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Article

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# Identification of Morpholino-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-ones as Non-steroidal Mineralocorticoid Antagonists

David W. Piotrowski,* Kentaro Futatsugi, Augstin Casimiro-Garcia, Liuqing Wei, Matthew F. Sammons, Michael Herr, Wenhua Jiao, Sophie Y. Lavergne, Steven B. Coffey, Stephen W. Wright, Kun Song, Paula M. Loria, Mary Ellen Banker, Donna N. Petersen, Jonathan Bauman

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KEYWORDS: nuclear hormone receptor, mineralocorticoid, non-steroidal

**Abstract**: A novel series of morpholine-based non-steroidal mineralocorticoid receptor antagonists is reported. Starting from a pyrrolidine HTS hit **9** that possessed modest potency but excellect selectivity versus related nuclear hormone receptors, a series of libraries led to identification of morpholine lead **10**. After further optimization, *cis* disubstituted morpholine **22** was discovered, which showed a 45-fold boost in binding affinity and corresponding functional potency compared to **13**. While **22** had high clearance in rat, it provided sufficient exposure at high doses to favorably assess in vivo efficacy (increased urinary $Na^+/K^+$ ratio) and safety. In contrast to rat, the dog and human MetID and PK profiles of **22** were adequate, suggesting that it could be suitable as a potential clinical asset.

## Introduction

The steroid hormone, aldosterone, discovered over 50 years ago, is the primary endogenous agonist for the mineralocorticoid receptor (MR). This receptor is a ligand-dependent transcription factor that belongs to the nuclear hormone receptor (NHR) super-family and is a regulator of sodium reabsorption in the kidney.[1] MR shares structural similarities with other NHRs that also recognize steroidal ligands including the progesterone receptor (PR), androgen receptor (AR), glucocorticoid receptor (GR), and estrogen receptor (ER). In the disease state, excessive levels of aldosterone activate the MR contributing to conditions such as congestive heart failure, hypertension and chronic kidney disease. Intervention with MR antagonists represents an attractive therapeutic option for the treatment of such diseases.[2] However, treatment with MR antagonists can also lead to elevation of circulating aldosterone levels, which can trigger subsequent genomic and non-genomic effects.[3] Nonetheless, the steroidal MR antagonists, spironolactone (**1**) and eplerenone (**2**)[4] (Figure 1), have proven beneficial in reducing the risk of death and hospitalization in patients with severe heart failure[5,6,7] and aid in blood pressure control and antiproteinuric effects in patients with diabetic nephropathy.[8,9,10,11] However, the broader use of these agents has been limited by their sex hormone related adverse effects. Compound **1** is associated with impotence, gynecomastia, and menstrual irregularities. These effects are commonly attributed to the low selectivity against other NHRs such as AR and PR.[12] Both of these steroidal agents are contraindicated for use in diabetic patients because of the potential risk of hyperkalemia. The accumulated body of data suggests that the limitations associated with steroidal agents can likely be mitigated with non-steroidal MR inhibitors.[13]

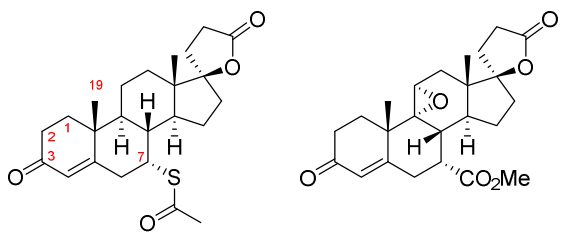



**Figure 1**. Marketed Steroidal MR Antagonist Drugs

Several selective non-steroidal agents have been or are currently being evaluated in human clinical trials (Figure 2). Preclinical and clinical data from a few of these MR antagonists suggests that mitigation of the risk of hyperkalemia might be possible. Among these compounds are finerenone (**3**),[14] esaxerenone (**4**)[15] and apararenone (**5**).[16] Compound **3** is the most advanced in clinical trials.[17,18] The first report of phase 2 study results showed that **3** had a reduction in hyperkalaemia and deterioration of renal function versus treatment with **1**. Biomarker data from this study showed a lower incidence of side effects that did not come at a cost of lower efficacy. This may, in part, be explained by differences in tissue distribution that could lead to reduction of observed hyperkalaemia: **3** distributes equally to kidney and heart in rats,[19] but **1** had at least six-fold higher concentration in kidney relative to cardiac tissue.[20,21] Taken together, these clinical results support the continued search for alternative structural classes of non-steroidal MR antagonists that have improved profiles.

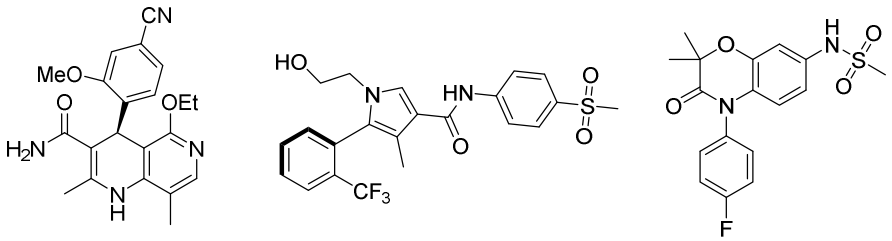
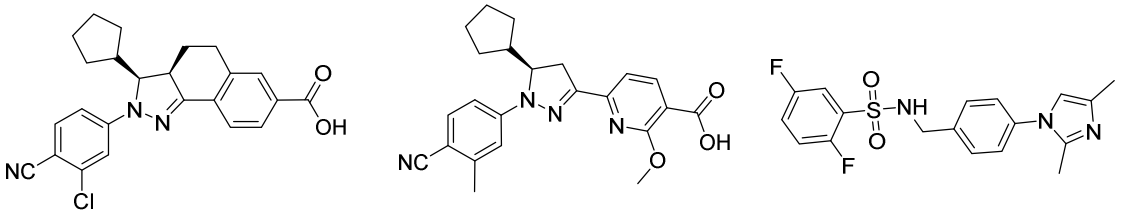



**Figure 2**. MR Antagonists, Phase 2 and Beyond

Previous reports from Pfizer have disclosed novel non-steroidal MR antagonists,[22] including the pyrazolines PF-03882845 (**6**) and **7** and the sulfonamide **8** (Figure 3). Of note, the conformationally restricted **6** was found to be a potent and selective antagonist with favorable pharmacokinetic profile that allowed for advancement to phase 1 clinical trials. However, **6** and related pyrazolines had the drawback of low solubility and empirically derived selectivity profiles.[22e,f]




PF-03882845 (**6**)
MR functional $IC_{50}$ = 21 nM
MR binding $IC_{50}$ = 9 nM
PR 46-fold selective
AR, GR, ER > 900-fold selective

**7**
MR functional $IC_{50}$ = 4.5 nM
MR binding $IC_{50}$ = 1.8 nM
PR, AR, GR, ER > 500-fold selective

**8**
MR functional $IC_{50}$ = 1311 nM
MR binding $IC_{50}$ = 191 nM

**Figure 3**. Pfizer MR Antagonists

**Results and Discussion**

The evaluation of the physicochemical property space, potency and selectivity data from all known MR antagonists at the time this work was conducted[23] suggested that **2** is an outlier (MW 414.5, logD 0.5, ~50% plasma protein binding) and could be used as an aspirational guide for discovery of potent and more selective compounds with modest dose requirements. Furthermore, knowledge gained from the co-crystal structure of the marginally selective **1**[24] showed that three main regions of interaction with MR exist: (1) H-bonding of the C3-keto group with Gln776 from H3 helix and Arg817 from H5 helix of MR, (2) occupancy of a lipophilic pocket which is lined with several lipophilic residues including Leu814, Leu938, Met845, Met 852, Cys849, Phe829 and Phe835, by the C7 thioacetyl group, (3) H-bonding of Thr945 or alternatively Asn770 to the lactone carbonyl (Figure 4). Furthermore, a variety of NHR modulators (agonists

or antagonists) make equivalent polar interactions in the region occupied by the A ring ketone of steroids (Figure 4, region 1). For example, in co-crystal structures both the 3-ketone of progesterone[25] or the nitrile of tanaproget[26] show interactions with Gln725/Arg766 (PR numbering) and the 3-ketone of testosterone[27] bound to AR shows interactions with Gln711/Arg752 (AR numbering). In principle, truncated analogs that are unable to make the polar interactions in region 1 and have enhanced interactions in regions 2 and 3 could lead to MR selective compounds. Thus, potency improvements could come from enhanced lipophilic interactions in region 2 while region 3 would serve to lock the ligand in place through H-bonding to Asn770 (Figure 5). Mindful of this possible binding mode, the discovery of a non-steroidal MR chemotype that had predictable selectivity over other NHRs was targeted. An in vitro screening cascade identical to that used for discovery of the previous Pfizer clinical candidate **6** and its back-up **7** was implemented to facilitate identification of a new orally bioavailable compound suitable for use in preclinical studies and beyond.[22e,f] New analogs were assessed in a panel of functional Gal4-based cellular assays to assess the transcriptional antagonist activity against MR, PR, GR and AR. Selected analogs were subsequently assessed for their ability to inhibit [$^3$H]-aldosterone in a lower-throughput MR filter binding assay.[22d,f]

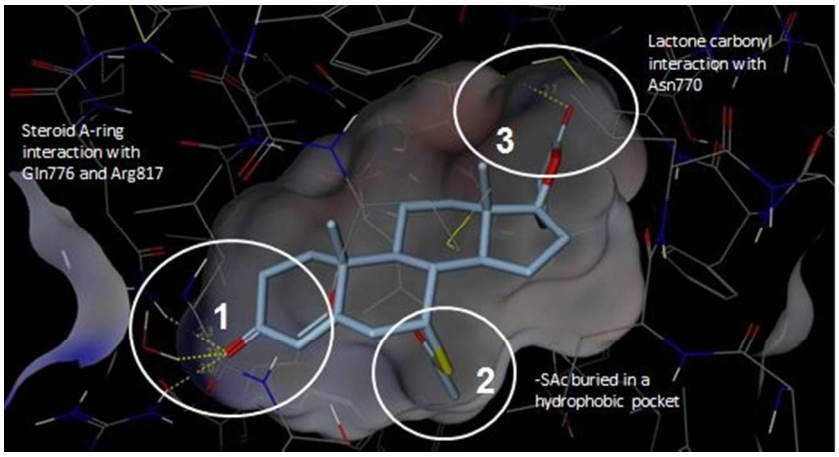



**Figure 4**. Crystal structure of **1** (2ab2) with key areas of interaction

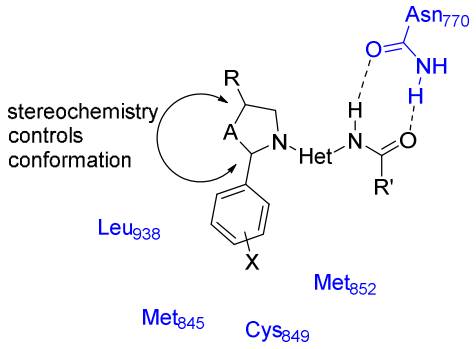
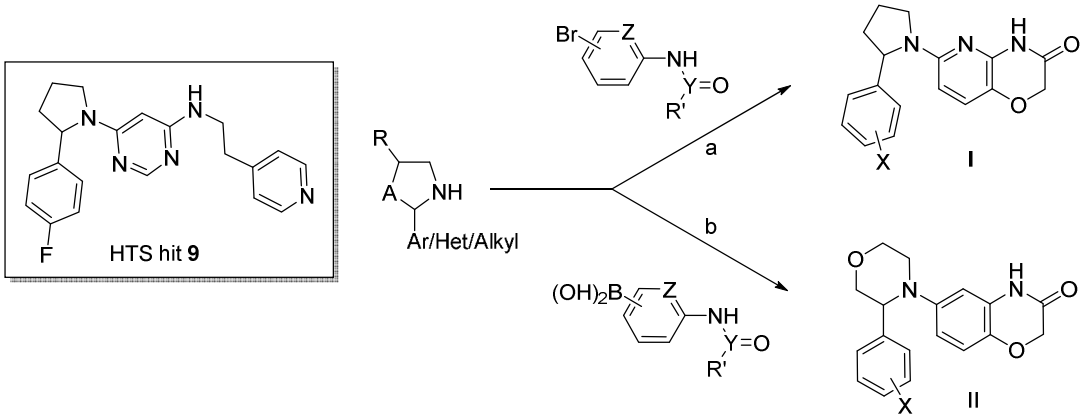



**Figure 5**. Depiction of the hypothetical binding mode of a generalized structure related to hit **9**

A suitable starting point for the aforementioned truncated series was generated by identification of a 2-arylpyrrolidine high throughput screening (HTS) hit **9** that was further refined with targeted libraries. Cyclic amines with small lipophilic groups alpha to the nitrogen were combined with (het)aryls containing a H-bond donor/acceptor motif capable of interacting with Asn770. Library-enabled chemistry suited for such C-N bond forming reactions included Buchwald-Hartwig and Chan-Lam couplings (Scheme 1). The libraries produced pyrrolidine and morpholine hits such as I and II (data not shown) and highlighted the importance of a benzo/pyrido-fused oxazinone moiety. Morpholine lead **10** was identified by this process (Table 1).

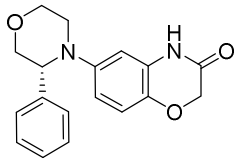
**Scheme 1.** Exploration of **9** by Parallel Chemistry



*Conditions for Buchwald-Hartwig and Chan-Lam libraries*: a) (het)aryl bromide, cyclic amine, NaOtBu, $Pd_2dba_3$, BINAP, DMSO, 60 $^oC$ b) arylboronic acid, $CH_2Cl_2$, pyridine, $Cu(OAc)_2$.

MR potency, selectivity, MW, eLogD and topological polar surface area (TPSA) indicated that **10** was a suitable lead (Table 1). In addition, the ADME (permeability, human liver microsomal (HLM) stability) and in vitro safety ([$^3$H]-dofetilide binding, cytochrome P450 (CYP) inhibition) assessments were adequate. However, the high intrinsic clearance in rat liver microsomes (RLM) highlighted an area for improvement to allow for potential selection of a representative analog for in vivo rat studies.

**Table 1.** In vitro Pharmacological, Absorption, Metabolism, and Safety Properties of **10**



| | **10**[b] |
|---|---|
| MW 310.4, elogD 2.7, TPSA 51 | |
| MR functional $IC_{50}$ (nM) | 583 |
| MR binding $IC_{50}$ (nM) | 401 |
| PR functional $IC_{50}$ (nM) | >10000 |
| GR functional $IC_{50}$ (nM) | >10000 |
| AR functional $IC_{50}$ (nM) | >10000 |
| RRCK (Papp, $10^{-6}$ cm/sec)[a] | 29.3 |
| RLM $Cl_{int}$ (μL/min/mg) | 223 |
| HLM $Cl_{int}$ (μL/min/mg) | 18.3 |

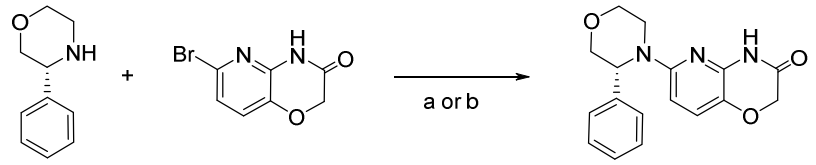
| | |
|---|---|
| CYP (pct inh at 3 μM) | 1A2, 2D6, 2C9, 3A4 < 10 % |
| [$^3$H]-dofetilide (pct inh at 10 μM) | 5 % |

[a]RRCK = Modified Madin-Darby Permeability Assay. [b]The corresponding (S)-enantiomer had an order of magnitude lower affinity and functional activity and was not pursued further.

With lead **10** in hand, we targeted specific modifications to three regions for optimization: (1) addition of small lipophilic substituents to the morpholine to fill the space occupied by C1 of the steroid A-ring and/or the C19 methyl of **1**, (2) insertion of N-atoms into the electron rich benzoxazinone moiety to adjust pKa and further reduce logD, and (3) addition of small lipophilic substituents to pendant phenyl ring to optimize non-polar interactions.

For analog work, a modular approach was desired so that the synthesis of more complex morpholine and halo benzo/pyrido-oxazinone fragments could be addressed separately. Despite the successful use of the Buchwald-Hartwig conditions for the libraries, further optimization was required for application of this methodology to singleton synthesis. A number of ligand/base combinations were screened to identify suitable conditions for broader use. Of note was the use of the electron-rich phosphine ligand *i*PrBiPPyPhos, along with LiO*t*Bu as base and HMPA as an additive. The latter two reagents were critical to keep the reactants in solution. For example, the unoptimized 8% yield (condition a, Scheme 2) for the coupling of morpholine **11** and bromide **12** was improved under these optimized conditions to provide an 86% yield of **13** (condition b, Scheme 2). With these improved C-N bond forming conditions in hand, attention was turned toward an efficient morpholine synthesis.

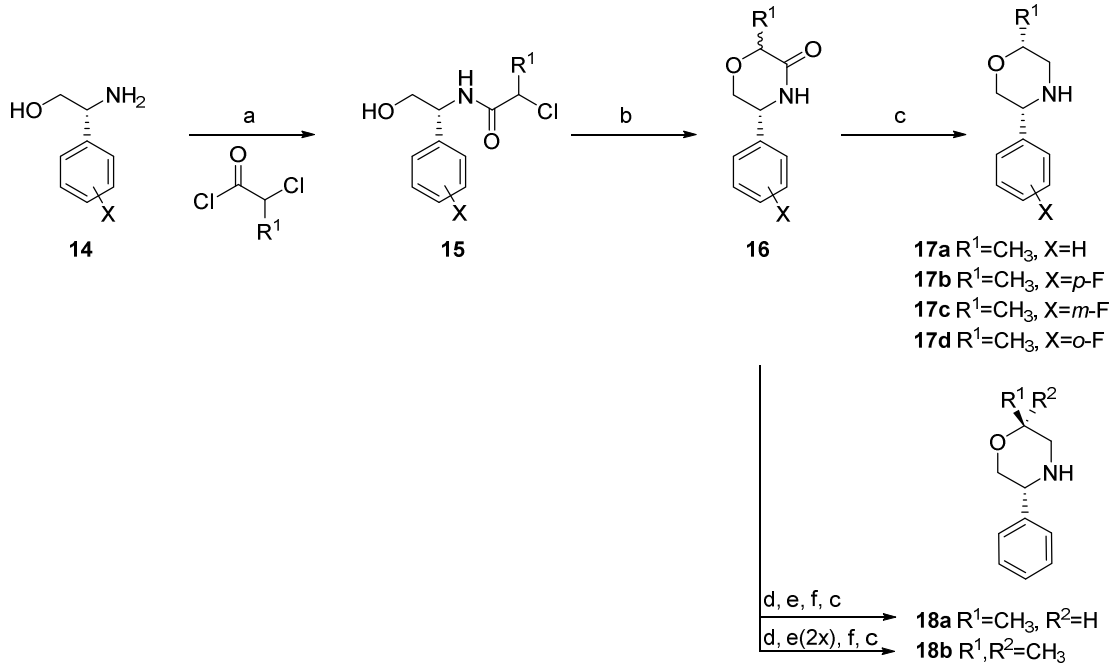
**Scheme 2**. Optimization of C-N Coupling




*Reagents and conditions*: (a) 2.5 mol% $Pd_2(dba)_3$, 5 mol% BiPPyPhos, 2 equiv. KOtBu, tAmylOH, 100 $^oC$, 8%. (b) 2.5 mol% $Pd_2(dba)_3$, 5 mol% iPr-BiPPyPhos, 6 equiv. LiOtBu, 5 equiv HMPA, tAmylOH, 60 $^oC$, 86%.

Several routes to access morpholinones and morpholines have been reported and were used to synthesize analogs to explore the early SAR.[28] Route improvements were made, as we have reported previously (Scheme 3),[29] which allowed for installation of the defined C5 stereocenter from commercially available (*R*)-2-phenylglycinols **14**, which could be acylated to provide amide **15**. Base promoted intramolecular Williamson ether synthesis on amide **15** provided lactam intermediate **16** that could be separated (*cis*/*trans* isomers) and further manipulated as required to provide morpholines with a range of C2 substituents. For example, the use of racemic 2-chloropropanoyl chloride for the acylation of (*R*)-2-phenylglycinol and cyclization provided a mixture of morpholinone diastereomers **16** that could be controlled by judicious choice of conditions.[29] Reduction of the morpholinone provided the required morpholines **17**. The *trans* morpholine isomer **18a** could be accessed from morpholinone **16** ($R^1$ = H) by an N-protection, methylation, deprotection and reduction sequence. The *gem*-dimethyl morpholine **18b** could be accessed using a similar sequence with the addition of a second methylation step (Scheme 3). The final analogs **22-24** and **28-34** were synthesized from morpholines **17a-d** and **18 a,b** by the previously described C-N coupling procedures (vide supra).

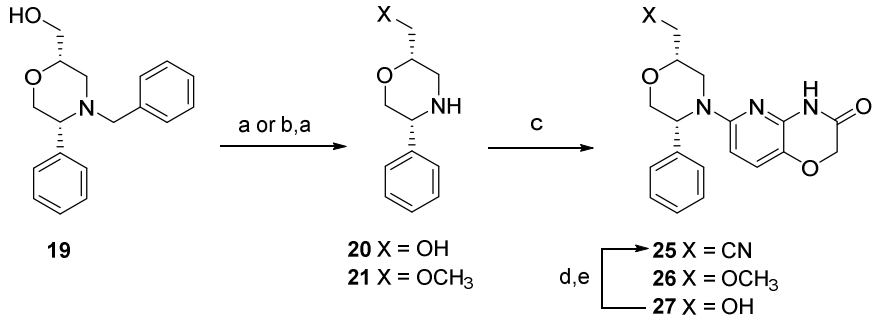
**Scheme 3**. Synthesis of Morpholine Intermediates




*Reagents and conditions*: a) $Et_3N$, 2-MeTHF, b) KOtBu, tBuOH, c) $LiAlH_4$ or Vitride®, toluene, d) *p*-methoxybenzyl chloride, NaH, DMF, e) LDA, $CH_3I$, THF, f) CAN, aq. $CH_3CN$

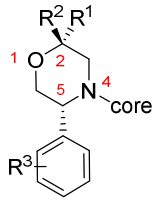
Compounds **25**-**27**, containing elaborated substituents at the 2-position of the morpholines, were synthesized starting from known morpholine intermediate **19**.[30] The hydroxymethyl group of **19** was manipulated either before or after the C-N coupling procedure (Scheme 4).

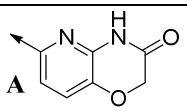
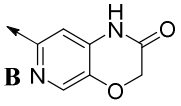
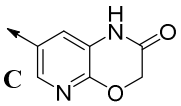
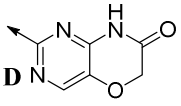
**Scheme 4**. Synthesis 2-Elaborated Morpholine Analogs

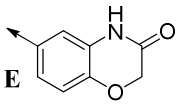



*Reagents and conditions*: a) 10% Pd/C, MeOH, $H_2$/50 psi, b) $CH_3I$, NaH, DMF, c) general C-N coupling procedure from Scheme 2, d) $Et_3N$, 1,2-dichloroethane, $(CH_3SO_2)_2O$, e) NaCN, DMF, 120 $^oC$.

**Table 2.** Potency and Properties of Selected Analogs




| Cmpd | $R^1$ | $R^2$ | $R^3$ | core | MR binding $IC_{50}$ (nM)[a] | MR functional $IC_{50}$ (nM)[a] | HLM $Cl_{int}$[b] | eLogD[c] |
|---|---|---|---|---|---|---|---|---|
| **13** | H | H | H |  **A** | 1181 | 1969 | 8.9 | 2.4 |
| **22** | $CH_3$ | H | H | **A** | 25 | 44 | 9.1 | 2.8 |
| **23** | H | $CH_3$ | H | **A** | 727 | 1217 | 24.3 | 2.7 |
| **24** | $CH_3$ | $CH_3$ | H | **A** | 86 | 407 | 15.2 | 3.3 |
| **25** | $CH_2CN$ | H | H | **A** | 56 | 98 | 11.9 | 2.3 |
| **26** | $CH_2OCH_3$ | H | H | A | 276 | 358 | 8.0 | 2.5 |
| **27** | $CH_2OH$ | H | H | **A** | >10000 | >10000 | 14.8 | 1.8 |
| **28** | $CH_3$ | H | H |  **B** | 109 | 252 | 12.6 | 2.5 |
| **29** | $CH_3$ | H | H |  **C** | 2225 | 2992 | 13.9 | 1.9 |
| **30** | $CH_3$ | H | H |  **D** | 113 | 266 | 8.0 | 2.7 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **31** | $CH_3$ | H | H |  | 13 | 24 | 15.8 | 3.1 |
| **32** | $CH_3$ | H | 2-F | **A** | 13 | 33 | 14.0 | 3.1 |
| **33** | $CH_3$ | H | 3-F | **A** | 19 | 55 | 9.6 | 3.1 |
| **34** | $CH_3$ | H | 4-F | **A** | 39 | 105 | 8.0 | 3.2 |

[a]Most values are reflective of n ≥ 3 experiments. Please see supporting information for the number of experiments and associated error. [b]intrinsic clearance μL/min/mg. [c]measured eLogD.[31]

We hypothesized that addition of substituents to the morpholine C2 position could increase potency through improved lipophilic interactions. We therefore prepared the *cis*-**22** and *trans*-**23** mono-methyl analogs as well as dimethyl analog **24**. Of significant note was the 45-fold boost in binding affinity of *cis*-**22** versus its unsubstituted counterpart **13**, while the dimethyl **24** and *trans*-**23** analogs had more modest affinity increases. The greater than 3-fold boost in binding affinity that might be expected from the increased lipophilicity suggested that the conformation of *cis*-2-methyl-5-phenylmorpholine enforced burial of both the methyl and phenyl groups in hydrophobic regions of MR; a so-called "magic methyl" effect.[32,33] This supposition was supported with computational assessments using ConfGen[34] and Jaguar[35]: *cis*-**22** revealed a strong axial phenyl preference (>5 kcal/mol), while *trans*-**23** preferred the equatorial phenyl (1.6 kcal/mol) over the axial phenyl conformer.[36] Furthermore, a small molecule crystal structure of **22** was obtained. The structure clearly displays the axial 5-phenyl group and the equatorial 2-methyl group on the morpholine.[37] Thus, subsequent analogs incorporated the *cis*-2,5-disubstituted morpholine moiety as a key feature. Attempts to independently increase or decrease bulk at the 2- or 5-positions of the morpholine led to compounds with reduced MR binding

affinity and/or increased HLM $Cl_{int}$ (data not shown). Some small substituents (CN **25,** OMe **26)** were tolerated on the C2 methyl but increased polarity (OH **27**) led to a reduction in binding affinity. However, the additional synthetic complexity introduced by these changes did not warrant further exploration of these analogs.

Next, we examined a number of core changes. The other pyridooxazinone isomers **28** and **29** and pyrimidinooxazinone **30** led to a 6- to 68-fold reduction in functional potency. The benzoxazinone **31** was made for comparative purposes. While **31** was slightly more potent than **22**, the electron-rich benzoxazinone moiety presented concerns about possible late-stage idiosyncratic toxicity[38] and minimal profiling was conducted on this compound. Other changes to the benzo/pyridooxazine core were tolerated (small substituents on aromatic or aliphatic portion, replacement of the ether oxygen with carbon or other heteroatoms) but lactam N-methylation essentially abolished binding affinity (data not shown). Again, the additional level of synthetic complexity combined with the lack of significant improvement precluded these analogs from further consideration.

Finally, using SAR data generated from the library compounds depicted in Scheme 1, a select number of substituted 5-aryl groups were examined. For illustrative purposes, the data for *o*-F **32**, *m*-F **33**, *p*-F **34** substituted analogs are shown in Table 2. Nearly equivalent functional potency was noted for these substituted analogs versus their unsubstituted counterpart **22**. Similar trends were noted for other mono-substituted and some di-substituted analogs (data not shown).[39] Taken together, **22** emerged as the compound with best balance of potency, selectivity and ADME properties.

We had previously established[22e,f] that a few different rodent models (Kagawa assay, blood pressure) could be used to support translation of in vitro potency to in vivo efficacy. Several

compounds in Table 2 were potent and selective, of modest elogD and had low intrinsic clearance in HLM but nevertheless suffered from high intrinsic clearance in RLM (selected data shown in Table 3). To better understand this disparity and explore a possible in vitro-in vivo PK correlation in rat, a group of analogs that spanned a range of elogD and RLM $Cl_{int}$ were selected for iv rat PK assessment regardless of their potency. In general, the rat PK profile of these compounds was characterized by high-to-very-high clearance, short $t_{½}$, and moderate-to-high volume of distribution. In order to further understand the high RLM $Cl_{int}$ findings, **22** was subjected to a metabolite identification (MetID) study using RLM, dog liver microsomes (DLM) and HLM. The results indicated a facile oxidation of the morpholine ring was the primary contributor to microsomal instability in rodents, while unchanged parent remained for DLM and HLM.[40] These data strongly suggested that the metabolic liabilities in rat would limit our ability to use **22** in many of the established in vivo models. The favorable MetID study in DLM prompted a dog pharmacokinetics study, where **22** exhibited moderate clearance, moderate volume of distribution, long half life and good bioavailability. Thus, there was a solid in vitro-in vivo PK correlation in dog. The similar MetID and microsomal stability profiles between dog and human suggested that the dog PK parameters were best suited for use in human PK projections.

**Table 3**. RLM $Cl_{int}$ and Rat Cl for Selected Analogs

| Cmpd | eLogD | RLM $Cl_{int}$[a] | Rat Cl[b] |
|---|---|---|---|
| **31** | 3.1 | 377 | 54 |
| **22** | 2.8 | 287 | 141 |
| **30** | 2.7 | 146 | 95 |
| **23** | 2.7 | >564 | 77 |

| | | | |
|---|---|---|---|
| **27** | 1.8 | 44 | 52 |

[a]μL/min/mg; [b]mL/min/kg after 1 mg/kg iv dose

Before commencing with the rat toxicology study, **22** was further assessed in an advanced battery of in vitro assays (Table 4). Of note was the high selectivity over CYP enzymes, selectivity over a wide panel of enzymes, receptors and ion channels and high selectivity over most other NHRs.

**Table 4**. Pharmacological, ADME and Safety Properties of **22**

| | |
|---|---|
| MW | 325.4 |
| eLogD | 2.80 |
| TPSA (Å$^2$) | 64 |

| | hFunctional $IC_{50}$ (nM)[a] | hBinding $IC_{50}$ (nM)[a] |
|---|---|---|
| MR | 44[b] | 25[c] |
| AR | >10000 | >10000 |
| GR | >10000 | >10000 |
| PR | >10000 | >10000 |
| ERα | n.d.[d] | >10000 |

microsomal intrinsic $Cl_{int}$ (μL/min/mg)

| | |
|---|---|
| rat | 287 |
| dog | 38.3 |
| human (fu, mic 0.80) | 6.9 |

cytochrome P450 % inh. at 3 μM

(1A2, 2C9, 2D6, 3A4) all < 20 %

thermodynamic solubility

pH 6.5, phosphate buffered saline, 5.5 μg/mL (17 μM)

plasma protein binding (% free, fu)

| | |
|---|---|
| rat | 15.0 |
| dog | 13.6 |
| human | 15.0 |

permeability ($P_{app}$ (cm/s))

| | |
|---|---|
| RRCK | AB $45.9 \times 10^{-6}$ |
| MDR1-MDCK | AB $24.9 \times 10^{-6}$ |
| | BA $24.7 \times 10^{-6}$ |

Genetox

Ames assay negative

hERG inhibition (patch clamp)

$IC_{50} > 100$ μM

CEREP panel (81 assays)

$IC_{50} > 10$ μM except GR binding in IM-9 cells ($IC_{50} = 597$ nM)

in vivo pharmacokinetics

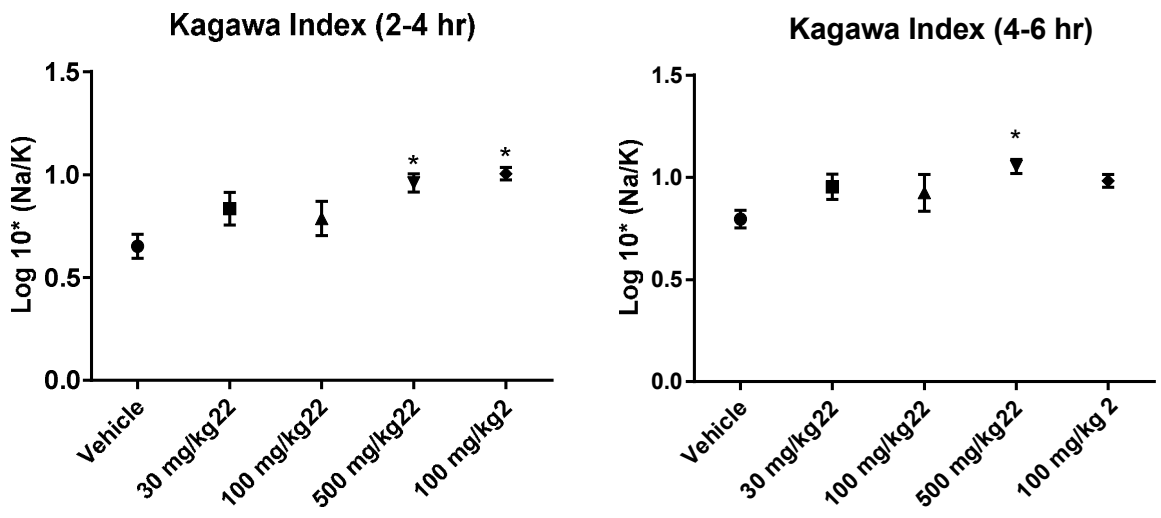
| Cl (mL/min/kg) | Vss (L/kg) | $t_{1/2}$ (h) | F (%) |
|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| rat | 141 | 6.15 | 0.65 | 4-16 |
| dog | 5.37 | 2.37 | 17.7 | 65 |
| projected human[e] | 3.75 | 2.37 | | 40 |

[a]Values are means of >3 experiments ± standard deviation. [b]$IC_{50}$ = 33 nM in a serum free version of the human MR assay and $IC_{50}$ = 3.2 nM in the rat MR assay. [c]Ki = 6.3 nM (derived from Cheng-Prusoff equation). [d]n.d. = not determined. [e]projected human PK scaled from dog.

Working under the assumption that sufficient exposure would be obtained at high doses by possible saturation of the clearance mechanisms, **22** was advanced into a 14-day rat toxicology study using both male and female rats with doses ranging from 30, 100, and 500 mg/kg.[41] The highlights from the study are briefly described. Based on AUC (ng•h/mL), free drug exposures at day 14 were between 79-3166x functional $IC_{50}$ (serum free, rat MR) in male rats and 1227-8250x $IC_{50}$ in female rats.[42] The expected compensatory increase in plasma aldosterone was noted at all doses. No reproductive tract findings were observed in the female rats.[43]

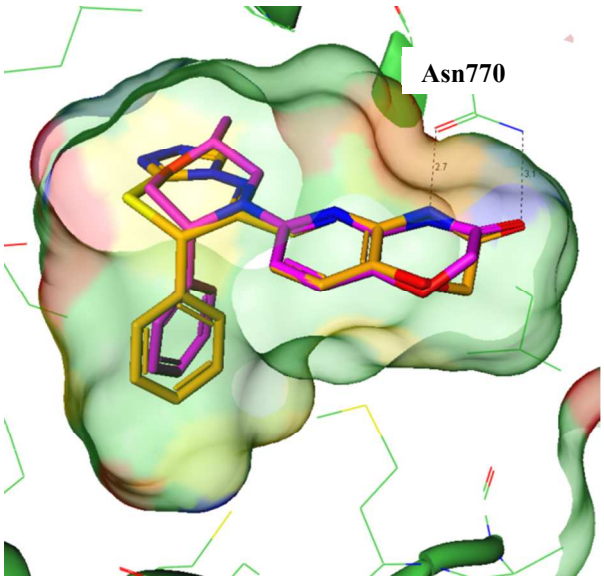
The higher exposure in female rats in the 14-day safety study provided the impetus to test **22** in an acute measure of urinary excretion of sodium and potassium.[44] Female rats were randomly assigned to treatment groups ($n$ = 7/group) to receive a single oral dose of vehicle, **22** (30, 100, or 500 mg/kg) or **2** (100 mg/kg), which was used as a positive control. Urine samples were collected at intervals of 0-2, 2-4, and 4-6 h post-dose for measurement of urinary sodium and potassium concentration. Treatment with 500 mg/kg of **22** and the positive control **2** resulted in a statistically significant increase in urinary $Na^+/K^+$ ratio at 2-4 h and 4-6 h post-dose (Figure 6). Average AUC concentrations at the doses of 30, 100 and 500 mg/kg were 2850, 9410 and 13500 ng•h/mL, respectively. These results confirmed that **22** acted as a MR antagonist.




*P< 0.05 vs. vehicle

**Figure 6**: $Na^+/K^+$ ratio (Kagawa index) for oral administration of **22** to female rats at 30, 100 and 500 mg/kg doses.

After this work had been completed,[45] the first X-ray co-crystal structure of a non-steriodal MR antagonist was disclosed.[46,47] Working under the assumption that our small molecule X-ray (ground state) structure approximates the bound conformation, the pyridooxazinone portion of **22** was overlaid with the benzoxazinone portion of the MR co-crystal structure (3vhv, Figure 7). Gratifyingly, the superimposed molecules show good overlap throughout the ligand binding domain with the *cis*-oriented 2-methyl and 5-phenyl moieties occupying key lipophilic pockets.[48]



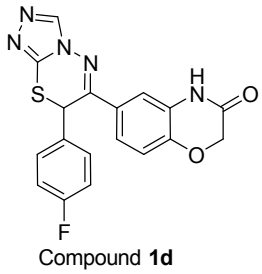




**Figure 7**: Overlay of **22** (magenta) with the co-crystal structure 3vhv (orange, Compound **1d** from reference 46a) with hydrogen bonds to Asn770 noted.

## Conclusions

In conclusion, a potent and orally available MR antagonist **22** was identified starting from lead **10**. The *cis* disubstituted morpholine **22** showed a 45-fold boost in binding affinity and commensurate functional potency versus the des-methyl morpholine **13**. While **22** had high clearance in rat, it provided sufficient exposure at high doses to favorably assess in vivo efficacy and safety. The dog and human MetID and PK profiles of **22** suggest that it could be suitable as a potential clinical asset.

## Experimental Section

### General

All chemicals, reagents and solvents were purchased from commercial sources when available and used without further purification. Nuclear magnetic resonance spectroscopy

(NMR) was recorded at 400 MHz ($^{1}$H) and 101 MHz ($^{13}$C) on Varian spectrometers unless otherwise noted. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. Mass spectrometry (MS) was performed via atmospheric pressure chemical ionization (APCI) or electron scatter (ES) ionization sources. Silica gel chromatography was performed primarily using a medium pressure Biotage or ISCO systems using columns pre-packaged by various commercial vendors including Biotage and ISCO. Microanalyses were performed by Quantitative Technologies Inc. and were within 0.4% of the calculated values. Purity of final compounds was assessed by reversed-phase HPLC with UV detection at 215 nM; all tested compounds were >95% purity, unless otherwise noted. The terms "concentrated" and "evaporated" refer to the removal of solvent at reduced pressure on a rotary evaporator with a bath temperature less than 60 °C. The abbreviation "min" and "h" stand for "minutes" and "hours" respectively.

**(*R*)-6-(3-Phenylmorpholino)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (10)**

A mixture of (*R*)-3-phenylmorpholine (292 mg, 1.8 mmol), tris(dibenzylideneacetone)dipalladium (0) (1.5 mg, 0.018 mmol), 6-bromo-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (342 mg, 1.5 mmol), 2-(2-dicyclohexylphosphanylphenyl)-*N,N*-dimethylaniline (1.5 mg, 0.36 mmol), lithium bis(trimethylsilyl)amide (1 M solution in hexanes, 3.3 mL) and THF (6 mL) in was stirred at 70 °C overnight. The reaction mixture was diluted with EtOAc and extracted with saturated aq. $NH_4Cl$. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with saturated aq. NaCl, dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by silica gel column chromatography

(gradient: 0–80 % EtOAc/heptanes) to afford 75 mg (16%) of a light yellow solid. $^1$H NMR ($CDCl_3$) δ 7.60 (br s, 1H), 7.30–7.14 (m, 5H), 6.75 (d, *J* = 8.6 Hz, 1H), 6.60 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.37 (d, *J* = 2.5 Hz, 1H), 4.51 (s, 2H), 4.15 (dd, *J* = 9.0, 3.5 Hz, 1H), 4.00–3.89 (m, 3H), 3.55 (dd, *J* = 11.5, 9.0 Hz, 1H), 3.32 (dt, *J* = 12.1, 2.7 Hz, 1H), 3.04 (ddd, *J* = 12.1, 9.3, 4.5 Hz, 1H).

**(*R*)-6-(3-Phenylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (13)**

Prepared from (*R*)-3-phenylmorpholine and 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one according to the general C-N coupling procedure to afford 125 mg (66%) of a solid. $^1$H NMR (*DMSO-d*$_6$) δ 10.86 (br s, 1H), 7.36–7.25 (m, 4H), 7.22–7.17 (m, 1H), 7.15 (d, *J* = 8.8 Hz, 1H), 6.20 (d, *J* = 8.8 Hz, 1H), 5.17 (t, *J* = 3.1 Hz, 1H), 4.47 (s, 2H), 4.16 (dd, *J* = 11.7, 2.0 Hz, 1H), 3.94 (dt, *J* = 10.7, 2.5 Hz, 1H), 3.87 (dd, *J* = 11.7, 3.7 Hz, 1H), 3.79 (dt, *J* = 13.1, 2.9 Hz, 1H), 3.63 (td, *J* = 11.1, 3.4 Hz, 1H), 3.37–3.29 (m, 2H).

**(2*R*,5*R*)-2-Methyl-5-phenylmorpholine (17a).[31] General procedure for the synthesis of *cis*-(2*R*,5*R*)-2-methyl-5-arylmorpholines.**

Step 1: A solution of 2-chloro-*N*-((*R*)-2-hydroxy-1-phenylethyl)propanamide (US 7629338, 60 g, 260 mmol) in *t*-BuOH (540 mL) was added to a stirred suspension of KO*t*-Bu (59.1 g, 527 mmol) in *t*-BuOH (920 mL) at rt. The reaction mixture was stirred for 1 h. The pH of the reaction mixture was adjusted to pH 4 by adding aq. HCl (1 N, 140 mL). The mixture was concentrated to remove the *t*-BuOH. EtOAc (1000 mL) and $H_2O$ (500 mL) were added. After the layers were separated, the organic layer was washed with saturated aq. NaCl (250 mL), dried over $Na_2SO_4$, filtered and concentrated to provide a solid. The solid was completely dissolved in

hot heptanes/EtOAc. The product precipitated upon cooling to rt overnight. The solid was filtered and dried to yield 33.75 g (67%). $^1$H NMR ($CDCl_3$) δ 7.42–7.29 (m, 5H), 6.75 (br s, 1H), 4.61 (q, *J* = 3.7 Hz, 1H), 4.34 (q, *J* = 7.0 Hz, 1H), 4.00 (dd, *J* = 11.9, 4.1 Hz, 1H), 3.84 (ddd, *J* = 11.9, 4.5, 0.8 Hz, 1H), 1.51 (d, *J* = 7.0 Hz, 3H).

Step 2: A solution of (2*R*,5*R*)-2-methyl-5-phenylmorpholin-3-one (32 g, 167.3 mmol) in toluene (600 mL) was added to an ice cooled solution of sodium bis(2-methoxyethoxy)aluminum hydride (65% wt in toluene, 300 mL, 1000 mmol). The reaction mixture was stirred at 5 $^o$C for 1 h and stirred at rt overnight. Aq. NaOH (2 M, 700 mL, 1390 mmol) was added to the reaction mixture, allowing the temperature to rise to 45 $^o$C. The solution was diluted with toluene (100 mL) and the layers were separated. The organic layer was washed with aq. $K_2CO_3$ (10%, 100 mL), dried over $Na_2SO_4$, filtered, and concentrated to afford 31.0 g (100%) of an oil. $^1$H NMR ($CDCl_3$) δ 7.52 (d, *J* = 7.4 Hz, 2H), 7.43–7.34 (m, 2H), 7.33–7.27 (m, 1H), 4.14–3.72 (m, 4H), 2.86–2.71 (dd, *J* = 12.0, 6.0 Hz, 1H), 1.88 (br s, 1H), 1.34 (d, *J* = 6.4 Hz, 3H); $^{13}$C NMR ($CDCl_3$) δ 141.5, 128.4, 127.7, 127.3, 70.7, 68.0, 57.3, 48.4, 17.4; FTIR ($cm^{-1}$) 3317 (w); HRMS Calcd. for $C_{11}H_{15}NO$ $(M+H)^+$ 178.1226; Found 178.1232; $[\alpha]_D^{20}$ 3.1 (*c* 1.01, MeOH).

**(2*R*,5*R*)-5-(4-Fluorophenyl)-2-methylmorpholine (17b)**

Prepared from (*R*)-2-amino-2-(4-fluorophenyl)ethanol according to the general procedure for the synthesis of *cis*-(2*R*,5*R*)-2-methyl-5-arylmorpholines (54.6 g, 90%) as a yellow oil. $^1$H NMR ($CDCl_3$) δ 7.52–7.45 (m, 2H), 7.03 (t, *J* = 8.8 Hz, 2H), 4.01 (dd, *J* = 11.3, 5.3 Hz, 1H), 3.89–3.83 (m, 2H), 3.78–3.83 (m, 1H), 2.92 (dd, *J* = 12.0, 3.2 Hz, 1H), 2.72 (dd, *J* = 12.0, 6.0 Hz, 1H), 1.30 (d, *J* = 6.4 Hz, 3H).

**(±)-*cis*-5-(3-Fluorophenyl)-2-methylmorpholine (17c)**

Prepared from racemic 2-amino-2-(3-fluorophenyl)ethanol according to the general procedure for the synthesis of *cis*-(2*R*,5*R*)-2-methyl-5-arylmorpholines. $^1$H NMR ($CDCl_3$) δ 7.33–7.22 (m, 3H), 6.99–6.92 (m, 1H), 4.08-3.97 (m, 1H), 3.89–3.79 (m, 3H), 2.89 (d, *J* = 11.7 Hz, 1H), 2.71 (dd, *J* = 11.7, 6.4 Hz 1H), 1.28 (d, *J* = 6.4 Hz, 3H).

**(±)-*cis*-5-(2-Fluorophenyl)-2-methylmorpholine (17d)**

Prepared from racemic 2-amino-2-(2-fluorophenyl)ethanol according to the general procedure for the synthesis of *cis*-(2*R*,5*R*)-2-methyl-5-arylmorpholines. $^1$H NMR ($CDCl_3$) δ 7.82–7.75 (m, 1H), 7.28–7.20 (m, 1H), 7.15–7.08 (m, 1H), 7.05–6.98 (m, 1H), 4.22–4.17 (m, 1H), 4.02 (ABq, *J* = 3.7 Hz, 2H), 3.90–3.75 (m, 1H), 2.82 (d, *J* = 11.7 Hz, 1H), 2.64 (dd, *J* = 11.7, 7.4 Hz, 1H), 1.77 (br s, 1H), 1.22 (d, *J* = 11.9 Hz, 3H).

**(2*S*,5*R*)-2-Methyl-5-phenylmorpholine (18a)**

Step 1: To a 0 °C solution of (*R*)-5-phenylmorpholin-3-one (US 7629338, 1 g, 5.64 mmol) in anhydrous DMF (5 mL) was added sodium hydride (60% dispersion in oil, 239 mg, 5.98 mmol). The mixture was stirred at rt for 15 min and then cooled to 0 °C before *p*-methoxybenzyl chloride (0.830 mL, 5.98 mmol) was added. The reaction mixture was stirred at rt for 4 h, diluted with EtOAc and washed with $H_2O$. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with saturated aq. NaCl, dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient: 20–50% EtOAc/heptanes) to provide 1.4 g (83%) of a white solid. $^1$H NMR (*DMSO-d*$_6$) δ 7.42–7.31 (m, 3H), 7.30–7.25 (m, 2H), 7.10–7.04 (m, 2H), 6.91–6.85 (m, 2H), 5.19 (d, *J* = 14.8 Hz,

1H), 4.47–4.38 (m, 1H), 4.28 (ABq, *J* = 16.4 Hz, 2H), 3.96 (dd, *J* = 11.9, 3.7 Hz, 1H), 3.74 (d, *J* = 11.3, 3.3 Hz, 1H), 3.73 (s, 3H), 3.36 (d, *J* = 14.8 Hz, 1H).

Step 2: To a solution of diisopropylamine (1.1 mL, 7.7 mmol) in THF (10 mL) at -78 °C was added *n*-BuLi (2.5 M in hexanes, 3 mL, 7.7 mmol). The solution was stirred at 0 °C for 15 min and then cooled to -78 °C. A solution of (*R*)-4-(4-methoxybenzyl)-5-phenylmorpholin-3-one (1.84 g, 6.2 mmol) in THF (10 mL) was added. After stirring at -78 °C for 30 min, methyl iodide (0.56 mL, 8.67 mmol) was added. The reaction mixture was warmed to rt overnight. The reaction mixture was poured into aq. HCl (1 N) and the mixture was extracted 3 x EtOAc. The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (gradient: 0–60 % EtOAc in heptanes) to provide 1.69 g (87%) of the desired compound containing 15% of the *cis* diastereoisomer. $^1$H NMR ($CDCl_3$) δ 7.44–7.35 (m, 3H), 7.22–7.17 (m, 2H), 7.02–6.96 (m, 2H), 6.84–6.80 (m, 2H), 5.44 (d, *J* = 14.4 Hz, 1H), 4.48–4.41 (m, 2H), 4.04 (dd, *J* = 12.2, 4.6 Hz, 1H), 3.81 (s, 3H), 3.67 (dd, *J* = 12.2, 7.9 Hz, 1H), 3.39 (d, *J* = 14.4 Hz, 1H), 1.59 (d, *J* = 7.4 Hz, 3H).

Step 3: To a solution of (*2S,5R*)-4-(4-methoxybenzyl)-2-methyl-5-phenylmorpholin-3-one (1.69 g, 1.57 mmol) in 50% acetonitrile/water (48 mL) was added ammonium cerium (IV) nitrate (6.04 g, 10.9 mmol). The reaction mixture was stirred at rt for 4 h, poured into aq. HCl (1 N) and extracted with EtOAc (2 x 100 mL). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (eluent: 50% EtOAc/heptanes) to provide 502 mg (48%) of a solid. $^1$H NMR ($CDCl_3$) δ 7.44–7.30 (m, 5H), 6.04 (br s, 1H), 4.82 (dd, *J* = 10.0, 4.5 Hz, 1H), 4.31–4.24 (m, 1H), 4.09–4.03 (m, 1H), 3.53 (dd, *J* = 11.9, 10.0 Hz, 1H), 1.54 (d, *J* = 6.8 Hz, 3H).

Step 4: Prepared by reduction of (*2S,5R*)-2-methyl-5-phenylmorpholin-3-one to give 382 mg (82%) of an oil. $^1$H NMR ($CDCl_3$) δ 7.42–7.25 (m, 5H), 3.92–3.84 (m, 2H), 3.78–3.73 (m, 1H), 3.73–3.67 (m, 1H), 3.52–3.43 (m, 1H), 3.06 (dd, *J* = 11.5, 2.3 Hz, 1H), 2.75 (dd, *J* = 11.5, 10.2 Hz, 1H), 1.20 (d, *J* = 6.3 Hz, 3H).

**(*R*)-2,2-Dimethyl-5-phenylmorpholine (18b)**

Prepared from (*2R,5R*)-2-methyl-5-phenylmorpholin-3-one using the methods described above. $^1$H NMR ($CDCl_3$) δ 7.44–7.38 (m, 2H), 7.34–7.22 (m, 3H), 3.67 (dd, *J* = 10.2, 3.7 Hz 1H), 3.45 (dd, *J* = 11.1, 3.7 Hz, 1H), 3.40 (ABq, *J* = 10.5 Hz, 1H), 2.68 (Abq, *J* = 11.7 Hz, 1H), 1.33 (s, 3H), 1.10 (s, 3H).

**6-((2*R*,5*R*)-2-Methyl-5-phenylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (22). General C-N coupling procedure.**

A mixture of tris(dibenzylideneacetone)dipalladium(0) (12.8 mg, 0.014 mmol) and 5-(diisopropylphosphino)-1',3',5'-triphenyl-1'*H*-1,4'-bipyrazole (prepared using the method described in *Org. Process Res. Dev.*, **2008**, *12*, 480-489, 13.4 mg, 0.028 mmol) in *t*-amyl alcohol (0.7 mL) in a sealed reaction vessel was stirred at rt under nitrogen for 30 min. (2*R*,5*R*)-2-methyl-5-phenylmorpholine (100 mg, 0.564 mmol), 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (129 mg, 0.564 mmol) and HMPA (0.516 g, 2.82 mmol) or DMSO (0.48 mL, 6.8 mmol) were added to the mixture followed by solid LiO*t*Bu (91.2 mg, 1.13 mmol) and a solution of LiO*t*Bu in *t*-amyl alcohol (1 M, 2.26 mL, 2.26 mmol). The reaction mixture was stirred at 60 $^o$C overnight. The solution was diluted with EtOAc and washed with saturated aq. $NH_4Cl$. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with

saturated aq. NaCl, dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by column chromatography on silica gel (gradient: 5–50% EtOAc/ heptanes). The resulting solid was triturated with acetonitrile to afford 31 mg (17%) from the HMPA reaction; 72 mg (39%) from the DMSO reaction. mp 203.8–204.7 $^{o}$C; $^{1}$H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.36–7.20 (m, 5H), 7.10 (d, *J* = 8.6 Hz, 1H), 6.13 (d, *J* = 8.8 Hz, 1H), 5.19 (d, *J* = 3.1 Hz, 1H), 4.55 (s, 2H), 4.41 (dd, *J* = 11.7, 1.6 Hz, 1H), 4.06 (dd, *J* = 11.8, 3.8 Hz, 1H), 3.91 (dd, *J* = 13.1, 3.1 Hz, 1H), 3.80–3.71 (m, 1H), 2.99 (dd, *J* = 13.1, 10.7 Hz, 1H), 1.27 (d, *J* = 6.2 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 166.4, 153.3, 139.8, 138.2, 130.8, 128.7, 128.0, 127.2, 126.7, 101.3, 72.3, 70.5, 67.8, 54.0, 47.4, 19.3; FTIR ($cm^{-1}$) 1701.4 (s); HRMS Calcd. for $C_{18}H_{19}N_3O_3$ $(M+H)^+$ 326.1505; Found 326.1498; HPLC: $t_R$ = 3.937 min, 99.75%; Anal. Calcd. for $C_{18}H_{19}N_3O_3$: C; 66.45, H; 5.89, N; 12.91. Found, C; 66.26, H; 5.62, N; 12.75. $[\alpha]_D^{20}$ -224.5 (*c* 0.392, MeOH).

**6-((2*S*,5*R*)-2-Methyl-5-phenylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (23)**

Prepared from (2*S*,5*R*)-2-methyl-5-phenylmorpholine and from 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one according to the general C-N coupling procedure to afford 47 mg (13%). $^{1}$H NMR ($CDCl_3$) δ 7.61 (br s, 1H), 7.34–7.17 (m, 5H), 6.94 (d, *J* = 8.6 Hz, 1H), 6.09 (d, *J* = 8.6 Hz, 1H), 4.53 (s, 2H), 4.27 (dd, *J* = 9.9, 4.2 Hz, 1H), 4.04–3.95 (m, 2H), 3.56 (dd, *J* = 11.8, 9.9 Hz, 1H), 2.81 (dd, *J* = 12.9, 10.1 Hz, 1H), 1.28 (d, *J* = 6.2 Hz, 3H).

**(*R*)-6-(2,2-Dimethyl-5-phenylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (24)**

Prepared from (*R*)-2,2-dimethyl-5-phenylmorpholine and 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one according to the general C-N coupling procedure to afford 9.3 mg (2.3%). $^{1}$H NMR ($CDCl_3$) δ 7.60 (br s, 1H), 7.35–7.25 (m, 5H), 7.03 (d, *J* = 8.8 Hz, 1H), 6.00 (d, *J* = 8.8 Hz, 1H),

4.98 (dd, *J* = 5.7, 5.7 Hz, 1H), 4.54 (s, 2H), 4.09–3.95 (m, 3H), 3.29 (d, *J* = 13.7 Hz, 1H), 1.29 (s, 3H), 1.27 (s, 3H). LC-MS (Method B): $t_R$ = 2.50 min. MS (ES+): 340.5 $(M+H)^+$.

**2-((2*R*,5*R*)-4-(3-Oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)-5-phenylmorpholin-2-yl)acetonitrile (25)**

A mixture of 6-((2*S,*5*R*)-2-(hydroxymethyl)-5-phenylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (57 mg, 0.17 mmol), DCE (5 mL), $Et_3N$ (38.6 μL, 0.273 mmol) and methanesulfonic anhydride (43.1 mg, 1.2 mmol) was stirred at 0 ºC for 2 h and at rt for 6 h. The reaction mixture was then partitioned between DCM (20 mL) and aq. NaOH (1 N, 20 mL). The organic layer was separated, washed with saturated aq. NaCl, dried over $MgSO_4$, filtered and concentrated to afford ((2*S,*5*R*)-4-(3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)-5-phenylmorpholin-2-yl)methyl methanesulfonate 35 mg (50%). The crude methanesulfonate (35 mg, 0.083mmol) was dissolved in DMF (1 mL) and treated with sodium cyanide (82 mg, 1.7 mmol). After heating at 120 ºC for 4 h, the mixture was partitioned between EtOAc (10 mL) and aq. NaOH (1 N, 10 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with saturated aq. NaCl (10 mL), dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (gradient: 0–100% EtOAc in heptanes) to provide 4.5 mg (15%) of a white solid. $^1$H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.36–7.24 (m, 5H), 7.13 (d, *J* = 8.8 Hz, 1H), 6.17 (d, *J* = 8.8 Hz, 1H), 5.31 (m, 2H), 5.19 (d, *J* = 2.2 Hz, 1H), 4.57 (s, 2H), 4.47 (dd, *J* = 11.7, 1.5Hz, 1H), 4.18–4.07 (m, 2H), 3.99–3.94 (m, 1H), 3.12 (dd, *J* = 13.2,11.0 Hz, 1H), 2.64 (dd, *J* = 6.1,2.0 Hz, 2H).

**6-((2*S*,5*R*)-2-(Methoxymethyl)-5-phenylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (26)**

Step 1: To a solution of ((2*S*,5*R*)-4-benzyl-5-phenylmorpholin-2-yl)methanol (**19**) (100 mg, 0.353 mmol) in DMF (2 mL) at 0 °C was added sodium hydride (17 mg, 60% dispersion in oil, 0.424 mmol). The solution was stirred at 0 °C for 30 min. Methyl iodide (0.068 mL, 1.06 mmol) was added. The solution was stirred overnight at rt. To the reaction mixture was added EtOAc. The mixture was extracted with saturated aq. $NH_4Cl$ and saturated aq. NaCl. The organic layer was dried over $Na_2SO_4$, filtered, concentrated and purified by column chromatography to afford 62 mg (59%). $^1$H NMR ($CDCl_3$) δ 7.49–7.44 (m, 2H), 7.37–7.18 (m, 8H), 4.05–3.95 (m, 2H), 3.83 (dd, *J* = 11.7, 8.4 Hz, 1H), 3.73 (dd, *J* = 11.7, 3.7 Hz, 1H), 3.67 (d, *J* = 13.5 Hz, 1H), 3.52–3.46 (m, 2H), 3.39 (s, 3H), 2.98 (d, *J* = 13.7 Hz, 1H), 2.73 (dd, *J* = 12.1, 3.1 Hz, 1H), 2.39 (dd, *J* = 12.1, 3.7 Hz, 1H).

Step 2: A mixture of (2*S,*5*R*)-4-benzyl-2-(methoxymethyl)-5-phenylmorpholine (350 mg, 1.18 mmol), MeOH (10mL), *p*-toluenesulphonic acid (452 mg, 2.35 mmol) and 10% Pd-C (50% water wet, 251 mg, 0.118 mmol) was hydrogenated in a Parr shaker for 1 h at 50 psi hydrogen. The mixture was filtered through Celite® and concentrated. The residue was dissolved in DCM and extracted with 4.3% aq. $NaHCO_3$. The layers were separated and the organic layer was washed with saturated aq. NaCl, dried over $Na_2SO_4$, filtered, and concentrated to provide 193 mg (79%) of (2*S,*5*R*)-2-(methoxymethyl)-5-phenylmorpholine (**21**) as a light yellow solid. $^1$H NMR (*DMSO-d*$_6$) δ 7.46–7.42 (m, 2H), 7.35–7.29 (m, 2H), 7.26–7.21 (m, 1H), 3.79–3.66 (m, 3H), 3.65–3.57 (m, 2H), 3.51 (dd, *J* = 10.2, 5.7 Hz, 1H), 3.26 (s, 3H), 2.83 (dd, *J* = 12.3, 3.5 Hz, 1H), 2.71 (dd, *J* = 12.2, 4.4 Hz, 2H).

Step 3: Prepared from (2*S*,5*R*)-2-(methoxymethyl)-5-phenylmorpholine (**21**) and from 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one according to the general C-N coupling procedure. The residue was dissolved in DMSO and purified by preparative HPLC Method B. Gradient: 75% water/ acetonitrile linear gradient to 100% acetonitrile in 8.5 min. LC-MS (Method A): $t_R$ = 2.78 min. MS (ES+): 361.11 $(M+H)^+$.

**6-((*2S,5R*)-2-(Hydroxymethyl)-5-phenylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (27)**

Step 1: ((2*S*,5*R*)-5-Phenylmorpholin-2-yl)methanol (**20**) was prepared by debenzylation of ((2*S*,5*R*)-4-benzyl-5-phenylmorpholin-2-yl)methanol. $^1$H NMR (*METHANOL-d*$_4$) δ 7.53–7.47 (m, 2H), 7.37–7.30 (m, 2H), 7.29–7.23 (m, 1H), 4.85 (s, 2H), 4.08–4.00 (m, 1H), 3.88-3.77 (m, 3H), 3.75–3.68 (m, 1H), 3.64 (dd, *J* = 10.9, 4.9 Hz, 1H), 2.87 (d, *J* = 4.9 Hz, 2H).

Step 2: Prepared from ((2*S*,5*R*)-5-phenylmorpholin-2-yl)methanol (**20**) and 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one according to the general C-N coupling procedure to afford 230 mg (67%). $^1$H NMR ($CDCl_3$) δ 8.84 (br s, 1H), 7.53–7.47 (m, 2H), 7.39–7.25 (m, 3H), 7.17 (d, *J* = 8.6 Hz, 1H), 6.39 (d, *J* = 8.6 Hz, 1H), 4.64–4.55 (m, 2H), 4.53 (d, *J* = 2.2 Hz, 2H), 4.21–4.14 (m, 1H), 4.01–3.91 (m, 2H), 3.82 (dd, *J* = 11.0, 2.9 Hz, 1H), 3.16 (d, *J* = 3.9 Hz, 2H).

**7-((2*R*,5*R*)-2-Methyl-5-phenylmorpholino)-1*H*-pyrido[3,4-*b*][1,4]oxazin-2(3*H*)-one (28)**

Prepared from (2*R*,5*R*)-2-methyl-5-phenylmorpholine and 7-chloro-1*H*-pyrido[3,4-*b*][1,4]oxazin-2(3*H*)-one according to the general C-N coupling procedure. The residue was dissolved in DMSO and purified by preparative HPLC Method A. Gradient: 90% water/

acetonitrile linear gradient to 100% acetonitrile in 8.5 min. LC-MS (Method A): $t_R$ = 1.92 min. MS (ES+): 326.17 $(M+H)^+$.

**7-((2*R*,5*R*)-2-Methyl-5-phenylmorpholino)-1*H*-pyrido[2,3-*b*][1,4]oxazin-2(3*H*)-one (29)**

Prepared from 7-bromo-1*H*-pyrido[2,3-*b*][1,4]oxazin-2-one and (2*R,*5*R*)-2-methyl-5-phenylmorpholine according to the general C-N coupling procedure. The residue was dissolved in DMSO and purified by preparative HPLC Method C. Gradient: 95% water/ acetonitrile linear gradient to 50% water/ acetonitrile in 8.5 min to 100% acetonitrile in 9.0 min, hold at 100% acetonitrile to 10.0 min. LC-MS (Method A): $t_R$ = 2.32 min. MS (ES+): 326.25 $(M+H)^+$.

**2-((2*R*,5*R*)-2-Methyl-5-phenylmorpholino)-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (30)**

Step 1: A mixture of 2-chloro-5-methoxypyrimidin-4-amine (WO2007/077961; 10.0 g, 62.5 mmol), DCM (600 mL) and boron tribromide (20 mL) was stirred at rt overnight. MeOH was added until the solution was homogenous. The solution was concentrated to give a mixture of 4-amino-2-chloropyrimidin-5-ol and 4-amino-2-bromopyrimidin-5-ol (8.0 g, 89%) as a yellow solid, which was used for the next step without further purification. $^1$H NMR (*DMSO-d*$_6$) δ 7.50 (s, 1H), 5.21 (s, 3H).

Step 2: A mixture of 4-amino-2-chloropyrimidin-5-ol and 4-amino-2-bromopyrimidin-5-ol (3.5 g, 24 mmol), DMF (50 mL), $K_2CO_3$ (1.66 g, 12 mmol) and ethyl bromoacetate (4.0 g, 24 mmol) was stirred at rt overnight. The mixture was diluted with water (50 mL) and extracted with EtOAc (5 x 100 mL). The organic layers were combined, washed with water (3 x 30 mL) and aq. NaCl, dried over $Na_2SO_4$ and concentrated. The residue was solidified from petroleum ether/EtOAc to give 3.0 g of a mixture of ethyl 2-(4-amino-2-chloropyrimidin-5-yloxy)acetate

and ethyl 2-(4-amino-2-bromopyrimidin-5-yloxy)acetate) as a solid. $^1$H NMR (*DMSO-d*$_6$) δ 7.63 (s, 1H), 4.83 (s, 2H), 4.18 (q, *J* = 6.8 Hz, 2H), 1.21 (t, *J* = 7.9 Hz, 3H).

Step 3: A mixture of ethyl 2-(4-amino-2-chloropyrimidin-5-yloxy)acetate and ethyl 2-(4-amino-2-bromopyrimidin-5-yloxy)acetate) (3.0 g, 13 mmol), DMF (35 mL) and $K_2CO_3$ (0.9 g, 6.5 mmol) was stirred at 60 $^o$C overnight. The mixture was diluted with water (30 mL) and extracted with EtOAc (8 x 50 mL). The organic layers were combined, washed with water (3 x 20 mL), saturated aq. NaCl, dried over $Na_2SO_4$, filtered and concentrated. The mixture was separated by preparative HPLC (Column: Kromasil Eternity-5-$C_{18}$ 30 x 150 mm; gradient: 5% acetonitrile/ water to 20% acetonitrile/ water over 12 min, hold 100% acetonitrile 2 min; modifier 0.225 % formic acid; wavelength 220 nm) and evaporated to afford 2-chloro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (60 mg) as a white solid and 2-bromo-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (263 mg) as a white solid. 2-chloro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one: $^1$H NMR (*DMSO-d*$_6$) δ 8.22 (s, 1H), 4.76 (s, 2H) and 2-bromo-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one: $^1$H NMR (*DMSO-d*$_6$) δ 8.17 (s, 1H), 4.75 (s, 2H).

Step 4: To a solution of 2-chloro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (100 mg, 0.539 mmol) in NMP (2 mL) was added (2*R,5R*)-2-methyl-5-phenylmorpholine (143 mg, 0.808 mmol) and $Et_3N$ (0.3 mL, 2 mmol). The mixture was heated to 200 °C under microwave irradiation for 1 h. The reaction was poured into aq. HCl (1 N) and EtOAc was added. The layers were separated. The organic layer was washed with saturated aq. NaCl, dried over $Na_2SO_4$, filtered, and concentrated. The crude material was purified by silica gel column chromatography (gradient: 0–30% heptanes/ acetone) to provide 75 mg (43%) as a crystalline solid. $^1$H NMR ($CDCl_3$) δ 7.99 (d, *J* = 1.0 Hz, 1H), 7.66 (br s, 1H), 7.46–7.41 (m, 2H), 7.34–7.29 (m, 2H), 7.26–7.22 (m,1H), 5.65 (d, *J* = 2.3 Hz, 1H), 4.59 (s, 2H), 4.49 (dd, *J* = 11.9, 1.2 Hz, 1H), 4.39 (dd, *J* =

13.5, 2.5 Hz, 1H), 4.00 (dd, *J* = 11.9, 3.7 Hz, 1H), 3.73–3.63 (m, 1H), 2.88 (dd, *J* = 13.7, 10.9 Hz, 1H), 1.24 (d, *J* = 6.2 Hz, 3H). LC-MS (Method B): $t_R$ = 2.20 min. MS (ES+): 327.5 $(M+H)^+$.

**6-((2*R*,5*R*)-2-Methyl-5-phenylmorpholino)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (31)**

Prepared from 6-bromo-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one and (2*R*,5*R*)-2-methyl-5-phenylmorpholine according to the general C-N coupling procedure to afford 123 mg (33%). $^1$H NMR (*DMSO-d*$_6$) δ 10.40 (s, 1H), 7.28–7.21 (m, 4H), 7.21–7.14 (m, 1H), 6.75 (d, *J* = 9.0 Hz, 1H), 6.46 (dd, *J* = 9.0, 2.9 Hz, 1H), 6.37 (d, *J* = 2.9 Hz, 1H), 4.72–4.68 (m, 1H), 4.41 (s, 2H), 4.15 (dd, *J* = 11.7, 1.6 Hz, 1H), 3.97 (dd, *J* = 11.7, 3.7 Hz, 1H), 3.80–3.71 (m, 1H), 3.33 (dd, *J* = 12.1, 3.1 Hz, 1H), 2.93 (dd, *J* = 12.1, 10.3 Hz, 1H), 1.21 (d, *J* = 6.2 Hz, 3H). LC-MS (Method B): $t_R$ = 2.40 min. MS (ES+): 325.5 $(M+H)^+$.

**6-((2*R*,5*R*)-5-(2-Fluorophenyl)-2-methylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (32)**

The *cis*-racemic compound was prepared from 5-(2-fluorophenyl)-2-methylmorpholine and 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one according to the general C-N coupling procedure to afford 59 mg (70%). The isomers were separated using supercritical fluid chromatography on Chiralcel OJ-H column 10 x 250 mm, mobile phase 85/15 carbon dioxide/MeOH, flow rate 10.0 mL/min. UV detection 210 nm. Peak 2: $t_R$ = 6.02 min. $^1$H NMR ($CDCl_3$) δ 7.71 (br s, 1H), 7.26–7.18 (m, 2H), 7.10–7.00 (m, 3H), 6.14 (d, *J* = 8.8 Hz, 1H), 5.30 (d, *J* = 11.9 Hz, 1H), 4.53 (s, 2H), 4.32 (d, *J* = 11.9 Hz, 1H), 4.09–4.04 (m, 2H), 3.83-3.73 (m, 1H), 3.13 (dd, *J* = 13.1, 10.7 Hz, 1H), 1.32 (d, *J* = 6.2 Hz, 3H).

**6-((2*R*,5*R*)-5-(3-Fluorophenyl)-2-methylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (33)**

The *cis*-racemic compound (60 mg, 28%) was prepared from 5-(3-fluorophenyl)-2-methylmorpholine and 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one followed by separation using supercritical fluid chromatography on Chiralcel OJ-H column 10 x 250 mm, mobile phase 80/20 carbon dioxide/MeOH and flow rate 10.0 mL/min. UV detection 210 nm. Peak 2: $t_R$ = 7.24 min. $^1$H NMR (*DMSO-d*$_6$) δ 10.87 (s, 1H), 7.37–7.29 (m, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 7.17–7.13 (m, 2H), 7.07–7.00 (m, 1H), 6.26 (d, *J* = 8.8 Hz, 1H), 5.29 (d, *J* = 2.9 Hz, 1H), 4.48 (s, 2H), 4.29 (d, *J* = 11.3 Hz, 1H), 4.00–3.88 (m, 2H), 3.71–3.61 (m, 1H), 3.17 (d, *J* = 5.3 Hz, 1H), 2.82 (dd, *J* = 13.1, 10.7 Hz, 1H), 1.16 (d, *J* = 6.2 Hz, 3H). LC-MS (Method B): $t_R$ = 1.77 min. MS (ES+): 344.4 $(M+H)^+$.

**6-((2*R*,5*R*)-5-(4-Fluorophenyl)-2-methylmorpholino)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (34)**

The racemic compound (18 mg, 7.5%) was prepared from 6-bromo-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one and (±)-*cis*-5-(4-fluorophenyl)-2-methylmorpholine followed by chiral separation using supercritical fluid chromatography on Chiralcel OJ-H column 10 x 250 mm, mobile phase 70/30 carbon dioxide/MeOH, flow rate 10.0 mL/ min. UV detection 210 nM. Peak 2: $t_R$ = 6.60 min. $^1$H NMR ($CDCl_3$) δ 7.63 (br s, 1H), 7.37–7.30 (m, 2H), 7.11 (d, *J* = 8.8 Hz, 1H), 7.02–6.94 (m, 2H), 6.12 (d, *J* = 8.8 Hz, 1H), 5.21 (d, *J* = 3.1 Hz, 1H), 4.56 (s, 2H), 4.36 (dd, *J* = 11.9, 1.2 Hz, 1H), 4.06 (dd, *J* = 11.8, 3.8 Hz, 1H), 3.85–3.79 (m, 1H), 3.78–3.70 (m, 1H), 3.50 (d, *J* = 5.1 Hz, 2H), 2.93 (dd, *J* = 12.8, 10.6 Hz, 1H), 1.28 (d, *J* = 6.0 Hz, 3H).

All the biological assays including the functional and binding assays for the nuclear hormone receptors MR, PR, GR, AR, ERα were performed as previously described.[22d,e,f]

**Effect of 22 on urinary sodium to potassium ratio ($Na^+/K^+$) in Wistar rats**. Female Wistar rats were received from Charles River laboratories at approximately 10 weeks of age (body weight ~ 400 g). Rats were singly housed in wire cages on a 12-hour light cycle and were provided standard laboratory chow diet and water *ad libitum* prior to and throughout the studies. Following a 1 week acclimation period, the animals were randomly assigned to treatment groups (n = 7/group) to receive a single oral dose (nanosuspension) of 30, 100, or 500 mg/kg **22**, vehicle (2% PVP and 0.025% SLS), or 100 mg/kg **2** used as a positive control in a dosing volume of 5 mL/kg. Urine samples were collected overnight prior to dosing and at intervals of 0-2, 2-4, 4-6 and 6-8 h post-dose for measurement of urinary sodium and potassium concentration. Data were converted to Log (10* $Na^+/K^+$) and were analyzed using ANOVA with Tukey post-hoc test. The plasma concentration of **22** was measured using LC-MS. The mean AUC of **22** at 30, 100 and 500 mg/kg were 2850, 9410 and 13500 ng•h/mL, respectively.

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**Abbreviations**. DLM, dog liver microsomes; HLM, human liver microsomes; MDCK Madin-Darby canine kidney; RLM, rat liver microsomes; RRCK, Ralph Russ canine kidney; TPSA, topological polar surface area.

**Supporting Information**

HPLC and LC-MS conditions, MR assay statistics, metabolism identification data, docking of **22** in 3vhv and molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

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3vhv, Compound **1d** from reference 46a is referred to in Figure 7.

**References**

(1) Williams, J. S.; Williams, G. H. 50th Anniversary of aldosterone. *J. Clin. Endocrinol. Metab*. **2003**, *88*, 2364−2372.

(2) For recent reviews on the role of aldosterone in the heart and kidney, see: (a) Catena, C.; Colussi, G.; Marzano, L.; Sechi, L. A. Aldosterone and the heart: from basic research to clinical evidence. *Horm. Metab. Res.* **2012**, *44*, 181−187. (b) Fourkiotis, V. G.; Hanslik, G.; Hanusch, F.; Lepenies, J.; Quinkle, M. Aldosterone and the kidney. *Horm. Metab. Res.* **2012**, *44*, 194−201.

(3) Funder, J. W. Aldosterone and the cardiovascular system: genomic and nongenomic effects. *Endocrinology*, **2006**, *147*, 5564–5567.

(4) Ménard, J. The 45-year story of the development of an anti-aldosterone more specific than spironolactone. *Mol. Cell. Endocrinol*. **2004**, *217*, 45−52.

(5) Pitt, B.; Zannad, F.; Remme, W. J.; Cody, R.; Castaigne, A.; Perez, A.; Palensky, J.; Wittes, J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *N. Engl. J. Med.* **1999,** *341*, 709–717.

(6) Pitt, B.; Remme, W.; Zannad, F.; Neaton, J.; Martinez, F.; Roniker, B.; Bittman, R.; Hurley, S.; Kleiman, J.; Gatlin, M. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N. Engl. J. Med.* **2003,** *348*, 1309−1321.

(7) Zannad, F.; McMurray, J. J. V.; Krum, H.; van, V. D. J.; Swedberg, K.; Shi, H.; Vincent, J.; Pocock, S. J.; Pitt, B. Eplerenone in patients with systolic heart failure and mild symptoms. *N. Engl. J. Med.* **2011,** *364*, 11–21.

(8) Rossing, K.; Schjoedt, K. J.; Smidt, U. M.; Boomsma, F.; Parving, H.-H. Beneficial effects of adding spironolactone to recommended antihypertensive treatment in diabetic nephropathy: a randomized, double-masked, cross-over study. *Diabetes Care* **2005**, *28*, 2106−2112.

(9) van den Meiracker, A. H.; Baggen, R. G.; Pauli, S.; Lindemans, A.; Vulto, A. G.; Poldermans, D.; Boomsma, F. Spironolactone in type 2 diabetic nephropathy: effects on proteinuria, blood pressure and renal function. *J. Hypertens.* **2006**, *24*, 2285−2292.

(10) Nielsen, S. E.; Persson, F.; Frandsen, E.; Sugaya, T.; Hess, G.; Zdunek, D.; Shjoedt, K. J.; Parving, H.-H.; Rossing, P. Spironolactone diminishes urinary albumin excretion in patients with type 1 diabetes and microalbuminuria: a randomized placebo-controlled crossover study. *Diabet. Med.* **2012**, *29*, e184–e190.

(11) Esteghamati, A.; Noshad, S.; Jarrah, S.; Mousavizadeh, M.; Khoee, S. H.; Nakhjavani, M. Long-term effects of addition of mineralocorticoid receptor antagonist to angiotensin II receptor blocker in patients with diabetic nephropathy: a randomized clinical trial. *Nephrol., Dial., Transplant.* **2013**, *28*, 2823−2833.

(12) Corvol, P.; Michaud, A.; Menard, J.; Freifeld, M.; Mahoudeau, J. Antiandrogenic effect of spirolactones: mechanism of action. *Endocrinology* **1975**, *97*, 52−58.

(13) (a) Martín-Martínez, M.; Perez-Gordillo, F. L.; Alvarez de la Rosa, D.; Rodríguez, Y. ; Gerona-Navarro, G.; Gonzalez-Muniz, R.; Zhou, M.-M. Modulating mineralocorticoid receptor with non-steroidal antagonists. New opportunities for the development of potent and selective ligands without off-target side effects. *J. Med. Chem.* **2017**, *60*, 2629−2650. (b) Kolkhof, P.; Bärfacker, L. Mineralocorticoid receptor antagonists: 60 years of research and development. *J. Endocrinol.* **2017**, *234*, T125–T140.

(14) Baerfacker, L.; Kuhl, A.; Hillisch, A.; Grosser, R.; Figueroa-Perez, S.; Heckroth, H.; Nitsche, A.; Ergueden, J.-K.; Gielen-Haertwig, H.; Schlemmer, K.-H.; Mittendorf, J.; Paulsen, H.; Platzek, J.; Kolkhof, P. Discovery of BAY 94-8862: a nonsteroidal antagonist of the mineralocorticoid receptor for the treatment of cardiorenal diseases. *ChemMedChem* **2012**, *7*, 1385−1403.

(15) Makiko, Y.; Makoto, T.; Eiko, S.; Hideo, T.; Masakatsu, K.; Takuo, W.; Nobuyuki, M.; Shin-Ichi, I.; Takashi, I. Pharmacokinetics, distribution, and disposition of esaxerenone, a novel, highly potent and selective non-steroidal mineralocorticoid receptor antagonist, in rats and monkeys. *Xenobiotica* **2016**, 1−14.

(16) (a) Iijima, T.; Yamamoto, Y.; Akatsuka, H.; Kawaguchi, T. Preparation of Benzoxazines and Related Nitrogen-Containing Heterobicyclic Compounds as Mineralocorticoid Receptor Modulators. PCT Int. Appl. WO 2007089034 A1, Aug 09, 2007. (b) Okabe, T.; Hamada, T.; Mitsuhashi, K.; Okamoto, Y.; Iijima, T.; Akatsuka, H.; Toyama, K.; Moroda, A.; Sugiura, Y. Method for Producing 1,4-Benzoxazine Compound. PCT Int. Appl. WO 2014024950 A1, Feb 13, 2014.

(17) (a) Heinig, R.; Kimmeskamp-Kirschbaum, N.; Halabi, A.; Lentini, S. Pharmacokinetics of the novel nonsteroidal mineralocorticoid receptor antagonist finerenone (BAY 94–8862) in individuals with renal impairment. *Clin. Pharm. Drug Dev.* **2016**, *5*, 488−501. (b) Lattenist, L.; Lechner, S. M.; Messaoudi, S.; Le Mercier, A.; El Moghrabi, S.; Prince, S.; Bobadilla, N. A.; Kolkhof, P.; Jaisser, F.; Barrera-Chimal, J. Nonsteroidal mineralocorticoid receptor antagonist finerenone protects against acute kidney injury–mediated chronic kidney disease. *Hypertension* **2017**, *69*, 870−878.

(18) Bramlage, P.; Swift, S. L.; Thoenes, M.; Minguet, J.; Ferrero, C.; Schmieder, R. E. Non-steroidal mineralocorticoid receptor antagonism for the treatment of cardiovascular and renal disease. *Eur. J. Heart Fail.* **2016**, *18*, 28−37.

(19) Pitt, B.; Kober, L.; Ponikowski, P.; Gheorghiade, M.; Filippatos, G.; Krum, H.; Nowack, C.; Kolkhof, P.; Kim, S.-Y.; Zannad, F. Safety and tolerability of the novel non-steroidal mineralocorticoid receptor antagonist BAY 94-8862 in patients with chronic heart failure and mild or moderate chronic kidney disease: a randomized, double-blind trial. *Eur. Heart J.* **2013**, *34*, 2453−2463.

(20) Platt, D.; Pauli, H. Studies on organ- and subcellular distribution of $^{3}$H-spironolactone in animals. *Arzneim. Forsch.* **1972**, *22*, 1801−1802.

(21) Kolkhof, P.; Borden, S. A. Molecular pharmacology of the mineralocorticoid receptor: prospects for novel therapeutics. *Mol. Cell. Endocrinol.* **2012**, *350*, 310−317.

(22) (a) Dietz, J. D.; Du, S.; Bolten, C. W.; Payne, M. A.; Xia, C.; Blinn, J. R.; Funder, J. W.; Hu, X. A number of marketed dihydropyridine calcium channel blockers have mineralocorticoid receptor antagonist activity. *Hypertension* **2008**, *51*, 742−748. (b) Arhancet, G. B.; Woodard, S. S.; Dietz, J. D.; Garland, D. J.; Wagner, G. M.; Iyanar, K.; Collins, J. T.; Blinn, J. R.; Numann, R. E.; Hu, X.; Huang, H.-C. Stereochemical requirements for the mineralocorticoid receptor antagonist activity of dihydropyridines. *J. Med. Chem.* **2010**, *53*, 4300−4304. (c) Arhancet, G. B.; Woodard, S. S.; Iyanar, K.; Case, B. L.; Woerndle, R.; Dietz, J. D.; Garland, D. J.; Collins, J. T.; Payne, M. A.; Blinn, J. R.; Pomposiello, S. I.; Hu, X.; Heron, M. I.; Huang, H.-C.; Lee, L. F. Discovery of novel cyanodihydropyridines as potent mineralocorticoid receptor antagonists. *J. Med. Chem.* **2010**, *53*, 5970−5978. (d) Futatsugi, K.; Piotrowski, D. W.; Casimiro-Garcia, A.; Robinson, S.; Sammons, M.; Loria, P. M.; Banker, M. E.; Petersen, D. N.; Schmidt, N. J. Design and synthesis of aryl sulfonamide-based nonsteroidal mineralocorticoid receptor antagonists. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6239−6242. (e) Meyers, M. J.; Arhancet, G. B.; Hockerman, S. L.; Chen, X.; Long, S. A.; Mahoney, M. W.; Rico, J. R.; Garland, D. J.; Blinn, J. R.; Collins, J. T.; Yang, S.; Huang, H.-C.; McGee, K. F.; Wendling, J. M.; Dietz, J. D.; Payne, M. A.; Homer, B. L.; Heron, M. I.; Reitz, D. B.; Hu, X. Discovery of (3*S*,3a*R*)-2-(3-chloro-4-cyanophenyl)-3-cyclopentyl-3,3a,4,5-tetrahydro-2*H*-benzo[*g*]indazole-7-carboxylic acid (PF-3882845), an orally efficacious mineralocorticoid receptor (MR) antagonist for hypertension and nephropathy. *J. Med. Chem.* **2010**, *53*, 5979−6002. (f) Casimiro-Garcia, A.; Piotrowski, D. W.; Ambler, C.; Arhancet, G. B.; Banker, M. E.; Banks, T.; Boustany-Kari, C. M.; Cai, C.; Chen, X.; Eudy, R.; Hepworth, D.; Hulford, C. A.; Jennings, S. M.; Loria, P. M.; Meyers, M. J.; Petersen,

D. N.; Raheja, N. K.; Sammons, M.; She, L.; Song, K.; Vrieze, D.; Wei, L. Identification of (*R*)-6-(1-(4-cyano-3-methylphenyl)-5-cyclopentyl-4,5-dihydro-1*H*-pyrazol-3-yl)-2-methoxynicotinic acid, a highly potent and selective nonsteroidal mineralocorticoid receptor antagonist. *J. Med. Chem.* **2014**, *57*, 4273−4288.

(23) Piotrowski, D. W. Mineralocorticoid receptor antagonists for the treatment of hypertension and diabetic nephropathy. *J. Med. Chem.* **2012**, *55*, 7957−7966.

(24) Bledsoe, R. K.; Madauss, K. P.; Holt, J. A.; Apolito, C. J.; Lambert, M. H.; Pearce, K. H.; Stanley, T. B.; Stewart, E. L.; Trump, R. P.; Willson, T. M.; Williams S. P. A ligand-mediated hydrogen bond network required for the activation of the mineralocorticoid receptor. *J. Biol. Chem*. **2005**, *280*, 31283–31293.

(25) Williams, S. P.; Sigler, P. B. Atomic structure of progesterone complexed with its receptor. *Nature* **1998**, *393*, 392−396.

(26) Zhang, Z.; Olland, A. M.; Zhu, Y.; Cohen, J.; Berrodin, T.; Chippari, S.; Appavu, C.; Li, S.; Wilhem, J.; Chopra, R.; Fensome, A.; Zhang, P.; Wrobel, J.; Unwalla, R. J.; Lyttle, C. R.; Winneker, R. C. Molecular and pharmacological properties of a potent and selective novel nonsteroidal progesterone receptor agonist tanaproget. *J. Biol. Chem*. **2005**, *280*, 28468–28475.

(27) Pereira, K. D.-T.; Cote. P.-L.; Cantin, L.; Blanchet, J.; Labrie, F.; Breton, R. Comparison of crystal structures of human androgen receptor ligand-binding domain complexed with various agonists reveals molecular determinants responsible for binding affinity. *Protein Sci.* **2006**, *15*, 987–999.

(28) (a) TenBrink, R. E. A method for the preparation of stereochemically defined ψ[CH2O] pseudodipeptides. *J. Org. Chem.* **1987**, *52*, 418−422. (b) Danklmaier, J.; Honig, H. Syntheses and structures of diastereomerically pure 2,6-disubstituted 3-morpholinones. *Liebigs Ann. Chem.*

**1988**, 851−854. (c) Norman, B. H.; Kroin, J. S. Alkylation studies of N-protected-5-substituted morpholin-3-ones. A stereoselective approach to novel methylene ether dipeptide isosteres. *J. Org. Chem*. **1996**, *61*, 4990−4998.

(29) Sammons, M.; Jennings, S. M.; Herr, M.; Hulford, C. A.; Wei, L.; Hallissey, J. F.; Kiser, E. J.; Wright, S. W.; Piotrowski, D. W. Synthesis of a cis 2,5-disubstituted morpholine by de-epimerization: application to the multigram scale synthesis of a mineralocorticoid antagonist. *Org. Process Res. Dev.* **2013**, *17*, 934−939.

(30) Breuning, M.; Winnacker, M.; Steiner, M. Efficient one-pot synthesis of enantiomerically pure 2-(hydroxymethyl)-morpholines. *Eur. J. Org. Chem.* **2007**, *13*, 2100–2106.

(31) Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A.; Gao, F. ElogD$_{oct}$: a tool for lipophilicity determination in drug discovery. 2. Basic and neutral compounds. *J. Med. Chem.* **2001**, *44*, 2490–2497.

(32) (a) Schonherr, H.; Cernak, T. Profound methyl effects in drug discovery and a call for new C-H methylation reactions. *Angew. Chem. Int. Ed.* **2013**, *52*, 12256−12267. (b) Leung, C. S.; Leung, S. S. F.; Tirado-Rives, J.; Jorgensen, W. L. Methyl effects on protein-ligand binding. *J. Med. Chem*. **2012**, *55*, 4489−4500.

(33) Kehler, J.; Rasmussen, L. K.; Jorgensen, M. Drug-Like Properties and Decision Making in Medicinal Chemistry. In *Textbook of Drug Design and Discovery*, 5th ed.; Stromgaard, K.; Krogsgaard-Larsen, P.; Madsen. U., Ed.; CRC Press: Boca Raton, 2017; Chapter 5.

(34) Watts, K. S.; Dalal, P.; Murphy, R. B.; Sherman, W.; Friesner, R. A.; Shelley, J. C. ConfGen: a conformational search method for efficient generation of bioactive conformers. *J. Chem. Inf. Model.*, **2010**, *50*, 534−546.

(35) Bochevarov, A. D.; Harder, E.; Hughes, T. F.; Greenwood, J. R.; Braden, D. A.; Philipp, D. M.; Rinaldo, D.; Halls, M. D.; Zhang, J.; Friesner, R. A. Jaguar: a high-performance quantum chemistry software program with strengths in life and materials sciences. *Int. J. Quantum Chem*. **2013**, *113*, 2110−2142.

(36) ConfGen was used to sample possible conformations of this compound. The lowest energy conformations with axial and equatorial phenyl were collected, and were used as the starting structures for further optimization by QM in Jaguar. The potential energy of the two conformations were calculated based on these optimized structures by QM in Jaguar as well.

(37) The crystal structure of **22** has been deposited into the Cambridge Crystallographic Data Center (CCDC 1573243).

(38) Kalgutkar, A. S.; Didiuk, M. T. Structural alerts, reactive metabolites, and protein covalent binding: how reliable are these attributes as predictors of drug toxicity? *Chem. Biodivers*. **2009**, *6*, 2115−2137.

(39) MR functional and binding data for selected library and patent literature analogs can be found in the Supporting Information.

(40) HPLC traces for MetID using human, dog and rat liver microsomes can be found in the Supporting Information.

(41) Oral dosing of **22** formulated in 2% polyvinyl pyrrolidone and 0.025% sodium lauryl sulfate to five Wistar Han rats/sex/group.

(42) Higher exposure of drugs in female rats has been attributed to gender-specific expression of CYP genes. See, Shapiro, B. H.; Agrawal, A. K.; Pampori, N. A. Gender differences in drug metabolism regulated by growth hormone. *Int. J. Biochem. Cell. Biol*. **1995**, *27*, 9−20.

(43) Other non-steroidal MR antagonists have been shown to be devoid of sex hormone related effects that have been noted for **1**. For example see, Nariai, T.; Fujita, K.; Mori, M.; Katayama, S.; Hori, S.; Matsui, K. SM-368229, a novel selective and potent non-steroidal mineralocorticoid receptor antagonist with strong urinary $Na^+$ excretion activity. *J. Pharmacol. Sci*. **2011**, *115*, 346–353.

(44) Brandish, P. E.; Chen, H.; Szczerba, P.; Hershey, J. C. Development of a simplified assay for determination of the antimineralocorticoid activity of compounds dosed in rats. *J. Pharmacol. Toxicol. Methods* **2008**, *57*, 155–160.

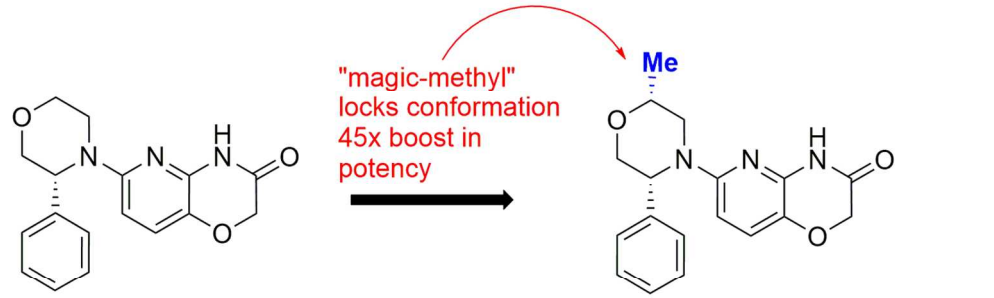
(45) Casimiro-Garcia, A.; Futatsugi, K.; Piotrowski, D. W. Preparation of Morpholine Compounds as Therapeutic Mineralocorticoid Receptor Antagonists. PCT Int. Appl. WO 2011141848 A1, Nov 17, 2011.

(46) (a) Hasui, T.; Matsunaga, N.; Ora, T.; Ohyabu, N.; Nishigaki, N.; Imura, Y.; Igata, Y.; Matsui, H.; Motoyaji, T.; Tanaka, T.; Habuka, N.; Sogabe, S.; Ono, M.; Siedem, C. S.; Tang, T. P.; Gauthier, C.; De, M. L. A.; Boyd, S. A.; Fukumoto, S. Identification of benzoxazin-3-one derivatives as novel, potent, and selective nonsteroidal mineralocorticoid receptor antagonists. *J. Med. Chem.* **2011**, *54*, 8616−8631. (b) Hasui, T.; Ohra, T.; Ohyabu, N.; Asano, K.; Matsui, H.; Mizukami, A.; Habuka, N.; Sogabe, S.; Endo, S.; Siedem, C. S.; Tang, T. P.; Gauthier, C.; De, M. L. A.; Boyd, S. A.; Fukumoto, S. Design, synthesis, and structure-activity relationships of dihydrofuran-2-one and dihydropyrrol-2-one derivatives as novel benzoxazin-3-one-based mineralocorticoid receptor antagonists. *Bioorg. Med. Chem.* **2013**, *21*, 5983−5994. (c) Hasui, T.; Ohyabu, N.; Ohra, T.; Fuji, K.; Sugimoto, T.; Fujimoto, J.; Asano, K.; Oosawa, M.; Shiotani, S.; Nishigaki, N.; Kusumoto, K.; Matsui, H.; Mizukami, A.; Habuka, N.; Sogabe, S.; Endo, S.; Ono, M.; Siedem, C. S.; Tang, T. P.; Gauthier, C.; De Meese, L. A.; Boyd, S. A.; Fukumoto, S.

Discovery of 6-[5-(4-fluorophenyl)-3-methyl-pyrazol-4-yl]-benzoxazin-3-one derivatives as novel selective nonsteroidal mineralocorticoid receptor antagonists. *Bioorg. Med. Chem.* **2014**, *22*, 5428−5445.

(47) For recent co-crystal structures of other MR antagonists, see (a) Nordqvist, A.; O'Mahony, G.; Friden-Saxin, M.; Fredenwall, M.; Hogner, A.; Granberg, K. L.; Aagaard, A.; Backstrom, S.; Gunnarsson, A.; Kaminski, T.; Xue, Y.; Dellsen, A.; Hansson, E.; Hansson, P.; Ivarsson, I.; Karlsson, U.; Bamberg, K.; Hermansson, M.; Georgsson, J.; Lindmark, B.; Edman, K. Structure-based drug design of mineralocorticoid receptor antagonists to explore oxosteroid receptor selectivity. *ChemMedChem* **2017**, *12*, 50–65. (b) Lotesta, S. D.; Marcus, A. P.; Zheng, Y.; Leftheris, K.; Noto, P. B.; Meng, S.; Kandpal, G.; Chen, G.; Zhou, J.; McKeever, B.; Bukhtiyarov, Y.; Zhao, Y.; Lala, D. S.; Singh, S. B.; McGeehan, G. M. Identification of spirooxindole and dibenzoxazepine motifs as potent mineralocorticoid receptor antagonists. *Bioorg. Med. Chem.* **2016**, *24*, 1384−1391.

(48) A docking study with **22** in 3vhv provided similar interactions with Asn770. See Supporting Information.




**13**

human MRa $IC_{50}$ = 1969 nM
human MR binding $IC_{50}$ = 1181 nM

**22**

human MRa $IC_{50}$ = 44 nM
human MR binding $IC_{50}$ = 25 nM
PRa, ARa, GRa $IC_{50}$ > 10000 nM

117x54mm (300 x 300 DPI)