**Inhibitors of the renal outer medullary potassium channel: a patent review.**


## Abstract

***Introduction:*** Hypertension represents a substantial cardiovascular risk factor. Among anti-hypertensive drugs, diuretics play an important role. Nevertheless, they present adverse effects like hypokalemia or hyperkalemia. In this panorama, inhibitors of the renal outer medullary potassium (ROMK) channels are emerging because they are predicted to give a diuretic/natriuretic activity higher than that provided by loop diuretics, without hypokaliemic and hyperkaliemic side effects.

***Areas covered:*** This article reviews the current literature, including all the patents published in the field of inhibitors of the ROMK channels for the treatment of hypertension, heart failure and correlated diseases. The patent examination has been carried out using electronic databases Espacenet.

***Expert opinion:*** Although anti-hypertensive drugs armamentarium enumerates a plethora of therapeutic classes, including diuretics, the novel class of ROMK inhibitors may find a place in this crowded market, because of the diuretic/natriuretic effects, devoid of worrying influence on potassium balance. The patent examination highlights, as a strength, the individuation of a successful template: almost all the compounds show noteworthy potency. However, only few selected compounds underwent an *in vivo* investigation of diuretic and anti-hypertensive activities, and no data on hERG channel are given in these patents.



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***Keywords:*** Diuretics, Heart failure, Hyperkalemia, Hypertension, Hypokalemia, Renal outer medullar potassium channels, ROMK inhibitors.

**Article highlights**

- Hypertension is a common chronic disease characterized by elevated systemic arterial blood pressure. It represents a risk factor for several cardiovascular accidents; hence, the therapy for hypertension is of fundamental importance.
- Heart failure is another widespread pathologic condition which leads to edematous states often treated with diuretic drugs.
- Diuretics agents have a key role, but could present adverse effects, such as for example, hypokalemia for loop and thiazide diuretics and hyperkalemia for amiloride.
- A new class of diuretics, inhibitors of the renal outer medullary potassium (ROMK) channels, is predicted to give a diuretic/natriuretic activity higher than that provided by loop diuretics, thanks to ROMK channels in TALHL and CDD.
- About twenty patent and 1700 compounds by Merck Sharpe & Dome have been examined: $IC_{50}$ values determined in *in vitro* electrophysiological, thallium flux and $^{86}Rb^{+}$ efflux assays are reported. Few selected compounds underwent deeper pharmacological characterization *in vivo* on rat diuresis and recording of blood pressure on spontaneously hypertensive rats.
- On the basis of these preliminary data, some selected compounds are promising prototypes of a newest class of diuretic agents useful for hypertension and heart failure.

**List of abbreviations:**

ROMK = renal outer medullary potassium

TALHL = thick ascending limb of Henle's loop

CCD = cortical collecting duct

CT = collecting tubule

ENaC = epithelial sodium channel

PKA = protein kinase A

$PIP_2$ = phosphatidylinositol 4,5-biphosphate

CFTR = cystic fibrosis transmembrane conductance regulator

HTS = high-throughput screening

CHO = Chinese hamster ovary

HEK = human embryonic kidney

SAR = Structure Activity Relationships

MSD = Merck Sharp & Dome

SHR = spontaneously hypertensive rats

BK = Large-conductance $Ca^{2+}$-activated potassium channels

EP = electrophysiology

## 1. Introduction

The renal outer medullary potassium (ROMK or Kir 1.1) channel belongs to the family of *inwardly rectifying* potassium channels and plays an important role in $K^+$ recycling in the thick ascending limb of Henle's loop (TALHL) across the luminal membrane and in $K^+$ secretion in the collecting tubule (CT) and especially in the cortical collecting duct (CCD) [1-3]. At the TALHL level, the ROMK channel mediates the $K^+$ efflux required by the $Na^+/K^+/2Cl^-$ co-transporter for NaCl transport, important for the urinary concentrating mechanism, and contributes to the TALHL transepithelial current flow and membrane potential necessary for paracellular $Na^+$ and $Ca^{2+}$ reabsorption (**Figure 1**) . Therefore, the pharmacological inhibition of the ROMK-mediated potassium flow into the lumen is coupled with an indirect inhibition of the $Na^+/K^+/2Cl^-$ co-transporter, without the potassium leak, typical of the direct inhibitors of this co-transporter (i.e. loop diuretics). Instead, on the principal cells of the CCD, the ROMK channel represents the fulcrum of the $K^+$ secretion pathway, coupled with the $Na^+$ uptake through the amiloride-sensitive-$Na^+$ channels (or the epithelial sodium channel, ENaC), and is essential for $K^+$ homeostasis [4] (**Figure 1**). Consistently, the pharmacological inhibition of the ROMK channels impedes the $K^+/Na^+$ exchange in the CDD. Even if the ROMK channels are the most important pathway for the $K^+$ secretion into the lumen, ROMK inhibition is not associated with the $K^+$ retention and consequent hyperkalemia (typical of ENaC inhibition), because of the compensatory activation of the large-conductance $Ca^{++}$-activated potassium channels (BK) (**Figure 1**) [5]. It is therefore evident that factors influencing the activity of the ROMK channel have a crucial role on $K^+$ secretion and renal concentrating mechanisms, and can deeply influence diuresis and blood pressure regulation.

Many endogenous intracellular factors regulate the ROMK channel: protein kinase A (PKA), phosphatidylinositol 4,5-biphosphate ($PIP_2$), ATP and pH. PKA-mediated phosphorylation on three separate sites regulates the channel opening process and is necessary for full channel function [6];

$PIP_2$ is an essential $K_{ir}$ channel cofactor, required to maintain the open state of all inward-rectifying potassium channels, and the ROMK channel tightly interacts with $PIP_2$ [7]. Moreover PKC-induced $PIP_2$ hydrolysis inhibits ROMK channel activity in *Xenopus* oocytes suggesting an explanation for PKC modulation of the ROMK channel in the collecting duct [8]. Differently from a classical ATP-sensitive potassium channel, the ROMK channel is inhibited by physiological concentration of cytosolic ATP so long as it is expressed with the cystic fibrosis transmembrane conductance regulator (CFTR) [9]**.** Finally, intracellular pH influences ROMK channel activity, because a cytosolic acidification inhibits the ROMK channel and induces a long-live closed state [10]. ROMK channels are localized in TALHL, CT and CCD, (**Figure 1**) which are the sites of action of three different classes of diuretics respectively: a) $Na^+/K^+/2Cl^-$ co-transporter, responsible for ~ 30% of salt reabsorption, represents the pharmacological target of furosemide (the prototypical loop diuretic); b) the $Na^+/Cl^-$ co-transporter, responsible for ~ 7% of salt reabsorption, is the pharmacological target of thiazide diuretics; c) the amiloride-sensitive epithelial $Na^+$ channels (ENaC), i.e. the pharmacological target of amiloride and triamterene. Due to the tight coupling between $Na^+$ reabsorption and $K^+$ secretion in the CCD, loop and thiazide diuretics are associated with hypokalemia, while amiloride induces hyperkalemia. On the contrary, the new class of diuretic drugs, able to inhibit selectively the ROMK channels, would be predicted to give a diuretic/natriuretic activity higher than that provided by loop diuretics (thanks to the ROMK channels in the TALHL), with reduced risk of hypokalemia typical of loop diuretics and thiazides [11], thanks to ROMK in CCD. Moreover, the observation that type II Bartter's syndrome, a rare autosomal recessive salt-wasting nephropathy characterized by polyuria, hypokalemia, metabolic alkalosis and hypotesion, caused by inactivating mutations in the ROMK channels [12], leads to reduced blood pressure values [13], reinforces the belief that the ROMK channels may be new targets for a new class of diuretic agents employable in hypertension and edematous states such as heart failure.

## 2. ROMK inhibitors

The first discovered ROMK inhibitor was a bee venom peptide, tertiapin, which was able to inhibit rat channel, but it was 100-fold less potent on human isoform [14,15]. In 2009, Lewis and colleagues, at the Vanderbilt University, through high throughput screening, discovered a small-molecule that was an inhibitor of the ROMK channel: VU590 (7,13-bis(4-nitrobenzyl)-1,4,10-trioxa-7,13-diazacyclopentadecane) [16]. Subsequently, the synthetic exploration of VU590, led to the identification of a structurally related small-molecule, VU591 (2,2$^{I}$-oxybis(methylene)bis(5-nitro-1*H*-benzo-[*d*]imidazole) [17] (**Figure 2**). VU590 and VU591 inhibit ROMK interacting with its intracellular pore with $IC_{50}$ values of 0.29 and 0.24 μM, respectively. As regards the other inwardly rectifying channels expressed in the kidney (see **Figure 1**), VU590 has no activity on Kir2.1 and Kir4.1, but inhibits Kir7.1; while, VU591 shows a higher selectivity for Kir1.1 over other Kir channels. Moreover, VU591 inhibits potassium transport (without effetcs on net sodium transport) in the experimental model of isolated-perfused rat collecting distal tubules, suggesting an encouraging basis for ROMK inhibition in further *in vivo* experiments [16,17]. Independently from the studies of the Vanderbilt's group, Merck researchers carried out an high-throughput screening (HTS) on about 1.5 M molecules and, among them, the 4-nitrophenethyl-piperazine (Compound 1, **Figure 3**) was early, but erroneously, indicated as a lead ROMK inhibitor and submitted to extensive studies for identifying its possible activity on other relevant potassium channels, such as $K_{ir}2.1$ (the inhibition of $K_{ir}2.1$ channels expressed in heart ventricle is recognized in long QT syndrome) and hERG (hERG inhibition is considered the main undesidered mechanism of QT prolonging cardiotoxic drugs, such as cisapride [18]. Unexpectedly, when Compound 1 was subjected to repurification by HPLC, the ROMK inhibitory activity was lost. Through a careful examination of the LC-MS features it was discovered that the activity was not due to Compound 1, but to a minor impurity that was present before the repurification: 1,4-bis(4-nitrophenethyl)piperazine (Compound 2). Compound 2 displayed good ROMK inhibitory activity

($IC_{50}$=0.052 μM) and excellent selectivity over the $K_{ir}2.1$ and $K_{ir}2.3$ channels ($IC_{50}$>100 μM). Unfortunately, it showed high potency on the hERG channel ($IC_{50}$=0.005μM) [19,20] (**Figure 3**). Starting from this lead compound, the Merck researchers' strategy focused on the identification of bioisosteric replacements of the nitro groups. Among them, benzonitriles, 5-benzo(2,1,3-oxadiazole) and 4-phthalide groups maintained an appreciable ROMK block, but only the 4-phthalide moiety (Compound 3) led to a very encouraging result, reducing the potency on hERG by about 20-fold (ROMK $IC_{50}$= 0.089 μM, hERG $IC_{50}$= 0.15 μM) (**Figure 4**). Other Structure Activity Relationships (SAR) showed that even small changes in the distance between the nitro groups (both in shortening and in lengthening), as well as methyl substitution in the core skeleton, resulted in loss of potency [19]. In a second generation of ROMK inhibitors (developed by Merck), researchers explored analogues of compound 2 in which both the nitrophenyl groups were replaced with bis-4-cyanophenyl, bis-5-benzo (2,1,3-oxadiazole) or bis-4-phthalide. The latter showed an appreciable ROMK inhibitory potency, being about 20-fold selective over the hERG channel (ROMK $IC_{50}$= 0.089 μM, hERG $IC_{50}$= 1.9 μM) (Compound 4, **Figure 4**). The hybridization of these three pharmacophores led to a series in which compound 5 represented the most potent ROMK inhibitor (ROMK $IC_{50}$= 0.30 μM, hERG $IC_{50}$= 0.43 μM) (**Figure 4**). Further investigations on compound 5 demonstrated that halides substituents (in *ortho* position to the nitrile group) gave derivatives almost equivalent to compound 3; while, an increase in the size of the substituents determined a decrease in ROMK inhibitory potency. Interestingly, among them, some analogues markedly improved the pharmacokinetic properties [19]. As a further attempt to replace the 4-nitrophenyl group with bioisosteric moieties, Merck researchers synthesized a series of new di-substituted piperazines characterized by a 4-phthalidyl ethyl group and a 4-(1H-tetrazol-1-yl)phenyl methyl amide, as N-substituents [21]. Different patterns of substitutions on the tetrazole phenyl ring or on the phthalide ring were exploited; nevertheless, the simpler unsubstituted 5-(2-(4-(2-(4-(1H-tetrazol-1yl)phenyl)acetyl)-piperazin-1-yl)ethyl)isobenzofuran-1(3H)-one (Compound 6, **Figure 4**) showed the most appreciable profile, because it maintained $K_{ir}1.1$ inhibitory activity, was selective

for $K_{ir}1.1$ over $K_{ir}2.1$, $K_{ir}2.3$, $K_{ir}4.1$ and $K_{ir}7.1$ and had a weakest inhibitory activity on hERG. This molecule, submitted to further pharmacological evaluation *in vivo,* showed good oral bio-availability and a dose-dependent increase in urinary flow and urinary sodium excretion after short-term oral administration to rats and dogs. This effect was comparable to that of hydrochlorotiazide (HCT) but, unlike HTC, compound 6 was not associated with the typical hypokalemia induced by the use of loop and thiazidic diuretics [22].

**3. Patenting activity of ROMK inhibitors**

Starting from the second decade of this century, Merck Sharp & Dome (MSD) company and some researchers of the MSD network, started an intense activity of patenting which led in a few years to about twenty patents. They cover approximately 1700 examples of molecules, mainly based on a common template constituted by "pharmacophore-linker-core-linker-pharmacophore". In order to demonstrate ROMK inhibitory activity, some different tests were carried out and in particular $^{86}Rb^+$ efflux assay that measures the ability of ROMK to permeate $^{86}Rb^+$ in response to the test compounds. $Rb^+$ is a $K^+$ mimetic cation which flows across the cell membranes through all the $K^+$ channels. Normally, CHO-DHFR cells, stably expressing hROMK (Kir1.1) and pre-loaded with $^{86}Rb^+$, show a time-dependent efflux of the isotope, after incubation with a $Rb^+$-free buffer. The rate of this efflux depends on the number of functional $K^+$ channels and is prevented in a concentration-dependent manner by the presence of a channel inhibitor, allowing to calculate the $IC_{50}$ of inhibitory activity for each test compound. Another ROMK functional assay reported in these patents is that based on the ability of $Tl^+$ (another $K^+$ mimetic cation) to permeate through open ROMK channels and determine an increase of fluorescence in a $Tl^+$-sensitive dye pre-loaded into the cells. According to this assay, performed through FluxOR Kit (Invitrogen), HEK293 cells stably expressing hROMK (hKir1.1), pre-loaded with the dye and then exposed to a thallium-containing medium, show a time-dependent increase in fluorescence which in proportional to the number of functional channels. The incubation of cells with a channel inhibitor leads to concentration-dependent attenuation of

fluorescence and allows to determine accurately the $IC_{50}$ values of inhibition due to the incubation of test compounds. Some authors highlight important limitations of $Tl^+$ flux-based high-throughput screen: the optical properties of the fluorescent probe could be affected by tested compounds. Besides, small-molecules could alter endogenous pathways of $Tl^+$ flux in HEK293 cells, giving a false-positive hits. Finally, $Tl^+$ has a low solubility in chloride-containing buffers and this implies the use of low concentrations of $Tl^+$ or of non-physiological buffers . However they conclude that the limitations of this useful assay could be exceeded by voltage clamp electrophysiology, which is considered the "gold-standard" method for ion channel pharmacology [23].

The third *in vitro* assay, employed to give $IC_{50}$ values for inhibitors, consisted in the measurements of the electrical current generated by the permeation of potassium through the channel. For these electrophysiological experiments, three different platforms: Ion Works, QPatch or manual patch clamp were used. To define a test compound as a ROMK inhibitor, it must show potencies of at least 1 μM or lower in one or more of the three above assays [24].

*In vivo* assays, concerning rat diuresis and the recording of systolic blood pressure on spontaneously hypertensive rats (SHR) after test compound administration, are reported in patents exhamined, but only for the fewest selected agents.

Diuretic efficacy was carried out on Sprague-Dawley rats which received a *per os* dosage of the selected compounds. Then animals urine was collected for four hours by the use of a metabolic cage. For studies on hypertension, SHR were implanted with a telemetric device able to record blood pressure for 30 seconds every 10 minutes. Hydrochlorothiazide (25mg/Kg/day, *per os*) was included as reference diuretic drug. The selected compounds were administered in a sigle oral gavage each day for a typical duration of three to fourteen days [25].

### 3.1 MSD Corp. WO2012058134 A1

Patent application publication number WO2012/058134 A1, describes ROMK inhibitors having the generic formula reported in **Figure 5A** wherein A and B are mono and/or bicyclic aromatic groups; $R^2$ is –H, -$C_{1-6}$ alkyl, -$C_{3-6}$ cycloalkyl, -$CF_3$, -$CH_2OH$, or –$CO_2R$, or $R^2$ can be joined up with $R^1$ or $R^{10a}$ to form a ring; $R^3$ is –H, -$C_{1-6}$ alkyl, -$C_{3-6}$ cycloalkyl, -OH, -F, -$OC_{1-3}$ alkyl, or –$CH_2OH$, or $R^3$ can be joined up with $R^{10b}$ to form a ring. The authors claim to have performed the three *in vitro* assays on cells and the two *in vivo* assays on rats (described in the previous paragraph) and they highlight the ROMK electrophysiological assay $IC_{50}$ (μM) for a selection of compounds showing levels of potency lower than 1 μM, (as required to be defined as a ROMK inhibitor). Examples of these selected compounds are shown in **Figure 5B** and **5C** [25].

### 3.2 MSD Corp. WO2013039802 A1

In this second patent MSD describes several compounds having, as a shared structure, the formula reported in **Figure 6A** characterized by a piperidine core. Examples of tested compounds were tested in the electrophysiology assay and found to have a therapeutic level of potency. In this patent, the *in vivo* method of rat diuresis was also described, but examples of promising compounds are highlighted through $^{86}Rb^+$ efflux assay which allowed to identify new molecules showing values of $IC_{50} \leq 0.2$ μM (such as the compound shown in **Figure 6B)** or through Thallium flux assay in which different compounds, (example **6C)** showed an $IC_{50} \leq 0.1$ μM [26].

### 3.3 MSD Corp. WO2013062900 A1 and WO2013062892 A1

The third patent of the MSD series shows compounds deriving from a general structure characterized by an oxo-piperazinic core, as reported in **Figure 7A**. Derivatives of this general formula underwent Thallium flux assay. Different examples showed appreciable levels of potency ($IC_{50}$ values lower than 0.25 μM): in particular compounds reported in **Figure 7B** and in **Figure 7C** were the most potent of this series [27]. Starting from the same general structure **7A,** but with different substituents, a further patent reported another series of potential ROMK inhibitors tested through $^{86}Rb^+$ efflux assay or Thallium flux assay. Among these examples, two structurally

correlated molecules showed the best $IC_{50}$ values: the compound reported in **Figure 7D** (that is a mixture of two enantiomers) and the compound shown in **Figure7E** (that is composed of two diastereomers) [28].

**3.4 MSD Corp. WO2012058116**

This patent describes ROMK inhibitors, having the generic formula shown in **Figure 8A,** characterized by a piperazine core, wherein $R^5$ and $R^6$ are independently -H, -$C_{1-6}$ alkyl or -$C(O)OC_{1-3}$alkyl; and X, $X^1$, Y and $Y^1$ are independently -H or -$C_{1-6}$ alkyl; or $Y^1$ can be joined up with $Z^2$ to form a fused ring system. In the series of examples described in this patent, two derivatives exhibited remarkable levels of potency determined respectively by $^{86}Rb^+$ efflux assay (as in the case of the two diastereomers reported in **Figure 8B**) or through Thallium flux assay (for compounds in **Figure 8C)** [29].

**3.5 MSD Corp. WO2014015495 A1**

Patent application publication number WO2014/015495 A1 (having an update in the number of examples in the patent application publication number US2014/0031349 A1), describes a series of compounds having the generic formula shown in **Figure 9A,** characterized by a aza-spirocyclic core, which have remarkable levels of potency determined by electrophysiological assay (compound described by **Figure 9B**) or Thallium flux assay (compound in **Figure 9C**). Four selected compounds, described in this patent, were tested also in the *in vivo* measurement of systolic pressure on SHR: in this assay, compounds administered *per os* at doses in the range of 0.3 to 10 mg/Kg promoted a typical reduction in the daily mean systolic blood pressure, ranging from 6 to 24 mmHg. An example of the compounds tested in vivo is reported in **Figure 9D** [30,31].

**3.6 MSD Corp. WO2010129379 and US20140142115.**

Patent application publication number WO2010/129379 (having an update in the patent application publication number US2014/0142115 A1), focuses on a piperazine core and describes compounds having the general formula represented in **Figure 10A.** Although several described derivatives showed appreciable level of ROMK inhibitory potency in the electrophysiology assay, as for

example compound in **Figure 10B,** there is a compound (reported in **Figure 10C)** which exhibited a remarkable profile in most of the biological assays, such as thallium flux, electrophysiology, rat diuresis evaluation and recording of systolic blood pressure on SHR. In particular, compound **10C**, which has a symmetrical structure, is a 5,5'-{piperazine-1,4-diylbis[(1R)-1-hydroxyethane-2,1-diyl]}bis(4-methyl-2-benzofuran-1 (3H)-one) and besides exhibiting very low levels of $IC_{50}$, is one of the selected derivatives able to induce a 2-9 fold increase in urine volume with respect to the vehicle. **10C** belongs to a restricted group of compounds able to determine a reduction of systolic pressure levels from 8 mmHg to 32 mmHg, when administered *per os* at doses ranging from 3 to 10 mg/Kg. [32,33].

**3.7 MSD Corp. WO2014085210 A1**

In this patent, examples with a piperidine-based core are illustrated and a general formula was identified in **Figure 11A.** ROMK inhibitors with good levels of potency in two in vitro assays, thallium flux and electrophysiology, were individuated: the compound shown in **Figure 11B** is a good example. However, also other compounds were highlighted because of an appreciable $IC_{50}$ value in the Thallium flux assay and an anti-hypertensive activity in the in vivo assay based on the measurement of systolic pressure on SHR. In fact, according to the results shown in this patent, compounds like **11C**, administered per os at doses in the range 0.3-10 mg/Kg, caused a reduction in daily mean systolic blood pressure ranging from 6 mmHg to 24 mmHg [34].

**3.8 MSD Corp. WO2013028474 A1 and WO2014099633 A2**

Patent WO2013/028474 A1 describes a series of ROMK inhibitors having a general formula characterized by a heterobicycle (one of the two fused rings is pirazine) and shown in **Figure 12A.** Some examples, like the compound reported in **Figure 12B,** are highlighted because of their low $IC_{50}$ values both in thallium flux and in electrophysiology assays. Instead, other compounds, as for example **12C**, are described as anti-hypertensive because of their ability to low systolic pressure in SHR, in a range of 7-21 mmHg, after oral administration of doses from 3mg/Kg to 10 mg/Kg [35].

A very similar general structure (**Figure 12D**) was described in patent WO2014/099633 A2, in which compound illustrated in **Figure 12E** results the best example as concerns $IC_{50}$ values both in thallium flux and in electrophysiology assays [36].

**3.9 MSD Corp. WO2014126944 A2 and US20140336177 A1**

Structural analogies with the general formulas previously described in patents WO2013/028474 A1 and WO2014/099633 A2 are detectable in two other subsequent patents: WO214/126944 A2 with general formula reported in **Figure 13 A,** and US2014/0336177 with the general formula shown in **Figure 13D.** From in vitro experiments, the example represented in **Figure 13B** emerges as a ROMK inhibitor with appreciable values of $IC_{50}$ both in the Thallium flux and the electrophysiology assays. Compound in **Figure 13C** and its analogues were selected for the in vivo recording of systolic blood pressure on SHR after oral administration in doses from 3mg/Kg to 10 mg/Kg: in this assay, the selected compounds were able to evoke a lowering of the systolic blood pressure of a 9,5-21 mmHg. In patent US2014/0336177, among several compounds deriving from the general formula **13D,** compounds **13E** showed an appreciable $IC_{50}$ value in the Thallium flux assay and a remarkable $IC_{50}$ value in the electrophysiological one. It was also selected for a deepening *in vivo* study on SHR. Like other selected compounds, it promoted a lowering of the systolic blood pressure of 7-25 mmHg after oral administration in doses from 3mg/Kg to 10 mg/Kg [37,38].

**3.10 MSD Corp. US20140309213 A1, US20140288042 A1 and US20140275020.**

Patents US2014/0309213 A1 and US2014/0288042 A1 describe a series of ROMK inhibitors having the same general formula (**Figure 14A**) but, despite this similarity, substituents and the core are characterized by different chemical moiety: for example, in the case of patent US2014/0309213 A1 the core could be a diazatricyclo, a diazabicyclo heptan, a diazabicyclo octane, a diaza-spiro octane, a oxa-diazabicyclo nonane or a piperazine. While, in the case of patent US2014/0288042 A1, we can find a diazaspiro nonane, a diazaspiro decane, a diazaspiro undecane, a diazabicyclo heptan, a pyrrolo-pyrrolo and a pyrrolo-piperidine. In both patents, ROMK inhibitors were tested

through thallium flux and electrophysiology assays and some compounds like those depicted in **Figure 14B** (described in patent US2014/0309213 A1) or the example represented in **Figure 14C** (highlighted in patent US2014/0288042 A1) showed an appreciable value of $IC_{50}$ in both the *in vitro* assays [39,40]. Patent US2014/0275020 presents ROMK inhibitors having the general formula (**Figure 14D**) very similar to that represented in **Figure 14A,** with different cores mainly represented by amino-pyrrolidin, amino-cyclo-pyrrolidin, amino-methyl pyrrolidin, amino-methyl-piperidine and azetidin moieties. Again compounds like, for example, those illustrated in **Figure 14E,** were tested through the two assays of Thallium flux and electrophysiology, and showed profiles compatible with a ROMK inhibitor [41].

### 3.11 MSD Corp. WO2014150132 A1 and WO2015017305 A1

These recent patents on ROMK inhibitors share a spirocyclic moiety at the core position. In particular in patent WO2014/150132 A1 we can find the general structure represented in **Figure 15A** and characterized by a diaza-spirodecanone core and by a tetrazole and an oxo-dihydrofuran as lateral pharmacophores. Two of the most representative derivatives described in this patent (**Figure 15B** and **C**) showed remarkable values of $IC_{50}$ obtained through thallium flux assay and anti-hypertensive activity on SHR when administered *per os* at doses in the range of 0.1-10 mg/Kg resulting in typical reductions in daily mean systolic blood pressure ranging from 5 to 33 mmHg. On the other hand, in patent WO2015/017305 A1, the spirocyclic moiety of the general formula shown in **Figure 15D** was mainly represented by diaza-spirodecano or diaza-spiro-undecan pirazine as in two of the most potent ROMK inhibitors highlighted in **Figure 15E** and **F** evaluated through the thallium flux assay [42,43].

## 4. Conclusions

The last five years represented a period of continuous flourishing in the field of synthesis and pharmacological characterization of ROMK inhibitors: about twenty patents covering approximately 1700 examples of molecules were presented by MSD. For all these molecules, at

least data on *in vitro* determination of $IC_{50}$ as ROMK inhibitors were shown and often the tested compounds showed $IC_{50}$ values lower than 1 μM in one or more of the three in vitro assays described (the necessary condition for defining a molecule as a ROMK inhibitor). Selected compounds of some patents (as for example compound represented in **Figure 10C**) were reported to display diuretic activity on a Sprague-Dawley rat diuresis model: in fact they were able to induce up to 9-fold increase in urine volume after an oral dose of 1-3 mg/Kg. Other compounds (as for example molecules reported in **Figure 9D, 10C, 11C, 12C, 13C, 13E, 15B** and **15C**) were reported to lower blood pressure (about 6-24 mmHg), after oral administration of doses in a range 0.1-10mg/Kg to SHR implanted with a telemetric device that allowed a continuous recording of systolic blood pressure [30-38; 42,43]. However although the pharmacokinetic *in vivo* or the activity on other types of potassium channels, were often unrevealed, clinical data should certainly be obtained in the future and the support of computational modeling studies could be useful to define ligand-ROMK interactions [44] , we can assert that this impressive development of novel selective ROMK inhibitors constitutes a promising basis for the birth of a newest class of diuretics.

## 5. Expert opinion

Presently, the area of marketed cardiovascular drugs is widely covered and especially anti-hypertensive drugs are well represented by several classes with a variety of mechanisms of action which allow to satisfactorily treat most patients with suitable therapies. Of course, every therapy presents weaknesses and adverse effects; this makes extremely interesting and still compelling every improvement of the pharmacological armamentarium in this field. Hence, although it seems very difficult to find a place for a new class of drugs in this area, this challenge is even more attractive and timely, and the work is harder than in other pharmacological fields. In the widest number of cardiovascular drugs, endowed with very heterogeneous mechanisms of action, diuretics are widely used, in monotherapy or in association with other drugs, in the pharmacological treatment of hypertension and heart failure (together with other non-cardiovascular uses). In

particular, treatment with diuretics, such as thiazide ones, often represents an effective first-line approach to hypertension [45]. Indeed, thiazide diuretics are equivalent to other classes of antihypertensive agents in reducing cardiovascular events in hypertensive patients and are considered even superior than beta-blockers and angiotensin-converting enzyme inhibitors in reducing stroke incidence. Loop diuretics, less used in hypertension, are more effective in improving the outcomes of heart failure. Even ENaC blockers, such as amiloride, are used for hypertension, often associated with other drugs. However, although the above currently used diuretic drugs are endowed with an overall positive risk-benefit balance, they are not devoid of worrying side effects. In fact, they cause clear class-related alteration of potassium homeostasis: loop and thiazide diuretics are associated with hypokalemia, while inhibitors of ENaC induce hyperkalemia. The novel class of ROMK inhibitors, described in the patents examined, was designed to act as diuretics. This new class of diuretic drugs is expected to give a diuretic/natriuretic activity higher than that provided by loop diuretics, with limited effects on potassium homeostasis [11]. Therefore, ROMK inhibitors can actually represent an improvement worth pursuing. In my opinion, this is the premise for believing that they may constitute, in the next few years, a new class of diuretics employable, alone or in combination with other drugs, in hypertension and edematous states such as heart failure. The patent examination highlights as a strength, the individuation of a successful chemical template: almost all the compounds, based on this template, showed high levels of potency in the inhibition of the ROMK channels. As a further strength, some of these compounds, for example compounds 10C, showed diuretic effects on Sprague-Dawley rats and anti-hypertensive activity on spontaneously hypertensive rats. However, in these early patents, the ROMK inhibitory activity of all the molecules was tested by means of suitable in vitro assays, but only very few selected compounds underwent an in vivo pharmacological evaluation of the possible effects on diuresis and blood pressure. Moreover, no data on the activity of these new molecular entities on other types of potassium channels, as for example the hERG are given in these patents and this knowledge could make the difference between a promising compound and a molecule with

dramatic adverse effects. Also pharmacokinetic data should be produced as soon as possible, in order to complete the profile candidates for possible clinical uses.

In conclusion, this new class of ROMK inhibitors is still at the beginning of its history; in the coming years, a more detailed pharmacological profile of these new molecules will be furnished and will allow a different approach to diuresis based on this new exciting mechanism of action.

**Declaration of interest**

The authors declare no conflict of interest.

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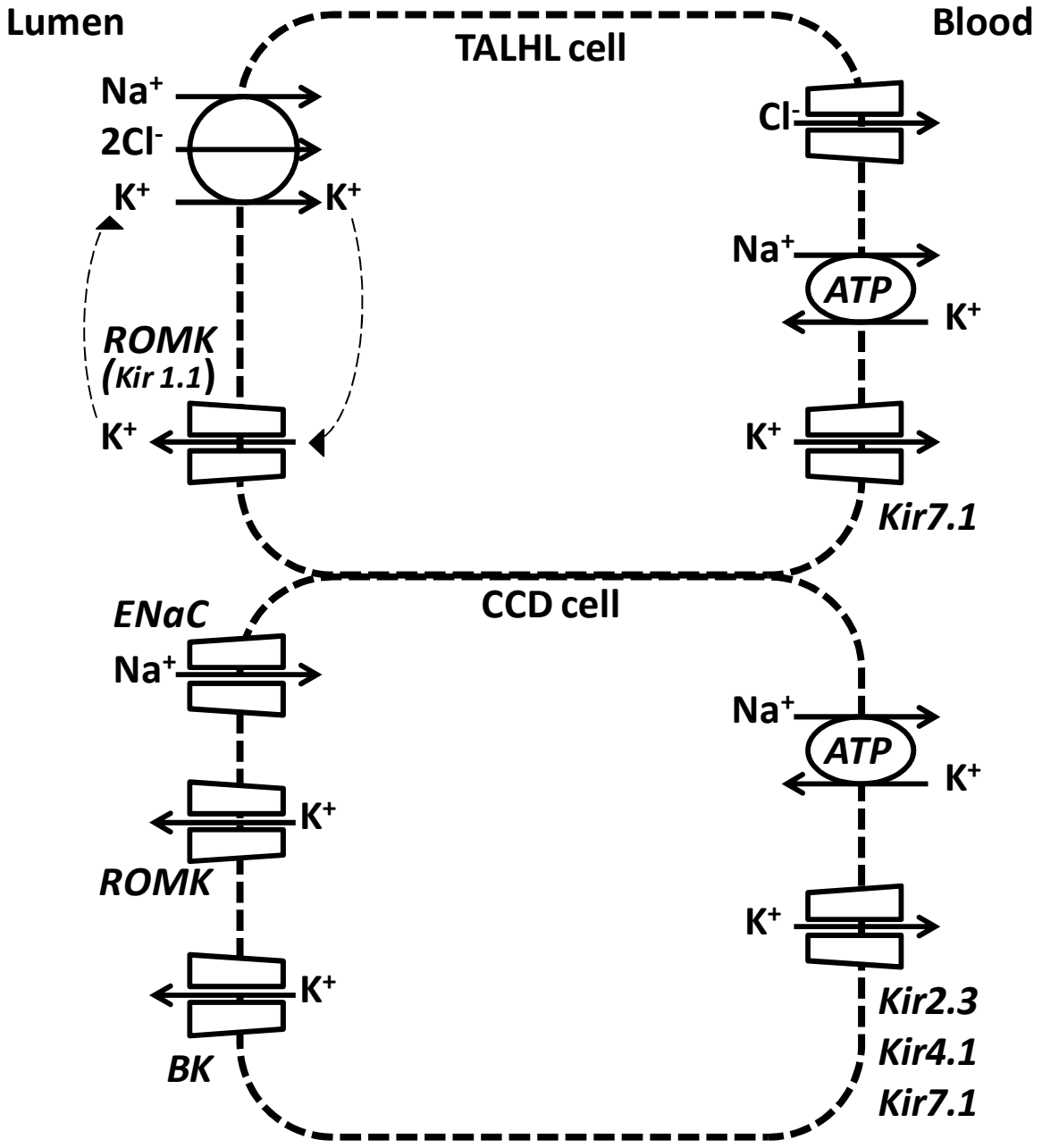
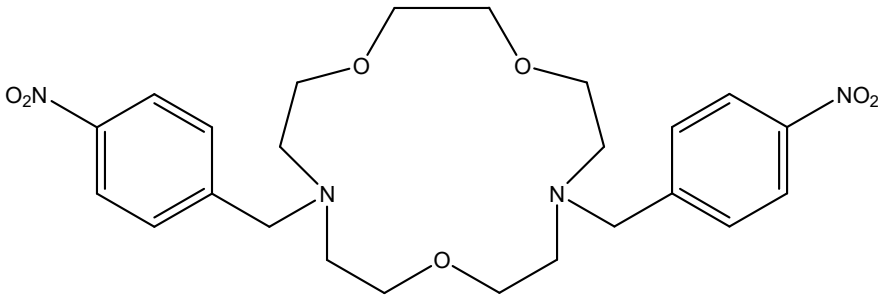
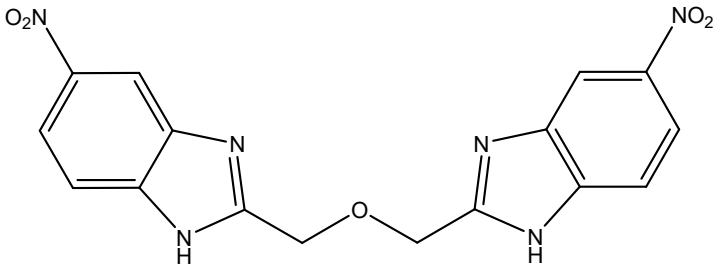



**Figure 1.** The role of ROMK channels in the thick ascending limb of Henle loop (TALHL) and cortical collecting duct (CCD) cells. Co-localization and interaction with the $Na^+/K^+/2Cl^-$ co-transporter, the $Na^+/K^+$-ATPase, the epithelial $Na^+$ channels (ENaC), the potassium channels like the "Big-conductance" $Ca^{2+}$-activated potassium channels (BK) and the "inwardly rectifying" Kir7.1, Kir2.3, Kir4.1.

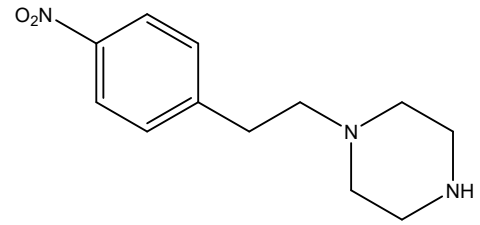



VU 590
ROMK $IC_{50}$ = 0.29 µM

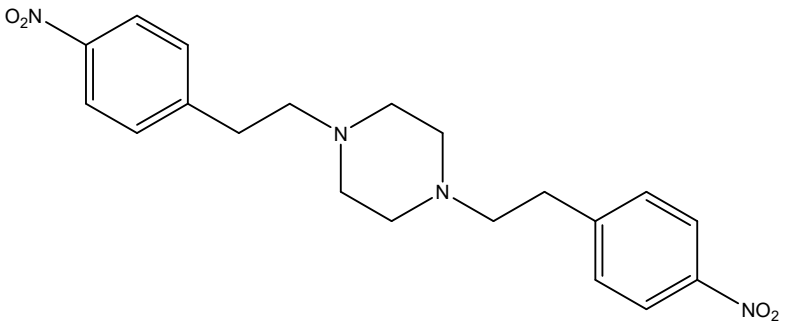



VU 591
ROMK $IC_{50}$ = 0.24 µM

**Figure 2.** Small-molecule ROMK inhibitors discovered at Vanderbilt University.




Compound 1.
ROMK $IC_{50}$= 5.2 µM
hERG $IC_{50}$= 0.19 µM
$K_{ir}$2.1 and $K_{ir}$2.3 $IC_{50}$ > 100 µM




Compound 2.
ROMK $IC_{50}$= 0.052 µM
hERG $IC_{50}$= 0.005 µM
$K_{ir}$2.1 and $K_{ir}$2.3 $IC_{50}$ > 100 µM

**Figure 3.** First ROMK inhibitors discovered by Merck through HTS (Compound 1) and, after careful examination of LC-MS, through the identification of impurity as the real ROMK inhibitor (Compound 2)

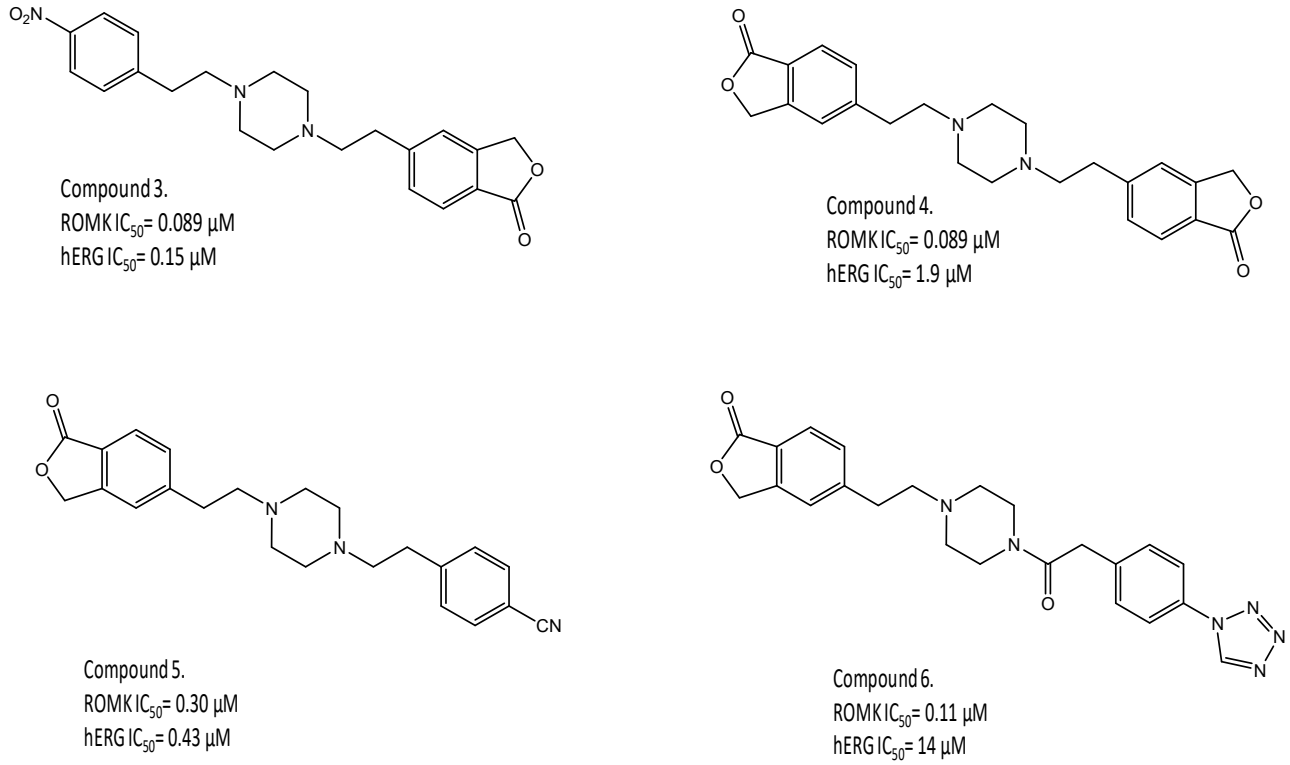



**Figure 4.** Compound 3,4,5,6 are ROMK inhibitors synthesized by Merck medicinal chemistry during SAR investigation on lead compound 2.

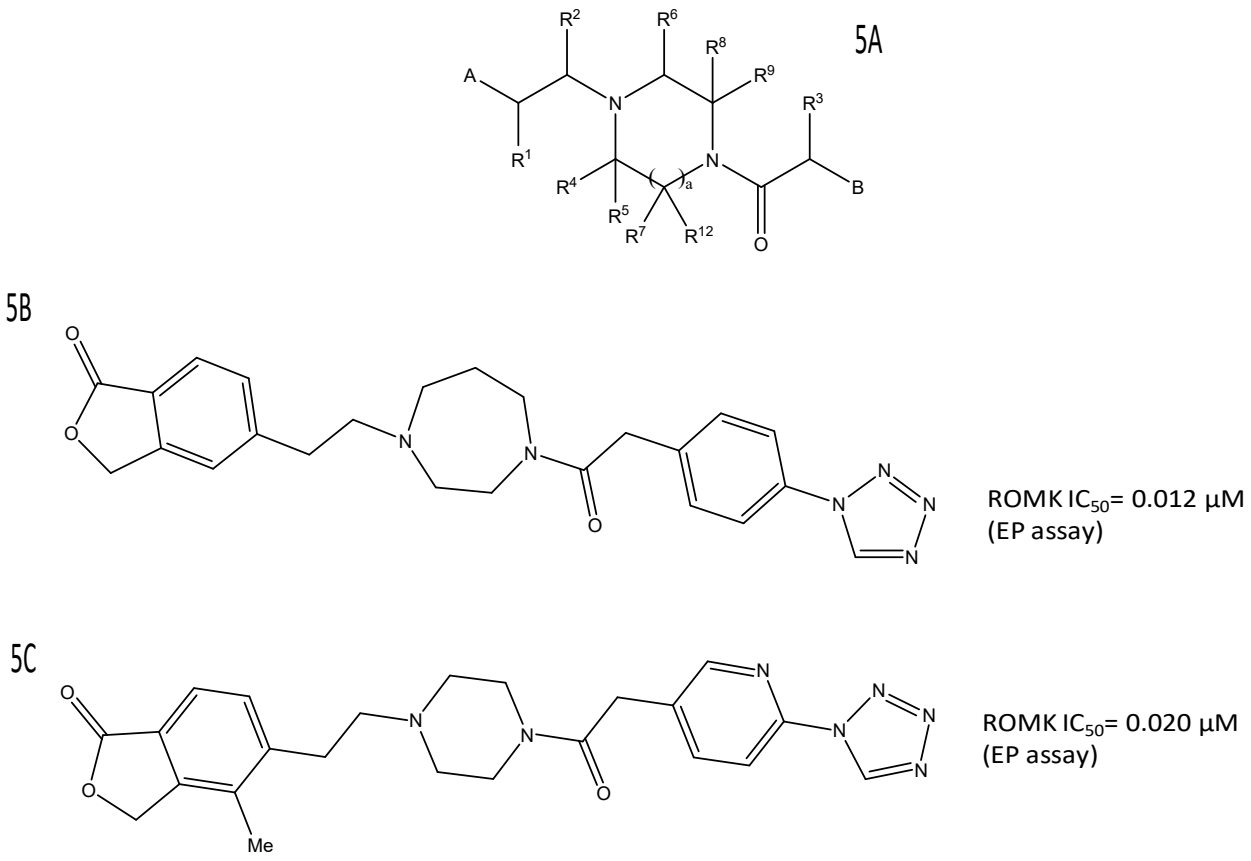



**Figure 5. A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO058134 A1; 2012. **B** and **C** are two examples of compounds highlighted in the patent through electrophysiological (EP) assay.

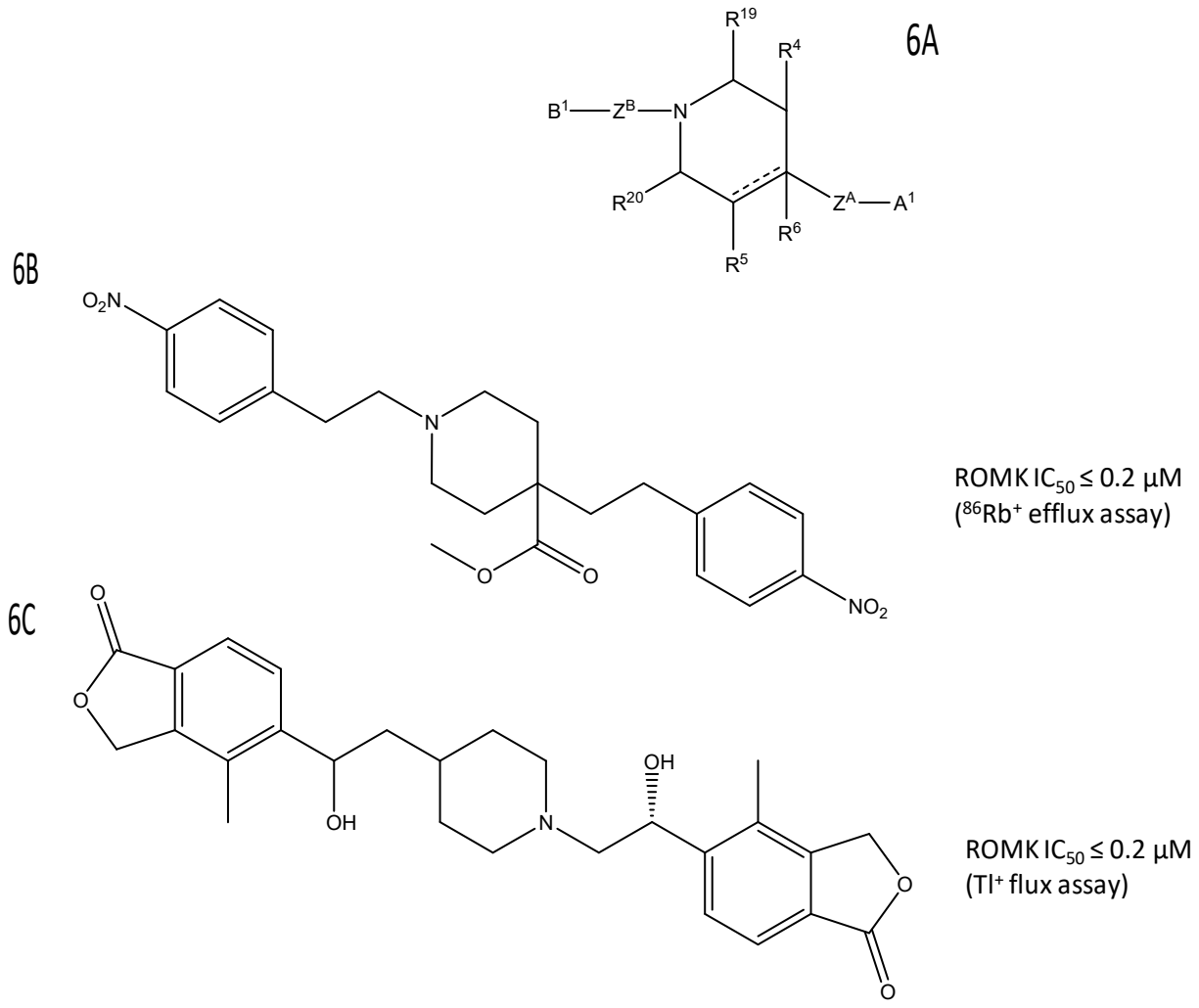



**Figure 6. A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO039802 A1; 2013. **B** and **C** are two examples of compounds highlighted in the patent through $^{86}Rb^{+}$ efflux and Thallium ($Tl^{+}$) assays, respectively.

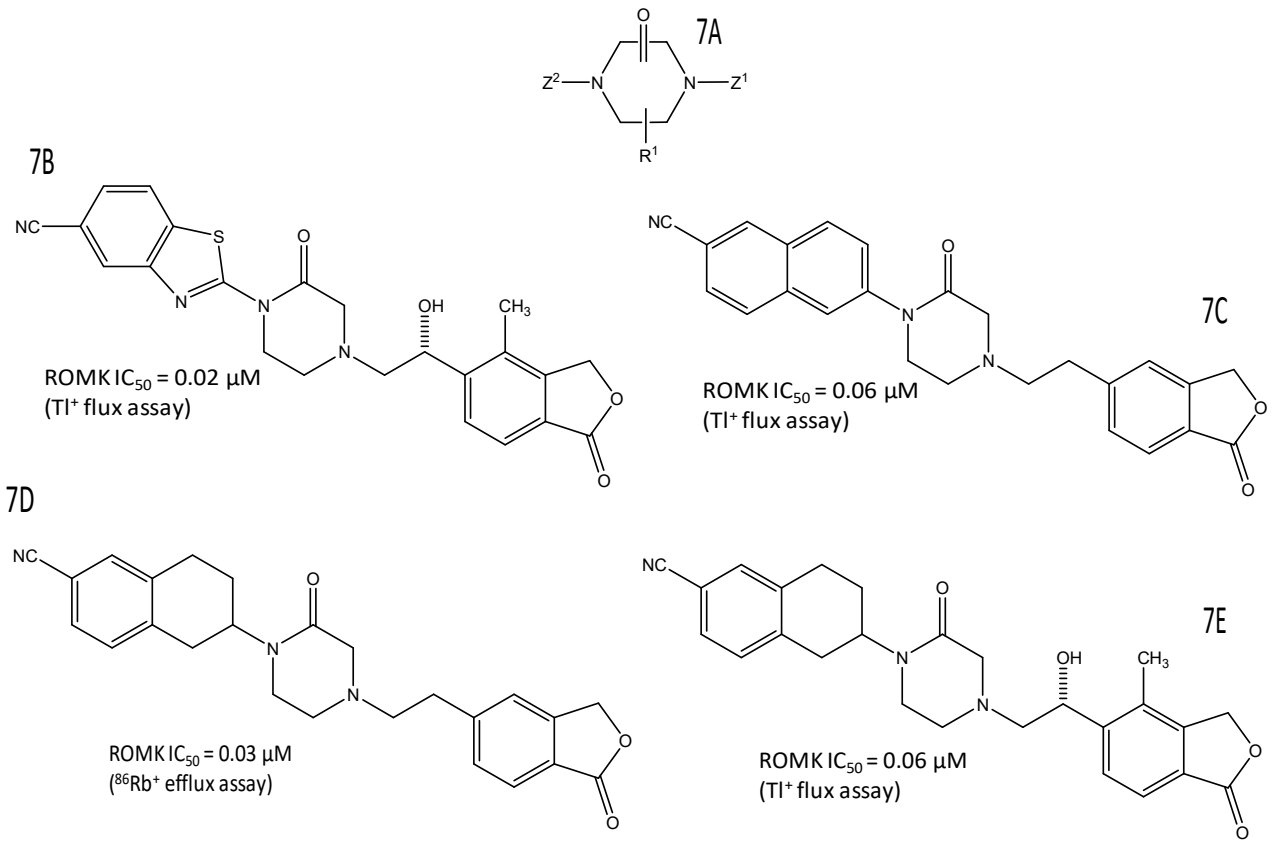



**Figure 7. A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel WO2013/062900 A1 and in WO2013/062892 A1. **B** and **C** are two examples of compounds highlighted in the patent WO2013/062900 A1 through Thallium ($Tl^+$) assay while **D** and **E** are two examples of compounds highlighted in the patent WO2013/062892 A1 through $^{86}Rb^+$ efflux and Thallium ($Tl^+$) assays, respectively.

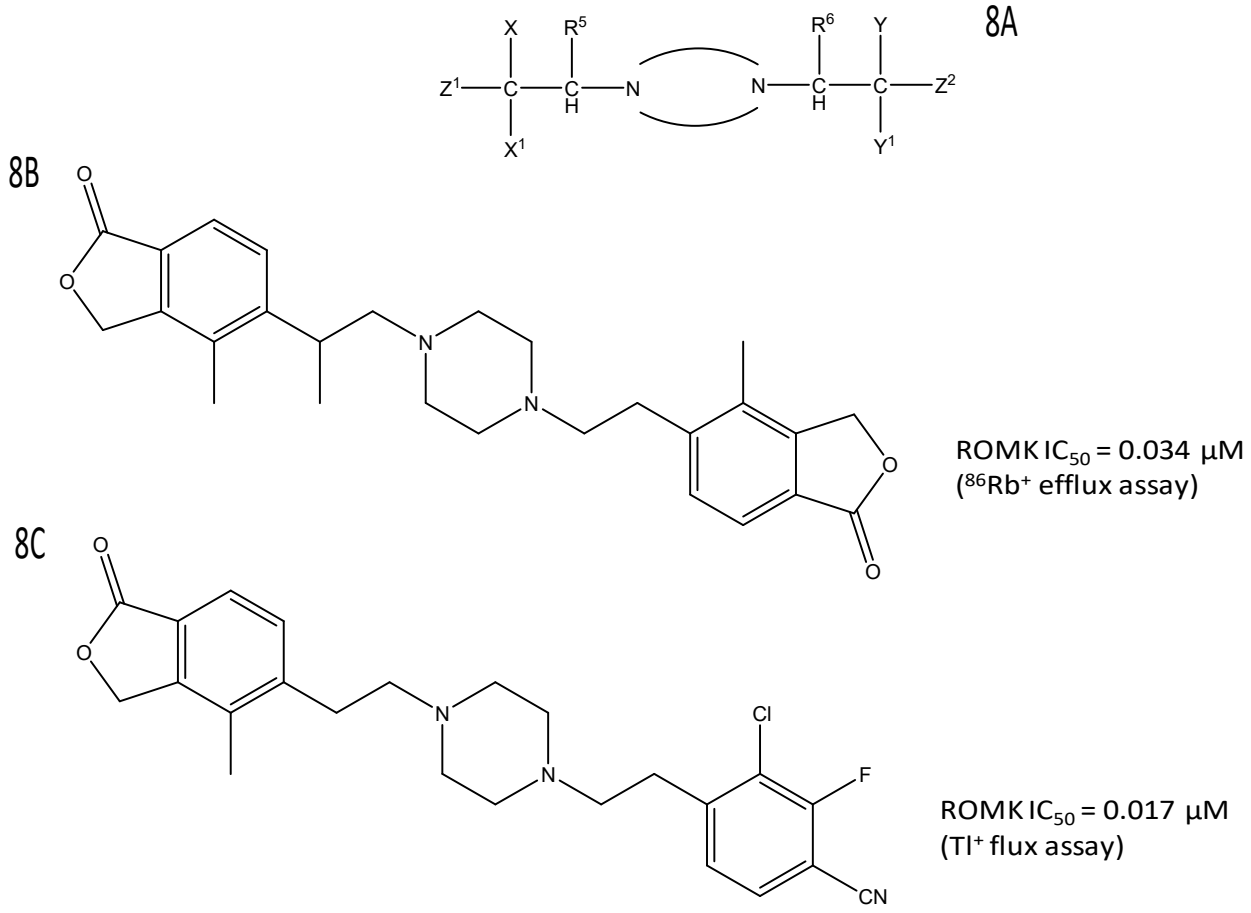



**Figure 8. A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2012/058116. **B** and **C** are two examples of compounds highlighted in the patent through $^{86}Rb^{+}$ efflux and Thallium ($Tl^{+}$) assays, respectively.

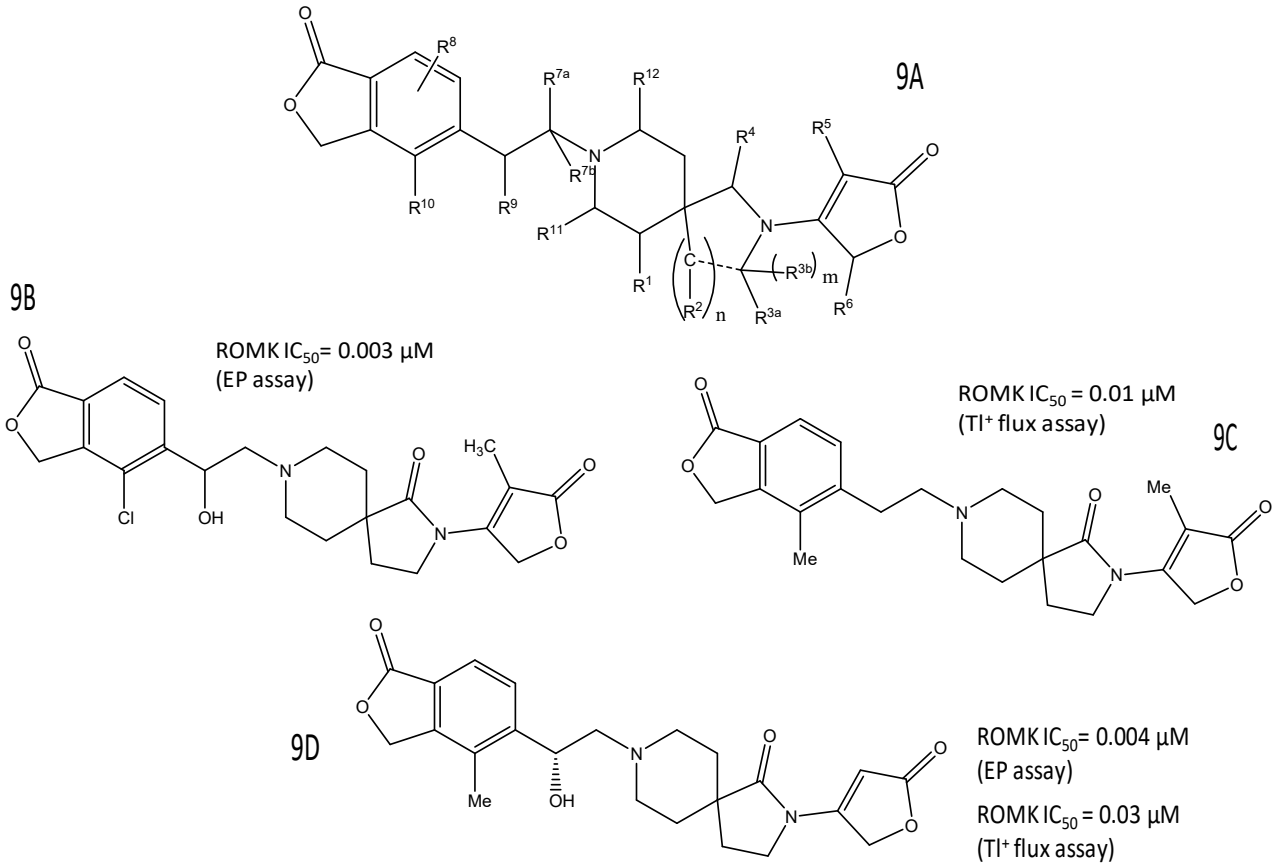



**Figure 9.A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2014/015495 A1 and US2014/0031349 A1. **B** and **C** two examples of compounds highlighted in the patent through electrophysiological and Thallium ($Tl^+$) assays, respectively. **D** is an example of compounds selected for the in vivo assay on SHR systolic pressure.

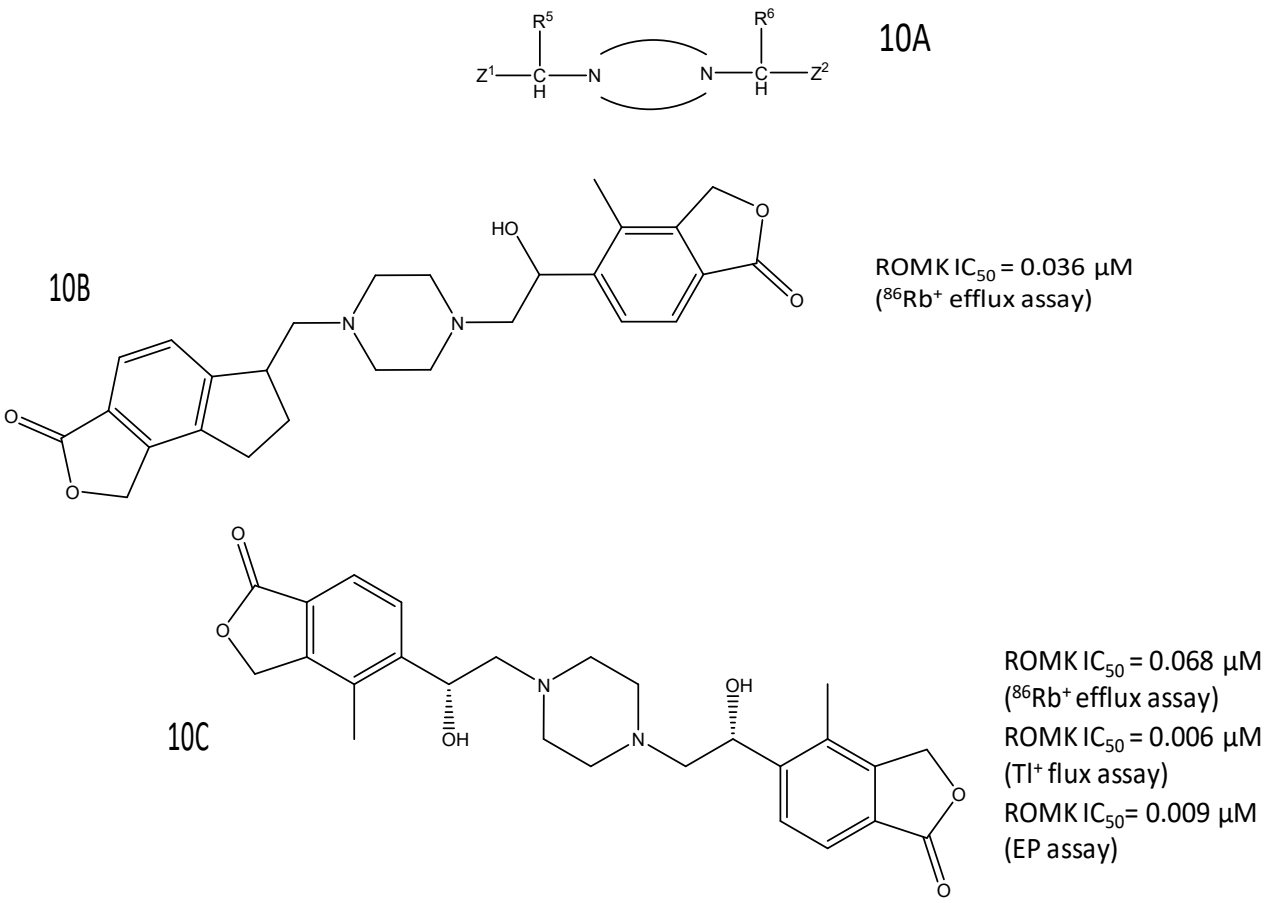



**Figure 10.A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2010/129379 and US2014/0142115 A1. **B** is an example of compounds showing remarkable $IC_{50}$ value in $^{86}Rb^{+}$ efflux assay and **C** is a 5,5'-{piperazine-1,4-diylbis[(1R)-1-hydroxyethane-2,1-diyl]}bis(4-methyl-2-benzofuran-1(3H)-one), example of a compound showing appreciable values in all the biological assays both in vitro and in vivo.

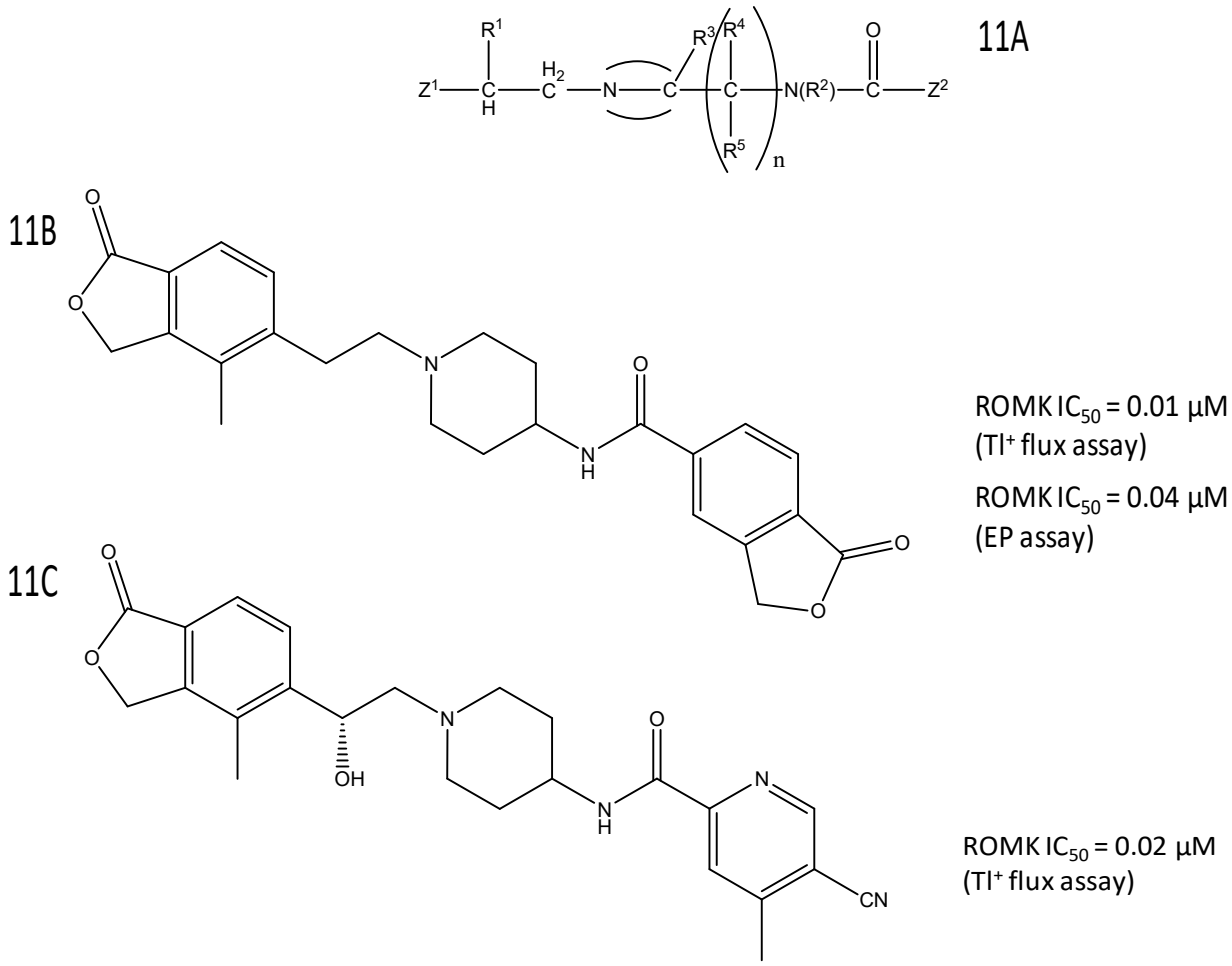



**Figure 11.A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2014/085210 A1. The **B** example shows appreciable values of $IC_{50}$ in thallium ($Tl^+$) and electrophysiology (EP) assays. The **C** example shows appreciable values of $IC_{50}$ in thallium ($Tl^+$) and anti-hypertensive activity on SHR in vivo.

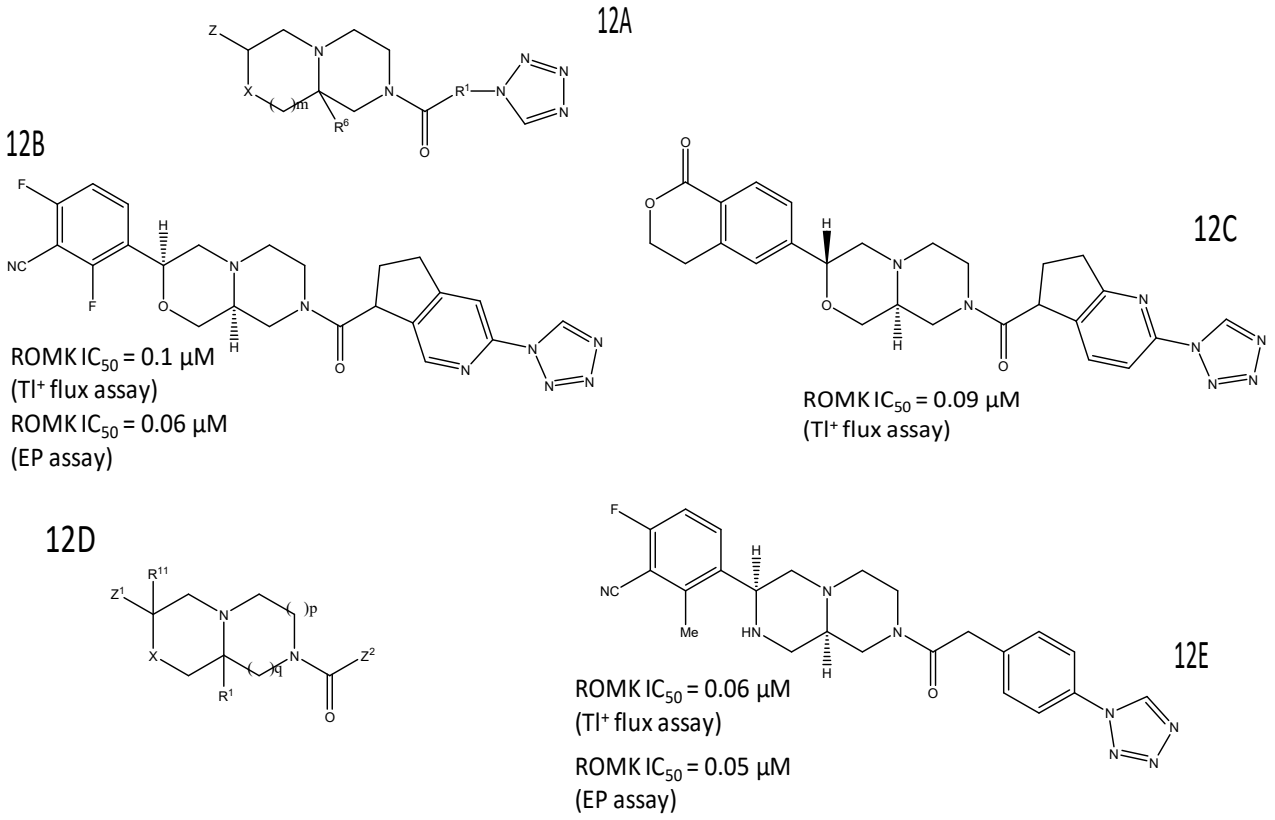



**Figure 12.A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2013/028474 A1. The **B** example shows appreciable values of $IC_{50}$ in Thallium (Th) and electrophysiology (EP) assays. The **C** example shows and anti-hypertensive activity on SHR in vivo. Figure **12D** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2014/099633 A2. The **12E** example shows appreciable values of $IC_{50}$ in Thallium ($Tl^{+}$) and electrophysiology (EP) assay.

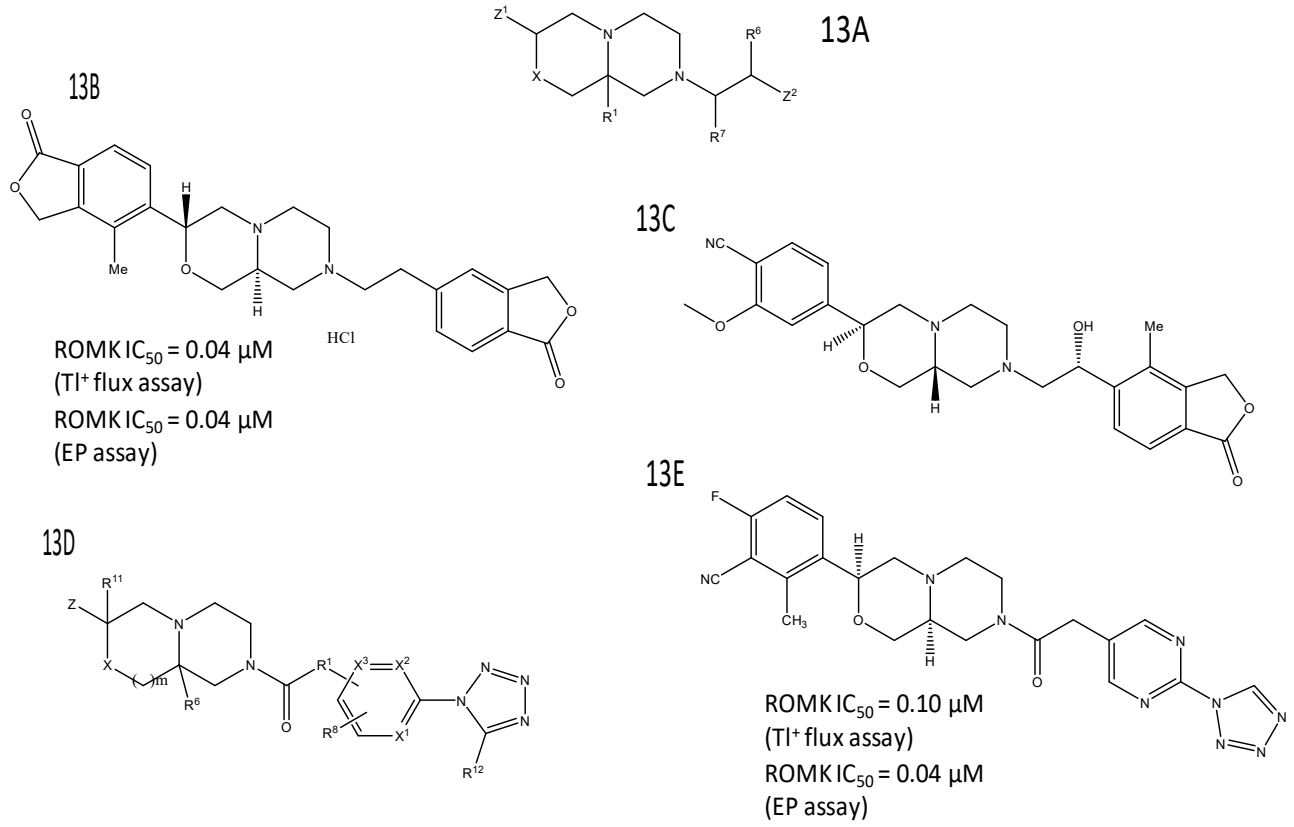



**Figure 13.A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2014/126944 A2. The **B** example shows appreciable values of $IC_{50}$ in Thallium ($Tl^{+}$) and electrophysiology (EP) assays. **The C** example shows and anti-hypertensive activity on SHR in vivo. Figure **13D** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. US2014/0336177 A1. The **13E** example shows appreciable values of $IC_{50}$ in Thallium ($Tl^{+}$), electrophysiology (EP) assays and anti-hypertensive activity on SHR in vivo.

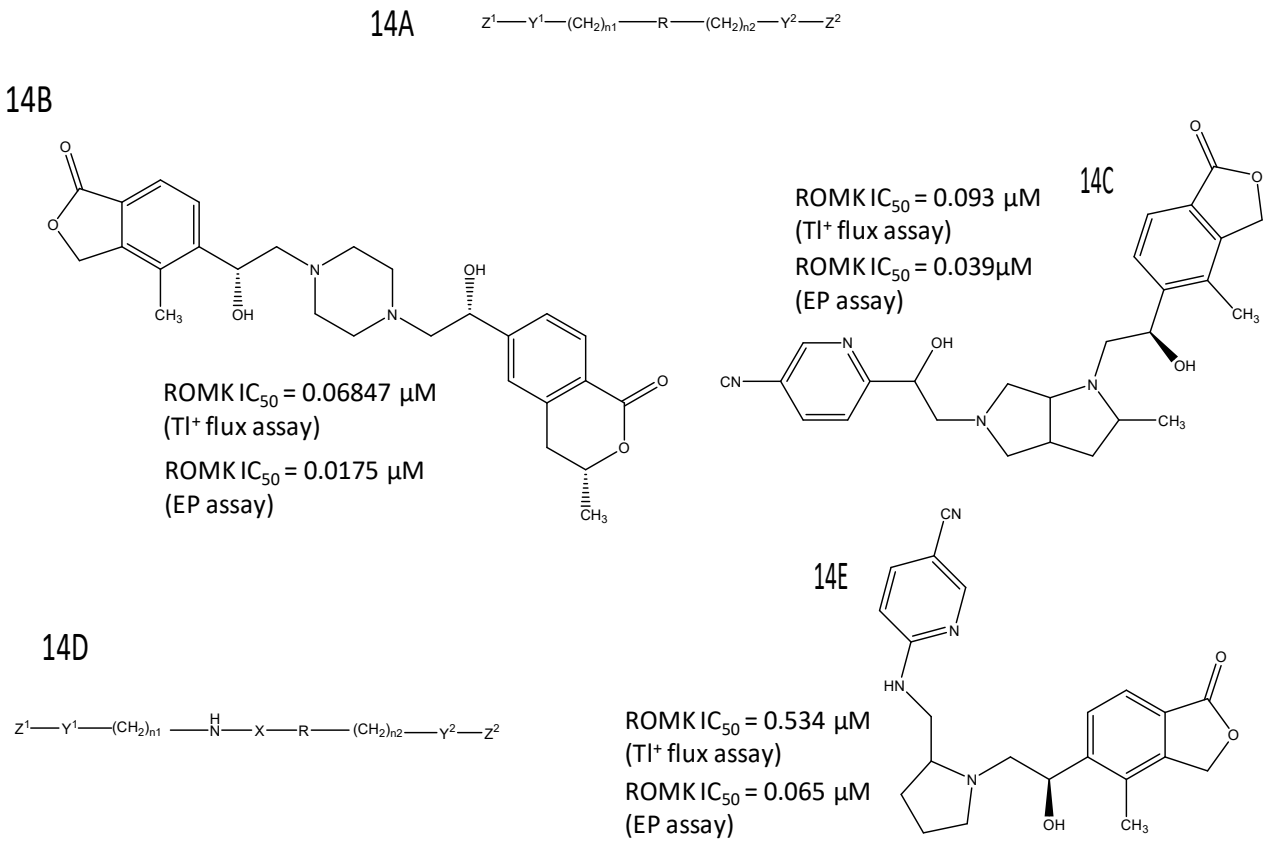



**Figure 14.A** The general formula reported in the patents by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. US2014/0309213 A1 and US2014/0288042 A1. The **B** example shows appreciable values of $IC_{50}$ in Thallium ($Tl^{+}$) and electrophysiology (EP) assays in the patent US2014/0309213 A1. The **C** example shows appreciable values of $IC_{50}$ in Thallium ($Tl^{+}$) and electrophysiology (EP) assays in the patent US2014/0288042 A1. **Figure 14D** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. US2014/0275020. The **E** example shows appreciable values of $IC_{50}$ in Thallium ($Tl^{+}$) and electrophysiology (EP) assays in this patent.

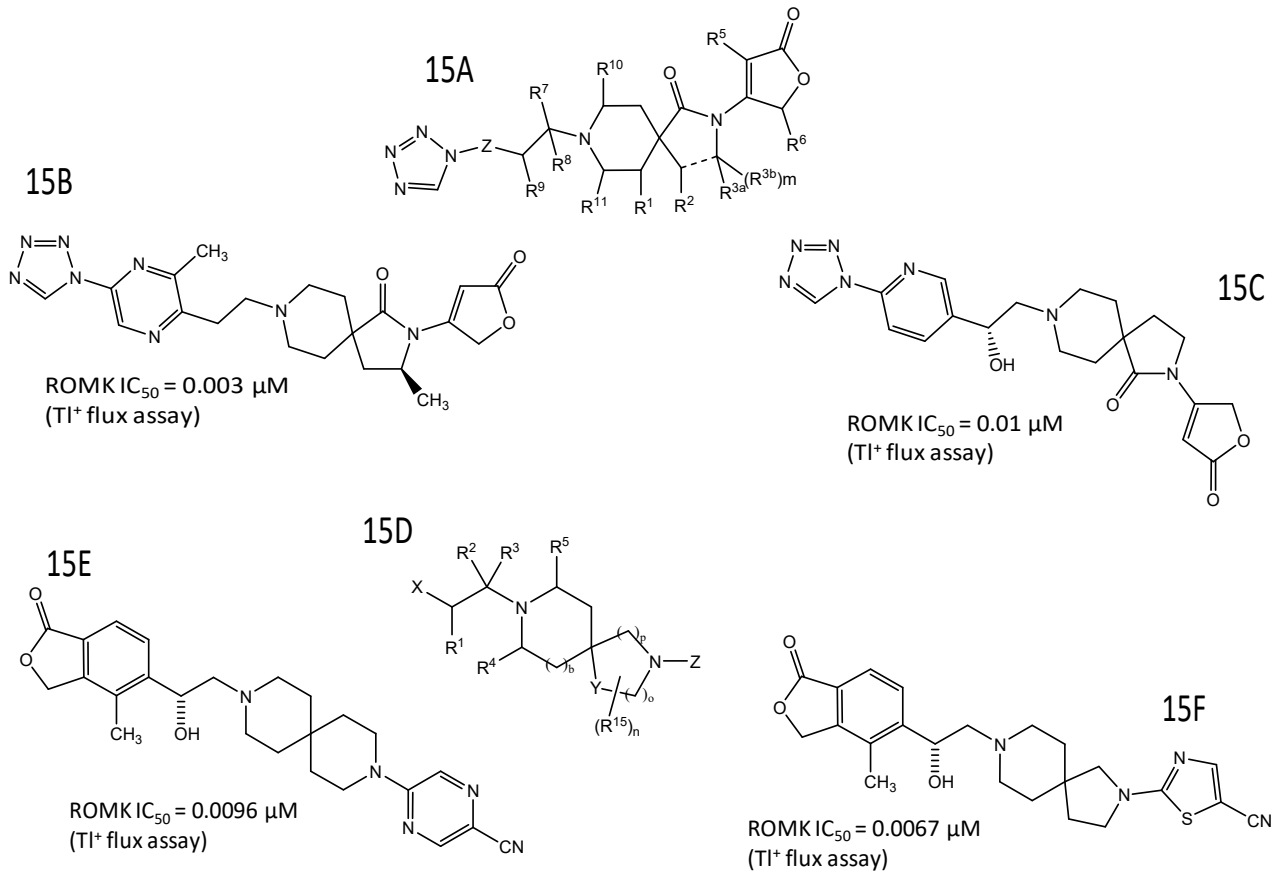



**Figure15.A** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2014/150132 A1. The **B** and **C** examples show appreciable values of $IC_{50}$ in Thallium ($Tl^{+}$) and anti-hypertensive activity on SHR in vivo. **Figure 15D** The general formula reported in the patent by Merck Sharp & Dohme Corp. Inhibitors of the renal outer medullary potassium channel. WO2015/017305 A1. The **E** and **F** examples show appreciable values of $IC_{50}$ in Thallium ($Tl^{+}$) flux assay in this patent.