2,5-Diketopiperazines as Potent, Selective, and Orally Bioavailable Oxytocin Antagonists. 3. Synthesis, Pharmacokinetics, and in Vivo Potency

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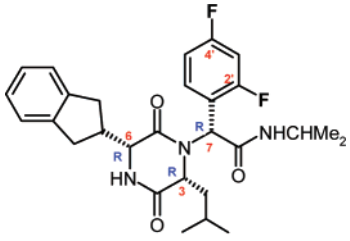
A short, efficient, and highly stereoselective synthesis of a series of (3*R*,6*R*,7*R*)-2,5-diketopiperazine oxytocin antagonists and their pharmacokinetics in rat and dog is described. Prediction of the estimated human oral absorption (EHOA) using measured lipophilicity (CHI log *D*) and calculated size (cMR) has allowed us to rank various 2,5-diketopiperazine templates and enabled us to focus effort on those templates with the greatest chance of high bioavailability in humans. This rapidly led to the 2′,4′-difluorophenyl-dimethylamide **25** and the benzofuran **4** with high levels of potency (pK_i) and good bioavailability in the rat and dog. Dimethylamide **25** is more potent (>20-fold) than **4** in vivo and has a high degree of selectivity toward the vasopressin receptors, >10000 for hV1a/hV1b and ∼500 for hV2. It has a good Cyp450 profile with no time dependent inhibition and was negative in the genotoxicity screens with a satisfactory oral safety profile in rats.

Introduction

Preterm labor is a major clinical problem leading to death and disability in newborns. It accounts for 10% of all births and causes 70% of all infant mortality and morbidity.[1] Oxytocin is a potent stimulant of uterine contractions and is responsible for the initiation of labor via the interaction with the oxytocin receptors in mammalian female uterus. Oxytocin antagonists have been shown to inhibit uterine contractions and delay preterm delivery. Hence, there is increasing interest in oxytocin antagonists as a consequence of their potential application in the prevention of preterm labor and premature birth. Although several tocolytics (uterine contraction inhibitors) have already been approved in clinical practice, they have harmful maternal or fetal side-effects.[2] The first clinically tested oxytocin antagonist, atosiban (Tractocile), 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 oxytocin/vasopressin V1a antagonist that has to be given by iv infusion and is unlikely to be suitable for long-term maintenance treatment, because it is not orally bioavailable.[3] Our target is a potent, orally active oxytocin antagonist with high levels of selectivity over the vasopressin receptor, which would safely delay labor by greater than 7 days and improve infant outcome.

We recently reported[4] the identification of a novel series of (3*R*,6*R*,7*R*)-2,5-diketopiperazine (DKP) derivatives with antagonist activity at the human oxytocin receptor (hOTR). The most potent of these was the 2′,4′-difluorophenyl derivative **1** with a pK_i = 8.9 at the human oxytocin receptor that had only a small shift in IC_{50} of 3.8-fold in the presence of HSA and was >1000-fold selective for the human oxytocin receptor relative to all three vasopressin receptors hV1aR, hV2R, and hV1bR. It had good rat pharmacokinetics with 46% bioavailability. It was also active in vivo in the rat (DR_{10} = 0.44 mg/kg iv).



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However, it had poor aqueous solubility and was prepared as the minor isomer in a short, nonstereospecific synthesis. Good pharmacokinetics in a second species could only be obtained by using a cyclodextrin formulation in the dog, which increased the bioavailability from 13% to 31%.

In this report we address these issues by using structure−property relationships to optimize the drug-like properties of this template, in particular using models to select structures having the potential for good human oral absorption. We describe how we investigated the modification of the amide function and developed a stereospecific, high yielding synthesis to these derivatives, as well as further investigating the effect of disubstitution in the aryl ring.

Chemistry

In our previous paper[4] we described the synthesis of 2,5-diketopiperazine secondary amides in two steps using a four-component Ugi reaction followed by ring closure. The compounds **2**−**9** which are the 3*R*,6*R*,7*R* isomers were prepared in ≤25% yield by this method (Figure 1, see Supporting Information).

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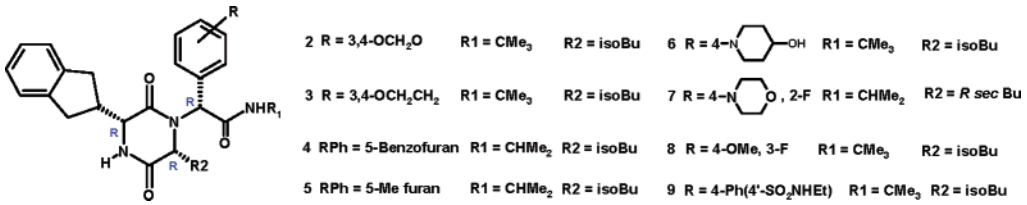
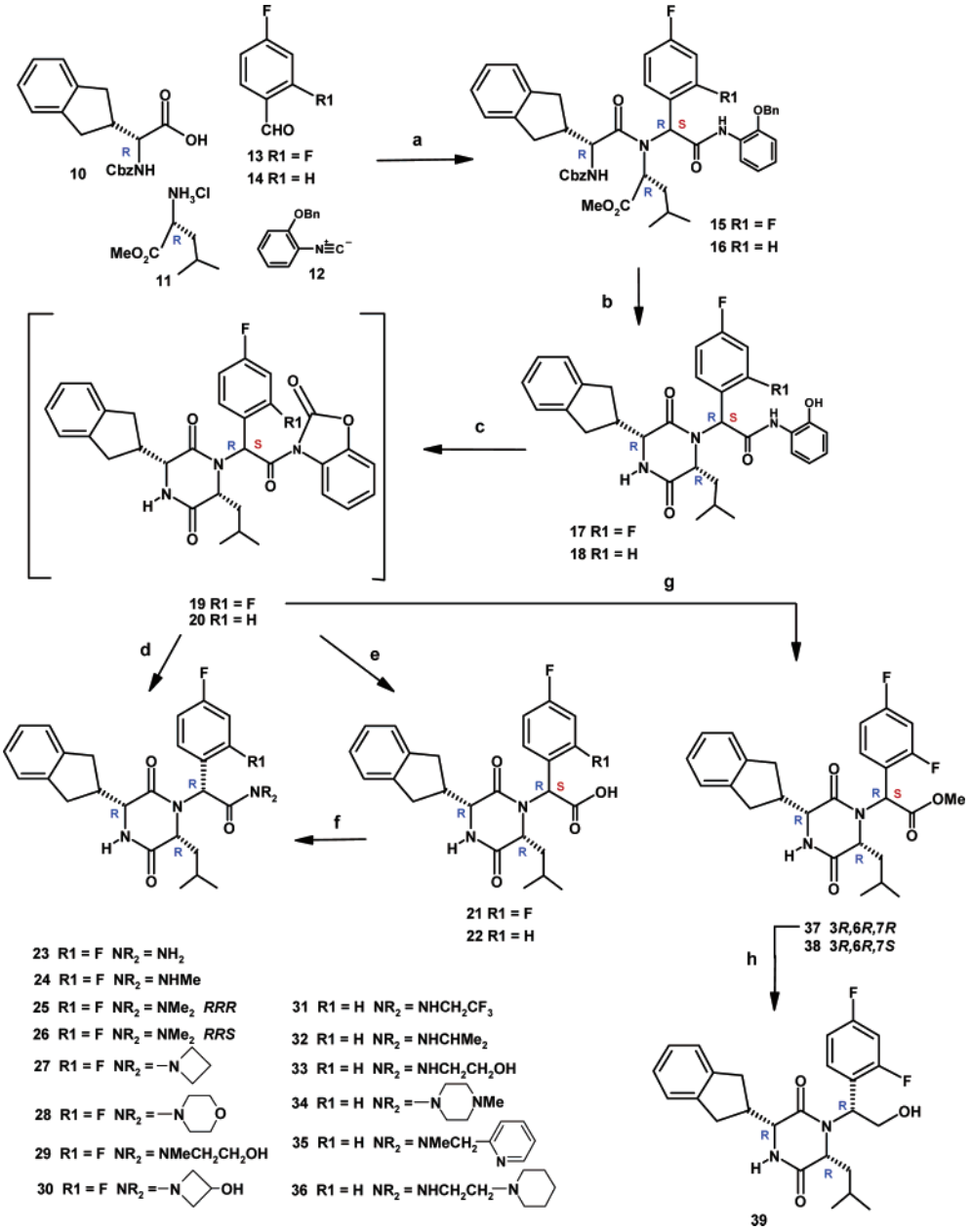


Figure 1. 2,5-Diketopiperazine secondary amides.

Scheme 1. [a] Synthesis of 2,5-Diketopiperazine Tertiary Amides, Acids, and Primary Alcohol

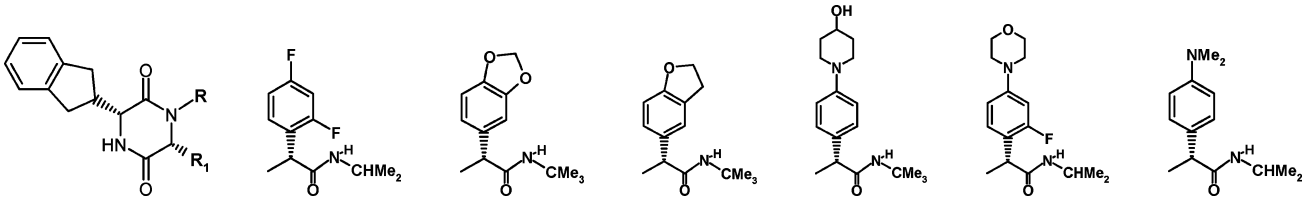


[a] Reagents and conditions: (a) D-Leu methyl ester hydrochloride, methanol, aryl aldehyde, Et_3N, room temp, 1−3 days; then Cbz-IndGly, 2-benzyloxyphenyl isocynanide, room temp, 24 h; (b) H_2, Pd/C, EtOAc, AcOH, 2−5 h; (c) CDI, DCM, 6 h; (d) R_2NH, room temp, 16 h; (e) Me_2CO, H^+, H_2O, 10 min; (f) Et_3N, DCM, MeCN then BOPCl, 10 min, followed by Me_2NH, room temp, 16 h; (g) MeOH; (h) $LiBHEt_3$ (3 equiv)/THF, −78 °C, 4 h.

A general synthetic procedure that enables the exocyclic amide function to be readily modified is outlined in Scheme 1. As a results of a sustained effort[5] we have achieved a high level of stereochemical control in the synthesis of the indane-2,5-diketopiperazine tertiary amides which has enable us to obtain >90% of our required 3*R*,6*R*,7*R* isomers. The four-component Ugi reaction of Cbz indanyl *R*-glycine **10**, *R*-aminoester (D-leucine methyl ester) **11**, convertible isonitrile[6] **12**, and the 2′,4′-difluorobenzaldehyde **13** gave the tripeptide intermediate **15**, which was followed by hydrogenation to remove the Cbz and benzyl groups, enabling cyclization to occur to give the phenolic 2,5-diketopiperazine **17**, in 70% yield from **13**. Only 25% of 2,5-diketopiperazine **17** was the (3*R*,6*R*,7*R*) isomer.

The cyclic carbamate **19** obtained from 1,1′-carbonyldiimidazole (CDI) and phenol **17** is an activated amide, which on hydrolysis with water gave the carboxylic acid **21** in 86% yield.

Table 1. Bioavailability of Mono- and Diaryl Substituted (3*R*,6*R*,7*R*)-2,5-Diketopiperazines in the Rat and Dog



	1	**2**	**3**	**6**	**7**	**40**[a]
R_1	isoBu	isoBu	isoBu	isoBu	R-secBu	isoBu
pK_i[b]	8.9	8.4	8.4	8.4	8.8	8.5
rat Cl/*F*%[c]	36/46	34/48	39/27	26/24	24/31	48/2
dog C/*F*%[c]	11/13	23/14	14/9	21/10	6/61	15/50
EHOA %[d]	60	48	46	19	33	46
CHI log *D*[e]	3.4	3.3	3.4	2.4	3.0	3.4
cMR[f]	13.51	14.54	14.85	16.61	15.69	14.78

[a] Prepared previously.[4] [b] Displacement of [^{3}H] oxytocin from hOTR by the test compound.[7] 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. [c] Rat ($n = 4$) and dog ($n = 3$) PK: Cl in mL min^{-1} kg^{-1}. [d] EHOA = estimated human oral absorption, eq 4. [e] An HPLC method-based measurement of lipophilicity.[8] [f] Calculated molar refraction (Daylight v 4.81).

Reaction of the acid **21** (activated with BOPCl) with dimethylamine gave the 3*R*,6*R*,7*R*-dimethylamide **25** in 37% yield (12% overall), together with 3*R*,6*R*,7*S*-dimethylamide **26** in 18% yield. This is a 2:1 ratio of (3*R*,6*R*,7*R*): (3*R*,6*R*,7*S*) isomers, and we sought to improve the ratio of isomers in favor of our required (3*R*,6*R*,7*R*) isomer **25**. We recognized that there were two activated amide steps in the preparation of **25** via the acid **21** and therefore investigated the reaction of the first activated amide **19** directly with dimethylamine. This reaction was much more selective and gave a 10:1 ratio of the (3*R*,6*R*,7*R*) **25**: (3*R*,6*R*,7*S*) **26** dimethylamides in 68% yield. The overall yield of 3*R*,6*R*,7*R*-dimethylamide **25** for the three stages from **13** is 40%. A similar 10:1 ratio of the (3*R*,6*R*,7*R*): (3*R*,6*R*,7*S*) isomers was achieved directly from the activated amide **19** with other secondary amines **27**−**30**,[5] whereas for methylamine and ammonia which furnished **24** and **23** the (3*R*,6*R*,7*R*): (3*R*,6*R*,7*S*) isomer ratio was 3:1. A similar sequence from 4-fluorobenzaldehyde **14** gave the monofluoro analogues **31**−**36** in similar overall yields. Reaction of the cyclic carbamate **19** with methanol gave a 2.4:1 ratio of the esters **37**:**38** in 93% yield. Reduction of the (3*R*,6*R*,7*R*) isomer **37** with $LiBHEt_3$ in THF gave the alcohol **39** in 56% yield.

Results and Discussion

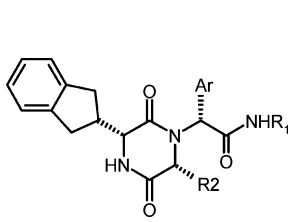
We recently [4] had success in improving the bioavailability of 4′-monosubstituted phenyl-2,5-diketopiperazines by further substitution in the 4-fluorophenyl ring giving the 2′,4′-difluorophenyl derivative **1** ($F = 46\%$ in rat). To exploit this, several further disubstituted aryl ring analogues were prepared. The best of these together with the 4′-monosubstituted derivatives **6** and **40** (Table 1) had good bioavailability in animals and good potency in the oxytocin binding assay (p$K_i = 8.4-8.9$). Those with the greatest bioavailability in the rat were the 2′,4′-difluorophenyl derivative **1** and the 3′,4′-methylenedioxyphenyl derivative **2**, while the dihydrobenzofuran **3** and the 4-(hydroxypiperidinyl)phenyl derivative **6** had moderate bioavailability in this species. The 4′-morpholino-2′-fluorophenyl derivative **7** and the 4′-dimethylaminophenyl derivative **40** had good bioavailability in the dog.

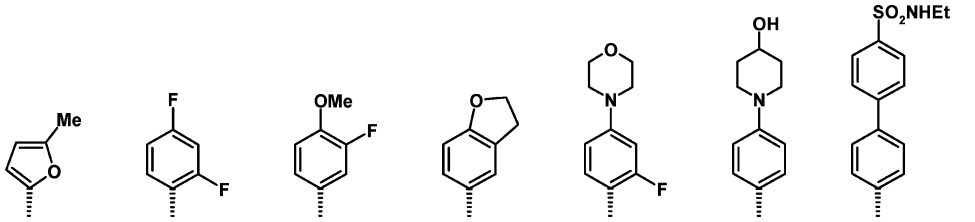
Our objective was to modify the amide function in order to further improve the bioavailability of these 2,5-diketopiperazine oxytocin antagonists. Most amides prepared previously were secondary amides so a range of tertiary amides were favored to further exploit substitution at this position. Also further modifications to the aryl ring were envisaged. However, we needed to choose one of these substituted phenyl derivatives (Table 1) to use to explore a range of amides. With the bioavailability of **1**, **2**, **3**, **6**, and **7** being good either in the rat or dog it was considered that some other estimation of how these would behave in humans would to be an advantage in choosing the best template to progress.

Several approaches to predicting oral bioavailability in humans have been investigated.[9] A key part of oral bioavailability is absorption across the gut wall by passive diffusion. Human intestinal drug absorption is largely driven by lipophilicity, hydrogen bonding, and size.[9] Although the most commonly used models, the Lipinski "rule of 5",[10] the molecular polar surface area model,[11] and the Abraham descriptors,[12] are very useful in general for selection of better compound libraries, they are less accurate in ranking compounds within this chemical series. We have found that the application of measured physicochemical data and models based on measured in vitro and physical chemical properties of the molecules are very helpful in selecting good candidate molecules within a chemical series. Also for the current 2,5-diketopiperazine compounds there is a poor correlation between the measured and calculated lipophilicity, possibly because the calculation methods employed are 2D based and do not compensate for potential conformational effects of these chirally pure molecules, which contain three asymmetric centers. It is possible that due to their conformation not all of the hydrophobic regions of the molecule are exposed to the solvent; this could result in measured values being significantly lower than calculated, which is consistent with our observations (see Supporting Information).

We have developed several types of high throughput HPLC-based measurements of lipophilicity, such as the Chromatographic Hydrophobicity Index (CHI) using C-18 stationary phases and fast acetonitrile CHI (ACN) or methanol CHI (MeOH) gradient.[8] The CHI values can be expressed in the log *D* scale and can be compared to the octanol/water log *D*s using the Abraham solvation equation approach.[13] This rapid measurement of lipophilicity has been used in the recently published equation[14] (1) that relates estimated human oral absorption (EHOA) to measured lipophilicity CHI (MeOH) and calculated size (cMR) using data for a range of drugs with known human oral absorption. The simplified version of the model calculated

Table 2. The Estimated Human Oral Absorption (EHOA) of Aryl Substituted (3*R*,6*R*,7*R*)-2,5-Diketopiperazines

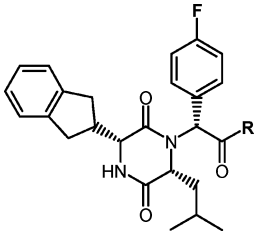


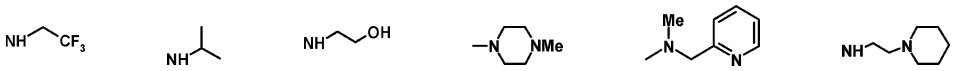


	5	**1**	**8**	**3**	**7**	**6**	**9**
R_1	$CHMe_2$	$CHMe_2$	CMe_3	CMe_3	$CHMe_2$	CMe_3	CMe_3
R2	isoBu	isoBu	isoBu	isoBu	R-secBu	isoBu	isoBu
EHOA%[a]	63	60	49	46	33	19	4
CHI log D[b]	3.3	3.4	3.4	3.4	3.0	2.4	3.4
cMR	13.16	13.51	14.58	14.85	15.69	16.61	18.62

[a] EHOA = estimated human oral absorption. [b] An HPLC method-based measurement of lipophilicity.[8] [c] Calculated molar refraction (Daylight v 4.81).

Table 3. The Estimated Human Oral Absorption (EHOA) of (3*R*,6*R*,7*R*)-4′-Fluorophenyl-2,5-diketopiperazine Amides





	31	**32**	**33**	**34**	**35**	**36**
EHOA%[a]	64	59	55	47	38	32
CHI log D[b]	3.3	3.21	2.3	2.7	3.2	2.0
cMR[c]	13.08	13.50	13.18	14.61	15.33	15.54

[a] EHOA = estimated human oral absorption. [b] An HPLC method-based measurement of lipophilicity.[8] [c] Calculated molar refraction (Daylight v 4.81).

using the data of 52 known drug molecules is shown by eq 2. For the 2,5-diketopiperazine series, a good correlation has been found between CHI (MeOH) and CHI log D as well as the number of positively chargeable groups (PosCh) values as is described by eq 3 (see Supporting Information).

$$\text{EHOA\%} = 0.0371\ \text{CHI (MeOH)} - 18.65\ \text{cMR} + 0.639\ \text{cMR}^2 + 103.8 \quad (1)$$

$$n = 46 \quad r = 0.73 \quad s = 18$$

$$\text{EHOA\%} = 1.31\ \text{CHI (MeOH)} - 10.93\ \text{cMR} + 88.6 \quad (2)$$

$$n = 52 \quad r = 0.81 \quad s = 19.7 \quad F = 15.9$$

$$\text{CHI (MeOH)} = 5.51\ \text{CHI log}\ D_{7.4} + 3.21\text{PosCh} + 72.0 \quad (3)$$

$$N = 70 \quad r^2 = 0.91 \quad s = 1 \quad F = 346$$

where n is the number of compounds, r is the multiple correlation coefficient, s is the standard error, and F is the Fisher test values.

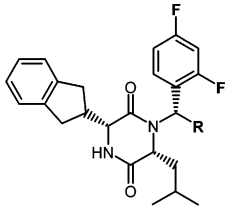
On the basis of eq 3 (which has been derived from the measured and calculated data of 70 2,5-diketopiperazines), the model described by eq 2 has been converted into eq 4, which can be considered as a local model for this series:

$$\text{EHOA \%} = 182.9 + 7.22\ \text{CHI log}\ D_{7.4} + 4.21\ \text{PosCh} - 10.93\ \text{cMR} \quad (4)$$

We measured the CHI log D for a range of 98 2,5-diketopiperazines and also calculated their cMRs and then derived their estimated human oral absorption (EHOA) from the above equation (see Supporting Information). This gave a range of absorption values 63% to 4% as illustrated in Tables 2 and 3. As bioavailability encompasses oral absorption less those effects due to transporter efflux or to first-pass elimination, the EHOA is the maximum possible bioavailability in the absence of these effects. Therefore, templates with high EHOA 63−45% may have high or low bioavailability, depending on their metabolic or efflux potential. Thus, the 2′,4′-difluorophenyl isopropylamide **1** has high rat bioavailability 46% and a high EHOA 60%; however, the 4′-dimethylaminophenyl isopropylamide **40**,[4] prepared previously with a high EHOA (46%), has low rat bioavailability (2%) possibly due to metabolism of the dimethylamino function (Table 1). However templates with low EHOA (<40%) would be expected to have low rat bioavailability. This enabled us to rank these 2,5-diketopiperazine oxytocin antagonists and work on those with the highest potential for human oral absorption and avoid those templates with low estimated human absorption**.** The data (Tables 2 and 3) showed that the highest EHOA would be achieved by keeping down the size of the aryl ring or the terminal N-substituent on the N^4-glycinamide group.

Therefore, to obtain the highest estimated human oral absorption, the priority was to focus on the smaller 2,5-diketopiperazine templates. What was required was to avoid large aryl rings in the exocyclic position and to choose a range of amides which were small in size. Therefore, from the disubstituted derivatives with good animal pharmacokinetics (Table 1), the difluorophenyl **1** and methylenedioxy **2** templates were chosen as starting points for a modification program and not the larger aryl morpholine **7** even though it had 61% bioavailability in dog. To this end first we made a range of small amides of the 2′,4′-difluorophenyl diketopiperazine as outlined in Table 4; all had good potency in the oxytocin binding

Table 4. Pharmacokinetics of (3*R*,6*R*,7*R*) -2′,4′-Difluorophenyl-2,5-diketopiperazines in the Rat and Dog



compd	R	pK_i	rat PK [b]			dog PK [c]			sol [d]	CHI
		hOTR[a]	AUC	Cl	*F*%	AUC	Cl	*F*%		log *D* [e]
			po			po				
1	$CONHCHMe_2$	8.9	2880	36	46	174	11	13	0.008	3.4
23	$CONH_2$	9.3	560	33	22	-	-	-	0.001	2.5
24	CONHMe	9.4	3690	32	98	82	13	11	0.089	2.9
25	$CONMe_2$	9.2	2971	15	53	770	7	51	0.083	3.3
27		9.2	660	22	17	710	4	37	0.001	3.3
28		8.9	2150	16	41	100	8	7	-	3.2
29	$CONMeCH_2CH_2OH$	9.9	2350	19	55	200	6	14	0.159	2.8
30		9.2	540	19	12	-	-	-	0.187	2.8
21*R*	CO_2H	9.2	805	53	30	23	21	5	0.216	1.7
39	CH_2OH	7.9	567	-	-	-	-	-	-	

[a] Displacement of [^{3}H] oxytocin from hOTR by the test compound.[7] 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 (h ng mL^{-1}) at 5 mg/kg, 5%DMSO/95%PEG400 formulation, Cl in mL min^{-1} kg^{-1}. [c] Dog PK ($n = 3$): AUC (h ng mL^{-1}) at 0.6 mg/kg, 5% DMSO/95% PEG400 formulation, Cl in mL min^{-1} kg^{-1}. [d] Solubility (mg/mL) a precipitation/HPLC-based measurement.[16] [e] An HPLC method-based measurement of lipophilicity.[8]

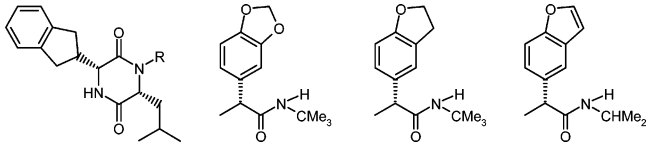
assay (pK_i = 8.9−10.0). The smallest tertiary amide, the 2′,4′-diflurophenyl *N*,*N*-dimethylamide **25** (EHOA 65%), was the best with increased bioavailability in both rat (53%) and dog (51%) and was the first time that good bioavailability in both species had been obtained with this template.

The smaller secondary amide **24** had a similar pharmacokinetic profile to the previous lead **1** with good bioavailability in the rat but not in the dog (Table 4). The azetidine tertiary amide **27** had moderate bioavailability in the dog, but only poor bioavailability in the rat. Smaller, more polar, amides **29** and **30** showed an increase in solubility; however, while the polar amides **28**, **29** maintained good rat pharmacokinetics, they exhibited poor pharmacokinetics in the dog, whereas the 3-hydroxy azetidine derivative **30** had poor bioavailability in the rat. Similarly the parent acid **21*R*** had moderate bioavailability in the rat, but only poor bioavailability in the dog.

Additional SAR in this series[4,15] has been developed by the discovery of the importance of the exocyclic carbonyl for good potency. Although the carboxylic acid **21*R*** has the same potency as the dimethylamide **25**, the primary alcohol **39**, prepared by reduction of the methyl ester **37**, resulted in a significant loss in potency indicating that the carbonyl of these exocyclic functions is important for good activity.

Aryl modifications to the 3′,4′-methylenedioxyphenyl lead **2** were also explored in order to increase bioavailability in the rat. Both saturated heterocyclic derivatives, the methylenedioxy **2** and the dihydrofuran **3**, had high clearance in the dog and rat, respectively (Table 5). In an attempt to minimize the metabolic liability of these bicyclic systems, we made the heteroaromatic benzofuran derivative **4**, which avoided increasing the overall size of the system (the estimated human oral absorption remained the same).

Table 5. Bioavailability of Heterocyclic (3*R*,6*R*,7*R*)-2,5-Diketopiperazines in the Rat and Dog



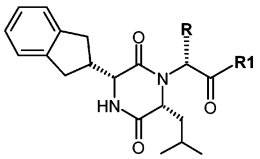
	2	**3**	**4**
pK_i [a]	8.4	8.4	9.0
rat Cl/*F*% [b]	34/48	39/27	9/68
dog Cl/*F*% [b]	23/14	14/9	6/95
EHOA% [c]	48	46	49

[a] Displacement of [^{3}H] oxytocin from hOTR by the test compound.[7] 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 ($n = 4$) and dog ($n = 3$) PK: Cl in mL min^{-1} kg^{-1}. [c] EHOA = estimated human oral absorption.

The benzofuran **4** had reduced clearance in both the rat and the dog relative to **2** and **3** and good bioavailability in both species (Table 5). Thus, we had two leads, the 2′,4′-difluorophenyl dimethylamide **25** and the benzofuran **4**, with high levels of potency (pK_i), good oral exposure in the rat and the dog, and good bioavailability in both species with low clearance.

Oxytocin Antagonist Potency and Selectivity vs Human Vasopressin Receptors. A further measure of the potency of our lead oxytocin antagonists **25** and **4** was established by their

Table 6. Inhibition of Whole Cell Oxytocin Activity[a] and Inhibition of OT binding at the Human OT (hOT) and Vasopressin Binding at the Human (V1a, V1b, and V2) Receptors[b]



			oxytocin	vasopressin		
compd	R	R1	hOTR, pK_i^b/fpK_i^a	hV1aR, pK_i^b	hV1bR, pK_i^b	hV2R, pK_i^b
25	2,4-diF-phenyl	NMe_2	9.2/8.8	<5.2	<5.2	6.5
4	5-benzofuran	NHiPr	9.0/8.4	<5.2	<5.2	5.3

[a] Functional OT antagonist activity measured by FLIPR assay; see Experimental Section.[17] [b] Displacement of [^{3}H] oxytocin from hOTR or vasopressin from hV1aR, hV1bR, and hV2R by the test compound. 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. 4.

functional oxytocin antagonist activity (**f**pK_i) in whole cells (by the FLIPR assay[17]) and is shown together with the binding inhibition data (pK_i) against the isolated recombinant oxytocin receptor (Table 6). In both assays, **25** is slightly more potent than **4**. Selectivity relative to human vasopressin receptors for dimethylamide **25** and isopropylamide **4** has been established by measuring their pK_i against the hV1aR, hV2R, and hV1bR (Table 6). Both lead oxytocin antagonists, **25** and **4**, have low affinity for all three human vasopressin receptors and OT receptor selectivity for **25** versus these receptors is >10000 for hV1a/hV1b and ~500 for hV2, while for **4** it is >6300 for hV1a/hV1b and ~7900 for hV2 (Table 6).

In Vivo Potency. The in vivo efficacy of the most promising compounds **25** and **4** was estimated using two anaesthetized rat models, where uterine contractions were elicited by iv bolus administration of oxytocin. In both methods, the reduction in intensity of uterine contractions in response to oxytocin was measured after iv bolus doses of **25** and **4**. In the first method, oxytocin was administered repeatedly at a constant dose, and the contractions together with the associated plasma concentrations of **25** and **4** were measured after increasing doses of the antagonists, enabling the determination of a plasma IC_{50}[18] (the concentration that caused 50% inhibition of response). In the second method, dose−response curves to iv administration of oxytocin were constructed after each increasing dose of **25** and **4**, and a DR_{10} value was determined (the antagonist dose that shifted the response to oxytocin 10-fold).[4]

The in vivo comparison of the two leads **25**, **4** and atosiban showed (Table 7) that the difluoro derivative **25** is of comparable efficacy to atosiban in both models, while the benzofuran **4** is 25−38-fold less active than **25** in the DR_{10} assay. In contrast, in vitro assessment using the rat receptor indicated that **25** and **4** have similar potency. Experiments carried out using the human receptor, where the potency of the compounds was determined with and without addition of HSA, suggested that the loss of activity in vivo is probably due to increased plasma protein binding. The presence of HSA reduced the activity of **4** by a factor of >11, while only reducing the activity of **25** by approximately 4.

Using a dose range of 0.01, 0.1, 0.5 mg/kg, the PK/PD relationship for **25** was determined ($n = 6$) by plotting the contractile response to oxytocin (0.3 μg/mL) against the plasma concentration fitted using an inhibitory sigmoidal C_{max} model, which gave a slope 1.36 and an IC_{50} of 110 ng/mL (227 nM) (Figure 2).

PK/PD comparisons of **25** with other oxytocin antagonists: atosiban, our previous oxytocin antagonist template benzoxazine **4c** (*N*-[(5-{[4-(2-oxo-2*H*-3,1-benzoxazin-1(4*H*)-yl)-1-piperidinyl]carbonyl}-1-benzofuran-2-yl)methyl]-2-[2-oxo-5-(trifluoromethyl)-1(2*H*)-pyridinyl]acetamide),[18] and Merck L-371257, in the rat uterine contractility model (Figure 3) using the same oxytocin challenge of 0.3 μg/mL throughout show the higher efficacy of **25** in this species.

The ability of **25** to inhibit OT-induced calcium mobilization in hOT-CHO cells has been demonstrated (measured using FLIPR,[20] Figure 4). The dimethylamide **25** was devoid of any agonist activity in this sensitive system and inhibited oxytocin (1 nM)-induced calcium mobilization with an IC_{50} of 3.7 nM (estimated pK_B 9.1).

Additional predevelopment studies revealed that **4** inhibited several Cyp450 isozymes, which was not the case with **25** (Table 8). Also no time-dependent inhibition[22] was observed for **25** against these five Cyp450 isozymes**.** The 2′,4′-diflurophenyl dimethylamide **25** was therefore chosen as the compound for further progression.

Further Predevelopment Studies. The 2′,4′-diflurophenyl dimethylamide **25** was shown not to be mutagenic in vitro in both the bacterial (HTFT/mini-Ames assay)[23] and mammalian cell (mouse lymphoma assay)[24] genotoxicity screens. A four day oral Safety Assessment study was carried out with **25** in female Sprague−Dawley rats at doses of 0, 30, 100, 300 (mg/kg/day) suspended in 0.5% (w/w) hydroxypropylmethyl-cellulose (HPMC) with 0.1% (w/w) Polysorbate 80 (Tween) in sterile water. There were no adverse clinical signs, no significant effects on body weight, food consumption, hematology, or clinical chemistry parameters at any dose level. Histopathological examination did not reveal any treatment-related effects at a dose up to 300 mg/kg/day.

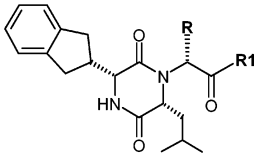
Conclusion

We have developed a short, efficient, and highly stereoselective synthesis of a series of (3*R*,6*R*,7*R*)-2,5-diketopiperazine amides. Disubstitution in the 7-aryl ring of the (3*R*,6*R*,7*R*)-2,5-diketopiperazines has produced a range of potent oxytocin antagonists with good oral bioavailablity in either the rat or dog. Property-based design has been used to enable us to focus effort on those templates with the greatest chance of high bioavailability in humans. Estimation of human oral absorption (EHOA), using measured lipophilicity (CHI log *D*) and calculated size (cMR), allowed us to rank a range of 2,5-diketopiperazine templates with the greatest chance of high bioavailability and suggested that 2,5-diketopiperazine templates which were small in size would be preferred. This led to the 2′,4′-diflurophenyl dimethylamide **25** and the benzofuran **4** with high levels of potency in the oxytocin binding assay (pK_i), good oral exposure in the rat and dog, and good bioavailability in both species with low clearance (Table 9). Evaluation in vivo has shown that **25** is (>20-fold) more potent than **4** and has comparable potency to atosiban (a marketed iv peptide oxytocin antagonist) in the rat, while being >60-fold more potent than the latter in vitro at the human receptor. Also **25** shows a high degree of selectivity toward the vasopressin receptors >10000 for hV1a/hV1b and ~500 for hV2 and has a good Cyp450 profile with no significant inhibition (IC_{50} > 49μM). It was negative in the genotoxicity screens and had a satisfactory safety profile warranting further consideration.

Experimental Procedures

General Procedures. Melting points were obtained using an Electrothermal digital melting point apparatus and are uncorrected.

Table 7. Potency of 2,5-Diketopiperazines **4**, **25** and Atosiban at the Rat and Human Oxytocin Receptor Compared with the Efficacy Obtained in the Rat Uterine Contractility Model



			rat iv				
compd	R	R1	DR_{10},[a] mg/kg	IC_{50},[b] nM	rOTR,[c] pK_i	hOTR,[d] pK_i	hOTR IC_{50}[e] shift + HSA
25	2,4-diF-phenyl	NMe_2	0.22	227	8.0	9.2	3.6
4	5-benzofuran	NHiPr	5.4-8.4	-	7.8	9.0	11.2
	atosiban		0.63	~186	7.2	7.4	-

[a] DR_{10} determination.[4] [b] Plasma IC_{50}.[18] [c] Displacement of [^{3}H] oxytocin from rOT by the test compound.[7d] Displacement of [^{3}H] oxytocin from hOTR by the test compound.[7] 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. [e] HSA shift = ratio of displacement of [^{3}H] oxytocin from hOTR by the test compound in the presence and absence of 50 mg/mL human serum albumin.[19]

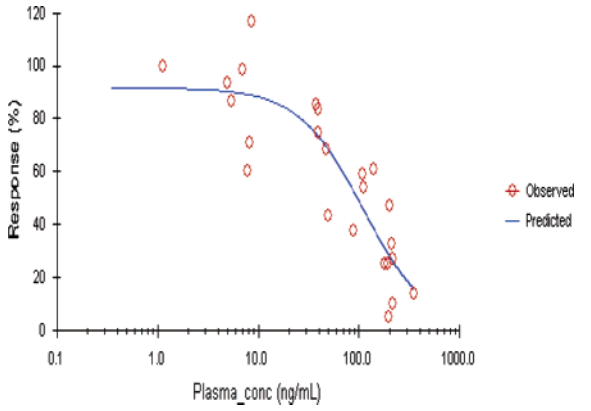


Figure 2. Inhibition of uterine contractions in the rat PK/PD model by **25**.

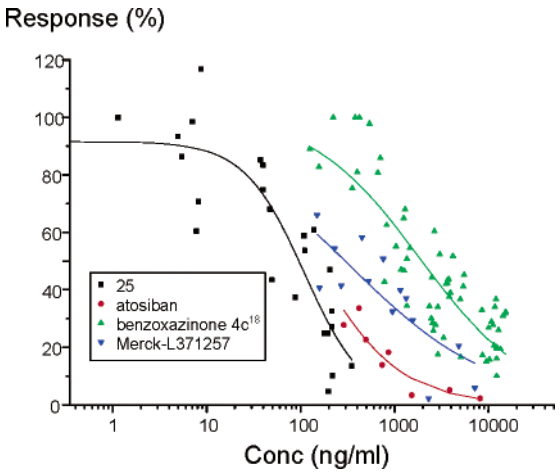


Figure 3. Comparison of in vivo efficacies for various oxytocin antagonists in the rat uterine contractility model.

All purifications by flash chromatography were performed using Kieselgel 60, Merck 9385 silica gel or using a Biotage Quad 3 system with Biotage silica cartridges. Hydrophobic frits refer to filtration tubes sold by Whatman. Preparative plate chromatography was performed using Whatman PK6F silica gel 60A plates eluting with ethyl acetate−cyclohexane or 2-propanol−dichloromethane mixtures. Monitoring of reactions by TLC used Merck 60 F254 silica gel glass backed plates (5 × 10 cm), eluted with mixtures of ethyl acetate and cyclohexane and visualized by UV light, followed by heating with aqueous phosphomolybdic acid. Analytical HPLC were run on a Hewlett-Packard 1090 HPLC instrument, equipped with a Intersil M column ODS2. Standard conditions were eluent system A (H_2O, 0.1% H_3PO_4) B (95% MeCN/H_2O, 0.1% H_3PO_4):

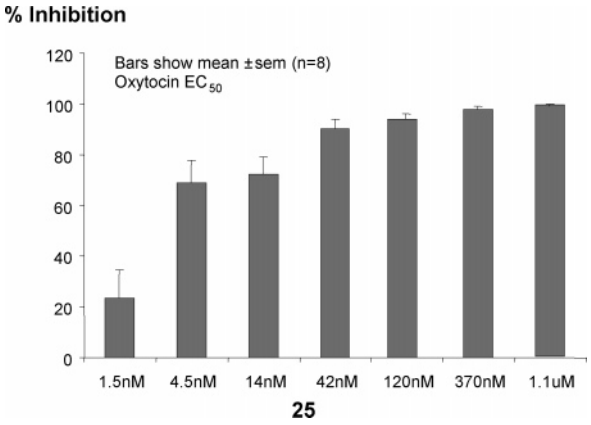
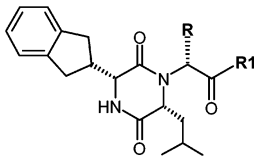


Figure 4. Inhibition of oxytocin (1 nM)-induced calcium signal on hOT-CHO cells.

Gradient 0%B 2 min, 0%-100% B 40 min,100% B 10 min; flow rate = 1 mL/min,, λ = 215 nm). Retention times (t_r) are given in minutes. LCMS were run on a Hewlett-Packard 1050 coupled with a Micromass Platform II equipped with a Supelco ABZplus column. Standard conditions were eluent system A (H_2O, 0.1% formic acid, 10 mmol ammonium acetate) B (MeCN, 0.05% formic acid): gradient **1** 100%A 0.7 min, 100%A to 100% B 3.5 min,100% B 3.5 min, 100% to 0%B 0.3 min; flow rate = 1 mL/min). Gradient **2** 100%A 0.7 min, 100%A to 100% B 4.2 min, 100% B 1.1 min, 100% to 0%B 0.2 min; flow rate = 1 mL/min). Gradient **3** 100%A 3 min, 100%A to 100% B 20 min, 100% B 5 min, 100% to 0%B 2 min; flow rate = 1 mL/min). All NMR spectra were run on a Bruker 400 MHz instrument generally as solutions in CDCl3 unless otherwise stated. IR spectra were recorded on a Bio-rad FTS7 spectrometer from thin films on NaCl plates, a KBr mix, or solutions in the solvent specified. Mass spectra were run on an electrospray Hewlett-Packard 5989B instrument. 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 anaylsis using a Chiracel OJ or Chiral Pak AD464 column with a UV detector λ = 215 nm and with eluents and flow rates as indicated in each case. Final organic solutions were dried 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 Butterworths.

(2*R*)-2,3-Dihydro-1*H*-inden-2-yl({[(phenylmethyl)oxy]carbonyl}amino)ethanoic Acid (10). *R*-Indanylglycine[4] (1.91 g, 10 mmol) was suspended in dioxane (10 mL) and water (10 mL). To this were added triethylamine (1.7 mL) and *N*-(benzyloxycarbonyloxy)succinimde (2.54 g), and the reaction mixture was stirred

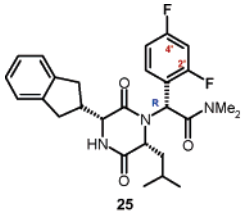
Table 8. Cyp450[21] Inhibition Profile



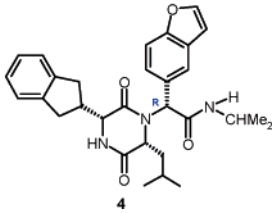
			IC$_{50}$, μM					
compd	R	R1	1A2	2C9	2C19	2D6	3A4-DEF[a]	3A4-PPR[a]
25	2, 4-diF-phenyl	NMe_2	64	49	>100	>100	89	62
4	5-benzofuran	NHiPr	73	3.5	>100	62	2.5	4.3

[a] Diethoxyfluorescein (DEF) and 7-{3-(4-phenylpiperazin-1-ylmethyl)benzyl}resorufin (PPR) are fluorogenic substrates for Cyp3A4.

Table 9. Profiles of 2,5-Diketopiperazines Oxytocin Antagonist **25** and **4**



25



4

		rat[b]				dog[c]				in vitro	rat in vivo efficacy		Cyp450[g]
compd	pK_i[a]	*F*%	Cl	$t_{1/2}$	V_{dss}	*F*%	Cl	$t_{1/2}$	V_{dss}	IC$_{50}$ shift + HSA[d]	DR$_{10}$, [e]mg/kg, iv	IC$_{50}$, nM,[f] iv	IC$_{50}$, μM, 2C9/3A4
25	9.2	53	15	1.1	1.2	51	7	2.1	1.1	3.6	0.22	227	>49
4	9.0	68	9	2	1.4	95	6	2.4	0.9	11.2	5.4−8.4		3.5/4.3
atosiban											0.63	~186	

[a] Displacement of [^{3}H] oxytocin from hOTR by the test compound.[7] 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 (h ng mL^{-1}) at 5 mg/kg, 5% DMSO/95% PEG400 formulation, Cl in mL min^{-1} kg^{-1}; V_{dss} in L kg^{-1}; $t_{1/2}$ in h. [c] Dog PK (n = 3): AUC (h ng mL^{-1}) at 0.6 mg/kg, 5% DMSO/95% PEG400 formulation, Cl in mL min^{-1} kg^{-1}; V_{dss} in L kg^{-1}; $t_{1/2}$ in h. [d] HSA shift = ratio of displacement of [^{3}H] oxytocin from hOTR by the test compound in the presence and absence of 50 mg/mL human serum albumin.[19] [e] DR$_{10}$ determination.[4] [f] Plasma IC$_{50}$.[18] [g] Cyp450[21] inhibition profile.

rapidly at room temperature for 2 days. The reaction mixture was poured into water (50 mL) and extracted with chloroform (3 × 30 mL). The combined organic phase was washed with 1 M hydrochloric acid (50 mL) and water (50 mL). This was dried over magnesium sulfate and the solvent removed in vacuo to give **10** (3.06 g, 94%): ^{1}H NMR ($CDCl_3$) δ 7.40−7.29 (m, 5H), 7.21−7.11 (m, 4H), 5.28 (d, 1H, J = 8.6 Hz), 5.11 (s, 2H), 4.57 (m, 1H), 3.14−2.79 (m, 5H); LCMS *m*/*z* 326 (MH$^+$) single component, Gradient 2 (t_R 3.35 min); Chiral HPLC: 100.00%, (t_R 11.77 min), (Chiral HPLC (Chiralpak AD, 20% EtOH/heptane (0.1% TFA) @ 215 nm) ee 100.0%, [Chiral HPLC of racemate shows clear separation of S and R isomers: 49.9%, (t_R 10.11 min) and 50.1% (t_R 11.70 min)]; HRMS calcd for for $C_{19}H_{19}NO_4$ (MH$^+$) 326.1392, found 326.1387; HPLC: 99.5% (t_R 13.9 min). $[\alpha]^{23}_d$ = −5.5° (*c* = 0.018 in MeOH, 10 cm cell).

(2*RS*)-2-(2,4-Difluorophenyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-*N*-(2-hydroxyphenyl)ethanamide (17). To a solution of D-leucine methyl ester hydrochloride **11** (1.45 g, 8 mmol) in methanol (10 mL) were added triethylamine (1.12 mL) and 2,4-difluorobenzaldehyde **13** (0.875 mL, 8 mmol). The mixture was stirred for 3 days before the acid **10** (2.6 g, 8 mmol) and 2-benzyloxyphenylisocynanide[6] **12** (1.76 g, 8.4 mmol) were sequentially added. The reaction mixture was left to stand for 24 h. The solvent was removed in vacuo, and the residue **15** was separated between ethyl acetate (200 mL) and water (200 mL). The organic phase was washed with brine. To this solution were added palladium on carbon (2.0 g) and acetic acid (10 mL), and the reaction mixture was stirred under an atmosphere of hydrogen for 2 h. The mixture was filtered through Celite, washed with water (3 × 100 mL), saturated sodium bicarbonate solution, and brine, and dried over magnesium sulfate. The solvent was evaporated, and the crude product was purified by flash column chromatography eluting with ethyl acetate−cyclohexane (1:1 to 2:1) to give as a mixture of diastereomers the phenol **17** (2.0 g, 46%): ^{1}H NMR (DMSO-d_6) δ 9.81, 9.76 (m, 1H), 9.55 (m, 1H), 8.65, 8.56 (d, 1H, J = 3.8 Hz), 7.90, 7.87 (d, 1H, J = 8 Hz), 7.54−7.38 (m, 1H), 7.21 (m, 3H), 7.12 (m, 2H), 6.99−6.74 (m, 3H), 6.48, 6.16 (s, 1H), 3.88, 3.47 (m, 1H), 3.83, 3.74 (dd, 1H, J = 4, 10 Hz), 3.07−2.94 (m, 2H), 2.91−2.71 (m, 3H), 1.77−1.54 (m, 2H), 1.46, 0.77 (m, 1H), 0.72, 0.65 (d, 3H, J = 7 Hz), 0.64 (d, 3H, J = 6 Hz); LCMS *m*/*z* 548 (MH$^+$) single component, Gradient 2 (t_R 3.59 min); HRMS calcd for for $C_{31}H_{31}F_2N_3O_4$ (MNa$^+$) 570.2180, found 570.2184; HPLC: 33% (t_R 14.81 min) and 66% (t_R 14.93 min).

The following were similarly prepared.

(2*RS*)-2-[(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-2-(4-fluorophenyl)-*N*-(2-hydroxyphenyl)ethanamide (18). Similarly prepared as for **17**, using 4-fluorobenzaldehyde, the phenol **18** was isolated as a white solid (73%): ^{1}H NMR (CD_3OD) δ 7.82 (m, 1H), 7.55 (m, 2H), 7.27−7.15 (m, 4H), 7.10 (m, 2H), 6.98 (m, 1H), 6.81 (m, 2H), 5.89 (s, 1H), 3.95 (d, 1H, J = 9.3 Hz), 3.74 (dd, 1H, J = 4.5, 10.5 Hz), 3.13−2.96 (m, 3H), 2.94−2.81 (m, 2H), 1.87−1.55 (m, 3H), 0.76 (d, 3H, J = 6.5 Hz), 0.59 (d, 3H, J = 6.5 Hz); LCMS *m*/*z* 530 (MH$^+$) single component, Gradient 2 (t_R 3.49 min); HRMS calcd for $C_{31}H_{32}FN_3O_4$ (MNa$^+$) 552.2275, found 552.2274; HPLC: 95% (t_R 14.82 min).

(2*RS*)-(2,4-Difluorophenyl)[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]ethanoic Acid (21). Carbonyldiimidazole (1.42 g, 1.6 equiv) was suspended in anhydrous dichloromethane (10 mL), and the suspension was left at room temperature for 15 min. The phenol **17** (3.00 g, 5.5 mmol) was then added, and the resultant solution was stirred at room temperature for 16 h. The resulting yellow solution was then evaporated under reduced pressure, and the residue was treated with a mixture of water−acetone (1:1, 10 mL). The mixture was stirred for 2 h, then the acetone was removed under reduced pressure and the residue was partitioned between dichloromethane (100 mL) and 0.1 M hydrochloric acid (100 mL). The organic phase was separated using a hydrophobic frit and then evaporated to low volume and

purified by flash column chromatography eluting with ethyl acetate−cyclohexane (1:1), then ethyl acetate, followed by ethyl acetate−methanol (1:1), to give acid **21** as a mixture of diastereomers (2.16 g 86%): ^{1}H NMR (CD_3OD): δ 7.94 (s, 0.4H), 7.44−7.33 (m, 1H), 7.08−6.80 (m, 7.3H), 5.92 (s, 0.7H), 5.53 (s, 0.3H), 3.93−3.88 (m, 0.6H), 3.75−3.67 (m, 1H), 3.53−3.48 (m, 0.4H), 2.97−2.56 (m, 5H), 1.81−1.71 (m, 0.4H), 1.62−1.38 (m, 2H), 0.80−0.70 (m, 0.6H), 0.62 (d, J = 6.5 Hz, 1.1H), 0.54 (d, J = 6.5 Hz, 1.9H), 0.49 (d, J = 6.5 Hz, 1.1H), 0.44 (d, J = 6.5 Hz, 1.9H); LCMS *m*/*z* 457 (MH^+) two component, Gradient 2 (t_R 3.47 and 3.68 min).

These were separated on a chiral reverse-phase column (Chiralcel OD, eluted with 15% propan-2-ol/heptane containing 0.1% TFA) to give the 2*R*,3*R*,6*R* acid **21*R*** diastereomer as a white solid (1.60 g, 64%): ^{1}H NMR ($CDCl_3$) δ 7.90 (m, 1H), 7.72 (m, 1H), 7.20−7.11 (m, 4H), 6.92 (m, 1H), 6.86 (m, 1H), 5.39 (s, 1H), 4.01 (m, 1H), 3.94 (dd, 1H, J = 3.8, 9.4 Hz), 3.15 (dd, 1H, J = 7, 15 Hz), 3.03 (m, 2H), 2.88 (m, 1H), 2.77 (dd, 1H, J = 9, 15 Hz), 1.92 (m, 1H), 1.85 (m, 1H), 1.55 (m, 1H), 0.91 (d, 3H, J = 6.5 Hz), 0.88 (d, 3H, J = 6.5 Hz); LCMS *m*/*z* 457 (MH^+) single component, Gradient 2 (t_R 3.4 min); HRMS calcd for for $C_{25}H_{26}F_2N_2O_4$ (MH^+) 457.1939, found 457.1942; HPLC: 99% (t_R 16.65 min).

The following were similarly prepared.

(2*RS*)-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl](4-fluorophenyl)ethanoic Acid (22). Similarly prepared as for **21**, the acid **22** as a mixture of diastereomers was isolated as a white solid (76%): ^{1}H NMR ($CDCl_3$): δ 7.79−7.72 (m, 1H), 7.46−7.05 (m, 1H), 7.08−6.80 (m, 8H), 5.49 (s, 0.3H), 5.38 (s, 0.7H), 4.07−3.90 (m, 1.7H), 3.75−3.67 (m, 0.3H), 3.17−2.73 (m, 5H), 1.83−1.62 (m, 2H), 1.29−1.19 (m, 1H), 0.82−0.62 (m, 6H); LCMS *m*/*z* 439 (MH^+) two component, Gradient 2 (t_R 3.36 and 3.49 min). A sample (50 mg) of this mixture was separated by preparative TLC to give the 2*R*,3*R*,6*R* **22*R*** diastereomer as a white solid (27 mg): ^{1}H NMR ($CDCl_3$): δ 7.89 (m, 1H), 7.42 (m, 2H), 7.20−7.05 (m, 6H), 5.44 (s, 1H), 4.02 (d, 1H, J = 4, 9.5 Hz), 3.93 (dd, 1H, J = 3.5, 11 Hz), 3.15−2.98 (m, 5H), 2.90−2.73 (m, 2H), 1.73 (m, 1H), 1.64 (m, 1H), 1.18 (m, 1H), 0.78 (d, 3H, J = 6.5 Hz), 0.63 (d, 3H, J = 6.5 Hz); LCMS *m*/*z* 439 (MH^+) single component, Gradient 2 (t_R 3.47 min); HRMS calcd for $C_{25}H_{27}FN_2O_4$ (MH^+) 439.2033, found 439.2030; HPLC: 98% (t_R 15.55 min).

(2*S*)-2-(2,4-Difluorophenyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-*N*,*N*-dimethylethanamide (26) and (2*R*)-2-(2,4-Difluorophenyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-*N*,*N*-dimethylethanamide (25). The acid **21** (0.724 g, 1.59 mmol), predried over P_4O_{10} in vacuo for 5 h, was dissolved in anhydrous dichloromethane−acetonitrile (1:1, 6 mL) and treated with triethylamine (0.223 mL) and BOP-Cl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.450 g, 1.77 mmol)), and the mixture was sonicated for 1 min to give a gelatinous mass. After 10 min at room temperature, a solution of dimethylamine in tetrahydrofuran (10 mL of 2 M solution) was added to give a clear solution, and this was left for 16 h at room temperature. The solvents were removed under reduced pressure, and the mixture was partitioned between dichloromethane and 0.1 M hydrochloric acid. The organic phase was separated using a hydrophobic frit and evaporated under reduced pressure. The crude product was purified by flash column chromatography eluting with ethyl acetate−cyclohexane (1:1) then ethyl acetate followed by ethyl acetate−methanol (9:1) to give the dimethylamide diastereomer **26** as a colorless solid (143 mg, 18%): ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 1H), 7.20 (m, 2H), 7.14 (m, 2H), 6.99−6.91 (m, 2H), 6.64 (d, 1H, J = 4 Hz), 6.32 (s, 1H), 3.95 (dd, 1H, J = 4, 10 Hz), 3.42 (m, 1H), 3.15 (m, 1H), 3.08 (m, 2H), 3.06 (s, 3H), 2.97 (m, 1H), 2.91 (s, 3H), 2.76 (m, 1H), 2.04 (m, 1H), 1.77 (m, 1H), 1.73 (m, 1H), 0.84 (d, 3H, J = 6.6 Hz), 0.52 (d, 3H, J = 6.6 Hz); Circular dichroism (CH_3CN) λ_{max} 226.2 nm, dE −3.90; E3457; LCMS *m*/*z* 484 (MH^+) single component, Gradient 2 (t_R 3.30 min); HRMS calcd for $C_{27}H_{31}F_2N_3O_3$ (MH^+) 484.2412, found 484.2405; HPLC: 99% (t_R 13.0 min).

The dimethylamide diastereomer **25** was isolated as a colorless solid (0.285 g, 36%) indentical in all respects as that prepared from phenol **17**.

(2*R*)-2-(2,4-Difluorophenyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-*N*,*N*-dimethylethanamide (25). Carbonyldiimidazole (4.80 g, 1.54 equiv) was suspended in anhydrous dichloromethane (40 mL), and the suspension was left at room temperature for 15 min. The phenol **17** (10.50 g, 19.17 mmol), predried in vacuo over P_4O_{10} for 24 h, was then added with stirring, and the resultant solution was stirred at room temperature for 6 h. The resulting yellow solution was then treated with a 2.0 M solution of dimethylamine in tetrahydrofuran (54 mL, 5.6 equiv), and the resulting mixture was stirred at room temperature for 16 h. The solvents plus residual dimethylamine were removed under reduced pressure, and the reaction mixture was taken up in dichloromethane (200 mL) and washed with 1 M hydrochloric acid (200 mL). The organic phase was separated using a hydrophobic frit and evaporated under reduced pressure to a gum (NMR indicated that the ratio of **25** to **26** was 10:1). The crude product was purified by flash column chromatography eluting with ethyl acetate−cyclohexane (2:1), then ethyl acetate, followed by ethyl acetate−methanol (9:1) to give the dimethylamide **25** as a colorless solid. (5.753 g, 62%). m.p 176−177 °C; $[\alpha]^{23}_D$ = −167° (*c*1.0, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.44 (dt, J = 8.5 Hz, 6.3 Hz, 1H, difluorophenyl-*6H*), 7.26−7.14 (m, 4H, indanyl-*arylH*), 7.02−6.91 (m, 2H, difluorophenyl-*5H,-3H*), 6.62 (broad s, 2H, NC*H*difluorophenyl, CON*H*), 4.09 (dd, J = 11.3 Hz, 3.0 Hz, 1H, NC*H*isobutyl), 3.98 (dd, J = 10.3 Hz, 4.5 Hz, 1H, NC*H*indanyl), 3.19−3.02 (m, 3H, indanyl-*3H,-1H*), 2.99 (s, 3H, CON*Me*Me), 2.93−2.81(m, 4H, indanyl-*2H*, CONMe*Me*), 2.80−2.72 (m, 1H, indanyl-*1H*), 1.61−1.51 (m, 2H, C*H*CHMe$_2$), CH_2CHMe_2), 0.75−0.64 (m, 4H, CH*H*CHMe$_2$, $CH_2CHMeMe$), 0.42 (d, J = 6.5 Hz, 3H, $CH_2CHMeMe$); Circular dichroism (CH_3CN) λ_{max} 205.8 nm, dE 12.04; E31054; λ_{max} 229.2 nm, dE −14.95; E3708; LCMS *m*/*z* 484 (MH^+) single component, Gradient 2 (t_R 3.43 min); HRMS calcd for $C_{27}H_{31}F_2N_3O_3$ (MNa^+) 506.2231, found 506.2225; HPLC 99% (t_R 13.76 min). Anal. ($C_{27}H_{31}F_2N_3O_3$) C, H, N.

The dimethylamide **26** was isolated as a white solid (0.578 g, 6%) indentical in all respects as that prepared from acid **21**.

The following compounds were similarly prepared.

(2*R*)-2-(2,4-Difluorophenyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]ethanamide (23). Similarly prepared as for **25**, using ammonia and phenol **17**, the amide **23** was isolated as a white solid (36%): ^{1}H NMR (CD_3OD) δ 7.58 (m, 1H), 7.19 (m, 2H), 7.11 (m, 4H), 6.19 (s, 1H), 4.00 (dd, 1H, J = 3.5, 11 Hz), 3.87 (d, 1H, J = 10 Hz), 3.13−2.93 (m, 3H), 2.87−2.74 (m, 2H), 1.67−1.52 (m, 2H), 0.90 (m, 1H), 0.71 (d, 3H, J = 6.5 Hz), 0.53 (d, 3H, J = 6.5 Hz); LCMS *m*/*z* 456 (MH^+) single component, Gradient 2 (t_R 3.30 min); HRMS calcd for $C_{25}H_{27}F_2N_3O_3$ (MNa^+) 478.1918, found 478.1928; HPLC 99% (t_R 12.86 min). Anal. ($C_{25}H_{27}F_2N_3O_3$) C, H, N.

(2*R*)-2-(2,4-Difluorophenyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-*N*-methylethanamide (24). Similarly prepared as for **25**, using methylamine and phenol **17**, the amide **24** was isolated as a white solid (41%): ^{1}H NMR ($CDCl_3$) δ 7.71 (dt, 1H, J = 6.5, 8.5 Hz), 7.60 (d, 1H, J = 4 Hz), 7.18 (m, 2H), 7.14 (m, 2H), 6.95 (m, 1H), 6.89 (m, 1H, J = 2.5, 8.5, 11 Hz), 6.12 (q, 1H, J = 5 Hz), 5.45 (s, 1H), 3.99 (dd, 1H, J = 3, 10 Hz), 3.93 (dd, 1H, J = 4, 9 Hz), 3.14 (dd, 1H, J = 7, 15 Hz), 3.03 (d, 2H, J = 8 Hz), 2.89 (m, 1H), 2.85 (d, 3H, J = 5 Hz), 2.80 (dd, 1H, J = 8.5, 15 Hz), 1.84 (m, 1H), 1.79 (m, 1H), 1.46 (m, 1H), 0.87 (d, 3H, J = 6.5 Hz), 0.80 (d, 3H, J = 6.5 Hz); LCMS *m*/*z* 470 (MH^+) single component, Gradient 2 (t_R 3.36 min); HRMS calcd for $C_{26}H_{29}F_2N_3O_3$ (MNa^+) 492.2075, found 492.2062; HPLC 99% (t_R 13.13 min).

(3*R*,6*R*)-1-[(1*R*)-2-(1-Azetidinyl)-1-(2,4-difluorophenyl)-2-oxoethyl]-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-piperazinedione (27). Similarly prepared as for **25**, using azetidine and phenol **17**, the amide **27** was isolated as a white solid (21%): ^{1}H NMR ($CDCl_3$) δ 7.65 (m, 1H), 7.24−7.11 (m, 4H), 6.99 (m,

1H), 6.91 (m, 1H), 6.80 (d, 1H, $J = 4$ Hz), 6.05 (s, 1H), 4.32 (m, 1H), 4.14 (m, 1H), 4.06 (m, 1H), 4.02−3.93 (m, 2H), 3.75 (m, 1H), 3.19−3.03(m, 3H), 2.86 (m, 1H), 2.77 (m, 1H), 2.34−2.14 (m, 2H), 1.63 (m, 1H), 1.55 (m, 1H), 0.87 (m, 1H), 0.70 (d, 3H, $J = 6.5$ Hz), 0.45 (d, 3H, $J = 6.5$ Hz); LCMS *m*/*z* 496 (MH^+) single component, Gradient 2 (t_R 3.53 min); HRMS calcd for $C_{28}H_{31}F_2N_3O_3$ (MNa^+) 518.2231, found 518.2241; HPLC 99% (t_R 13.81 min); Anal. ($C_{28}H_{31}F_2N_3O_3$_$0.5H_2O$) C, H, N.

(3*R*,6*R*)-1-[(1*R*)-1-(2,4-Difluorophenyl)-2-(4-morpholinyl)-2-oxoethyl]-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-piperazinedione (28). Similarly prepared as for **25,** using morpholine and phenol **17**, the amide **28** was isolated as a white solid (61%): ^{1}H NMR ($CDCl_3$) δ 7.43 (dt, 1H, $J = 6$, 8.5 Hz), 7.25−7.13 (m, 4H), 7.01 (m, 1H), 6.96 (m, 1H), 6.89 (d, 1H, $J = 4$ Hz), 6.63 (s, 1H), 4.06 (m, 1H), 3.99 (dd, 1H, $J = 4.5$, 10 Hz), 3.76−3.56 (m, 5H), 3.39 (m, 1H), 3.30 (m, 1H), 3.19−3.01(m, 4H), 2.87 (m, 1H), 2.77 (m, 1H, $J = 9$, 15 Hz), 1.56 (m, 2H), 0.72−0.63 (m, 4H), 0.43 (d, 3H, $J = 6.5$ Hz); LCMS *m*/*z* 526 (MH^+) single component, Gradient 2 (t_R 3.35 min); HRMS calcd for $C_{29}H_{33}F_2N_3O$ (MNa^+) 548.2337, found 548.2357; HPLC 95% (t_R 13.54 min).

(2*R*)-2-(2,4-Difluorophenyl)-2-[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-*N*-(2-hydroxyethyl)-*N*-methylethanamide (29). Similarly prepared as for **25,** using 2-(methylamino)ethanol and phenol **17**, the amide **29** was isolated as a white solid (54%): ^{1}H NMR ($CDCl_3$) δ Rotamers 7.63−7.48 (m, 1H), 7.26−7.12 (m, 4H), 7.04−6.84 (m, 3H), 6.61, 6.57 (s, 1H), 4.07−3.53 (m, 6H), 3.19−2.99 (m, 3H), 3.00, 2.92 (s, 3H), 2.90−2.71 (m, 2H), 1.67−1.51 (m, 2H), 0.96−0.76 (m, 1H), 0.69 (m, 3H), 0.40, 0.34 (d, 3H, $J = 6.3$ Hz); LCMS *m*/*z* 514 (MH^+) single component, Gradient 2 (t_R 3.27 min); HRMS calcd for for $C_{28}H_{33}F_2N_3O_4$ (MH^+) 514.2517, found 514.2523; HPLC: 98% (t_R 13.08 min).

(3*R*,6*R*)-1-[(1*R*)-1-(2,4-Difluorophenyl)-2-(3-hydroxy-1-azetidinyl)-2-oxoethyl]-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-piperazinedione (30). Similarly prepared as for **25**, using azetidin-3-ol[25] and phenol **17**, the amide **30** was isolated as a colorless solid (45%): ^{1}H NMR ($CDCl_3$) δ Rotamers 7.58 (m, 1H), 7.25−7.10 (m, 4H), 7.00 (m, 1H), 6.92 (m, 1H), 6.11, 6.05 (s, 1H), 4.70−4.15 (m, 4H), 4.05−3.92 (m, 3H), 3.84, 3.59 (m, 1H), 3.23−2.99 (m, mH), 2.90−2.74 (m, 2H), 1.65−1.50 (m, 2H), 0.90, 0.74 (m, 1H), 0.69 (m, 3H), 0.47, 0.42 (d, 3H, $J = 6.3$ Hz); LCMS *m*/*z* 512 (MH^+) single component, Gradient 2 (t_R 3.2 min); HRMS calcd for for $C_{28}H_{31}F_2N_3O_4$ (MH^+) 512.2361, found 512.2370; HPLC: 98% (t_R 12.85 min).

(2*R*)-2-[(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-2-(4-fluorophenyl)-*N*-(2,2,2-trifluoroethyl)ethanamide (31). Similarly prepared as for **25**, using (2,2,2-trifluoroethyl)amine and phenol **18**, the amide **31** was isolated as a white solid (27%): ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 2H), 7.23−7.10 (m, 6H), 6.74 (d, 1H, $J = 3.5$ Hz), 6.45 (t, 1H, $J = 6.5$ Hz), 5.14 (s, 1H), 4.06−3.82 (m, 4H), 3.16 (dd, 1H, $J = 7$, 14.5 Hz), 3.04 (m, 2H), 2.89 (m, 1H), 2.78 (dd, 1H, $J = 9$, 14.5 Hz), 1.85 (m, 1H), 1.77 (m, 1H), 1.47 (m, 1H), 0.87 (d, 3H, $J = 6.5$ Hz), 0.82 (d, 3H, $J = 6.5$ Hz); LCMS *m*/*z* 520 (MH^+) single component, Gradient 2 (t_R 3.48 min); HRMS calcd for $C_{27}H_{29}F_4N_3O_3$ (MH^+) 520.2223, found 520.2220; HPLC: 99% (t_R 14.2 min).

(2*R*)-2-[(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-2-(4-fluorophenyl)-*N*-(1-methylethyl)ethanamide (**32**). Similarly prepared as for **25**, using isopropylamine and phenol **18**, the amide **32** was isolated as a white solid (18%): ^{1}H NMR ($CDCl_3$) δ 7.46−7.41 (m, 2H), 7.25−7.08 (m, 6H), 6.51 (d, 1H, $J = 3.5$ Hz), 6.60 (d, 1H, $J = 7.5$ Hz), 5.10 (s, 1H), 4.09 (m, 1H), 3.98−3.91 (m, 2H), 3.16 (dd, 1H, $J = 7$, 15 Hz), 3.07 (m, 2H), 2.90 (m, 1H), 2.87 (dd, 1H, $J = 8.5$, 15 Hz), 1.83 (m, 1H), 1.73 (m, 1H), 1.43 (m, 1H), 1.12 (d, 6H, $J = 6.5$ Hz), 0.84 (d, 3H, $J = 6.5$ Hz), 0.79 (d, 3H, $J = 6$ Hz); LCMS *m*/*z* 480 (MH^+) single component, Gradient 2 (t_R 3.31 min); HRMS calcd for $C_{28}H_{34}FN_3O_3$ (MH^+) 480.2662, found 480.2657; HPLC 99% (t_R 13.85 min).

(2*R*)-2-[(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-2-(4-fluorophenyl)-*N*-(2-hydroxyethyl)ethanamide (**33**). Similarly prepared as for **25**, using 2-aminoethanol and phenol **18**, the amide **33** was isolated as a white solid (54%): ^{1}H NMR ($CDCl_3$) δ 7.45 (m, 2H), 7.23−7.09 (m, 6H), 7.05 (d, 1H, $J = 3.5$ Hz), 6.05 (t, 1H, $J = 5.5$ Hz), 4.96 (s, 1H), 3.97 (dd, 1H, $J = 4$, 10 Hz), 3.87−3.81 (m, 2H), 3.65−3.53 (m, 2H), 3.23−3.13 (m, 2H), 3.03 (m, 2H), 2.90 (m, 1H), 2.79 (dd, 1H, $J = 9$, 15 Hz), 1.89 (m, 1H), 1.83 (m, 1H), 1.64 (m, 1H), 0.89 (d, 3H, $J = 6.5$ Hz), 0.83 (d, 3H, $J = 6.5$ Hz); LCMS *m*/*z* 482 (MH^+) single component, Gradient 2 (t_R 3.02 min); HRMS calcd for $C_{27}H_{32}FN_3O_4$ (MH^+) 482.2455, found 482.2459; HPLC >98% (t_R 12.22 min).

(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-1-[(1*R*)-1-(4-fluorophenyl)-2-(4-methyl-1-piperazinyl)-2-oxoethyl]-6-(2-methylpropyl)-2,5-piperazinedione (**34**). Similarly prepared as for **25**, using *N*-methylpiperazine and phenol **18**, the amide **34** was isolated as a white solid (34%): ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 2H), 7.25−7.12 (m, 6H), 6.54 (s, 1H), 6.31 (d, 1H, $J = 4$ Hz), 4.12 (m, 1H), 3.98 (dd, 1H, $J = 4.5$, 10.5 Hz), 3.68 (m, 2H), 3.36 (m, 1H), 3.21−3.01 (m, 4H), 2.87 (m, 1H), 2.75 (dd, 1H, $J = 9$, 15 Hz), 2.41 (m, 1H), 2.30 (m, 2H), 2.23 (s, 3H), 1.95 (m, 1H), 1.53 (m, 1H), 1.43 (m, 1H), 0.62 (d, 3H, $J = 6.5$ Hz), 0.57 (m, 1H), 0.41 (d, 3H, $J = 6.3$ Hz); LCMS *m*/*z* 521 (MH^+) single component, Gradient 2 (t_R 2.62 min); HRMS calcd for $C_{30}H_{37}FN_4O_3$ (MH^+) 521.2928, found 521.2919; HPLC 98% (t_R 10.13 min).

(2*R*)-2-[(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-2-(4-fluorophenyl)-*N*-methyl-*N*-(2-pyridinylmethyl)ethanamide (35). Similarly prepared as for **25**, using methyl(2-pyridinylmethyl)amine and phenol **18**, the amide **35** was obtained as a white solid (19%): ^{1}H NMR ($CDCl_3$) δ 8.50 (m, 1H), 7.67 (m, 1H), 7.16, 7.48 (m, 2H), 7.25−7.06 (m, 8H), 6.50, 6.48 (s, 1H), 6.31 (m, 1H), 4.74, 4.58, 4.34 (m, 2H), 4.15 (m, 1H), 4.02 (m, 1H), 3.22−3.04 (m, 3H), 3.02, 2.88 (s, 3H), 2.94−2.73 (m, 2H), 1.55 (m, 1H), 1.47 (m, 1H), 0.67 (m, 1H), 0.63 (m, 3H), 0.39 (m, 3H); LCMS *m*/*z* 543 (MH^+) single component, Gradient 2 (t_R 3.28 min); HRMS calcd for $C_{32}H_{35}FN_4O_3$ (MH^+) 543.2771, found 543.2769; HPLC 99% (t_R 13.63 min).

(2*R*)-2-[(3*R*,6*R*)-3-(2,3-Dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]-2-(4-fluorophenyl)-*N*-[2-(1-piperidinyl)ethyl]ethanamide (**36**). Similarly prepared as for **25,** using 2-(1-piperidinyl)ethanamine and phenol **18**, the amide **36** was isolated as a white solid (30%): ^{1}H NMR (CD_3OD) δ 7.46 (m, 2H), 7.14−7.07 (m, 4H), 7.02 (m, 2H), 5.64 (s, 1H), 3.91 (dd, 1H, $J = 3.5$, 11 Hz), 3.81 (d, 1H, $J = 9.5$ Hz), 3.33−3.17 (m, 2H), 3.02−2.88 (m, 3H), 2.74 (m, 2H), 2.39−2.28 (m, 6H), 1.54−1.41 (m, 6H), 1.35 (m, 2H), 0.97 (m, 1H), 0.62 (d, 3H, $J = 6.5$ Hz), 0.43 (d, 3H, $J = 6.3$ Hz); LCMS *m*/*z* 549 (MH^+) single component, Gradient 2 (t_R 2.63 min); HRMS calcd for $C_{32}H_{41}FN_4O_3$ (MH^+) 549.3241, found 549.3237; HPLC 98% (t_R 10.09 min).

Methyl (2*R*)-(2,4-Difluorophenyl)[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]ethanoate (37) and Methyl (2*S*)-(2,4-Difluorophenyl)[(3*R*,6*R*)-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-dioxo-1-piperazinyl]ethanoate (38). Carbonyldiimidazole (0.324 g, 1.6 equiv) was suspended in anhydrous dichloromethane (4 mL), and the suspension was left at room temperature for 15 min. The phenol **17** (0.800 g, 1.46 mmol) was then added with stirring, and the resultant solution was left at room temperature for 16 h. The mixture was then treated with methanol (10 mL) and left at room-temperature overnight. The solvents were removed under reduced pressure, and the residue was purified by preparative plate chromatography eluting with ethyl acetate−cyclohexane (1:3) to give the 2*R*,3*R*,6*R* methyl ester **37** (0.453 g, 66%): ^{1}H NMR ($CDCl_3$) δ 7.69−7.61 (m, 1H, difluorophenyl-*6H*), 7.26−7.15 (m, 4H, indanyl-*arylH*), 6.95−6.82 (m, 2H, difluorophenyl-*5H,-3H*), 6.70 (broad d, $J = 3.5$ Hz, 1H, CON*H*), 5.32 (s, 1H, NC*H*difluorophenyl), 3.98−3.91 (m, 2H, NC*H*isobutyl, NC*H*indanyl), 3.81 (s, 3H, CO_2Me), 3.20−2.74 (m, 5H, indanyl-*3H, -1H, -2H)*, 1.99−1.80 (m, 2H, C*H*HCHMe$_2$, CH$_2$C*H*Me$_2$), 1.59−1.51 (m, 1H, CH*H*CHMe$_2$), 0.93 and 0.89 (2d, $J = 6.5$ Hz, 6H, CH$_2$CH*Me$_2$*); LCMS *m*/*z* 471 (MH^+) single component, Gradient 2 (t_R 3.42 min); HRMS calcd

for $C_{26}H_{29}F_2N_2O_4$ (MH^+) 471.20899, found 471.20956; HPLC 98% (t_R 14.46 min).

The 2*S*,3*R*,6*R* methyl ester **38** was isolated as a colorless solid (0.180 g, 26%): ^{1}H NMR ($CDCl_3$) δ 7.37−7.29 (m, 1H, difluorophenyl-*6H*), 7.26−7.15 (m, 4H, indanyl-*arylH*), 7.97−6.87 (m, 2H, difluorophenyl-*5H,-3H*), 6.46 (broad d, J = 3.3 Hz, 1H, CON*H*), 5.78 (s, 1H, NC*H*difluorophenyl), 4.02 (dd, J = 9.5 Hz, 4.3 Hz, 1H, NC*H*indanyl), 3.81 (s, 3H, CO_2Me), 3.67 (dd, J = 10.3 Hz, 3.8 Hz, 1H, NC*H*isobutyl), 3.20−2.75 (m, 5H, indanyl-*3H,-1H, −2H)*, 1.80−1.61 (m, 3H, CH_2CHMe_2, CH_2CHMe_2), 0.81 and 0.67 (2d, J = 6.3 Hz, 6H, CH_2CHMe_2); LCMS *m*/*z* 471 (MH^+) single component, Gradient 2 (t_R 3.42 min); HRMS calcd for $C_{26}H_{29}F_2N_2O_4$ (MH^+) 471.20899, found 471.20953; HPLC 95% (t_R 14.27 min).

(3*R*,6*R*)-1-[(1*R*)-1-(2,4-Difluorophenyl)-2-hydroxyethyl]-3-(2,3-dihydro-1*H*-inden-2-yl)-6-(2-methylpropyl)-2,5-piperazinedione (39). The methyl ester **37** (360 mg, 0.765 mmol) was dissolved in anhydrous tetrahydrofuran ((10 mL), and the solution was stiirred under nitrogen at −78 °C. Lithium triethylborohydride (2.30 mL, 3 equiv) of a 1.0 M solution in tetrahydrofuran was added dropwise via syringe over 1 min to the solution, which was stirred at −78 °C for 4 h then allowed to warm to room temperature. After 30 min at room temperature, the mixture was again cooled to −78 °C and quenched by addition of saturated aqueous ammonium chloride (5 mL) added via syringe over 1 min to give a white suspension. This was allowed to warm to room temperature with stirring and was left at room-temperature overnight. The mixture was diluted with dichloromethane (100 mL), and the organic phase was separated using a hydrophobic frit and evaporated under reduced pressure to give the crude product which was purified by flash column chromatography eluting with dichloromethane, then ethyl acetate−cyclohexane (1:2), followed by ethyl acetate−cyclohexane (1:1) and then ethyl acetate. The ethyl acetate fractions were combined and evaporated under reduced pressure to give the alcohol **39** as a white solid (190 mg, 56% yield): ^{1}H NMR ($CDCl_3$) δ 7.56−7.49 (m, 1H, difluorophenyl-*6H*), 7.26−7.15 (m, 4H, indanyl-*arylH*), 6.93−6.77 (m, 3H, difluorophenyl-*5H,-3H*, CO*NH*), 4.76 (dd, J = 7.3 Hz, 4.5 Hz, 1H, NC*H*difluorophenyl), 4.41−4.32 and 4.15−4.06 (2m, 2H, difluorophenylCHCH_2), 3.96 (dd, J = 9.5 Hz, 4.0 Hz, 1H, NC*H*indanyl), 3.76 (dd, J = 10.0 Hz, 6.0 Hz, 1H, NC*H*isobutyl), 3.50−3.44 (m, 1H, O*H*), 3.20−2.99 and 2.92−2.75 (2m, 5H, indanyl-*3H,-1H, −2H)*, 1.98−1.80 (m, 2H, C*H*HCHMe$_2$, CH_2CHMe_2), 1.63−1.55 (m, 1H, CH*H*CHMe$_2$), 0.95 and 0.94 (2d, J = 6.5 Hz, 6H, CH_2CHMe_2); LCMS *m*/*z* 443 (MH^+) single component, Gradient 2 (t_R 3.21 min); HRMS calcd for $C_{25}H_{29}F_2N_2O_3$ (MH^+) 443.21408, found 443.21427; HPLC 98% (t_R 13.37 min).

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Supporting Information Available: C, H, N analysis for compounds **4**, **23**, **24**, **25**, and **27** and the CD spectra of **25** and **26**. Experimental and spectroscopic data for compounds **2**−**9**. Lipophilicity calculations and measurements, absorption models, and estimated human oral absorption (EHOA) tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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, U.K., 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.; 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, P. G. 2,5-Diketopiperazines as potent, selective and orally bioavailable oxytocin antagonists 2: Synthesis, chirality and pharmacokinetics. *J. Med. Chem.* **2005**, *48*, 6956−6969.

(5) Davies, D. E. Stereospecific synthesis of 2,5-diketopiperazines *Tetrahedron Lett.* **2006**, *47*, in press.

(6) 2-Benzyloxyphenylisocynanide isonitrile was prepared by the method of Obrecht, R.; Herrmann, R.; Ugi, I. Isocyanide synthesis with phosphoryl chloride and diisopropylamine. *Synthesis* **1985**, *4*, 400−402.

(7) For human OT binding assay method, see the following: Wyatt, P. G.; Hickin, G.; Miller, N. D.; Allen, M. J.; Chilcott, J.; Woollard, P. M. Structure−Activity relationship investigations of a potent and selective benzodiazepine oxytocin antagonist. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1301−1305. Human V1a, V1b, and V2 binding assay method is identical to the hOT method except 10 μg of V1a-CHO membrane was used and 0.5 nM [^{3}H] vasopressin was used as ligand (K_d = 0.79 nM), 10 μg of V1b-CHO membrane was used and 1 nM [^{3}H] vasopressin was used as ligand (K_d = 3.9 nM), and 10 μg of V2-CHO membrane was used and 0.5 nM [^{3}H] vasopressin was used as ligand (K_d = 3.8 nM).

(8) 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.

(9) (a) Van De Waterbeemd, H.; Jones, B. C. Predicting oral absorption and bioavailability. *Prog. Med. Chem.* **2003**, *41*, 1−59. (b) Lajiness, M. S.; Vieth, M.; Erickson, J. Molecular properties that influence oral drug-like behaviour *Curr. Opin. Drug Discuss. Dev.* **2004**, *7*, 470−477.

(10) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeny, P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3−25.

(11) Clark, D. E. Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 1. Prediction of intestinal absorption. *J. Pharm. Sci.* **1999**, *88*, 807−814.

(12) (a) Abraham, M. H.; Zhao, Y. H.; Le, J.; Hersey, A.; Eddershaw, P. J.; Luscombe, C. N.; Reynolds, D. P.; Beck, G.; Sherborne, B.; Cooper, I. On the mechanism of human intestinal absorption. *Eur. J. Med. Chem.* **2002**, *37*, 595−605. (b) Zhao, Y. H.; Le, J.; Abraham, M. H.; Hersey, A.; Eddershaw, P. J.; Luscombe, C. N.; Butina, D.; Beck, G.; Sherborne, B.; Cooper, I.; Platts, J. A. Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure−activity relationship (QSAR) with the Abraham descriptors. *J. Pharm. Sci.* **2001**, *90*, 749−784. (c) Zhao, Y. H.; Abraham, M. H.; Le, J.; Hersey, A.; Luscombe, C. N.; Beck, G.; Sherborne, B.; Cooper, I. Rate-limited steps of human oral absorption and QSAR studies. *Pharm. Res*. **2002**, *19*, 1446−1457.

(13) Du, C. M.; Valko, K.; Bevan, C.; Reynolds, D.; Abraham, M. H. Characterizing the selectivity of stationary phases and organic modifiers in reversed-phase high-performance liquid chromatographic systems by a general solvation equation using gradient elution. *J. Chromatog. Sci.* **2000**, *38*, 503−511.

(14) Valko, K. Measurements and predictions of physicochemical properties. In *High-Throughput ADMETox Estimation*; Darvas, F.; Dormain, G., Eds.; Eaton Publishing: Westbrough, MA, 2002; Chapter 1, pp 1−24.

(15) 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.

(16) 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.

(17) Determination of antagonist affinity at human oxytocin-1 receptors using FLIPR. Cell Culture. Adherent Chinese Hamster Ovary (CHO) cells, stably expressing the recombinant human oxytocin-1 (hOT) receptor, were maintained in culture in DMEM:F12 medium (Sigma, cat. no. D6421), supplemented with 10% heat inactivated fetal calf serum (Gibco/Invitrogen, cat. no. 01000−147), 2 mM L-glutamine (Gibco/Invitrogen, cat. no. 25030−024) and 0.2 mg/mL G418 (Gibco/Invitrogen, cat. no. 10131−027). Cells were grown as monolayers under 95%:5% air:CO_2 at 37 °C and passaged every 3−4 days using TrypLE Express (Gibco/Invitrogen, cat no. 12604−013). Measure-

ment of $[Ca^{2+}]_i$ using the FLIPR. CHO-hOT cells were seeded into black-walled clear-base 384-well plates (Nunc) at a density of 10000 cells per well in culture medium as described above and maintained overnight (95%:5% air:CO_2 at 37 °C). After removal of culture medium, cells were incubated for 1 h at 37 °C in Tyrode's medium (NaCl, 145 mM; KCl, 2.5 mM; HEPES, 10 mM; glucose, 10 mM.; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.5 mM) containing probenacid (0.7 mg/mL), the cytoplasmic calcium indicator, Fluo-4 (4 μM; Teflabs, Austin, TX) and the quenching agent Brilliant Black (250 μM; Molecular Devices, UK). Cells were then incubated for an additional 30 min at 37 °C with either buffer alone or buffer containing OT antagonist, before being placed into a FLIPR (Molecular Devices, UK) to monitor cell fluorescence (λ_{ex} = 488 nm, λ_{EM} = 540 nm) before and after the addition of a submaximal concentration of oxytocin (EC_{80}). Data Analysis. Functional responses using FLIPR were analysed using Activity Base Version 5.0.10.

(18) 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.

(19) Ratio of the IC_{50} hOT in the presence of 50 mg/mL HSA to IC_{50} hOT in the absence of 50 mg/mL HSA. Method as in ref 7. The ligand [^{3}H]oxytocin was made up in buffer containing 100 mg/mL HSA. HSA was obtained from Sigma (Poole, Dorset, U.K.).

(20) Agonist responses were measured according to ref 17 except that test compound was added to cells in FLIPR reader in place of oxytocin EC_{80}.

(21) Microsomes, from human lymphoblastoid cell lines transfected with cDNA for specific cytochrome P450 enzymes, were obtained from Gentest Corporation (Woburn, MA). Reaction progress was monitored using fluorescent substrates. Each test compound was dissolved in methanol at 5 mM and serially diluted to determine IC_{50} values. A positive control was included for each isozyme.

(22) Time-dependent inhibition assays were preformed as described in ref 21, except that 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.

(23) 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.

(24) 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.

(25) Prepared by the method of Chatterjee, S. S. and Triggle, D. J. Synthesis of azetidin-3-ol. *J. Chem. Soc., Chem. Commun.* **1968**, *2*, 93.

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