ACS Medicinal Chemistry Letters

Letter

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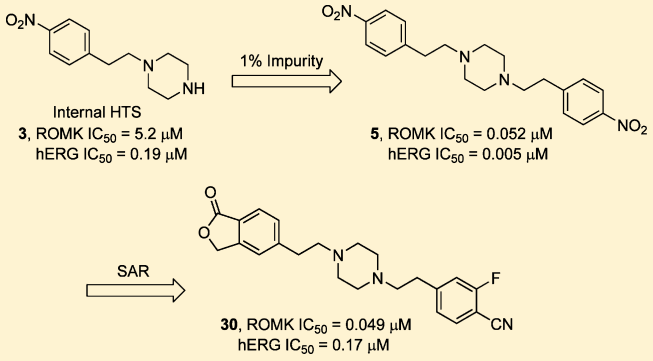
Discovery of Selective Small Molecule ROMK Inhibitors as Potential New Mechanism Diuretics

Haifeng Tang,*[,†] Shawn P. Walsh,[†] Yan Yan,[†] Reynalda K. de Jesus,[†] Aurash Shahripour,[†] Nardos Teumelsan,[†] Yuping Zhu,[†] Sookhee Ha,[¶] Karen A. Owens,[⊥] Brande S. Thomas-Fowlkes,[§] John P. Felix,[§] Jessica Liu,[§] Martin Kohler,[§] Birgit T. Priest,[§] Timothy Bailey,[§] Richard Brochu,[§] Magdalena Alonso-Galicia,[‡] Gregory J. Kaczorowski,[§] Sophie Roy,[‡] Lihu Yang,[†] Sander G. Mills,[†] Maria L. Garcia,[§] and Alexander Pasternak[†]

Departments of [†]Medicinal Chemistry, [‡]Hypertension, [§]Ion Channels, [⊥]Preclinical DMPK, and [¶]Chemistry Modeling, Merck Research Laboratories, Rahway New Jersey 07065, United States

Supporting Information

ABSTRACT: The renal outer medullary potassium channel (ROMK or $K_{ir}1.1$) is a putative drug target for a novel class of diuretics that could be used for the treatment of hypertension and edematous states such as heart failure. An internal high-throughput screening campaign identified 1,4-bis(4-nitrophenethyl)piperazine (**5**) as a potent ROMK inhibitor. It is worth noting that this compound was identified as a minor impurity in a screening hit that was responsible for all of the initially observed ROMK activity. Structure−activity studies resulted in analogues with improved rat pharmacokinetic properties and selectivity over the hERG channel, providing tool compounds that can be used for in vivo pharmacological assessment. The featured ROMK inhibitors were also selective against other members of the inward rectifier family of potassium channels.



KEYWORDS: *hypertension, heart failure, ROMK, $K_{ir}1.1$, KCNJ1, diuretics, potassium channel, inward rectifier, K_{ir}, hERG, high-throughput screening (HTS)*

Hypertension or high blood pressure is a common chronic medical condition in which the systemic arterial blood pressure is elevated. According to a recent survey, hypertension affects about one-third of the adult population in the United States, and the incidence rate increases with age.[1] Persistent hypertension is a major risk factor for stroke, myocardial infarction, heart failure, and arterial aneurysm and is a leading cause of chronic kidney failure.[2] The incidence of these diseases can be substantially reduced by managing blood pressure, whether through change in life style or use of medication. Although a large number of drugs are available for the treatment of hypertension, approximately half of patients do not reach optimal blood pressure control, and clinicians often need to use combination therapies for achieving target blood pressure levels.[3] The market for antihypertensive agents is highly fragmented with diuretics being among the most prescribed classes of medications. Within the diuretic class, thiazides, such as hydrochlorothiazide (HCTZ), are the most widely prescribed for treating hypertension and can be used as first line therapy to treat uncomplicated hypertension or as add-on therapy. However, the use of this type of diuretic is associated with hypokalemia (serum potassium concentration <3.5 mEq/L) and elevations in fasting blood glucose,[4] which limit the doses used clinically to those that are suboptimal to achieve natriuresis and blood pressure lowering.[5] Currently, no new diuretics have been disclosed to be in development, but new diuretics with superior blood pressure efficacy to HCTZ and suitability for combination therapy may provide hypertensive patients with better alternatives to achieve target blood pressure levels.

The renal outer medullary potassium channel (ROMK, $K_{ir}1.1$, encoded by the *KCNJ1* gene)[6,7] is expressed in two regions of the kidney: the thick ascending loop of Henle (TALH) and the cortical collecting duct (CCD).[8] At the TALH, ROMK participates in potassium recycling across the luminal membrane, which is critical for the function of the furosemide-sensitive $Na^+/K^+/2Cl^-$ cotransporter, the rate-determining step for salt reuptake in this part of the nephron. At the CCD, ROMK provides a pathway for potassium secretion that is tightly coupled to sodium uptake through the amiloride-sensitive epithelial sodium channel.[9] The fact that ROMK is expressed in both the TALH and the CCD suggests

Received: January 5, 2012
Accepted: March 28, 2012
Published: March 28, 2012

that ROMK inhibitors may provide superior diuretic/natriuretic efficacy as compared to loop diuretics, which exert their effects in the TALH alone. Bartter's syndrome type II patients (*KCNJ1* homozygotes), who lack ROMK expression, have a phenotype characterized by renal salt wasting, hypotension, and mild hypokalemia.[10] Similar observations have also been reported from studies with the ROMK knock out mice.[11,12] In addition,

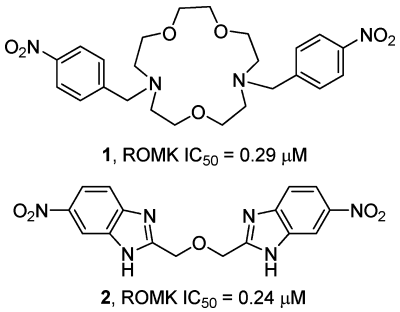


Figure 1. Vanderbilt ROMK inhibitors.

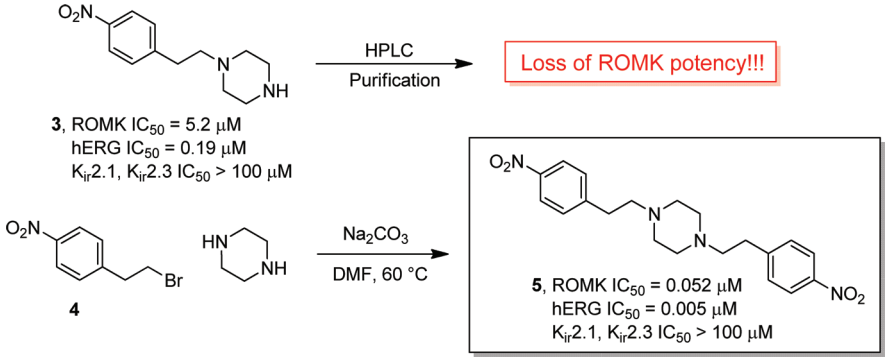
KCNJ1 heterozygote carriers from the Framingham heart study exhibit reduced blood pressure and a reduced risk of hypertension at age 60, when compared with matched controls.[13] Thus, selective ROMK inhibitors with increased efficacy and reduced liabilities over the currently used diuretics could represent a novel class of diuretics for the treatment of hypertension and edematous states such as heart failure.[14]

At the time this project was initiated, small molecule ROMK inhibitors had not been disclosed. More recently, scientists at Vanderbilt University reported a moderately potent small molecule ROMK inhibitor based on a 1,4,10-trioxa-7,13-diazacyclopentadecane scaffold (**1**, reported IC_{50} = 0.29 μM, Figure 1). Within the inward rectifier family of potassium channels, compound **1** is selective over $K_{ir}2.1$ and $K_{ir}4.1$ but inhibits the $K_{ir}7.1$ channel.[15] Further work by the Vanderbilt team led to the synthesis of **2**, which has similar potency to **1** but is selective over $K_{ir}7.1$.[16] To date, there are no reports of in vivo pharmacological assessment of ROMK using small molecule inhibitors.

To develop ROMK inhibitors as new mechanism diuretics, suitable tool compounds are needed to study ROMK pharmacology. High-throughput screening (HTS) of our internal sample collection (~1.5 M compounds) using a membrane potential-based fluorescent assay revealed a few small molecule hits.[17,18] Among them, compound **3** was selected for further evaluation (Scheme 1). This compound showed moderate inhibitory potency on ROMK, IC_{50} = 5.2 μM, in a functional $^{86}Rb^+$ efflux assay from CHO cells stably expressing the human ROMK channel,[19] but it is more potent on the cardiac voltage-gated potassium channel hERG.[20] Importantly, it demonstrated excellent selectivity against the related inward rectifier channel $K_{ir}2.1$ (IC_{50} > 100 μM) in a functional fluorescence assay that measures the ability of thallium to permeate through open $K_{ir}2.1$ channels stably expressed in HEK293 cells. $K_{ir}2.1$ is present in the heart ventricle, and inhibition of this channel could cause long QT syndrome.[21,22] In addition, compound **3** was also selective against the inward rectifier channel $K_{ir}2.3$ (IC_{50} > 100 μM) in a functional $^{86}Rb^+$ efflux assay from CHO cells stably expressing the human $K_{ir}2.3$ channel.[23] While HPLC and NMR analysis indicated good purity of the compound sample, ROMK inhibitory activity was lost when the sample was subjected to repurification by HPLC. Careful examination of the LC-MS features led to the identification of a minor sample impurity with a molecular weight of 384. On the basis of this molecular weight, it was speculated that the impurity could represent the symmetrical compound **5**. Thus, **5** was synthesized via double alkylation of piperazine with 1-(2-bromoethyl)-4-nitrobenzene (**4**) and evaluated in the ROMK $^{86}Rb^+$ efflux assay where it displayed good ROMK inhibitory activity (ROMK IC_{50} = 0.052 μM). However, this compound also displayed high potency on the hERG channel (hERG IC_{50} = 0.005 μM). Similar to hit **3**, compound **5** retained excellent selectivity over the $K_{ir}2.1$ and $K_{ir}2.3$ channels (IC_{50} > 100 μM).

With a potent small molecule ROMK lead in hand, exploratory chemistry was initiated to improve its selectivity over the hERG channel and to identify replacements for the nitro groups. Early SAR studies indicated that both nitro groups were critical for ROMK inhibitory activity, as deletion of one nitro group led to total loss of ROMK potency (**6**, Table 1). To our satisfaction, synthesis of a focused small library led to the identification of a few pharmacophores, which could be used to replace the nitro groups. For instance, replacing the nitrophenyl with a benzonitrile moiety led to compound **7** with similar ROMK potency and hERG selectivity, whereas the related 3-cyanophenyl analogue, **8**, is a less active ROMK inhibitor. The 5-benzo(2,1,3-oxadiazole) moiety was explored as a nitrophenyl isostere and led to compound **9** with slightly reduced ROMK activity and a similar selectivity profile against hERG. In contrast, the 2-oxazole analogue **10** was much less active on ROMK, and the 5-pyrimidine analogue **11** was inactive. Most interestingly, the nitrophenyl group can be replaced with a 4-phthalide group to afford compound **12**.[24] While maintaining

Scheme 1. Identification of the ROMK Lead



dx.doi.org/10.1021/ml3000066 | ACS Med. Chem. Lett. 2012, 3, 367−372

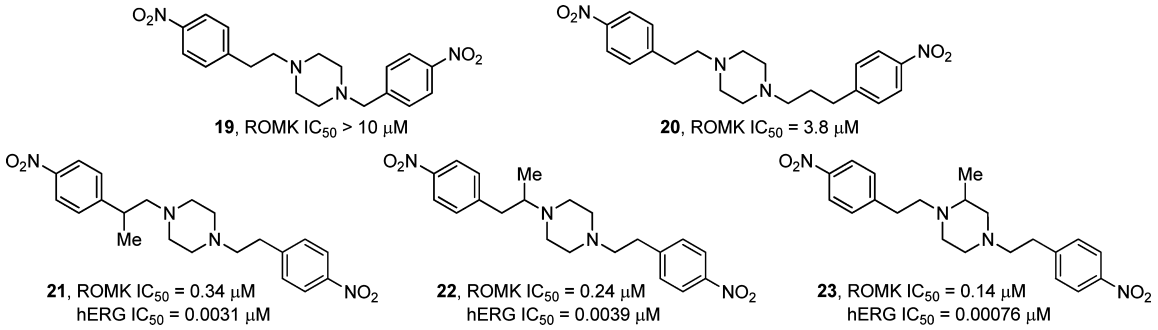


Figure 2. Modification of the core scaffold.[19]

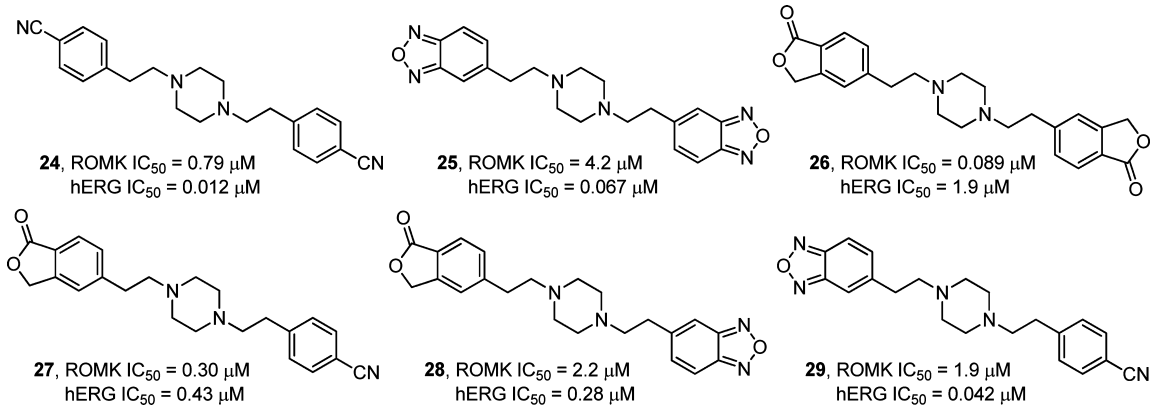
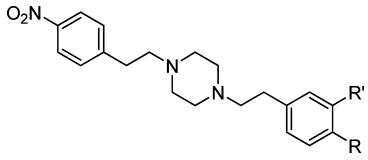


Figure 3. Second generation ROMK inhibitors.[19]

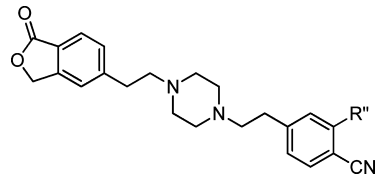
Table 1. Replacement of the Nitro Group[19]



compd	R, R′	IC$_{50}$ (μM) ROMK	IC$_{50}$ (μM) hERG
6	H, H	>10	ND[b]
7	CN, H	0.079	0.009
8	H, CN	3.8	0.022
9[a]	5-benzo(2,1,3-oxadiazole)	0.17	0.025
10	2-oxazole, H	4.3	0.047
11	5-pyrimidine, H	>10	ND
12[a]	4-phthalide	0.089	0.15
13	COOEt, H	2.9	0.019
14	F, H	3.1	0.023
15	Cl, H	>10	ND
16	CF_3, H	>10	ND
17	OMe, H	4.6	0.007
18	SO_2Me, H	9.4	0.15

[a]R, R′, and phenyl together represent the named fused bicyclic ring. [b]ND, no data.

Table 2. SAR on the Benzonitrile Ring[19]



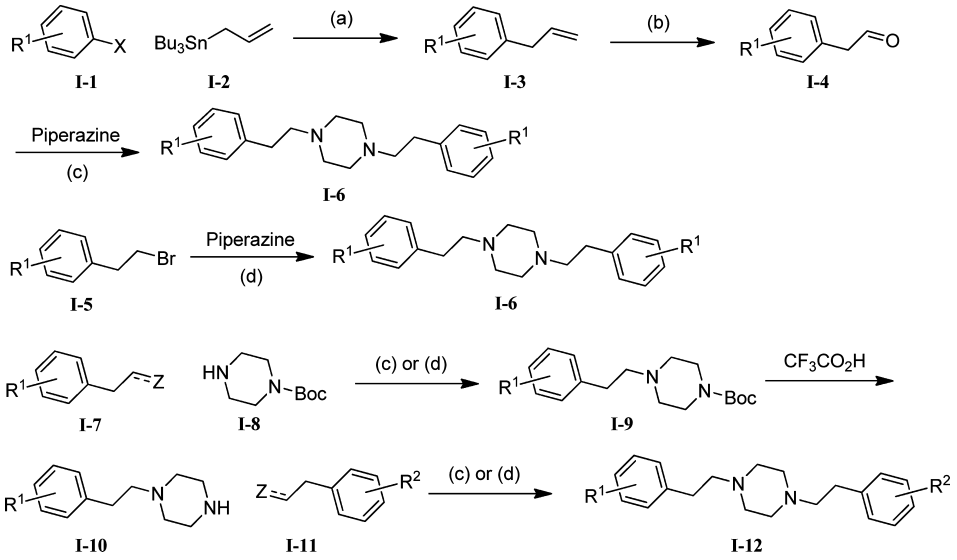
compd	R″	IC$_{50}$ (μM) ROMK	IC$_{50}$ (μM) hERG
27	H	0.30	0.43
30	F	0.049	0.17
31	Cl	0.075	0.12
32	Br	0.099	0.56
33	OMe	0.089	1.6
34	OEt	0.053	0.67
35	OCF_3	0.62	0.11
36	*O*-cyclopropyl	0.28	0.47
37	SMe	0.55	0.46
38	NHMe	0.43	0.67
39	SO_2Me	12	4.7

similar ROMK inhibitory potency to **5**, compound **12** is now slightly selective for ROMK over the hERG channel. In comparison, the acyclic ethyl ester analogue **13** is a weak ROMK inhibitor. Although fluoride (**14**) can serve as a weak pharmacophore, analogues with chloride (**15**) or trifluoromethyl (**16**) are much less active on ROMK. In addition, we also identified a few other weak pharmacophores, such as methoxy (**17**) and methylsulfone (**18**).

In a parallel effort, we attempted to modify the core scaffold of **5** and found that the distance between the nitro groups is critical for ROMK inhibitory activity (Figure 2). Shortening the distance by one carbon resulted in total loss of potency; similarly, lengthening the distance by one carbon also led to significant loss of potency (**19** and **20**). In addition, methyl substitution was introduced in the core skeleton, but all methyl-substituted analogues were less potent on ROMK (**21**–**23**).

dx.doi.org/10.1021/ml3000066 | ACS Med. Chem. Lett. 2012, 3, 367–372

Scheme 2[a]



[a]Synthesis: (a) $Pd(PPh_3)_4$, LiCl, toluene, 120 °C. (b) O_3, MeOH, −78 °C, Me_2S. (c) $NaCNBH_3$, MeOH, cat. HOAc. (d) Et_3N or Na_2CO_3, DMF, 60 °C.

Table 3. ROMK $^{86}Rb^+$ Efflux Assay vs ROMK EP Assay

	IC_{50} (μM)	
compd	ROMK Rb^+	ROMK EP
5	0.052	0.024
26	0.089	0.026
30	0.049	0.030

Having identified several nitrophenyl pharmacophore replacements, we next explored analogues in which both nitrophenyl groups were replaced (Figure 3). Interestingly, compound **24**, the bis-4-cyanophenyl analogue of **5**, represents a moderately potent ROMK inhibitor. The bis-5-benzo(2,1,3-oxadiazole) analogue **25** is also a less potent ROMK inhibitor. The most interesting analogue is the bis-4-phthalide, **26**, which displays similar ROMK potency to **5**, but it is about 20-fold selective over the hERG channel. These three pharmacophores were further hybridized. Among the hybrids, compound **27** was the most potent ROMK inhibitor.

Using **27** as a template, we explored the SAR of the phenyl ring, as a variety of substituted phenyl carbonitriles can be accessed from commercial sources. As shown in Table 2, halides can be tolerated *ortho* to the nitrile group. A decrease in ROMK inhibitory potency and hERG selectivity was observed with an increase in the size of the halides (**30**−**32**). Small alkoxy substituents, methoxy (**33**) and ethoxy (**34**), are also tolerated at the *ortho* position, while larger alkoxy (**35** and **36**) and methylthio ether (**37**) substitutions generally led to less active ROMK inhibitors. In comparison, a small decrease in ROMK potency was observed when the carbonitrile was substituted with methylamine (**38**) at the *ortho* position. Substitution with a larger hydrophilic group methylsulfone (**39**) led to significant loss of potency.

In addition to the ROMK $^{86}Rb^+$ efflux assay, a ROMK electrophysiology (EP) assay was also established.[25] A selected set of compounds was tested in the EP assay, further confirming the functional activity of this lead class (Table 3). These compounds were also evaluated on other members of the inward rectifier family of potassium channels. Just like compound **5**, compounds **26** and **30** are selective over $K_{ir}2.1$ and $K_{ir}2.3$. Furthermore, none of these compounds display any significant activity toward two other inward rectifier channels, $K_{ir}4.1$ and $K_{ir}7.1$,[26] supporting the selectivity of this lead class of ROMK inhibitors in the inward rectifier family of potassium channels.

In addition to enhancing the selectivity of ROMK inhibitors over hERG, we were able to significantly improve the preclinical pharmacokinetic (PK) properties of this lead class (Table 4). As compared to the original molecule lead **5**, which

Table 4. Rat PK Properties (1 mg/kg iv and 2 mg/kg po)

	compd	
	5	**30**
Cl (L/kg)	261	59
$AUCN_{po}$ (μM h kg/mg)	0.023	0.46
half-life (h)	1.9	1.5
F_{po} (%)	14	64

featured low oral exposure in rat due to high clearance and low oral bioavailability, compound **30** displays reduced clearance and acceptable oral exposure in rat with a half-life of 1.5 h. Because **30** retains good inhibitory potency against the rat ROMK channel (IC_{50} = 0.055 μM),[27] it can serve as a tool compound allowing the evaluation of small molecule ROMK inhibitors in in vivo rat models of hypertension.

The synthesis of the above molecules is relatively straightforward. The C−N bonds are generally formed via reductive amination of the aldehyde with piperazine or alkylation with the alkyl bromide where commercially available (Scheme 2). The aldehyde (**I-4**), in turn, was synthesized from the corresponding aryl halide (**I-1**, *X* = Br or I) via routine transformations, most commonly via palladium-catalyzed allylation, followed by ozonolysis and reductive workup with dimethylsulfide. If the target structure is symmetrical, it can be accessed in one single operation via a double reductive amination or double alkylation with piperazine from **I-5**. Otherwise, N-Boc piperazine (**I-8**) was used as starting material

dx.doi.org/10.1021/ml3000066 | ACS Med. Chem. Lett. 2012, 3, 367−372

to form the C−N bonds in a stepwise manner. The formation of the first C−N bond gave rise to (**I-9**), which was then treated with trifluoroacetic acid to remove the Boc group. The resulting amine (**I-10**) was subjected to a second C−N bond formation to yield the product (**I-12**).

In conclusion, we have discovered a class of selective small molecule ROMK inhibitors starting from a high-throughput screen of our internal sample collection. The program lead **5** was identified after isolation of a minor impurity from a screening hit. Compound **5** was selective over other members of the inward rectifier family of potassium channels but was about 10-fold more potent on the hERG channel. Medicinal chemistry efforts led to the identification of a number of pharmacophores more suitable for drug development as compared with the original nitrophenyl groups. Further SAR also enabled us to improve the selectivity of this class of ROMK inhibitors over the hERG channel as well as the preclinical PK properties. To that end, compound **30** can serve as a practical tool compound in animal models of diuresis and hypertension. Future work will be centered in further enhancing the selectivity over the hERG channel and in determining the in vivo effects of selective ROMK inhibitors in preclinical animal models.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of key compounds and the $^{86}Rb^+$ assay protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 732-594-2932. E-mail: haifeng_tang@merck.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Vincent Tong for high-resolution mass spectroscopy analysis of the key compounds.

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(18) IC_{50} values were determined from 10-point concentration−response curves where each point was run in triplicate.

(19) Assays were usually run at least twice, and the results were generally within 20% of each other. Please see the Supporting Information for the $^{86}Rb^+$ efflux assay protocol.

(20) All compounds were tested in the ^{35}S-MK499 binding assay to gauge their ability to inhibit the hERG channel. For a reference on the assay protocol and correlation between the MK499 binding assay and the hERG assay, please see Schmalhofer, W. A.; Swensen, A. M.; Thomas, B. S.; Felix, J. P.; Haedo, R. J.; Solly, K.; Kiss, L.; Kaczorowski, G. J.; Garcia, M. L. A Pharmacologically Validated, High-Capacity, Functional Thallium Flux Assay for the Human Ether-a-go-go Related Gene Potassium Channel. *Assay Drug Dev. Technol.* **2010**, *8* (6), 715−726.

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(23) $K_{ir}2.3$ is expressed at the basolateral membrane of the principal cells at the CCD.

(24) This pharmacophore was initially discovered in a related ROMK inhibitor series that will be described in due course.
(25) The assay protocol will be published in a separate communication later.
(26) The $K_{ir}4.1$ and $K_{ir}7.1$ assays are functional fluorescence assays that measure the ability of thallium to permeate through open $K_{ir}4.1$ and $K_{ir}7.1$ channels stably expressed in HEK293 cells, respectively. The three compounds showed less than 50% inhibition at concentrations up to 100 μM.
(27) This lead class showed very similar potency for human and rat ROMK channels. Potency against rat ROMK channel is measured by a functional fluorescence assay that measures the ability of thallium to permeate through open Rat ROMK channels stably expressed in HEK293 cells.