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Mineralocorticoid receptor antagonists: Identification of heterocyclic amide replacements in the oxazolidinedione series

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ABSTRACT

Novel potent and selective mineralocorticoid receptor antagonists were identified, utilizing heterocyclic amide replacements in the oxazolidinedione series. Structure–activity relationship (SAR) efforts focused on improving lipophilic ligand efficiency (LLE) while maintaining nuclear hormone receptor selectivity and reasonable pharmacokinetic profiles.

The mineralocorticoid receptor (MR) is a member of the nuclear hormone receptor (NHR) super-family and regulates gene expression involved in cardiovascular disease.[1] The natural endogenous hormone aldosterone activates MR leading to chronic kidney disease, hypertension and congestive heart failure via electrolyte imbalance.[2] The only two marketed MR antagonists are the steroids spironolactone and eplerenone, which have demonstrated positive effects in the treatment of patients with the aforementioned conditions (Fig. 1).[3] However, selectivity for MR versus other members in the NHR super-family (members include: androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR), and progesterone receptor (PR)) is essential to avoid undesired side effects. Spironolactone, for example, has sex hormone related side effects (gynecomastia and menstrual irregularities) due to AR and PR activity.[4] In contrast, eplerenone is very selective for MR versus the other NHR members, but at the price of reduced MR potency and b.i.d dosing.[5]

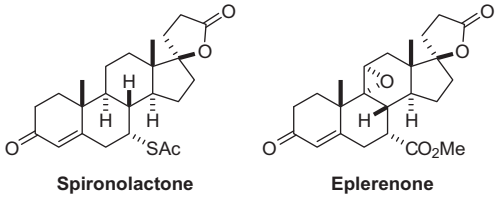

Figure 1. Marketed steroidal MR antagonists.

Due to the limitations of the steroid MR antagonists, we[6] and others[7] have initiated discovery programs targeting non-steroidal MR antagonists. Herein we report our continuing investigation of a novel oxazolidinedione (ODO) class of potent MR antagonists, focusing on efforts toward the discovery of heterocyclic replacements for the amide moiety in compound **1** (Fig. 2). Compound **1** was identified through an in-house high-throughput screen utilizing a commercial PathHunter™ assay[8] and demonstrated modest MR potency (IC_{50} = 6 μM).[9]

As previously disclosed,[10] initial efforts focused on amide substitution and identification of N-3 substitution, with appropriate

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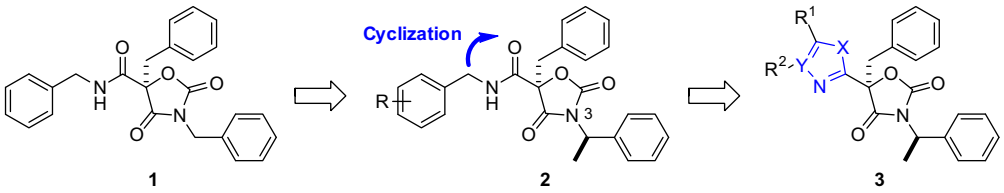
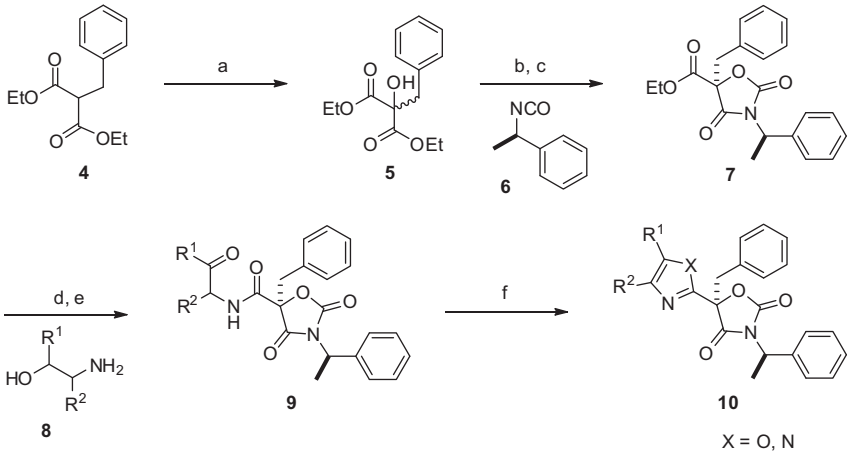


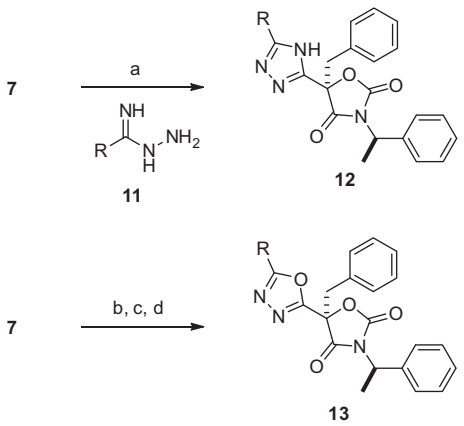
Figure 2. Evolution of ODO class MR antagonists.



Scheme 1. Synthesis of oxazole and imidazole ODO MR antagonists. Reagents and conditions: (a) CsF, DMF, Air, 40 °C, 3 days, 71%; (b) NaOH, 4 Å ms, THF, 0 °C, 5 min. then **6**, 0 °C to rt, 4 h, 46%; (c) Chiral SFC (AD column); (d) **8**, EtOH, 70 °C, 2 h; (e) DMP, DCM, rt, 30 min. 40–82% over two steps; (f) oxazole: I_2, PPh_3, TEA, 5 min, then **9**, DCM, 1 h or imidazole: NH_4OAc, AcOH, 100 °C, 40 h, 25–90% over two steps.

R stereochemistry, to identify MR antagonists **2**. We envisioned cyclization of the amide moiety would afford novel antagonists (**3**), with improved physicochemical, ADME, and safety profiles by attempting to improve the lipophilic ligand efficiency (LLE).[11]

The synthesis of oxazole and imidazole MR antagonists is depicted in Scheme 1.[12] Diethyl malonate derivative **4** was oxidized by treatment with cesium fluoride in the presence of air to afford racemic hydroxymalonate **5**. The hydroxymalonate was converted to enantiopure oxazolidinedione **7** by first condensation with chiral isocyanate **6** in the presence of sodium hydroxide followed by purification via chiral supercritical fluid chromatography (SFC). Treatment of key intermediate **7** with substituted hydroxylethyl amines **8**, provided cyclization precursors **9** after oxidation of the alcohol with Dess–Martin periodinane. The final oxazole targets



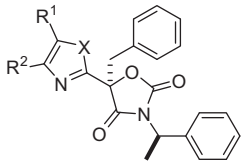
Scheme 2. Synthesis of triazole and oxadiazole ODO MR antagonists. Reagents and conditions: (a) **11**, DIEA, EtOH, 70 °C, 1 h, 30–75%; (b) anhydrous hydrazine, MeOH, sonication, 1 h; (c) RCOOH, DIEA, HATU, DCM, rt, 1.5 h; (d) I_2, PPh_3, TEA, 5 min, then product from step c, DCM, 1 h, 40–90% over three steps.

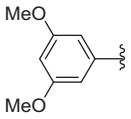
10 were prepared by adding intermediates **9** to a freshly prepared solution of iodine, triphenylphosphine and triethylamine. Alternatively, treatment of intermediates **9** with ammonium acetate in hot acetic acid afforded imidazoles **10**.

Highlighted in Scheme 2 is the synthesis of triazole and oxadiazole MR antagonists. The 1,3,4-triazole derivatives **12** were synthesized in a single step from key intermediate **7** by heating with amino-hydrazides in ethanol. 1,3,4-Oxadiazoles **13** were prepared in three steps from **7** by first acyl-hydrazide formation using anhydrous hydrazine, followed by amide bond generation using a variety of acids. Finally, cyclization utilizing the procedure for synthesizing the oxazoles (addition of the product from the previous step to a freshly prepared solution of iodine, triphenylphosphine and triethylamine) provided 1,3,4-oxadiazoles **13**.

Our initial focus was to identify heterocyclic amide replacements (Table 1) to simultaneously improve not only liver microsome (LM) stability but also LLE with respect to the previously disclosed 3,5-dimethoxy benzyl amide analog (IC_{50} = 54 nM; LLE = 2.54; *c*log*P* = 4.73; H, R LM% at 1 μM incubation after 0.5 h = 0, 0).[6d] While oxazole region-isomers **10a** and **10b** had a significant loss in potency and LLE, gratifyingly, each compound demonstrated an increase in microsomal stability when compared to the amide analog. We could improve the intrinsic potency and LLE by making the benzyl substitution (**10c**); however, not surprisingly this came at the expense of the microsomal stability. Switching to phenyl imidazole **10d** improved the intrinsic potency and LLE, while showing similar LM stability compared to the oxazoles; however, benzyl substituted imidazole **10e** had a similar profile to oxazole **10c**. Addition of the 3,5-dimethoxy phenyl substitution (**10f**) significantly improved the intrinsic potency and LLE with respect to the other cyclized analogs, while maintaining some of the microsomal stability improvements gained from cyclization. Unfortunately, the oxazoles and imidazoles did not achieve our goal of improved LLE compared to their respective amide analogs.

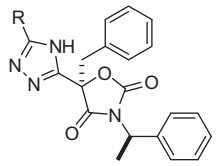
Table 1
SAR of amide replacement: oxazole and imidazole

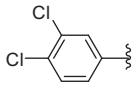
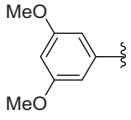


Compd	R1	R^2	X	IC_{50}[a] (μM)	LLE[b]	*c*log*P*	LM[c] H, R
10a	H	Ph	O	1.70	1.21	4.55	73, 15
10b	Ph	H	O	1.11	1.4	4.55	73, 29
10c	Bn	H	O	0.24	2.32	4.29	0, 0
10d	Ph	H	NH	0.24	1.91	4.70	62, 4
10e	Bn	H	NH	0.27	2.12	4.44	0, 0
10f		H	NH	0.068	2.38	4.79	43, 4

[a] Values are the average of two experiments, each in 10-point titrations.
[b] LLE = pIC_{50}—*c*log*P*.
[c] % Remaining at 0.5 h and 1 μM incubation in human (H) and rat (R) liver microsomes (LM).

Table 2
SAR of amide replacement: 1,3,4-triazole



Compd	R	IC_{50}[a] (μM)	LLE[b]	*c*log*P*	LM[c] H, R
12a	Ph	1.44	1.39	4.45	78, 29
12b	Bn	0.66	1.99	4.19	63, 7
12c		1.32	0.58	5.30	88, 41
12d		0.67	2.06	4.11	74, 37

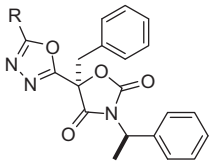
[a] Values are the average of two experiments, each in 10-point titrations.
[b] LLE = pIC_{50}—*c*log*P*.
[c] % Remaining at 0.5 h and 1 μM incubation in human (H) and rat (R) liver microsomes (LM).

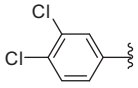
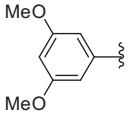
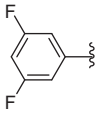
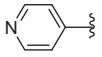
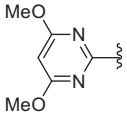
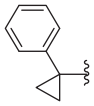
With the increased LM stability imparted by cyclization (as exemplified by analogs in Table 1), we next turned our attention to improving the LLE profile, by attempting to maintain potency while reducing *c*log*P* via introduction of a third heteroatom in our cyclized ring. To this end, we first investigated the addition of a nitrogen atom to generate 1,3,4-triazoles as depicted in Table 2. In general, the analogs in Table 2 had lower *c*log*P* and improved microsomal stability, but these attributes came at the cost of reduced intrinsic potency and reduced LLE. For example, 1,3,4-triazole **12d** when compared to imidazole **10f** showed a 10-fold reduction in intrinsic potency with a 0.68 log unit improvement in *c*log*P*, leading to a 0.32 reduction in LLE. However, **12d** demonstrated an improved microsomal stability (*H*, *R* = 74, 37) versus **10f** (*H*, *R* = 43, 4) after 0.5 h microsomal incubation in both human and rat microsomes.

While we observed improved microsomal stability with the oxazoles, imidazoles, and 1,3,4-triazoles compared to the amide analogs, we were still left with the challenge of improving the LLE and reducing the *c*log*P* in order to be in a more desirable drug-like space. By calculating *c*log*P* prior to synthesis, we knew that the 1,3,4-oxadiazole amide replacement would, in general, have a 0.5–1.5 *c*log*P* reduction when compared to the previous heterocyclic and amide analogs. With this in mind, we synthesized 1,3,4-oxadiazoles as depicted in Scheme 2, with representative examples highlighted in Table 3. In general, the 1,3,4-oxadiazoles showed similar potency to the imidazoles; both had improved potency relative to the 1,3,4-triazoles and oxazoles, with improved LLE due to improved *c*log*P* (e.g. **13a** and **13b** vs **10a**–**e** and **12a**,**b**) as predicted. Unfortunately, these improvements in LLE came at the price of generally reduced rat microsomal stability compared to the 1,3,4-triazoles, however, the human microsomal stability was maintained. Interestingly, while we saw similar potency with the 3,4-dichlorophenyl **12c** compared to the unsubstituted phenyl **12a** in the 1,3,4-triazoles, the 3,4-dichlorophenyl-1,3,4-oxadiazole **13c** had a 20-fold loss in potency compared to **13a**.

As was previously noted, addition of 3,5-methoxyphenyl substitution provided a boost in potency, which also held true for 1,3,4-oxadiazole **13d**, leading to an improved LLE. Compound **13d** was

Table 3
SAR of amide replacement: 1,3,4-oxadiazole



Compd	R	IC_{50}[a] (μM)	LLE[b]	*c*log*P*	LM[c] H, R
13a	Ph	0.38	3.09	3.33	82, 0
13b	Bn	0.16	3.74	3.07	7, 0
13c		6.74	0.5	4.67	—
13d		0.039	3.95	3.46	64, 28
13e		0.27	2.92	3.65	74, 1
13f		1.53	3.79	2.03	—
13g		0.13	4.12	2.76	31, 0
13h		0.47	3.85	2.47	—
13i		0.082	3.58	3.50	57, 2

[a] Values are the average of two experiments, each in 10-point titrations.
[b] LLE = pIC_{50}—*c*log P.
[c] % Remaining at 0.5 h and 1 μM incubation in human (H) and rat (R) liver microsomes (LM).

Table 4
Selectivity and PK[a] profile of select compounds **13d** and **13i**

Property	13d	13i
NHR selectivity IC_{50}[b] (μM): AR, ER, GR, PR	>5	>5
Ion channel IC_{50} (μM): Ca, IKr, Na	>13	>6
CYP IC_{50} (μM): 3A4, 2D6, 2C9	>50	>19
AUCN iv (μM h kg/mg)	3.7	4.4
Cl (mL/min/kg)	7.7	7.9
Vd (L/kg)	1.2	1.0
$T_{1/2}$ (h)	2.4	2.8
F (%)	44	89
Rat plasma protein binding (% bound)	0.2	0.5

[a] Formulation: 1 mg/mL PEG 200: water (70:30). iv dose: 1 mg/kg ($n = 2$). po dose: 2 mg/kg ($n = 3$). Blood concentrations were determined by LC/MS/MS following protein precipitation with acetonitrile.
[b] Values are the average of two experiments, each in 10-point titrations.

also the only compound that maintained similar human and rat microsomal stability in this subclass, when compared to the 1,3,4-triazoles. With these positive attributes, we continued our SAR evaluation by investigating alternate 3,5-phenyl substitution, introduction of nitrogen, and a combination thereof as exemplified by **13e**, **13f**, and **13g**, respectively.[13] Unfortunately, these analogs had reduced intrinsic potency leading to reduced LLE (**13e** and **13f**) and/or decreased rat microsomal stability (**13e** and **13g**). We investigated non-aromatic substitution, but in all cases this led to significant loss in intrinsic potency with **13h** being the most potent and highest LLE non-aromatic substitution compound synthesized. All other non-aromatic analogs demonstrated significant erosion of LLE. In an effort to block the presumed benzylic metabolism of **13b**, we introduced cyclopropyl substitution (**13i**), which improved human metabolic stability and modestly increased potency but at the expense of increased *c*log*P*.

Based on improved in vitro data for **13d** in the phenyl 1,3,4-oxadiazole series and **13i** in the benzyl 1,3,4-oxadiazole series, we chose to profile both molecules in greater detail (Table 4). Both **13d** and **13i** had similar nuclear hormone receptor selectivity (IC_{50} >5 μM), ion channel activity (IC_{50} >6 μM), and cytochrome P450 (CYP) activity (IC_{50} >19 μM). The rat pharmacokinetic (PK) profile for both analogs were also very similar; each compound had low clearance, moderate half-life, and reasonable oral bioavailability.

In conclusion, improved analog profiles, with respect to the amides, was achieved by cyclization of the amide to form heterocycles in the oxazolidinedione series. The heterocycles maintained intrinsic MR potency while showing improved LLE, and increased metabolic stability. The 1,3,4-oxadiazole amide replacement, exemplified by both **13d** and **13i**, also demonstrated significant selectivity versus other nuclear hormone receptors, ion channels, and CYP's while having reasonable rat PK profiles. Further study of the ODO series will be reported in due course.

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