Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

# Pyridyl-2,5-Diketopiperazines as Potent, Selective, and Orally Bioavailable Oxytocin Antagonists: Synthesis, Pharmacokinetics, and In Vivo Potency

Alan D. Borthwick,*[,†,▽] John Liddle,*[,†] Dave E. Davies,[†] Anne M. Exall,[†] Christopher Hamlett,[†] Deirdre M. Hickey,[†] Andrew M. Mason,[†] Ian E. D. Smith,[†] Fabrizio Nerozzi,[†] Simon Peace,[†] Derek Pollard,[†] Steve L. Sollis,[†] Michael J. Allen,[§] Patrick M. Woollard,[‡] Mark A. Pullen,[⊥] Timothy D. Westfall,[⊥] and Dinesh J. Stanislaus[#]

[†]Departments of Medicinal Chemistry and [‡]DMPK, Cardiovascular and Urogenital Centre of Excellence for Drug Discovery, GlaxoSmithKline Research and Development, Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts SG1 2NY, U.K.

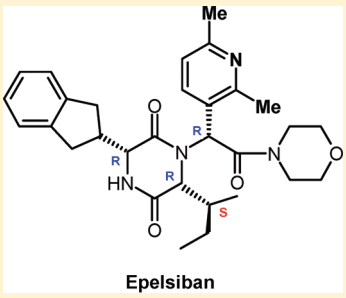
[§]Department of Assay Development and Compound Profiling, Harlow Research 2, GlaxoSmithKline, New Frontiers, Science Park, Third Avenue, Harlow, Essex CM19 5AD, U.K.

[⊥]Department of Urogenital Biology, Cardiovascular and Urogenital Center of Excellence for Drug Discovery, GlaxoSmithKline Pharmaceuticals, King of Prussia, Pennsylvania, United States

[#]Safety Assessment, GlaxoSmithKline Pharmaceuticals, King of Prussia, Pennsylvania, United States

Supporting Information

**ABSTRACT:** A six-stage stereoselective synthesis of indanyl-7-(3′-pyridyl)-(3*R*,6*R*,7*R*)-2,5-diketopiperazines oxytocin antagonists from indene is described. SAR studies involving mono- and disubstitution in the 3′-pyridyl ring and variation of the 3-isobutyl group gave potent compounds ($pK_i$ > 9.0) with good aqueous solubility. Evaluation of the pharmacokinetic profile in the rat, dog, and cynomolgus monkey of those derivatives with low cynomolgus monkey and human intrinsic clearance gave 2′,6′-dimethyl-3′-pyridyl *R-sec*-butyl morpholine amide Epelsiban (**69**), a highly potent oxytocin antagonist ($pK_i$ = 9.9) with >31000-fold selectivity over all three human vasopressin receptors hV1aR, hV2R, and hV1bR, with no significant P450 inhibition. Epelsiban has low levels of intrinsic clearance against the microsomes of four species, good bioavailability (55%) and comparable potency to atosiban in the rat, but is 100-fold more potent than the latter in vitro and was negative in the genotoxicity screens with a satisfactory oral safety profile in female rats.



Epelsiban

## INTRODUCTION

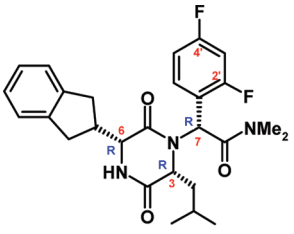
Preterm labor is a major clinical problem leading to death and disability in newborns and accounts for 10% of all births and causes 70% of all infant mortality and morbidity.[1] Oxytocin (OT) is a potent stimulant of uterine contractions and is responsible for the initiation of labor via the interaction with the OT receptors in the mammalian uterus. OT antagonists have been shown to inhibit uterine contractions and delay preterm delivery. So there is increasing interest in OT antagonists because of their potential application in the prevention of preterm labor. Although several tocolytics have already been approved in clinical practice, they have harmful maternal or fetal side effects.[2] The first clinically tested OT antagonist atosiban has a much more tolerable side effect profile and has recently been approved for use in Europe. However, atosiban is a peptide and a mixed OT/vasopressin V1a receptor antagonist that has to be given by iv infusion and is not suitable for long-term maintenance treatment, as it is not orally bioavailable.[3] Hence there has been considerable interest in overcoming the shortcomings of the peptide OT antagonists by identifying orally active nonpeptide OT antagonists with a higher degree of selectivity toward the vasopressin receptors (V1a, V1b, V2) with good oral bioavailability. Although several templates have been investigated as potential selective OT antagonists, few have achieved the required selectivity for the OT receptor vs the vasopressin receptors combined with the bioavailability and physical chemical properties required for an efficacious oral drug.[4] Therefore our objective was to design a potent, orally active OT antagonist with high levels of selectivity over the vasopressin receptor with good oral bioavailability in humans that would delay labor safely by greater than seven days and with improved infant outcome, as shown by a reduced combined morbidity score.

We recently reported[5] the identification of a novel series of (3*R*,6*R*,7*R*)-2,5-diketopiperazine (2,5-DKP) derivatives with antagonist activity at the human oxytocin receptor (hOTR). The most potent of these was the 2,4-difluorophenyl dimethylamide **1**,

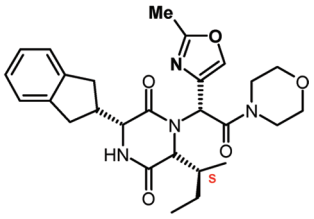
Received: September 27, 2011
Published: January 12, 2012

 © 2012 American Chemical Society  dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783–796

which has good in vitro ($pK_i$ = 9.2) and in vivo ($IC_{50}$ = 227 nM) potency and is 20-fold more potent than atosiban in vitro. Compound **1** also has good pharmacokinetics with bioavailability >50% in both the rat and the dog. Moreover, it is >500-fold selective over all three human vasopressin receptors (hV1aR, hV2R, and hV1bR) and has an acceptable P450 profile. In addition, it has a satisfactory safety profile in the genotoxicity screens and in the four day oral toxicity test in rats.




**1**




**106**

However, **1** had poor aqueous solubility and high intrinsic clearance in human and cynomolgus monkey liver microsomes, so a compound was required that retained high antagonist potency and excellent pharmacokinetics in animal species seen with **1** but was more soluble and with improved human intrinsic clearance to decrease the risk of low bioavailability in humans. Our first approach was to replace the 7-aryl ring with a five-membered heterocycle, which led to the oxazole Retosiban (**106**) as our clinical candidate.[6] As a backup to **106**, an alternative replacement of the 7-aryl ring with a six-membered heterocycle was considered and in this report we describe how we investigated the modification of the 7-aryl ring to the 7(3′-pyridyl) ring and optimized substitution in this ring as well as modifying the isobutyl group to obtain good potency, lower intrinsic clearance in human microsomes, and good pharmacokinetics in animal species.

## CHEMISTRY

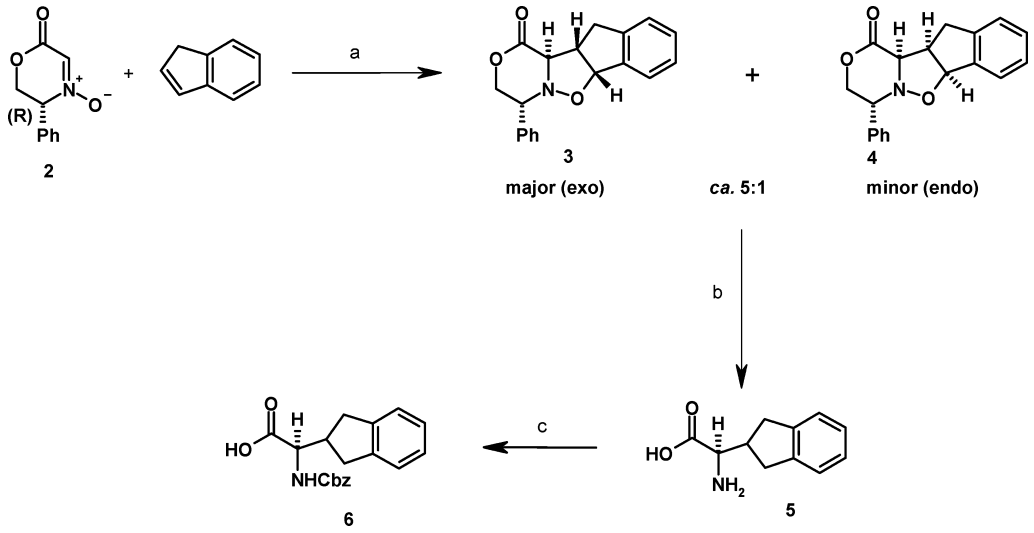
Our previous synthesis[9] of chirally pure *R*-indanylglycine **5** was a five-step synthesis from 2-iodoindane involving a dynamic kinetic resolution of a racemic hydantion intermediate with D-hydantoinase followed by hydrolysis of the resulting urea with D-carbamoylase. Although this is an efficient synthesis, it is reasonably long and uses solid-phase enzymes on a plant scale. A shorter route would have advantages. A very short route (two-stage process) was developed using the method of Long and Baldwin[7] from commercially inexpensive indene. A 1,3 dipolar addition of the *R*-nitrone **2**[8] to indene gave a 75% yield of the exo and endo cycloadducts **3** and **4** in a ratio of 5:1. The cycloadducts **3** and **4** were unstable on silica and were purified by recrystallization. Hydrogenolysis of the cycloadduct mixture of **3** and **4** using Pearlman's catalyst at 60 psi (~4 atm) gave *R*-indanylglycine **5** in 70% yield, not optimized, which on Cbz-protection gave *N*-benzyloxycarbonyl-D-indanylglycine **6** in 95% yield (Scheme 1).

The synthesis of indane 2,5-diketopiperazine tertiary amides developed previously[5] was used to make a range of amides for each of the substituted pyridine templates as outlined in Scheme 2. The four-component Ugi reaction of *Z* indanyl *R*-glycine **6**, with the *R*-aminoesters **8**−**13**, convertible isonitrile **7**, and the pyridylaldehydes **14**−**19**, gave the tripeptide intermediates **20**−**31**, which was followed by hydrogenation to remove the *Z* and benzyl groups, enabling cyclization to occur to give the phenolic 2,5-DKPs **32**−**43** in good yield.

The cyclic carbamates obtained from carbonyldiimidazole and phenol's **32**−**43** were treated directly with a range of amines, which gave the required *RRR* amides **44**, **45**, **47**−**59**, and **61**−**69** in high yield. The 6-OH derivative **46** was obtained in 91% yield by acid hydrolysis of the 6-MeO derivative **44** and the *N*-oxide **60** was prepared in 72% yield by oxidation of **58** with *m*-chloroperbenzoic acid. The acids **70**, **71**, and **72** were prepared by hydrolysis of the cyclic carbamates, obtained from carbonyldiimidazole and phenols **37**, **39**, and **43**, with aqueous acetone in high yield. The *RRR* carboxamides **69**, and **73**−**78** were prepared by treating these with acids **70**, **71**, and **72** with carbonyldiimidazole followed by the required amines and separating the required *RRR* isomers.

The synthesis of an array of *ortho*-substituted 7-phenyl-2,5-diketopiperazine secondary amides is outlined in Scheme 3.

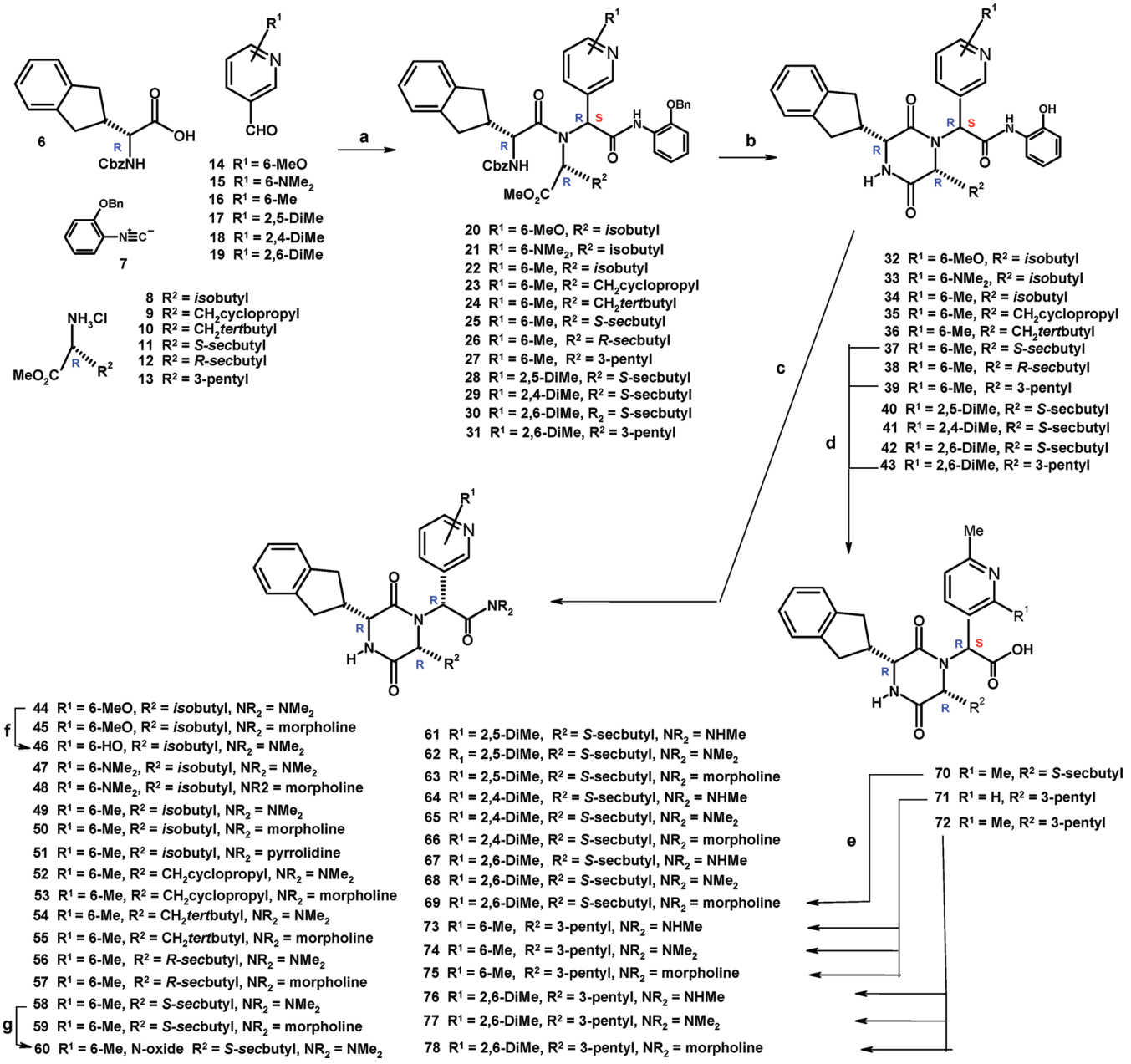
**Scheme 1**[a]




[a]Reagents and conditions: (a) $CDCl_3$, 61 °C, 3 h; (b) $Pd(OH)_2/C$, dioxane−TFA 10:1, $H_2$ (60 psi), 55 °C, 24 h; (c) dioxane−$H_2O$ 1:1, $Et_3N$, *N*-(benzyloxycarbonyloxy)succinimde, room temp, 2 days.


dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783−796

Scheme 2[a]




[a]Reagents and conditions: (a) *R*-amino methyl ester hydrochloride, methanol/trifluoroethanol (1:1), pyridyl aldehyde, $Et_3N$, room temp, 20 h; then Cbz-IndGly, 2-benzyloxyphenylisocynanide, room temp, 4 days; (b) $H_2$, Pd/C, EtOAc, AcOH, 18 h; (c) CDI, DCM, 16 h; then $R_2NH$, room temp, 16 h; (d) CDI, DCM, 20 h, then $Me_2CO$, $H^+$, $H_2O$, 20 h; (e) CDI, DCM, 18 h; then $R_2NH$, room temp, 18 h; (f) NaI, $Me_3CCOCl$, MeCN, reflux, 4 h, then $H_2O$ 10 min; (g) *m*-chloroperbenzoic acid, $CHCl_3$ room temp, 16 h.

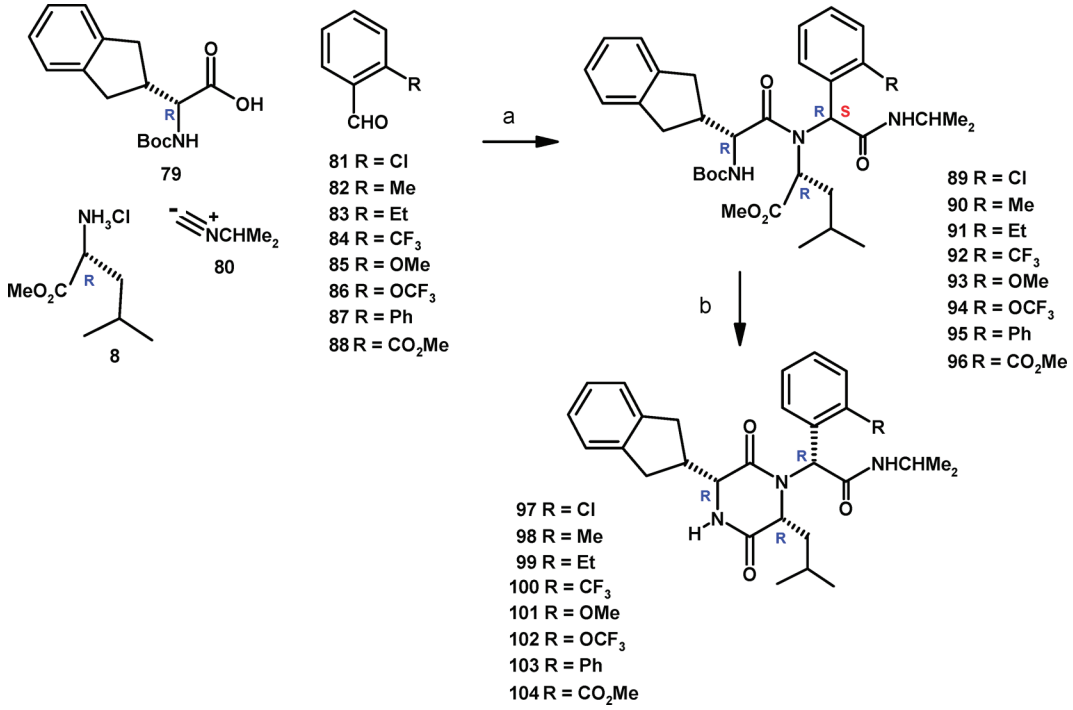
These *ortho*-substituted phenyl isopropylamides **97**−**104** were prepared by a four-component Ugi reaction where the imines formed by the initial reaction of the *ortho*-substituted aryl aldehydes **81**−**88** and amine **8** D-leucine methyl ester reacted with the isopropyl isonitrile **80** and the resulting intermediate imminium ion then further reacted with the acid **79** (boc-protected indanyl *R*-glycine), which rearranged to give the tripeptides **89**−**96**. In the second-stage deprotection of the Boc-amine in **89**−**96** with TFA followed by cyclization in the presence of $Et_3N$ gave a 1:3−2:3 mixture[9] of the *RRR*/*RRS* isomers. The *RRR* isomers **97**−**104** were isolated in ≤11% yield (see Supporting Information).

## RESULTS AND DISCUSSION

The aim was to discover a compound equally potent and more soluble than the 2,4-difluorophenyl dimethylamide **1** with acceptable pharmacokinetics in animal species and with reduced human intrinsic clearance[10] to decrease the risk of low bioavailability in humans. Pyridyl rings are known bioisosteres of fluorobenzene, so derivatives based on this ring system would be expected to be similarly active and would enable the production of more soluble OT antagonists. However, the *ortho* pyridyl derivative **105** (Table 1) was found to readily epimerize[11] at the exocyclic position due to the electron withdrawing properties of the *ortho* nitrogen atom. We reasoned that the *meta* analogues would be less susceptible

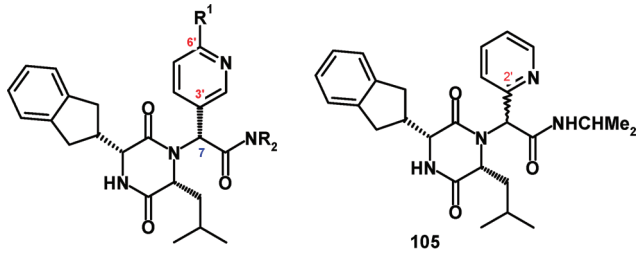

dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783−796

**Scheme 3**[a]




[a]Reagents and conditions: (a) $Et_3N$, methanol, room temp, 16 h; (b) TFA, $CH_2Cl_2$, 3 h, then $Et_3N$, room temp, 16 h.

**Table 1. Inhibition of Oxytocin Binding at the Human Oxytocin Receptors, Solubility and the Pharmacokinetic Profile of 6′-Pyridyl substituted (3*R*,6*R*,7*R*)-2,5-Diketopiperazines in the Rat and Dog**[a]




| compd | $R^1$ | $NR_2$ | hOTR $pK_i$[a] | rat PK[b] AUC po | rat PK[b] Cl | rat PK[b] $F$% | dog PK[c] AUC po | dog PK[c] Cl | dog PK[c] $F$% | sol aq[d] | CHI Log $D$[e] | Serum Al hum[f] | Serum Al rat[g] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 44 | MeO | $NMe_2$ | 9.4 | 2229 | 23 | 62 | 158 | 8 | 13 | 0.216 | 2.7 | 92 | 86 |
| 45 | MeO |  | 9.6 | 2128 | 35 | 88 | | | | 0.249 | 2.7 | 76 | 78 |
| 46 | OH | $NMe_2$ | 8.5 | | | | | | | >0.240 | 1.3 | 61 | 37 |
| 47 | $NMe_2$ | $NMe_2$ | 9.6 | 135 | | | | | | 0.233 | 2.6 | 74 | 82 |
| 48 | $NMe_2$ |  | 9.5 | 80 | | | | | | 0.235 | 2.9 | 70 | 71 |
| 49 | Me | $NMe_2$ | 8.8 | 2675 | 18 | 57 | 454 | 6 | 26 | >0.236 | 2.1 | 83 | 86 |
| 50 | Me |  | 9.4 | 811 | | | 53 | 17 | 10 | >0.253 | 2.1 | 67 | 62 |
| 51 | Me |  | 9.3 | 920 | 33 | 36 | | | | 0.241 | 2.3 | 76 | 70 |
| 1[j] | | | 10.1[h] | 2971 | 15 | 53 | 770 | 7 | 51 | 0.083 | 3.4 | 94 | 99 |

[a]Displacement of [$^3$H] oxytocin from hOTR by the test compound.[12] $pK_i$ values are reported as mean values of three or more determinations and all results were obtained in binding assays with a standard deviation in $pK_i$ of less than 0.25. [b]Rat PK ($n$ = 4): AUC (ng·h mL$^{-1}$) at 5 mg/kg, 5% DMSO/95%PEG400 formulation, Cl in mL min$^{-1}$ kg$^{-1}$. [c]Dog PK ($n$ = 3): AUC (ng·h mL$^{-1}$) at 0.6 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL min$^{-1}$ kg$^{-1}$. [d]Solubility (mg mL$^{-1}$) a precipitation/HPLC based measurement.[13] [e]An HPLC method based measurement of lipophilicity.[14] [f]% Human serum albumin binding.[15] [g]% Rat serum albumin binding.[15] [h]Value obtained for **1** when used as internal standard in OT binding assay for 6′-pyridyl 2,5-DKP's, previous value $pK_i$ = 9.2, see ref 5. [j]Previous published data see ref 5.


dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783−796

to epimerization, so the initial target became a series of 6′-substituted-3′-pyridyl derivative where the substituents were non-electron-withdrawing and the *ortho* position to the N atom was blocked to avoid potential metabolic problems (Table 1). Also, the 6′-substituents in the pyridyl derivatives are equivalent to the 4′-position in the aryl series, which is the optimal substitution position for potency in the monosubstituted phenyl derivatives.[9]

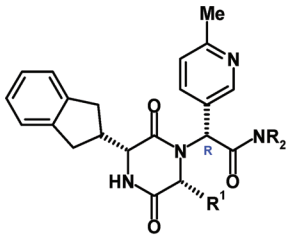
**Monosubstituted Pyridyl *iso*-Butyl Derivatives.** We initially made the dimethylamides (**44**, **47**, **49**) and morpholine amides (**45**, **48**, **50**) in the 6′-MeO, 6′-$NMe_2$, and 6′-Me-3-pyridyl series. All had better solubility, a lower log *D*, and lower protein binding to human and rat serum albumin than **1** (Table 1). All had a similar level of potency ($pK_i$ 9.6−8.8), however, the 6-hydroxy derivative **46** was less potent ($pK_i$ 8.5) and all were less potent than **1**. Both the 6′-MeO and 6′-Me derivatives (**44**, **45**) and (**49**, **50**) had better exposure in the rat than the 6′-$NMe_2$ derivatives (**47** and **48**), and the best bioavailability in the rat was achieved by **44**, **45**, and **49**. However, **44** had poor bioavailability in the dog.

The best overall pharmacokinetic profile in rat and dog was achieved by the 6′-Me-3′-pyridyl dimethylamide **49**. Nevertheless, it was 20-fold less potent than **1**. Further amide derivatives of 6′-methyl pyridyl 2,5-DKP template, the morpholine **50** and the pyrrolidinyl **51**, were inferior in terms of oral exposure in the rat compared to the dimethylamide **49**. Also, although **49** had good rat and moderate dog bioavailability with low intrinsic clearance in these species, its intrinsic clearance in cynomolgus monkey and human microsomes is high (Table 2). A similar pattern was seen with **1**. To increase the chance of good bioavailability in cynomolgus monkey and humans, a decrease in intrinsic clearance in microsomes of these species was sought.

**Modification of *iso*-Butyl in 6′-Me-Pyridyl Derivatives.** In an attempt to improve potency and intrinsic clearance in cynomolgus monkey and human microsomes, we then investigated modification of the 3-isobutyl group (Table 2). As expected, all maintained the improved solubility compared to **1**. The cyclopropylmethyl dimethylamide **52** and morpholine derivative **53** were 3 and 10 times less potent than the corresponding isobutyl derivative **49** and **50** (Table 1). In contrast, both the tertiarybutylmethyl derivatives **54** and **55** had a better potency than **49**; however, they had a worst oral exposure in the rat than **49** although they had a better intrinsic clearance in human microsomes than **49**.

In the natural ligand oxytocin, the 3-Ile is one of the key amino acids required for agonist potency and we have rationalized from extensive SAR[6,9] that the 3-isobutyl group in our 2,5-diketopiperazine oxytocin antagonists mimics the 3-Ile interaction of the natural ligand. However, the alpha group in the 3-Ile amino acid of oxytocin is an *S-sec*-butyl while the 3-isobutyl group in **49** is achiral. By changing the 3-isobutyl group in **49** for a chiral *sec*-butyl seen in the natural ligand, it was hoped that a better fit with the receptor could be achieved

**Table 2. Pharmacokinetic Profile of 6′Me-3-Pyridyl Substituted (3*R*,6*R*,7*R*)-2,5-Diketopiperazines in the Rat and Dog[a]**




isobutyl c-$PrCH_2$ t-$BuCH_2$ sec-*R*Bu sec-*S*Bu

| compd | $R^1$ | $NR_2$ | hOTR | intrinsic Cl[b] | | | | rat PK[c] | | | dog PK[d] | | | sol aq[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $pK_i$[a] | rat | dog | cyno monkey | human | AUC po | Cl | *F*% | AUC po | Cl | *F*% | |
| **49** | isoBu | $NMe_2$ | 8.8 | 3 | 3 | 21 | 11 | 2675 | 18 | 57 | 454 | 6 | 26 | >0.236 |
| **52** | c-$PrCH_2$ | $NMe_2$ | 8.3 | | | | | | | | | | | 0.230 |
| **53** | c-$PrCH_2$ | −N◯O | 8.3 | | | | | | | | | | | >0.258 |
| **54** | t-$BuCH_2$ | $NMe_2$ | 10.2 | 3 | 1 | 8 | 6 | 618 | | | | | | >0.242 |
| **55** | t-$BuCH_2$ | −N◯O | 10.1 | 1 | 9 | 21 | 4 | 770 | 20 | 18 | 21 | 24 | 5 | 0.213 |
| **56** | sec-*R*Bu | $NMe_2$ | 9.0 | 1 | 1 | 11 | 3 | 3355 | 23 | 82 | | | | >0.237 |
| **58** | sec-*S*Bu | $NMe_2$ | 9.7 | 1 | 1 | 9 | 2 | 2270 | 19 | 62 | 347 | 10 | 39 | >0.254 |
| **57** | sec-*R*Bu | −N◯O | 9.3 | 1 | 2 | 8 | 4 | 1590 | 39 | 44 | 138 | 20 | 31 | >0.280 |
| **59** | sec-*S*Bu | −N◯O | 9.8 | 1 | 2 | 6 | 3 | 1450 | 27 | 54 | 55 | 23 | 14 | >0.268 |
| **60** | sec-*S*Bu | $NMe_2$N -oxide | 9.0 | 1 | - | 1 | 1 | 24 | 28 | <1 | 73 | 14 | 11 | >0.242 |
| **1** | | | 10.1[f] | 2 | 7 | 22 | 14 | 2971[g] | 15[g] | 53[g] | 770[g] | 7[g] | 51[g] | 0.083[g] |

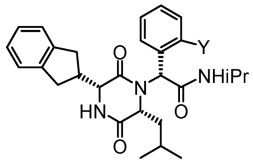
[a]Displacement of [$^3$H] oxytocin from hOTR by the test compound.[12] $pK_i$ values are reported as mean values of three or more determinations, and all results were obtained in binding assays with a standard deviation in $pK_i$ of less than 0.25. [b]Microsomal intrinsic clearance (mL $min^{-1}$ g $liver^{-1}$).[16] [c]Rat PK ($n$ = 4): AUC (ng·h $mL^{-1}$) at 5 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL $min^{-1}$ $kg^{-1}$. [d]Dog PK ($n$ = 3): AUC (ng·h $mL^{-1}$) at 0.6 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL $min^{-1}$ $kg^{-1}$. [e]Solubility (mg $mL^{-1}$) a precipitation/HPLC based measurement.[13] [f]Value obtained for **1** when used as internal standard in OT binding assay for 6′-pyridyl 2,5-DKP's, previous value $pK_i$ = 9.2, see ref 5. [g]Previous published data see ref 5.


dx.doi.org/10.1021/jm201287w | *J. Med. Chem.* 2012, 55, 783−796

and a subsequent increase in potency. The *R-sec*-butyl and the *S-sec*-butyl derivatives of **49** were prepared. Both the *S-sec*-butyl dimethylamide **58** and morpholine amide **59** were more potent than the corresponding amides **56** and **57** in the *R-sec*-butyl series (Table 2) and more potent than the 3-isobutyl dimethylamide **49**. These four derivatives also had a better intrinsic clearance in human microsomes than **49**. The *N*-oxide **60** of the *S-sec*-butyl dimethylamide **58** was 5-fold less active than the parent **58**, and it had a much lower oral exposure in the rat than **58**. Of these four derivatives, the *S-sec*-butyl dimethylamide **58** had the better pharmacokinetic profile across both species and had better bioavailability in the dog than **49** (Table 2). Also, **58** had a better intrinsic clearance in cynomolgus monkey and human microsomes than the 3-isobutyl dimethylamide **49** and was better across all four species than **1**. Although **58** was greater in its in vitro potency than **49**, it was however still less potent than **1**. The aim was to try and maintain the good in vitro and in vivo pharmacokinetic profile and solubility shown by **58** and increase potency further. Two ways forward were envisaged.

**Dimethyl-Pyridyl *S-sec*-Butyl Amides.** One was to further explore the group at the 3-position and the other was to modify the pyridine ring. Further substitution of the 3′-pyridyl ring to give disubstituted derivatives by maintaining the 6′methyl group and substituting the 2′, 4′, or 5′ position was considered. Although the effect of *ortho* F substitution on the 4′-F-phenyl ring to give 2′,4′-di-F-phenyl ring derivatives (e.g., **1**) was previously shown[9] to be an advantage in terms of potency and rat PK, no systematic investigation of substitution at the *ortho* position on potency had ever been carried out. Therefore, the effect of *ortho* substitution on the aromatic group was investigated first with the hope that this would be transferable to the pyridine system. An array of 7-phenyl substituted (3*R*,6*R*,7*R*)-2,5-diketopiperazines containing *ortho*-substituted phenyl groups were prepared (Table 3).

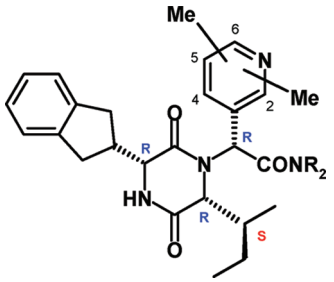
**Table 3. Inhibition of Oxytocin Binding at the Human Oxytocin Receptors by 2′ Phenyl Substituted (3*R*,6*R*,7*R*)-2,5-Diketopiperazines**




| compd | Y | fp$K_i$ [a] | compd | Y | fp$K_i$ [a] |
|---|---|---|---|---|---|
| **97** | Cl | **8.4** | **101** | OMe | **7.5** |
| **98** | Me | **8.1** | **102** | $OCF_3$ | **7.4** |
| **99** | Et | **7.8** | **103** | Ph | **7.4** |
| **100** | $CF_3$ | **7.5** | **104** | $CO_2Me$ | **6.8** |

[a]Functional OT antagonist activity measured by FLIPR assay, see ref 5.

**Table 4. Inhibition of Oxytocin Binding at the Human Oxytocin Receptor and the Pharmacokinetic profile of *S-sec*-Butyl Dimethyl Substituted Pyridyl (3*R*,6*R*,7*R*)-2,5-Diketopiperazines in the Rat and Dog[a]**




| compd | pyridyl substituents | $NR_2$ | hOTR | intrinsic Cl [b] | | | | rat PK [c] | | | dog PK [d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | p$K_i$ [a] | rat | dog | cyno monkey | human | AUC po | Cl | *F* % | AUC po | Cl | *F* % |
| **58** | 6-Me | $NMe_2$ | 9.7 | 1 | 1 | 9 | 2 | 2270 | 19 | 62 | 347 | 10 | 39 |
| **61** | 2,5-DiMe | NHMe | 8.9 | 1 | 3 | 5 | 3 | | | | | | |
| **62** | 2,5-DiMe | $NMe_2$ | 9.1 | 1 | 2 | 13 | 5 | 1369 | | | | | |
| **63** | 2,5-DiMe | –N○ | 9.1 | | | | | | | | | | |
| **64** | 4,6-DiMe | NHMe | 10.2 | 1 | 4 | 20 | 7 | 407 | 23 | 11 | | | |
| **65** | 4,6-DiMe | $NMe_2$ | 10.4 | 1 | 1 | 32 | 9 | 2685 | | | 140 | 12 | 17 |
| **66** | 4,6-DiMe | –N○ | 10.3 | 1 | 4 | 16 | 1 | 1112 | | | | | |
| **67** | 2,6-DiMe | NHMe | 9.4 | 1 | 5 | 6 | 5 | 1730 | 25 | 51 | 58 | 22 | 13 |
| **68** | 2,6-DiMe | $NMe_2$ | 10.1 | 1 | 1 | 14 | 7 | 4725 | 15 | 84 | 305 | 14 | 44 |
| **69** | 2,6-DiMe | –N○ | 9.9 | <1 | <1 | 4 | 5 | 3450 | 13 | 55 | 69 | 19 | 13 |

[a]Displacement of [$^3$H] oxytocin from hOTR by the test compound.[12] p$K_i$ values are reported as mean values of three or more determinations and all results were obtained in binding assays with a standard deviation in p$K_i$ of less than 0.25. [b]Microsomal intrinsic clearance (mL min$^{-1}$ g liver$^{-1}$).[16] [c]Rat PK ($n$ = 4): AUC (ng·h mL$^{-1}$) at 5 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL min$^{-1}$ kg$^{-1}$. [d]Dog PK ($n$ = 3): AUC (ng·h mL$^{-1}$) at 0.6 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL min$^{-1}$ kg$^{-1}$.


dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783–796

These were all in the 3-isobutyl/isopropyl amide series due to ease of synthesis and availability of starting material. Those compounds with the highest potency were the chloro **97** and methyl **98** derivatives (Table 3). Because of the activity of Cl at the 2-position in a pyridine ring, a series of dimethyl pyridine templates were prepared with a methyl group in the *ortho* position. These were the 2′,6′-dimethyl, the 4′,6′-dimethyl, and the 2′,5′-dimethyl pyridines (Table 4).

The impact of the *ortho* methyl group on potency was achieved with the 2′,6′-dimethyl pyridines (**68** and **69**) and 4′,6′-dimethyl pyridines (**64**, **65**, and **66**) but not with the 2′,5′-dimethyl pyridines (**61**, **62**, and **63**), which were less active than the 6′-methyl pyridines **58** and **59**. This confirms that disubstitution *para* and *ortho* is preferred. The increase in potency seen with the 4′,6′-dimethyl and 2′,6′-dimethyl pyridine series indicates that the *ortho* methyl group puts the 6′-methylpyridine ring in a conformation that makes this template have a more favorable interaction at the oxytocin receptor. We had previously rationalized from detailed SAR[6] that the *R* aryl ring in the exocyclic aryl amide restricts the conformational mobility of the leucine (3-isobutyl) group, which facilitates its interaction at the oxytocin receptor. Hence the *ortho* methyl group in the 6′-methylpyridine ring which increases potency probably augments this interaction at the receptor.

The 2′,5′-dimethylpyridine dimethylamide **62** also had an inferior oral exposure in the rat than the corresponding dimethylamides **68** and **65** in the other two series. A comparison of the pharmacokinetic profile of the 4′,6′-dimethyl and 2′,6′-dimethyl pyridines (Table 4) showed that the monomethylamide **67** and the dimethylamide **68** in the 2′,6′-dimethyl series had a better oral exposure in the rat than the corresponding analogues **64** and **65** in the 4′,6′-dimethyl series. Also, the intrinsic clearance against the microsomes of all four species for the 2′,6′-dimethyl pyridines was low (in single figures) and generally better than that of the 4′,6′-dimethyl pyridines. Within the 2′,6′-dimethyl series, the secondary amide **67** and both the tertiary amides **68** and **69** had good pharmacokinetic profiles in the rat, all with bioavailability >50%. Both 2′,6′-dimethyl pyridines **68** and **69** were more potent in vitro than the 6′-methyl pyridine **58**, and both have a superior oral exposure in the rat than **58**. The dimethylamide **68** had the best pharmacokinetic profile in the rat and the dog.

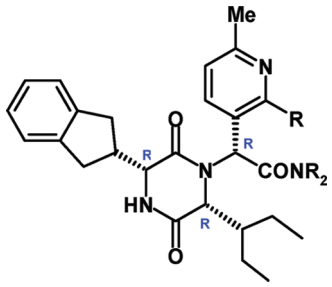
**Mono and Di-Me Pyridyl 3-Pentyl Derivatives.** The other way forward to improve potency was to further explore the group at the 3-position. The *S-sec*-butyl derivatives (**58** and **59**) had increased potency over the isobutyl derivative **49**, while the *R-sec*-butyl derivatives (**56** and **57**) had a more comparable potency to the latter (Table 2).

This was taken further by combining the structural elements of each of these in the more bulky 3-pentyl derivatives (Table 5). All the 3′-pyridine-2,5-diketopiperazine-3-pentyl tertiary amide derivatives had increased potency over **68**, and all had good oral exposure in the rat. However, apart from secondary amide **73**, all had an inferior intrinsic clearance in human microsomes and a lower oral exposure in the rat than **68**. The methylamide **73** had the best intrinsic clearance in this series and a good pharmacokinetic profile in the rat and the dog. However, its overall profile was slightly inferior to that of the *S-sec*-butyl-2′,6′-dimethyl pyridine dimethylamide **68**.

**Requirements for Further Progression of a Oxytocin Antagonist Drug.** The desirable features that an oral oxytocin drug should also display are the following:

(1) Sufficient pharmacokinetics in the cynomolgus monkey to enable preterm labor to be evaluated.
(2) High OT antagonist potency and good selectivity vs human vasopressin receptors.
(3) High in vivo OT antagonist potency with no significant P450 inhibition.
(4) Minimal adverse effects.
(5) Ease of synthesis and formulation.

**Table 5. Inhibition of Oxytocin Binding at the Human Oxytocin Receptor and the Pharmacokinetic Profile of 3-Pentyl Substituted Pyridyl (3*R*,6*R*,7*R*)-2,5-Diketopiperazines in the Rat and Dog[a]**

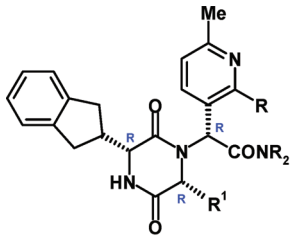



| compd | R | $NR_2$ | hOTR | intrinsic Cl[b] | | | | rat PK[c] | | | dog PK[d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $pK_i$[a] | rat | dog | cyno monkey | human | AUC po | Cl | *F* % | AUC po | Cl | *F* % |
| 73 | H | NHMe | 10.0 | 1 | 2 | 8 | 4 | 1470 | 17 | 28 | 173 | 22 | 38 |
| 74 | H | $NMe_2$ | 10.3 | 2 | 1 | 12 | 11 | 1810 | 20 | 36 | 255 | 19 | 47 |
| 75 | H |  | 10.6 | 1 | 5 | 18 | 12 | 1383 | 23 | 55 | | | |
| 76 | Me | NHMe | 10.3 | 2 | | 31 | | 1245 | | | | | |
| 77 | Me | $NMe_2$ | 10.5 | 3 | 1 | 29 | 18 | | | | | | |
| 78 | Me |  | 10.5 | 1 | 1 | 14 | 11 | 2326 | 15 | 42 | | | |

[a]Displacement of [$^3$H] oxytocin from hOTR by the test compound.[12] $pK_i$ values are reported as mean values of three or more determinations and all results were obtained in binding assays with a standard deviation in $pK_i$ of less than 0.25. [b]Microsomal intrinsic clearance (mL/min/g liver).[16] [c]Rat PK ($n$ = 4): AUC (ng·h mL$^{-1}$) at 5 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL min$^{-1}$ kg$^{-1}$. [d]Dog PK ($n$ = 3): AUC (ng·h mL$^{-1}$) at 0.6 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL min$^{-1}$ kg$^{-1}$.–


dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783–796

**Table 6. Inhibition of Oxytocin Binding at the Human Oxytocin Receptors and the Pharmacokinetic Profile of *S*-*sec*-Butyl and 3-Pentyl Pyridyl (3*R*,6*R*,7*R*)-2,5-Diketopiperazines in the Rat and Cynomolgus Monkey[a]**



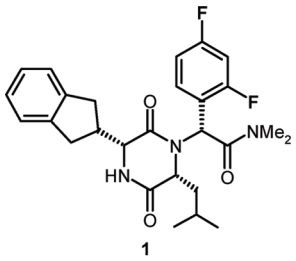

3-pentyl


sec-SBu

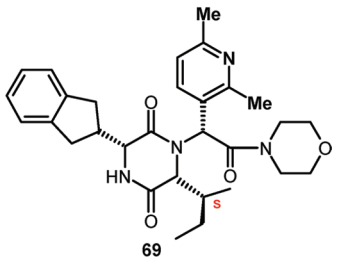
| compd | R | $R^1$ | $NR_2$ | hOTR | intrinsic Cl[b] | | | | rat PK[c] | | | monkey PK[d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $pK_i$[a] | rat | dog | cyno monkey | human | AUC po | Cl | *F*% | AUC po | Cl | *F*% |
| **1** | | | | 10.1 | 2 | 7 | 22 | 14 | 2971[e] | 15[e] | 53[e] | 16 | 24 | 1.5 |
| **58** | H | sec-*S*Bu | $NMe_2$ | 9.7 | 1 | 1 | 9 | 2 | 2270 | 19 | 62 | | | |
| **68** | Me | sec-*S*Bu | $NMe_2$ | 10.1 | 1 | 1 | 14 | 7 | 4725 | 15 | 84 | 17 | 20 | 1 |
| **69** | Me | sec-*S*Bu |  | 9.9 | <1 | <1 | 4 | 5 | 3450 | 13 | 55 | 116 | 26 | 7-10 |
| **73** | H | 3-pentyl | NHMe | 10.0 | 1 | 2 | 8 | 4 | 1470 | 17 | 28 | 29 | 24 | 4 |
| **78** | Me | 3-pentyl |  | 10.5 | 1 | 1 | 14 | 11 | 2326 | 15 | 42 | 11 | nd | nd |

[a]Displacement of [$^3$H] oxytocin from hOTR by the test compound.[12] $pK_i$ values are reported as mean values of three or more determinations and all results were obtained in binding assays with a standard deviation in $pK_i$ of less than 0.25. [b]Microsomal intrinsic clearance (mL min$^{-1}$ g liver$^{-1}$).[16] [c]Rat PK ($n$ = 4): AUC (ng·h mL$^{-1}$) at 5 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL min$^{-1}$ kg$^{-1}$. [d]Cynomolgus monkey PK ($n$ = 4): AUC (ng·h mL$^{-1}$) besylate salt formulated in 0.5% methylcellulose/0.1% Tween 80 in water and administered as a solution at nominal dose of 2 mg/kg free base, Cl in mL min$^{-1}$ kg$^{-1}$. [e]Previous published data see ref 5.

**Table 7. Potency of 2,5-Diketopiperazines 1, 69, and Atosiban at the Human Oxytocin Receptor Compared with the Efficacy Obtained in the Rat Uterine Contractility Model and Inhibition of OT Binding at the Human OT (hOT) and Vasopressin Binding at the Human (V1a, V1b, and V2) Receptors[a]**




1




69

| compd | oxytocin receptor | vasopressin receptors | | | oxytocin antagonism | sol aq[c] | CHI Log *D*[d] | human serum Al[e] % |
|---|---|---|---|---|---|---|---|---|
| | hOT | hVIa | hVIb | hV2 | in-vivo rat $IC_{50}$ nM[b] | | | |
| | $pK_i$[a] | $pK_i$[a] | $pK_i$[a] | $pK_i$[a] | | | | |
| **1** | 10.1 | 6.2 | <4.9 | 7.4 | 227[f] | 0.083[f] | 3.4[f] | 94[f] |
| **69** | 9.9 | <5.2 | <5.1 | 5.4 | 192 | 0.232 | 2.2 | 70 |
| atosiban[g] | 7.9 | 9.8 | 7.4 | 6.5 | ~186 | | | |

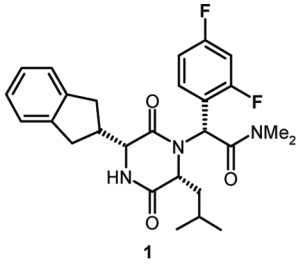
[a]Displacement of [$^3$H] oxytocin from hOTR or vasopressin from hV1aR, hV1bR, and hV2R by the test compound.[12] $pK_i$ values are reported as mean values of three or more determinations and all results were obtained in binding assays with a standard deviation in $pK_i$ of less than 0.25, see ref 5. [b]Plasma $IC_{50}$.[17] [c]Solubility (mg mL$^{-1}$) a precipitation/HPLC based measurement.[13] [d]An HPLC method based measurement of lipophilicity.[14] [e]% Human serum albumin binding.[15] [f]Previous published data, see ref 5. [g]Previous published data, see ref 22.

*Requirement 1: Sufficient Pharmacokinetics in the Cynomolgus Monkey.* Because preterm labor would be evaluated in the cynomolgus monkey post candidate selection, pharmacokinetics in this species was considered as a requisite for the progression of lead compounds. Of the five lead 7-(3′-pyridyl)-2,5-diketopiperazines **58**, **68**, **69**, **73**, and **78**, the 2′,6′-dimethyl-3′-pyridines morpholine amide **69** had the best intrinsic clearance in the three animal species and the best oral exposure and bioavailability in the cynomolgus monkey (Table 6). Compound **69** was also better than **1** in its oral exposure and bioavailability in the cynomolgus monkey, with low microsomal intrinsic clearance in all four species (Table 6), and a lower log *D*, lower binding to human serum albumin, and a better solubility than **1** (Table 7).

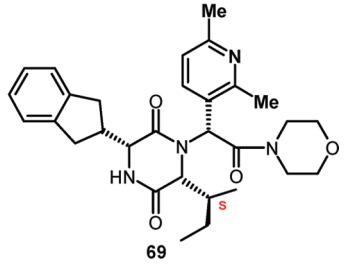
*Requirement 2: High Oxytocin Antagonist Potency and Selectivity vs Human Vasopressin Receptors.* A measure of the potency of our lead OT antagonist **69** compared to the previous lead **1** was established by their binding inhibition data


dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783–796

Table 8. Cyp450[18] Inhibition and Microsomal Intrinsic Clearance[16] Profile of 2,5-Diketopiperazines 1 and 69




1




69

| property | 1 [c] | 69 |
|---|---|---|
| **Cyp450** [a] | | |
| 1A2 | >100 | >100 |
| 2C9 | 12 | >100 |
| 2C19 | 38 | >100 |
| 2D6 | 66 | >100 |
| 3A4 DEF [b] | 5 | >100 |
| 3A4 7BQ [b] | 31 | >100 |
| **Intrinsic CL** [d] | | |
| rat | 2 | <1 |
| dog | 7 | <1 |
| cyno monkey | 22 | 4 |
| human | 14 | 5 |

[a] $IC_{50}$ μM Cypex.[18] [b] Diethoxyfluorescein (DEF) and 7-benzyloxyquinoline (7BQ) are fluorogenic substrates for Cyp3A4. [c] Previous published data, see ref 6. [d] Microsomal intrinsic clearance (mL $min^{-1}$ g $liver^{-1}$).[16]

($pK_i$) against the isolated recombinant human oxytocin receptors (Table 7). The morpholine amide **69** has comparable activity to the previous lead **1** in vitro at the human oxytocin receptor in the binding assay. Selectivity relative to human vasopressin receptors for **69** and **1** (Table 7) has been established by measuring their $pK_i$ against hV1aR, hV2R, and hV1bR.

Both OT antagonists, the dimethylamide **1** and the morpholine amide **69** (Table 7) are >500-fold selective for the hOTR relative to all three vasopressin receptors hV1aR, hV2R, and hV1bR. The morpholine amide **69** was more selective than the dimethylamide **1** against the human vasopressin receptors hV1aR and hV2R and was also >50000-fold, >63000-fold, and >31000-fold selective for the human OT receptor relative to the three vasopressin receptors hV1aR, hV1bR, and hV2R.

*Requirement 3: High In Vivo Potency and No Significant Cyp 450 Inhibition.* The in vivo efficacy of the most promising compound **69** was estimated in an anaesthetized rat model (see ref 5), where uterine contractions were elicited by iv administration of oxytocin. The reduction in uterine contractility was measured after subsequent iv administrations of increasing doses of **69** (0.1, 0.3, and 1 mg/kg iv). The method used for the measurement of in vivo activity was the plasma $IC_{50}$ determination[17] [the concentration that causes 50% inhibition of response to the standard OT dose (0.3 ug/kg iv)]. The in vivo comparison of the lead **69**, and previous lead **1** is outlined in Table 7. The morpholine amide **69** has comparable potency ($IC_{50}$ = 192 nM) to the previous lead **1** and atosiban (a marketed iv, peptide OT antagonist) in vivo in the rat. In contrast, **69** is 100-fold more potent than atosiban at the human receptor in vitro.

*Cyp 450 Inhibition.* Additional predevelopment studies revealed that **1** inhibited several P450 isozymes, which was not the case with **69** (Table 8). Also no time-dependent inhibition[19] was observed for **69** against these five P450 isozymes. The 2′,6′-dimethyl-3-pyridyl morpholine amide Epelsiban **69** was therefore chosen as the compound which fulfilled the selection criteria for further development.

*Requirement 4: Minimal Adverse Effects.* The 2′,6′-dimethyl-3-pyridyl morpholine amide **69** was shown not to be mutagenic in vitro in both the bacterial (HTFT/mini-Ames assay)[20] and mammalian cell (mouse lymphoma assay)[21]genotoxicity screens. A seven-day oral safety assessment study was carried out with **69** in female Sprague−Dawley rats at doses suspended in 0.5% (w/w) methylcellulose/0.1% (w/w) Tween 80 in sterile water. There was no effect on body weight or food consumption, no adverse clinical signs, and histological findings did not reveal any treatment-related effects at doses up to 100 mg/kg/day. The coverage in toxicity studies based on the minimum effective dose (MED) allowed the further progression of **69**.

*Requirement 5: Ease of Synthesis and Formulation.* The 6*R*(indanyl),3*R*(*S*-*sec*-butyl),7*R*(2′,6′-dimethyl-3′-pyridyl)-2,5-diketopiperazine morpholine amide **69** can be synthesized in six high-yield reaction steps from indene. The 2,5-diketopiperazine **69** is a crystalline powder mp 140 °C (2.5% heptane) with molecular mass of 518.7 Da, and a white crystalline besylate salt **69B** mp 179−183 °C with molecular mass of 676.8 Da has been identified. The compound is ionizable in aqueous solution ($pK_a$ = 5.78) and is moderately lipophilic (log *D* = 2.55 at pH 7.4). Potential advantages over **106** include greater solubility 33 mg/mL in water at 35 °C. No significant change in terms of assay, impurity profiles, and appearance was observed when the solid-state stability of **69B** was investigated for up to 3 months at 50 °C at ambient room humidity. Also, **69B** is chemically stable in aqueous solutions covering the pH range 2−6 and when exposed to light at room temperature for up to 7 days. The oral bioavailability of at 5 mg/kg formulated in aqueous solution in the rat was acceptable (62%), and exposure was approximately proportional when administered in the same formulation at 100 mg/kg (7-day rat toxicity study). The good aqueous solubility may offer more solution formulation options, and formulation in solid form would not be expected to result in any substantial loss of bioavailability compared to formulations in aqueous solution.

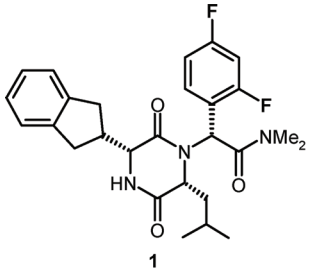
**Comparison of Lead Compounds.** Although the oxytocin antagonist Epelsiban **69** is comparable in potency to **1** in vitro and in vivo, it is more selective against the human vasopressin receptors hV1aR and hV2R and has better aqueous solubility (Table 9). Compared to **1**, Epelsiban **69** has a better P450 profile with no significant inhibition $IC_{50}$ > 100 μM against five P450 isozymes and a better microsomal intrinsic clearance profile, which was low in all four species and especially in the cynomolgus monkey and human microsomes (Table 8). Also, it has better pharmacokinetics than **1** in the cynomolgus monkey (Table 9). In contrast, it is comparable to Retosiban **106**[6] in terms of its pharmacokinetic profile in the rat and cynomolgus monkey, and in its intrinsic clearance in cynomlogus monkey and human microsomes, but is 5-fold more potent against the OT receptor, is more selective against the human vasopressin receptors, especially hV2, and is more soluble in its salt form (Table 9).

## CONCLUSION

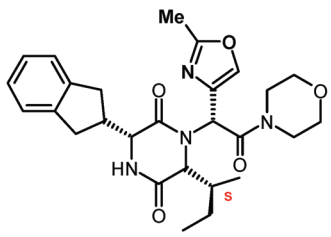
We have shortened the synthesis of indanyl (3*R*,6*R*,7*R*)-2,5-diketopiperazine OT antagonists to six stages by reducing the five-stage synthesis of the *R*-indanylglycine to two stages from indene. SAR studies have shown that increased aqueous solubility was possible with a range of 6′-substituted 7-(3′-pyridyl)-(3*R*,6*R*,7*R*)-2,5-diketopiperazine amides, and the


dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783−796

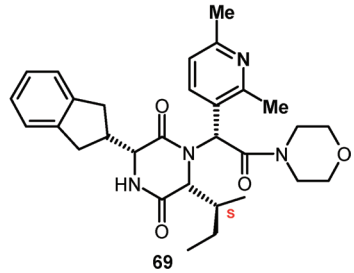
**Table 9. Comparison of the Oxytocin Antagonist Potency, Selectivity vs the Human Vasopressin Receptors, Intrinsic Clearance in Two Species, and Pharmacokinetic Profile in Rat and Cynomolgus Monkey for 1, 106, 69**




1




106




69

| compd | hOTR p$K_i$[a] | hVIa p$K_i$[a] | hVIb p$K_i$[a] | hV2 p$K_i$[a] | $IC_{50}$[b] nM | intrinsic Cl[c] cy | intrinsic Cl[c] hu | rat PK[d] AUC po | rat PK[d] Cl | rat PK[d] $t_{1/2}$ | rat PK[d] $F$% | monkey PK[e] AUC po | monkey PK[e] Cl | monkey PK[e] $F$% | sol aq[g] | CHI Log $D$[i] | HSA[j] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 10.1 | 6.2 | <4.9 | 7.4 | 227[k] | 22 | 14 | 2971[k] | 15[k] | 1.1[k] | 53[k] | 16 | 24 | 1.5 | 0.08[k] | 3.4[k] | 94[k] |
| **106** | 9.2[l] | <4.9[l] | <5.0[l] | 6.0[l] | 180[l] | 6 | 3 | 5465[l] | 19[l] | 1.4[l] | ~100[l] | 45-131 | 26 | 10 | >0.22[l] | 2.2[l] | <80[l] |
| **69** | 9.9 | <5.2 | <5.1 | 5.4 | 192 | 4 | 5 | 3450 | 13 | 1.2 | 55 | 116 | 26 | 7-10[f] | 33[h] | 2.2 | 70 |
| **atosiban** | 7.9[l] | 9.8[l] | 7.4[l] | 6.5[l] | ~186[l] | | | | | | | | | | | | |

[a]Displacement of [$^3$H] oxytocin from hOTR or vasopressin from hV1aR, hV1bR, and hV2R by the test compound.[12] p$K_i$ values are reported as mean values of three or more determinations, and all results were obtained in binding assays with a standard deviation in p$K_i$ of less than 0.25, see ref 5. [b]Plasma $IC_{50}$ nM in the rat.[17] [c]Microsomal intrinsic clearance (mL min$^{-1}$ g liver$^{-1}$) (cy = cynomolgus monkey, hu = human).[16] [d]Rat PK ($n$ = 4): AUC (ng·h mL$^{-1}$) at 5 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL min$^{-1}$ kg$^{-1}$; $t_{1/2}$ in h. [e]Cynomolgus monkey PK ($n$ = 3): AUC (ng·h mL$^{-1}$) administered as a solution at nominal dose of 2.0 mg/kg in 1% DMSO and 6% hydroxypropyl $\beta$ cyclodextrin (Cavitron) in water (pH 3.5−4). [f]Cynomolgus monkey PK ($n$ = 4): AUC (ng·h mL$^{-1}$) besylate salt formulated in 0.5% methylcellulose/0.1% Tween 80 in water and administered as a solution at nominal dose of 2 mg/kg free base, Cl in mL min$^{-1}$ kg. [g]Solubility (mg mL$^{-1}$), a precipitation/HPLC based measurement.[13] [h]Solubility in water for besylate salt. [i]An HPLC method based measurement of lipophilicity.[14] [j]% Human serum albumin binding.[15] [k]Previous published data, see ref 5. [l]Previous published data, see ref 22.

6′-methyl-3′-pyridyl dimethylamide derivative **49** was the best in terms of PK in the rat and dog. Also variation of the isobutyl group showed that the 6-methyl *R-sec*-butyl derivative **58** maintained good rat and dog pharmacokinetics and had good intrinsic clearance in cynomolgus monkey and human microsomes. Substitution at the *ortho* position of the 7-phenyl ring of (3*R*,6*R*,7*R*)-2,5-diketopiperazines with a range of groups has shown that the chloro and methyl groups were the best at increasing potency. Substitution at the *ortho*-position of the 7-(3′-pyridyl)-(3*R*,6*R*,7*R*)-2,5-diketopiperazine gave the 2′,6′, the 4′,6′, and the 2′,5′-dimethyl-3-pyridyl-2,5-diketopiperazine templates of which the 2′,6′-dimethyl-3′-pyridyl derivatives **68** and **69** were best in terms of potency and pharmacokinetic profile in the rat. Although further modification of the *S-sec*-butyl group of the 2′,6′-dimethyl-3′-pyridyl 2,5-diketopiperazine template to the 3-pentyl analogues gave a further increase in potency, these analogues were worse in terms of pharmacokinetics especially in the cynomolgus monkey. The 2′,6′-dimethyl-3′-pyridyl morpholine amide Epelsiban **69** had low intrinsic clearance in all four species, a good pharmacokinetic profile in the rat with a bioavailability of 55%, and the best oral exposure and bioavailability in the cynomolgus monkey. Evaluation in vitro has shown that Epelsiban **69** is 100-fold more potent at the hOTR than atosiban (a marketed iv peptide OT antagonist) with comparable potency to the latter in vivo in the rat. Also, **69** is >31000-fold selective versus the human vasopressin receptors (V1aR, V2R, and V1bR) and has a good P450 profile with no significant inhibition $IC_{50}$ > 100 $\mu$M and had good aqueous solubility. In addition, it was negative in the genotoxicity screens and had a satisfactory safety profile in the seven-day oral toxicity test in female rats.

## EXPERIMENTAL PROCEDURES

**General Procedures.** Melting points were obtained using an Electrothermal digital melting point apparatus and are uncorrected. Purification using silica or basic alumina cartridges refers to chromatography carried out using a Combiflash Companion with Redisep cartridges supplied by Presearch. Hydrophobic frits refer to filtration tubes sold by Whatman. SPE (solid phase extraction) refers to the use of cartridges sold by International Sorbent Technology Ltd. All purifications by flash chromatography were performed using Kieselgel 60, Merck 9385 silica gel. Preparative plate chromatography were performed using WhatmanPK6F silica gel 60A plates eluting with ethyl acetate-cyclohexane or 2-propanol−dichloromethane mixtures. Monitoring of reactions by TLC used Merck 60 $F_{254}$ silica gel glass backed plates (5 cm × 10 cm), eluted with mixtures of ethyl acetate and cyclohexane, and visualized by UV light, followed by heating with aqueous phosphomolybdic acid. Purity for final compounds was greater than 95% and was measured using combustion analysis or analytical HPLC conducted on a Supelcosil LCABZ+PLUS column (3.3 cm × 4.6 mm ID), eluting with 0.1% $HCO_2H$ and 0.01 M ammonium acetate in water (solvent A) and 0.05% $HCO_2H$ 5% water in acetonitrile (solvent B), using the either elution gradient 1, 0−0.7 min 0%B, 0.7−4.2 min 0−100%B, 4.2−5.3 min 100%B, 5.3−5.5 min 0%B, or elution gradient 2, 0−0.7 min 0%B, 0.7−4.2 min 0−100%B, 4.2−4.6 min 100%B, 4.6−4.8 min 0%B at a flow rate of 3 mL/min. The mass spectra (MS) were recorded on a Waters ZQ 2000 mass spectrometer using electrospray positive [(ES + ve to give $MH^+$ and $M(NH_4)^+$ molecular ions] or electrospray negative [(ES − ve to give $(M - H)^-$ molecular ion] modes. $^1$H NMR spectra were recorded using a Bruker DPX 400 MHz spectrometer using tetramethylsilane as the external standard. CD spectra were recorded in acetonitrile on a Jasco J-720A spectropolarimeter. Optical rotations were taken with a Perkin-Elmer model 241 polarimeter. Enantiomeric excess (% ee) was determined by chiral HPLC analysis using a Chiracel OJ or Chiral Pak AD464 column with a UV detector $\lambda$ = 215 nm and with eluents and flow rates as indicated in each case. Final organic solutions were dried


dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783−796

over $MgSO_4$ before filtration and evaporation using a Buchi Rotavapor. Ambient temperature was 20 °C. All solvents used were Fisons analytical reagents except for Pentane (Aldrich Chemical Co.) and anhydrous THF (Fluka sureseal). All other reagents were usually obtained from Aldrich, Fluka, or Lancaster. Elemental microanalyses were determined by the Microanalytical Laboratory, GlaxoSmithKline Stevenage.

All animal studies were ethically reviewed and carried out in accordance with the Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare, and Treatment of Laboratory Animals.

**(4*R*,6a*R*,11a*S*,11b*R*)-4-Phenyl-3,4,6a,11,11a,11b-hexahydro-1*H*-indeno[2′,1′:4,5]isoxazolo[3,2-*c*][1,4]oxazin-1-one (3).** The nitrone (5*R*)-5-phenyl-5,6-dihydro-2*H*-1,4-oxazin-2-one 4-oxide[7] **2** (0.500 g, 2.615 mmol) and indene (2 mL) were dissolved in dry chloroform (20 mL), and the mixture was heated at reflux under nitrogen for 3 h. The mixture was then evaporated under reduced pressure, and the gummy solid residue was stirred with cyclohexane to remove excess indene. The crude solid contained the major cycloadduct **3** and the minor cycloadduct **4** in a ratio of 5:1, LCMS $m/z$ 308 ($MH^+$) two components, gradient 2 (major component $t_R$ 3.19 min, minor component $t_R$ 3.27 min). The crude solid was recrystallized (×2) from ethyl acetate/cyclohexane to give the major exo cycloadduct **3** as colorless needles (0.302 g, 38%); mp 220–222 °C (dec). $^1$H NMR ($CDCl_3$) $\delta$ 7.52–7.21 (m, 9H, Ar*H*), 5.75 (d, $J$ = 7.6 Hz, 1H, ArC*H*O), 4.40–4.18 (m, 3H, PhC*H*C*H*$_2$O), 3.91 (d, $J$ = 9.1 Hz, 1H, NC*H*COO), 3.85–3.77 (m, 1H, ArCH$_2$C*H*), 3.41–3.31 (m, 2H, ArC*H*$_2$CH). LCMS $m/z$ 308 ($MH^+$) single component, gradient 2 ($t_R$ 3.20 min).

The mother liquors from the recrystallization were evaporated under reduced pressure and a small portion was purified by reverse-phase HPLC to give an impure sample of the minor endo cycloadduct **4** (contaminated with the exo cycloadduct et al.) as a white solid (estimated yield of endo cycloadduct from nitrone 8%). LCMS $m/z$ 308 ($MH^+$), gradient 2 ($t_R$ 3.27 min). The cycloadducts showed some instability on silica.

**(2*R*)-Amino(2,3-dihydro-1*H*-inden-2-yl)ethanoic Acid (5).** The cycloadduct **3** (100 mg, 0.325 mmol) and 20% palladium hydroxide on carbon (250 mg) were hydrogenated in 1,4-dioxan/trifluoroacetic acid (10:1 v:v) (11 mL) at 60 psi over atmospheric pressure and 50 °C using a Parr hydrogenator and a hydrogen generator for 2 h. The crude reaction mixture was filtered with suction (using a glass microfibre filter), and the residue was blown down under nitrogen and triturated under diethyl ether to give **5** as an off-white solid (26 mg, 41%). LCMS $m/z$ 192 ($MH^+$) single component, gradient 2 ($t_R$ 1.32 min). $^1$H NMR (DMSO-$d_6$) $\delta$ 8.42–8.32 (broad s, 3H, exchangeables), 7.26–7.12 (m, 4H, Ar*H*), 4.12–4.06 (m, 1H, NC*H*CO$_2$), 3.06–2.80 (m, 5H, Ar(C*H*$_2$)$_2$C*H*); only one enantiomer was present as shown by chiral HPLC comparison with authentic (*R*)-2-indanylglycine[9] and the racemic material (Chirobiotic T column, 25 cm, eluted with 20% acetonitrile in water containing 0.1% tetraethylammonium acetate (pH 4.1), single component ($t_R$ 5.41 min) (<0.5% (*S*) enantiomer present).

**(2*R*)-2,3-Dihydro-1*H*-inden-2-yl({[(phenylmethyl)oxy]carbonyl}amino)ethanoic Acid (6).** This was prepared from **5** as described previously.[5]

**2-{(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-[(1*S*)-1-methylpropyl]-2,5-dioxo-1-piperazinyl}-2-(2,6-dimethyl-3-pyridinyl)-*N*-(2-hydroxyphenyl)acetamide (42).** 2,6-Dimethylpyridine-3-carboxaldehyde **19** (2.00 g, 16.1 mmol)) and D-alloisoleucine methyl ester hydrochloride **11** (2.93 g, 16.1 mmol)) in methanol (50 mL) and 2,2,2-trifluoroethanol (50 mL) were treated with triethylamine (2.24 mL, 16.1 mmol), and the mixture was stirred under nitrogen at room temperature for 20 h. (2*R*)-[(Benzyloxycarbonyl)amino](2,3-dihydro-1*H*-inden-2-yl)ethanoic acid **6** (5.24 g, 16.1 mmol) and 2-benzyloxyphenylisonitrile **7** (3.37 g, 16.1 mmol) were added, and the mixture was stirred at room temperature under nitrogen for 4 days. The mixture was concentrated under reduced pressure and then partitioned between ethyl acetate (150 mL) and water (150 mL) plus saturated aqueous sodium hydrogen carbonate (6 mL). The aqueous phase was back-extracted with ethyl acetate (50 mL), and the combined organic extracts were washed successively with semisaturated aqueous solutions of sodium hydrogen carbonate (100 mL), ammonium chloride (100 mL), and sodium chloride (100 mL), dried ($MgSO_4$), and evaporated under reduced pressure to give the crude product (12.01 g). This was purified on a Redisep silica column (330 g) eluted with 20–50% ethyl acetate in cyclohexane to afford 7.46 g of methyl *N*-[(2*R*)-2-(2,3-dihydro-1*H*-inden-2-yl)-2-({[(phenylmethyl)oxy]carbonyl}amino)acetyl]-*N*-[1-(2,6-dimethyl-3-pyridinyl)-2-oxo-2-({2-[(phenylmethyl)oxy]phenyl}amino)ethyl]-D-alloisoleucinate **30** as a pair of diastereomers. LCMS $m/z$ 797 ($MH^+$) two components, gradient 2 ($t_R$ 3.88 and 3.96 min). This was dissolved in ethanol (150 mL) and acetic acid (10 mL) and the mixture was hydrogenated at 1 atm of $H_2$ over 10% palladium on carbon (Degussa type) (1.8 g wetted with water 1:1 w:w) for 18 h. The reaction mixture was evaporated under reduced pressure, and the residue was partitioned between ethyl acetate (250 mL) and water (50 mL) with saturated aqueous sodium hydrogen carbonate added until the aqueous phase was basic (pH 8). The aqueous phase was extracted with ethyl acetate 2 (50 mL), and the combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate/water 3:1 (100 mL) and then with saturated brine (50 mL) before being dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified on a Redisep silica column (120 g) eluted with 0–10% methanol in ethyl acetate to give **42** as a pair of diastereomers (2.94 g, 34%). $^1$H NMR ($CDCl_3$) $\delta$ 8.39, 8.34 (m, 1H), 7.78 (d, 1H, $J$ = 8 Hz), 7.63, 7.53 (m, 1H), 7.45 (d, 1H, $J$ = 8 Hz), 7.22–7.03 (m, 5H), 6.97 (m, 1H), 6.85 (m, 1H), 6.00, 5.55 (s, 1H), 4.18, 4.10 (m, 1H), 4.00–3.91 (m, 1H), 3.22–2.75 (m, 5H), 2.63, 2.56, 2.55, 2.51 (s, 6H), 1.72 (m, 1H), 1.58 (m, 1H), 1.14–0.88 (m, 4H), 0.84, 0.71 (t, 3H, $J$ = 7 Hz). LCMS $m/z$ 541 ($MH^+$) two components, gradient 2 ($t_R$ 2.75 and 2.81 min). HRMS calcd for $C_{32}H_{36}N_4O_4$ ($MH^+$) 541.28093, found 541.28103. HPLC: two components 35% and 58% ($t_R$ 10.93 and 11.37 min).

The compounds **32**–**41** and **43** were similarly prepared; see Supporting Information for experimental and spectroscopic details.

**(2*R*)-2-{(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-[(1*S*)-1-methylpropyl]-2,5-dioxo-1-piperazinyl}-2-(2,6-dimethyl-3-pyridinyl)-*N*-methylethanamide (67).** The phenol (**42**) (0.400 g, 0.74 mmol) and carbonyldiimidazole (0.192 g, 1.18 mmol) in dry dichloromethane (10 mL) were stirred at room temperature overnight. The mixture was treated with a 2 M solution of methylamine in tetrahydrofuran (1.849 mL, 3.70 mmol) and left to stand overnight at room temperature. The solvents were blown down, and the residue was purified on a Redisep silica column (35 g) eluted with 0–10% methanol in ethyl acetate followed by further purification on a Kromasil KR100–10-C18 reverse-phase column eluted with aqueous acetonitrile (20–45% MeCN) containing 0.1% formic acid. This gave after freeze-drying from 1,4-dioxane the amide **67** (102 mg, 30%) as a white lyophilizate. $^1$H NMR ($CDCl_3$) $\delta$ 7.63 (d, $J$ = 8.1 Hz, 1H, pyridyl-4*H*), 7.25–7.14 (m, 4H, indanyl-*arylH*), 7.05 (d, $J$ = 8.1 Hz, 1H, pyridyl-5*H*), 6.56 (d, $J$ = 2.8 Hz, lactam-N*H*), 5.95 (q, $J$ = 4.4 Hz, 1H, CONHMe), 5.35 (s, 1H, NC*H*pyridyl), 4.07 (dd, $J$ = 9.9 Hz, 3.3 Hz, 1H, NC*H*indanyl), 3.89 (d, $J$ = 4.3 Hz, 1H, NC*Hsec*-butyl), 3.19–2.71 (m, 8H, indanyl-3*H*,-1*H*,-2*H*, CONH*Me*), 2.57–2.53 (m, 6H, pyridyl-2*Me*, -6*Me*), 1.82–1.67 (m, 2H, C*H*HMe, C*H*MeCH$_2$), 1.20–1.05 (m, 1H, CH*H*Me), 0.99 (d, $J$ = 7.1 Hz, 3H, CH*Me*), 0.90 (t, $J$ = 7.3 Hz, 3H, CH$_2$*Me*). LCMS $m/z$ 463 ($MH^+$) single component, gradient 2 ($t_R$ 2.44 min). HRMS calcd for $C_{27}H_{34}N_4O_3$ ($MH^+$) 463.27037, found 463.27036. HPLC: 100% ($t_R$ 9.793 min).

The following compounds **49**, **58**, and **69** were similarly prepared.

**(2*R*)-2-[(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-*N*,*N*-dimethyl-2-(6-methyl-3-pyridinyl)ethanamide (49).** Similarly prepared as **67**, using dimethylamine and phenol **34**, the amide **49** was obtained as a white solid (50%). $^1$H NMR ($CDCl_3$) $\delta$ 8.58 (d, $J$ = 2.2 Hz, 1H, pyridyl-2*H*), 7.66 (dd, $J$ = 8.0 Hz, 2.2 Hz, 1H, pyridyl-4*H*), 7.26–7.14 (m, 5H, pyridyl-5*H*, indanyl-*arylH*), 6.72 (d, $J$ = 4.0 Hz, 1H, lactam-N*H*), 6.44 (s, 1H, NC*H*pyridyl), 4.07 (dd, $J$ = 11.8 Hz, 2.5 Hz, 1H, NC*H*isobutyl), 4.00 (dd, $J$ = 10.3 Hz, 4.5 Hz, 1H, NC*H*indanyl), 3.20–3.03 (m, 3H, indanyl-3*H*, -1*H*), 2.99 (s, 3H, CONM*e*Me),


dx.doi.org/10.1021/jm201287w | J. Med. Chem. 2012, 55, 783–796

2.93−2.74 (m, 4H, CONMe*Me*, indanyl-*2H*, indanyl-*1H*), 2.61 (s, 3H, pyridyl-*6Me*), 1.63−1.44 (m, 2H, C*H*HCHMe$_2$, CH$_2$C*H*Me$_2$), 0.72−0.61 (m, 4H, CH*H*CHMe$_2$, CH$_2$CHM*e*Me), 0.36 (d, *J* = 6.5 Hz, 3H, CH$_2$CHMe*Me*). LCMS *m*/*z* 463 (MH$^+$) single component, gradient 2 ($t_R$ 2.92 min). HRMS calcd for $C_{27}H_{34}N_4O_3$ (MH$^+$) 463.27037, found 463.27010. HPLC: 100% ($t_R$ 10.294 min).

**(2*R*)-2-{(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-[(1*S*)-1-methylpropyl]-2,5-dioxo-1-piperazinyl}-*N*,*N*-dimethyl-2-(6-methyl-3-pyridinyl)ethanamide (58).** Similarly prepared as **67**, using dimethylamine and phenol **37**, the amide **58** was obtained, as a white solid (25%). $^1$H NMR (CD$_3$OD) $\delta$ 8.57 (d, *J* = 2.2 Hz, 1H, pyridyl-*2H*), 7.83 (dd, *J* = 8.3 Hz, 2.5 Hz, 1H, pyridyl-*4H*), 7.43 (d, *J* = 8.0 Hz, 1H, pyridyl-*5H*), 7.21−7.08 (m, 4H, indanyl-*arylH*), 6.31 (s, 1H, NC*H*pyridyl), 3.95 (d, *J* = 10.0 Hz, 1H, NC*H*indanyl), 3.90 (d, *J* = 5.8 Hz, 1H, NC*Hsec*-butyl), 3.10−3.00 (m, 3H, indanyl-*3H*, *-1H*), 2.94 and 2.93 (2s, 6H, CON*Me*$_2$), 2.87−2.73 (m, 2H, indanyl-*2H*, indanyl-*1H*), 2.58 (s, 3H, pyridyl-*6Me*), 1.64−1.54 (m, 1H, C*H*HMe), 1.39−1.27 (m, 1H, C*H*MeCH$_2$), 0.92−0.80 (m, 1H, CH*H*Me), 0.70 (t, *J* = 7.3 Hz, 3H, CH$_2$*Me*), 0.58 (d, *J* = 7.0 Hz, 3H, CH*Me*). LCMS *m*/*z* 463 (MH$^+$) single component, gradient 2 ($t_R$ 2.82 min). HRMS calcd for $C_{27}H_{34}N_4O_3$ (MH$^+$) 463.27037, found 463.27018. HPLC: 100% ($t_R$ 10.547 min).

The freebase **58** was treated with 2N hydrochloric acid, and the resulting solution evaporated in vacuo to give the hydrochloride **58B** as a white solid. $^1$H NMR (DMSO-$d_6$) $\delta$ 8.76 (broad s, 1H, pyridyl-*2H*), 8.51 (d, *J* = 3.5 Hz, 1H, lactam-N*H*), 8.33 (broad d, *J* = 8.1 Hz, 1H, pyridyl-*4H*), 7.86 (d, *J* = 8.5 Hz, 1H, pyridyl-*5H*), 7.23−7.09 (m, 4H, indanyl-*arylH*), 6.11 (s, 1H, NC*H*pyridyl), 3.99 (d, *J* = 2.5 Hz, 1H, NC*Hsec*-butyl), 3.81 (dd, *J* = 8.6 Hz, 3.3 Hz, 1H, NC*H*indanyl), 3.00−2.73 (m, 14H, indanyl-*3H*, *-1H*, CON*Me*$_2$, indanyl-*2H*, indanyl-*1H*, pyridyl-*6Me*), 1.74−1.63 (m, 1H, C*H*HMe), 1.56−1.46 (m, 1H, C*H*MeCH$_2$), 0.98−0.85 (m, 4H, CH*H*Me), 0.73 (t, *J* = 7.3 Hz, 3H, CH$_2$*Me*). LCMS *m*/*z* 463 (MH$^+$) single component, gradient 2 ($t_R$ 2.79 min). HPLC: 100% ($t_R$ 10.40 min). HRMS calcd for $C_{27}H_{34}N_4O_3$ (MH$^+$) 463.27037, found 463.27018; circular dichroism (CH$_3$CN) $\lambda_{max}$227.8 nm, dE −5.31, E5795; $\lambda_{max}$255.6 nm, dE 0.77, E3881; $\lambda_{max}$280.0 nm, dE −0.97, E3756.

**(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-1-[(1*R*)-1-(2,6-dimethyl-3-pyridinyl)-2-(4-morpholinyl)-2-oxoethyl]-6-[(1*S*)-1-methylpropyl]-2,5-piperazinedione (69).** Similarly prepared as **67**, using morpholine and phenol **42**, the amide **69** was obtained, after freeze-drying from 1,4-dioxane as a white solid (23%). Identical in all respects with that obtained from acid hydrochloride **70**.

The compounds **44**, **45**,**47**, **48**, **50**−**57**, **59**, and **61**−**68** were similarly prepared; see Supporting Information for experimental and spectroscopic details.

**(2*R*)-2-[(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-*N*,*N*-dimethyl-2-(6-oxo-1,6-dihydro-3-pyridinyl)ethanamide (46).** A solution of the 2-methoxypyridyl derivative **44** (76 mg, 0.165 mmol), sodium iodide (30 mg, 0.2 mmol), and trimethylacetyl chloride (22.4 $\mu$L, 0.182 mmol) in dry acetonitrile (7 mL) was refluxed for 4 h and then water (700 $\mu$L) was added and the mixture stirred for a further 10 min. The mixture was diluted with dichloromethane (15 mL), washed with 2N HCl (5 mL), a saturated aqueous solution of sodium hydrogen carbonate (5 mL) and brine (5 mL), dried (MgSO$_4$), and evaporated to give after freeze-drying from 1,4-dioxane **46** as a white solid (70 mgs, 91%). $^1$H NMR (CDCl$_3$) $\delta$ 12.36 (broad s, 1H, pyridone N*H*), 7.53−7.45 (m, 2H, pyridone-*2H*, *-4H*), 7.38 (broad d, *J* = 3.0 Hz, lactam-N*H*), 7.25−7.14 (m, 4H, indanyl-*arylH*), 6.67 (d, *J* = 9.5 Hz, pyridone-*5H*), 6.27 (s, 1H, NC*H*pyridone), 4.20 (dd, *J* = 12.1 Hz, 3.5 Hz, 1H, NC*H*isobutyl), 3.99 (dd, *J* = 10.31 Hz, 4.5 Hz, 1H, NC*H*indanyl), 3.20−2.77 (m, 11H, indanyl-*3H*, *-1H*,*-2H*, CON*Me*$_2$), 1.74−1.54 (m, 2H, C*H*HCHMe$_2$, CH$_2$C*H*Me$_2$), 1.01−0.92 (m, 1H, CH*H*CHMe$_2$), 0.74 (d, *J* = 6.5 Hz, 3H, CH$_2$CH*Me*Me), 0.64 (d, *J* = 6.5 Hz, 3H, CH$_2$CHMe*Me*). LCMS *m*/*z* 465 (MH$^+$) single component, gradient 2 ($t_R$ 2.68 min). HRMS calcd for $C_{26}H_{32}N_4O_4$ (MH$^+$) 465.24963, found 465.24938. HPLC: 100% ($t_R$ 9.839 min).

**(2*R*)-2-{(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-[(1*S*)-1-methylpropyl]-2,5-dioxo-1-piperazinyl}-*N*,*N*-dimethyl-2-(6-methyl-1-oxido-3-pyridinyl)ethanamide (60).** To a solution of the 2-methyl pyridine **58** (0.232 g, 0.5 mmol) in chloroform (10 mL) was added to *m*-chloroperbenzoic acid (∼50%, 0.345 g, ∼1 mmol) and the reaction mixture stirred at room temperature for 4 h. Methanol was added and the mixture applied to an aminopropyl SPA column (10 g), which was eluted with chloroform/methanol (1: 1) to give after evaporation of the required fractions a white solid (0.300 g). This was purified on a Redisep silica column (12 g) eluted with ethyl acetate/methanol (9:1 to 4:1) to give the *N*-oxide **60** as white solid (0.172 g, 72%). $^1$H NMR (CDCl$_3$) $\delta$ 8.47 (s, 1H, pyridyl-*2H*), 7.39 (d, *J* = 8.0 Hz, 1H, pyridyl-*4H*), 7.32 (d, *J* = 8.0 Hz, 1H, pyridyl-*5H*), 7.26−7.14 (m, 5H, indanyl-*arylH*), 6.07 (s, 1H, NC*H*pyridyl), 4.10−4.03 (m, 3H, NC*H*indanyl and NC*Hsec*-butyl), 3.19−3.10 (m, 3H, indanyl-*3H*,*-1H*), 2.98 and 2.96 (2s, 6H, CON*Me*$_2$), 2.91−2.80 (m, 2H, indanyl-*2H*, indanyl-*1H*), 2.55 (s, 3H, pyridyl-*6Me*), 1.60 (m, 1H, C*H*HMe), 1.39 (m, 1H, C*H*MeCH$_2$), 0.99 (m, 1H, CH*H*Me), 0.79−0.73 (m, 6H, CH$_2$*Me* and CH*Me*). LCMS *m*/*z* 479.4 (MH$^+$) single component, gradient 2 ($t_R$ 2.59 min). HRMS calcd for $C_{27}H_{35}N_4O_4$ (MH$^+$) 479.2658, found 479.2656. HPLC: 95% ($t_R$ 9.906 min).

**{(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-[(1*S*)-1-methylpropyl]-2,5-dioxo-1-piperazinyl}(2,6-dimethyl-3-pyridinyl)-acetic Acid Hydrochloride (70).** The phenol **42** (24.25 g, 45 mmol) and carbonyldiimidazole (11.7 g, 72 mmol) were dissolved in dry dichloromethane (200 mL) and left to stand under nitrogen for 20 h. The solvent was removed in vacuo, and the residue was dissolved in acetone (200 mL) and 2N hydrochloric acid (20 mL). After stirring for 20 h, the solvent was removed in vacuo and the residue was dissolved in methanol (50 mL). The solution was applied to an aminopropyl cartridge (2 × 70 g) and eluted with methanol (250 mL) and then 10% acetic acid in methanol (250 mL). The required fractions were combined and evaporated in vacuo. The residue was treated with 2N hydrochloric acid and the resulting solution evaporated in vacuo to give the acid hydrochloride **70** as a tan solid (12.21 g, 56%). $^1$H NMR (CDCl$_3$) $\delta$ 8.66, 8.60 (d, 1H, *J* = 3.8 Hz), 8.19, 7.96 (d, 1H, *J* = 8 Hz), 7.74, 7.68 (d, 1H, *J* = 8 Hz), 7.22 (m, 2H), 7.13 (m, 2H), 5.53, 5.24 (s, 1H), 4.24, 3.81 (m, 1H), 4.08−4.00 (m, 1H), 3.13−2.84 (m, 4H), 2.81−2.67 (m, 7H), 2.14, 1.82 (m, 1H), 1.46, 1.38 (m, 1H), 1.12, 0.92 (d, 3H, *J* = 7 Hz), 1.07 (m, 1H), 0.88, 0.58 (t, 3H, *J* = 7 Hz). LCMS *m*/*z* 450.3 (MH$^+$) single components, gradient 2 ($t_R$ 2.41 min). HRMS calcd for $C_{26}H_{31}N_3O_4$ (MH$^+$) 450.23873, found 450.23854. HPLC: two components 48% and 48% ($t_R$ 9.104 and 9.172 min).

The compounds **71** and **72** were similarly prepared; see Supporting Information for experimental and spectroscopic details.

**(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-1-[(1*R*)-1-(2,6-dimethyl-3-pyridinyl)-2-(4-morpholinyl)-2-oxoethyl]-6-[(1*S*)-1-methylpropyl]-2,5-piperazinedione (69).** A suspension of the acid hydrochloride **70** (5.0 g, 10.3 mmol) in dry dichloromethane (50 mL) was treated with carbonyldiimidazole (2.6 g, 16 mmol), and the reaction mixture was stirred under nitrogen for 18 h. Morpholine (4.8 mL, 55 mmol) was added, and the resultant solution was left to stand under nitrogen for 18 h. The solvent was removed in vacuo, and the residue was separated between ethyl acetate and water. The organic phase was washed with brine and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo, and the residue was dissolved in dichloromethane. This was applied to a basic alumina cartridge (240 g) and eluted using a gradient of 0−7.5% methanol in diethyl ether (9CV), 7.5−10% methanol in diethyl ether (1CV), and 10% methanol in diethyl ether (1CV). The required fractions were combined and evaporated in vacuo to give **69** as a white solid (2.4 g, 45%). Recystallisation from ethyl acetate/hexane (1:3) gave colorless needles (75%) mp 140 °C. $^1$H NMR (CDCl$_3$) $\delta$ 7.49 (d, *J* = 7.8 Hz, 1H, pyridyl-*4H*), 7.26−7.15 (m, 4H, indanyl-*arylH*), 7.10 (d, *J* = 8.1 Hz, 1H, pyridyl-*5H*), 6.68 (s, 1H, NC*H*pyridyl), 6.49 (d, *J* = 2.8 Hz, 1H, lactam-N*H*), 4.10 (dd, *J* = 10.1 Hz, 4.0 Hz, 1H, NC*H*indanyl), 4.01 (d, *J* = 4.5 Hz, NC*Hsec*-butyl), 3.75−2.71 (m, 13H, 8× morpholinyl-*H*, indanyl-*3H*, *-1H*, *-2H*), 2.62 and 2.58 (2s, 6H, pyridyl-*2Me*,*-6Me*), 1.64−1.52 (m, 1H, C*H*HMe), 0.98−0.79 (m, 2H, CH*H*Me, C*H*MeCH$_2$), 0.70 (t, *J* = 7.1 Hz, 3H, CH$_2$*Me*), 0.45 (d, *J* = 6.8 Hz, 3H, CH*Me*). LCMS *m*/*z* 519 (MH$^+$) single component,


dx.doi.org/10.1021/jm201287w | *J. Med. Chem.* 2012, 55, 783−796

gradient 2 ($t_R$ 2.70 min). HRMS calcd for $C_{30}H_{38}N_4O_4$ ($MH^+$) 519.29658, found 519.29667. HPLC: 100% ($t_R$ 10.388 min).

To a warm solution of **69** (2.66 g, 5.1 mmol) in acetone (40 mL) was added a solution of benzene sulfonic acid (0.81 g, 5.1 mmol) in acetone (40 mL), and the resulting solution was heated to boiling and allowed to cool to room temperature during 48 h. The resulting crystals were filtered off, air-dried on the filter pad to give the besylate (3.214 g, 92.6%) as white crystals of **69B** mp 179−183 °C. $^1$H NMR ($CD_3OD$) $\delta$ 8.30 (d, 1H, $J$ = 8.1 Hz, pyridyl-*4H*), 7.84−7.80 (m, 2H, $PhSO_3^-$ 2× *ortho*-H), 7.78 (d, $J$ = 8.3 Hz, 1H, pyridyl-*5H*), 7.45−7.38 (m, 3H, $PhSO_3^-$ 2× *meta*-H, *para*-H), 7.23−7.09 (m, 4H, indanyl-*arylH*), 6.08 (broad s, 1H, NC*H*pyridyl), 4.00 (d, $J$ = 4.6 Hz, 1H, NC*H*sec-butyl), 3.92 (d, $J$ = 9.9 Hz, 1H, NC*H*indanyl), 3.78−3.39 and 3.14−2.80 (m, 13H, 8× morpholinyl-*H*, indanyl-*3H*, *-1H*, *-2H*)), 2.79 and 2.78 (2s, 6H, pyridyl-*2Me*, *-6Me*), 1.85−1.74 (m, 1H, C*H*HMe), 1.59−1.48 (m, 1H, CH*H*Me), 1.15−1.01 (m, 1H, C*H*MeCH$_2$), 0.92 (d, $J$ = 6.3 Hz, 3H, CH*Me*), 0.85 (t, $J$ = 7.3 Hz, 3H, CH$_2$*Me*). LCMS $m/z$ 519 $MH^+$ single components, $t_R$ 2.72 min; circular dichroism ($CH_3CN$) $\lambda_{max}$ 225.4 nm, dE −15.70, E15086; $\lambda_{max}$ 276 nm, dE 3.82, E5172. HRMS calcd for $C_{30}H_{38}N_4O_4$ ($MH^+$) 519.2971, found 519.2972. Anal. ($C_{30}H_{38}N_4O_4 \cdot C_6H_6O_3S \cdot 3.0H_2O$) C, H, N, S.

The compounds **73**−**78** were similarly prepared; see Supporting Information for experimental and spectroscopic details.

## ■ ASSOCIATED CONTENT

### Supporting Information

C, H, N, analysis for compounds **59B**, **68**, and **69B**. Experimental and spectroscopic details for similarly prepared compounds **32**−**41**, **43**, **44**, **45**, **47**, **48**, **50**−**57**, **59**, **61**−**68**, **71**, **72**, and **73**−**78**. Array chemistry for compounds **95**−**102**. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*For A.D.B.: phone, +44(0)7772112742; E-mail, alan.d.borthwick@drugmoldesign.com. For J. L.: phone, +44(0)1438 763643; E-mail, John.2.Liddle@GSK.com.

### Present Address

▽DrugMolDesign, 15 Temple Grove, London NW11 7UA, U.K.

## ■ ABBREVIATIONS USED

Cyno, cynomolgus; CHILog *D*, chromatographic hydrophobicity index logarithm of the distribution coefficient; 2,5-DKP, 2,5-diketopiperazine; FLIPR, fluorometric imaging plate reader; hOTR, human oxytocin receptor; hV1aR, human arginine vasopressin receptor 1A; hV1bR, human arginine vasopressin receptor 1B; hV2R human arginine vasopressin receptor 2; HSA, human serum albumin; HTFT, high throughput fluctuation test; MED, minimum effective dose; OT, oxytocin; P450, human cytochrome P450 enzyme; PK, pharmacokinetics; p$K_i$, $-\log_{10}K_i$; SAR, structure−activity relationships

## ■ REFERENCES

(1) Goldenberg, R. L.; Rouse, D. Prevention of premature birth. *N. Engl. J. Med.* **1998**, *339*, 313.

(2) Enkin, M.; Kierse, M.; Neilson, J.;et al. *Preterm Labour: A Guide to Effective Care in Pregnancy and Childbirth*, 3rd ed.; Oxford University Press: Oxford, UK, 2000; pp 211−225.

(3) (a) Bossmar, T. Treatment of preterm labor with the oxytocin and vasopressin antagonist atosiban. *J. Perinat. Med.* **1998**, *26*, 458−465. See also (b) Coomarasamy, A.; Knox, E. M.; Gee, H.; Khan, K. S. Oxytocin antagonists for tocolysis in preterm labour—a systematic review. *Med. Sci. Monit.* **2002**, *8*, RA268−RA273.

(4) Borthwick, A. D. Oral Oxytocin Antagonists. *J. Med. Chem.* **2010**, *53*, 6525−6538.

(5) Borthwick, A. D.; Davies, D. E.; Exall, A. M.; Hatley, R. J. D.; Hughes, J. A.; Irving, W. R.; Livermore, D. G.; Sollis, S. L.; Nerozzi, F.; Valko, K. L.; Allen, M. J.; Perren, M.; Shabbir, S. S.; Woollard, P. M.; Price, M. A. 2,5-Diketopiperazines as Potent, Selective and Orally Bioavailable Oxytocin Antagonists 3: Synthesis, Pharmacokinetics and in Vivo Potency. *J. Med. Chem.* **2006**, *49*, 4159−4970.

(6) Borthwick, A. D.; Liddle, J. The design of orally bioavailable 2,5 diketopiperazine oxytocin antagonists: from concept to clinical candidate for premature labour. *Med. Res. Rev.* **2011**, *31*, 576−604.

(7) Long, A.; Baldwin, S. W. Enantioselective syntheses of homophenylalanine derivatives via nitrone 1,3-dipolar cycloaddition reactions with styrenes. *Tetrahedron Lett.* **2001**, *42*, 5343−5345.

(8) Tamura, O.; Gotanda, K.; Yoshino, J.; Morita, Y.; Terashima, R.; Kikuchi, M.; Miyawaki, T.; Mita, N.; Yamashita, M.; Ishibashi, H.; Sakamoto, M. Design, Synthesis, and 1,3-Dipolar Cycloaddition of (5*R*)-[and (5*S*)]-5,6-Dihydro-5-phenyl-2*H*-1,4-oxazin-2-one *N*-Oxides as Chiral (*E*)-Geometry-Fixed $\alpha$-Alkoxycarbonylnitrones. *J. Org. Chem.* **2000**, *65*, 8544−8551.

(9) Borthwick, A. D.; Davies, D. E.; Exall, A. M.; Livermore, D. G.; Sollis, S. L.; Nerozzi, F.; Allen, M. J.; Perren, M.; Shabbir, S. S.; Woollard, P. M.; Wyatt. 2,5-Diketopiperazines as Potent, Selective and Orally Bioavailable Oxytocin Antagonists 2: Synthesis, Chirality and Pharmacokinetics. *J. Med. Chem.* **2005**, *48*, 6956−6969.

(10) Obach, R. S. The prediction of human clearance from hepatic microsomal metabolism data. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 36−44.

(11) Wyatt, P. G.; Allen, M. J.; Borthwick, A. D.; Davies, D. E.; Exall, A. M.; Hatley, R. J. D.; Irving, W. R.; Livermore, D. G.; Miller, N. D.; Nerozzi, F.; Sollis, S. L.; Szardenings, A. K. 2,5-Diketopiperazines as Potent and Selective Oxytocin Antagonists 1: Identification, Stereochemistry and Initial SAR. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2579−2582.

(12) McCafferty, G. P.; Pullen, M. A.; Wu, C.; Edwards, R. M.; Allen, M. J.; Woollard, P. M.; Borthwick, A. D.; Liddle, J.; Hickey, D. M.; Brooks, D. P.; Westfall, T. D. Use of a novel and highly selective oxytocin receptor antagonist to characterize uterine contractions in the rat. *Am. J. Physiol.* **2007**, *293*, R299−R305.

(13) Valko, K. Separation Methods in Drug Synthesis and Purification. In *Handbook of Analytical Separations*; Valko, K., Ed.; Elsevier: Amsterdam, 2000; Vol. *1*, Chapter 12 , pp 535−583.

(14) Chromatographic hydrophobicity index (CHI) utilizes the gradient retention time from rapid gradient reverse-phase elution to measure lipophilicity. See the following: Valko, K.; Du, C. M.; Bevan, C.; Reynolds, D. P.; Abrahams, M. H. Rapid method for the estimation of octanol/water partition coefficient (log $P_{oct}$) from gradient RP-HPLC retention and a hydrogen bond acidity term ($\Sigma\alpha$2H). *Curr. Med. Chem.* **2001**, *8*, 1137−1146.

(15) HSA and RSA binding methods using Chromtech Immobilized HSA and RSA HPLC columns see: Valko, K.; Nunhuck, S.; Bevan, C.; Abraham, M. H.; Reynolds, D. P. Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationships with octanol/water and immobilized artificial membrane lipophilicity. *J. Pharm. Sci.* **2003**, *92*, 2236−2248.

(16) Intrinsic clearance protocol: NADP regeneration buffer for use in incubations was prepared fresh on the assay day. It contained 7.8 mg glucose-6-phosphate (monosodium salt), 1.7 mg NADP and 6 units glucose-6-phosphate dehydrogenase per 1 mL of 2% sodium bicarbonate. Microsomes (human, female; cynomolgus monkey, female; dog, female; rat, female) were prepared in pH 7.4 phosphate buffer. The final protein concentration in the incubation mixture was 0.5 mg/mL. The microsomal solution containing the compound was transferred to a microplate and was prewarmed at 37 °C prior to initiation of incubations. All incubations were initiated by addition of the NADP regeneration system to the prewarmed microsomes and the mixtures were incubated at 37 °C. Following 0, 3, 6, 12, and 30 min incubation, aliquots were taken and added to acetonitrile containing an internal standard. For determination of the rate of metabolism,

incubations were performed at a compound concentration of 0.5 $\mu$M. The concentration of solvent in the incubation was not greater than 0.5%. The rate of metabolism was determined by using HPLC-MS/MS analysis where disappearance of the parent compound was calculated from the start of the incubation.

(17) Wyatt, P. G.; Allen, M. J.; Chilcott, J.; Gardner, C. J.; Livermore, D. G.; Mordaunt, J. E.; Nerozzi, F.; Patel, M.; Perren, M. J.; Weingarten, G. G.; Shabbir, S; Woollard, P. M.; Zhou, P. Identification of potent and selective oxytocin antagonists. Part 2: further investigation of benzofuran derivatives. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1405−1411.

(18) P450 Inhibition: Human cytochrome P450 enzymes expressed in *Escherichia coli* (Bactosomes) were obtained from Cypex Ltd. (Dundee, UK). Reaction progress was monitored using fluorescent substrates. Each test compound was dissolved in DMSO/methanol at 1.65 mM and serially diluted to determine $IC_{50}$ values. A positive control was included for each isozyme.

(19) Time-dependent inhibition assays were preformed as described in (18), except the fluorescence in each well was measured for 30 min at 1 min intervals. Each assay plate also contained a CYP3A4 time-dependent positive control.

(20) Reifferscheid, G.; Heil, J.; Oda, Y.; Zahn, R. K. A microplate version of the SOS/umu-test for rapid detection of genotoxins and genotoxic potentials of environmental samples. *Mutat. Res.* **1991**, *253*, 215−222.

(21) Clive, D.; Caspary, W.; Kirby, P.; Krehl., R.; Moore, M.; Mayo, J.; Oberly, T. J. Guide for performing the mouse lymphoma assay for mammalian cell mutagenicity. *Mutat. Res.* **1987**, *189*, 143−156.

(22) Liddle, J.; Allen, M. J.; Borthwick, A. D.; Brooks, D. P.; Davies, D. E.; Edwards, R. M.; Exall, A. M.; Hamlett, C.; Irving, W. R.; Mason, A. M.; McCafferty, G. P.; Nerozzi, F.; Peace, S.; Philp, J.; Pollard, D.; Pullen, M. A.; Shabbir, S. S.; Sollis, S. L.; Westfall, T. D.; Woollard, P. M.; Wu, C.; Hickey, D. M. The discovery of GSK221149A: a potent and selective oxytocin antagonist. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 90−94.