Designed Solubilizing Groups

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ROCK Inhibitors 2. Improving Potency, Selectivity and Solubility through the Application of Rationally Designed Solubilizing Groups.

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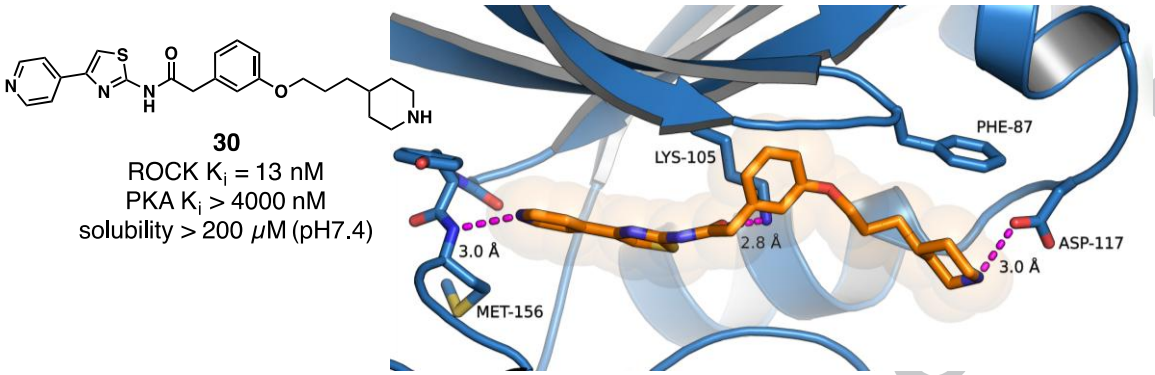
Abstract

Solubilizing groups have been frequently appended to kinase inhibitor drug molecules when solubility is insufficient for pharmaceutical development. Such groups are usually located at substitution sites that have minimal impact on target activity. In this report we describe the incorporation of solubilizing groups in a class of Rho kinase (ROCK) inhibitors that not only confer improved solubility, but also enhance target potency and selectivity against a closely related kinase, PKA.

Keywords

solubilizing group, structure-based design, rho kinase, ROCK

Graphical Abstract



In the period since the introduction of imatinib (**1**) in 2001 as the first explicitly designed therapeutic kinase inhibitor, more than 30 kinase inhibitor drugs have since been approved by the US FDA. Of these kinase-targeted drugs, 19 contain chemical moieties specifically incorporated for the purpose of enhancing solubility, since the parent structure is lacking sufficient solubility for pharmaceutical development (Figure 1: for a complete illustration of the current FDA-approved kinase inhibitor drugs see Figure S1 in the Supplementary file). These solubilizing groups are typically attached to a substitution point on the parent molecule that possesses a high degree of SAR tolerance and usually neither enhances nor interferes with target potency. The binding implications of these solubilizing features have been well described and illustrated in a recent review by Wu *et al*.[1] For example, the X-ray crystal structure of gefitinib (**2**) (PDB: 2ITY) bound to its target, EGFR, shows the solubilizing morpholinopropoxy group to be extended away from the protein surface, not making contact with the protein (Figure 2). Furthermore, gefitinib (**2**) reportedly inhibits EGFR with IC_{50} = 9 nM, while the analogous 6-methoxy compound has IC_{50} = 23 nM,[2] indicating that the solubilizing group does not significantly contribute to binding. Interestingly, the X-ray structure of imatinib (**1**) bound to its target Abl (PDB: 1IEP) shows the solubilizing methylpiperazine to be in contact with protein backbone residues (Figure 3). The binding data shows that Bcr-Abl inhibition by imatinib (1: IC_{50} = 38 nM) is approximately 5-fold improved over the unsubstituted (4-methyl) compound (IC_{50} = 200 nM).[3] However, with the exception of the related Bcr-Abl inhibitors nilotinib and ponatinib, the solubilizing appendages of kinase-inhibitor drugs extend into solvent space, not providing any additional value in terms of binding interactions as illustrated by Wu *et al*.[1]

Figure 1. FDA approved kinase inhibitors containing solubilizing features (highlighted in red).

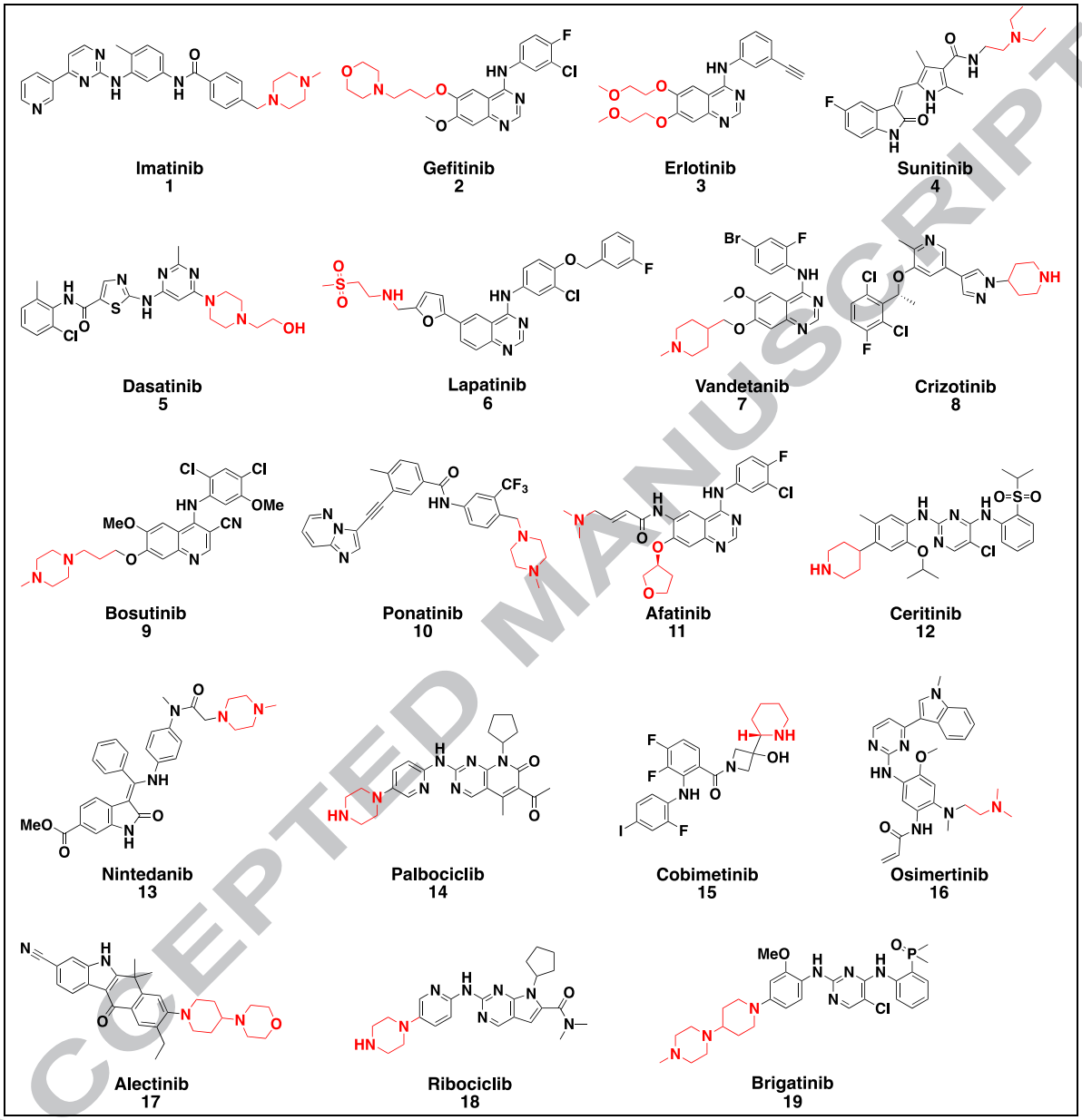


Figure 2. **A**. Gefitinib (**2**: R = morpholinopropyl); **B**. EGFR inhibitory activity of **2** compared with the non-solubilized parent; **C**. X-ray structure of gefitinb (PDB: 2ITY) showing the solubilizing morpholinopropyl group extending away from protein surface.

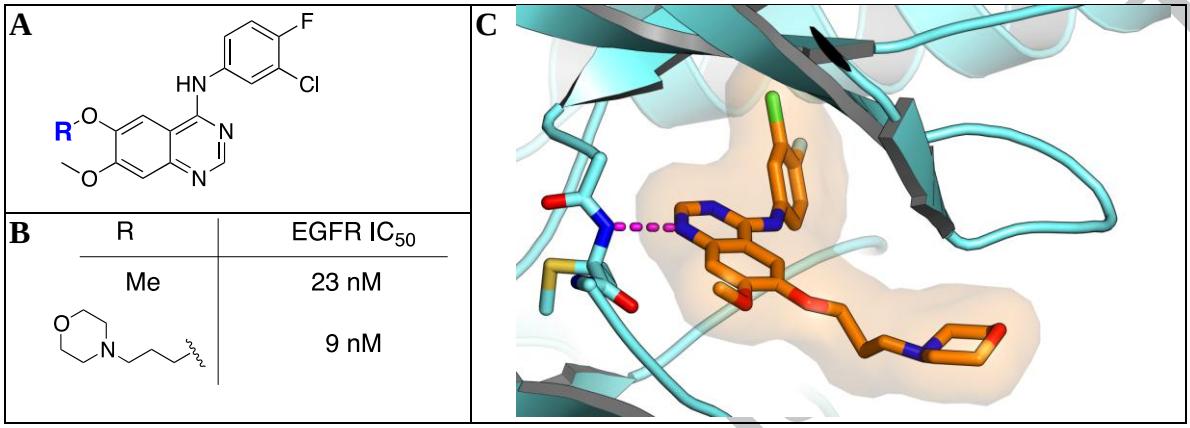
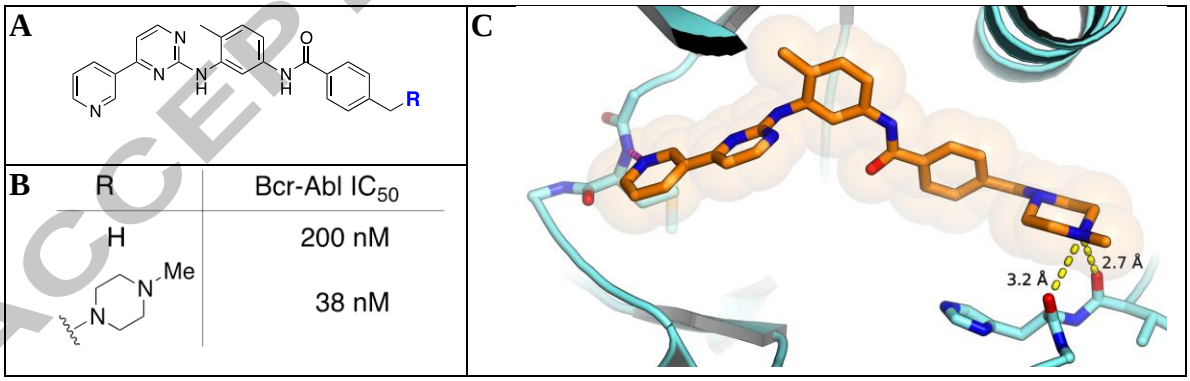


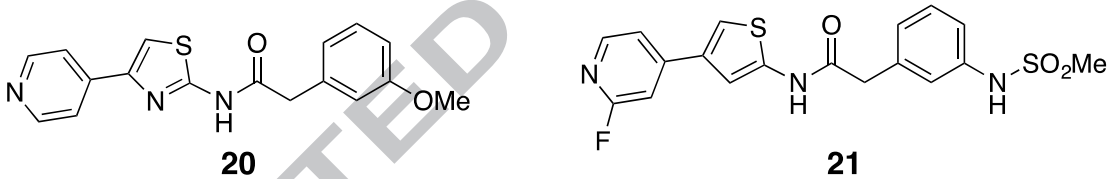
Figure 3. **A**: Imatinib (**1**: R = 4-methylpiperazinyl); **B**. Bcr-Abl inhibitory activity of **1** compared with non-solubilized parent; **C**. X-ray structure of imatinib (PDB: 1IEP) showing the methylpiperazinyl group in close proximity (2.7-3.2 Å) to the backbone carbonyls of Ile360 and His361.



The Rho-associated kinases, ROCK1 and ROCK2 are highly homologous Ser/Thr kinases, activated by binding of the small GTPase Rho, that act on a variety of substrates, many of which are implicated in smooth muscle contractility. ROCK inhibitors are

currently under clinical development for a number of therapeutic applications, most notably for the treatment of glaucoma. These inhibitors have been summarized in a recent review, which also thoroughly covers the ROCK inhibitor literature.[4] We have recently reported our approach to the discovery and design of pyridyl-thiazole and pyridyl-thiophene ROCK inhibitors, exemplified by compounds **20** and **21** shown in Figure 4.[5] X-ray crystallography and molecular modeling facilitated the optimization of this series of molecules from the early lead (**20**) to an optimized molecule (**21**) possessing properties suitable for pharmacological evaluation following oral dosing. During the course of this work key challenges included enhancing potency and selectivity, and improving solubility.

Figure 4.



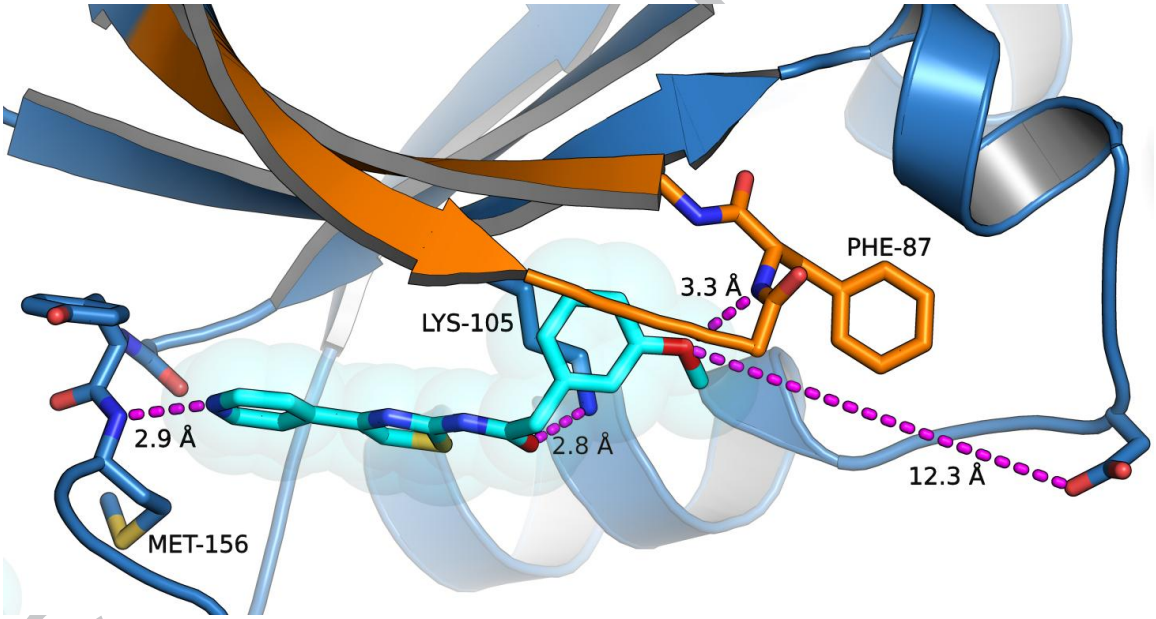
20
ROCK K_i = 83 nM
PKA K_i = 1400 nM

21
ROCK K_i = 10 nM
PKA K_i = 3000 nM

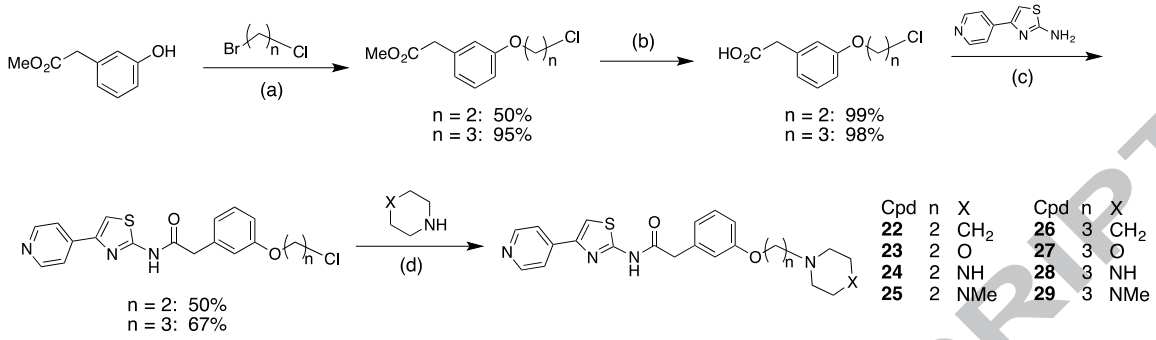
Figure 5 illustrates the X-ray structure of compound **20** bound to ROCK. Notable interactions include hydrogen bonds between the pyridine nitrogen and Met156 (2.9 Å), the amide carbonyl and the sidechain of catalytic Lys105 (2.8 Å), and the methoxy oxygen and the backbone NH of Phe87 (3.3 Å). In addition, we noted that Asp117, some 12 Å distant from the methoxy group, might offer an additional binding opportunity. Not only could this residue engage in an ionic interaction with a pendant basic moiety, such

as piperidine or piperazine, but the analogous residue in PKA, a closely related AGC-family kinase[6] and a key anti-target, is Gln84. The greater steric requirements and neutral nature of this side chain should enhance selectivity for ROCK. To access this region of the target, we prepared 3-substituted phenylacetic acid derivatives bearing extended solubilizing groups and incorporated these into ROCK inhibitors, as illustrated in Schemes 1 and 2.

Figure 5. X-ray structure of **20** bound to ROCK1 (PDB ID: 4YVE). The Gly-rich loop is shown in orange and the distal outer loop is shown in blue.

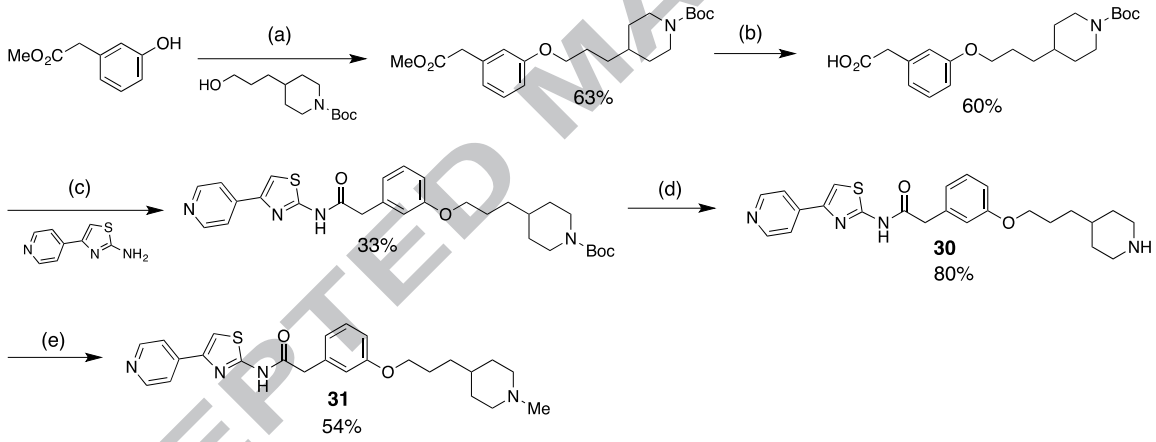


Scheme 1. Preparation of compounds **22** – **29**.



(a) K_2CO_3, acetone, reflux; (b) 1N NaOH, dioxane; (c) Ms-Bt, Et_3N, THF, reflux; (d) DMSO, 70 °C

Scheme 2. Preparation of compounds **30** and **31**.



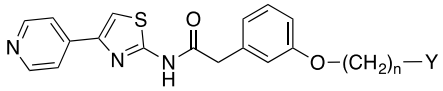
(a) DIAD, PPh_3, THF; (b) 2N NaOH, MeOH; (c) Ms-Bt, Et_3N, THF, reflux; (d) TFA, CH_2Cl_2; (e) 37% HCHO, HCOOH, MeOH, reflux

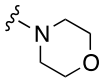
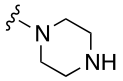
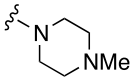
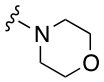
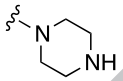
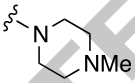
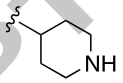
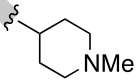
N-Piperidine, -piperazine and -morpholine compounds **22-29** were prepared as shown in Scheme 1. Methyl 3-hydroxyphenylacetic acid was alkylated with 2-chloro-1-bromo-ethane, or 3-chloro-1-bromopropane, under basic conditions in acetone at reflux for 24 h. Subsequent base hydrolysis yielded the phenylacetic acids, which were coupled to 4-

(pyridin-4-yl)thiazol-2-amine using 1-methanesulfonylbenzotriazole (Ms-Bt),[7] as previously described.[5] Conversion to the final products was accomplished by treatment with the appropriate secondary amine in DMSO at 60-90°C. Products were purified by preparative HPLC. The preparation of 4-substituted piperidine compounds **30** and **31** is shown in Scheme 2. Methyl 3-hydroxyphenylacetic acid was alkylated with *N*-Boc-4-(3-hydroxypropyl)piperidine under Mitsunobu conditions, the product hydrolyzed and coupled as described above. Boc deprotection using TFA gave compound **30**, while Eschweiler-Clarke methylation gave compound **31**.

Evaluation of the effects of the solubilizing groups is summarized in Table 1.

Table 1. ROCK1 and PKA inhibition by solubilized ROCK inhibitors.



Compd	n	Y	ROCK1 Ki, nM[a]	PKA Ki, nM[a]	Solubility, μM[b]
20	0	Me	83[c]	1400[c]	22
22	2		510	580	>200
23	2		430	>4000	>200
24	2		47	>4000	>200
25	2		88	>4000	>200
26	3		220	>4000	>200
27	3		240	>4000	>200
28	3		51	>4000	>200
29	3		45	>4000	>200
30	3		13	>4000	>200
31	3		10	>4000	>200

[a] Enzyme inhibition assays were performed as previously described[5]

[b] Solubility measured in 10 mM phosphate buffer (pH 7.4)

[c] K_i data generated contemporaneously with compounds **22** to **31** and differs slightly from previously reported data[5]

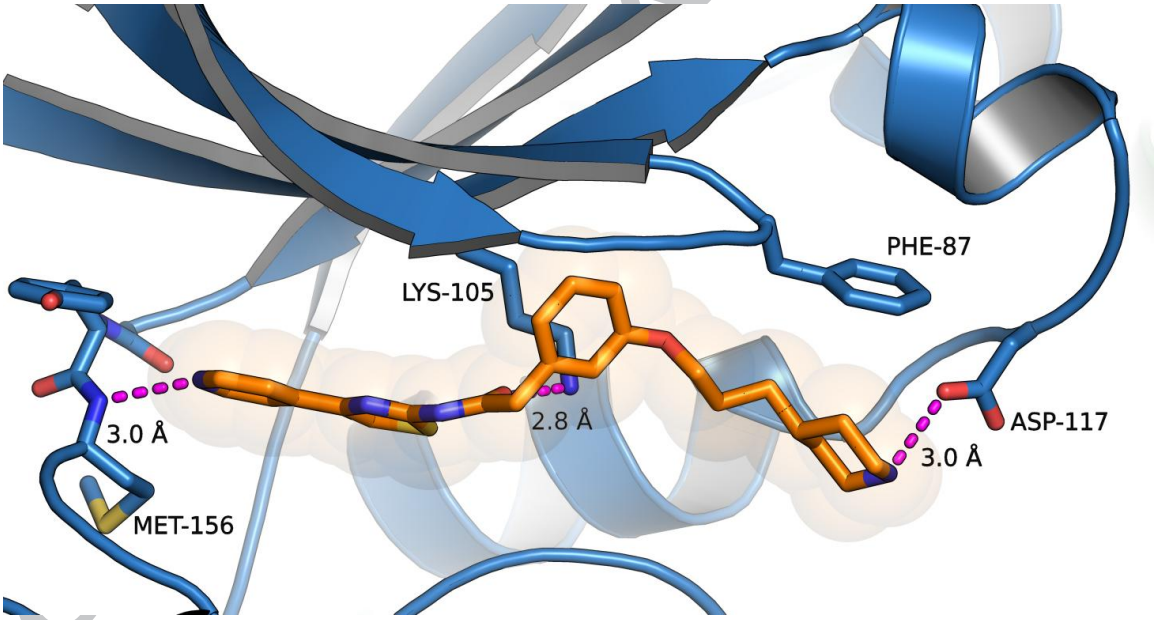
The introduction of a solubilizing side-chain, attached at the 3-position of the phenylacetamide led to compounds with enhanced potency relative to the parent molecule **20**. With a 2-carbon linker, the piperidine (**22**) and morpholine (**23**) derivatives

were *ca*. 5- to 6-fold less potent than **20**, indicating that neither occupancy of the above-described space, nor the presence of a basic, solubilizing center offers any particular advantage to ROCK inhibition. The analogous piperazine derivatives **24** and **25** were comparable to **20** in inhibitory potency, suggesting that activity could be restored, though not improved relative to **20**. Compounds **24** and **25** showed superior selectivity against PKA relative to **20**. Extending the linker to 3 carbon atoms in compounds **26** to **29** generally shows enhanced target potency, relative to the 2-carbon linked analogs. Thus both piperidine (**26**) and morpholine (**27**) derivatives are approximately 2- to 3-fold improved relative to the 2-carbon analogs, but not improved compared to **20**. Again this supports the hypothesis that space occupancy and basicity are neither advantageous, nor particularly detrimental. However, compounds **28** to **29**, in which a basic nitrogen atom exists at the terminus of the molecule show modestly enhanced potency relative to the parent molecule **20**. In particular, the most basic compounds, the piperidine derivatives **30** and **31** exhibit the greatest enhancement in potency, approximately 8-fold relative to **20**. These results suggest that the size of the substituent and the presence of the remote basic center contribute to the improved inhibitory activity. In all cases activity towards PKA remains >4 μM, again suggesting that the enhanced activity towards ROCK is due to specific interactions that are not achieved with a closely related kinase. In addition all molecules bearing a solubilizing group show enhanced solubility (> 10-fold) relative to **20** in pH 7.4 phosphate buffer (Table 1).

The X-ray crystal structure of **30** bound to ROCK supports our hypothesis. As shown in Figure 6, the piperidine nitrogen of **30** is in close proximity (2.9 Å) with the side chain of Asp117, while the rest of the molecule binds in the same conformation observed with

compound **20** and, in general, makes the same contacts with the enzyme as described above. However the Gly-rich loop has moved upwards (as illustrated), possibly to accommodate the carbon chain of the solubilizing group, resulting in the apparent loss of an H-bond between the loop and the meta-O atom. This might explain why greater gains in activity were not observed. Furthermore compound **30** showed no significant inhibition towards 20 kinases tested (all >1 μM), while compound **28** showed no inhibition of 145 kinases tested, including 20 AGC-family kinases (Tables S2, S3 in Supplementary Material).

Figure 6. Compound **30** bound to ROCK1 (PDB ID: 5HVU)



In summary, we have illustrated a strategy for the introduction of solubilizing groups that accomplishes more than mere solubility enhancement. By identifying key residues proximal to the active site, solubility, enhanced potency and greater target selectivity were all accomplished by introducing a soluble moiety that provides additional unique

interactions. Through the application of structure-guided design, we have developed a series of potent, selective and soluble inhibitors of ROCK. Modeling and X-ray crystallographic methods helped to identify a key amino acid (Asp117) that was targeted to provide an additional binding interaction with inhibitors bearing an appended solubilizing group. Connection of a solubilizing piperidine or piperazine through a 3-carbon linker gave potent, selective and soluble inhibitors of ROCK. This work illustrates a basis for future design of kinase inhibitors bearing solubilizing groups that accomplish potency and selectivity improvements, in addition to enhanced solubility.

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Highlights

- Solubilizing groups are used to enhance solubility, potency and selectivity
- Structure-based design used to target key interactions with solubilizing groups
- Approximately half of FDA-approved kinase inhibitor drugs contain a solubilizing group