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Pyrazolopyridazine alpha-2-delta-1 ligands for the treatment of neuropathic pain

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ABSTRACT

Optimization of the novel alpha-2-delta-1 ligand **4** provided compounds **37** and **38** which have improved DMPK profiles, good in vivo analgesic activity and in vitro selectivity over alpha-2-delta-2. An in-house P-gp prediction programme and the MetaSite® software package were used to help solve the specific problems of high P-gp efflux and high in vivo clearance.

Alpha-2-delta is an auxiliary subunit of voltage-gated calcium channels that was originally reported to modify the activity of the channel formed by the alpha-1 protein.[1] Binding to alpha-2-delta was suggested to inhibit the flux of calcium into the neuron[2,3] and subsequently inhibit the evoked release of various neurotransmitters.[4–6] More recent data suggests that alpha-2-delta is important in the trafficking of calcium channels from the endoplasmic reticulum to the cell membrane.[7] Alpha-2-delta is up-regulated after nerve injury and is believed to play a significant role in establishing the sensitization which causes neuropathic pain,[8] therefore alpha-2-delta ligands have become attractive targets for the discovery of neuropathic pain treatments.[9] Indeed, this mechanism is believed to be the mode-of-action of the currently marketed compounds gabapentin and pregabalin (**1** and **2**, Fig. 1).[10–13]

Gabapentin and pregabalin are non-selective ligands of two of the four known alpha-2-delta subtypes, alpha-2-delta-1 and alpha-2-delta-2.[14] Studies on the distribution and differential effects[9] of the two subtypes have suggested that the analgesia produced by these compounds is due to interaction with alpha-2-delta-1 and the major side effects of sedation, dizziness and ataxia are caused by interaction with alpha-2-delta-2. Our aim was to find novel alpha-2-delta-1 selective ligands to provide patients with relief from neuropathic pain without the side effects suffered during treatment with the current gold-standard medicines.

A knowledge-based design approach (which will be the subject of a future publication) starting from the known alpha-2-delta ligand **3**[15–18] (Fig. 1) gave rise to the amino-substituted pyrazolopy-

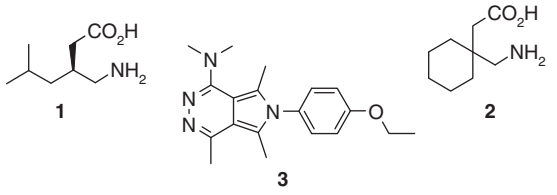
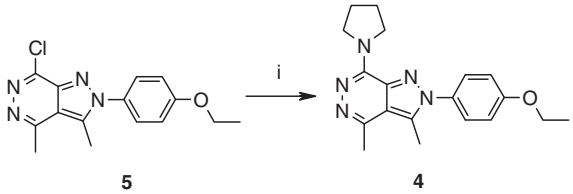


Figure 1. Gabapentin (**1**), pregabalin (**2**) and alpha-2-delta-1 ligand (**3**).



Scheme 1. Reagents and conditions: (i) pyrrolidine, NEt_3, EtOH, 140 °C MW, 100%.

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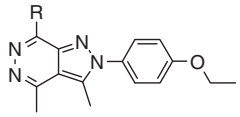
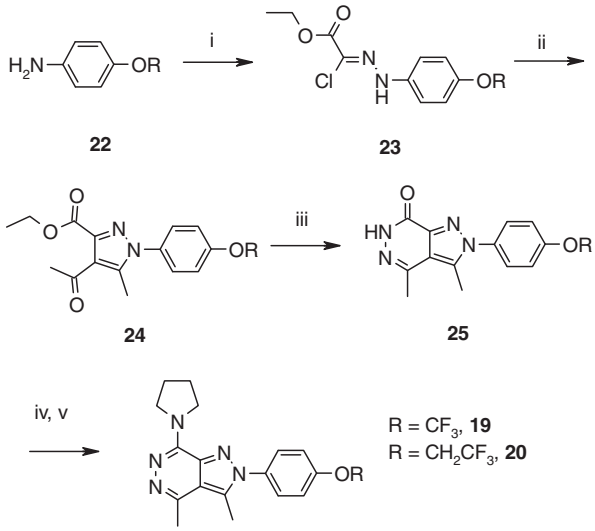
Table 1
Effect of pyridazine amino group on alpha-2-delta-1 potency



Compound	R	*c* log *P*	IC_{50} (nM)	
			α2δ1	α2δ2
3	–NMe_2	5.1	28	501
4		3.7	2	22
6		3.2	1	59
7	–NMe_2	3.6	7	158
8	–NHMe	3.4	22	537
9		4.3	42	<1000
10		4.9	22	2131



Scheme 2. Reagents and conditions: yields R = CF_3/R = CH_2CF_3: (i) (a) isoamyl nitrite, TFA, MeCN, 0 °C; (b) ethyl 2-chloro-3-oxobutanoate, NaOAc, ethanol, H_2O, 53%/96%; (ii) 2,4-pentadione, NaH, THF, 81%/74%; (iii) $N_2H_4{\cdot}H_2O$, EtOH, 100 °C, 82%/94%; (iv) $POCl_3$, 120 °C, 46%/24%; (v) pyrrolidine, NEt_3, 160 °C MW, 31%/53%.

ridazine compound **4**, prepared from the commercially available chloride **5** as shown in Scheme 1. This Letter, describes the subsequent optimization of this template to develop a selective alpha-2-delta-1 ligand for further profiling.

Exploration of the SAR of the pyrazolopyridazine series commenced with the variation of the amino moiety on the pyridazine ring. Compounds **6–10** (Table 1) were prepared analogously to **4** (Scheme 2), employing the requisite amines for the chloride displacement.

Reducing the amino-ring size to give the azetidine **6** retained potency and moderately improved selectivity. The acyclic dimethylamino derivative **7** had lower potency at both receptor subtypes and the removal of one methyl group to give **8** reduced these potencies further. Both the larger-ring derivatives piperidine **9** and azepine **10** also had significantly lower potency than compound **4**. This indicated the smaller-ring pyrrolidine and azetine groups were optimal in this position.

DMPK profiling of **4** gave a moderate in vitro intrinsic clearance in rat microsomes of 3.5 ml/min/g. However, this translated to a high in vivo rat clearance of 84 ml/min/kg. To reduce this high clearance, the lipophilicity of this template was lowered through the introduction of polar groups into the amino-ring substituent to give compounds **11–16**, Table 2.

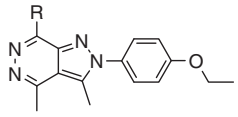
Piperazine **11** and 1,4-diazepine **13** were less potent than the piperidine **9** and azepine **10**, respectively, but did show low intrinsic clearance (both <0.5 ml/min/g). This corresponded to a moderate in vivo rat clearance of 47 ml/min/kg in the case of **13**. *N*-Methyl piperazine **12** gave a higher intrinsic clearance of 2.5 ml/min/g but a similar in vivo clearance of 57 ml/min/kg and 4-hydroxypiperidine **14** showed similar values to **13**. The 3-(*S*)-aminopyrrolidine **15** gave a lower in vivo clearance of 34 ml/min/kg but the 3-(*R*)-hydroxypyrrolidine **16** was much less metabolically stable with a clearance of 78 ml/min/kg, despite the low in vitro value (<0.5 ml/min/g). This showed that low in vitro clearance was not always predictive of low in vivo clearance in this series. The similar plasma protein binding data for these compounds (**15**: 19% uBl, 4% uBr; **16**: 14% uBl, 8% uBr) did not help to explain the discrepancy. It is notable that alcohol **16** showed remarkably higher selectivity over alpha-2-delta-2 (approximately 300-fold) compared to amine **15** (fivefold) and that their opposite enantiomers were equipotent to **15** and **16**.

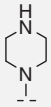
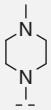
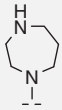
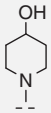
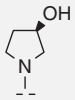
Compound **14** was progressed the Complete Freund's Adjuvant (CFA)[19] pharmacodynamic model of pain to establish whether this novel series of alpha-2-delta-1 selective ligands is effective in vivo. Pleasingly, **14** showed a dose-related reversal of induced hypersensitivity with an ED_{50} of 5.9 mg/kg that was not statistically different from pregabalin (dosed at 10 mg/kg) at the top dose of 30 mg/kg. No overt side effects were observed in the study. This experiment also revealed a moderate brain:blood ratio of 0.17:1 despite in vitro Kbb measurement[20] giving a ratio of 5:1. This discrepancy could be explained by the high P-glycoprotein (P-gp) efflux ratio[21] of 9:1.

P-gp efflux ratios were measured for a sub-set of the compounds shown in Table 2. The less polar parent compound **4** proved to not be a P-gp substrate (efflux ratio of 1.4:1) whereas the more polar compounds **11**, **13**, **15** and **16** were all substrates, giving ratios of 21:1, 14:1, 29:1 and 9:1, respectively, demonstrating that this liability had arisen due to the introduction of the polar groups. In an effort to address this issue, prospective target compounds were analysed by the in-house P-gp prediction programme which uses the Abraham BetaH, Abraham Alpha, total charge, *c* log *D* at pH 7.4 and *c* log *P* to calculate the probability of the test compound being a substrate for P-gp. Table 3 shows that this model correctly predicted the high P-gp efflux ratios of compounds **14**, **15** and **16** and the low P-gp efflux ratios of compounds **4** and **8**. This model also predicted that the putative methoxy derivatives **17** and **18** would be borderline P-gp substrates. These compounds were duly prepared and tested, giving P-gp efflux ratios below the accepted in-house threshold value of 2:1 and so were deemed to be non-substrates.

In addition to being non-P-gp substrates, compounds **17** and **18** showed good potency and greater than 10-fold selectivity over alpha-2-delta-2. Both compounds exhibited moderate to high in vivo clearance in rat of 51 and 61 ml/min/kg, respectively, which was a significant improvement compared with compound **4** but this was still predicted to limit exposure in higher species. Further optimization was clearly required to reduce in vivo clearance to a more acceptable level and to guide this effort an in silico analysis of compound **4** using the MetaSite® software package was undertaken.[22] MetaSite® uses computational methods to calculate the accessibil-

Table 2
Effect of pyridazine amino group on in vitro and in vivo clearance



Compound	R	*c* log *P*	IC_{50} (nM)		CLi (ml/min/g)	CLb (ml/min/kg)
			α2δ1	α2δ2		
4		3.7	2	22	3.5	84
11		2.9	213	1000	<0.5	–
12		3.4	169	1071	2.5	57
13		2.9	58	1000	<0.5	47
14		2.2	32	398	<0.5	55
15		2.5	2	10	0.8	34
16		2.4	5	1585	<0.5	78

ity and reactivity of each atom of the test substrate towards the reactive heme of a range of CYP450 enzymes. The product of these two values is the probability of metabolism at that specific atom. Figure 2 shows the resulting plot for compound **4** which clearly identifies the CH_2 of the ethoxy group as the most likely site of metabolism by the CYP450 3A4 isoform.

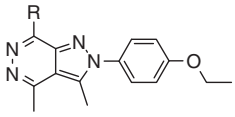
To explore the potential for improving metabolic stability by blocking this ethoxy group, the trifluoromethoxy, trifluoroethoxy and isopropoxy analogues **19**–**21** were synthesized. Compounds **19** and **20** were prepared by the route described in Scheme 2, starting from the trifluoromethoxy aniline and the trifluoroethoxy aniline, respectively. Alkoxyaniline **22** underwent a Japp–Klingemann[23] reaction with ethyl 2-chloroacetoacetate to give the chloro-hydrazone **23**. Chloride displacement with acetoacetone and subsequent ring-closure gave the pyrazole **24** which was treated with hydrazine to form the pyridazine ring of intermediate **25**.[24] Chlorination and subsequent displacement with pyrrolidine gave the pyrazolopyridazines **19** and **20**.

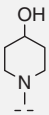
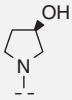
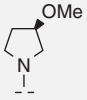
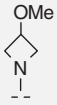
The Japp–Klingemann reaction failed with isoproxy aniline so analogue **21** was provided by the alternative route outlined in Scheme 3. 2,4-Pentanedione **26** was treated with oxalyl chloride and magnesium chloride to give the 4-acetyl-5-methyl-2,3-furandione **27**.[25] Pyrazole **28** was then formed by treatment of **27** with 4-isoproxyphenylhydrazine. The final three steps of pyridazine formation with hydrazine, chlorination and displacement proceeded as described above to provide compound **21**.

All three analogues **19**, **20** and **21** showed reduced in vitro clearance in rat microsomes compared with compound **4**, but this improvement was at the expense of an approximate 10-fold drop in potency (Table 4). The trifluoroethoxy analogue **20** was taken forward to an in vivo rat PK study, giving a CLb of 49 ml/min/kg, again a significant improvement compared with **4** (84 ml/min/kg). These results correlate well with the outcome of the MetaSite® analysis, suggesting the ethoxy group is a major liability for metabolism. Further analogues **30** and **31** that omitted the 4-ethoxyphenyl group in question were prepared by the route described in Scheme 2. The 2,4-dichlorophenyl derivative **30** and the 4-chloro-2-trifluoromethoxyphenyl derivative **31** both had reduced in vitro rat clearance while retaining acceptable levels of alpha-2-delta-1 potency. Compound **30** was progressed to an in vivo rat PK study and gave a similar moderate CLb value to compound **20** (51 ml/min/kg).

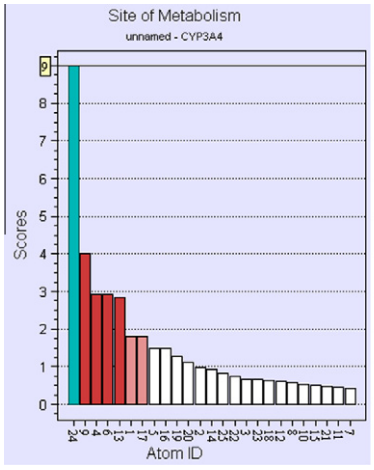
Returning to the Metasite results, the C-3 and C-4 methylene groups on the pyrrolidine ring were also identified as potential sites of metabolism.[26] Substitutions on the 3-position of the pyrrolidine had already been explored by the compounds described in Tables 2 and 3 which identified the 3-(*R*)-methoxypyrrolidine and the 3-methoxyazetidine R fragments as advantageous for metabolic stability. In addition to these, the 3-(*R*)-3-fluoro-and 3,3-difluoro-pyrrolidine analogues **32** and **33** were prepared (Table 5). Compound **32** gave a rat microsomal clearance of 2.4 ml/min/g and a reduced rat in vivo clearance of 56 ml/min/kg (the (*S*) enantiomer of **32** gave a higher CLi of 6.7 ml/min/g). Interestingly, the

Table 3
Predicted P-gp score and measured P-gp efflux ratio



Compound	R	*c* log *P*	IC_{50} (nM)		Predicted P-gp score[a]	P-gp efflux ratio[b]
			α2δ1	α2δ2		
4		3.7	2	22	0.38	1.4:1
8	–NHMe	3.6	22	537	0.06	1.4:1
14		2.2	32	398	−0.40	9.1:1
15		2.5	2	10	−0.68	29:1
16		2.4	5	1585	−0.36	9:1
17		3.2	6	79	−0.01	1.8:1
18		3.4	5	126	−0.03	1.8:1

[a] P-gp score calculated by an unpublished in-house method, <−0.20 predicts a substrate with high confidence, −0.20 to 0.00 predicts a borderline substrate, 0.00–0.20 predicts a borderline non-substrate and >0.20 predicts a non-substrate with high confidence.
[b] P-gp efflux ratio determined by measuring the permeation through a monolayer of hMDR-MDCK (type II) cells in the presence and absence of a known P-gp ligand.[21]



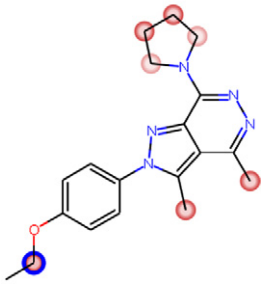
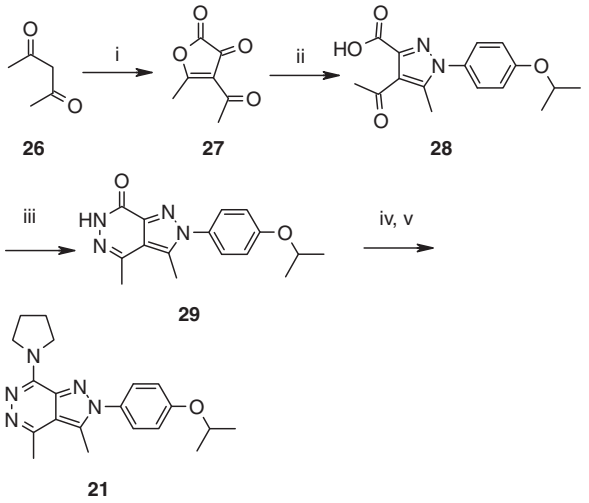


Figure 2. Metasite output showing the most likely sites of metabolism of compound **4** by CYP450 3A4. Each atom on the test compound is ranked in the histogram (and by shading on the structure) according to the calculated probability of metabolism. Higher score (darker shading) = higher probability of metabolism.

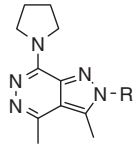


Scheme 3. Reagents and conditions: (i) oxalyl chloride, $MgCl_2$, Et_2O, −5 °C, 64%; (ii) 4-*iso*-propoxyphenylhydrazine, PhMe, 60 °C, 89%; (iii) $N_2H_4{\cdot}H_2O$, EtOH, 100 °C, 87%; (iv) $POCl_3$, 120 °C, 54%; (v) pyrrolidine, NEt_3, 120 °C MW, 37%.

difluoro analogue **33** gave a much higher rat microsomal clearance of 10.8 ml/min/g so was not progressed to an in vivo study.

Unfortunately, the combination of these amine groups with the promising phenyl groups was not always successful (Table 6). The 3-(*R*)-3-fluoropyrrolidine amino group in combination with 4-trifluoroethoxyphenyl fragment gave compound **34** which had low rat microsomal clearance but was not progressed due to potent hERG inhibition (316 nM). The 3-methoxyazetidine amino group gave a large drop in potency when combined with the 4-trifluoroethoxyphenyl (**35**) and 2,4-dichlorophenyl (**36**) groups, but pleasingly the 4-chloro-2-trifluoromethoxyphenyl derivative **37** retained the high potency of the parent compound. The in vitro

Table 4
PK profiles of substituted phenyl analogues



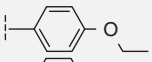
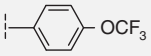
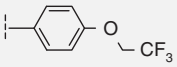
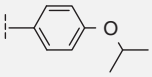
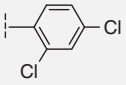
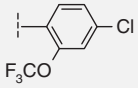
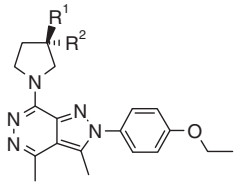
Compound	R	*c* log *P*	IC_{50} (nM)		CLi(r) (ml/min/g)	CLb(r) (ml/min/kg)
			α2δ1	α2δ2		
4		3.7	2	22	3.5	84
19		4.3	79	1995	0.8	–
20		4.0	20	398	<0.5	49
21		4.0	32	316	0.8	–
30		4.5	25	631	1.0	51
31		5.1	3	794	0.6	–

Table 5
Profiles of fluoro-pyrrolidine analogues



Compound	R^1	R^2	*c* log *P*	IC_{50} (nM)		CLi(r) (ml/min/g)	CLb(r) (ml/min/kg)
				α2δ1	α2δ2		
4	H	H	3.7	2	22	3.5	84
32	F	H	3.7	3	85	2.4	56
33	F	F	4.0	25	794	10.8	–

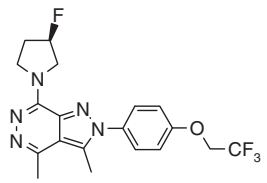
rat clearance of this analogue was below 0.5 ml/min/g and this translated to an in vivo rat clearance of 52 ml/min/kg. This was still higher than desired but lower than the value for compound **18** (61 ml/min/kg), showing this phenyl substitution has had an additional clearance lowering effect over that provided by the 3-methoxyazetidine group. This positive effect was probably tempered by the increase in lipophilicity going from compound **18** (*c* log *P* = 3.4) to compound **37** (*c* log *P* = 4.7). This particular phenyl substitution also had the advantage of increasing the selectivity over alpha-2-delta-2. This appeared to be a general trend with the 2-trifluoromethoxy-, difluoromethoxy- and methoxy-phenyl analogues (data not shown).

Further SAR exploration of the phenyl ring led to the discovery of the *ortho*-fluoro-*para*-ethoxyphenyl group which in combination with the 3-methoxyazetidine amino group gave compound **38**. This analogue had good potency, acceptable selectivity and a low to moderate in vivo rat clearance of 37 ml/min/kg. Further profiling of compounds **37** and **38** showed no problematic activity at the hERG channel (IC_{50} of 4 and 6 μM, respectively) and that **37** was a non-P-gp substrate. Both compounds reversed CFA induced hypersensitivity, **37** giving full reversal when dosed at 30 mg/kg and **38** giving full reversal at 10 mg/kg—the same level of in vivo potency as the gold-standard alpha-2-delta-1 ligand pregabalin (Fig. 3). No side effects were observed in the study and both compounds showed improved blood:brain ratios compared to compound **14** (0.94:1 for **37** and for **38** 0.92:1 compared with 0.17:1 for **14**). Analysis of the blood and brain samples from this study showed that the unbound concentrations in blood and brain are similar for **37** and **38** and so do not help to explain the difference between their in vivo potencies.[27]

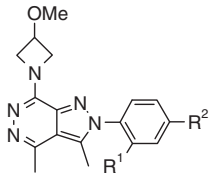
The overall profiles of **37** and **38** show they are promising potential development candidates that could test the hypothesis that alpha-2-delta-1 selective ligands can provide effective pain relief without the common side effects of the currently marketed treatments.[28]

In summary, the novel pyrazolopyridazine alpha-2-delta-1 ligand **4** has been optimized by variation of the amino group and the phenyl substituents, with guidance from in silico tools, to provide compounds **37** and **38**. These compounds have improved DMPK profiles, in vivo activity in the CFA pain model and in vitro selectivity for alpha-2-delta-1 over alpha-2-delta-2.

Table 6
Profiles of analogues **34**–**38**



34



35-38

Compound	R^1	R^2	*c* log *P*	IC_{50} (nM)		CLb(r) (ml/min/kg)
				α2δ1	α2δ2	
4	—	—	3.7	2	22	84
34	—	—	4.0	32	631	—
35	H	$-OCH_2CF_3$	3.7	126	1995	—
36	Cl	Cl	4.2	200	6310	—
37	$-OCF_3$	Cl	4.7	8	3981	52
38	F	OEt	3.6	8	200	37

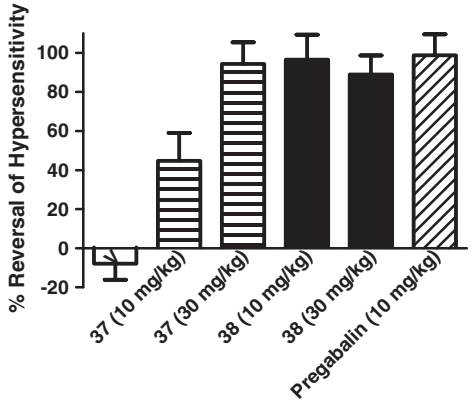


Figure 3. CFA data for compounds **37**, **38** and pregabalin.

References and notes

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26. An in vivo metabolism study of compound **4** largely confirmed the Metasite® predictions. The major metabolites found in urine were the result of O-dealkylation, oxidation on the ethyl chain and oxidation of the pyrrolidine ring and those found in bile were predominantly associated with glucuronidation of the desethyl metabolite and again oxidation of the pyrrolidine ring.
27. Compound **37**: 30 mg/kg: [uBl] = 0.84 μM, [uBr] = 0.20 μM; **37** 10 mg/kg: [uBl] = 0.46 μM.
    Compound **38**: 30 mg/kg: [uBl] = 0.91 μM, [uBr] = 0.23 μM; **38** 10 mg/kg: [uBl] = 0.48 μM.
28. Preliminary in vivo selectivity data was provided by the rotarod model of sedation which showed compound **14** had no effect up to a dose of 300 mg/kg. This correlates to at least a threefold window between pain relief and sedation once the non-linear relationship between exposures and dose at the higher concentrations of 30 and 300 mg/kg is taken into account. Compound **14** had an in vitro selectivity for alpha-2-delta-1 over alpha-2-delta-2 of approximately 10-fold.