CHAPTER 6

Recent Advances in Mineralocorticoid Receptor Antagonists

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Contents

1. INTRODUCTION

The mineralocorticoid receptor (MR) is a member of the nuclear hormone receptor (NHR) superfamily and is structurally related to the progesterone receptor (PR), androgen receptor (AR), estrogen receptor (ER),

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and glucocorticoid receptor (GR) [1]. Aldosterone is the primary physiologic steroid hormone that binds to MR and promotes sodium and water reabsorption with potassium excretion. Other endogenous steroids such as cortisol can also bind to MR with high affinity. Excessive levels of aldosterone result in deleterious conditions including hypertension and cardiac hypertrophy. There is extensive clinical validation for treating hypertension and congestive heart failure with spironolactone and eplerenone, both of which are steroid-based antagonists of MR. However, these agents have side effects such as gynecomastia (off-target), hyperkalemia (on-target) and drug–drug interactions that limit their safety and effectiveness, thus providing a need for MR antagonists with superior profiles. Recent evidence suggests that MR blockade, when given in combination with standard therapy (*e.g.*, ACE inhibitors), reduces proteinuria in patients with renal disorders such as diabetic nephropathy and chronic kidney disease. In recent years, there has been a renewed interest in identifying nonsteroid-based antagonists of MR. Significant progress has been made toward identifying agents with greater selectivity against other steroid receptors such as AR and PR and greater potency compared to known steroid-based MR antagonists [2].

2. ALDOSTERONE AND MR BIOLOGY

MR is expressed in epithelial tissues notably in the distal convoluted tubules and cortical collecting ducts of the kidney. Its expression is also detected in lung, colon, and liver. MR is also expressed in nonepithelial tissues such as heart and brain [3]. Aldosterone, through binding to MR, promotes renal sodium reabsorption and potassium secretion in the distal nephron, distal colon, and salivary and sweat glands. Under conditions of abnormally elevated aldosterone levels as in patients with congestive heart failure, aldosterone-mediated sodium and water retention leads to an elevation in blood pressure due to inappropriate intravascular volume expansion [4].

Aldosterone can also regulate blood pressure by actions in the brain and directly on the vascular wall [5]. Evidence also indicates that inappropriate levels of aldosterone, in the presence of moderate to high sodium levels, mediate significant damage in nonepithelial tissues [6]. Elevated aldosterone levels, which are normally low (<1 nmol/L) [7], are linked to endothelial dysfunction, vascular inflammation, and myocardial fibrosis [2,8]. In patients with congestive heart failure, an increase in aldosterone-mediated sodium and water retention leads to inappropriate intravascular volume expansion and clinical symptoms consistent with hypervolemia [2]. While these effects were initially thought to be due to blood pressure elevation, the realization that functional MR is expressed

in blood vessels has extended the role of aldosterone beyond sodium and water balance to direct and pleiotropic effects in the vasculature [9]. The primary damaging effects of aldosterone in the vasculature appear to lie in its induction of vascular inflammation and fibrosis. These effects occur not only in the heart but also in other organs such as the kidney and brain [3,10]. Plasma aldosterone levels among patients with ST segment-elevation myocardial infarction (STEMI) are associated with early and late adverse clinical outcomes, including mortality. The association between high aldosterone levels and late mortality is independent of age, heart failure, and reperfusion status. These results underline a pivotal role for aldosterone in the setting of STEMI [11].

Aldosterone ($K_D = 1$ nM) [12] acting via MR thus plays a crucial role in the pathophysiology of hypertension and ischemic heart disease [13]. In addition to aldosterone, the endogenous glucocorticoid cortisol can also bind to MR with similar affinity [12]. *In vitro*, both aldosterone and cortisol activate MR with EC_{50} values of 1 and 3 nM, respectively [14]. In epithelial cells *in vivo*, MR is protected from cortisol activation by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), the enzyme that converts cortisol into inactive cortisone. Inactivation of 11β-HSD2, which occurs in the syndrome of apparent mineralocorticoid excess, allows cortisol to function as an MR agonist to increase sodium reabsorption. In some nonepithelial tissues, including the heart and specific regions of the brain, 11β-HSD2 is not coexpressed with MR. Thus in these tissues, or under conditions of glucocorticoid excess, MR is presumably occupied by the higher levels of glucocorticoids that are present raising the possibility of a role for glucocorticoids in MR activation [15,16].

3. RAAS PATHWAY, MR ANTAGONISTS VERSUS ACE, ARB THERAPY

The renin–angiotensin aldosterone system (RAAS) is the major hormonal system that regulates blood pressure by controlling salt and water homeostasis and is a major target for therapy in patients with cardiovascular disease. Renin, an aspartyl protease synthesized in the juxtaglomerular cells of the kidney, performs the first rate limiting step of the conversion of angiotensinogen to angiotensin I, which is then converted to angiotensin II (A-II) by angiotensin-converting enzymes (ACEs). A-II acts via its receptors expressed in the adrenal cortex to release aldosterone. Activation of RAAS plays a significant role in the pathophysiology of various disease states including cardiac, vascular, and renal complications due to hypertension, vascular smooth muscle and cardiac hypertrophy, and fibrosis [17].

ACE inhibitors (ACEis) and angiotensin receptor blockers (ARBs) are well-established pharmacological options for the treatment of hypertension. However, ACE and A-II receptors are upstream of aldosterone signaling, and their blockade alone is not sufficient to sustain a long-term reduction in aldosterone due to a phenomenon termed ''aldosterone escape'' which leads to a gradual reactivation of the aldosterone signaling cascade [9].

3.1. Steroid-based MR antagonists in the clinic—Clinical results

Spironolactone and eplerenone are the only two MR antagonists on the market at present with spironolactone being more potent on MR but less selective against other steroid receptors than eplerenone [6]. In clinical trials, the beneficial effects of spironolactone (**1**) and eplerenone (**2**) (IC_{50} = 24 and 990 nM, respectively), in a cell-based Gal4 response element controlled luciferase reporter assay in CHO-K1 cells [18]) have been demonstrated in the treatment of hypertension and heart failure. Eplerenone was more effective in lowering blood pressure compared to losartan in patients with low-renin hypertension [19]. Spironolactone when added to multidrug regimens that included a diuretic and an ACEi or ARB has also been shown to provide better than anticipated benefit in patients with resistant hypertension [20]. These results have suggested that MR antagonists are also useful as an add-on therapy for hypertension.

In heart failure, the Randomized Aldactone Evaluation Study (RALES) reported in 1999 demonstrated that a low dose of spironolactone, when added to therapy that included an ACEi, significantly improved survival [21]. The Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) investigated the addition of eplerenone to an ACEi or ARB and beta blocker in patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure. The results from this study demonstrated a significant improvement in survival and reduced hospitalization among these patients [22]. These intervention studies support a role for aldosterone in directly contributing to the development and/or progression of cardiovascular disease.

The recent publication of the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) has affirmed a broader potential for clinical use of MR antagonists. In this study, eplerenone reduced both the risk of death and hospitalization among patients with chronic systolic heart failure and mild symptoms [23]. These data also suggest that investigation of MR antagonism is warranted in other cardiovascular diseases. Ongoing studies of this therapy in patients with diastolic dysfunction and acute myocardial infarction could expand the use of MR antagonists in these additional indications [24].

In addition to their use in hypertension and heart failure, recent evidence suggests MR antagonists may also be useful in treatment of diabetic nephropathy. A recent study [25] showed that addition of spironolactone to a regimen including maximal ACE inhibition provided greater renoprotection than a maximal dose of ACEi-based monotherapy in patients with diabetic nephropathy. Additionally, it was found that the benefit did not appear to be solely dependent on reduced time-integral blood pressure (BP) burden as assessed by 24-h ambulatory BP monitoring. These studies suggest that large-scale randomized trials are needed to determine whether spironolactone or other MR antagonists added onto an ACEi-based regimen will be safe and effective for reducing the incidence of end stage renal disease (ESRD) in patients with diabetic nephropathy [25].

While both compounds show significant benefit and have become a mainstay in the therapy of cardiovascular disease, both have limitations. Spironolactone is not very selective against other steroid receptors such as AR and PR. In particular, its anti-progesterone and anti-androgen properties lead to unwanted side effects such as gynecomastia, breast pain, menstrual irregularities, and impotence, thus limiting its use [2,11]. In cells (Gal4 receptor-LBDs transfected into CHO-K1), the IC_{50} values of spironolactone for MR, GR, AR, and PR are 24, 2400, 77, and 740 nM, respectively [18]). For eplerenone, the IC_{50} values are 990, 22,000, 21,000, and 31,000 nM, respectively. The sex hormone-related side effects found with spironolactone have been significantly reduced with eplerenone most likely because of its improved AR and PR profile [6]. However, it is less potent than spironolactone, is predominantly metabolized by cytochrome P450 3A4 (CYP3A4) and coadministration with drugs that inhibit CYP3A4 may require a reduction in the dose. In addition, eplerenone has been associated with gastrointestinal intolerance [5]. The major adverse effect of MR antagonism by either spironolactone or eplerenone is an induction of clinically relevant potassium levels [2].

4. STRUCTURAL FEATURES OF THE LIGAND BINDING DOMAIN OF MR

Similar to other members of the NHR family, MR has three major functional domains, namely a N-terminal activation function 1 (AF-1) domain, a DNA binding domain (DBD), and a C-terminal activation function 2 (AF-2) domain. The AF-1 domain is the least conserved among other NHRs and possesses a ligand independent transactivation function. The DBD is the most conserved among nuclear receptor members and is responsible for mediating sequence-specific DNA binding. The ligand binding domain (LBD), containing the AF-2 domain, is also relatively well conserved

among other steroid receptors and is responsible for ligand binding, dimerization, and ligand-dependent activation. All three MR domains are highly conserved across species [3].

Among steroid receptors, MR has the highest homology to the GR in terms of primary amino acid sequence. The MR LBD consists of 251 amino acids and has high sequence homology with the AR, PR, and GR LBDs. Crystal structures have been reported for the wild-type and mutant MR bound to various steroid ligands including cortisone, progesterone, deoxycorticosterone, aldosterone, and spironolactone [26–29]. As expected from sequence homology [30], the three-dimensional structure of the MR LBD shares remarkable structural similarity to the crystal structures of the GR, AR, PR, and ER LBDs [31]. The LBD of MR consists of 11 α helices (H1, H3–H12) and two short β sheets arranged around a central hydrophobic pocket, with helices 3, 4, 5, 6, 7, and 11 providing the amino acids that line the binding pocket.

The ligand binding pocket in all of the reported crystal structures is fully enclosed. Even though the unliganded (apo form) crystal structure has not been reported, previous studies with other NHRs suggest that the ligand binding pocket of the apo form is partially exposed to solvent with H12 randomly distributed. Upon agonist binding, H12 adopts the position indicated in the crystal structures where it interacts with helices 3, 5, and 11. This forms a hydrophobic groove on the surface of the LBD enabling coactivator recruitment. The high stability of the agonist bound MR complexes has facilitated their purification and crystallization. However, the low stability of antagonist bound complexes has prevented characterization of the antagonist conformation of the LBD to date. Spironolactone bound crystal structures were solved using the S810L mutant where a single mutation converts the antagonist (for the wild-type MR) to an agonist (for the S810L mutant MR) [32–34].

The binding mode of spironolactone is depicted in Figure 1. The lactone carbonyl forms a hydrogen bond with the amide H–N of Asn-770, while the A-ring carbonyl oxygen forms hydrogen bonds with Gln-776, Arg-817, and a crystallographic water.

5. CURRENT MEDICINAL CHEMISTRY EFFORTS

5.1. Dihydropyridines

Recent efforts in screening compound collections for MR antagonist activity have led to the identification of several chemotypes including the 1,4-dihydropyridine (DHP) class of calcium channel blockers (CCBs) as MR antagonists. The original report demonstrated that these frequently used antihypertensive agents compete with aldosterone in binding to the MR

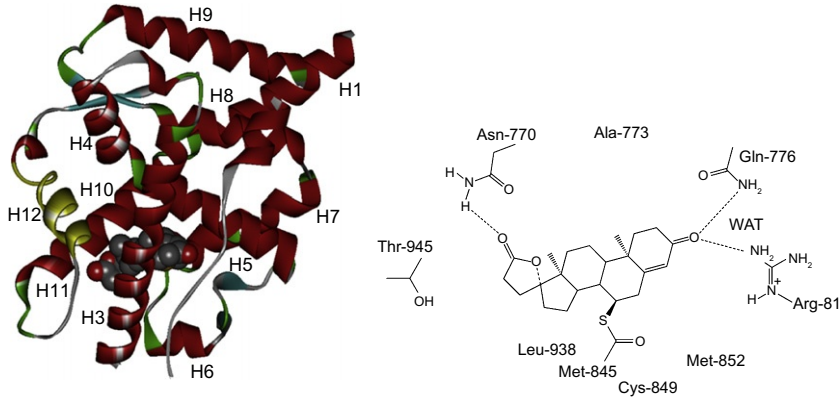
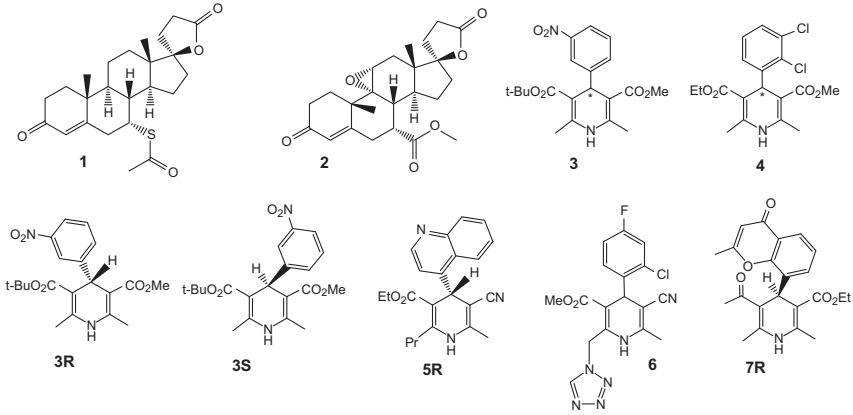


Figure 1 The 3D structure of the LBD of the MR with bound spironolactone (left) and schematic drawing of the binding mode (right).

LBD, block binding of coactivators such as SRC1-4a, and inhibit aldosterone-induced gene expression [35,36]. In these studies, several DHPs including mebudipine (**3**) and felodipine (**4**) were shown to possess similar IC_{50} values (126–450 nM) to eplerenone (IC_{50} = 135 nM, cell-based Gal4 response element controlled luciferase reporter assay in HUH7 cells). However, the DHP compounds as a class have significantly higher intrinsic potencies as CCBs compared to their MR antagonist potency. For example, the MR IC_{50} value of **3** is 126 nM (Gal4), and the IC_{50} for Ca^{2+} channel (CC) inhibition is 20 nM (thoracic rat cell line). Thus, blood levels of currently prescribed human doses of CCBs are probably not sufficient to greatly affect peripheral MR binding. However, it does suggest that a dual-acting compound may have synergistic benefits in BP control.

Although the first DHPs were achiral molecules, the search for vasodilators with a longer duration of action led to the discovery of chiral DHPs with nonidentical ester groups in the 3 and 5 positions (*e.g.*, **4**). While more recent DHPs are marketed as racemic mixtures, the absolute configuration required at the C4 position for CCB activity has been established [37]. In an effort to determine if a specific configuration is required for MR activity, a chiral resolution of **3** was performed and through evaluation of small molecule X-ray crystal structures, it was demonstrated that opposite enantiomers are responsible for MR and CCB activity [36]. Thus, the (+)-stereoisomer of mebudipine (**3R**) has an IC_{50} = 46 nM for MR inhibition (cell-based Gal4 response element controlled luciferase reporter assay in HUH7 cells) and an IC_{50} = 607 nM for Ca^{2+} channel (CC) inhibition (thoracic rat cell line). The (−)-enantiomer (**3S**) has an MR IC_{50} = 540 nM and a CC IC_{50} = 0.5 nM. Replacement of the methyl ester with a nitrile improved metabolic stability and along

with other changes, led to the discovery of **5R**. This compound has an $IC_{50} = 10$ nM in the MR Gal4 assay and is over 220-fold selective versus the CC. NHR selectivity ranges from 30× for GR to 100× for AR/PR and 1000× for ER vs MR. Pharmacokinetic (PK) data (Sprague–Dawley (SD) rats, iv 2 mg/kg) showed moderate clearance (10 mL/min/kg) and adequate half-life (6.5 h).



While there are no published X-ray co-crystal structures of DHPs with the MR LBD, a proposed structure based on both the wild-type and S810L MR crystal structures suggests that the N–H of the DHP ring forms an H-bond to Asn770 and the ester group fills the R-face hydrophobic pocket. The opposite (*S*) configuration cannot adapt an orientation that can accommodate these key interactions (Figure 2).

In another report by the same authors, further optimization of the cyano DHP series led to the identification of **6** (MR $IC_{50} = 64$ nM for

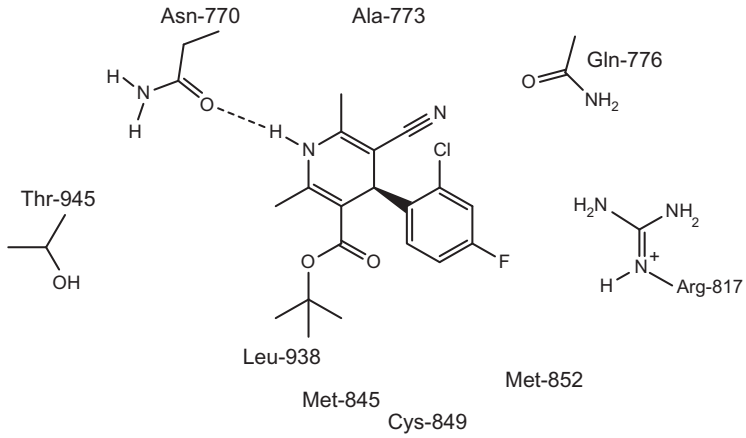


Figure 2 The proposed mode of binding for 1,4-dihydropyridines.

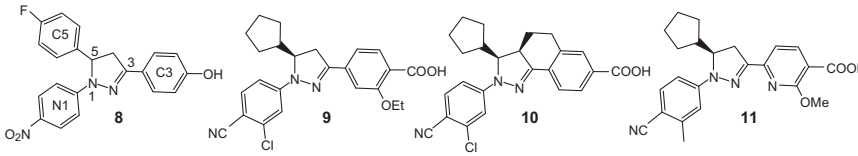
MR inhibition, cell-based Gal4 response element in HUH7 cells) which had improved aqueous solubility (31 μM), limited CYP inhibition liability (IC_{50} = 1.2, 1.9, >10 for CYP3A4, 2C9, 2D6, respectively) and rat liver microsomal stability (60% remaining after 30 min), although human liver microsomal stability was low (3% remaining after 30 min) [38,39]. NHR selectivity ranged from 20× for GR to 42× for AR and >156× for PR versus MR. The cell-based potency, NHR selectivity, and measured profiling parameters of **6R** were similar to that of racemic **6**. The CC inhibition data for **6** and **6R** were not reported. The PK parameters in SD rat (iv, 5 mg/kg, Cl = 14 mL/min/kg, $t_{1/2}$ = 4.8 h) were sufficiently favorable to advance compound **6** into *in vivo* studies. In these studies, **6** lowered systolic BP when administered at a dose of 60 mg/kg p.o, b.i.d. for 21 days to Dahl SS rats fed a high salt diet. A trend toward a reduction of albumin levels, although not statistically significant, was suggestive of protection against kidney damage.

In a related report by a different group, **7R** was identified as a highly potent, selective MR antagonist with *in vitro* and *in vivo* activity similar to spironolactone [18]. This compound had an IC_{50} = 28 nM in the MR Gal4 assay and was 70-fold selective versus the CC. NHR selectivity ranged from 195× versus GR, 160× versus AR, and 322× versus PR. Interestingly, it was shown that **7R** is a full, functional antagonist of both the Gal4-MR_{WT} LBD fusion protein and the Gal-4MR_{S810L} mutant LBD expressed in CHO-K1 cells. Both eplerenone and spironolactone were functional agonists when tested using the Gal-4MR_{S810L} mutant LBD. When dosed orally to conscious rats, **7R** increased the urinary Na^+/K^+ ratio in a dose-dependent manner, with a significant effect at the 1 mg/kg dose. Spironolactone showed a similar trend, but statistical significance was reached at a dose of 10 mg/kg. Finally, in the absence of a **7R** X-ray co-crystal structure, an Ala-scanning mutagenesis approach was used to determine critical residues for binding of **7R** to the MR LBD compared with spironolactone. For example, MR containing Gly instead of Ala^{773} and Ala instead of Thr^{945} gave IC_{50} (binding affinity) for BR-4628 of 960 and 30 nM, respectively. The IC_{50} (WT MR) of BR-4628 = 34 nM in this assay. For these same mutations, spironolactone IC_{50} = 84 and 444 nM, respectively (IC_{50} (WT MR) for spironolactone = 74 nM in this assay). These data collectively suggest that while key interactions (such as with Asn770) are critical for binding of both **7R** and spironolactone, **7R** does present a unique binding mode to the LBD compared with known steroids.

5.2. Pyrazoline derivatives

Pyrazolines represent another chemotype possessing MR antagonist activity identified *via* high-throughput screening efforts [40,41]. The screening hit **8** was found to have acceptable potency (MR IC_{50} = 460 nM)

in an aldosterone-induced activation of a luciferase reporter driven by the MR LBD in HUH7 cells. Unwanted features included high lipophilicity (aqueous solubility ≤ 3 μM) and inhibition of the hERG channel (>30% inhibition at 10 μM in a dofetilide-Cy3B competitive binding assay). Unacceptable functional groups were replaced through optimization of each position on the pyrazoline ring ultimately leading to the discovery of **9** (MR IC_{50} 6 nM) and **10** (MR IC_{50} 9 nM). Both compounds were selective for MR versus AR (>1000×) and GR (>1000×) but were less selective versus PR (335× for **9**, 46× for **10**). The stereochemistry at C5 of the pyrazoline ring was found to be critical for MR antagonist potency. Thus, for the compounds where both isomers were tested, the (*R*)-configuration was 50- to 60-fold more potent in the MR cell assay than the corresponding (*S*)-isomer.



A unique feature of these compounds is the tolerance for a charged carboxylate in the LBD of the MR. Among the functional groups tested, only the carboxylate demonstrated reduced hERG channel inhibition while maintaining potent MR antagonist activity. Through investigation of an induced fit model based on an overlay of the native MR/corticosterone crystal structure with **10**, the authors postulate that the presence of the carboxylate causes a movement of Phe941, with disruption of the Leu960 and Asn770 sidechains by the cyclopentyl group. The cyanophenyl ring is believed to occupy a similar position to the A/B ring system of steroid modulators.

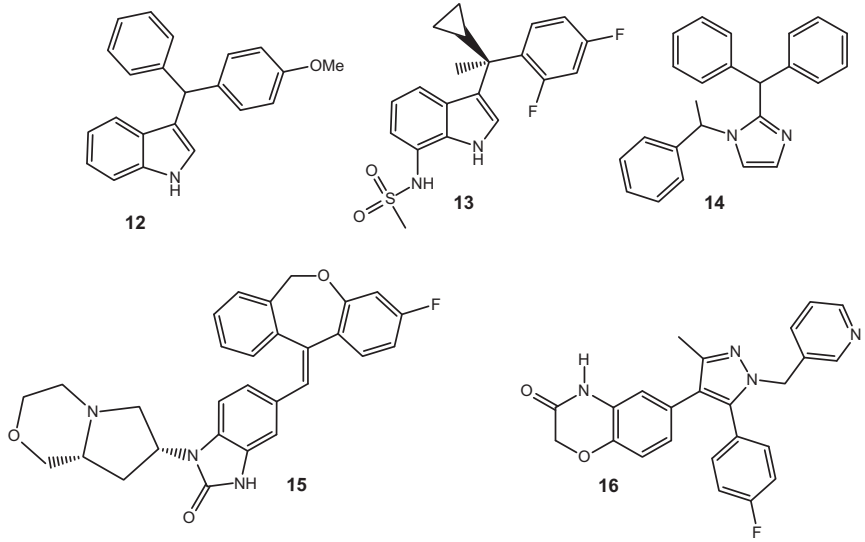
A recent patent application exemplifies **11** and close analogs containing a substituted pyridine ring with very limited biological data given [42].

Compound **10** (PF-3882845) administered at doses of 10, 40, and 100 mg/kg p.o. for 21 days to Dahl SS rats, a preclinical model of salt-induced hypertension and nephropathy, was shown to decrease urinary albumin, reduce blood pressure, and protect against kidney damage. Based on its PK properties and preclinical safety profile, compound **10** was chosen to advance into clinical studies for diabetic nephropathy.

5.3. Indole sulfonamides and related structures

Indoles represent a further MR antagonist chemotype identified through screening of in-house compound collections [43]. In this series, **12** was the starting point for structure–activity relationship (SAR) development leading to the identification of **13,** which displayed a preference for the

S stereoisomer for binding to the MR. A methyl sulfonamide was ultimately selected for substitution of the indole at the 7-position driven by SAR data that suggested the need for an H-bond donor. A dialkyl substituent revealed a narrowly defined hydrophobic pocket that appears to optimally accommodate a cyclopropyl(methyl) side chain. Finally, the 2,4-difluorophenyl substituent gave a threefold improvement in MR binding compared to the 4-difluorophenyl. Compound **13** potently binds the MR receptor ($K_i = 0.5$ nM), is a functional antagonist ($K_b = 19$ nM), and showed some improvement in NHR selectivity. In salt loaded, uninephrectomized, aldosterone-induced SD rats administered with **13** (10 mg/kg) for 14 days p.o., an 80% decrease in blood pressure compared to vehicle was observed. Eplerenone at the same dose gave a 30% decrease compared to vehicle.



In a recent report, imidazole **14**, a modest antagonist of MR with a binding K_i of 285 nM discovered as a screening hit, afforded modest changes in Na^+/K^+ ratios when administered to SD rats at a dose of 30 mg/kg s.c. followed by administration of 0.9% saline (acute mode) [44,45].

5.4. Benzimidazole derivatives

A highly selective and potent benzimidazolone MR antagonist **15** was recently reported [46]. In a MR binding assay, **15** demonstrated a K_i of 0.4 nM with >1000-fold binding selectivity versus AR, GR, and PR. In HEK293 cells singly transfected with either MR or other NHRs, **15** gave IC_{50} values of 21, 924, >10,000, and >10,000 nM for MR, PR, GR, and AR,

respectively, in antagonist mode, thereby demonstrating significant NHR selectivity. In a human MR (hMR) competitive antagonist assay utilizing HEK293 cells, **15** gave a K_b of 5.1 nM, demonstrating potent hMR antagonist activity. In an *in vivo* model of adosterone-mediated renal disease using male uninephrectomized SD rats fed a high salt diet, **15** reduced urinary protein excretion by 60% when orally administered at a dose of 10 mg/kg/day for 28 days, demonstrating durable and potent *in vivo* renal protection. In the same animal model, **15** was also shown to reduce the hypertensive effects of aldosterone (administered *via* alzet pump at a rate of 0.75 μg/h, s.c.) compared to vehicle in a dose-dependent manner when given p.o. (1–30 mg/kg) for 14 days.

5.5. Other MR antagonist structures

Over the past few years, several compositions of matter patent applications covering benzofused oxazinones as NHR modulators and, specifically, MR antagonists have emerged [47–49]. In one recent narrow composition of matter application [50], limited MR binding inhibition data on selected compounds (inhibition at 10 μM) were disclosed suggesting that these compounds possess MR antagonist activity. Compound **16** is one example. Future disclosures will shed light on the value of these compounds to the field.

6. CONCLUSIONS

The past several years have seen a resurgence in targeting MR for intervention in cardiovascular disease. Known limitations of existing therapy with the steroid-based MR antagonist eplerenone and spironolactone due to off-target effects or poor efficacy have led drug discovery groups to look for highly potent, selective nonsteroid-based antagonists.

Recent findings strongly suggest that MR antagonism can play a major role not only in lowering blood pressure but also in sparing the kidney from damage due to diseases such as diabetic nephropathy and, more broadly, chronic kidney disease. Given the high rate of occurrence of type 2 diabetes in an aging, overweight population, these diseases represent a potentially huge unmet medical need. MR antagonism has also been shown to be cardioprotective, reducing mortality rates among patients recovering from acute myocardial infarction. Hyperkalemia is a potential mechanism-based side effect of MR antagonism, and it remains to be seen whether this new generation of selective, nonsteroidal MR antagonists can demonstrate an improved therapeutic window versus the steroid MR antagonists. In summary, these nonsteroidal MR antagonists will need to

demonstrate a clearly superior profile to effectively displace current steroidal generics as standard of care for these indications.

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