Chapter

Advances in the Development of Non-steroidal Mineralocorticoid-receptor Antagonists

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Abstract

The mineralocorticoid receptor (MR) belongs to the nuclear receptor superfamily and regulates body fluid and electrolyte balance. In the last years, much effort has been put into the development of non-steroidal MR antagonists that overcome the side effects of the marketed steroid drugs, and can be used for the treatment of hypertension and heart failure, among others. Initially, MR was identified in epithelial cells, however it also plays important roles in non-epithelial tissues. In this sense, it is of interest to discover ligands that might induce different MR conformational changes, leading to specific coregulator interactions, which could confer tissue-specific effects. Different series of non-steroidal ligands with diverse central scaffolds has been described, which shows antihypertensive and cardiorenal protective effects. This review covers a description of different non-steroidal MR antagonist families, with special focus on compounds under clinical development. The analysis of the three-dimensional (3D) structures of non-steroidal MR antagonists in complex with the MR ligand-binding domain (LBD), recently reported, highlights the interactions crucial for binding. The structure-activity relationships of known ligands, together with the insights provided by the 3D structures of ligand - LBD MR complexes, could help in the development of non-steroidal MR antagonists with improved properties.

Keywords: mineralocorticoid receptor, MR antagonist, structure-activity relationship, clinical trials

1. Introduction

The mineralocorticoid receptor (MR) transduces the effects of the steroid hormone aldosterone on mineral ion homeostasis, extracellular volume, and blood pressure mainly by regulating kidney Na^+ reabsorption and K^+ and H^+ excretion [1]. In addition, MR can also act as a high-affinity receptor for glucocorticoids. In aldosterone target tissues such as the kidney distal nephron, glucocorticoid-mediated activation of MR is limited by co-expression of 11-β-hydroxysteroid dehydrogenase type II (11ßHSD2), which enzymatically limits access of glucocorticoids to the

receptor [2]. High corticosteroid circulating levels can overcome this mechanism, producing mineralocorticoid-like effects. Furthermore, MR tissue distribution is much broader than originally expected, and in many cells it is unclear if there are any mechanisms at all providing aldosterone selectivity over glucocorticoids [3]. It is thus reasonable to assume that both aldosterone and glucocorticoids can activate MR in a cell type-specific fashion.

The role of MR in blood pressure regulation and K^+ homeostasis has been therapeutically exploited using steroid analogs with competitive inhibitory activity. This strategy is commonly used for treating primary aldosteronism and additional clinical situations where a decrease in extracellular volume is advantageous, such as essential hypertension and edema associated with congestive heart failure or cirrhosis [4]. The interest in MR antagonists has greatly increased in the past two decades. The unexpectedly broad tissue distribution of MR prompted research on possible additional physiological and pathological roles for this receptor [5]. It is now clear that MR has important contributions to the development of fibrosis [6], inflammation [7], and oxidative stress [8], greatly expanding the potential role of MR in human disease. Aldosterone/MR signaling associated with high NaCl intake produces cardiac, vascular, and renal injury independent of changes in blood pressure [9]. The benefits of MR antagonists in patients with heart failure resulted in the approval of their use to treat this condition [10]. Furthermore adipocyte MR activation may be implicated in obesity and metabolic syndrome, opening new possible applications to MR antagonists [11]. New roles of MR in ocular or skin diseases have led to new uses of MR antagonists [5, 12].

Unfortunately, therapeutic interventions aimed at limiting MR actions are hampered by adverse side effects. Ligand-binding domain (LBD) sequence conservation between MR and glucocorticoid, progesterone, and androgen receptors (GR, PR, and AR) implies frequent ligand cross-reactivity. For instance, spironolactone is structurally related to progesterone and acts as a PR agonist and AR antagonist, leading to frequent adverse sexual side effects. This has largely been solved by the use of eplerenone, a second-generation MR antagonist with weaker affinity for AR and PR [13]. The use of MR antagonists is further complicated by their potassium-sparing characteristics. While this activity is desirable in the context of hypertensive patients treated with loop diuretics [14], it is associated with higher mortality in patients with heart failure [15]. Therefore, there is a clear need for developing selective MR modulators that preferably have a nonsteroidal nature and may present selective beneficial actions without undesired side effects [4].

This review aims to delineate the different chemical structural families that have led to MR nonsteroidal antagonists. For a more comprehensive data regarding different compounds in each family, a recent review by Martin-Martinez et al. can be of interest [16]. In this chapter, we also focused on those compounds that entered clinical trials and in the known three-dimensional (3D) structure of nonsteroidal compounds bound to the LBD of MR.

2. Nonsteroidal MR ligands

An active search for nonsteroidal MR antagonists has been carried out to overcome the side effects observed with steroidal drugs. In general, starting from a high-throughput screening (HTS), an initial hit compound is identified. Next, a hit to lead optimization process, quite frequently through a structure-based design, leads to compounds with improved binding affinity and pharmacokinetic (PK) properties. Examples of different nonsteroidal MR antagonist families are included in this section. In addition, to facilitate a better understanding through the

manuscript, the 2D structures of compounds under clinical trials and/or known 3D structures are described in Sections 3 and 4.

The 1,4-dihydropyridine ring (DHP) has proven to be a rather interesting scaffold and have been explored by Pfizer, Bayer Pharma, and Merck (**Figure 1**). Their studies showed the importance of the stereochemistry at DHP C4 [17, 18], as well as a free DHP amino group [18, 19]. Pfizer described a series of DHP with a phenyl group at C4, with small, nonpolar substituents like F, Cl, or CF_3 suitable at this ring [19]. Although a voluminous substituent at C2 led, in general, to lower affinity, the incorporation of tetrazolmethyl group as in **1** resulted in better physicochemical properties while maintaining potency and selectivity over other nuclear hormone receptors (NHRs, IC_{50} > 300 nM for PR, GR, and AR). Compound **1** reduces blood pressure and renal injury in rats. On the other hand, Bayer Pharma, starting from an HTS, and the subsequent optimization, identified DHP **2** (BR-4628) with a chromenone at DHP C4 as a potent MR antagonist with more that 150-fold selectivity over GR, AR and PR and a good PK profile in rats [18]. BR-4628 has been proposed to be a bulky antagonist with a passive mechanism. Docking studies shown that BR-4628 5-acetyl and C6-methyl groups protrude toward the MR helix H12. However, there is also a loss of contacts within this region compared to steroid agonist, which might account for the inability of this complex to recruit co-regulators [20]. Interestingly, for several steroid antagonists, a mechanism based on loss of contacts has been proposed with helix H12 leading to a destabilization of the AF2 region, which is involved in co-regulator interaction [21]. The moderate selectivity of BR-4628 versus L-type Ca^{2+} channels prevented further development. Afterwards, Bayer studies led to a series of heterobicyclic analogs, from which a naphthyridine derivative, named finerenone (**17**, see **Figure 5**), entered clinical trials. DHP has also been the focus of Merck, which patented a series of sub-micromolar binding affinity DHP, as derivative **3** [22].

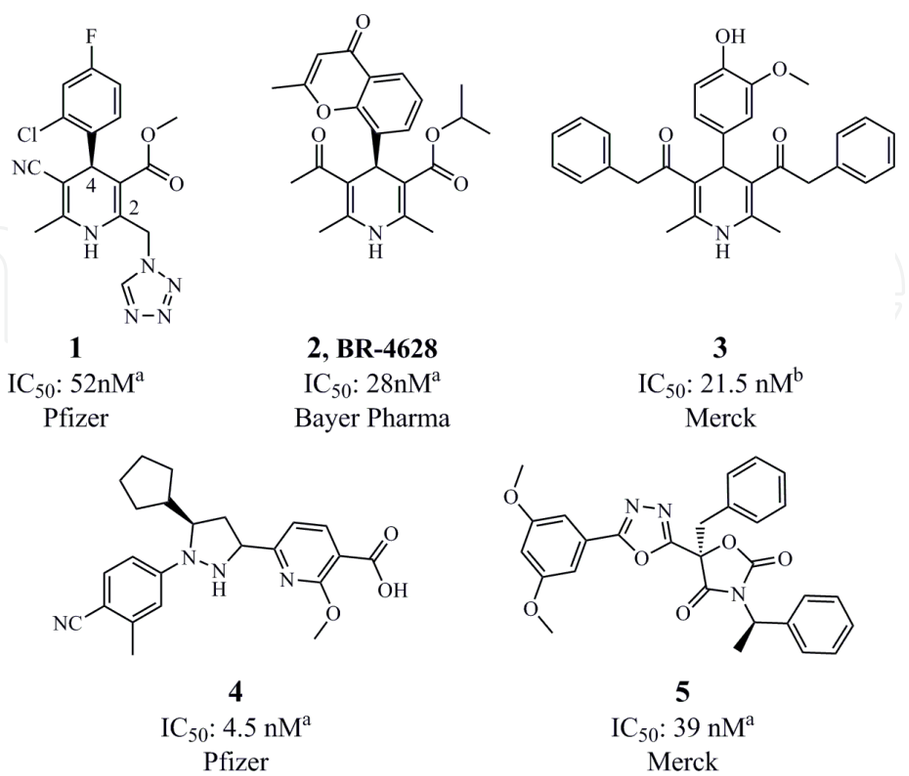


Figure 1.
DHP and five-membered heterocyclic rings as scaffolds in MR ligands. [a]Cell-based assays. [b]Competitive binding assays.

Five-membered heterocyclic rings have also proven to be quite successful moieties in the search of nonsteroidal MR antagonists. In particular, the pyrrole ring is found in a series of MR ligands developed by Exelixis and Daiichi Sankyo, such as CS-3150 that entered clinical trials (**18**, **Figure 5**) [23]. On the other hand, the pyrazoline ring has been explored by Pfizer, as in compound **4** (**Figure 1**), where the R enantiomer showed higher potency. Compound **4**, with more than 500-fold selectivity over PR, and at least 2000-fold over GR and AR, behaves as an antagonist increasing the urinary Na^+/K^+ ratio in rats [24]. The 3-phenyl-pyrazoline cyclization led to a series of conformational restricted pyrazoline derivatives, one of them entered clinical trials, PF-3882845 (**14**, **Figure 5**) [25]. Additionally, starting from an HTS, Merck identified a series of MR antagonists based on the oxazolidine-2,4-dione scaffold. Several derivatives showed potent MR affinity, as compound **5** (**Figure 1**), which has significant selectivity versus other NHRs (IC_{50}, PR, AR, GR > 5 μm) [26].

On the other hand, it is worth mentioning the benzoxazinone-derived MR ligands, which have been also broadly explored. An analysis of the X-ray crystal structure of compounds with this bicycle bound to MR LBD provided insights regarding their binding determinants to MR, as explained below (Section 4). In general, in these series there is an additional aromatic ring linked to position 6 of the benzoxazinone moiety, either through linear linkers as in Novartis compound **6** [27] or heteroaromatic rings, like in Takeda derivatives **7**, **19**–**22** (**Figures 2** and **6**) [28, 29]. Compound **7** behaves as antagonist and shows good selectivity over GR, PR, and AR (IC_{50} > 2.5 μM). It is able to decrease urinary Na^+/K^+ and has a blood pressure-lowering effect similar to that of spironolactone in a DOCA-salt hypertensive rat model. Interestingly, a carbonyl linker led to AstraZeneca benzoxazinone derivative AZD9977, which is currently in clinical trials (**15**, **Figure 5**). In a recent publication, AstraZeneca describes the thorough structure and property studies that led to the identification of this clinical candidate [30]. Additionally, a benzoxazinone derivative with a rather different pattern of substituents was developed by Mitsubishi Tanabe Pharma leading to apararenone (**16**, **Figure 5**), which is also in clinical trials .

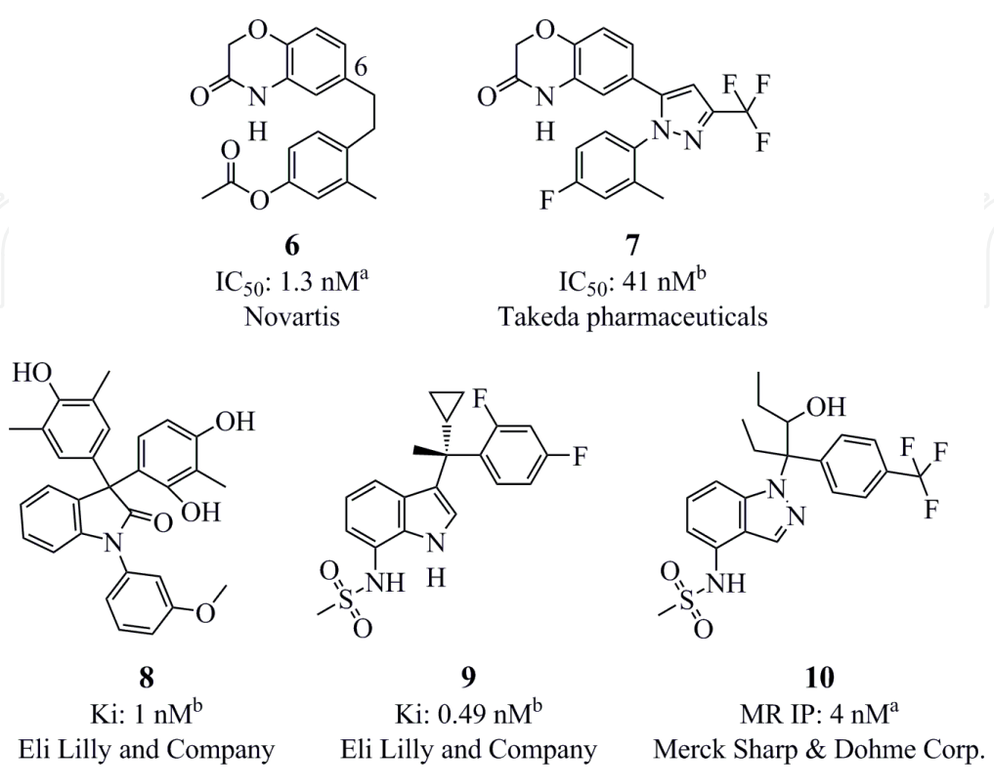
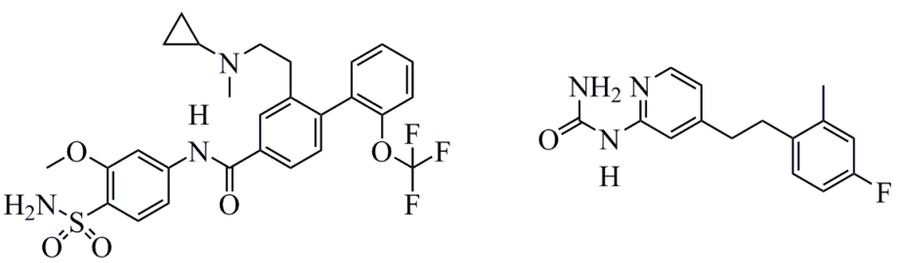


Figure 2.
Benzoxazinone-, indole-, and indazole-derived MR ligands. [a]Cell-based assays. [b]Competitive binding assays.

The indole group was also found as part of two series of potent MR antagonists identified by screening methodologies by Eli Lilly (**Figure 2**). One of them included a 3,3-bisaryloxoindol as central scaffold, where, for example, derivative **8** showed good selectivity over GR, PR, and AR (more than 390-fold) [31]. The other series contained an indole ring, as in compound **9**, which was more potent than eplerenone in lowering blood pressure in a rat hypertension model [32]. A related series containing an indazole ring and an aryl sulfonamide were developed by Merck Sharp & Dohme Corp., for example, compound **10** (stereochemistry not disclosed) that showed a good PK profile in rats [33].

Aryl sulfonamide and urea moieties were also found in compounds **11** and **12**, developed by Sumitomo Dainippon Pharma and Boehiringer Ingelheim, respectively (**Figure 3**) [34, 35]. Recently, a novel byaryl sulfonamide-based MR antagonist was identified in an HTS by AstraZeneca (compound **27**, **Figure 8**). A combination of structure–activity relationship (SAR) exploration and X-ray crystal structure determination subsequently guided the design of related MR antagonist **28** and **29** (**Figure 8**) [36].

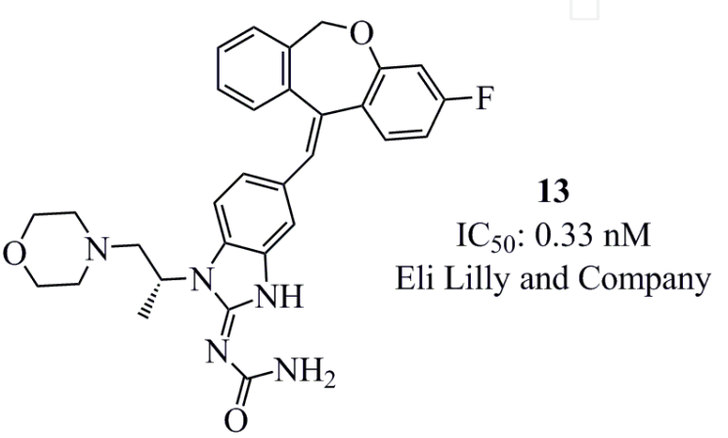
Tricyclic scaffolds have also been studied, particularly those containing a central six- or seven-membered ring flanked by differently substituted phenyl moieties. These derivatives are frequently described within patents, and for some of them only scarce pharmacological data is available [16]. Thus, Eli Lilly has developed different families with interesting properties, among them the dibenzooxepine **13** (**Figure 4**), an MR antagonist, with more than 800-fold selectivity over GR, AR, and PR. Compound **13** was studied in combination with tadalafil, a



11
IC_{50}: 22 nM
Dainippon Sumimoto Pharma

12
IC_{50}: 4.7 nM
Boehringer Ingelheim
International GMBH

Figure 3.
Aryl sulfonamide and urea functionalities in MR ligands. IC_{50} from competitive binding assays.



13
IC_{50}: 0.33 nM
Eli Lilly and Company

Figure 4.
A tricyclic scaffold in MR antagonist. IC_{50} from competitive binding assays.

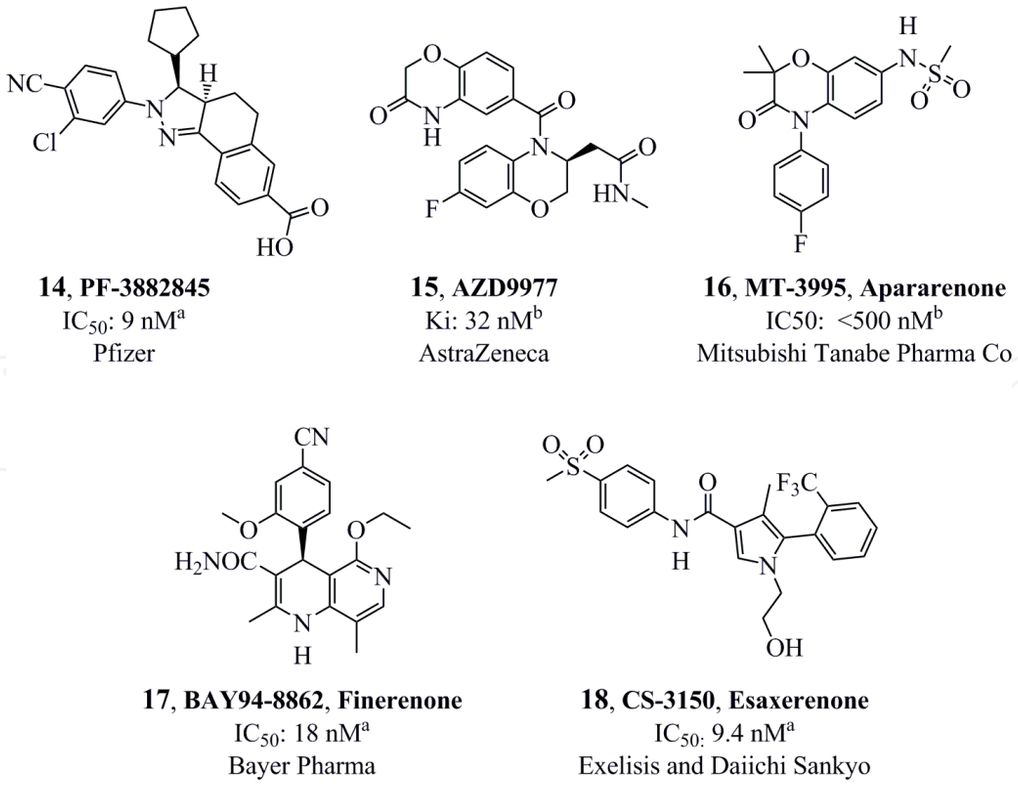


Figure 5.
MR antagonist that entered clinical trials. [a]Cell-based assays. [b]Binding-based assays.

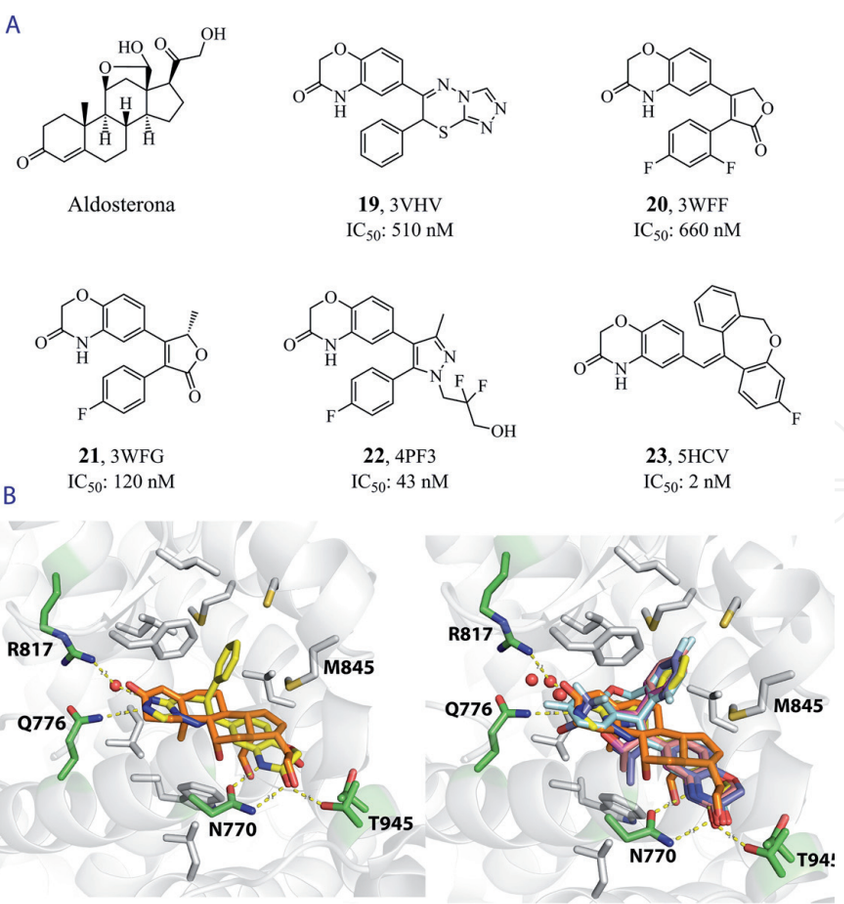


Figure 6.
2D and 3D structures of MR benzoxazine-3-one-based antagonist complexes. (A) 2D molecular structures. (B) Superimposition of the X-ray crystal structures of MR LBD in complex with aldosterone (in orange) and compound ***19*** *(in yellow) (left) and compounds* ***19*** *(in yellow),* ***20*** *(in red),* ***21*** *(in pink),* ***22*** *(in blue), and* ***23*** *(in cyan) (right) [75]. The hydrogen bonds and water molecules are depicted as yellow dashed lines and red spheres, respectively. IC_{50} from competitive binding assays.*

mild vasodilator, for the treatment of patients with resistant hypertension. These studies showed that their combination led to a greater blood pressure reduction in patients than monotherapies [37]. Some websites associated this compound with LY2623091, which entered clinical trials [38, 39]. Recently, other tricyclic derivatives, with a seven-membered heterocyclic central ring and a benzoxazinone moiety linked to this ring, have been described by Vitae Pharmaceuticals. An example of these latter derivatives is compound **23** whose X-ray crystal structure bound to MR LBD has been solved (**Figure 6**) [40].

3. Nonsteroidal MR antagonists that entered clinical trials

The effort devoted to the search of nonsteroidal MR antagonist has led, to the best of our knowledge, to seven compounds entering clinical trials [41–43]. However, two of them, namely, LY2623091 from Eli Lilly and PF03882845 from Pfizer, have been discontinued. Both derivatives are nonsteroidal MR antagonist, selective, and oral bioavailable. LY262309 entered two phase II clinical studies, for patients with chronic kidney disease (CKD) or high blood pressure [44–46], but according to Adis Insight database, this compound was discontinued [47]. PF03882845 (**14**, **Figure 5**) entered phase I clinical studies in patients with type 2 diabetic nephropathy; however the study terminated prematurely due to strategic reasons, according to Pfizer [48, 49].

Recently, AZD9977 from AstraZeneca and KBP-5074 from KBP Biosciences have entered clinical trials. AZD9977 (**15**, **Figure 5**) is a partial MR antagonist in vitro, with cardiorenal protection [50]. It separates organ-protective effects from urinary electrolyte excretion in rodent models, likely reducing hyperkalemia risk. This profile seems to be due to a different pattern of interactions with MR, particularly affecting Met777, which influences the AF2 surface. The AF2 region is key for co-regulator interaction, and a different recruitment compared to eplerenone was observed for this compound. Initial clinical studies showed that AZD9977 was safe and well tolerated; however, compared with rodents, in humans the effects on urinary Na^+/K^+ were similar to eplerenone [51]. Yet four phase I clinical trials have been completed with this compound, all in the United Kingdom, the last ones in June 2018 [52]. On the other hand, KBP-5074 is a highly selective and potent MR antagonist. Phase I and IIa studies have been completed in the United States [53]. These studies evaluated safety, tolerability, and PK in healthy volunteers and patients with CKD or renal impairment. A phase IIb trial started in April 2018 for patients with uncontrolled hypertension and advanced CKD.

To date, the most advanced compounds are esaxerenone (CS-3150), finerenone (BAY94-8862), and apararenone (MT-3995). Apararenone (**16**, **Figure 5**, **Table 1**) [42, 54, 55], developed by Mitsubishi Tanabe Pharma Corporation, has completed seven phase II clinical trials for the treatment of diabetic nephropathy; some studies include patients also with albuminuria or with albuminuria and moderately decreased in glomerular filtration rate. There is an active phase II clinical trial in patients with nonalcoholic steatohepatitis (NASH), which will be completed by April 2019.

Finerenone (**17**, **Figure 5**) is a potent, oral bioavailable MR antagonist from Bayer, with more than 500-fold selectivity over AR, PR, or GR [18]. Structural studies indicated that MR Ala773 and Ser810 are the reasons behind its selectivity. These studies also suggested that it is a bulky antagonist that inactivates MR producing a protrusion of LBD helix H12 and avoiding the recruitment of coactivators [56]. Furthermore, it has been found that finerenone modulates MR cofactor binding different from eplerenone. This selective modulation has been suggested as the molecular basis from the different clinical behavior of finerenone compared with eplerenone [57]. Interestingly, it behaves as an antagonist of S810L MR, a mutant that leads to a severe form of familiar hypertension. In preclinical models

NCT number	Phase	Enrollment	Study start/completion	Location
Efficacy and safety of MT-3995 in patients with NASH				
NCT02923154	II	40	September 2016/April 2019	Japan
Efficacy and safety of MT-3995 in patients with diabetic nephropathy				
NCT02517320	II	293	July 2015/January 2017	Japan
An extended treatment study of MT-3995 in patients with diabetic nephropathy				
NCT02676401	II	241	February 2016/August 2017[a]	Japan

[a]*Primary completion.*

Table 1.
Apararenone (MT-3995) phase II clinical trials completion after 2016 [55].

NCT number	Phase	Enrollment	Study start/completion	Location
Efficacy and safety of finerenone in subjects with T2DM and DKD				
NCT02540993 FIDELIO-DKD	III	4800	September 2015/October 2019	Global
Efficacy and safety of finerenone in subjects with T2DM and the clinical diagnosis of DKD				
NCT02545049 FIGARO-DKD	III	6400	September 2015/February 2020	Global
Efficacy and safety of oral doses of BAY94-8862 in subjects with T2DM and the clinical diagnosis of diabetic nephropathy				
NCT01874431 ARTS-DN	II	823	June 2013/August 2014	Global
NCT01968668 ARTS-DN Japan	II	96	October 2013/November 2014	Japan
Phase IIb safety and efficacy of different oral doses of BAY94-8862 in subjects with worsening chronic heart failure and left ventricular systolic dysfunction and either T2DM with or without CKD or CKD alone				
NCT01807221 ARTS-HF	II	1058	June 2013/December 2014	Global
NCT01955694 ARTS-HF-Japan	II	72	November 2013/February 2015	Japan
BAY94–8862 dose finding trial in subjects with chronic heart failure and mild (part A) or moderate (part B) CKD				
NCT01345656 ARTS	II	458	May 2011/July 2012	Global

Table 2.
Finerenone (BAY94-8862) phase III and phase II ARTS clinical trials [68].

finerenone showed better cardiorenal end-organ protection than spironolactone or eplerenone [58]. Its good properties prompted to further advance it to clinical trials (**Table 2**). In a phase II trial (mineralocorticoid receptor antagonist tolerability study (ARTS)) including subjects with chronic heart failure and mild or moderate CKD, finerenone was at least as effective as spironolactone in decreasing ventricular remodeling but with lower incidence of hyperkalemia and renal adverse effects [59, 60]. The fact that finerenone distributes equally between cardiac and renal tissues in rats, whereas spironolactone and eplerenone show a higher kidney

accumulation, might explain the lower incidence of hyperkalemia. The lower accumulation, together with its minimal renal elimination, might open opportunities for the treatment of patients with renal impairment [61, 62]. A subsequent phase II study (ARTS-diabetic nephropathy (ARTS-DN)) analyzed the safety and efficacy of finerenone in subjects with type 2 diabetes mellitus (T2DM) and diabetic nephropathy. In this study the urinary albumin to creatinine ratio (UACR) decreased in patients treated with finerenone compared to placebo, with no significant differences in adverse effects observed between both groups [63, 64]. In the ARTS-heart failure trial (ARTS-HF), different doses of finerenone and eplerenone were compared for patients with worsening heart failure with concomitant T2DM and/or CKD. Finerenone (10–20 mg dose) showed better outcome, including death, cardiovascular hospitalization, or emergency visit [65]. There are two large trials on going (FIDELIO-DKD, FIGARO-DKD) including subjects with T2DM and diabetic kidney disease (DKD) at doses of 10 or 20 mg of finerenone.

Recently, the administration of finerenone to a rat model of metabolic syndrome (Zucker fa/fa) showed that finerenone exerted cardiac protection, as it has been previously described for spironolactone. However, only finerenone afforded renal protection [66, 67].

Esaxerenone (**18**, **Figure 5**) is a highly potent and selective MR antagonist, with at least 1000-fold higher selectivity over AR, PR, or GR. It has also long-lasting oral activity, longer than steroidal drugs [23]. In addition, it has shown antihypertensive and cardiorenal protective effects in Dahl salt-sensitive hypertensive rats with superior potency than spironolactone or eplerenone and no apparent hyperkalemia [69]. The similar balanced distribution of esaxerenone to the kidney and heart in rats might be the reason of its higher organ-protective effects than marketed drugs. A subsequent study was performed with a model of hypertensive rats, based on a synthetic mineralocorticoid, deoxycorticosterone acetate (DOCA), that induces hypertension and renal injury in combination with salt loading (DOCA rats). In this model, esaxerenone was able to prevent hypertension and the development of renal damage. It has also been suggested that its beneficial actions on renal injury cannot

NCT number	Phase	Enrollment	Study start/ completion	Location
Study of CS-3150 in patients with severe hypertension				
NCT02808026	III	20	June 2016/February 2017	Japan
Study of CS-3150 in hypertensive patients with type 2 diabetes and albuminuria				
NCT02807974	III	51	June 2016/March 2017	Japan
Study of CS-3150 in combination with ARB or ACE inhibitor in hypertensive patients with moderate renal impairment				
NCT02807987	III	58	June 2016/May 2017	Japan
Long-term study of CS-3150 as monotherapy or in combination with other antihypertensive drug in Japanese patients with essential hypertension				
NCT02722265	III	368	March 2016/July, 2017	Japan
Study of CS-3150 in patients with essential hypertension				
NCT02890173 ESAX-HTN	III	1001	September 2016/July, 2017	Japan

Table 3.
Esaxerenone (CS-3150) phase III clinical trials [72].

be only attributed to its antihypertensive effect but also to direct renal protection through antifibrotic, anti-inflammatory, and antioxidant actions. Interestingly, this compound is also able to restore the established renal damage in DOCA rats [70]. Esaxerenone was identified by Exelixis' research collaboration with Daiichi Sankyo. In 2006 they signed an agreement in which Daiichi Sankyo was granted a worldwide license. To date, five phase III studies have been completed related to hypertension, some of them in addition to type 2 diabetes and albuminuria or moderate renal impairment. The combination with other antihypertensive therapies has also been studied (**Table 3**). In February 2018, Exelixis announced that Daiichi Sankyo had submitted a regulatory application to the Japanese Pharmaceutical and Medical Devices Agency for esaxerenone to be approved for the treatment of hypertension [71].

4. Structural determinants for nonsteroidal MR antagonists binding to MR LBD

To the best of our knowledge, to date 12 X-ray crystal structures of nonsteroidal ligands bound to MR LBD have been solved. Nine of these reported structures correspond to MR antagonists within the benzoxazinone moiety derivatives class (**Figures 6A** and **7A**) (PDB IDs: compound **15**, 1.82 Å 5MWP [50]; **19**, 1.35 Å 3VHV [28]; **20**, 2.05 Å 2WFF [29]; **21**, 1.40 Å 3WFG [29]; **22**, 1.10 Å 4PF3 [73]; **23**, 2.5 Å 5HCV [40]; **24**, 1.54 Å 6GEV [30]; **25**, 1.8 Å 6GG8 [30]; and **26**, 1.71 Å 6GGG [30]), whereas the remaining three correspond to a sulfonamide aryl moiety (PDB IDs: **27**, 1.86 Å 5L7E; **28**, 2.01 Å 5L7G; and **29**, 2.12 Å 5L7H) (**Figure 8A**) [36].

The X-ray crystal structure of $MR_{C808S/S810L}$-LBD double mutant in complex with compound **19** first revealed the binding mode of benzoxazine-3-one derivatives. The NH group and the carbonyl oxygen of this moiety form hydrogen bonds to Asn770. In addition, the nitrogen atoms of the triazole scaffold establish hydrogen bonds to residue Gln776 and through a water molecule to Arg817 (**Figure 6B**). Similarly, aldosterone, the main MR hormone, is also engaged in hydrogen bonding with both Gln776 and Arg817 through its C3-ketone moiety, as well as with Asn770 through its C21-hydroxyl group (**Figure 6**) [74].

On the other hand, compound **20**, which was reported later [29], also forms hydrogen bonds to Asn770 through the NH group and the carbonyl oxygen of benzoxazine as described for compound **19**. In addition, compound **20** forms a new hydrogen bond to Thr945 through the carbonyl oxygen of the benzoxazine-3-one moiety (**Figure 6B**). The carbonyl oxygen in the dihydrofuran-2-one scaffold establishes hydrogen bonds to residues Arg817 and Gln776 through a water molecule. Overall, compound **20** binds to MR LBD in a similar way as compound **19**. Based on these structural considerations, compound **21**, a dihydrofuran-2-one derivative, was subsequently developed. As expected, the binding mode of compound **21** was similar to that of compounds **19** and **20** (**Figure 6B**).

Later, compound **22**, a benzoxazine-3-one derivative with an azole central ring as core scaffold, was developed [73]. This molecule is a highly potent MR antagonist and also shows remarkable selectivity over other steroid hormone receptors. In addition, it exhibits good PK profile and very low partial agonistic activity [3]. The azole central ring of compound **22** was selected to avoid the formation of water-mediated hydrogen bonding networks, which is known to contribute to partial agonistic activity of some previously reported benzoxazine-3-one derivatives [29, 73]. As expected, the binding mode of compound **22** is similar to that of compounds **19–21**, as observed by the solution of the X-ray crystal structure of its complex with MR LBD (PDB ID: 4PF3) (**Figure 6B**). The NH group and the carbonyl oxygen of the benzoxazine-3-one moiety form hydrogen bonds to Asn770 and Thr945, and the 4-fluorobenzene ring

occupies the α-face hydrophobic pocket. The ligand 2,2-difluoropropyl-3-hydroxy moiety points out toward the residues Gln776 and Arg817. Its hydroxyl group directly forms a hydrogen bond to Gln776. The two fluorine atoms do not form any specific hydrogen bonding interactions suggesting that the major contribution of these fluorine atoms to the binding is hydrophobic.

In 2016, Vitae Pharmaceuticals developed compound **23**, a benzoxazine-3-one derivative with a tricyclic scaffold. The X-ray crystal structure of compound **23** in complex with MR LBD showed again a binding mode similar to compounds **19**–**22**. Similarly, the benzoxazinone moiety is engaged in three hydrogen bonds, two with Asn770, through its NH and CO groups, and another one with Thr945 through its carbonyl oxygen (**Figure 6B**). The tricyclic structure does not engage in any hydrogen bonding with MR LBD [40].

Recently, compound **15** (AZD9977) has been identified as a novel and selective benzoxazine-3-one-based partial MR antagonist [50]. The X-ray crystal structure of compound **15** in complex with $MR_{C808S/C910S}$-LBD double mutant revealed the molecular determinants of its high affinity and selectivity for MR (5MWP). Likewise, compound **15** benzoxamide moiety is also involved in hydrogen bonds with Asn770 and Thr945, whereas compound **15**'s amide extension forms hydrogen bonds with

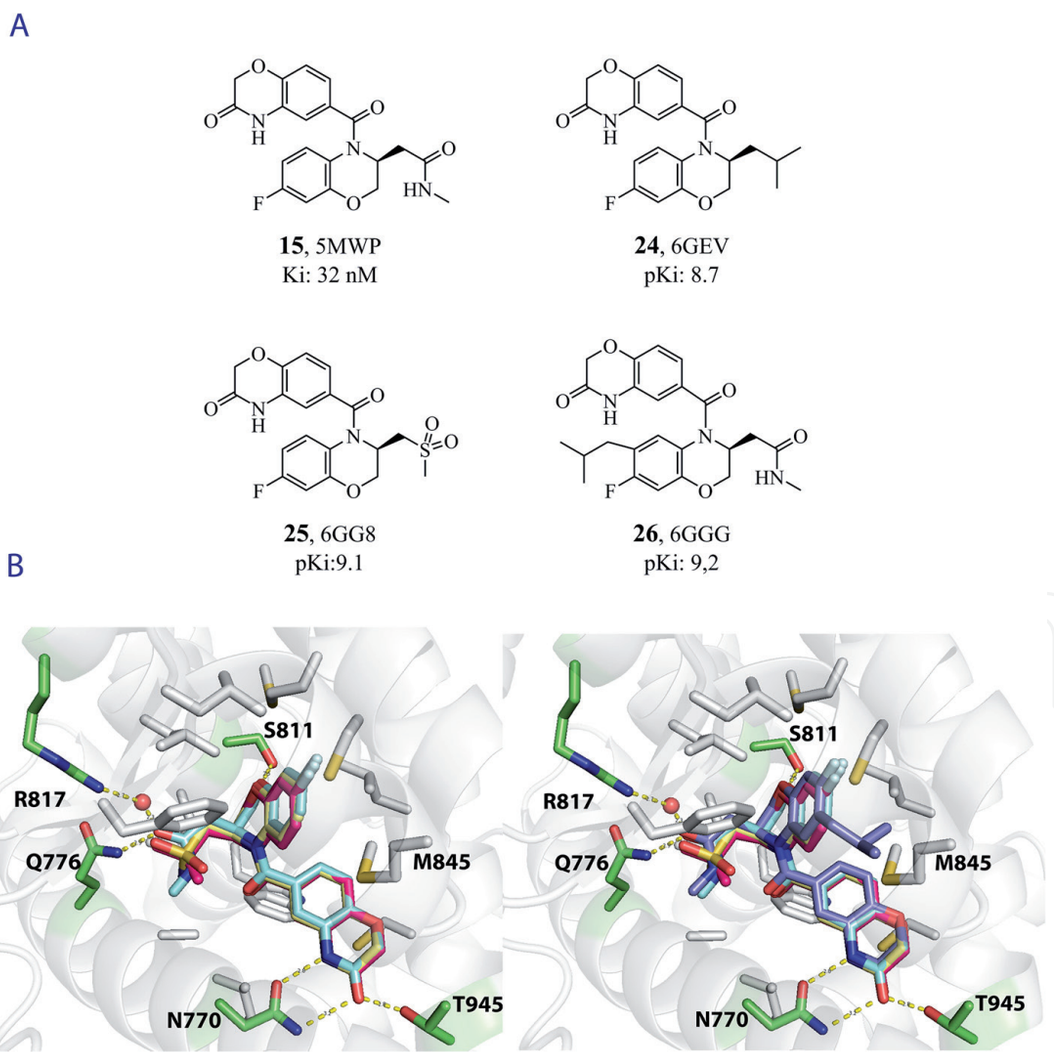


Figure 7.
*2D and 3D structures of AstraZeneca benzoxazine-3-one-based antagonist complexes. (A) 2D molecular structures. (B) Superimposition of the X-ray crystal structures of MR LBD bound to compounds **15** (in cyan), **24** (in red), and **25** (in light yellow) on the left and **15** (in cyan), **24** (in red), **25** (in light yellow), and **26** (in purple) on the right [30, 50]. The hydrogen bonds and water molecules are depicted as yellow dashed lines and red spheres, respectively. Ki from competitive binding assays.*

Gln776, Arg817, and Ser810 (**Figure 6B**). An ordered water molecule is also found in the ligand-binding pocket, but it does not seem to mediate hydrogen binding between this compound and MR LBD. The 3,4-dihydro-2H-1,4-benzoxazine oxygen interacts through hydrogen bonding with Ser811. Within the steroid receptor family, Ser811 is unique to MR, which might contribute to the selectivity of compound **15**. The identification of **15** was guided by structure-based design, and in this process three X-ray structures were solved, namely, those of MR LBD bound to derivatives **24**, **25**, and **26**. As it is shown in **Figure 7**, the binding mode of these compounds is rather similar. However, as expected, derivative **24** is not able to interact with Gln776 or Arg817. On the other hand, the isobutyl substituent of **26** causes a rearrangement resulting in an extension of helix H7 and a reposition of helix H6 [30].

Regarding the sulfonamide aryl-based nonsteroidal MR antagonists, in 2017, Nordqvist et al. solved the first X-ray crystal structure of this scaffold-containing derivatives through hit compound **27** in complex with MR LBD (PDB ID: 5L7E) [36]. The 3D structure revealed that the isoxazole is located close to residues Gln776 and Arg817 (**Figure 8**). On the other side of the ligand-binding pocket, compound **27**'s sulfonamide NH also interacts directly with Asn770 through hydrogen bonding. One of the sulfonamide oxygen atoms interacts with both Asn770 and Thr945 through water-mediated hydrogen bond. Interestingly, compound **27** folds back upon itself, pivoting on the sulfonamide motif, to form an intramolecular packing interaction between the two phenyl moieties acquiring a U-shaped binding mode. Following the superimposition of its X-ray crystal structure and that of

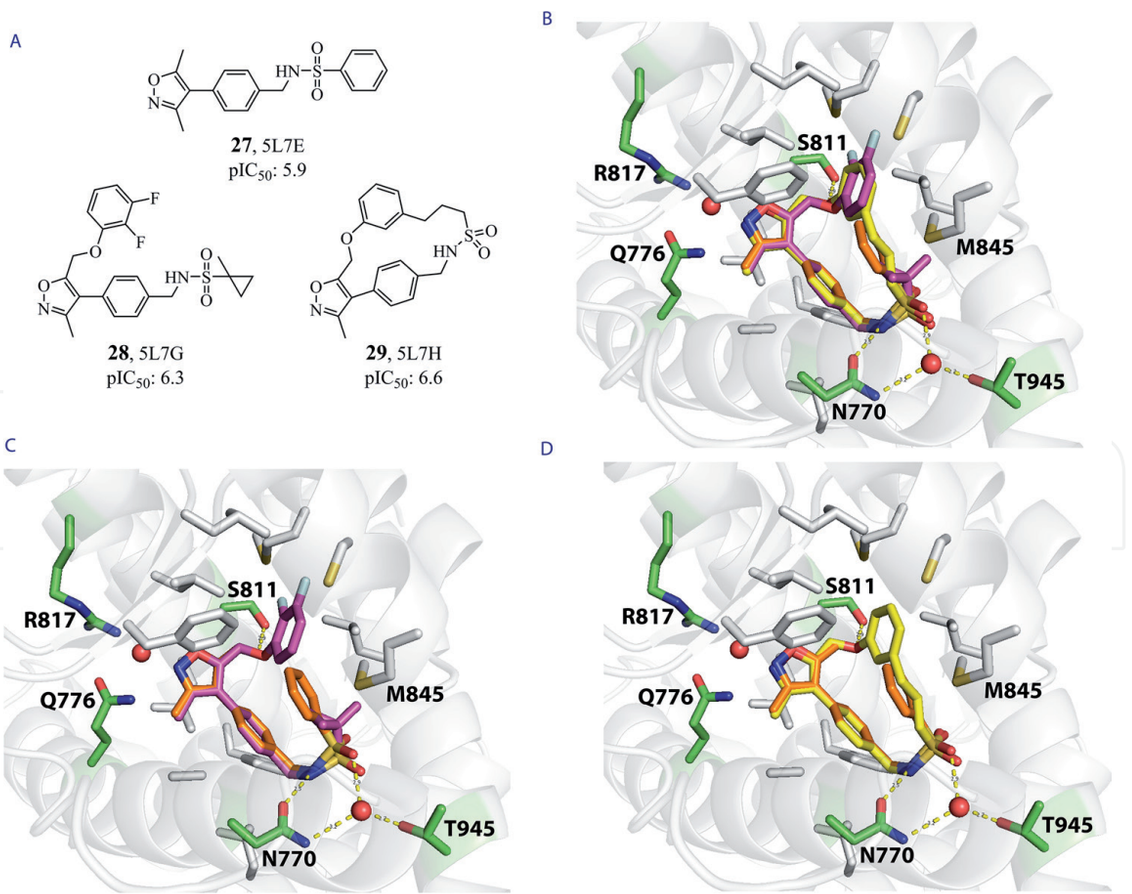


Figure 8.
*2D and 3D structures of MR sulfonamide aryl-based antagonist complexes. (A) 2D molecular structures. Superimposition of the X-ray crystal structures of MR LBD in complex with (B) compounds **27** (in orange), **28** (in magenta), and **29** (in yellow), (C) compounds **27** and **28**, and (D) compounds **27** and **29** [75]. The hydrogen bonds and water molecules are depicted as yellow dashed lines and red spheres, respectively. IC_{50} from human MR reporter gene assays.*

compound **21** (3WFG), it was reasoned to expand the ligand from the isoxazole 5-methyl substituent toward the area around Met852 which is occupied by a phenyl ring of compound **21**. Thus, compound **28** was developed with increased binding affinity toward MR and selectivity over PR and GR. The X-ray crystal structure of MR LBD and compound **28** complex (5L7G) verified that the proposed isoxazole orientation of hit compound **27** was retained and the side chain of Met852 moved to accommodate the 2,3-difluorophenoxymethyl moiety (**Figure 8C**). Afterwards, a deeper analysis of ligand binding modes inspired the design of compound **29**, the first potent macrocyclic oxosteroid receptor antagonist. Despite the conformation constraints imposed by the macrocyclization, the X-ray crystal structure of the complex between MR LBD and compound **29** (5L7H [36]) disclosed that the ligand interactions to the receptor stayed the same as those exhibited by compounds **27** and **28** (**Figure 8D**). It is worth noting that the water molecule mediating hydrogen bond between compounds **27**–**29** and Ans770 and Thr945 is displaced by the benzoxazine scaffold in compounds 15 and **19**–**25** (**Figures 6** and 7). For these latter compounds, the benzoxazine scaffold forms a bidentate hydrogen bonding with Asn770 and an additional hydrogen bond with Thr945.

5. Conclusions

Since 1959 in which the steroid MR antagonist spironolactone was introduced in the market [42], continuous research has been carried out in attempting to overcome the undesired side effects of this drug. Eplerenone, a second-generation MR antagonist, although more selective for MR, still increases the incidence of hyperkalemia. For this reason, the research was turned toward a third generation of compounds, comprising nonsteroidal antagonists within different chemotypes, which show in general lower side effects. Several examples of this third generation have entered clinical trials as compiled in this review. However, in spite of the last decades of advances, there are still important questions that need further research. Thus, for example, the structural requirements needed for ligands to discriminate the recruitment of different co-regulators and, hence, to fine-tune the transcription of selected genes are still poorly understood. Undoubtedly, a larger body of knowledge in this field will contribute significantly to the future development of novel MR antagonists with improved properties.

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