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ROCK inhibitors 4: Structure-activity relationship studies of 7-azaindole--based rho kinase (ROCK) inhibitors

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ABSTRACT

Rho kinase (ROCK) inhibitors are of therapeutic value for the treatment of disorders such as hypertension and glaucoma, and potentially of wider use against diseases such as cancer and multiple sclerosis. We previously reported a series of potent and selective ROCK inhibitors based on a substituted 7-azaindole scaffold. Here we extend the SAR exploration of the 7-azaindole series to identify leads for further evaluation. New compounds such as **16, 17, 19, 21** and **22** showed excellent ROCK potency and protein kinase A (PKA) selectivity, combined with microsome and hepatocyte stability.

Introduction

The Rho-associated protein kinases (ROCK) are serine-threonine-kinases of the AGC-family and are involved in the regulation of vital cellular processes including motility, division, differentiation and contraction.[1] The two isoforms, ROCK I and ROCK II, are highly homologous (92% sequence identity) in their ATP-binding domain and share a 65% overall sequence identity.[2] Upon activation by GTP-bound Rho, ROCK phosphorylates various protein targets, such as myosin light chain[3] and myosin light chain phosphatase,[4] LIM kinase,[5] and zipper-interacting kinase (ZIPK)[6] which lead to cell signaling that promotes changes in cell motility, adhesion,[7] stress fiber formation,[7] and force generation through smooth muscle contraction[8]. ROCK inhibitors are of potential therapeutic value for the treatment of glaucoma[9] and other diseases.[10–13]

To date, three ROCK inhibitors have been approved for clinical use: fasudil is approved for the treatment of cerebral vasospasm[14] in Japan and China, and ripasudil and netarsudil are approved for the treatment of glaucoma in Japan and the U.S. respectively.[15,16]

We previously reported a new series of very potent ROCK inhibitors derived from a 3-substituted-7-azaindole scaffold exemplified by compounds **1** and **2** which exhibited excellent potency against ROCK, good selectivity against the closely related AGC family kinase PKA, and good CYP inhibition profiles (Fig. 1).[17] In our continued efforts to identify potent and selective ROCK inhibitors for clinical development, we further explored the SAR of the 3-substituted-7-azaindole series.

Results and discussion

In our previous reports we described a pyridine series of ROCK inhibitors[18] and the development of a related 7-azaindole-derived series.[17] During the course of this work we explored the SAR of the central heterocycle ring in the context of the pyridine series. In this work we re-evaluated this ring in the context of the 7-azaindole series, as well as explored the effects of substituents on the 7-azaindole group.

We first explored the replacement of the thiazole ring of compound **1** with five- and six-membered heterocyclic ring systems including regioisomeric thiazoles, thiophenes and pyrimidines, as well as thiadiazole (Fig. 2). Based on our previous studies,[17,18] we incorporated the *m*-methanesulfonamide phenylacetyl group to interact with the glycine-rich P-loop region of the ATP-binding pocket.

Enzyme inhibition data for the replacement of the thiazole ring of compound **1** with various five- and six-membered heterocycles is summarized in Table 1. Most of these new analogs showed strong inhibition of ROCK-1with K_i values typically below 100 nM and good selectivity against PKA. In particular, regioisomeric thiazole **3**, thiophene regioisomer **4**, and thiadiazole **6** all displayed >30-fold selectivity against PKA while thiophene regioisomer **5** showed the weakest selectivity against PKA. Both pyrimidine regioisomers **7** and **8** showed good ROCK potency and somewhat reduced PKA selectivity with respect to the other 5-membered heterocycles in the series. Overall, the replacement of the thiazole moiety of compound **1** with various five- and six-membered heterocycles did not further improve either ROCK potency

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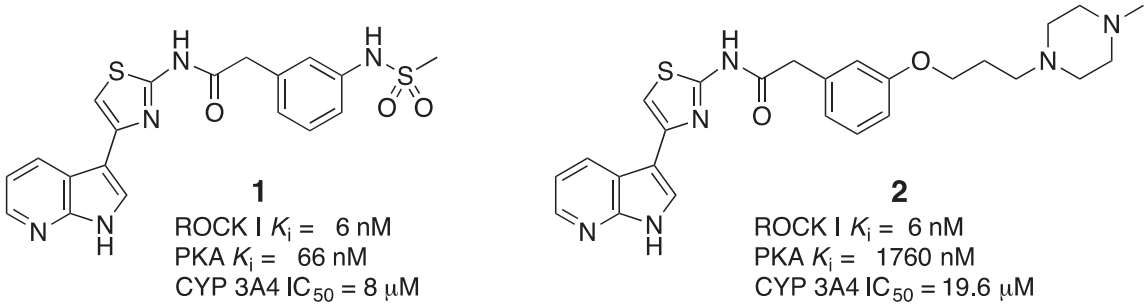


Fig. 1. Structures of ROCK I inhibitors **1** and **2**.[17]

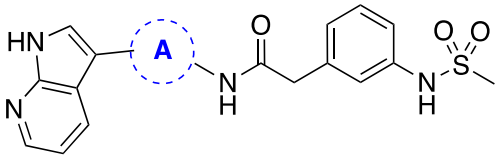


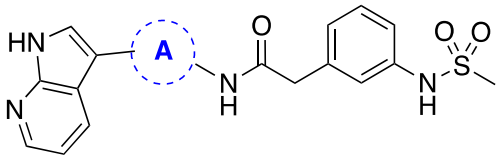
Fig. 2. Replacement of compound **1** thiazole with 5- and 6-membered heterocycles.

or PKA selectivity. Methyl-substituted thiazole **9** showed reduced ROCK potency and PKA selectivity. Furthermore, most of these new analogs showed reduced cellular activity in the THP-1 cell migration assay. Thiadiazole **6** showed decreased cell potencies possibly due to poor cell permeability and/or low compound solubility.

We next explored 7-azaindole substituents at the 4- and 5-positions with groups including F, Cl, Br and OH and the results are outlined in Table 2. Most of the compounds showed excellent ROCK enzyme potency and PKA selectivity except the 4-chloro analog **13**, which showed very poor selectivity against PKA. Fluorine-substituted analogs **10** and **11** showed improved THP-1 migration cellular potencies compared with other substituted azaindoles. This observation prompted us to synthesize additional fluorine-substituted analogs at the 4- and 5- positions of the 7-azaindole ring.

In previous studies[17], we reported that 7-azaindole ROCK inhibitors containing *m*-substituted phenylacetamides bearing solubilizing groups such as piperazines and piperidines, attached *via* two- and three-carbon linkers, can increase both ROCK potency and PKA selectively due to an additional binding interaction with the acidic side chain of Asp177 in ROCK. The corresponding residue in PKA (Gln84) is both neutral and larger, thus conferring desirable potency, selectivity and solubility properties[17,18]Therefore, we synthesized compounds containing solubilizing groups bearing the beneficial 4 - and 5-fluorine substituted 7-azaindoles with the aim of further enhancing cellular potency and PKA selectivity. The results are shown in Table 3. The 4- and 5-fluorine

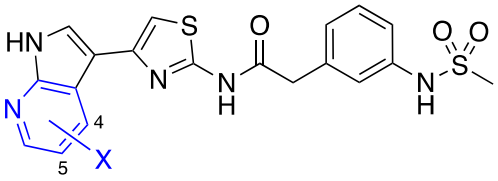
Table 1
ROCK inhibition and PKA selectivity data for ring A replacements.



Compound	A	ROCK I K_i (μM)[a]	PKA Selectivity	THP-1 migration	CYP 3A4	LLE[b]
			PKA K_i /ROCK I K_i	IC_{50} (μM)	IC_{50} (μM)	(ROCK I)
1		0.006	11	0.02	8.3	6
3		0.01	31	0.37	12	6.1
4		0.008	39	0.34	6	5.8
5		0.1	1	ND	5	4.7
6		0.007	34	1.52	100	7
7		0.048	23	8	48	5.9
8		0.007	20	0.82	100	6.7
9		0.052	31	ND	5	4.6

[a] Minimum Significant Ratio (MSR)19 = 3.6; i.e., compounds that have a difference in K_i of at least a factor of 3.6 are considered significantly different[19]
[b] LLE: Lipophilic ligand efficiency (LLE = pKi - clogP)[20]

Table 2
ROCK inhibition and PKA selectivity data for substituted 7-azaindoles.



Compound	X	ROCK I K_i (μM)	PKA Selectivity PKA K_i /ROCK I K_i	THP-1 migration IC_{50} (μM)	CYP 3A4 IC_{50} (μM)	LLE (ROCK I)
1	H	0.006	11	0.02	8	6
10	4-F	0.004	31	0.08	36	6.3
11	5-F	0.003	53	0.07	6	6.3
12	5-OH	0.007	107	1.3	5	6.4
13	4-Cl	0.092	2	ND	8	4.4
14	5-Cl	0.011	46	0.31	8	5.3
15	5-Br	0.022	32	10	35	4.8

substituted 7-azaindoles containing 2 and 3 carbon linked *N*-methyl piperazine (**19, 16, 17**) and three-carbon linked piperidine (**21** and **22**) showed >140-fold PKA selectivity. Compound **22** showed the highest PKA selectivity (>480-fold) while maintaining excellent ROCK potency. However, these new analogs displayed reduced cellular potency compared with respect to unsubstituted 7-azaindoles (*cf* **2**, **18** and **20**).

The metabolic stability of selected compounds was next evaluated in human and rat hepatocytes (Table 4). Generally, these compounds displayed moderate stability in rat hepatocyte and good stability in human hepatocytes. In addition, these compounds show no inhibition of the hERG channel (all are >30 μM).

In vivo rat PK profiling of select compounds is summarized in Table 5. All compounds bearing a basic solubilizing chain showed high plasma clearance (>65), high volumes of distribution (>10) and longer $T_{1/2}$ (>3 h) except compound **2**. Compounds **1** and **18** were also evaluated by oral dosing and showed good oral bioavailability.

Table 4
In vitro hepatic stability and hERG profiles for selected compounds.

Compound	**1**	**2**	**11**	**18**	**21**	**22**
Rat Cl_{int} (μL/min/10^6 cells)	6.7	26.8	11.7	32	11.2	20.5
Human Cl_{int} (μL/min/10^6 cells)	4	7.5	4.3	3.3	5.7	6.3
hERG IC_{50} (μM)	>30	>30	>30	>30	>30	>30

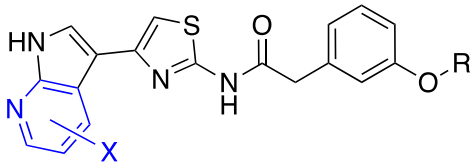
Table 5
Rat IV (1 mpk) and PO (3 mpk) PK profiles of selected compounds.

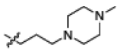
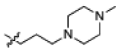
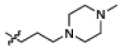
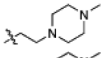
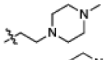
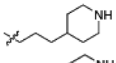
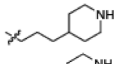
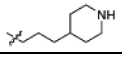
Compound	**1**	**2**	**18**	**20**	**21**	**22**
Rat IV PK (1 mg/kg)						
CL (mL/min/kg)	7	114	71	128	68	66
$T_{1/2}$ (hr)	2	0.71	3.2	5.4	5.5	3.8
V_{ss} (L/kg)	0.6	10	14	24	19	8.7
$AUC_{0\text{-}inf}$ (μg.h/mL)	2.4	0.15	0.24	0.13	0.25	0.25
Rat PO PK (3 mg/kg)						
$AUC_{0\text{-}inf}$ (μg.h/mL)	9.2		0.6			
C_{max} (μg/mL)	0.9		0.06			
%F	38		46			

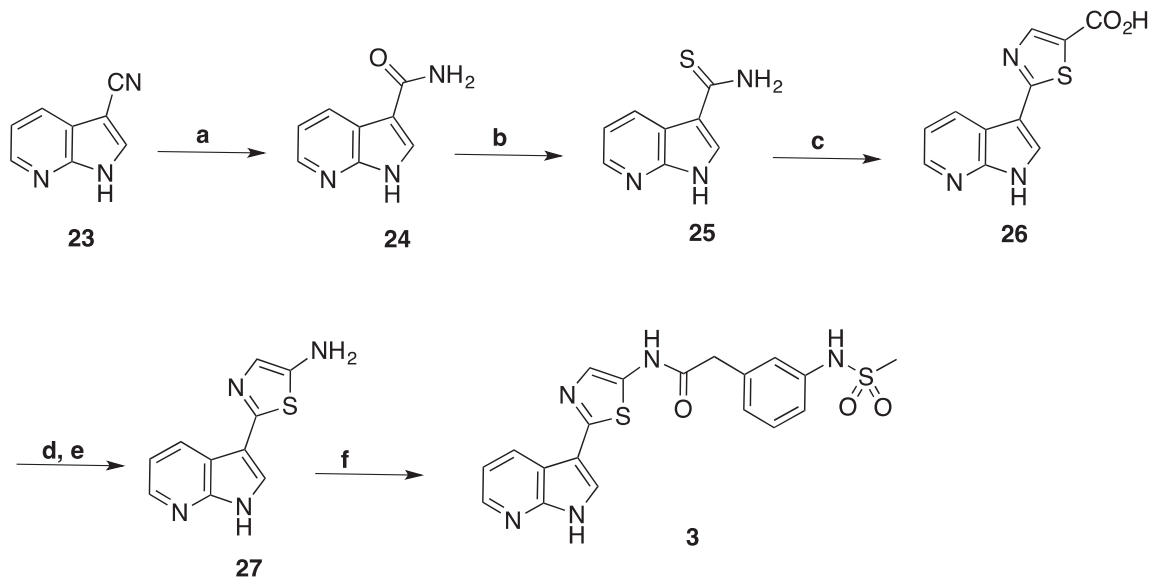
Chemistry

The syntheses of the described inhibitors are illustrated in Schemes 1–5. Regioisomeric thiazole **3** was synthesized in six steps as illustrated in Scheme 1. 3-Cyano-7-azaindole **23** was first converted to carboxamide **24** by treatment with concentrated sulfuric acid. Reaction of **24** with Lawesson's reagent afforded thiocarboxamide **25** which was converted to thiazolocarboxylic acid **26** by heating with bromopyruvic acid in ethanol. Curtius rearrangement of **26** with DPPA and *t*-BuOH and followed by removed of Boc group with TFA afforded aminothiazole **27**. The coupling of **27** with *m*-methylsulfonamidophenylacetic acid in the presence of $BtSO_2CH_3$[21] afforded desired target compound **3**.

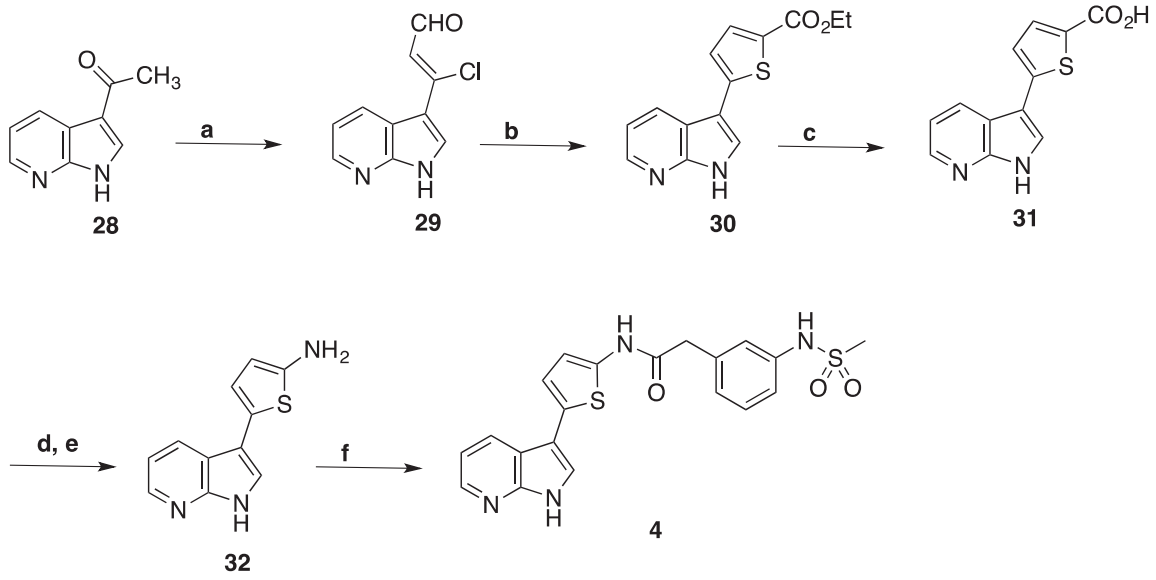
Table 3
Selected data for 4- and 5-fluoro-7-azaindoles with solubilizing groups.



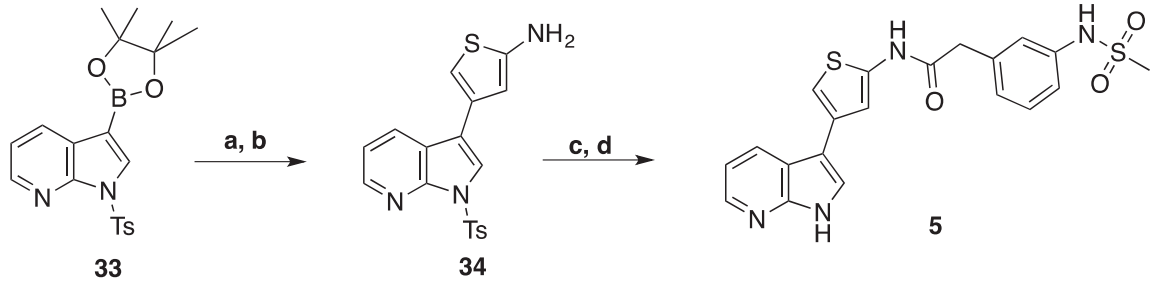
Compound	R	X	ROCK I K_i (μM)	PKA Selectivity PKA K_i /ROCK I K_i	THP-1 migration IC_{50} (μM)	CYP 3A4 IC_{50} (μM)	LLE (ROCK I)
2		H	0.006	293	0.02	19	4.9
16		4-F	0.01	180	0.4	ND	4.4
17		5-F	0.005	240	0.26	ND	4.7
18		H	0.006	50	0.02	18	4.9
19		5-F	0.003	320	0.4	27	5
20		H	0.005	17	0.02	21	4.4
21		4-F	0.003	143	0.05	100	4.7
22		5-F	0.001	480	0.14	ND	4.9



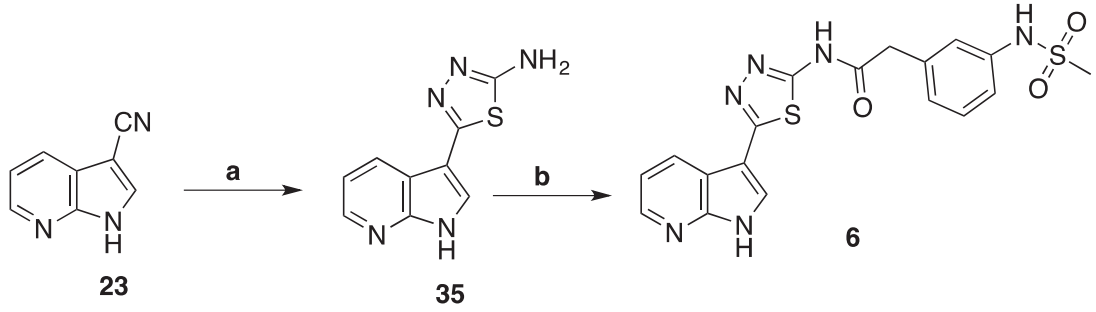
Scheme 1. Synthesis of 3-thiazolo-7-azaindole **3**. Reagents and conditions: (a) c.H_2SO_4, RT, 18 h; (b) Lawesson's reagent, THF, 70 °C; (c) bromopyruvic acid, EtOH, reflux, 2 h; (d) DPPA, *t*-BuOH, DIPEA, 90 °C, 2 h; (e) TFA, DCM, RT, 3 h; (f) *m*-methylsulfonamidophenylacetic acid, $BtSO_2CH_3$, Et_3N, THF, DMF, 180 °C, μW, 15 min.



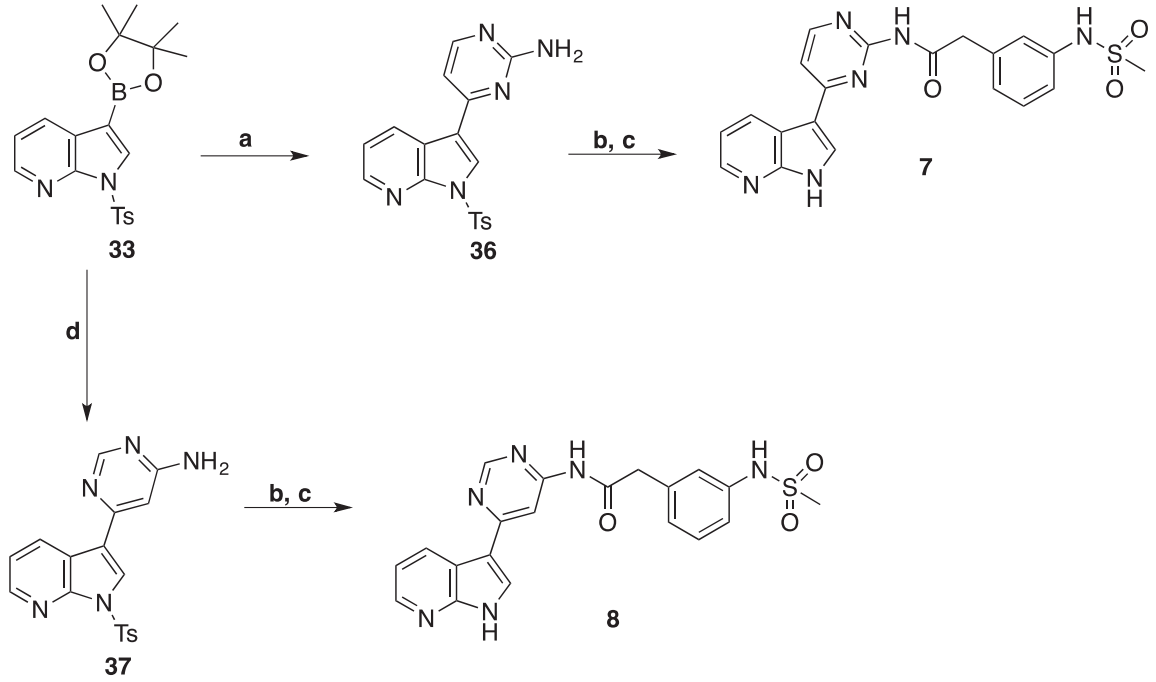
Scheme 2. Synthesis of thiopheno-7-azaindole **4**. Reagents and conditions: (a) DMF, $POCl_3$, 60 °C, 4 h; (b) ethyl 2-mercaptoacetate, NaOEt, EtOH, reflux, 90 min; (c) 1 M NaOH, dioxane, 80 °C, 90 min; (d) DPPA, *t*-BuOH, DIPEA, 90 °C, 2 h; (e) TFA, DCM, RT, 3 h (f) *m*-methylsulfonamidophenylacetic acid, $BtSO_2CH_3$, Et_3N, THF, DMF, 180 °C, μW, 15 min.



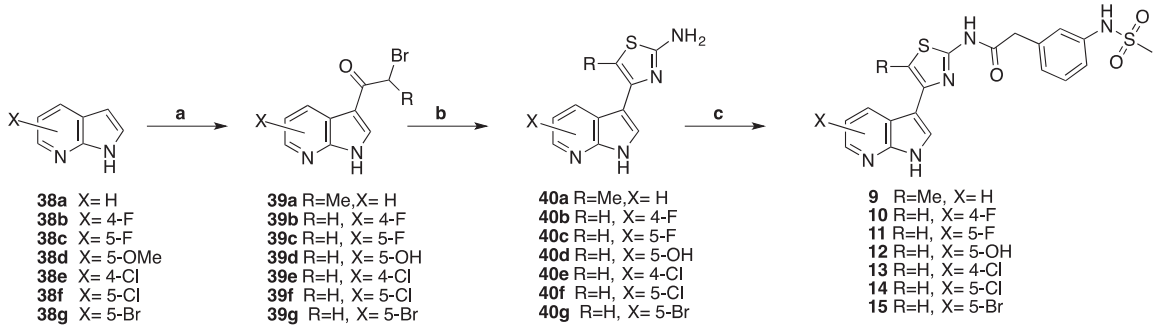
Scheme 3. Synthesis of thiophene-7-azaindole **5**. Reagents and conditions: (a) *t*-butyl-4-bromothiophen-2-ylcarbamate, K_2CO_3, $Pd(PPh_3)_4$, K_2CO_3, DME, 160 °C, μW, 30 min; (b) TFA, DCM, RT, 1 h; (c) *m*-methylsulfonamidophenylacetic acid, $BtSO_2CH_3$, Et_3N, THF, DMF, 180 °C, μW, 15 min; (d) LiOH, THF, water.



Scheme 4. Synthesis of thiadiazolo-7-azindole **6**. Reagents and conditions: (a) thiosemicarbazide, TFA, 100 °C, 2 h; (b) *m*-methylsulfonamidophenylacetic acid, $BtSO_2CH_3$, Et_3N, THF, DMF, 180 °C, μW, 15 min.



Scheme 5. Synthesis of 3-pyrimidine-7-azaindoles **7** and **8**. Reagents and conditions: (a) 4-bromopyrimidine 2-amine, $Pd(dppf)_2.Cl_2$. DCM, dioxane; (b) *m*-methylsulfonamidophenylacetic acid, $BtSO_2CH_3$, Et_3N, THF, DMF, 180 °C, μW, 15 min; (c) 4 N HCl, dioxane, CH_3CN, 80 °C, 3 h; (d) 6-bromopyrimidine-4-amine, $Pd(dppf)_2.Cl_2$. DCM, dioxane.



Scheme 6. Synthesis of substituted 3-thiazolo-7-azaindoles **9**–**15**. Reagents and conditions: (a) bromoacetyl bromides, $AlCl_3$, CH_2Cl_2, 50 °C, 1 h; (b) thiourea, ethanol, 70 °C, 1 h; (c) *m*-methylsulfonamidophenylacetic acid, $BtSO_2CH_3$, Et_3N, THF, DMF, 180 °C, μW, 15 min.

Compound **4** was synthesized in six steps as outlined in Scheme 2. The reaction of 3-acetyl-7-azaindole **28** with $POCl_3$ afforded compound **29**, which was reacted with ethyl 2-mercaptoacetate and sodium ethoxide in EtOH to afford thiophene ethylester **30**. The hydrolysis of ester **30**, followed by Curtius rearrangement and removal of the Boc group afforded compound **32**. Coupling of **32** with *m*-methylsulfonamidophenylacetic acid in the presence of $MeSO_2Bt$ afforded target compound **4**.

Regioisomeric thiophene **5** was synthesized in four steps as outlined in Scheme 3. Suzuki coupling of boronate ester **33** with *tert*-butyl-4-bromothiophen-2-ylcarbamate afforded compound **34**. Coupling of **34** with *m*-methylsulfonamidophenylacetic acid in the presence of MeS-O_2Bt, followed by removal of the tosyl group afforded target compound **5**.

Thiadiazole **6** was synthesized in two steps as outlined in Scheme 4. 3-Cyano-7-azaindole, **23** was reacted with thiosemicarbazide in TFA to afford aminothiadiazole **35** which was coupled with *m*-methylsulfonamidophenylacetic acid to afford target compound **6**.

The syntheses of pyrimidine containing compounds **7** and **8** are outlined in Scheme 5. Suzuki coupling of boronate ester **33** with 4-bromopyrimidine-2-amine or 6-bromopyrimidine-4-amine afforded aminopyrimidines **36** or **37** respectively. Amide coupling, followed by removal of the tosyl group with HCl, afforded target compounds **7** and **8**.

3-Thiazolo-7-azaindoles **9**–**15** were prepared in three steps as shown in Scheme 6. Friedel-Crafts acylation of commercially available azaindoles **38a-g** with bromoacetyl bromide in DCM afforded 3-(2-bromoacetyl)-7-azaindole **39a-g** in good yields. Hantzsch thiazole synthesis of azaindoles **39a-g** and thiourea afforded aminothiazoles **40a-g**. Amidations of compounds **40a-g** with *m*-methylsulfonamidophenylacetic acid were performed as previously described to afford final products **9**–**15** in good yields.

Conclusion

We synthesized a series of 7-azaindole-based ROCK inhibitors and evaluated their biological activity. Many of these compounds exhibited excellent ROCK potency and good selectivity against the closely related AGC kinase, PKA. In addition, top compounds showed good microsomal and hepatocyte stability, lacked hERG inhibition and good overall CYP 3A4 inhibition profiles. *In vivo* PK in rats indicated that compounds with solubilizing chains are rapidly cleared from plasma and highly distributed, consistent with other kinase inhibitors bearing solubilizing groups. Replacement of the central thiazole or thiophene ring with other heterocycles did not enhance compound profiles. Azaindole substituents, most notably 4-F and 5-F, may offer an improved overall profile and warrant further investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127721.

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