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# Identification of Spirooxindole and Dibenzoxazepine Motifs as Potent Mineralocorticoid Receptor Antagonists

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ABSTRACT

Mineralocorticoid receptor (MR) antagonists continue to be a prevalent area of research in the pharmaceutical industry. Herein we report the discovery of various spirooxindole and dibenzoxazepine constructs as potent MR antagonists. SAR analysis of our spirooxindole hit led to highly potent compounds containing polar solubilizing groups, which interact with the helix-11 region of the MR ligand binding domain (LBD). Various dibenzoxazepine moieties were also prepared in an effort to replace a known dibenzoxepane system which interacts with the hydrophobic region of the MR LBD. In addition, an X-ray crystal structure was obtained from a highly potent compound which was shown to exhibit both partial agonist and antagonist modes of action against MR.



## 1. Introduction

The mineralocorticoid receptor (MR), one of the 48 members of the nuclear hormone receptor (NHR) superfamily, is responsible for regulating gene expression. Aberrant activation of MR leads to a variety of medical conditions such as cardiovascular disease, chronic kidney disease and hypertension. Discovered over 50 years ago, aldosterone is considered the major endogenous ligand for MR. Thus, elevated levels of aldosterone ultimately cause an increase in blood pressure through the inhibition of natriuresis leading to elevated levels of sodium in the blood. [1-2]

MR is part of an NHR subfamily commonly referred to as the estrogen receptor-like family which consists of estrogen (ER), glucocorticoid (GR), progesterone (PR) and androgen (AR) receptors. The ligand binding domains (LBDs) of GR and AR are structurally homologous to the LBD of MR. These high structural homologies present a challenge in developing motifs that selectively bind to MR in the LBD in order to circumvent any potential side effects resulting from other estrogen receptor-like interactions.

To date, only two steroidal MR antagonists, spironolactone (**1**) and eplerenone (**2**), are marketed as drugs and used for the treatment of heart failure and hypertension (Figure 1). Discovered over 50 years ago and still a widely used medicine, spironolactone suffers from a lack of selective binding to MR leading to a variety of unwanted side effects such as gynecomastia/feminization in men and menstrual irregularities in women. The more current MR antagonist, eplerenone, also known by its trade name Inspra®, was brought to market by Pfizer in 2002. Although this newer steroidal medicine does possess good selectivity to the MR LBD, it suffers from a lack of potency and is therefore taken orally twice daily. There is a clear unmet medical need for new MR antagonists without harmful side effects due to poor selectivity while maintaining high levels of potency for better dosing regimens and patient compliance.[3]

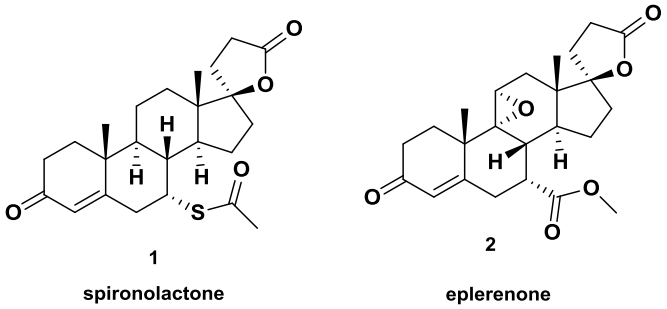



**Figure 1**. Marketed MR antagonist drugs

A variety of non-steroidal MR antagonists have been disclosed in the patent and journal literature over the past ten years (Figure 2).[4] The first crystal structure of a nonsteroidal MR antagonist (**4b**) in the MR LBD was elucidated just three years ago by a team from Takeda.[4b] Other chemotypes have also been disclosed including compound **3b** from Eli Lilly[4c] as well as compounds **5** and **6** which were discovered by Dainippon Sumitomo[4d] and Novartis,[4e] respectively. In addition, Bayer's BAY-94-8862, also known as Finerenone (**6b**), is currently in clinical trials as an MR antagonist for the treatment of heart failure.[4i] Of the several structural motifs in the literature possessing MR antagonist activity, we were particularly drawn to compounds **3-6** as a starting point for our efforts. Utilizing Contour®, our structure-based design platform[4h], as a molecular modeling tool, our goal for this project was to develop novel, potent and selective nonsteroidal MR antagonists.

When Vitae initiated the MR project, there were ten liganded MR structures in the PDB (Protein Data Bank), all of which were steroid ligands that bound very similarly to MR.[5] However, in the structure of the S810L mutant of MR with bound spironolactone (PDB code, 2OAX),[5d] the Met-852 sidechain swings away, creating a new pocket to accommodate the thioester sidechain of spironolactone. We hypothesized that this new pocket could be useful for optimizing off-target selectivity. Therefore, 2OAX was used to model various molecular designs. First, we docked two known non-steroidal MR antagonists from the literature: compound **3a**[4f] and **4a**[4a] (Figure 2).

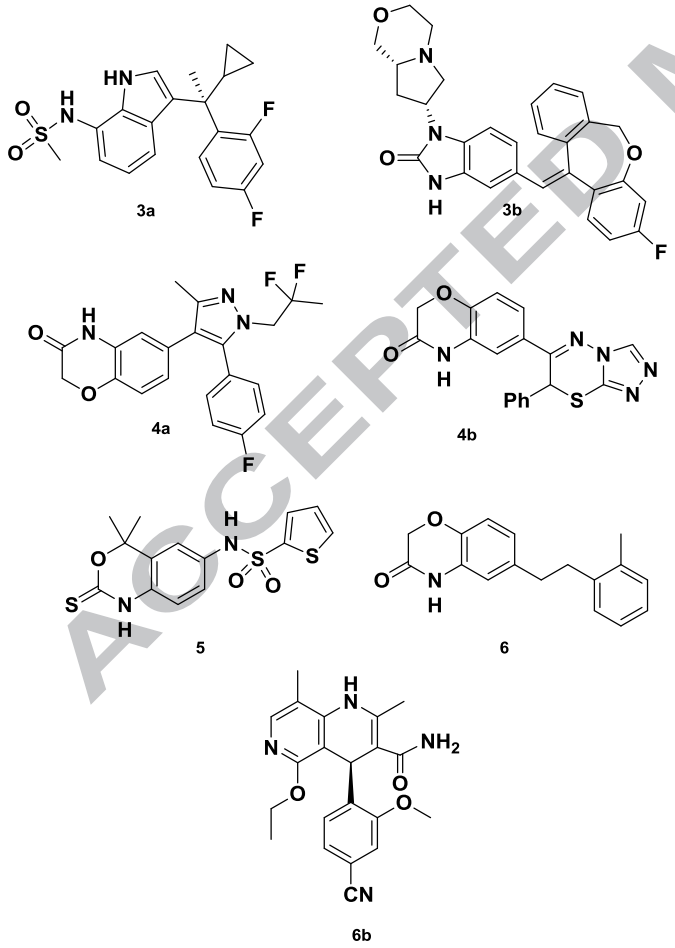



**Figure 2**. MR antagonists from literature and patents

The proposed binding modes based on the molecular modeling for these two compounds are shown in Figures 3 and 4. These binding modes appeared to be consistent with the binding mode of tanaproget with the progesterone receptor (PR) as revealed in the crystal structure (PDB code, 1ZUC).[6] If the proposed binding modes for compounds **3a** and **4a** are correct, one

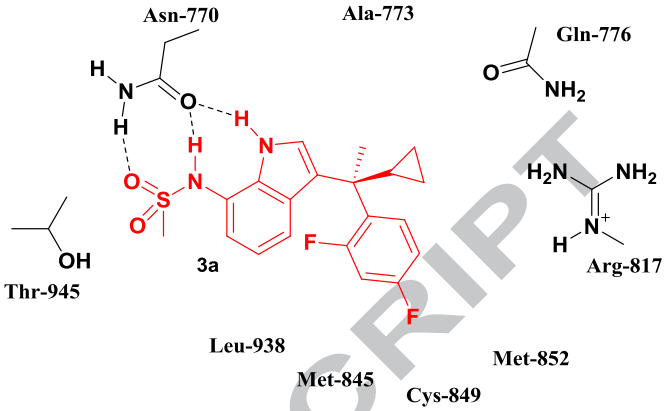



**Figure 3**. The proposed binding mode for compound **3a**.

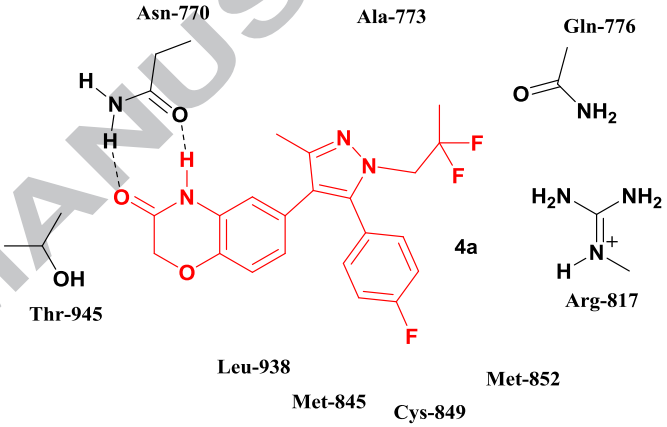



**Figure 4**. The proposed binding mode for compound **4a**.

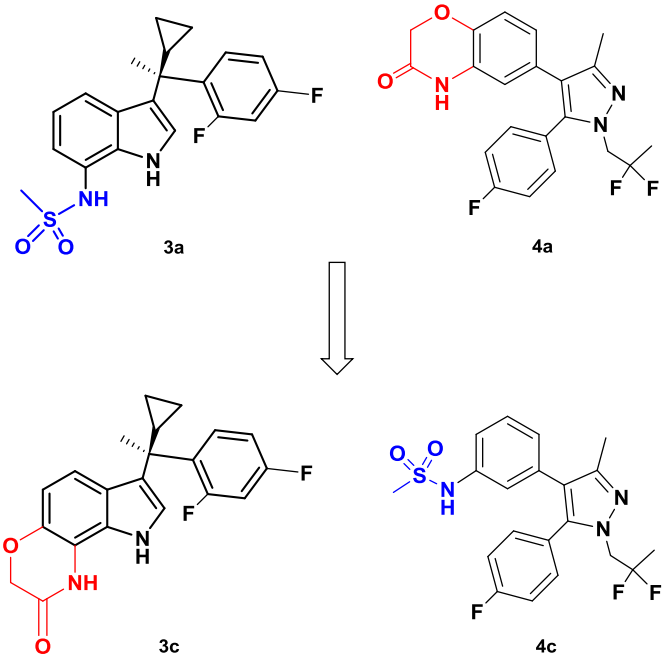



**Figure 5**. Hybrid structures **3c** and **4c**

can imagine hybrid structures **3c** and **4c** (Figure 5) as potential ligands for MR. To test this hypothesis, compound **4c** was synthesized and was found to bind to MR with a $K_i$ of 390 nM

and showed inhibition of MR activity in a cellular Gal4 assay ($IC_{50}$ = 889 nM), suggesting the validity of our proposed binding model. Moreover, the binding mode of **4a** was subsequently confirmed crystallographically by Takeda.[4b, g]

To generate ideas, we computationally grew various structures to fill the ligand binding pocket starting from the benzoxazinone fragment (Figure 6). One of the best scoring structures generated had an aromatic ring attached to the benzoxazinone moiety through a two-atom linker as shown in Figure 7. Interestingly, 2,4-difluorophenyl compound **7** (Figure 7) which came out of this analysis was quite similar to compound **6** (Figure 2) claimed in an earlier Novartis patent.[4e]

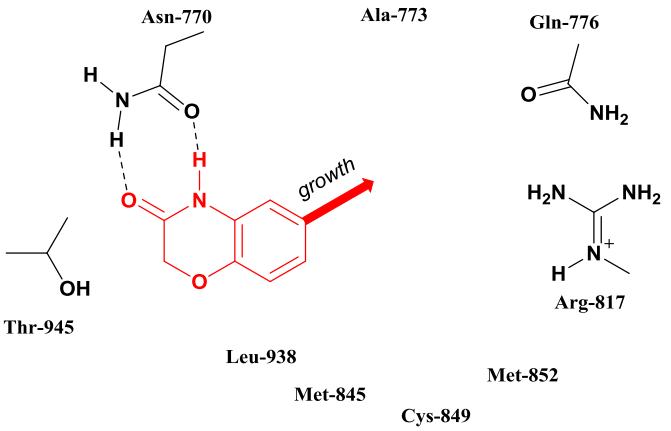



**Figure 6**. Growth from benzoxazinone moiety.

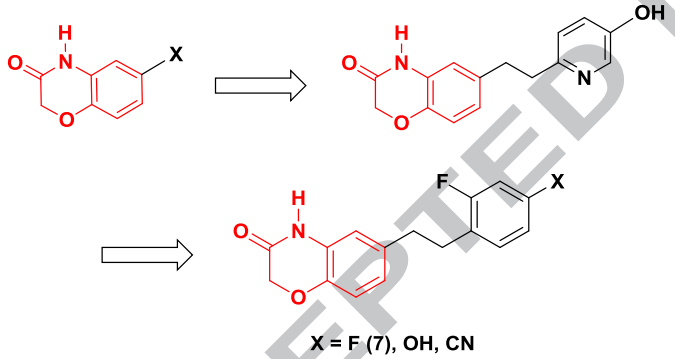



**Figure 7**. Development of phenethyl scaffold from Contour®

## 2. Chemistry and SAR Analysis

Compound **7** was prepared in two steps from commercially available bromobenzoxazinone **8** via Sonogashira coupling followed by hydrogenation to give this target which exhibited good binding and cellular potency in our MR assays (Figure 8). SAR analysis of compound **7** was performed in an attempt to gain insight into the effects of changing the phenethyl sidechain shape in the hydrophobic pocket of the MR LBD where the Met-845, Met-852 and Cys-849 reside. Interestingly, more rigid templates housing either an olefin or alkyne resulted in a 7- to 13-fold loss in binding potency (**12-14**). Apparently, the conformational flexibility of the saturated phenethyl sidechain was more compatible with the ligand binding pocket giving rise to its tighter binding affinity. It is also interesting to note that incorporation of an oxygen into the phenethyl sidechain (**15**) resulted in a ~50-fold loss in potency compared to **7**, presumably due to the conformational preference of phenethyl versus phenoxymethyl.

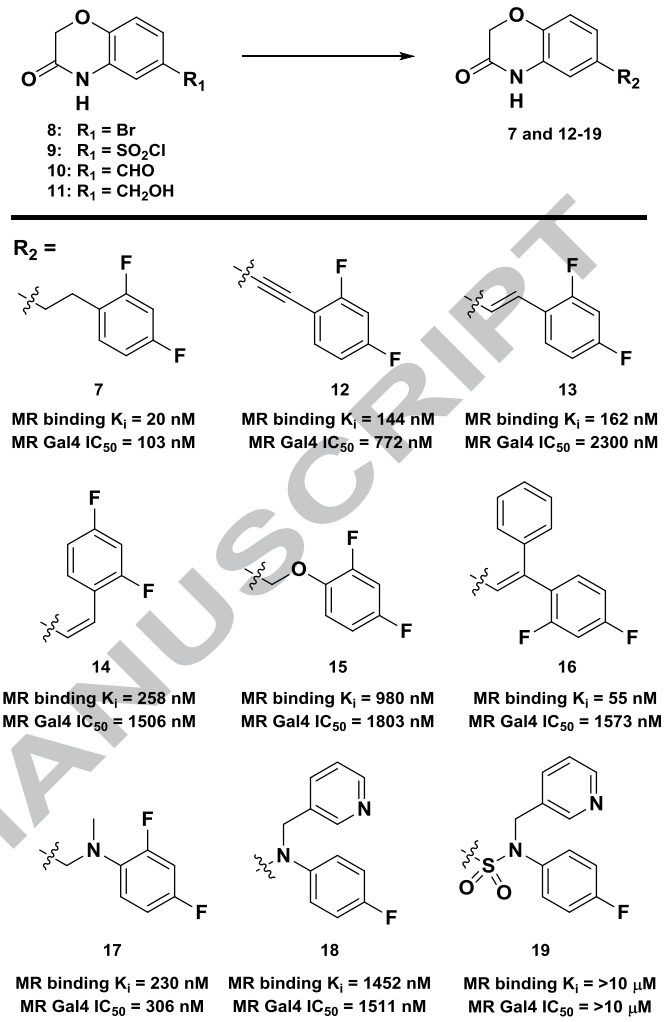
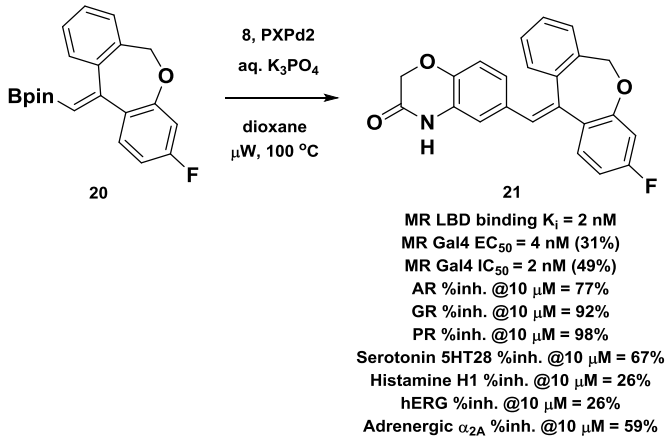


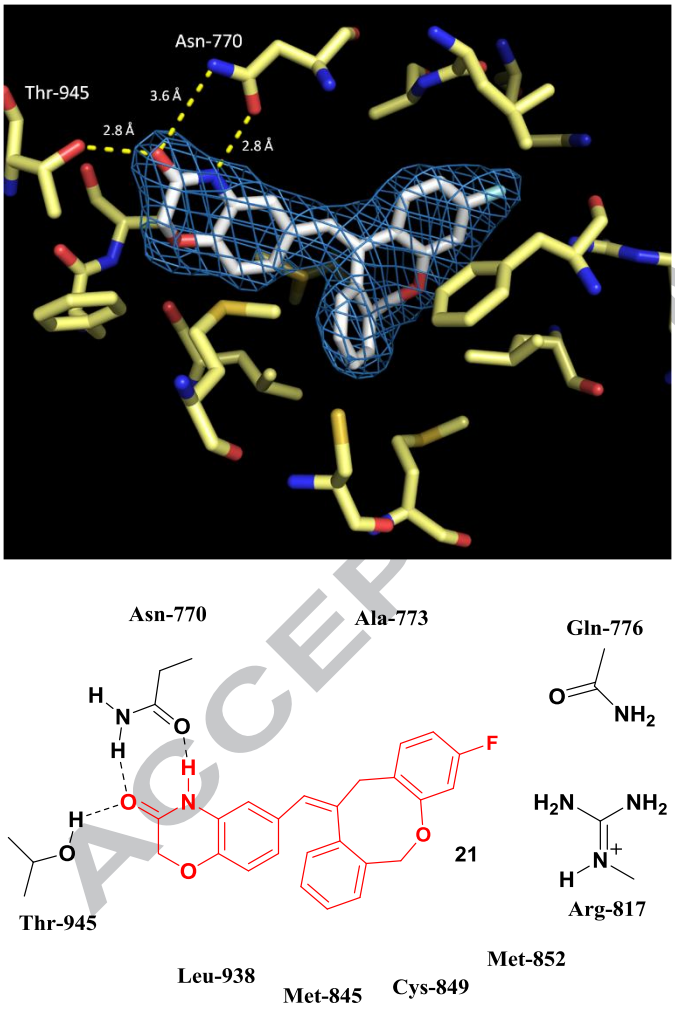

**Figure 8.** Modifications of the Hydrophobic Region. Efficacy levels for compounds **7**, **12-18** were 100%.

Therefore, various bifurcated constructs were identified and synthesized in order to fill more space in the hydrophobic pocket in an attempt to improve the binding affinity. From this analysis, compound **16** exhibited a binding potency of 55 nM which was roughly a 3- to 5-fold improvement from **13** and **14** but was still 3-fold less potent than **7** and suffered a significant shift in the cellular assay. Initially, more polar aniline motifs, such as **17**, showed promising data with virtually no shift when comparing the binding and cellular potencies. However, larger substituents on the amine (**18**) as well as sulfonamide incorporation (**19**) led to diminished potencies.

By comparing compound **3b** (Figure 2) with the phenethyl scaffold, it became clear that one of the fused benzene rings of the tricyclic dibenzoxepane moiety was filling the Met-852 pocket. We decided to combine the tricyclic system contained in compound **3b** with the Asn-770 interacting benzoxazinone scaffold seen in **4a**. Hence, coupling of compound **8** with known boronate ester **20**[4c] gave compound **21** which exhibited excellent binding and cellular potency (Scheme 1). Although this compound had exquisite potency, it was shown to behave as both a partial agonist as well as a partial antagonist.




**Scheme 1.** Discovery of potent partial MR antagonist **21.** PXPd2 - dichloro[di-*tert*-butyl(chloro)phosphine] palladium(II) dimer




**Figures 9**. The binding pose of **21** in the MR ligand binding pocket. The electron density map for **21** is contoured at 1.0 σ (PDB code 5HCV). The hydrogen bonds between **21** and the Asn-770 and Thr-945 residues are indicated along with a ChemDraw rendering of the crystal structure.

We subsequently obtained an X-ray crystal structure of MR bound to **21** at 2.5 Å (Figure 9, PDB Code 5HCV). X-ray structural analysis of compound **21** in the MR LBD clearly indicated hydrogen bonding interactions between the benzoxazinone *cis*-amide moiety and the amide side chain of Asn-770, in addition to the hydroxyl group of Thr-945. Moreover, MR bound with **21** existed as a trimer in the crystal form. The overall conformations observed were very similar among the three monomers except for minor differences in the side chain conformations as well as small differences in the intermolecular hydrogen bonding distances.

A 6-nanosecond molecular dynamics simulation was performed on MR with bound **21** to probe the conformational flexibility of the complex. The overall structure was quite stable during the course of the simulation. The complex appeared to oscillate between two main ensembles of conformations: one with and one without the hydrogen bonding between the carbonyl oxygen of **21** and the OH of the Thr-945, while both had the Asn-770 interaction.

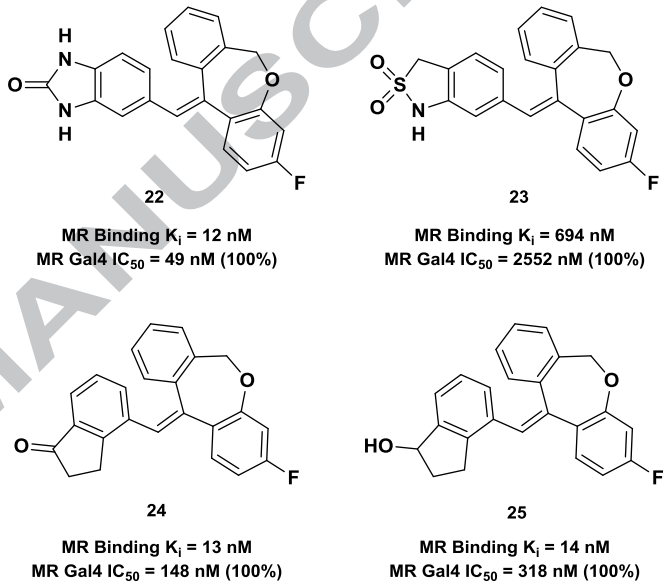
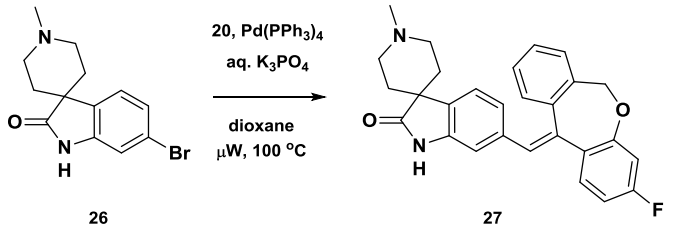



**Figure 10.** Modifications of the hydrophilic region

Although compound **21** suffered from a lack of selectivity, in addition to CYP (cytochrome P450) and off-target[7] liabilities, we decided to explore other Asn-770 interacting scaffolds to try and improve upon these areas while maintaining high levels of potency. Thus, other bicyclic systems were also examined with tricyclic scaffold **20** which exhibited reasonable levels of binding and cellular potencies (Figure 10, **22-25**). However, all of these compounds suffered from a lack of selectivity to AR, PR, GR and ER. Based on these results, we speculated that the pyrrolo-oxazine substituent in compound **3b** was necessary for achieving high levels of selectivity as well as improving the physical properties of the molecule.

Therefore, we explored the feasibility of preparing compounds with substitution directed towards the helix-11 region of the MR LBD, similar to that of the pyrrolo-oxazine substituent in compound **3b**. Ideally, a polar substituent with functionality amenable to rapid SAR was preferred. This led us to consider an oxindole construct bearing a spirocyclic piperidine due to its intrinsic polarity in addition for the opportunity to perform various derivatizations of the amine functionality. Thus, Suzuki coupling of commercially available spirooxindole **26** with **20** gave compound **27** which possessed not only excellent binding and cellular potency but better selectivity against AR, PR, GR and ER (Scheme 2). Although this compound still suffered from off-target activities[7] as well as CYP and RLM (rat liver microsome) issues, the improved selectivity against the NHRs

was encouraging and therefore we sought to overcome these liabilities through SAR exploration on the nitrogen of the piperidine ring system.



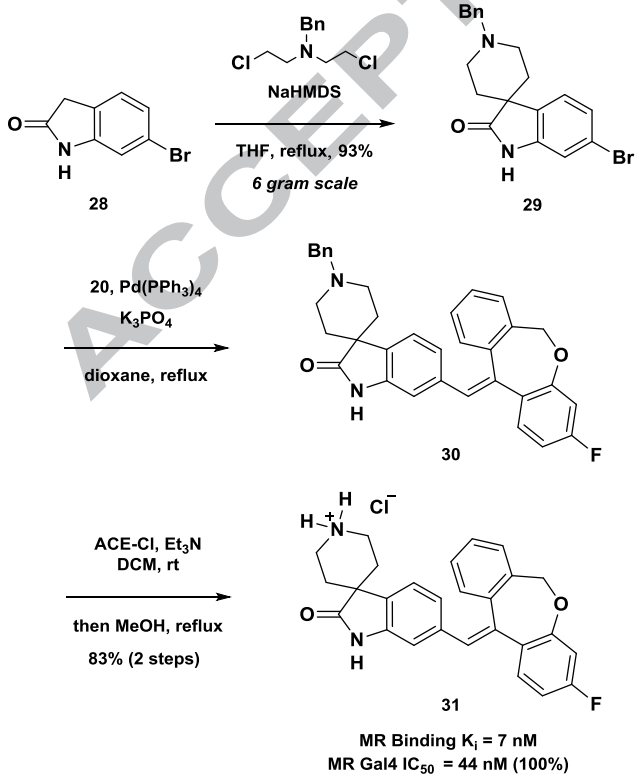

26

27

MR Binding $K_i$ = 6 nM
MR Gal4 $IC_{50}$ = 19 nM (99%)
<40% inh. @10 μM for AR, GR, PR and ER
CYP 2C9 $IC_{50}$ = 2828 nM
CYP 3A4 $IC_{50}$ = 7040 nM
CYP 2D6 $IC_{50}$ = 7624 nM
Serotonin 5HT28 %inh. @10 μM = 87%
Histamine H1 %inh. @10 μM = 97%
hERG %inh. @10 μM = 78%
Adrenergic $\alpha_{2A}$ %inh. @10 μM = 100%
Rat Liver Microsomes = 31 min
ClogD = 3.21

**Scheme 2.** Discovery of potent spirooxindole compound **27**

Consequently, benzyl protected spirooxindole **29** was prepared in one step from commercially available bromo-oxindole **28** in excellent yield on a multi-gram scale (Scheme 3).[8] Suzuki coupling of **29** with **20** gave compound **30** followed by benzyl deprotection using 1-chloroethyl chloroformate[9] afforded unprotected piperidine **31** as the HCl salt. Various modifications of the piperidine were performed introducing a variety of polar substituents. This SAR analysis led to the identification of three compounds (**32-34,** Figure 11) which showed good binding and cellular potencies as well as excellent selectivities (400 to >1000 fold) against GR, PR, and ER. In addition, these compounds had reasonable CYP profiles and excellent HLM stabilities. It is interesting to note that polar groups such as a carboxylic acid and




28

29

30

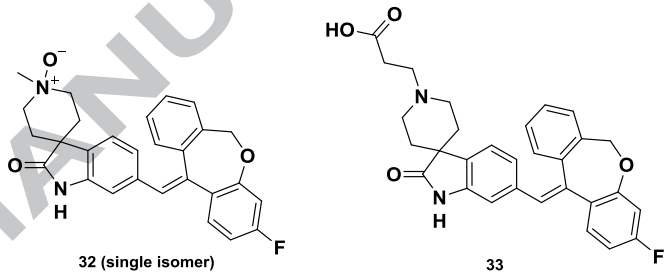
31

MR Binding $K_i$ = 7 nM
MR Gal4 $IC_{50}$ = 44 nM (100%)

**Scheme 3**. Gram scale synthesis of spirooxindole amine **31**

dimethylamine were tolerated in these molecules without any significant loss in potency.[9b] However, further analysis still showed some level of off-target activity for the 4 panel PanLabs screen.[7] Subsequently, we found that Eli-Lilly's compound **3**b (bottom of Figure 11) had a similar profile when tested in our in-house assays. In an effort to circumvent these off-target issues, a number of other spirocyclic systems were prepared (**35-41**, Figure 12). Although these compounds still maintained high levels of potency they still suffered from the same off-target activities.

At this stage, we decided to investigate potential modifications of the tricyclic system in the hydrophobic region. Based on earlier efforts, it was clear to us that the tricyclic dibenzoxepine system was adequately filling the desired space in the hydrophobic region leading to high levels of potency. Therefore, we investigated various tricyclic heterocyclic motifs which would mimic the space filling ability of the dibenzoxepane system but provide more polarity. We reasoned that an increase in polarity could lead to a better off-target profile.



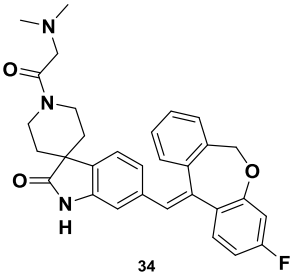

32 (single isomer)

MR Binding $K_i$ = 19 nM
MR Gal4 $IC_{50}$ = 44 nM (100%)
>37,000 nM Gal4 assay vs. PR, GR, ER
CYP 2C9 $IC_{50}$ = 16986 nM
CYP 3A4 $IC_{50}$ = 26139 nM
CYP 2D6 $IC_{50}$ = >30000 nM
Serotonin 5HT28 %inh. @10 μM = 66%
Histamine H1 %inh. @10 μM = 65%
hERG %inh. @10 μM = 55%
Adrenergic $\alpha_{2A}$ %inh. @10 μM = 60%
Rat Liver Microsomes = >60 min
ClogD = 3.8



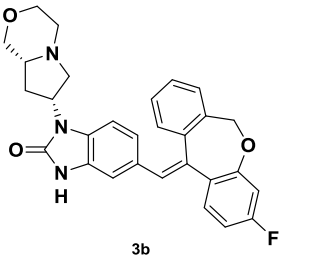

33

MR Binding $K_i$ = 16 nM
MR Gal4 $IC_{50}$ = 29 nM (98%)
>12,000 nM Gal4 assay vs. PR, GR, ER
CYP 2C9 $IC_{50}$ = 12572 nM
CYP 3A4 $IC_{50}$ = 25988 nM
CYP 2D6 $IC_{50}$ = 29880 nM
Serotonin 5HT28 %inh. @10 μM = 36%
Histamine H1 %inh. @10 μM = 60%
hERG %inh. @10 μM = 32%
Adrenergic $\alpha_{2A}$ %inh. @10 μM = 75%
Rat Liver Microsomes = >60 min
ClogD = 1.3




34

MR Binding $K_i$ = 6 nM
MR Gal4 $IC_{50}$ = 6 nM (100%)
>5,000 nM Gal4 assay vs. PR and GR
>40,000 nM vs. ER
CYP 2C9 $IC_{50}$ = 3259 nM
CYP 3A4 $IC_{50}$ = 4778 nM
CYP 2D6 $IC_{50}$ = 7754 nM
Serotonin 5HT28 %inh. @10 μM = 79%
Histamine H1 %inh. @10 μM = 77%
hERG %inh. @10 μM = 98%
Adrenergic $\alpha_{2A}$ %inh. @10 μM = 88%
Rat Liver Microsomes = 53 min
ClogD = 3.9




3b

MR Binding $K_i$ = 9 nM
MR Gal4 $IC_{50}$ = 23 nM (100%)
>25,000 nM Gal4 assay vs. PR, GR, ER
CYP 2C9 $IC_{50}$ = 1600 nM
CYP 3A4 $IC_{50}$ = 5810 nM
CYP 2D6 $IC_{50}$ = >30000 nM
Serotonin $5HT_{28}$ %inh. @$_{10}$ μM = 62%
Histamine H1 %inh. @10 μM = 49%
hERG %inh. @10 μM = 62%
Adrenergic $\alpha_{2A}$ %inh. @10 μM = 76%
Rat Liver Microsomes = 34 min
ClogD = 3.4

**Figure 11.** Discovery of lead compounds in spirooxindole series

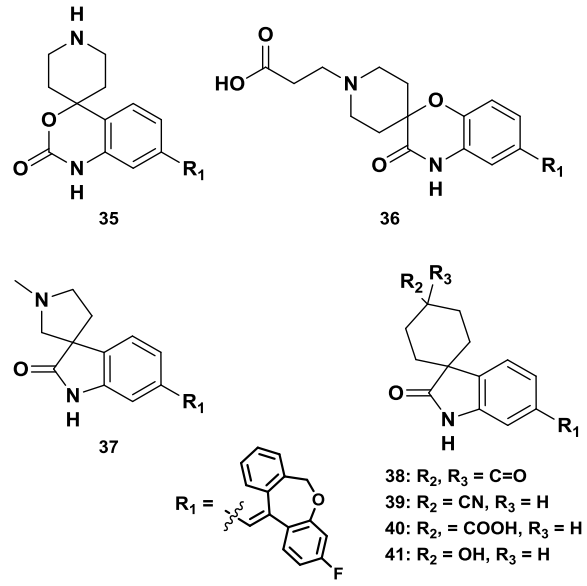



**Figure 12.** Analogs and modifications of the spirooxindole hydrophilic region

We were immediately drawn to various dibenzoxazepane systems of type **43** (Figure 13). Although there is literature precedence for such motifs,[10] installation of such constructs into our spirocyclic scaffolds did not seem like a trivial task. Indeed, this hypothesis was confirmed when attempted couplings of either sulfonyl or acid chlorides **44** (bottom of Figure 13) with various tricyclic systems (**45)** suffered from very low conversions (<5%) to the desired sulfonamides and amides (**46**). Alternatively, we investigated the feasibility of a late-stage 7-membered ring cyclization event to form the dibenzoxazepane system. Since intramolecular C-N bond formations are known for medium-size rings[11], we thought that this was a reasonable strategy to pursue.

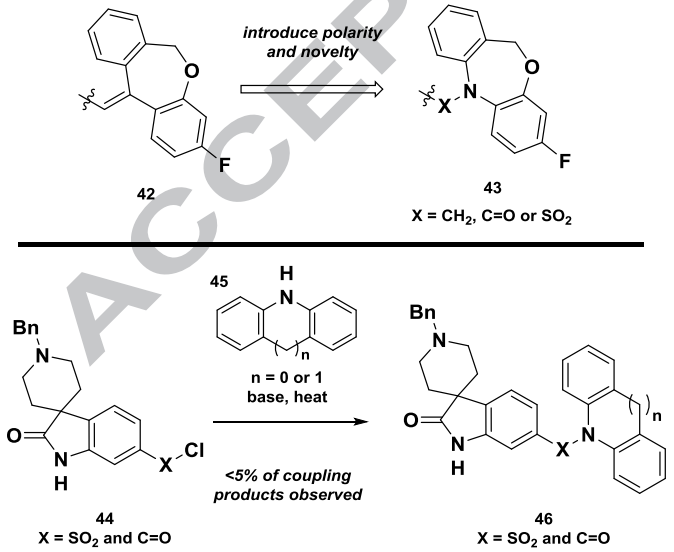
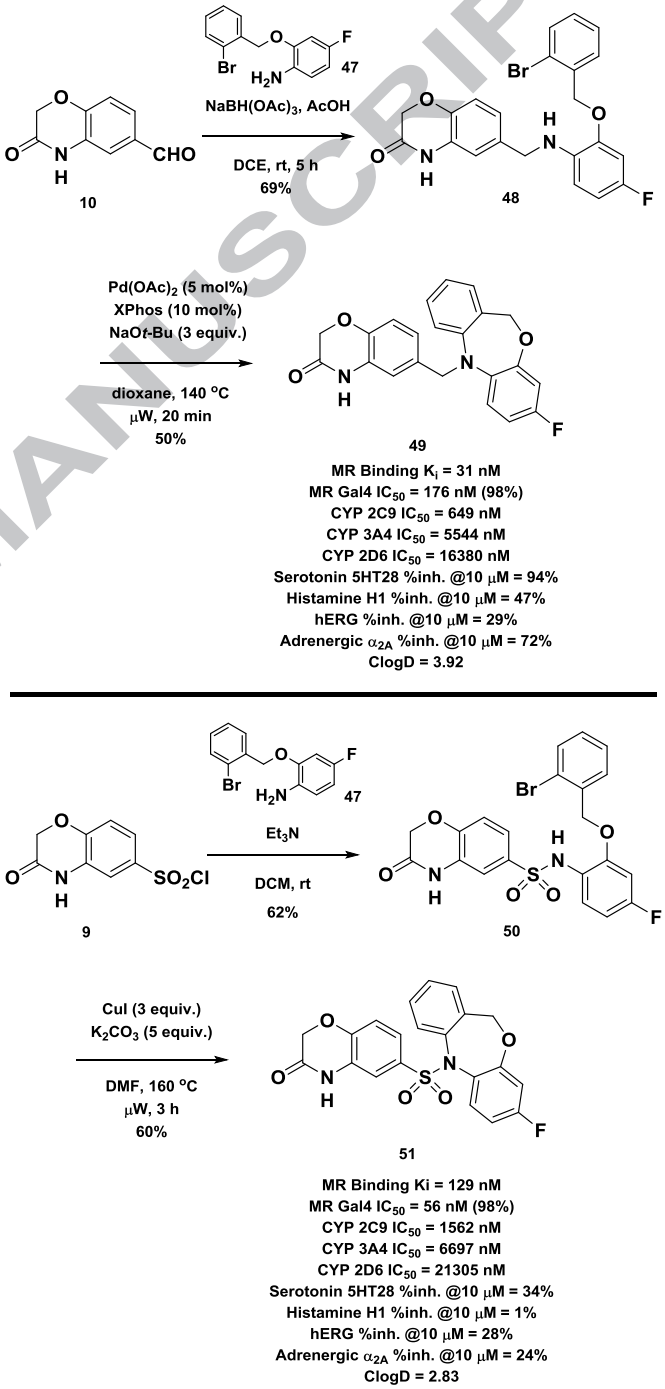



**Figure 13.** Modification of the dibenzoxepane system

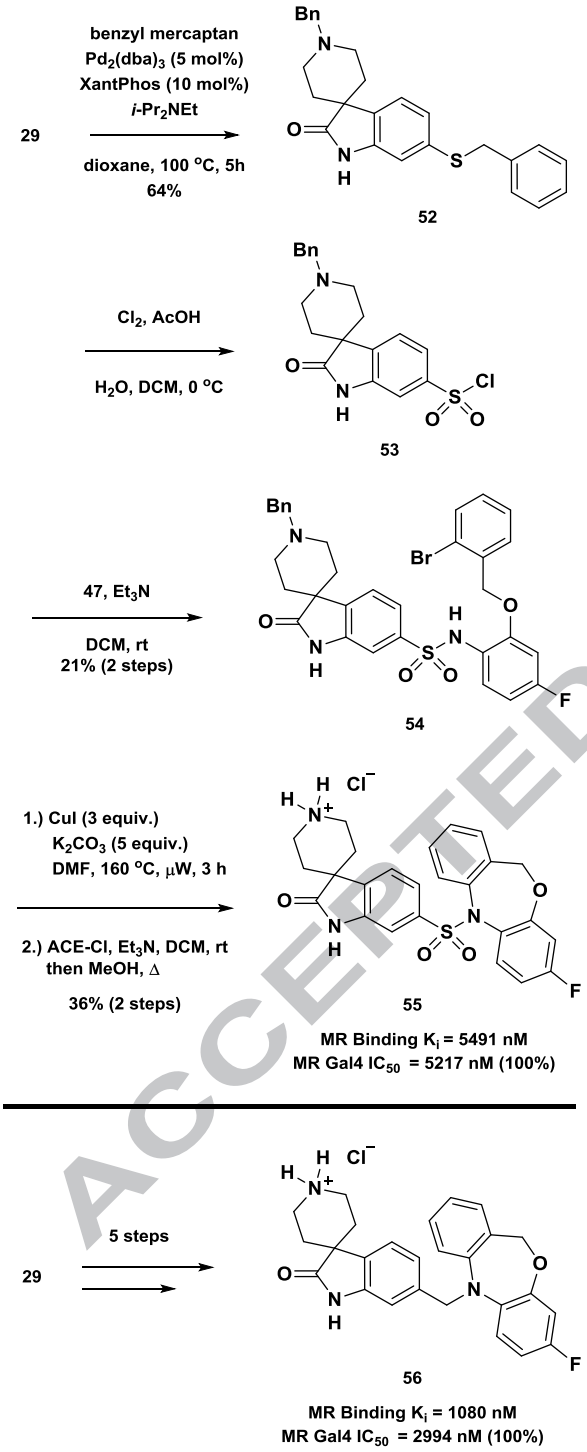
Thus, coupling of known aniline **47** with commercially available benzoxazinones **10** and **9** gave benzylic amine **48** and sulfonamide **50** respectively (Scheme 4). Various conditions were investigated for the 7-membered ring cyclizations via C-N bond formation. For benzylic amine substrate **48**, $Pd(OAc)_2$-catalyzed conditions employing XPhos with NaO*t*-Bu as the base gave a smooth conversion to tricyclic dibenoxazepane system **49** in 50% yield. Alternatively, sulfonamide substrate **50** underwent clean and rapid cyclization to **51** using CuI with $K_2CO_3$ as the base. Although these compounds suffered from a loss in potency, we were encouraged by the off-target profile of sulfonamide **51**. Thus, incorporation of a sulfonamide in the hydrophobic region seemed to alleviate the previous off-target liabilities seen in compound **21**.




**Scheme 4.** Synthesis of dibenzoxazepanes via 7-membered cyclization

We decided to prepare our previously explored spirooxindole templates with the dibenzoxazepane scaffolds in the hydrophobic region (Scheme 5). Thus, bromide **29** was

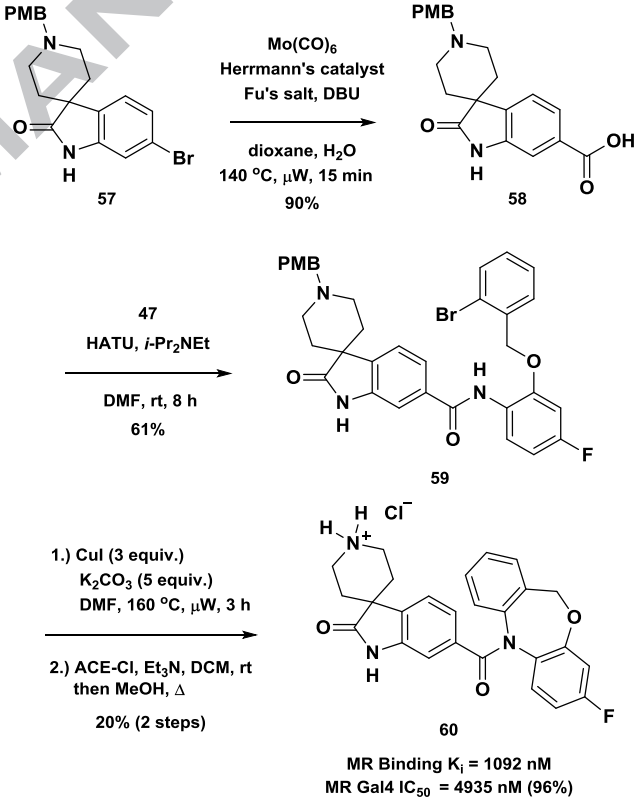
converted to the corresponding benzylic thioether via Pd-catalyzed coupling to give compound **52** in 64% yield. An oxidation/chlorination[12] sequence was employed using chlorine gas in the presence of aq. AcOH to give sulfonyl chloride **53** which was taken without purification and coupled with known aniline **47** to give sulfonamide substrate **54** in 21% yield from thioether **52**. Cyclization of **54** proceeded without incident to give compound **55** after benzyl deprotection.




**Scheme 5.** Synthesis of dibenzoxazepines **55** and **56**

Unfortunately, comparison of **55** with earlier spirooxindole compound **31** (Scheme 3), showed close to a 1000-fold loss in potency. The benzyl amine analog of **55**, analogous to compound **49**, was also prepared from bromide **29** via conversion to the corresponding aldehyde followed by reductive amination and Pd-catalyzed ring closure to afford tricylic amine system **56** after benzyl deprotection. However, this compound also suffered from a significant loss in potency when compared to that of **31**.

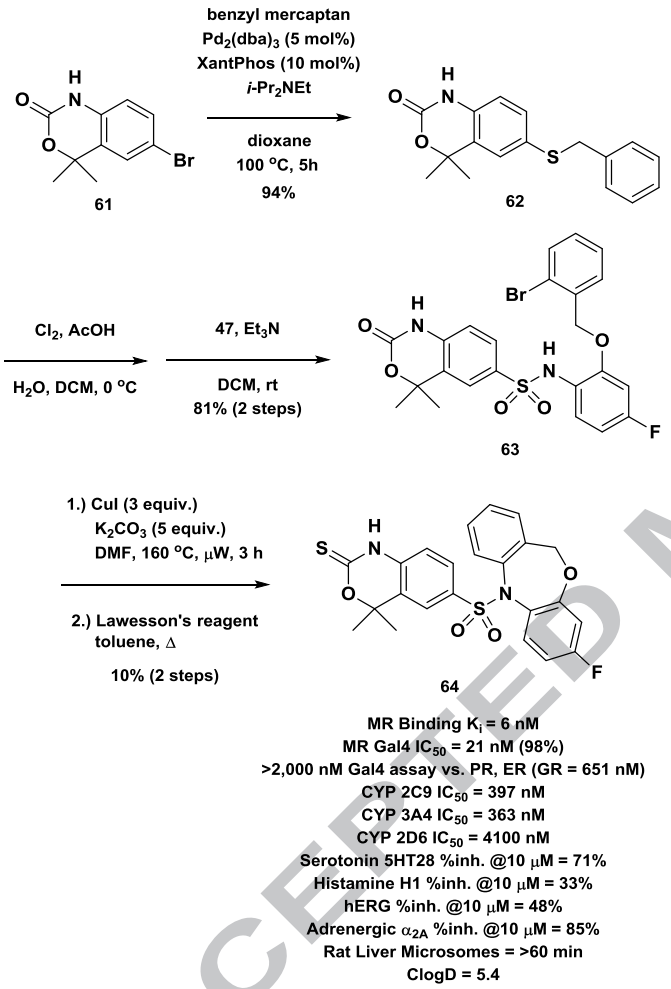
Although we had not explored the amide construct (Figure 13, **46**, R = C=O) for the benoxazinone system containing the new tricyclic motif, we reasoned that this cyclization could also proceed as smoothly as the transformation from **50** to **51**. Thus, carboxylic acid **58** was prepared in excellent yield from bromide **57** using a Pd-catalyzed carbonylation method employing $Mo(CO)_6$ as the carbon monoxide source (Scheme 6) .[13] HATU-promoted coupling of **58** with known aniline **47** gave **59** which was subjected to the previously used $CuI/K_2CO_3$ conditions to give **60** after benzyl deprotection. However, this compound also suffered from a significant loss in potency compared to that of **31**. Based on this SAR analysis, it appeared to us that these types of compounds housing the more polar dibenzoxazepane ring system in the hydrophobic pocket were adopting a different conformation compared to that of **42** (Figure 13). However, these conformational differences are not definitive based on our models and we can only hypothesize that this is the reason for the substantial loss in potency.




**Scheme 6.** Synthesis of dibenzoxazepine **60**

A group from Sumitomo reported in a patent[4d] compound **5** (Figure 2), for which we modeled its sulfonamide in a similar region of the MR LBD. It also exhibited a *para* relationship between the sulfonamide and the Asn-770 interacting NH group. The cyclic thiocarbamate motif of **5** was then incorporated with our tricyclic sulfonamide scaffold. Conversion of commercially available bromide **61** proceeded in high yield to the corresponding thioether (**62**, Scheme 7). Oxidation/chlorination sequence followed by sulfonamide formation with **47** gave compound **63** in 81% yield from **62**. Similar to our earlier conditions, $CuI/K_2CO_3$-promoted

cyclization afforded the desired tricyclic motif which was subjected to Lawesson's reagent to give final target **64**. Although the cyclic carbamate analog of **63** (not shown) exhibited a moderate level of potency, thiocarbamate **64** showed a very high level of binding and cellular potency. Even though this compound shows moderate a level of selectivity, in addition to some off-target liabilities and CYP issues, we feel that further optimizations to **64** (molecular weight of 470 g/mol) could lead to a more promising series. Based on our results from compounds **32-34** (Figure 11), introduction of polar functionality at the gem-dimethyl region of **64** could potentially circumvent some of these issues.




**Scheme 7.** Synthesis of cyclic thiocarbamate **64**

## 3. Conclusion

In conclusion, various spirooxindole and dibenzoxazepine constructs were identified as potent MR antagonists. Optimizations of our spirooxindole hit led to highly potent compounds containing polar solubilizing groups (i.e compounds **32-34** containing an N-oxide, carboxylic acid and dimethyl amine, respectively) which interact with the helix-11 region of the MR LBD. Various dibenzoxazepine moieties were also prepared in an effort to replace a known dibenzoxepane system (from compound **3b**) which interacts with the hydrophobic region of the MR LBD. However, significant losses in potency were observed in most cases with the exception of compound **64** which replaced the spirooxindole template with a cyclic thiocarbamate. In addition, an X-ray crystal structure was obtained from a highly potent compound (**21**) which was shown to exhibit both partial agonist and antagonist modes of action against MR.

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## Supplementary Material

Experimental details for the synthesis of compounds, biological assays and molecular modeling studies are available free of charge via the Internet at http://www.sciencedirect.com.

# Identification of Spirooxindole and Dibenzoxazepine Motifs as Potent Mineralocorticoid Receptor Antagonists

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