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Fragment-based discovery of 6-substituted isoquinolin-1-amine based ROCK-I inhibitors

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ABSTRACT

Fragment-based NMR screening of a small literature focused library led to identification of a historical thrombin/FactorXa building block, **17A**, that was found to be a ROCK-I inhibitor. In the absence of an X-ray structure, fragment growth afforded 6-substituted isoquinolin-1-amine derivatives which were profiled in the primary ROCK-I IMAP assay. Compounds **23A** and **23E** were selected as fragment optimized hits for further profiling. Compound **23A** has similar ROCK-1 affinity, potency and cell based efficacy to the first generation ROCK inhibitors, however, it has a superior PK profile in C57 mouse. Compound **23E** demonstrates the feasibility of improving ROCK-1 affinity, potency and cell based efficacy for the series, however, it has a poor PK profile relative to **23A**.

Rho kinase (ROCK) belongs to the AGC family of serine/threonine kinases that are activated via interaction with the small GTP-binding protein RhoA. Two isoforms have been described, ROCK-I and ROCK-II, which are highly homologous (65% overall homology; 92% identity in kinase domains) and exhibit absolute identity in their ATP-binding site.[1] ROCK is ubiquitously expressed across a range of cell types within various tissues and is implicated in a number of important physiological functions including regulation of smooth muscle contraction, cytoskeleton re-arrangement, cell migration and proliferation and inflammatory responses.[2]

Accumulating clinical and pre-clinical evidence indicates that ROCK is an important target for several cardiovascular diseases including angina pectoris, hypertension, coronary vasospasm, restenosis after percutaneous coronary intervention and arteriosclerosis.[3] Clinical studies using the ROCK inhibitor Fasudil have demonstrated efficacy and safety in treating cerebral vasospasm, ischemic stroke and stable angina. Furthermore, pre-clinical studies suggest additional therapeutic potential for ROCK inhibitors in a wide range of diseases including cancer, glaucoma and CNS diseases such as Alzheimer's disease and neuropathic pain.[4]

First generation ROCK inhibitors, including hydroxyl-Fasudil (the active metabolite of Fasudil) and Y-27632, have been known for a number of years (Fig. 1).[5]

More recently, there have been extensive research efforts towards identifying novel classes of potent and selective ROCK inhibitors.[6] To this end, specialized screening techniques including fragment-based hit discovery are being utilized to identify novel, tractable low-MW hit compounds.[7] Herein we describe a fragment-based approach which afforded a novel low molecular weight ROCK inhibitor with improved pharmacokinetics relative to the first generation compounds, for the potential treatment of atherosclerosis.

A small library of 69 compounds was created by fragmenting a database of kinase inhibitors that included existing ROCK

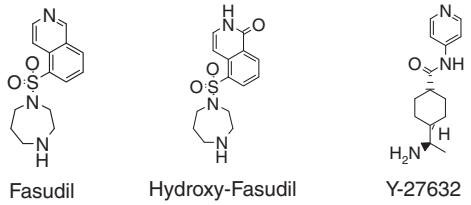

Figure 1. First generation ROCK inhibitors.

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inhibitors such as Y-27632 and Fasudil. Stock solutions were prepared (500 μM or 100 μM) and saturation transfer NMR used to screen for binders.[7] Forty-one fragments bound to ROCK-I and NMR competition binding experiments with Y-27632 showed that 16 bound in a similar manner to the ATP-binding site (see Fig. 2).

The NMR competition experiments suggested that fragments **10**–**16**, which are structurally related to both Fasudil and Y-27632, were the most potent ATP binders (Table 1). Fragments **10**, **13** and **14** were prioritised based on analysis of the literature, which indicated their potential to deliver a novel series of ROCK-I inhibitors.[9]

Fragments **10**, **13** and **14** were used as templates for similarity and sub-structure searches of the corporate building block collection, with a focus on identifying bulk quantities of non-commercially available cores.

One such building block, **17A** (Fig. 3), was derived from a historical thrombin inhibitor project.[10] With a pK_a of ~7.6, the amino isoquinoline moiety exists, at physiological pH, in equilibrium between the neutral and protonated forms.[11] It was envisaged that the neutral form would be able to bind to the kinase's hinge region and when tested in the ROCK-I IMAP®,[12] assay **17A** exhibited a pIC_{50} of 5.11 ± 0.03.

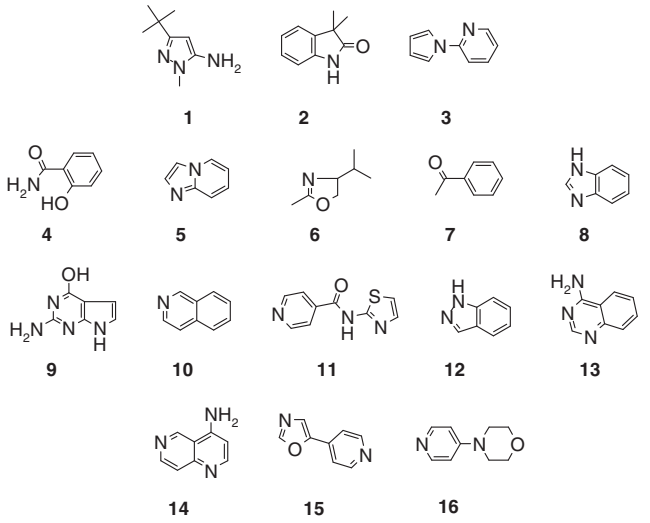


Figure 2. Fragments **1**–**16**, identified from ROCK-I NMR competition binding experiments with Y-27632.

Table 1
A comparison of the results from the ROCK-1 NMR competition experiments

Fragment	% drop in intensity of binding signals on addition of Y-27632
1	20
2	25
3	30
4	34
5	38
6	40
7	60
8	64
9	68
10	70
11	>80
12	>80
13	>80
14	>80
15	>80
16	>80

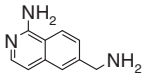


Figure 3. Thrombin 6-(aminomethyl)isoquinolin-1-amine fragment **17A**.

Since the 6-substituted isoquinolin-1-amine core was relatively unexplored in the kinase arena at the time, it was felt that elaborating this fused bicyclic ring system would offer good scope for generating a novel series. Fragment-based approaches benefit enormously from biostructural information but at that time no X-ray structure for ROCK was available. However, extensive use had been made of PKA for co-crystallization of ROCK-I ligands, so to guide fragment growth efforts, compound **17A** was docked into a PKA-based homology model of ROCK-I (Fig. 4). The preferred binding mode indicated the isoquinolin-1-amine core co-ordinating with the protein backbone in the ATP-binding site in a conformation identical to that observed for the adenine ring from the X-ray structure of a non-hydrolysable ATP mimetic, rather than that adopted by the isoquinoline ring of Fasudil.[13]

In this orientation the pendant aminomethyl group is placed in the ribose binding region[14] normally exploited by hydrophobic substituents in other inhibitor series. So we designed one library in which hydrophobic substituents were appended to the aminomethyl group (Table 2). Additionally the docking model suggested moving the basic amino group further from the bicyclic core, in order to provide better access to the phosphate binding region of the ATP-binding site. So plans for a second library were drawn up with positively charged substituents to explore this hypothesis (Table 4). The first fragment growth library from **17A** was achieved via reductive amination of *N*-(6-formylisoquinolin-1-yl)benzamide **18** with a variety of amines, followed by deprotection as described in Scheme 1.

The *N*-(6-formylisoquinolin-1-yl)benzamide **18** was prepared by Knoevenagel condensation of 3-bromobenzaldehyde **19** with malonic acid in refluxing ethanol in the presence of pyridine to afford 3-bromocinnamic acid, which was treated with thionyl chloride in refluxing toluene to give the acid chloride **20**. Acid chloride **20** was dissolved in acetone and the resulting solution added to a mixture of sodium azide in water/acetone at 0 °C. After stirring vigorously for 1 h, the reaction mixture was poured onto ice-water and the 3-bromocinnamoyl azide precipitated as a white solid. A solution of the azide in dichloromethane was then added dropwise to preheated (140 °C) diphenyl ether to give the presumed Curtius-re-arrangement product, with evolution of nitrogen

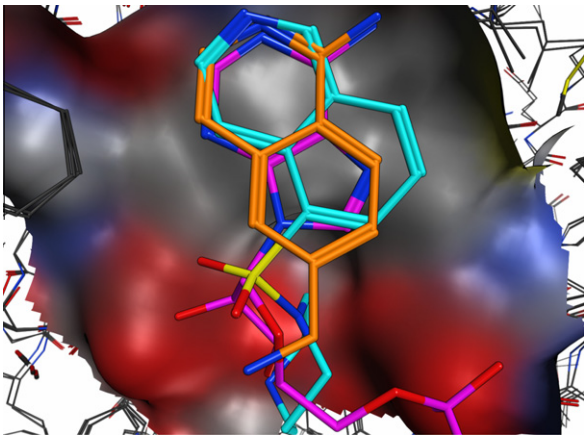
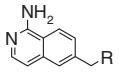
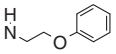
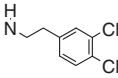
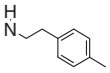
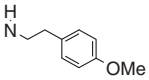
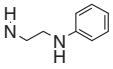
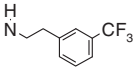
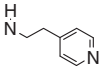
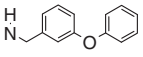
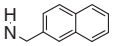


Figure 4. Docking of fragment **17A** (orange) into the ROCK-I homology model alongside modeled Fasudil (cyan) and ATP mimetic (pink).

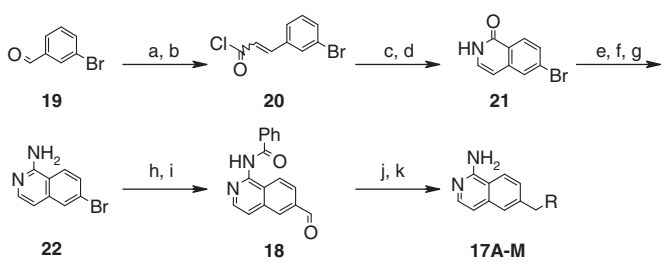
Table 2
ROCK-I IMAP SAR for R amines, **17A–M**



Compounds	R	ROCK-I (IMAP) pIC_{50}[a]
17A	NH_2	5.11 ± 0.03
17B		6.37 ± 0.07
17C		6.23 ± 0.3
17D		6.11 ± 0.04
17E		6.09 ± 0.04
17F		6.00 ± 0.03
17G		5.89 ± 0.05
17H		5.83 ± 0.09
17I		5.77 ± 0.05
17J		5.73 ± 0.04
17K		6.24 ± 0.09
17L		5.46 ± 0.04
17M		5.17

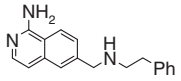
[a] pIC_{50} values are shown as mean ± standard deviation from $n = 2–5$ separate experiments (except where $n = 1$).

gas. The dichloromethane was removed under reduced pressure and the resulting diphenyl ether isocyanate solution heated (250–260 °C) to afford 6-bromoisoquinolinone **21**. Conversion of **21** to 1-amino-6-bromoisoquinoline **22** involved treatment with $POCl_3$, followed by reaction with phenol and potassium hydroxide and then ammonium acetate. The aminoisoquinoline **22** was *N*-benzoyl protected, the bromine lithiated and then quenched with *N*,*N*-dimethylformamide to afford aldehyde **18**.



Scheme 1. Synthesis of *N*-(6-formylisoquinolin-1-yl)benzamide **18** and reductive amination, followed by deprotection to afford 6-(aminomethyl)isoquinolin-1-amine based ROCK-I inhibitors **17A–O**. Reagents and conditions: (a) $CH_2(CO_2H)_2$, EtOH, pyridine, 16 h, reflux; (b) $SOCl_2$, toluene, 1 h, reflux; (c) NaN_3, H_2O, acetone, 1.5 h, 0 °C; (d) Ph_2O, 2 h, 140 °C then 2 h, 250–260 °C; (e) $POCl_3$, $CHCl_3$, 2 h, reflux; (f) PhOH, KOH, xylene, 4 h, reflux; (g) NH_4OAc, 160 °C; (h) $(PhCO)_2O$, pyridine, 1 h, 125 °C; (i) *n*-BuLi, THF, −78 °C, DMF added and allowed to warm to 0 °C; (j) THF, amine, MP-cyanoborohydride resin; (k) AcOH, 6 N HCl, 24 h at 104 °C.

Table 3
Further in vitro profiling of **17G**


17G

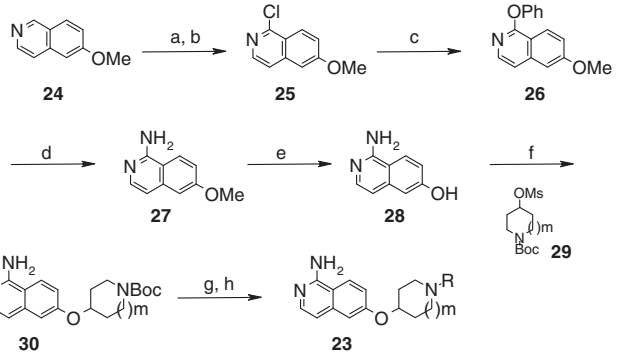
	17G[a]
ROCK-I pK_i	7.2
ROCK-I IMAP pIC_{50}	5.89 ± 0.05
ROCK-II IMAP pIC_{50}	6.5
LE/LipE	0.48/3.0
THP migration pIC_{50}	<4.5
hERG (Dofetilide) pK_i	5.5

[a] pIC_{50} values are shown as mean ± standard deviation from $n = 2–5$ separate experiments (except where $n = 1$).

A diverse array of reductive aminations were investigated and a number of 6-(aminomethyl)isoquinolin-1-amine derivatives prepared for SAR evaluation in the ROCK-I IMAP assay. Compounds which afforded a pIC_{50} >5 in the ROCK-I IMAP assay, **17B–O**, are shown in Table 2. Compounds with significantly improved potency compared to **17A** were identified such as **17B**, **17C**, **17G** and **17K**. These compounds were further profiled in vitro in order to evaluate the scope for further optimization.

The data for compound **17G** is shown in Table 3. As expected from the close ATP-site homology, a similar ROCK-II IMAP potency was obtained. However, **17G** was poorly active in the THP cell based (MCP-1) migration assay, which provides an in vitro understanding of MCP-1-induced transendothelial migration of monocytes in unstable atherosclerotic lesions. The compound also possessed significant hERG binding.

As a key element of the design of our second fragment-growing library based on extending the linker to the basic amine, ether linked cyclic amines were prioritised. Cyclic amines are highly represented within the ROCK literature and it was rationalized that linking readily available piperidines to a 6-hydroxy-isoquinolin-1-amine core would provide compounds with reduced rotational bonds and possibly improved ligand-lipophilicity efficiency values relative to compounds **17A–M**. Piperidine derivatives of 6-(oxy)isoquinolin-1-amine, **23A–F**, were readily accessible from the route described in Scheme 2. The 6-hydroxy-isoquinolin-1-amine core **28** was prepared from commercially available **24**. Oxidation of **24** with mCPBA, followed by treatment with $POCl_3$ afforded **25**, which was converted to **26** following reaction with phenol and potassium hydroxide. Phenoxy derivative **26** was then treated with ammonium acetate to afford **27**, which was deprotected



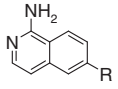
Scheme 2. Synthesis of 6-(oxy)isoquinolin-1-amine **25A–F** based ROCK inhibitors. Reagents and conditions: (a) mCPBA, DCM; (b) $POCl_3$, 6 h, 90 °C; (c) PhOH, KOH, 3 h, 140 °C; (d) NH_4OAc, 16 h, 150 °C; (e) BBr_3, DCM; (f) PS-BEMP, MeCN, 160 °C; (g) TFA, DCM; (h) HOAc, CH_3CN, RCHO, $NaBH(OAc)_3$.

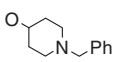
with boron tribromide to afford **28**. PS-BEMP mediated S_N2 displacement of the readily prepared mesylates **29** afforded ether **30**. *N*-Boc deprotection of **30** afforded compounds **23A–C**. Reductive amination with the appropriate amine **23A–C** (R = H) provided compounds **23D–F**.

The SAR for **23A–F**, in the ROCK-I IMAP assay is shown in Table 4. The potencies obtained for piperidines **23A** and **23B** were encouraging and improved following N-benzylation to afford **23D** and **23E**. Compounds **23A**, **23B**, **23D** and **23E** represented a significant improvement over the original fragment hit **17A** illustrating the benefit of employing an extended rigidified linker between the isoquinolin-1-amine and the basic amine. As predicted by the homology model, the importance of the basic nitrogen on the piperidine ring is illustrated by the inactivity of compound **23G** relative to **23A**.

In order to evaluate the scope for further optimization, compound **23A** and its N-benzylated derivative **23E** were selected for further in vitro and in vivo pharmacokinetic profiling (Table 5). Compound **23A** had comparable binding affinity, potency and cell based efficacy to the first generation ROCK inhibitors hydroxyl-Fasudil and Y-27632 (Table 6), which were benchmarked in the same assay formats. The LiPE and selectivity over hERG for **23A** was improved relative to **17G**. Also unlike **17G**, **23A** showed activity in the THP migration assay. Compound **23E** exhibited higher activity than **23A** in both the IMAP and THP assays, but its hERG activity was also high. In the C57 mouse, **23A** had an excellent in vivo PK profile relative to the first generation ROCK-I inhibitors (Tables 5 and 6). In contrast, the more lipophilic *N*-benzyl derivative **23E**, had poor in vivo bioavailability with high clearance and volume in the Wistar rat. Compound **23A** was screened in duplicate at 10 μM in Invitrogen's SelectScreen® profiling service. Out of 245 kinases, **23A** inhibited 25 with >80% inhibition. These kinases included PKB, CK1, DYRK3, EPHA8, FES, CRK4, HGK, PDGFRA, PLK3, PKA, PKD, PKC, PRKG, PRKX, RET, ROCK-I, ROCK-II, SGK2, SNFILK2, Tie2 and RSE.

Table 4
ROCK-I IMAP SAR for R^2 amines, **23A–F**



Compounds	R	ROCK-I (IMAP) pIC_{50}[a]
23A		5.67 ± 0.16
23B		5.71 ± 0.02
23C		5.44 ± 0.18
23D		6.62 ± 0.09
23E		6.62 ± 0.01
23F		5.37 ± 0.01
23G		<4

[a] pIC_{50} values are shown as mean ± standard deviation from $n = 2–5$ separate experiments (except where $n = 1$).

Table 5
Profiling of compound **23A** and the N-benzylated derivative **23E** selected optimized fragments

	23A[a]	**23E**[a]
ROCK-I pK_i	7.4	n.d.
ROCK-I IMAP pIC_{50}	5.67 ± 0.16	6.62 ± 0.01
ROCK-II IMAP pIC_{50}	5.95 ± 0.04	7.04 ± 0.09
THP migration pIC_{50}	5.3	6.11 ± 0.21
LE/LipE	0.58/3.9	−/2.4
HLM/RLM/MLM (min, $T_{1/2}$)	All >120	12/22/>120
Rat Heps (min, $T_{1/2}$)	70	27
Caco-2($>5 \times 10^{-6}$ cm/s) A–B/B–A	6.3/3.6	2.2/1.5
Cyp inhibition (2C19, 3A4, 1A2, 2D6, 2C9)	Clean	All >3 μM
MW	243	333
c log *P*/*c* log *D*	2/−1	4.2/3.2
hERG (Dofetilide) pK_i	4.7	6.1
	C57 mouse	Wistar rat
Bioavailability (*F*%)	>75	7
Clp (ml/min/kg)	9.5	295
V_{ss} (L/kg)	11	8.2
$T_{1/2}$ (h)	5.8	8.2

[a] pIC_{50} values are shown as mean ± standard deviation from $n = 2–5$ separate experiments (except where $n = 1$).

Table 6
Benchmarking of first generation ROCK inhibitors

	Fasudil[a]	HO-Fasudil[a]	Y-27632[a]
ROCK-I pK_i	7.0	7.5	7.6
ROCK-I IMAP pIC_{50}	5.5 ± 0.1	6.12 ± 0.02	6.06 ± 0.02
ROCK-II IMAP pIC_{50}	5.87 ± 0.02	6.24 ± 0.06	6.61 ± 0.09
THP (MCP1) pIC_{50}	5.16 ± 0.09	5.16 ± 0.31	5.58 ± 0.26
hERG (Dofetilide) pK_i	4.6	5.1	4.1
C57 mouse			
Bioavailability (*F*%)	3.6	57	52
Clp (ml/min/kg)	318	34	50.9
V_{ss} (L/kg)	1.8	2.4	3.8
$T_{1/2}$ (h)	0.02	0.75	1.67

[a] pIC_{50} values are shown as mean ± standard deviation from $n = 2–5$ separate experiments (except where $n = 1$).

In conclusion, a historical thrombin building block **17A**, was identified by first conducting a focused NMR screen of fragments from known ROCK and other kinase inhibitors, and then mining the corporate building block collection for novel cores. Then by employing a fragment growth and linker modification strategy, a ROCK-I inhibitor **23A** was discovered with favorable properties relative to known ROCK-I inhibitors. This approach highlights the utility of focused literature-based fragment screening, followed by substructure and similarity searches to identify related cores suitable for fragment growth to afford novel compounds. In particular, **23A** has superior in vivo pharmacokinetics in C57 mouse compared to the first generation ROCK inhibitors, as well as similar ROCK-I affinity and cell based efficacy. Although **23E** demonstrated improved affinity and cell based efficacy, it suffered from poor in vivo bioavailability with a high clearance and volume in the rat. Compounds **23A** and **23E** were considered suitable starting points for further optimization into a lead candidate, with the goal of improving ROCK-I potency and selectivity, whilst maintaining good in vivo PK. This hit to lead candidate optimization program will be described in a subsequent communication.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.060.

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