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Perspective

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Mineralocorticoid Receptor Antagonists for the Treatment of Hypertension and Diabetic Nephropathy

Miniperspective

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INTRODUCTION

Since the discovery of aldosterone over 50 years ago, many facets of its biological action have been elucidated.[1] Aldosterone is the primary endogenous agonist ligand for the mineralocorticoid receptor (MR), which is a regulator of sodium reabsorption in the kidney. The physiological disease state, that is, the action of excessive levels of aldosterone on the MR, results in conditions such as congestive heart failure, hypertension, and chronic kidney disease.[2] Cortisol can, in a disease-state dependent manner, also activate the MR. For example, in the cases of congestive heart failure and essential hypertension, cortisol levels appear to play an important role.[3] The MR, encoded by the gene NR3C2, is a steroid nuclear hormone and part of the greater nuclear hormone receptor (NHR) superfamily.[4] It is a ligand-dependent transcription factor that binds to mineralocorticoid response element to regulate gene transcription. The knowledge gained in the past few decades has revealed the beneficial effects of treatment with steroidal MR antagonists. New nonsteroidal agents that can diminish the harmful effects of excess aldosterone through blockade of the MR are being sought for their potential cardiovascular and renal protective effects. Reviews on MR antagonists[5] and other drugs that exert their action through the renin−angiotensin−aldosterone system (RAAS) have recently been published.[6] Several articles in a full issue of *Molecular and Cellular Endocrinology* are devoted to mineralocorticoids and the mineralocorticoid receptor[7] that give a historical perspective on aldosterone and MR[7a] and cover the recent progress on structural determinants of ligand binding,[7c] function, and tissue selectivity. Furthermore, with an increased understanding of the pharmacology of current MR antagonists, a greater appreciation of its role in disease has emerged.[7] With these recent reviews in mind, this Miniperspective will focus primarily on the medicinal chemistry aspects of the identification and optimization of MR antagonists.

NHRs can pose a significant challenge to the medicinal chemist. The requirement to maintain high selectivity and balance of agonist/antagonist activity is a screening intensive venture. A strict monitoring of ADME and physicochemical properties will then provide the highest probability of maintaining high potency, good selectivity, low off-target pharmacology, solubility, and safety. In addition to controlling physical properties as a guide to safer molecules,[8] understanding the nature of structurally related receptors, progesterone receptor (PR), androgen receptor (AR), estrogen receptor (ER), and glucocorticoid receptor (GR) is the next most important task for the medicinal chemist. In terms of sequence homology, GR, PR, and AR are each closer to one another compared to MR in terms of both the full-length sequence and for the cofactor binding helices in the ligand binding domain (LBD). However, for the LBD for which several cocrystal structures have been published and where the medicinal chemist would turn for structure-based information, the homology between GR and MR is closest and AR appears most different.[9] A more detailed discussion of the impact of structural knowledge on function and design will be addressed later.

STEROIDAL DRUGS

Two mineralocorticoid antagonists, spironolactone and eplerenone,[10] are marketed agents currently used for congestive heart failure and as antihypertensives. In addition, drospirenone is a progestogen with anti-mineralocorticoid properties (see Figure 1).[11] The former agents have side effects such as gynecomastia,

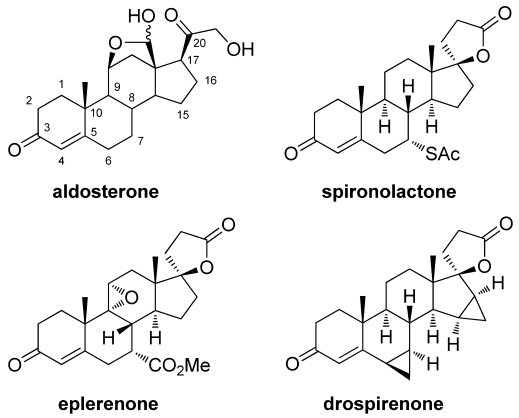

Figure 1. Aldosterone and related marketed steroidal antagonists.

menstrual irregularities, testicular atrophy, or hyperkalemia that have limited their use. For example, a likely explanation for the adverse effects of spironolactone is the lack of selectivity for the mineralocorticoid receptor, primarily from undesired action at androgen receptors.[12] This highlights the need for an effective screening funnel that includes a thorough assessment of the relative affinity of all new MR antagonists over related steroid hormone receptors to avoid anti-androgen and progestogen activities. Spironolactone is extensively metabolized to sulfur-containing products (7-α-(thiomethyl)spirolactone and 6-β-hydroxy-7-α-(thiomethyl)spirolactone) as well as canrenone. Each of the metabolites has a terminal half-life of >12 h, while the half-life for spironolactone is about 1.5 h. It is believed that the

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mixture of spironolactone and metabolites contributes to the therapeutic effect of the drug. The combination of active metabolites with longer half-lives, high potency, and 90% plasma protein binding contributes to a reasonable dose (typical dose for hypertension, 25 mg). As such, the receptor selectivity issue pointed toward the path to discovery of eplerenone.[9] Key structural changes, namely, incorporation of a C9−C11 epoxide and use of a C7 ester, into eplerenone resulted in increased selectivity that served to minimize undesired side effects but also result in a 20- to 30-fold reduction in affinity for human MR. The loss in affinity corresponds to a compound that is 50−75% as potent as spironolactone in vivo. Upon oral dosing, eplerenone is well absorbed with a half-life 3.8 h. An inactive ring-opened lactone metabolite that is chemically and enzymatically interconvertible with eplerenone exists in equilibrium. While less potent than spironolactone, the favorable physical properties (MW = 414.5, log D = 1) and lower plasma protein binding (49%) counterbalance the lower potency to still allow for a reasonably low dose (typical dose for hypertension, 50 mg, once or twice daily).[13] In the eplerenone heart failure efficacy and survival study (EPHESUS) there was reduced morbidity and mortality and effective blood pressure lowering in heart failure patients.[14,15] In general, a lower incidence of sex hormone related side effects have been noted with eplerenone because of the improved selectivity profile. However, spironolactone and eplerenone are associated with hyperkalemia.

HYPERKALEMIA

The normal serum level of potassium is 3.5−5 mequiv/L. Hyperkalemia is defined as a condition in which serum potassium is >5.5 mequiv/L. The combination of reduced renal function and the use of other RAAS drugs often puts patients with renal insufficiency, diabetes mellitus, and advanced heart failure at higher risk of hyperkalemia.[16] Aldosterone acts in the renal cortical collecting ducts to effect reabsorption of sodium and water in exchange for potassium. Blocking the action of aldosterone can cause hyperkalemia, which is an established adverse effect for spironolactone and eplerenone. Thus, monitoring of serum and dietary sources of potassium and the potential for simultaneous use of a loop diuretic can be considered. Several possible mechanistic explanations ranging from reduced aldosterone availability, insulin induced shift of potassium from intracellular space, and a predisposition of electrolyte disturbances have been offered for the increased risk of hyperkalemia in diabetic patients, but the causes are still not well understood. An analysis of the risk of hyperkalemia with add-on mineralocorticoid antagonist therapy was the subject of a recent review.[17]

STEROIDOGENESIS HYPOTHESIS

Another complicating factor with the use of steroid based agents is the potential for inhibition of the steroidogenesis pathway. It is known that spironolactone can bind to and inhibit steroidogenic cytochrome P450 enzymes.[18] It has recently been demonstrated that in human adrenocortical H295R cells, spironolactone inhibited steroid production while eplerenone had no effect on production of aldosterone or cortisol in this system. Furthermore, the metabolic instability of steroids in the presence of CYPs can affect the magnitude and duration of action and can alter their pharmacological and toxicological profiles in relevant organs.[19] These results suggest that the nonspecific actions of spironolactone not only are inhibition of androgen receptor but also include an effect on steroid hormone biosynthesis. On the other hand, eplerenone has an improved selectivity profile with respect to other steroidal receptors. In H295R cells, eplerenone did not appear to inhibit the steroidogenesis pathway at pharmacological concentrations.[20]

STRUCTURAL INFORMATION FROM STEROIDS

The use of correspondence analysis,[21] homology modeling,[22] induced fit docking,[23] and crystallography[24] has been used to understand the binding mode, to exploit differences across steroid receptors, and as a means to begin to predict selectivity. Structural information currently available to the medicinal chemist for most receptors, especially MR, is limited to the LBD. A cursory examination of the LBDs reveals that they are largely defined by mobile hydrophobic residues that can adopt various conformations when interacting with lipophilic ligands. Another common feature of the LBD pocket is the presence of polar residues that lock the ligand in via a hydrogen bond network. The two hydrophilic groups on opposite ends of the steroids are able to hydrogen-bond to polar side chains in the pocket.[25] Specifically for MR, Gln776 from H3 helix and Arg817 from H5 helix make hydrogen bonds with the 3-keto group, which is contained in nearly all MR binding steroids (see Figure 2). The Gln776 residue appears to be essential for ligand binding

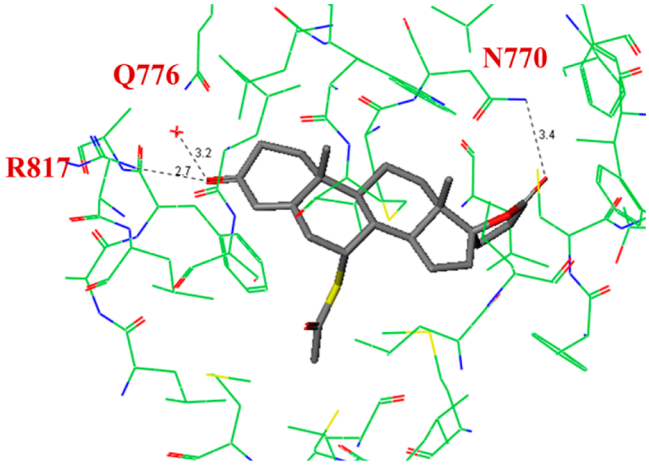

Figure 2. Cocrystal structure of spironolactone in MR (2OAX).

based on mutation data.[26] Although related steroid 5-α-pregnan-20-one does not contain a C-3 carbonyl group, it has been shown to be an antagonist of MR S810L.[27] At the D-ring end of the steroid, key hydrogen bonding residues are Thr945 from H11 helix and Asn770 from H3 helix. From most of the current data it appears that the latter residue is critical for stabilizing the agonist conformation of the LBD, since ligands, such as progesterone, that do not establish this contact are functional antagonists.[5] A distinguishing feature of MR is the existence of a pocket occupied by the C7 group of spironolactone (-SAc). Induced-fit docking of eplerenone into the MR S810L receptor showed the C7 ester in this same pocket. This pocket is lined with several hydrophobic (Leu, Phe, Met) residues. Despite significant effort by several companies, a steroidal compound that is more selective and equal to or more potent than the spironolactone has not successfully progressed through clinical trials. Over the past decade, there has been an effort across several companies to identify nonsteroidal agents with higher potency, selectivity, and safety compared to their steroidal counterparts.

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Chart 1. Physicochemical Property Space of NHRs and a Marketed MR Steroid

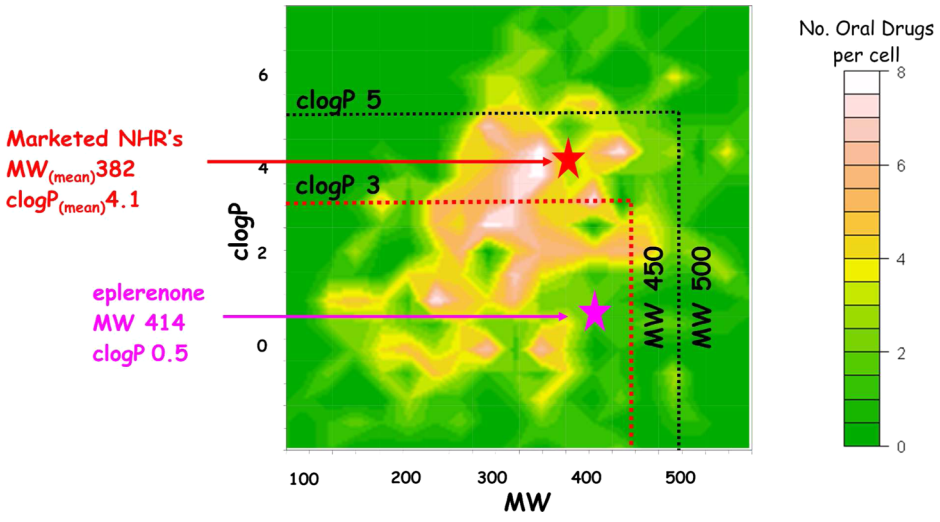
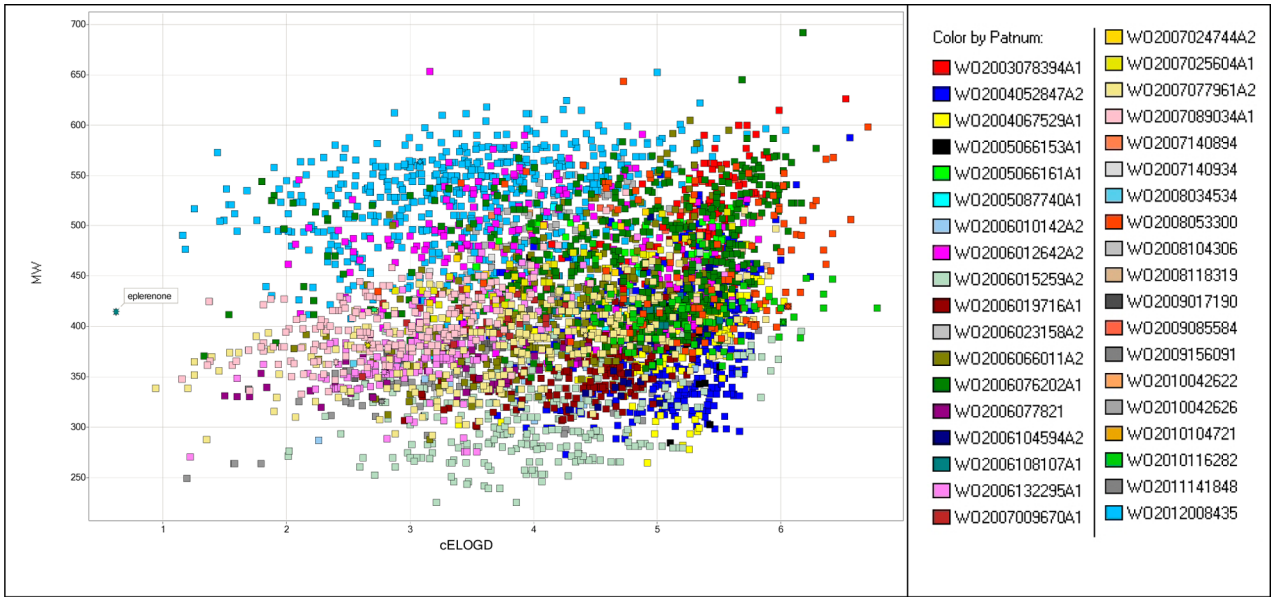


Chart 2. MW vs cElogD of Exemplified MR Compounds from the Patent Literature



NONSTEROIDAL MOLECULES

Additional chemotypes as well as new details and variants of known chemotypes have been reported since the last reviews of nonsteroidal agents.[28] An important consideration for the design of new nonsteroidal compounds should include a thorough evaluation of expected physical chemical property space. One should not only consider the nuclear hormone target class but also include lessons from the marketed steroids and all chemotypes from the patent literature. On the basis of reported data connecting target and required physical properties, a few trends appear to be emerging.[29] The analysis by Sutherland and Vieth showed that marketed NHR ligands have a mean MW of 382 and a mean clogP of 4.1. Not all of the compounds in the NHR set are neutral molecules. Therefore, an evaluation the same set of NHR compounds taking ionization state into account was also done. Prediction of the log *D* using a statistical model based on Pfizer experimental log *D* data provided a mean cElogD of 3.8. In a similar vein, Morphy's analysis showed that optimized analogues from the NHR target family had among the highest median clogP, with a mean clogP of 5.0 for NHR antagonists. An increase in MW was observed during the optimization of compounds from all the families.[30] The physicochemical properties of both spironolactone (MW = 416.6, cElogD = 2.7) and eplerenone (MW = 414.5, cElogD = 0.6) are on the high side and low side of the mean NHR MW and NHR cElogD, respectively. Chart 1 pictorially depicts Sutherland and Vieth data highlighting where the average NHR compound and the optimized drug eplerenone lie with respect to other oral drugs. The significantly lower cElogD for eplerenone coupled with its relatively high free fraction (~50%) makes these suitable properties worthy of greater attention in the design of new nonsteroidal antagonists. The merits of working in optimal physicochemical property space, especially when supported by affirmative data (vide supra), cannot be understated, since this will increase the probability of attaining many desirable attributes of a drug: solid form, aqueous solubility, reduced polypharmacology, and minimal P450 inhibition/induction among others.

Finally, analysis of the exemplified compounds from the patent literature revealed a mean MW of 417 and cElogD of 4.5. Greater

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Chart 3. (a, Left) Average MW vs Patent Application Number and (b, Right) Average cElogD vs Patent Application Number

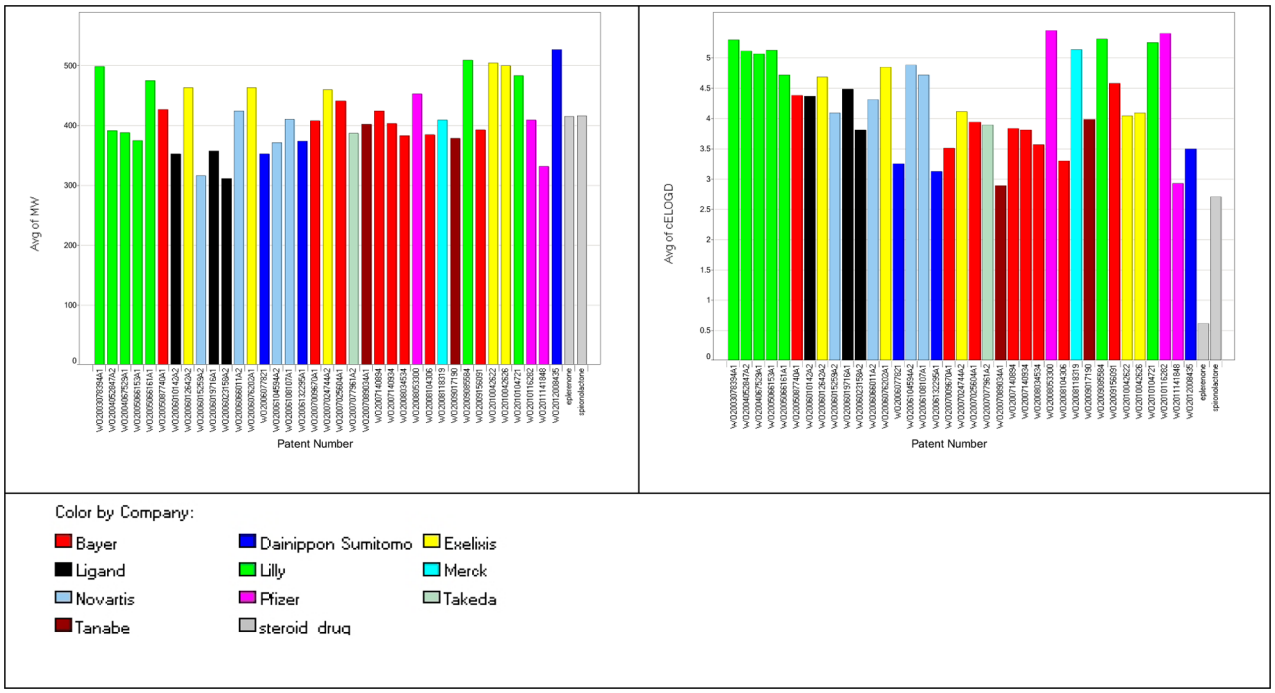
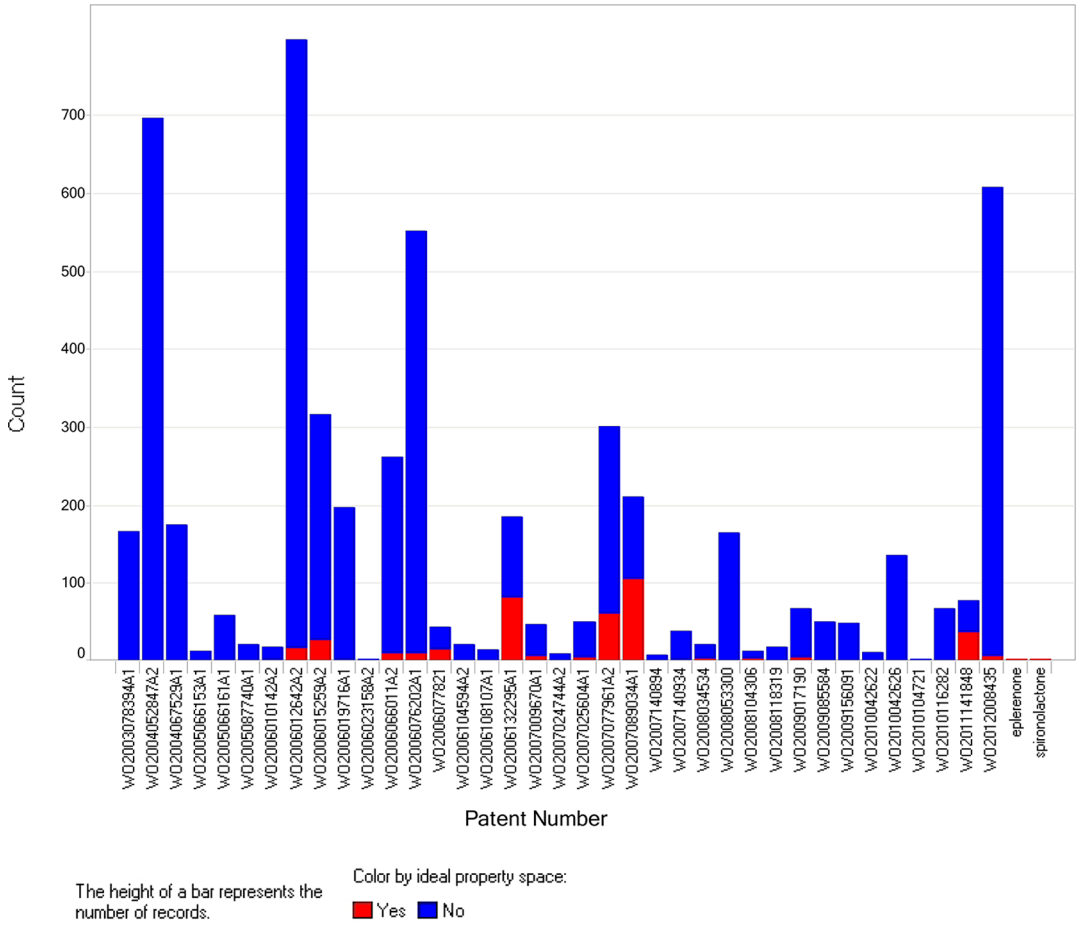


Chart 4. Compound Count vs Patent Application Number



than 50% of the compounds lie in space where MW > 500 and clogP > 5 (see Chart 2).[31] These results are not surprising, as the patent literature set covers a range of compounds from weakly active hits through potent clinical candidates. The average MW from each patent application, roughly defining one particular chemotype, ranges from as low as 312 all the way to 527 (see

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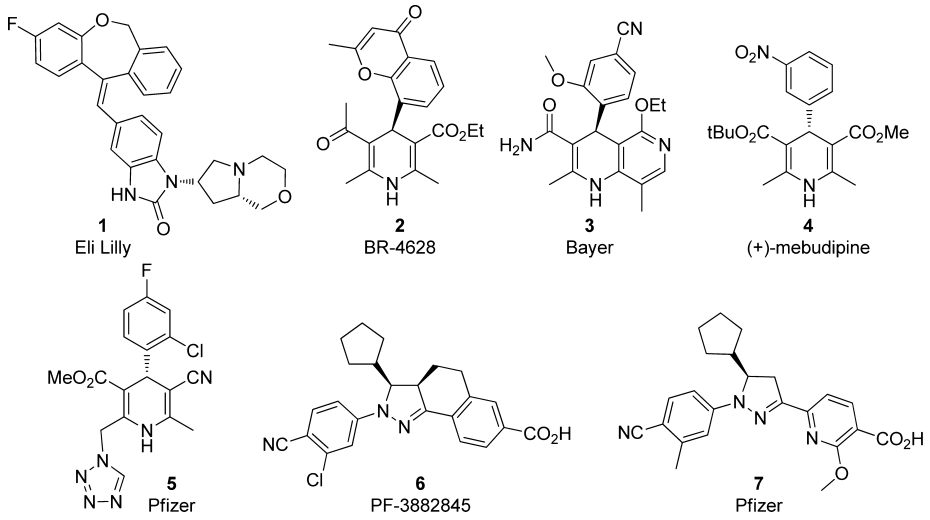

Figure 3. Eli Lilly, Bayer, and Pfizer nonsteroidal MR antagonists.

Chart 3a). The higher MW is not a particular concern for this set of unoptimized compounds but can signal the presence of less ligand efficient cores. The average cElogD from each patent application ranges from 3.4 to 5.5, which is in stark contrast to the cElogD of both marketed steroids (see Chart 3b). The higher clogP/cElogD for some chemotypes can prove problematic. As noted in reviews on druglike properties, lipophilicity can have an impact on several aspects of an oral drug profile including solubility, permeability, absorption, plasma protein binding, metabolism, toxicity, and binding affinity for the molecular target of interest.[6] Inappropriate use of lipophilicity to drive potency can prove costly in terms of the greater potential to elicit undesired toxicological outcomes. However, the use of average clogP/cElogD values clearly does not provide a complete picture. Examination of property space within each application showed that a significant number of compounds within selected applications do indeed fall in the desirable property space roughly defined by the marketed steroids (see Chart 4; WO2006/132295, WO2007/077961, WO2007/089034, WO2011/141848). Thus, this analysis shows that both MW and clogP/cElogD have been controlled for some of the newer chemotypes, inferring that nonsteroidal MR antagonists need not necessarily occupy typical NHR territory and targeting the property space of eplerenone is a worthy goal.

CHEMOTYPES

Eli Lilly has disclosed several different chemotypes that have been discussed in previous reviews. One such example is compound **1** from the dibenzooxepine class (Figure 3). Of note, this compound is the subject of a single compound patent application, WO2010/104721, with a K_i of 0.40 nM in a MR binding assay with demonstrated selectivity over AR, GR, and PR in binding assays (K_i of 1170, 669, and 478 nM, respectively). In a preclinical assay, this compound had a lower increase of urinary Na^+/K^+ ratio when compared to vehicle, suggesting a reduced likelihood of producing hyperkalemia. The structure of their clinical candidate has not been disclosed. LY2623091 is listed in the Lilly pipeline as a phase 2 clinical candidate for treatment of chronic kidney disease.[32] It has also been assessed for its effect on renal potassium clearance after multiple oral dosing.[33]

Bayer has a series of patent applications covering both steroidal and nonsteroidal chemical matter. Noteworthy are the several patent applications on a class of MR-selective dihydropyridines (DHP) such as BR-4628 (**2**)[34] and **3**.[35] In a recent publication, **2** was disclosed as a potent and selective MR antagonist (MR IC_{50} of 28 nM; PR, AR, GR IC_{50} of 9020, 4440, 5470 nM, respectively) that was devoid of significant L-type Ca^{2+} channel antagonism and retained its antagonist character at the MR S810L mutant.[35] In WO2008/104306, a selected group of 1,4-dihydro[1,6]naphthyridines were separated by chiral chromatography. A single-crystal X-ray structure identified **3** as the *S*-enantiomer with MR IC_{50} = 16 nM and IC_{50} > 1000 nM against the L-type Ca^{2+} channel. The structure of their clinical candidate, BAY 94-8862, has not been disclosed. This compound is listed in the Bayer pipeline as a clinical candidate for treatment of chronic heart failure and mild to moderate chronic kidney disease, currently in phase 2.[36]

The MR antagonistic properties of the DHP class have also been disclosed by other companies. A series of papers from Pfizer showed that several marketed DHPs have MR antagonistic properties and can be docked into the LBD of MR in a pose that partially overlaps with steroidal antagonists and that the MR antagonist properties and the Ca^{2+} channel blocking properties largely reside in opposite enantiomers. Significant modifications were required to transform the lead mebudipine (**4**) into **5**.[37] In this case, **5** had sufficient potency at MR (IC_{50} = 52 nM) along with modest selectivity against other receptors (approximately 6- to 140-fold selective against AR, GR, PR) and exposure to allow for demonstration of blood pressure lowering in Dahl salt sensitive rats.

A second series of pyrazoline compounds has also been disclosed by Pfizer. Starting from a neutral HTS hit that was plagued by poor solubility and a propensity to inhibit the hERG channel, optimization of compounds from this series was progressed by changing the ionization state through incorporation of a carboxylic acid. After adjustment of substituents at each position of the pyrazoline core, compounds with a balance of potency, selectivity, and reasonable pharmacokinetic profiles were found. PF-3882845 (**6**)[38] was identified as a conformationally restricted variant characterized by high MR potency (IC_{50} of 9 nM, functional assays), favorable selectivity (AR, GR IC_{50} of >8910 and 10 000 nM and PR IC_{50} of 416 nM) and a good pharmacokinetic profile. Blood pressure attenuation significantly greater than eplerenone, reduction in urinary albumin, and renal protection were demonstrated in preclinical models. In addition, on the basis of measures of serum potassium

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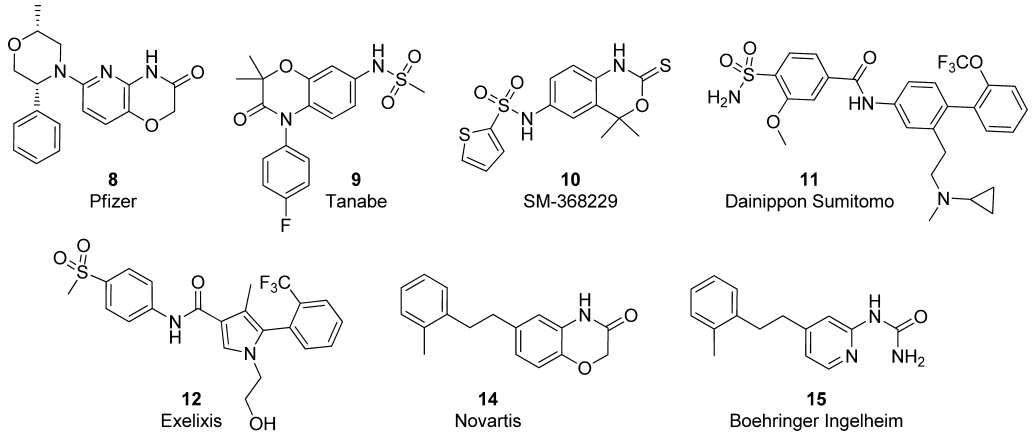

Figure 4. Nonsteroidal MR antagonists.

levels relative to eplerenone, it was surmised that **6** may have reduced risk in inducing hyperkalemia.[38] Preclinical and clinical data with eplerenone and **6** were used to establish the rat-to-human translatability of urinary Na^+/K^+ ratio (acute) and plasma aldosterone level (chronic) biomarkers.[39] Compound **6** is in phase 1 clinical trials to assess safety and tolerability in patients with diabetic nephropathy.[29] A large scale synthesis to support preclinical toxicology studies of a second compound from this series, **7**, was disclosed.[40] The MR potency was reported as IC_{50} = 4.2 nM (functional assay). Additional details will be reported in due course. Recent patent applications have disclosed additional variants of this chemotype.[41] A third Pfizer chemotype, represented by **8**, has been disclosed in the patent literature.[42] The MR potency (IC_{50} = 44 nM, functional assay) and single crystal X-ray structure confirming the cis stereochemistry were reported.

Mitsubishi Tanabe has two patent applications covering compounds related to benzoxazines such as **9** (Figure 4). In general, representatives from this series have slightly lower MW and cElogD (avg MW = 400, avg cElogD = 2.9) and are conformationally rigid. Data reported in the patent application for **9** indicate that the MR IC_{50} is <500 nM in a rat binding assay. MT-3995 (structure not disclosed) is listed on the Mitsubishi Tanabe Web site as a clinical candidate for hypertension, currently in a phase 1 trial in Europe.[43]

Dainippon Sumitomo has described work on a benzoxazin-2-thione series in the patent literature and publications. Of note is their work on SM-368229 (**10**)[44a] that showed strong MR inhibitory activity with IC_{50} of 21 nM (rat binding assay) and 130 nM (functional assay) with reasonable selectivity against other receptors (approximately 18- to 231-fold selective against PR, AR, GR). In preclinical studies in rats, **10** was as good as or superior to marketed steroids at increasing the urinary Na^+/K^+ ratio and preventing an increase in systolic blood pressure. The selectivity profile appeared to correlate with in vivo data. Very weak antiandrogenic effects in male rats and no progestagenic effect in the estrus cycle of female rats were seen when **10** was tested at high doses (100 and 300 mg/kg). Compound **10** also harbors some partial agonist activity at MR. It has been hypothesized that combination of the strong MR antagonistic activity coupled with the partial agonist property gives rise to antihypertensive efficacy with minimal effect on serum potassium level in preclinical animal models.[44] A patent application covering a new biphenylamide series, represented by compound **11** (MR IC_{50} = 22 nM, binding assay), was recently published.[45]

Exelixis has disclosed a series of heterocyclic biphenylamides in the patent literature, a large majority of which were *N*-phenylpyrroleamides. The identification of three different atropisomeric compounds, represented by **12**, appears to have been selected for further investigation. Data reported in the patent application demonstrated potent MR antagonist property (IC_{50} = 2.4 nM, functional assay) for the active atropisomer.[46] A selective and potent MR antagonist, XL550 (structure not disclosed), for the treatment of hypertension, congestive heart failure, and end organ protection was out-licensed to Daiichi Sankyo. It is currently listed as being in a phase 1 clinical trial for hypertension.[47]

A series of benzoxazin-3-ones was the subject of a recent manuscript from Takeda.[48] Optimization efforts, starting from an HTS hit, were conducted with the aid of a docking model and scaffold hopping. Compound **13** (Figure 5) was identified as a

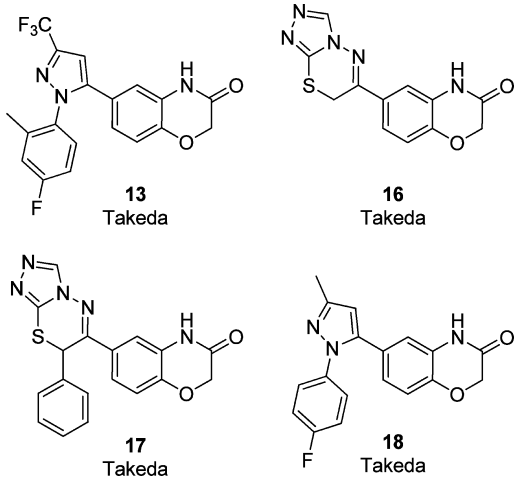

Figure 5. Takeda nonsteroidal MR antagonists.

potent MR antagonist with IC_{50} = 41 nM (binding) and IC_{50} = 43 nM (functional), with demonstrated selectivity over AR, GR, and PR in binding assays (IC_{50} of >10 000 and IC_{50} of 1800 and 1900 nM, respectively). The acceptable rat PK profile (CL = 1328 mL h^{-1} kg^{-1}, Vd = 4.6 L/kg, F = 51%) allowed for assessment in preclinical models. This compound not only exhibited a significant antihypertensive effect but also increased urinary Na^+/K^+ ratio in a dose-dependent manner. Significantly, the first nonsteroidal cocrystal structure was solved for a related structure. A more detailed analysis of this work will be provided in a later

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section. Acyclic variants of this chemotype have been disclosed by Novartis **14**[49] and Boehringer Ingleheim **15**.[50] Compound **15** was disclosed as a potent MR antagonist with IC_{50} = 7.1 nM (binding) and IC_{50} = 19 nM (functional).

MR BINDING SITE

As stated in the section on steroidal MR antagonists, the endogenous agonists for the selectivity targets, PR, AR and GR, anchor the ligand in the LBD through hydrogen-bond interaction of the 3-keto group with residues Gln (H3 helix) and Arg (H5 helix). In addition, some but not all of the recently disclosed nonsteroidal antagonists have cyano, nitro, or carbonyl groups that are well-known[51] or proposed[35,37a] substituents that can interact with the Gln776 and Arg817 of MR to mimic the 3-keto group interaction. On the basis of modeling and docking into the LBD of steroidal crystal structures, the DHPs (**2**, (+)-**4**, **5**) and pyrazoline **6** have been proposed to interact with one or both of Gln776 and Arg817. Several of the other disclosed structures do not have an obvious mimic of the 3-keto group. Specifically, compounds **1** and **8**−**13** fall into this category. These compounds might point the way to an alternative binding motif that exploits different interactions, relative to the interactions exploited by steroids, pyrazolines, and DHPs, that result in a higher degree of binding energy and more efficient molecules. The latter statement appears to be borne out with some of the lower MW/lower cElogD compounds such as **3**, **8**, **10** and to some extent **9**. These molecules stand apart as being more ligand efficient (LE): **3**, LE = 0.39; **8**, LE = 0.44; **10**, LE = 0.49.[52] In addition, from reported data for **10** and **13**, adequate selectivity over PR, AR, and GR was achieved suggesting that optimal and likely MR specific polar interactions are in play.

USE OF CRYSTAL STRUCTURES FOR MR ANTAGONIST DESIGN

The knowledge and understanding about the structure of MR continue to emerge. The receptor has three major functional domains: an N-terminal domain (NTD), a DNA-binding domain (DBD), and the ligand-binding domain (LBD) that resides in the C-terminal hinge region. The NTD also contains transcriptional activation functions, known as activation function 1 (AF-1) and, embedded within the LBD, activation function 2 (AF-2). It is through the AF-1 and AF-2 regions that a range of transcription enhancers (coactivators) or transcription repressors (corepressors) interact with the nuclear hormone receptor. These coregulators interact in a highly selective manner, which is likely an important contributor of mineralocorticoid selectivity. The knowledge in this field is still emerging and therefore less predictive but could point the way toward more receptor selective compounds in the future.

The preponderance of the structural knowledge lies with the LBD. The LBD contains a number of helices that are arranged around a central hydrophobic pocket where the ligands interact. All MR crystal structures to date are based on a mutated form of the LBD portion of the receptor. In particular, the clinically relevant S810L mutant has led to the determination of several cocrystal structures.[53] Individuals that harbor the S810L mutation are known to have early onset hypertension. The mutation lies in the MR LBD and alters an amino acid that is conserved in MRs across species and is not found in other nuclear receptors. The S810L mutation results in modified receptor specificity, rendering progesterone and other steroids lacking 21-hydroxyl groups as partial agonists. Of significant note, spironolactone is also a potent agonist of MR S810L. In addition, wild type MR cocrystal structures have been reported with agonists, aldosterone, and deoxycorticosterone, as well as the weak agonist progesterone.[54] A clear benefit of access to these structures is the identification of the key polar amino acid residues that interact with the endogenous steroid ligand aldosterone.[55] The interactions achieved for the binding mode of aldosterone with MR are consistent with how other endogenous ligands bind their cognate steroid receptors. The antagonist spironolactone is generally believed to bind in a passive mode where it inhibits proper stabilization of the coactivator-binding conformation, thus preventing transcription from occurring. To date, there are no MR cocrystal structures in the so-called antagonist mode.

FIRST FULL LENGTH NHR CRYSTAL STRUCTURE

It has become increasingly clear that interactions at the LBD only reflect part of a very complicated full picture. This is, in part, because most structural efforts have focused on crystallization of domains (DBD, LBD) in isolation from each other. The ability to visualize a full-length NHR, revealing domain−domain interactions that will likely alter how future agents are designed, will be an important step forward. To this end, a full length crystal structure of the PPARγ−RXRα heterodimer bound to its DNA site, coactivator peptides, and ligands of both receptors has been reported. This structure revealed that interdomain interactions between surfaces of the PPARγ LBD can alter the DNA binding of both receptors. This provided the first structural evidence that LBD and DBD do indeed interact, which is in contrast to previous suppositions.[56] There have been several other studies that clearly show that interdomain contact between individual domains of the steroid receptors occurs in a ligand- and cell-specific manner. Furthermore, invoking domain−domain interactions, for example, N/C interaction, can offer an explanation for the tissue-specific actions of endogenous ligands, cortisol, and aldosterone at the MR.[57] A low resolution, full length crystal structure of AR has been reported.[58]

FIRST NONSTEROIDAL CRYSTAL STRUCTURE

Despite the incomplete view of the full MR, progress has been made on structure elucidation of the LBD. The publication of the first nonsteroidal crystal structure by the Takeda group is worthy of further comment.[48] Compound **16** (Figure 5), discovered through HTS, was of modest potency and not selective versus PR. A docking model based on the cocrystal structure of MR with spironolactone suggested that the benzoxazinone and triazolothiadiazine moieties of **16** form hydrogen-bonding interactions with respective polar residues, Asn770 and Gln776, at each end of the steroid binding pocket. This is not unlike the binding of PR agonist tanaproget,[59] which makes similar polar interactions and might explain the equipotent PR binding affinity for **16**. The Takeda group hypothesized that filling the C7 lipophilic pocket occupied by the -SAc moiety of spironolactone would increase the binding affinity by hydrophobic interaction. After assessment of several different lipophilic groups, it was found that the phenyl analogue **17** exhibited good MR potency (IC_{50} = 510 nM) and improved PR selectivity (IC_{50} > 10 000 nM). Figure 6 depicts the cocrystal structure of **17** with MR and overlay of **18**. The crystal structure showed the expected hydrogen bond interactions with Asn770 through benzoxazinone and with Gln776 and Arg817 through triazole nitrogens. The phenyl group occupied the hydrophobic pocket. The improvement in selectivity over PR led

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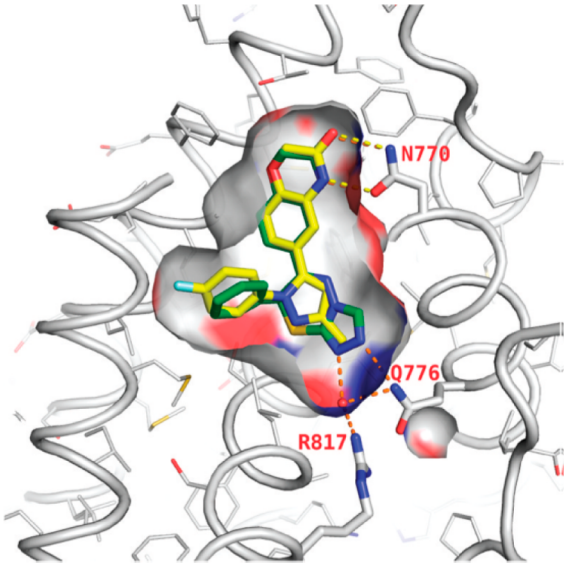

Figure 6. Overlay of **18** (yellow) and compound **17** (green) in MR. Compound **18** was docked into the X-ray crystal structure of MR/**17** complex (3VHV). The hydrogen bonds are shown as orange dotted lines. Figure reproduced with permission from *Journal of Medicinal Chemistry* (Hasui et al. *J. Med. Chem.* **2011**, *54*, 8616).[48] Copyright 2011 American Chemical Society.

to the conclusion that the corresponding hydrophobic pockets of AR, PR, and GR are smaller than that of MR. Scaffold hopping away from the triazolothiadiazine to pyrazole led to **13** that had *increased* binding affinity despite removal of the hydrogen-bonding interactions with Gln776 and Arg817. Importantly, these results suggested that the presumed strong binding interaction with Gln776 and Arg817 was not the dominant polar interaction. Instead, the hydrogen-bonding interaction with Asn770 appears more effective at locking the compound into the receptor, thus maximizing binding energy gained through the hydrophobic interaction of the phenyl group. The first nonsteroidal structure has shed light on the binding mode of compounds related to **13**. The information gained from this structure could point the way to new potent and selective antagonists.

FUTURE OF MR ANTAGONISTS

The advent of the first X-ray crystal structure of a nonsteroidal MR antagonist and the increased number of compounds in clinical trials give hope for the discovery of well-tolerated, low dose agents for the treatment of hypertension, chronic kidney disease, and diabetic nephropathy. Hyperkalemia remains as a potential mechanism based side effect. Hints from preclinical data, however, suggest that some nonsteroidal structures have a reduced risk in this area. Data from clinical trials will hopefully shed light on the possibility of attaining an agent with reduction of or freedom from hyperkalemia risk. However, much remains to be learned about the structure and function of MR. A caveat on the X-ray crystal structures must be kept in mind; the current structures are of a single, isolated domain (LBD), and nearly all structures use the mutated receptor (MR S810L). With the determination of the first full length crystal structure of an NHR, there is now hope that steroid receptor structures will follow. This should shed light on the interdomain interactions and the involvement of the other domains that have been demonstrated to play an important role in determining the recruitment of cofactors and the potential of achieving tissue specificity.[60] Tissue selective agents should lie in the future for MR antagonists. The discovery of such agents will come through a combination of understanding tissue specific pharmacology, pharmacokinetics, and pharmacodynamics and increased structural knowledge. Specific to the latter point, identification of non-LBD binding sites that result in blockade of receptor signaling will likely be required.[61]

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ABBREVIATIONS USED

MR, mineralocorticoid receptor; NHR, nuclear hormone receptor; RAAS, renin−angiotensin−aldosterone system; AR, androgen receptor; GR, glucocorticoid receptor; PR, progesterone receptor; ER, estrogen receptor; DBD, DNA binding domain; AF-1, activation function 1; AF-2, activation function 2; DHP, dihydropyridine; NTD, N-terminal domain

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