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3,3-Bisaryloxindoles as mineralocorticoid receptor antagonists

David A. Neel,* Matthew L. Brown, Peter A. Lander, Timothy A. Grese, Jean M. Defauw, Robert A. Doti, Todd Fields, Sally Ann Kelley, Stephon Smith, Karen M. Zimmerman, Mitchell I. Steinberg and Prabhakar K. Jadhav

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA

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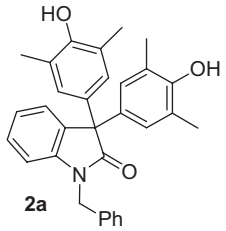
Abstract—Syntheses and SAR studies of 3,3-bisaryloxindole analogues provided potent mineralocorticoid receptor (MR) antagonists that were selective over other steroid nuclear hormone receptors.

Nuclear hormone receptors (NHRs) are a superfamily of ligand-activated transcriptional regulators that are involved in many physiological and pathological processes. The steroid receptor subfamily of NHRs, use classical steroid hormones as natural ligands to regulate gene expression. Steroid NHRs include the glucocorticoid receptor (GR), progesterone receptor (PR), androgen receptor (AR), estrogen receptor (ER), and mineralocorticoid receptor (MR). MRs located in the kidney, are used as the primary means to regulate body sodium, potassium, and water balance by aldosterone.[1] More recently, aldosterone has been shown to mediate deleterious cardiovascular effects through MRs in brain, heart, and vascular tissue.[2]

Congestive heart failure (CHF) continues to be a major contributor to morbidity and mortality. Standard therapies include digitalis, ACE inhibitors, diuretics, and β-blockers. These agents can help relieve symptoms and prolong life, but there continues to be a need for novel agents that target the underlying pathophysiological basis of the disorder. The randomized aldactone evaluation study (RALES) has demonstrated that spironolactone, an MR antagonist, when added to standard therapy, reduces risk of death by 30% and improves cardiac function of patients with severe CHF.[3] The renin–angiotensin–aldosterone-system (RAAS) is responsible for maintaining blood pressure and volume homeostasis. Upstream inhibitors of the RAAS such as angiotensin converting enzyme (ACE) inhibitors and angiotensin II (AII) blockers do not prevent the rise of aldosterone levels over time, a phenomenon known as 'aldosterone escape' (RESOLVD study).[4] These elevated levels of aldosterone result in increased cardiovascular risk for CHF patients and accentuate the potential utility of MR antagonists. Moreover, a recent review suggests that MR antagonists might be useful in other vascular diseases, rather than simply those with elevated levels of aldosterone.[5]

Spironolactone and more recently, eplerenone,[6] are the only therapeutic options available to antagonize the mineralocorticoid receptor. Spironolactone lacks selectivity against other steroid nuclear hormone receptors, primarily AR and PR. Long term use is limited due to undesirable side effects such as gynecomastia in men and menstrual irregularity in women.[7] Eplerenone is more selective, but less potent than spironolactone in vitro.[8]

Our objective was to identify a non-steroidal human MR (hMR) antagonist and thereby diminish the adverse effects of elevated levels of aldosterone under pathological conditions. In order to avoid the deleterious side



2a

* Corresponding author. E-mail: neel_david_a@lilly.com

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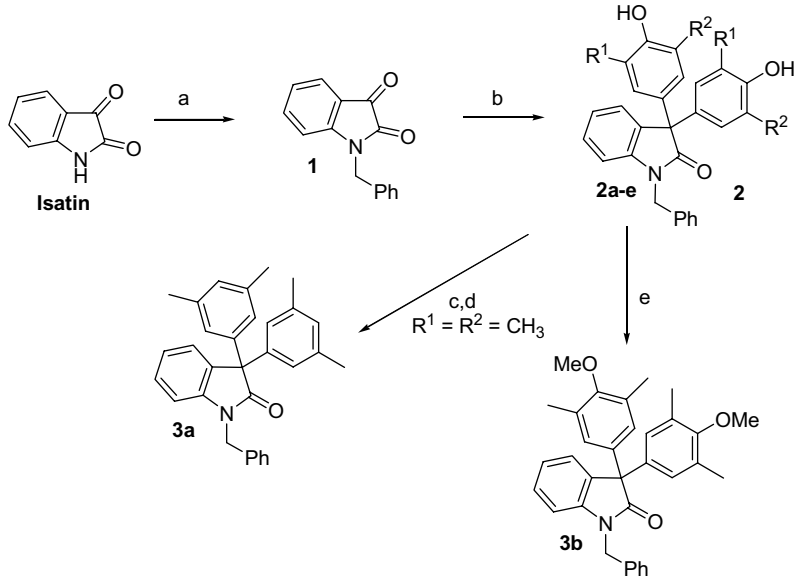
effects of spironolactone, such an antagonist must be selective for hMR over other steroid nuclear hormone receptors. Initial screening identified oxindole derivative **2a** as a ligand for human MR (hMR) and SAR studies were initiated to improve potency and selectivity. To our knowledge, these are the first non-steroidal MR antagonists.

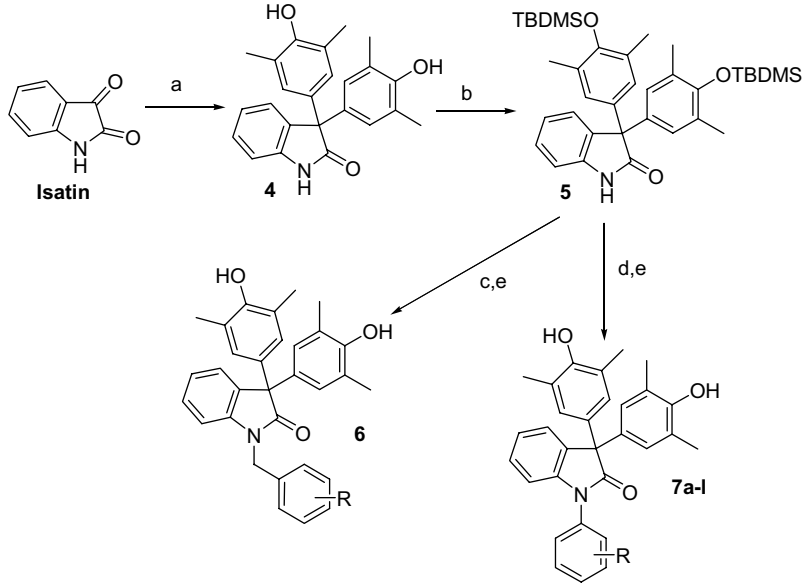
3,3-Bisaryloxindoles were obtained using superacid mediated coupling methodology reported by Olah and co-workers[9] (Scheme 1). The dicationic intermediate generated by subjecting *N*-benzylisatin **1** to trifluoromethanesulfonic acid was condensed with 2 equivalents of a phenol to provide **2a**–**e**. Deoxygenation to **3a** was accomplished by triflation of **2a** and subsequent Pd(0) catalyzed hydrogenolysis.[10]

Treatment of isatin with 2,6-dimethylphenol (Scheme 2) in a Friedel–Crafts procedure[11] provided **4**. Silyl protection of the phenols followed by alkylation and deprotection gave compounds with substituted benzyl groups (**6**). N-Arylation of **5** with phenyl boronic acids and cupric acetate led to compounds **7a**–**l**.[12]

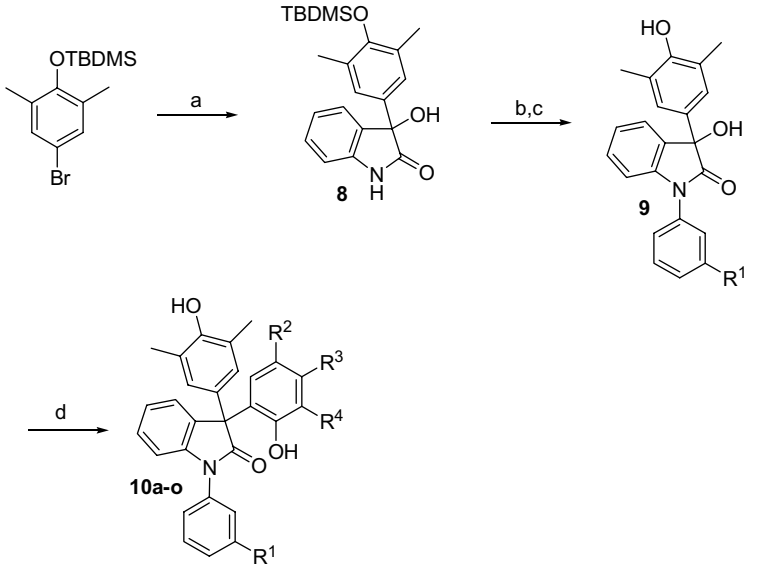
The unsymmetrical bisaryloxindoles **10** were obtained as shown in Scheme 3. Isatin was treated with 2 equiv of



Scheme 1. Reagents and conditions: (a) KO-*t*-Bu, BrBn, 84%; (b) excess phenol, TfOH, 50–70%; (c) 2,4,6-collidine, Tf_2O, 67%; (d) *n*-Bu_3N, $Ph_2P(CH_2)_3PPh_2$, $PdCl_2(PPh_3)_2$, HCO_2H, DMF, 58%; (e) Cs_2CO_3, MeI, DMF, 40 °C, 76%.



Scheme 2. Reagents and conditions: (a) AcOH, $AlCl_3$, 86%; (b) imidazole, DMF, TBDMSCl, 75%; (c) KO-*t*-Bu, XCH_2R, 51–66%; (d) $RPhB(OH)_2$, $Cu(OAc)_2$, TEA, 4 Å sieves, 15–52%; (e) TBAF, 86%.



Scheme 3. Reagents and conditions: (a) *n*-BuLi/THF/−70 °C, then isatin, 80%; (b) $R_1PhB(OH)_2$, $Cu(OAc)_2$, TEA, 4 Å sieves, 64%; (c) TBAF, 86%; (d) TFA, substituted phenol, 61–75%.

the lithio adduct of (4-bromo-2,6-dimethylphenoxy)-*tert*-butyldimethylsilane to provide **8**. Coupling with an appropriately substituted phenylboronic acid followed by deprotection of the phenol provided intermediate **9**. Formation of the carbocation in trifluoroacetic acid in the presence of an excess of a phenol gave **10a**–**o**.

A radioligand binding assay was used to identify human MR (hMR) antagonists. Full length hMR was isolated from a human kidney cDNA library and hMR protein was expressed in Sf9 cells using the baculovirus expression vector system (PharMingen). Affinity of compounds for hMR was determined using vacuum filtration methods. IC_{50} values defined as the concentration of test compound required to decrease [3*H*]aldosterone binding by 50% were determined using non-linear regression curve fitting. Inhibition constants (K_i) were calculated from these IC_{50} values using the Cheng–Prusoff equation.[13] Where compounds exhibited weak affinity and full inhibition was not achieved at 3 μM, IC_{50} could not be calculated, and % inhibition at 1 μM test compound is reported. All data points represent means of at least two determinations unless otherwise indicated.

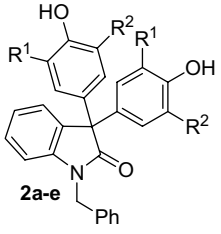
A panel of related steroid nuclear hormone receptors was used to determine hMR selectivity. These included binding assays for human glucocorticoid receptor (hGR), human androgen receptor (hAR), human progesterone receptor (hPR) and human estrogen receptor (hERα, hERβ).

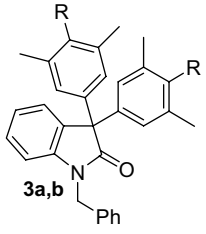
Functional activity was determined using COS-7 cells co-transfected with an expression vector containing full length hMR and a luciferase reporter plasmid responsive to hMR activation. Ability of test compounds to inhibit the agonist response of aldosterone on hMR was determined in the absence and presence of test compound and measured by luciferase luminesence. Neutral antagonist affinity (K_b) was estimated using the ratio of the EC_{50} in the presence and absence of 1 μM test compound using the Schild equation, $K_b = [\text{antagonist}]/(\text{dose ratio} - 1)$.[14] Non-linear regression analysis was used to estimate EC_{50} values.

We quickly recognized that simple changes to the bis-dimethyl phenol resulted in dramatic loss of hMR affinity (Table 1). The biological activity of **2a** was surprisingly sensitive to any changes to the methyl groups as shown by **2b** and **e**. Other changes to these symmetrical molecules, wherein the hydroxyls were removed (**3a**), or capped with methyls (**3b**), resulted in complete loss of activity.

Extensive substitution on the N-benzyl group of **2a** (giving compounds of type **6**) resulted in only modest improvements in potency or selectivity (data not shown). However, when the phenyl ring was directly attached to the oxindole core, there was a dramatic

Table 1. hMR Binding of compounds **2a**–**e** and **3a** and **b**

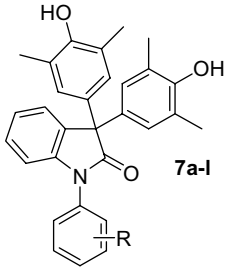




Cpd	R^1	R^2	hMR K_i (nM)	Cpd	R	hMR K_i (nM)
2a	Me	Me	65	**3a**	H	16%[a]
2b	H	H	22%[a]	**3b**	OMe	0%[a]
2c	Me	H	423			
2d	F	F	16%[a]			
2e	Cl	Cl	701			

[a] Percent inhibition at 1 μM antagonist concentration.

Table 2. Steroid NHR binding of compounds **7a–l**



Cpd	R	hMR K_i (nM)	hGR	hPR	hAR	hERα	hERβ
7a	H	27	147	44%[a]	41%[a]	733	>1100
7b	2-Cl	15	188[b]	36	51%[a]	179	354
7c	2-Me	24	115	218	594	>1100	1044
7d	3-Me	7	84	138	306	183	844
7e	4-Me	20	180[b]	297	425	315	890
7h	3-OMe	0.6	51	715	402	242	930
7i	4-OMe	39	158	36%[a]	316	402	>1100
7j	3-OEt	3	224	284	277	196	926
7k	3-CF_3	12	275[b]	194	390	163	316
7l	4-CF_3	156	145	821	43%[a]	771	1016

[a] Percent inhibition at 1 μM antagonist concentration.
[b] One determination.

improvement in potency and selectivity (Table 2). *meta*-Substitution provided the best combination of potency and selectivity as exemplified by **7d**,**h**, and **j**.

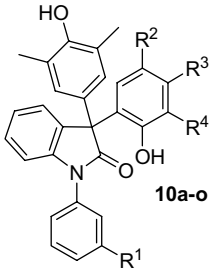
The unsymmetrical phenols (Table 3) provided compounds with the optimum selectivity. In particular, the resorcinol analogues, **10d**,**e**,**g**, and **o** were less potent at hGR than any of the other oxindoles in this series. We speculate that the *ortho*-phenol provides a rigid conformer through hydrogen bonding to the carbonyl of the oxindole, allowing for optimum discrimination between the binding pockets of hMR and hGR. Also notable is the lack of space available for larger substituents. Ethyl **10i**, was dramatically less potent than methyl **10h**. The resorcinol analogues, **10d**,**e**,**g**, and **o** had the best selectivity across the panel of steroid NHRs tested. Compound **10o** was separated by chiral chromatography into its (+)- and (−)-enantiomers. The (−)-enantiomer was significantly more potent and selective.

Table 4. Transcriptional activity of select compounds

Cpd	hMR K_i (nM)	K_b (nM)
7d	7	56
7h	0.6	26
10a	1	89
10d	3	150
10f	3	181
10g	2	93
10o(−)	1	41
Spironolactone	2	18

The functional activity of a select group of ligands is shown in Table 4. The resorcinol analogue **10o(−)** as

Table 3. Steroid NHR binding of compounds **10a–o**



Cpd	R^1	R^2	R^3	R^4	hMR K_i (nM)	hGR	hPR	hAR	hERα	hERβ
10a	OMe	H	Me	Me	1	165	485	539	>1100	>1100
10b	OMe	Me	H	Me	2	66	453	44%[a]	>1100	>1100
10c	OMe	Me	H	H	3	62	573	44%[a]	>1100	>1100
10d	OMe	H	OH	H	3	793[b]	678	19%[a]	>1100	>1100
10e	OMe	H	OH	Me	1	693[b]	395	1105	>1100	>1100
10f	OCF_3	H	Me	Me	1	470	762	29%	>1100	>1100
10g	OCF_3	H	OH	Me	2	519	796	1203	335	896
10h	OCF_3	Me	H	H	7	843[b]	217	41%	>1100	>1100
10i	OCF_3	Et	H	H	395	72	378	47%	>1100	>1100
10j	Me	H	Me	Me	8	100	206	40%	>1100	>1100
10k	Me	Me	H	H	6	146[b]	228	53%	>1100	>1100
10l	Me	H	Me	H	9	79	251	52%	>1100	>1100
10m	Me	F	H	OMe	130	143	448	50%	346	>1100
10n	Me	F	H	Me	6	77	310	242	650	>1100
10o	Me	H	OH	Me	2	334	245	37%	579	>1100
10o(−)	Me	H	OH	Me	1	582	33%	16%[a]	359	>1100
10o(+)	Me	H	OH	Me	40	686[b]	49%	20%	519	>1100

[a] Percent inhibition at 1 μM antagonist concentration.
[b] One determination.

well as **7d** and **h** had the greatest potency as antagonists. There was no agonist or partial agonist activity observed.

In summary, we have identified a series of 3,3-bisaryloxindoles that are potent non-steroidal hMR antagonists. These ligands display excellent selectivity for hMR over hGR, hPR, hAR, and hER. The best combination of hMR potency and selectivity was demonstrated with analogues wherein one of the aryl groups was a resorcinol derivative. On the basis of our results, further studies to investigate the potential of selective non-steroidal hMR antagonists to treat CHF are warranted.

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