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Discovery of novel non-steroidal reverse indole mineralocorticoid receptor antagonists

Anthony K. Ogawa [a,*], Ellen Vande Bunte [a], Rudrajit Mal [a], Ping Lan [a], Zhongxiang Sun [a], Alejandro Crespo [b], Judyann Wiltsie [a], Joseph Clemas [a], Jack Gibson [a], Lisa Contino [a], JeanMarie Lisnock [a], Gaochao Zhou [a], Margarita Garcia-Calvo [a], Nina Jochnowitz [a], Xiuying Ma [a], Yi Pan [a], Patricia Brown [a], Beata Zamlynny [a], Thomas Bateman [a], Dennis Leung [b], Ling Xu [b], Xinchun Tong [b], Kun Liu [a], Martin Crook [a], Peter Sinclair [a]

[a] Early Development and Discovery Sciences, Merck and Co., 2000 Galloping Hill Rd., Kenilworth, NJ 07033, USA
[b] Early Development and Discovery Sciences, Merck and Co., 126 E. Lincoln Ave., Rahway, NJ 07065, USA

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ABSTRACT

Reported herein are a series of reverse indoles that represent novel non-steroidal mineralocorticoid receptor (MR) antagonists. The key structure–activity relationships (SAR) are presented below. This reverse indole series is exemplified by a compound that demonstrated efficacy in an acute natriuresis rodent model comparable to marketed MR antagonists, spironolactone and eplerenone.

Mineralocorticoid receptor antagonists (MRA) have traditionally been prescribed as hypotensive drugs that were intended to blunt the potential for aldosterone escape as a means of greater control over of the RAAS signaling pathway.[1] The first generation MRA, spironolactone, demonstrated good efficacy, but was beset with adverse effects (AE), such as gynocomastia and impotence in male patients.[2] The observed AE profile was attributed to a lack of nuclear hormone receptor selectivity, which was overcome with the approval of eplerenone in 2002.[3]

Recent clinical trials involving both spironolactone and eplerenone have demonstrated a strong link between MR antagonism and positive outcome benefits to heart failure patients, albeit with an elevated risk of hyperkalemia that is believed to be mechanism-related.[4] The observed benefits to mortality, cardiovascular adverse events and hospitalization were independent of a hypotensive effect long presumed to be the primary benefit of an MRA.

Over the past decade, a number of reports have detailed efforts to identify a next generation, non-steroidal MRA.[5,6] Reported herein are the background rationale and discovery of a potent and selective reverse indole MRA class that has demonstrated acute PD efficacy (Fig. 1).

Initial efforts sought to expand on the SAR established around a number of efficient central scaffolds.[7] For example, qualitative analysis of literature examples featuring 3,7-disubstituted indole and 4,7-disubstituted benzoxazine scaffolds, e.g., suggested that the analogous display of key functional groups from N1 and C4 of an indole central scaffold could be successful.[8] Further, alkylation at the indole N1-position facilitated the synthesis of more structurally diverse pendant hydrophobic functionality, which would, subsequently, enable SAR refinement.

To this end, the SAR derived from an initial basis set of compounds **1**–**7**,[9] supported the aforementioned hypothesis that the 'reverse indole' scaffold could yield MR antagonists of high lipophilic ligand efficiency (LLE), as well as providing evidence that a second hydrogen bond donor was not required for activity. The initial array of MRAs also revealed subtle but potentially significant determinants of activity, as seen from the improved activity for compounds **5**–**6**, which contain a conformational-directing *ortho*-Cl atom. Lastly, the loss in activity observed for biaryl compound, **7**, suggests a steric limit to the binding pocket.

Having established preliminary aryl ring SAR, the next goal involved gaining a similar understanding with respect to indole

* Corresponding author.

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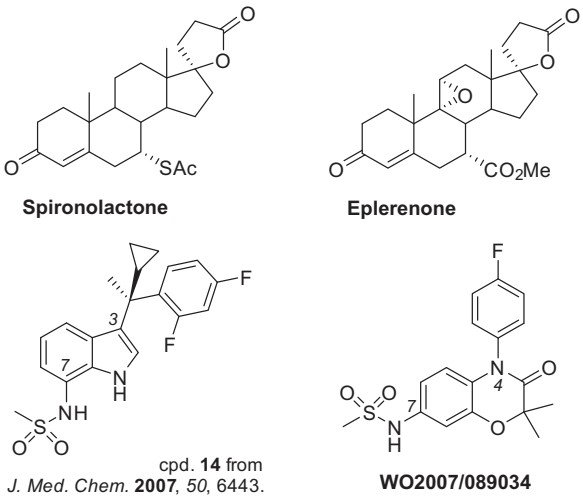


Figure 1. Known mineralocorticoid receptor antagonists.

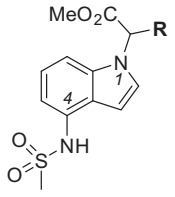
substitution (**8**–**14**). A brief SAR examination revealed two significant findings. First, although replacement of the sulfonamide with a nitrile (**11**) led to a significant improvement in activity, the off-target activity at other NHR precluded its employment. The second finding was that the sulfonamide SAR was also highly sensitive as close homologs (**13**–**14**) resulted in erosion of activity (Tables 1 and 2).

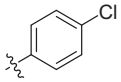
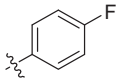
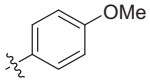
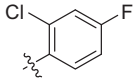
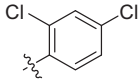
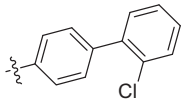
Despite the knowledge gained from probing the indole and aryl ring SAR, the conclusions largely supported what was previously established in the literature. Less well known, however is the SAR governing substitution at the benzylic carbon linking the aforementioned moieties. Further, the reverse indole scaffold readily lent itself to rapid SAR development, and more significantly, unsymmetrical substitution (see Table 3).

The introduction of a methyl substituent to form a quaternary carbon (**15**) applied a common strategy to improve potency by restricting rotation.[10] The functional activity for **15** affirmed this strategy, but expanding the SAR revealed a separate and distinct preference for small compact lipophilic substituents, highlighted by compounds **16**, **18**, and **20**. Extending the hydrophobic surface proved to be deleterious (**17**), and introducing polar functionality in the form of a methoxymethyl group, also had an adverse effect on activity.

Chromatographic separation of the enantiomers from racemic **16**, indicated a clear preference for one stereoisomer (**21**), which was tentatively assigned the *R*-stereochemistry based on correlation studies using Vibrational Circular Dichroism (VCD).[11] Also

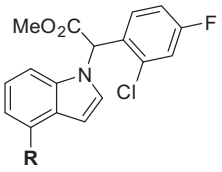
Table 1
In vitro SAR for the pendant phenyl ring, compounds **1**–**7**



Compd[a]	R	hMR NH Pro IC_{50}[b] (nM)	LLE[c]
1	Ph	4000	–
2		1700	1.8
3		10,000	2.1
4		9800	–
5		530	2.9
6		80	3.8
7		3920	1.4

[a] Compounds are racemic.
[b] Values are the average of two 10-pt titrations.
[c] LLE = $pIC_{50} - c\log P$.

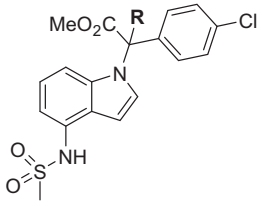
Table 2
In vitro SAR at the indole C4-position, compounds **8**–**14**



Compd[a]	R	hMR NH Pro IC_{50}[b] (nM)	LLE[c]	Other NHR counterscreens
8	H	2700	1.4	
9	F	2310	1.2	
10	Cl	940	1.3	AR_{ag} = 58 nM[d] (76%)
11	CN	200	2.9	GR_{antag} = 0.3 μM[d] AR_{ag} = 9 nM[d] (76%)
12	CO_2Et	4800	1.1	
5	$NHSO_2Me$	530	2.9	GR_{antag} = 3.2 μM[d]
13	$NHC(O)CF_3$	7900	1.4	
14	$NHSO_2CF_3$	2120	2.1	

[a] Compounds are racemic.
[b] Values are the average of two 10-pt titrations.
[c] LLE = $pIC_{50} - c\log P$.
[d] Value is from a single 10-pt titration.

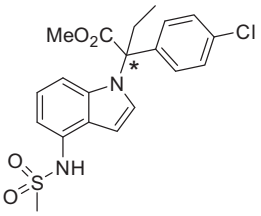
Table 3
In vitro SAR for the α-alkyl substituents, compounds **17**–**22**



Compd[a]	R	hMR NH Pro IC_{50}[b] (nM)	LLE[c]
2	H	1860	1.8
15	Me	200	3.2
16	Et	60	3.4
17	Bn	275	2.3
18	$CH_2{}^cPr$	30	–
19	CH_2OMe	1900	–
20	CH_2CN	70	4.3

[a] Compounds are racemic.
[b] Values are the average of two 10-pt titrations.
[c] LLE = $pIC_{50} - c\log P$.

Table 4
Comparison of in vitro data for α-ethyl compounds **21–22**



Compd	Chirality[a]	hMR NH Pro IC_{50}[b] (nM)	LLE[c]	Other NHR counterscreens
16	Racemic	90	3.4	—
21	*R*	15	3.3	PR_{antag} = 3.6 μM[d]
22	*S*	330	1.8	PR_{antag} = 0.4 μM[e]

[a] Absolute stereochemistry inferred from VCD correlations.
[b] Values are the average of two 10-pt titrations.
[c] LLE = pIC_{50} – *c*log*P*.
[d] Value is from one 10-pt titration.
[e] Value is from three 10-pt titrations.

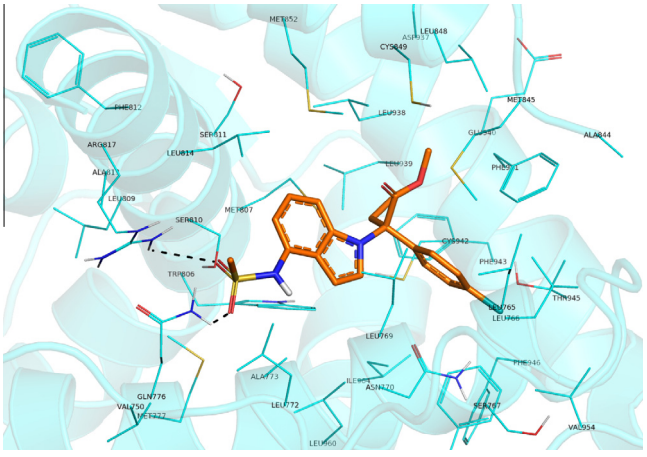


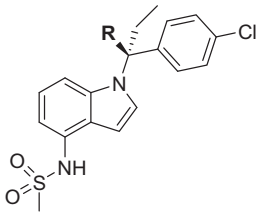
Figure 2. Predicted binding mode of compound **21** within MR pocket.

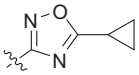
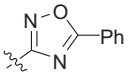
gratifying was that the off-target PR antagonism exhibited the opposite stereochemical preference (see Table 4).

Figure 2 shows the predicted binding mode of compound **21** obtained by molecular modeling using MR X ray structure 2A3I.[8] The indole core binds in the hydrophobic middle region of the pocket and it is placed in the same plane as aldosterone's sterol ring.[12] The C4-sulfonamide makes hydrogen bonds with canonical MR resides Q766 and R817 (resembling the 3-keto group of aldosterone). The methyl ester moiety binds in the similar region where eplerenone ester does, interacting with residues located in helix 7 (M845, L848, C849 and M852). The ethyl group points down towards residues located in helix 10 (L938 and L939). Finally, the chlorophenyl ring stacks between residues located in helix 3 (N770 and L766) and helix 10 (F941). This orientation is predicted to prevent the formation of the canonical hydrogen bond network (between the ligand, N770 in helix 3 and T945 in helix 10) required for receptor activation,[12] thus conveying the antagonism behavior to the series.

The synthesis of compound **16** follows a general route to reverse indole MRA, in which the appropriate phenylacetate is treated with NBS to afford the corresponding α-bromophenylacetate that is alkylated with 4-nitroindole. Reduction of the nitro group of **16a**, followed by Boc-protection yields intermediate **16b**. It should be noted the early stage reduction and protection

Table 5
Comparison of in vitro data for α-ethyl compounds **23–28**

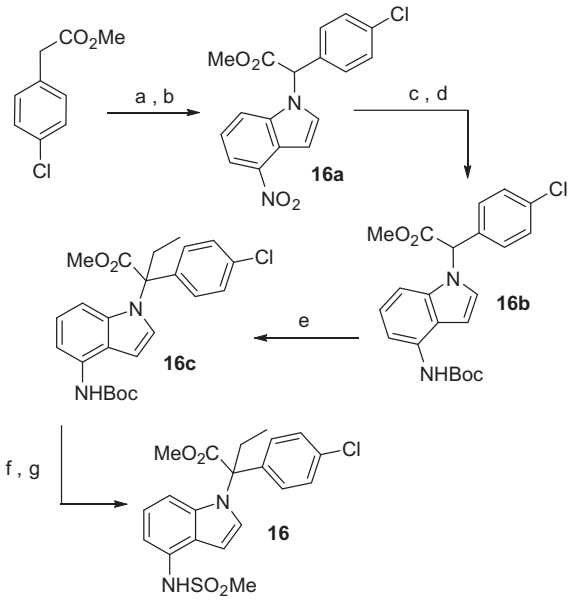


Compd	R	hMR NH Pro IC_{50}[a] (nM)	LLE[b]	Other NHR counterscreens
23	—C(O)NHMe	115	3.8	GR_{antag} = 3.2 μM (31%)[c]
24	—C(O)$NHCH_2CF_3$	97	2.7	GR_{antag} 27%
25	—C(O)NH^{t}Bu	960	1.6	—
6		33	3.2	GR_{antag} = 5.9 μM (48%)[c]
7		1900	3.9	PR_{antag} = 1.5 μM [c]
28[12]	—CH_2OH	21	4.2	GR_{antag} >10 μM; PR_{antag} = 6.7 μM

[a] Values are the average of two 10-pt titrations.
[b] LLE = pIC_{50} – *c*log*P*.
[c] Value is from one 10-pt titration.

Table 6
Rat PK for compounds **23** and **28**

Compd	Cl (mL/min/kg)	V_{dss} (L/kg)	$t_{½}$ (h)	$AUCN_{po}$ (μM h kg/mg)	%*F*
23	67	2.8	0.6	0.13	21
28	41	4.1	1.5	0.67	65



Scheme 1. Synthesis of compound **16**. Reagents and conditions: (a) NBS, AIBN, 80 °C; (b) 4-nitroindole, NaH, DMF, 0 0C; (c) 5% Pt–C, H_2 (1 atm), EtOAc; (d) Boc_2O; (e) addition of **16b** in DMF to NaH, DMF, 0 °C, then iodoethane; (f) TFA; (g) MsCl.

was necessary to facilitate alkylation to generate the α-quaternary ester functionality, as attempts to directly convert **16a** resulted in fragmentation. Conversion of **16b** to **16c** proved to also require

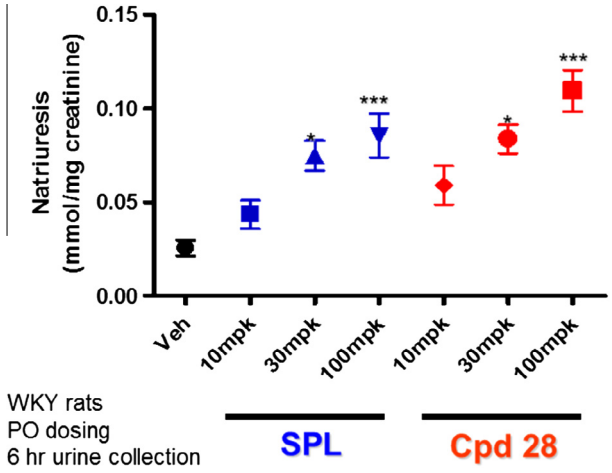


Figure 3. Acute pharmacodynamic evaluation of spironolactone and compound **28** in oral dosed WKY rats.

care, as slow reverse addition of starting material to base was required to further suppress fragmentation. With **16c** in hand, the synthesis was readily completed by exchange of the Boc-protecting group for the desired methylsulfonamide moiety to afford compound **16** (Tables 5 and 6).

Elaboration of the ester moiety in **21** remained as a clear avenue to positively impact activity. The SAR for both simple amides, as well as 1,2,4-oxadiazole bioisosteres, revealed a similar trend as previously observed for other substituents.[13] There was a clear preference for compact aliphatic groups (**23**–**24**, **26**) over sterically larger groups (**25**, **27**). Reduction of the ester to the primary alcohol yielded compound **28**,[14] which exhibited a better off-target profile along with excellent lipophilic ligand efficiency (Scheme 1).

Confirmation of in vivo efficacy followed from initial pharmacokinetic evaluation of key compounds.[15] Intravenous and oral dosing of compounds **23** and **28** in rats affirmed the potential for the series to achieve oral efficacy. Despite modest clearance for both representative compounds, both compounds **23** and **28** exhibited suitable half-life and oral exposures to support investigation of their effect in an acute rat natriuresis model (Fig. 3).

An important experiment to validate the lead series involved confirmation of in vivo efficacy in an animal model. A well characterized pharmacodynamic model for assessing target engagement in vivo involves quantifying the natriuretic effect in rats treated with an MR antagonist.[16] WKY rats on an established low-salt diet were treated with compound **28** in a dose dependent manner, and the urine collected over 6 h was analyzed for cumulative electrolyte output. The effect of compound **28** was benchmarked against a comparable dose titration of spironolactone (SPL) and demonstrated a comparable natriuretic effect. The acute pharmacodynamic data demonstrating comparable efficacy for compound **28** versus a well-established benchmark like spironolactone affirms the promise for the reverse indole series of MR antagonists.

References and notes

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7. (a) Kawaguchi, T. et al. PCT Int. Appl., WO 2007/089034.; (b) Bell, M. G. et al *J. Med. Chem.* **2007**, *50*, 6443; (c) Casimiro-Garcia, A. et al *J. Med. Chem.* **2014**, *57*, 4273.
8. Putative design structures were modeled based on docking experiments performed starting from the 1.95 Å MR structure with bound corticosterone (PDB code 2A3I: Li, Y.; Suino, K.; Daugherty, J.; Xu, H. E. *Mol. Cell* **2005**, *19*, 367;). Structures were prepared by removal of waters, addition of hydrogens, and restrained energy minimization using Macromodel (Macromodel 9.8.107, Schrödinger LLC, New York, NY). Molecular docking was performed by employing our in-house docking routine FLOG (Miller, M. D.; Kearsley, S. K.; Underwood, D. J.; Sheridan, R. P. *J. Comput. Aided Mol. Des.* **1994**, *8*, 153.), which ranked precalculated conformations of the target molecules in a 6 Å grid centered on the crystallographic ligand. Conformations were generated using our in-house metric matrix distance geometry algorithm JG (Kearsley, S. K. Merck & Co., unpublished).; The conformations were subjected to energy minimization with Macromodel using the MMFFs force field (Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720.). A representative docking pose (data not shown) was selected by visual inspection and was subjected to restrained energy minimization (using Macromodel) to produce a putative model.
9. MR antagonist IC_{50} data were determined using a commercially available cell-based PathHunter™ protein–protein interaction assay that measured the ability of compounds to antagonize full-length human MR binding to a coactivator peptide. Similar PathHunter™ assays were applied to determine other nuclear hormone antagonist activities. Average potency in PathHunter™assay: spironolactone 11 nM and eplerenone 244 nM. http://www.discoverx.com/nhrs/prod-nhrs.php.
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13. Amides and heterocycles were prepared by derivatization of compound **17c**. WO2012/097744.
14. *Additional off-target data:* other NHR selectivity IC_{50} >10 μM; Ion Channel IC_{50} (Ikr, Na) >30 μM; CYP (3A4, 2D6, 2C8, 2C9) IC_{50} >13 μM.
15. *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.
16. Procedures were modified based on semilal references: Kagawa, C. M. et al *Science* **1957**, *126*, 1015; Bicking, J. B. et al *J. Med. Chem.* **1965**, *8*, 638. Age-matched male WKY rats ($N = 6$ per group) were fed a low salt diet and maintained under a reverse dark-light cycle environment. Animals were dosed PO using Imwitor:tween as a vehicle (2 mL/kg) at the doses indicated, and urinary electrolytes were analyzed. Jugular vein bleeds were performed at 6 h to determine terminal drug levels (data not shown).