Discovery of a Potent and Selective ROMK Inhibitor with Improved Pharmacokinetic Properties Based on an Octahydropyrazino[2,1-c][1,4]oxazine Scaffold

Yuping Zhu, Reynalda K. de Jesus, Haifeng Tang, Shawn P. Walsh, Jinlong Jiang, Xin Gu, Nardos Teumelsan, Aurash Shahripour, Barbara Pio, Fa-Xiang Ding, Sookhee Ha, Birgit T. Priest, Andrew M. Swensen, Magdalena Alonso-Galicia, John P. Felix, Richard M. Brochu, Timothy Bailey, Brande Thomas-Fowlkes, Xiaoyan Zhou, Lee-Yuh Pai, Caryn Hampton, Melba Hernandez, Karen Owens, Juliann Ehrhart, Sophie Roy, Gregory J. Kaczorowski, Lihu Yang, Emma R. Parmee, Kathleen Sullivan, Maria L. Garcia, Alexander Pasternak

PII: S0960-894X(16)31103-9
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.10.064
Reference: BMCL 24367

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 19 August 2016
Revised Date: 19 October 2016
Accepted Date: 21 October 2016

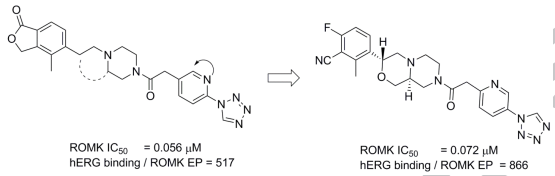
Please cite this article as: Zhu, Y., de Jesus, R.K., Tang, H., Walsh, S.P., Jiang, J., Gu, X., Teumelsan, N., Shahripour, A., Pio, B., Ding, F-X., Ha, S., Priest, B.T., Swensen, A.M., Alonso-Galicia, M., Felix, J.P., Brochu, R.M., Bailey, T., Thomas-Fowlkes, B., Zhou, X., Pai, L-Y., Hampton, C., Hernandez, M., Owens, K., Ehrhart, J., Roy, S., Kaczorowski, G.J., Yang, L., Parmee, E.R., Sullivan, K., Garcia, M.L., Pasternak, A., Discovery of a Potent and Selective ROMK Inhibitor with Improved Pharmacokinetic Properties Based on an Octahydropyrazino[2,1-c][1,4]oxazine Scaffold, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.10.064

Graphical Abstract

Discovery of a Potent and Selective ROMK Inhibitor Based on a Octahydropyrazino[2,1-c][1,4]oxazine Scaffold with Improved Pharmacokinetic Properties

Leave this area blank for abstract info.

Yuping Zhu, Reynalda K. de Jesus, Haifeng Tang, Shawn P. Walsh, Jinlong Jiang, Xin Gu, Nardos Teumelsan, Aurash Shahripour, Pio Barbara, Fa-Xiang Ding, Jessica Frie, Sookhee Ha, Birgit T. Priest, Andrew M. Swensen, Magdalena Alonso-Galicia, John P. Felix, Richard M. Brochu, Timothy Bailey, Brande Thomas-Fowlkes, Xiaoyan Zhou, Lee-Yuh Pai, Caryn Hampton, Melba Hernandez, Karen Owens, Juliann Ehrhart, Sophie Roy, Gregory J. Kaczorowski, Lihu Yang, Emma R. Parmee, Kathleen Sullivan, Maria L. Garcia, Alexander Pasternak



Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Discovery of a Potent and Selective ROMK Inhibitor with Improved Pharmacokinetic Properties Based on an Octahydropyrazino[2,1-c][1,4]oxazine Scaffold

Yuping Zhu[a,*], Reynalda K. de Jesus[a], Haifeng Tang[a], Shawn P. Walsh[a], Jinlong Jiang[a], Xin Gu[a], Nardos Teumelsan[a], Aurash Shahripour[a], Barbara Pio[a], Fa-Xiang Ding[a], Sookhee Ha[a], Birgit T. Priest[b], Andrew M. Swensen[b], Magdalena Alonso-Galicia[b], John P. Felix[b], Richard M. Brochu[b], Timothy Bailey[b], Brande Thomas-Fowlkes[b], Xiaoyan Zhou[c], Lee-Yuh Pai[c], Caryn Hampton[c], Melba Hernandez[c], Karen Owens[d], Juliann Ehrhart[e],Sophie Roy[c], Gregory J. Kaczorowski[b], Lihu Yang[a], Emma R. Parmee[a], Kathleen Sullivan[b], Maria L. Garcia[b], Alexander Pasternak[a]

[a] Discovery Chemistry, Merck Research Laboratories, Kenilworth, NJ 07033, United States
[b] Department of Pharmacology, Merck Research Laboratories, Kenilworth, NJ 07033, United States
[c] Department of Cardiometabolic Diseases, Merck Research Laboratories, Kenilworth, NJ 07033, United States
[d] Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck Research Laboratories, Kenilworth, NJ 07033, United States
[e] Department of Safety Assessment & Laboratory Animal Resources, Merck Research Laboratories, Kenilworth, NJ 07033, United States

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

riKeywords:
hERG
ROMK
Hypertension
Heart failure
Diuresis
Natriuresis

ABSTRACT

Following the discovery of small molecule acyl piperazine ROMK inhibitors, the acyl octahydropyrazino[2,1-c][1,4]oxazine series was identified. This series displays improved ROMK/hERG selectivity, and as a consequence, the resulting ROMK inhibitors do not evoke QTc prolongation in an *in vivo* cardiovascular dog model. Further efforts in this series led to the discovery of analogues with improved pharmacokinetic profiles. This new series also retained comparable ROMK potency compared to earlier leads.

Hypertension affects up to 25% of the adult population in industrialized countries, about one billion people world-wide, and the incidence increases with age. Despite the large number of medications available, blood pressure remains largely uncontrolled so that clinicians need to use combination therapies for achieving target blood pressure levels. Diuretics, such as hydrochlorothiazide (HCTZ), are widely prescribed as first line therapy to treat uncomplicated hypertension, or as add-on therapy.[1] No new diuretics are known to be in development despite the importance of these therapies.

The Renal Outer Medullary Potassium Channel (ROMK, Kir1.1) is a member of the family of inward rectifying potassium channels[2] that plays a critical role in regulating salt and water homeostasis. ROMK is mainly expressed on the apical membrane of epithelial cells lining two nephron segments: the thick ascending loop of Henle (TALH) and the cortical collecting duct (CCD).[3] At the TALH, ROMK participates in potassium recycling across the luminal membrane, providing potassium cations required for function of the furosemide-sensitive $Na^+/K^+/2Cl^-$ co-transporter, the rate-determining step for salt reuptake in this part of the nephron. At the CCD, ROMK plays a critical role in potassium homeostasis and provides a pathway for potassium secretion that is tightly coupled to sodium uptake through the amiloride-sensitive epithelial sodium channel.[4] Based on the dual site of action of ROMK and the potential for differential pharmacokinetic (PK) and physicochemical properties, we hoped that novel ROMK inhibitors would provide improved efficacy with reduced liabilities, such as hypokalemia, compared to the current standard-of-care diuretics. Human genetics, for example homozygote loss of function ROMK expression (Bartter's syndrome type II) and genetic ablation of ROMK in rodents support these expectations, and suggest that selective ROMK inhibitors will represent a new class of diuretic and antihypertensive agents.[5]

Small molecule inhibitors of ROMK were first described by Denton at Vanderbilt University.[6] In 2013, a novel ROMK inhibitor **1** (Fig. 1) with good ROMK functional potency and hERG selectivity was described by our group.[7] This effort resulted in the first pharmacological proof-of-biology, confirming in a rat diuresis model that small molecule ROMK inhibitors represent a new class of novel mechanism diuretics.[8] More recently, SAR efforts led to the discovery of a 4-*N*-tetrazole heteroaryl acetyl series with an improved ROMK/hERG ratio.[9] The best compound in this series, **2** (Fig. 1)**,** demonstrated a comparable diuretic effect to **1** in rats with no detectable QTc effects in an *in vivo* cardiovascular dog model[9]. Because of the correlation between QTc prolongation caused by hERG inhibition and torsade de pointes that could degenerate into ventricular fibrillation and cause sudden cardiac death, hERG liabilities need to be considered during drug development.[10] Despite the improved selectivity over the hERG channel, short half-lives and high clearance rates remained an issue in these series (Table 1). Since compounds with longer half-lives would be expected to have more sustained pharmacodynamics (PD) effects,[11] the focus of our effort was to improve preclinical PK profiles, while retaining or improving ROMK potency and selectivity over hERG. In this communication, we will describe SAR efforts to block metabolic soft spots and to introduce conformational constraints which led to improved ROMK inhibitor PK profiles and the identification of a novel morpholine-fused piperazine scaffold.

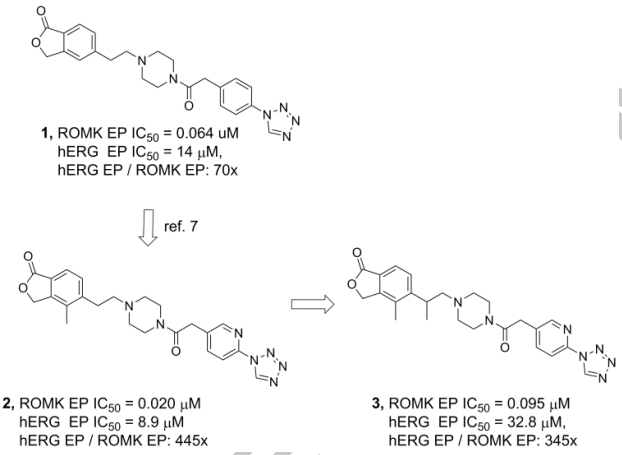


Figure 1

Discovery of 4-*N*-tetrazole heteroaryl acetyl ROMK inhibitor **2** with ROMK and hERG electrophysiology (EP) data[12]

For all compounds herein, ROMK activity was determined, as previously described, using one or more of three functional assays[12]: $^{86}Rb^{+}$ flux in CHO cells stably expressing ROMK, thallium flux in HEK-293 cells stably expressing ROMK, or electrophysiology (EP). Potency on the hERG channel was determined by measuring displacement of ^{35}-S MK499 binding from membranes prepared from HEK-293 cells stably expressing hERG.[13]

Table 1:
Sprague Dawley (SD) rat PK properties for selected ROMK inhibitors (0.5 mpk iv and 1 mpk po)

	Compound		
	1	**2**	**3**
Cl (L/Kg)	40	45	29
$AUCN_{PO}$ (μM h Kg/mg)	0.34	0.84	0.45
$t_{1/2}$ (h)	0.62	0.44	0.96
F_{po} (%)	33	38	28

As described in our previous work,[9] benzylic substitution with methyl on the left side of the piperazine (**3**) resulted in a compound with reduced clearance rate and slightly improved half-life, although with 5-fold lower ROMK potency as compared to **2** (Figure 1, Table 1). The reduced clearance rate suggested a possible metabolic soft spot centered on the benzylic position; hepatocyte stability studies on compound **1** do show left-side oxidative metabolites, although the site of oxidation was not definitively demonstrated. Our initial follow-up to **3** was to introduce steric hindrance at the benzylic methyl site to block possible metabolism[14] with the aim of maintaining/improving ROMK potency. Previous work indicated that reducing piperazine basicity, through incorporation of an amide linkage, led to attenuation of hERG channel potency.[7] We calculated the basicity of fusedbicyclic piperazine scaffolds incorporating an -*O*-, -*S*- and -*NH*- (Fig. 2) and found a predicted reduction of basicity with -*O*- or -*S*- containing bicycles and an increased basicity for a -*N*- containing bicycle. In addition to steric and electronic modulation, we hoped that incorporating these features together in ring systems would make the scaffold more rigid, and potentially less susceptible to oxidative metabolism.

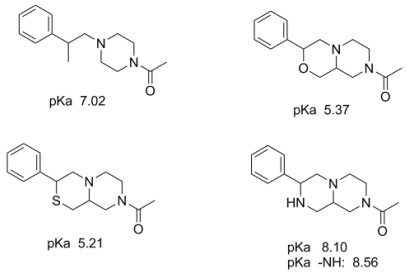
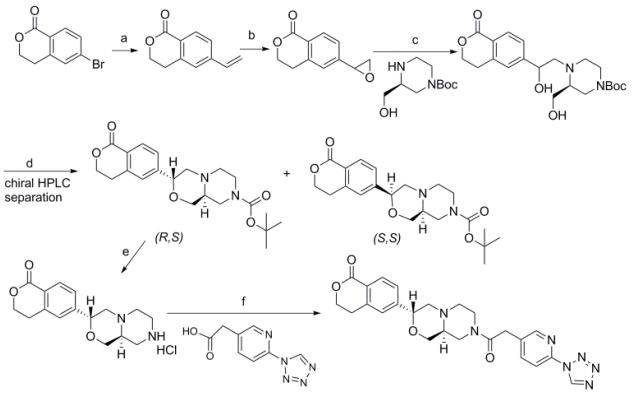


Figure 2

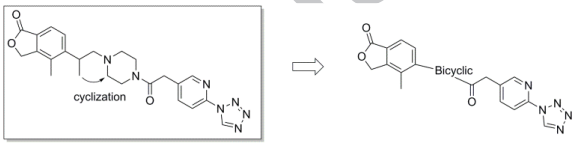
Calculated pKa of fused bicyclic surrogates

To test this hypothesis, several cyclized cores incorporating -*O*-, -*S*- and -*NH*- were prepared. A synthesis of a morpholine-fused bicyclic analog is illustrated in Scheme 1.



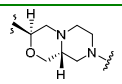
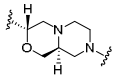
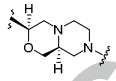
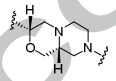
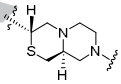
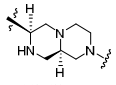
Scheme 1. Synthesis of morpholine-fused bicyclic analogs
Reagents and conditions: (a) Potassium vinyltrifloroborate, $PdCl_2$(dppf)-CH_2Cl_2, triethylamine (TEA), ethanol (EtOH), reflux, 10 hr, 100%; (b) mCPBA, CH_2Cl_2, 0 °C, 70% yield; (c) EtOH, 100 °C, MW, 40%; (d) Cyanomethylenetributyl phosphorane, Benzene, 100 °C, 18 hr; HPLC separation, ChiralPac® AD 4.6x250 mm, 25 °C, 19% (*R,S*), 7% (*S,S*) (e) 4 *N* HCl/Dioxane, 18 hr, 25 °C, 100% (f) EDC, HOBt, DCM, 25 °C, 3 hr, 63%.

Using the chemistry outlined in Scheme 1, or with some variations, a series of analogues were prepared to explore the effect of cyclized cores on ROMK potency, selectivity over hERG, and PK profiles. While most analogues containing the -*O*-cyclized cores had similar or better ROMK potency than the uncyclized compound **3**, compounds with the -*S*- and -*N*-containing cores resulted in loss of ROMK inhibitory activity (Table 2). Surprisingly, ROMK electrophysiology potency for the four diastereomers **4**-**7** was quite similar despite the fact that different orientations for the phthalide pharmacophore were expected. We speculate that the *cis*-isomers may readily flip to a morpholine boat conformation, resulting in a similar positioning of the phthalide pharmacophore. Compounds with reduced basicity (**4**-**7**) had similar or better selectivities over hERG (303-fold to 690-fold) than the uncyclized analog **3** (345-fold). A compound with increased basicity, **9**, had reduced selectivity (157-fold). The stronger hERG potency of **8** (IC_{50} 8.9 □M) led to diminished interest in the fused thiomorpholines. Data for **6** (642-fold) and **7** (870-fold) confirmed an improvement of ROMK selectivity over hERG compared to the uncyclized analog **3** (345-fold). The (*R,S*) isomer **5** had a similar selectivity (303-fold) compared to **3** (345-fold).



3 4-7

Table 2: ROMK inhibitors with fused bicyclic core

#	Bicycle =	ROMK* EP IC_{50} (μM)	hERG binding* IC_{50} (μM)	hERG binding / ROMK EP (fold)	hERG EP* IC_{50} (μM)	hERG EP/ ROMK EP (fold)
4	 *(S,R)*	0.105	53.4	508	NA	NA
5	 *(R,S)*	0.065	21.1	325	19.7	303
6	 *(S,S)*	0.088	26.8	305	56.5	642
7	 *(R,R)*	0.086	59.4	690	74.9	870
8	 *(R,S)*	0.100	8.9	89	NA	NA
9	 *(S,S)*	0.140	22.0	157	NA	NA

*Control compound used in all assays; STD < 20%

Compounds **5**-**7** were further evaluated in SD rats (0.5 mpk iv and 1 mpk po, Table 3) in order to determine their pharmacokinetic (PK) profiles. Compounds **5** and **7** possessed the most favorable overall features, namely good *in vitro*

potency, >100x *in vitro* selectivity over hERG, excellent oral bioavailability, with reduced clearance rates, and higher exposures than **3**.

Table 3: SD rat PK properties for selected ROMK inhibitors (0.5 mg/kg IV and 1.0 mg/kg PO)

	Compounds			
	3	**5**	**6**	**7**
Cl (L/Kg)	29	14	54	20
$AUCN_{po}$ (μM h Kg/mg)	0.45	2.05	0.32	2.02
$t_{1/2}$ (h)	0.96	1.0	0.67	0.80
F_{po} (%)	28	82	46	100

We next focused on modifying the left side of the mopholine containing bicyclic core of **5-7**, hoping to identify compounds with an extended half-life and equivalent or improved selectivity over hERG. We explored replacement of the phthalide with pharmacophores which had been successfully used in our previous work.[7] The compounds were prepared in a similar fashion as described in Scheme 1. Compounds **10** and **21** had improved ROMK selectivity over hERG (1,332-fold to 548-fold) compared to **5** (303-fold), (Table 4). Compounds **12**, and **16** were comparable to **5** (303-fold). Further PK st0.85udies (Table 5) of compounds with similar or better ROMK/hERG selectivity revealed that most displayed comparable or improved PK profiles to those of **5**-**7**. Compounds **16** and **21** had the longest half-lives (1.46 and 1.64 h, respectively) with good oral bioavailabilities (69% and 67%, respectively). SAR trends indicated that in the morpholine fused bicyclic series, 6-fluoro-2-methyl-benzonitrile (in **12** and **16**) and 2-methyl-benzonitrile (in **13**, **17** and **21**) were the best left side moieties with respect to improving half-life. However, the isochromanone pharmacophore (in **10**), provided the best ROMK / hERG selectivity.

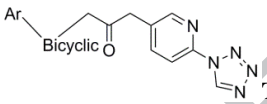
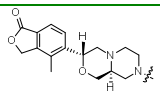
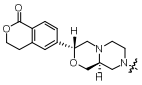
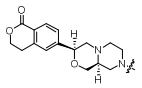
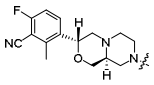
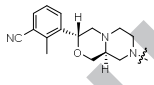
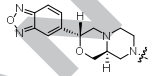
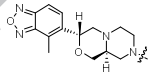
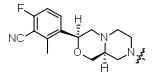
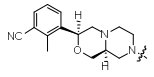
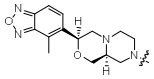
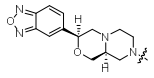
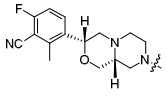
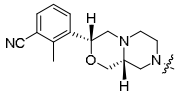


Table 4: SAR of left-side pharmacophores

=	Bicycle =	ROMK* EP IC_{50} (μM)	hERG EP* IC_{50} (μM)	hERG EP/ ROMK EP (fold)
5		0.065	19.7	303
10		0.057	75.9	1,332
11		0.205	NA	NA
12		0.036	~10	~278
13		0.050	NA	NA
14		0.045	>10	>222
15		0.072	>10	>139
16		0.041	12.5	305
17		0.112	17.6	157
18		0.083	~10	~120
19		0.104	NA	NA
20		0.060	>10	>167
21		0.027	14.8	548

*Control compound used in all assays; STD < 20%

Table 5
SD rat PK properties for selected ROMK inhibitors
(0.5 mg/kg IV and 1.0 mg/kg PO)

	Compound				
	10	**12**	**16**	**18**	**21**
Cl (L/Kg)	17	22	14	26	13
$AUCN_{po}$ (μM*h*kg/mg)	1.4	1.0	1.8	1.8	2.0
$t_{1/2}$ (h)	0.85	1.05	1.46	0.97	1.64
F_{po} (%)	65	57	69	100	67

Having optimized the left side of the scaffold, we next explored the right side. Small molecule inhibition of the hERG channel is often associated with π-stacking and hydrophobic interactions between inhibitors and aromatic residues in the hERG cavity.[15] Accordingly, we tried to change the position of the nitrogen within the the phenyl ring or incorporate an additional nitrogen to see if this would modulate hERG activity. We found that in the isochromanone series (Table 6), compound **10** had the best *in vitro* profile (potency and hERG selectivity). The PK profiles of analogs **23** and **24** had no obvious advantage over **10** (Table 7).

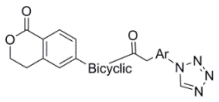
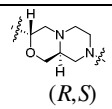
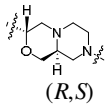
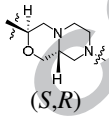
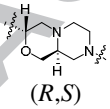


Table 6: SAR of isosteres

#	Bicyclic=	Ar =	ROMK* EP IC_{50} (μM)	hERG* EP IC_{50} (μM)	hERG EP/ROMK EP (fold)
10	 (*R*,*S*)		0.057	75.9	1,332
22	 (*R*,*S*)		0.080	>30	>375
23	 (*S*,*R*)		0.165	41.5	252
24	 (*R*,*S*)		0.260	>90	>346

*Control compoud used in all assays; STD < 20%

Table 7
SD rat PK properties selected ROMK inhibitors
(0.5 mg/kg IV and 1.0 mg/kg PO)

	Compounds		
	10	**23**	**24**
Cl (L/Kg)	17	15	49
$AUCN_{po}$ (μM h Kg/mg)	1.4	2.25	0.18
$t_{1/2}$ (h)	0.85	0.75	0.49
F_{po} (%)	65	98	12

In the 6-fluoro-2-methyl-benzonitrile series, the pyridine regioisomer of the (*R*,*S*) and (*S*,*S*) bicyclic series (analogs **25** and **28**), the pyrimidine analog (**27**), and the pyridazine analog (**30**) showed similar ROMK/hERG selectivity (**25** 278-fold, **28** 284-fold, **27** 303-fold, **30**, 392-fold, Table 8). The pyrimidine analog **29** had the best selectivity for ROMK.

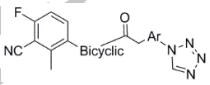
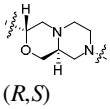
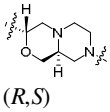
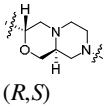
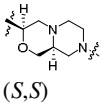
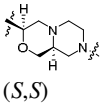
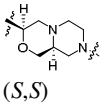
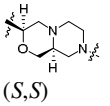


Table 8:SAR of isosteres

*Control compound used in all assays; STD < 20%

#	Bicyclic=	Ar =	ROMK* EP IC_{50} (μM)	hERG* EP IC_{50} (μM)	hERG EP/ ROMK EP (fold)
25	 (*R*,*S*)		0.073	20.3	278
26	 (*R*,*S*)		0.018	5.9	328
27	 (*R*,*S*)		0.033	~10	~303
28	 (*S*,*S*)		0.070	19.9	284
29	 (*S*,*S*)		0.039	25.2	646
30	 (*S*,*S*)		0.086	33.7	392
31	 (*S*,*S*)		0.107	12.3	115

Compounds with good ROMK potency, hERG EP (IC_{50} >10
Compounds with good ROMK potency, hERG EP (IC_{50} >10 μM)

and ROMK/hERG selectivity (>200) were further examined to determine their pK profiles in SD rats (Table 9). A general trend towards increased half-life was observed in the (*R*,*S*) bicyclic series when the nitrogen was positioned adjacent to the acetamide linker (**25**) or when the number of nitrogens in the ring was increased (**27**). Although compound **27** had a slightly better PK profile with a half-life in SD rats of 1.4 hr and good oral bioavailability, the potency in hERG EP (IC_{50} ~10 μM) made it less favorable than compound **25** (hERG EP IC_{50} 20.3 μM, ROMK/hERG selectivity 278-fold). An opposite effect was observed in the (*S,S*) bicyclic series (**16**, **28-29**). Although compound **29** had the largest selectivity ratio in the 6-fluoro-2-methyl-benzonitrile series, the relative shorter half-life made it less attractive than **25**.

Table 9SD or Wistar rat PK properties selected ROMK inhibitors

	Compounds					
	12	**25**	**27**	**16**	**28**	**29**
Dose iv/po (mg/kg)	0.5/1	1/2	0.5/1	0.5/1	0.5/1	1/2
Cl (L/Kg)	22	21	12.4	14	20.7	19.7
$AUCN_{po}$ (μM h Kg/mg)	1.0	0.79	4.64	1.80		1.01
$t_{1/2}$ (h)	1.05	1.5	1.4	1.46	0.96	1.36
F_{po} (%)	57	46	~100	69	68	54

Given its favorable rat PK profile, potent ROMK activity and good selectivity over hERG, compound **25** was selected for further evaluation in functional assays for other related inward rectifying potassium channels (Kir2.1, Kir4.1 and Kir7.1, all IC_{50} >100 μM) as well as the cardiac channels Nav1.5 and Cav1.2 (both with IC_{50} > 30 μM) and was found to have excellent selectivity for ROMK over other ion channels. Selectivity of **25** was further evaluated in a panel of 115 off-target enzyme and radioligand binding assays at Ricerca Biosciences, LLC. The sole finding with >50% inhibition at 10 μM was the somatostatin sub-type 2 receptor, with an IC_{50} of 4.1 μM (~60-fold *in vitro* selectivity for ROMK). Compound **25** was also evaluated for inhibition of a panel of CYP's (3A4, 2D6, 2C9) and found to have no significant inhibition at the concentrations tested (IC_{50}'s >50 μM). Microsome stability (% parent @ 45 min) for **25** was determined to be 85% in rat, 82% in dog, 72% in rhesus and 89% in human. Hepatocyte stability (%parent @ 90 min) for **25** was determined to be 92% in rhesus and 99% in human, indicating minimal in vitro metabolism. Plasma protein binding (% free fraction) for **25** was determined to be 48% in rat, 52% in dog, 60% in rhesus, and 58% in human plasma. Compound **25** had excellent permeability in LLC-PK1 cells (Papp 28). Inhibitor **25** was selected for further PK profiling in two additional species (dog and rhesus monkey; Table 10). The compound had PK profiles across preclinical species consistent with oral QD dosing. In addition, the human half-life projection of **25** based on observed preclinical PK in rat, dog and rhesus monkeys, (by applying allometric scaling) was estimated to be 24 hr (with some uncertainty given the significantly longer dog half-life, despite similar metabolic profiles between species). This is a significantly longer projected half-life than the most commonly used loop diuretics, furosemide (human half-life = 0.5-2 hr) and torsemide (human half-life = 3 hr).[16] The enhanced half-life may provide a PK-PD advantage with regard to the peak diuretic effects associated with loop diuretics, by providing a reduction in peak-to-trough exposures.

Table 10
PK properties of **25** in dog and rhesus monkey

Species	Dose IV (PO)	Cl (L/Kg)	$AUCN_{po}$ (μM h Kg/mg)	$t_{1/2}$ (h)	F_{po} (%)
Dog (Beagle)	1 (2)	2.5	16.7	17.3	100
Rhesus	1 (2)	4.0	6.0	5.0	67

To assess its pharmacodynamic effects, **25** was dosed orally QD for 3 days in spontaneously hypertensive rats (SHR) at 3, 10 and 30 mg/kg (Table 11).[17] Compound **25** demonstrated better efficacy than HCTZ for 24 hour diuresis at day 1, and a comparable blood pressure lowering at day 3 at all three doses.

Table 11
Diuresis and blood pressure lowering of **25** in SHR

Compound (Dose, mg/kg)	**25** (3)	**25** (10)	**25** (30)	**HCTZ** (25)
Diuresis fold-increase*24 hr	1.4	1.6	1.8	1.05
δSBP (mmHg)* (day 3)	-9	-16	-16	-12

*Compared to vehicle; n = 5 per group, on day 3 for blood pressure lowering and day 1 for diuresis

To determine how the *in vitro* hERG selectivity translated *in vivo*, compound **25** was further evaluated in an anesthetized cardiovascular guinea pig (GP) model (n = 3).[18] No significant change in the QTc interval was observed following IV infusion of **25**, with a maximal average peak unbound plasma concentration of 83 μM.

In conclusion, following the discovery of compound **3** and the initial preclinical validation of small molecule ROMK inhibitors as a novel diuretic agents in rats, we set out to discover new ROMK inhibitors with comparable ROMK potency, low risk for QTc effects, and improved pharmacokinetic properties. The most promising candidate to emerge from this work, **25**, had significantly improved half-life in three preclinical species, with comparable ROMK potency and an improved ROMK/hERG selectivity ratio over previously reported compound **3**. *In vivo* evaluation of **25** demonstrated a blood pressure lowering effect comparable to HCTZ at a lower dose (10 mpk vs. 25 mpk of HCTZ) and no detectable QTc effects when evaluated in an *in vivo* cardiovascular GP model. Future development of this series of ROMK inhibitors will be the subject of subsequent publications.

References and notes

1. Tamargo, J.; Segura, J.; Ruilope, L. M. Expert Opin. Pharmacother. **2014**, *15(4)*, 527.
2. (a) Ho, K.; Nichols, C. G.; Lederer, W. J.; Lytton, J.; Vassilev, P. M.; Kanazirska, M. V.; Hebert, S. C. *Nature* **1993**, *362*, 31; (b) Shuck, M. E.; Bock, J. H.; Benjamin, C. W.; Tsai, T. D.; Lee, K. S.; Slightom, J. L.; Bienkowski, M. J. *J. Biol. Chem.* **1994**, *269*, 24261.
3. (a) Xu, J. Z., Hall, A. E., Peterson, L. N., Bienkowski, M. J., Eessalu, T. E., Hebert, S. C. *Am. J. Physiol.* **1997**, *273*, F739-F748; (b) A., Wade, J. B., Ecelbarger, C. A., Palmer, L. G., and Frindt, G. *J. Am. Soc. Nephrol.* **1997**, *8*, 1823-1830. (c) Kohda, Y., Ding, W., Phan, E., Housini, I., Wang, J., Star, R. A.; Huang, C. L. *Kidney Int.* **1998**, *54*, 1214; (d) Hebert, S. C.; Desir, G.; Giebisch, G.; Wang, W. *Physiol. Rev.* **2005**, *85*, 319.
4. (a) Reinalter, S. C.; Jeck, N.; Peters, M.; Seyberth, H. W. *Acta Physiol. Scand.* **2004**, *181*, 513; (b) Wang, W. *Curr. Opin. Nephrol. Hypertens.* **2004**, *13*, 549. (c)Ji, W.; Foo, J. N.; O'Roak, B. J.; Zhao, H.; Larson, M. G.; Simon, D. B.; Newton-Cheh, C.; State, M. W.; Levy, D.; Lifton, R. P. *Nat. Genet.* **2008**, *40*, 592.
5. (a) Hebert SC. Bartter syndrome. *Curr Opin Nephrol Hypertens* **2003**;*12*:527-32. (b) Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, et al. *Nat. Genet*. **2008**;*40(5)*:592-9.
6. (a) Lewis, L. M.; Bhave, G.; Chauder, B. A.; Banerjee, S.; Lornsen, K. A.; Redha, R.; Fallen, K.; Lindsley, C. W.; Weaver, C. D.; Denton, J. S. *Mol. Pharmacol.* **2009**,*1094*, 76; (b) Bhave, G.; Chauder, B. A.; Liu, W.; Dawson, E. S.; Kadakia, R.; Nguyen, T. T.; Lewis, L. M.; Meiler, J.; Weaver, D. D.; Satlin, L. M.; Lindsley, C. W.; Denton, J. S. *Mol. Pharmacol.* **2011**, *79*, 42.
7. Tang, H.; de Jesus, R. K.; Walsh, S. P.; Zhu, Y.; Yan, Y. Priest, B. T.; Swensen, A. M.; Alonso-Galicia, M.; Felix, J. P.; Brochu, R. M.; Bailey, T.; Thomas-Fowlkes, B.; Zhou, X.; Pai, L.-Y.; Hampton, C.; Hernandez, M.; Owens, K.; Roy, S.; Kaczorowski, G. J.; Yang, L.; Garcia, M. L.; Pasternak, A. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5829.
8. Garcia, M. L.; Priest, B. T.; Alonso-Galicia, M.; Zhou, X.; Felix, J. P.; Brochu, R. M.; Bailey, T.; Thomas-Fowlkes, B.; Liu, J.; Swensen, A.; Pai. L.-Y.; Xiao, J.; Hernandez, M.; Hoagland, K.; Owens, K.; Tang, H.; de Jesus, R. K.; Roy, S.; Kaczorowski, G. J.; Pasternak, A. *J Pharmacol Exp Ther.* **2014**; *348*, 153-164.
9. Walsh, S. P.; Shahripour, A.; Tang, H.; de Jesus, R. K.; Teumelsan, N.; Zhu, Y.; Jessica Frie; Priest, B. T.; Swensen, A.; Alonso-Galicia, A.; Felix, J.P.; Brochu, R. M.; Bailey, T.; Thomas-Fowlkes, B.; Zhou, X.; Pai, L.; Hampton, C.; Hernandez, m.; Owens, K.; Ehrhart, J. Roy, S.; Kaczorowski, G.; Yang, L.; Gacia, M.; Pasternak, A. . *Bioorg. Med. Chem. Lett. Submitted.*
10. (a) Abott, G. W.; Sesti, F.; Splawski, I.; Buck, M. E.; Lehmann, M. H.; Timothy, K. W.; Keating, M. T.; Goldstein, S. A. *Cell* **1999**, *97*, 175-187. (b) Fermini, B.; Fossa, A. A. *Nat. Rev. Drug Discovery* **2003**, *2*, 439-447. (c) Keating, M. T. Sanguinetti, M. C. *Cell* **2001**, *104*, 569-580. (d) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *49*, 5029-5046.
11. Singh SS, *Curr. Drug. Metab*. **2006**, 7 (2), 165-82.
12. Felix, J. P.; Priest, B. T.; Solly, S.; Bailey, T.; Brochu, R. M.; Liu, C. J.; Kohler, M. G.; Kiss, L.; Alonso-Galicia, M.; Tang, H.; Pasternak, A.; Kaczorowski, G. J.; Garcia, M. L. *Assay Drug Dev. Technol*. **2012**, *10*, 417.
13. Wang, J.; Della Penna, K.; Wang, H.; Karczewski, J.; Connolly, T. M.; Koblan, K. S.; Bennett, P. B.; Salata, J. J. *Am. J. Phys.* **2003**, *284*, 256.
14. D.C. Evans, T.A. Baille, *Curr. Oppin. Drug Disc. Develop.* **2005**, *8*, 44-50
15. Jamieson, C.; Moir, E.M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *49*, 5029-5046.
16. Vadivelan, M.; Dabhi, A. *Indian Journal of Clinical Practice*, **2013**, *24 (4)*, 385-388.
17. For details on the SHR model please see reference 8
18. (a) Terrar DA, Wilson CM, Graham SG, Bryant SM, Heath BM, *J Pharmacol Toxicol Methods.* **2007**, *56(2)*,171-85. (b) Testai L, Calderone V, Salvadori A, Breschi MC, Nieri P, Martinotti E**.** *J Appl Toxicol.* **2004,** *24(3)*, 217-22.