Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2609–2624

BIOORGANIC & MEDICINAL CHEMISTRY

Parallel Synthesis and Biological Activity of a New Class of High Affinity and Selective δ-Opioid Ligand

D.R. Barn,[a] W.L. Caulfield,[a] J. Cottney,[a] K. McGurk,[b] J.R. Morphy,[a,*] Z. Rankovic[a] and B. Roberts[c]

[a]*Organon Laboratories Ltd, Newhouse, ML1 5SH, Scotland, UK*
[b]*N.V. Organon, Molenstraat 110, 5430 BH, Oss, The Netherlands*
[c]*AstraZeneca, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK*

Received 6 October 2000; revised 11 January 2001; accepted 15 January 2001

Abstract—A considerable number of research papers describing the synthesis and testing of the delta opioid receptor (DOR) ligands, SNC-80 and TAN-67, and analogues of these two compounds, have been published in recent years. However, there have been few reports of the discovery of completely new structural classes of selective DOR ligand. By optimising a hit compound identified by high throughput screening, a new series of tetrahydroisoquinoline sulphonamide-based delta opioid ligands was discovered. The main challenge in this series was to simultaneously improve both affinity and physicochemical properties, notably aqueous solubility. The most active ligand had an affinity (IC_{50}) of 6 nM for the cloned human DOR, representing a 15-fold improvement relative to the original hit **1** (IC_{50} 98 nM). Compounds from this new series show good selectivity for the DOR over μ and κ opioid receptors. However the most active and selective compounds had poor aqueous solubility. Improved aqueous solubility was obtained by replacing the phthalimide group in **1** by basic groups, allowing the synthesis of salt forms. A series of compounds with improved affinity and solubility relative to **1** was identified and these compounds showed activity in an in vivo model of antinociception, the formalin paw test. In the case of compound **19**, this analgesic activity was shown to be mediated primarily via a DOR mechanism. The most active compound in vivo, **46**, showed superior potency in this test compared to the reference DOR ligand, TAN-67 and similar potency to morphine (68% and 58% inhibition in Phases 1 and 2, respectively, at a dose of 10 mmol/kg i.v.).

Introduction

Opioid receptors are classified into three major subtypes: μ, κ and δ. Most currently used μ-opioid analgesic drugs, such as fentanyl and its analogues, exhibit a range of serious side effects including respiratory depression, dependence and muscle rigidity. Extensive studies over the past decade have shown that δ-opioid receptor (DOR) agonists produce antinociception in animal models of pain whilst appearing to lack the characteristic side-effect profile of μ-opioid receptor (MOR) agonists.[1,2] For this reason the DOR is an attractive target for the design of novel opioid analgesics. A number of peptide agonists that display high affinity and selectivity for the DOR have been reported, but their clinical use has been hampered by poor metabolic stability and low systemic availability. Over the last few years, selective non-peptide agonists and antagonists for the DOR have been discovered (Fig. 1). The first non-peptide antagonist to possess reasonable selectivity for the DOR, naltrindole (NTI), was discovered by applying the message-address concept.[3] More recently, a highly selective peptidomimetic antagonist, H-Dmt-Tic-OH, based upon the N-terminal message domain of the endogenous enkephalins, was discovered.[4] One of the first non-peptide agonists to show good affinity and selectivity at the DOR, TAN-67, is structurally related to naltrindole.[5] However, a second agonist, SNC-80, is clearly unrelated to the morphinoid skeleton.[6] Despite their structural diversity, all these selective delta opioid ligands have one feature in common, a basic amine. Earlier reports of non basic opioids are few, such as the discovery that casomorphin-derived hexapeptides, such as N-t-Boc-Tyr-Pro-Gly-Phe-Leu-Thr-OH, are delta opioid selective antagonists.[7] Recently we published the structure of a new class of delta opioid ligand **1**, which lacks a protonatable nitrogen.[8] Very recently the structures of cyclic

*Corresponding author: Fax: +44-01698-736187; e-mail: r.morphy@organon.nhe.akzonobel.nl

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00017-7

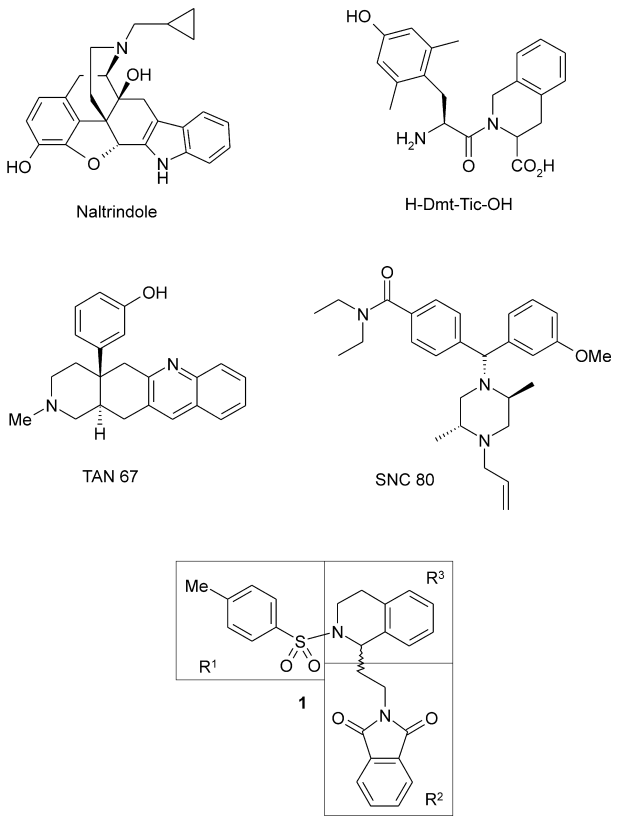


Figure 1. Selective ligands for the delta opioid receptor.

peptides, which are also non basic ligands at the DOR, have also been published.[9] Compound **1**, identified through high throughput screening of the Organon compound collection, was attractive for reasons of novelty, high selectivity over the other opioid receptor sub-types and synthetic accessibility. The main disadvantage, particularly from the perspective of an intravenously-administered analgesic, was its poor aqueous solubility (<0.1 mg/mL). This paper describes our efforts to optimise both the affinity and physicochemical properties of **1**.

Results and Discussion

Chemistry

Compound **1** was synthesised in three steps, starting from 2-phenethylamine **2** and *N*-(3-hydroxypropyl)phthalimide **4** (Scheme 1, Method A). 2-phenethyl amine was tosylated using tosyl chloride in pyridine. *N*-(3-hydroxypropyl)phthalimide was oxidised using pyridinium chlorochromate in dichloromethane. Pictet Spengler cyclisation in neat TFA provided **1** in 89.8% overall yield. An alternative route to **1** started from *N*-(2-carboxyethyl)phthalimide **6** and involved conversion to the amide **7**, followed by a Bischler-Napieralski reaction (Scheme 1, Method B). Protonation and hydrogenation produced the tetrahydroisoquinoline **10**[10] and finally tosylation gave **1** in 65% overall yield. Method A was used to make changes in the tetrahydroisoquinoline (THIQ) and phthalimide regions. Method B was used to make changes in the aryl-sulphonamide region.

Compound **1** was also synthesised using the solid phase routes shown in Scheme 2 (Methods C and D). This novel methodology by which a range of cyclic imides can be formed by cyclative cleavage has recently been published.[8] Method D allowed the synthesis of a two-dimensional library in which both the phthalimide and tosyl groups could be varied simultaneously.

We attempted to synthesise a larger amount of **20** using a more traditional solution phase method, opening of the anhydride followed by thermolytic ring closure (Scheme 3, Method E). However decarboxylation of regioisomer **17** occurred at the intermediate carboxylic acid stage, giving a mixture of the amide **19** and the imide **20**.[11]

A library of 312 amides was synthesised using a solution phase route, utilising solid phase reagents and scavengers (Scheme 4, Method F). The primary amine **15** was converted to 12 different amides **21** using carboxylic acids and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC). After a simple purification by aqueous extraction, the Boc group was removed using 25% trifluoroacetic acid (TFA) in dichloromethane (DCM). In the second diversity-generating step, the free secondary amine was converted to various sulphonamides using sulphonyl chlorides, in the presence of resin-bound piperidine. A slight excess of sulphonyl chloride (1.25 mol equiv) was used to drive the reaction to completion, and this was scavenged using aminomethyl-polystyrene resin. The 312 amides **22** were obtained in an average yield of 29.3 mg (84%) and 81.4% purity, as measured by HPLC. Each of the 312 products was analysed by flow injection MS, and 60 products were selected at random and structures confirmed by ^{1}H NMR.

Structure–activity relationships. For the purposes of simplicity, we divided the molecule into three fragments: the aryl sulphonamide region (R^1), the phthalimide side chain region (R^2) and the tetrahydroisoquinoline (THIQ) region (R^3) (Fig. 1). We explored the basic SAR in each of these regions, by conducting deletion studies, before embarking on the synthesis of hit optimisation libraries.

Region R^1. The 4-toluene sulphonamide group is extremely important for affinity (Table 1). Complete removal of the tosyl group or replacement of the aromatic ring by a methyl group, compounds **27** and **26** respectively, produce a complete loss of affinity. More conservative changes such as replacement of the sulphonyl group by a carbonyl, **24**, or removal of the 4-methyl group, **23**, also cause all activity to be lost.

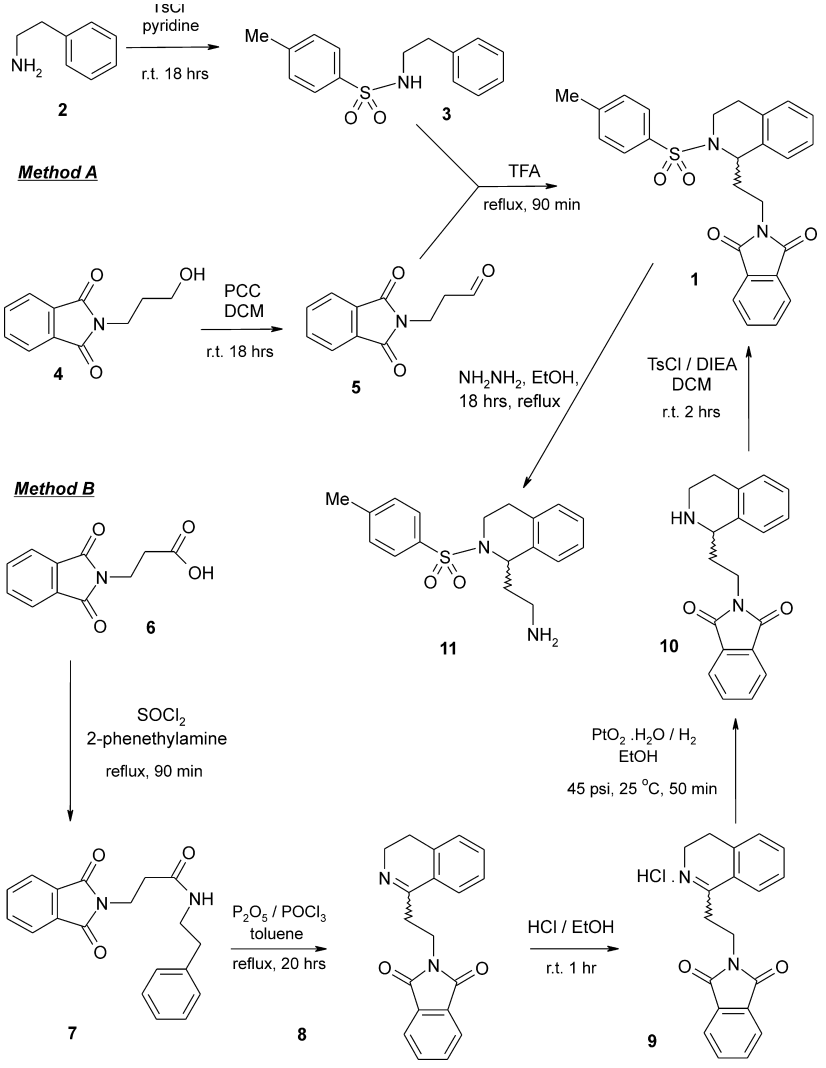
Region R^2. The phthalimide-containing side chain is also important for activity, but the SAR appears to be somewhat less tight in this region compared to R^1 (Table 2). Whereas substitution of the phthalimidoethyl group by a methyl group **29** causes a 25-fold decrease in

activity, its replacement by an acetamidoethyl **28** gives only a 3-fold drop in affinity suggesting that the aromatic ring is not an essential pharmacophoric group. Extension of the side chain by one carbon **30** gives a slight decrease in affinity, but a one carbon reduction **31** has a more profound effect, with a near 30-fold attenuation.

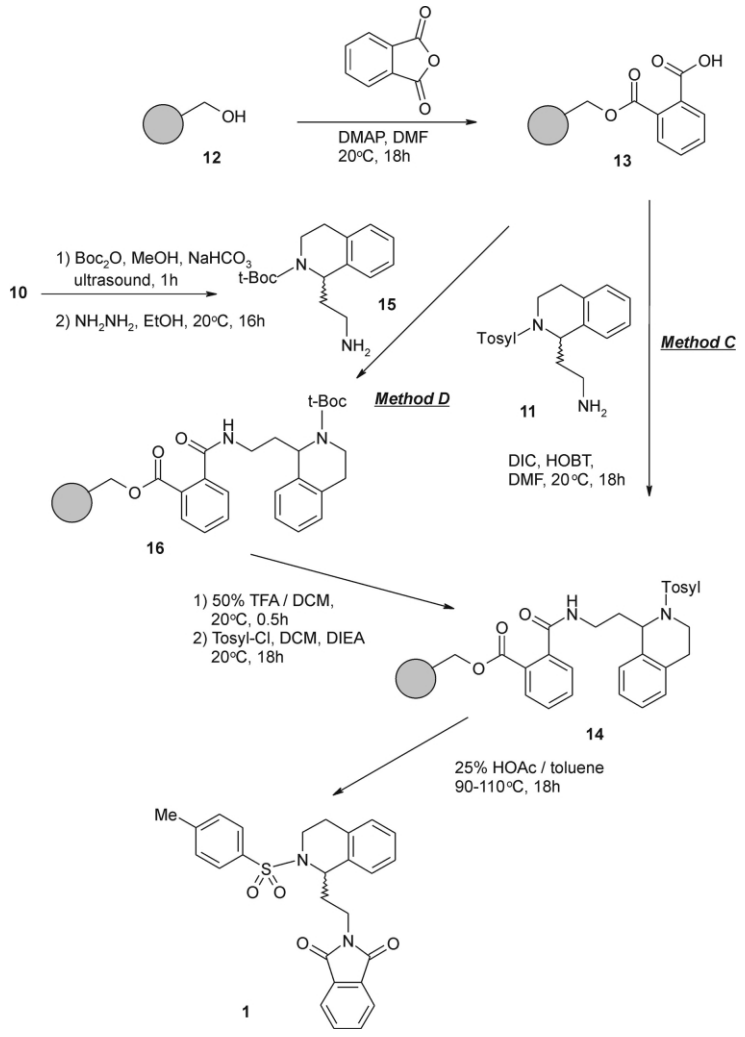
Region R^3. Aromatic substituents on the THIQ ring were not well tolerated (Table 3). The substituents were limited to electron rich groups by the synthetic route, since the Pictet-Spengler cyclization does not permit electron-deficient aromatic precursors to be used. The most active analogue was the 7-hydroxy compound **32**, but the affinity was 3-fold lower than the unsubstituted parent. Ethers in the 5-, 6- or 7- positions are slightly less active (**33**, **35** and **34** respectively), whereas an alkyl group in the 7-position (**37**) or a dihydroxy-substituted ring (**36**) gives analogues that are virtually inactive. Removal of the THIQ aromatic ring **39**, or its replacement by an imidazole **40**, led to compounds with little or no affinity.

Overall, the preliminary SAR study suggests that the tosyl group is very important for activity. In particular, a para-substituent and the SO_2 group appear to be crucial and these features were therefore retained in the subsequent hit optimisation libraries. There appeared to be more scope for varying the phthalimide group and it was felt that this region offered the best opportunity for manipulating the physicochemical properties of the compound. Substituents on the THIQ ring appear to offer no advantage in terms of affinity.

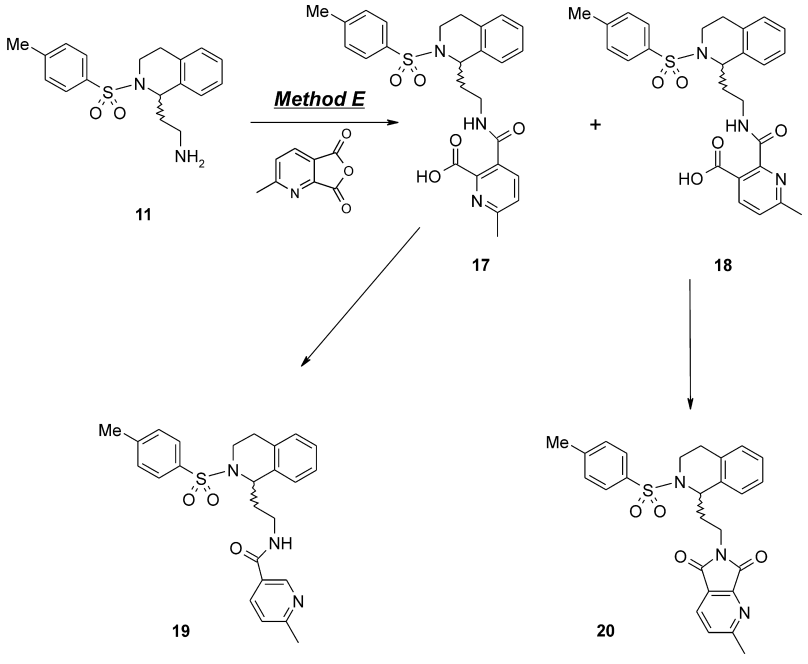
In the first library, we concentrated on the R^2 region, making modest changes to the structure of **1**, by changing the phthalimide group for other cyclic imides, whilst keeping the tosyl group constant (Table 4). Compound **1** was remade as an internal standard within the library and was found to exhibit similar affinity: 112 nM for **41{1}** compared to 98 nM for **1** (Table 4). The SAR apparent from testing the library at the DOR suggests that an electron withdrawing substituent in the 3-position on the phthalimide ring is favoured, since both the 3-nitro **41{3}** and 3-fluoro **41{5}** derivatives



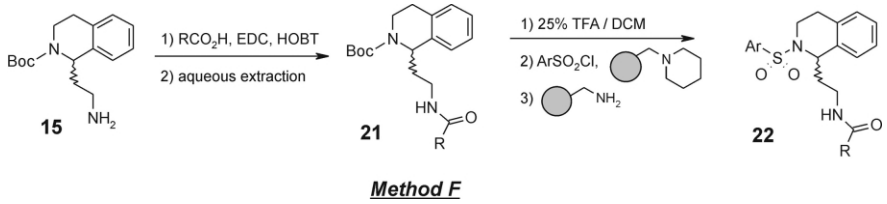
Scheme 1.



Scheme 2.



Scheme 3.



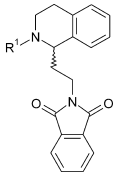
Scheme 4.

were 2-fold more active than **1**. The presence of a second fluorine atom, in the 6-position **41{6}**, gave a further increase in affinity to 16 nM, representing a 6-fold improvement relative to **1**. In contrast, the 3,6-dichloro derivative **41{9}** was much less active, possibly indicating that two electron withdrawing substituents are only well tolerated if of small size. The pyridine derivative **41{2}** was 4-fold more active than **1**, whereas the pyrazine **41{12}** was 2-fold less active. Substituents in the 4-position are generally less well tolerated. For example, the 4-nitro **41{4}** and 4-fluoro derivatives **41{13}** were less active than **1**, and much less active than the analogues with the equivalent substituent in the 3-position. Derivatives containing both 4- and 5-substituents showed very poor activity, such as the dichloro-compound **41{10}** and the 3,4-naphthyl derivative **41{15}**. The tetrafluoro-analogue **41{10}** gave only moderate activity relative to **41{6}**, again suggesting that 4- and 5-substituents are not well tolerated. The only compound containing a 3,4-disubstitution pattern, **41{14}**, was poorly active. The combination of a nitrogen in the 3-position of the ring, with a 4-methyl substituent **41{16}**, provided good affinity. In this case, the beneficial effect of the ring nitrogen seems to counteract the generally detrimental effect of introducing a 4-substituent.

The SAR for the succinimides within the library suggests that small non-polar substituents are well tolerated. For a single substituent in the 3-position, the order of activity is: Me **41{28}** > Ph **41{27}** > HCONH **41{24}** > $PhCH_2OCONH$ **41{21}**. Two methyl groups, either on the same **41{26}** or different **41{29}** carbons, are also tolerated. Where a second saturated ring is fused to the succinimide ring, small rings appear to provide higher affinity, although the SAR is generally quite flat: cyclopropyl **41{25}** > *trans*-cyclohexyl **41{20}** > *cis*-cyclohexenyl **41{18}** > bicycloheptene **41{22}** > *cis*-cyclohexyl **41{19}** > bicyclooctene **41{23}**.

Overall, this first library produced eight compounds with higher affinity than **1**. Perhaps the most interesting new compound was **41{16}**, which was 3-fold more active than **1**, and due to the presence of a pyridine ring offered the prospect of improved water solubility relative to the parent phthalimide.

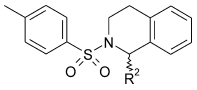
Table 1. Biological data for ligands with variations in the R^1 position[a]



	R^1	IC_{50} δ (nM)[b]
1	$4\text{-}Me(C_6H_4)SO_2-$	98
23	$C_6H_5SO_2-$	7044
24	$4\text{-}Me(C_6H_4)CO-$	> 10,000
25	$C_6H_5CH_2-$	> 10,000
26	$MeSO_2-$	> 10,000
27	H	> 10,000

[a]Compounds were made from **10** (Scheme 1).
[b]IC_{50} values were determined from the log concentration-inhibition curves (at least 6 points) and are mean values of at least 2 experiments.

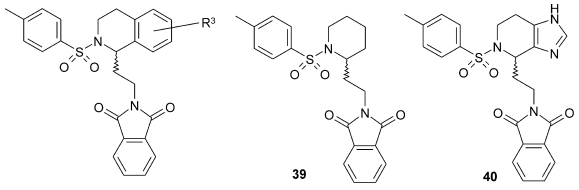
Table 2. Biological Data for Ligands with variations in the R^2 position



	R^2	IC_{50} δ (nM) [c]
1	$PhtCH_2CH_2$[a]	98
28	$MeCONHCH_2CH_2-$[b]	293
29	Me−[a]	2400
30	$PhtCH_2CH_2CH_2-$[a]	197
31	$PhtCH_2-$[a]	2700

[a]Compounds were made using Method A (Scheme 1).
[b]Compounds were made from **11** (Scheme 1); Pht = Phthalimido.
[c]IC_{50} values were determined from the log concentration-inhibition curves (at least six points) and are mean values of at least two experiments.

Table 3. Biological data for ligands with variations in the R^3 position[a]

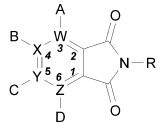


	R^3	IC_{50} δ (nM)[b]
1	H	98
32	7-OH	296
33	5-OMe	510
34	7- OMe	570
35	6- OMe	1400
36	$6,7\text{-}(OH)_2$	6900
37	7-Me	9313
38	$7\text{-}(OCH_2C_6H_4CN)$	568
39	—	> 10,000
40	—	8000

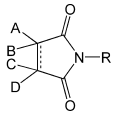
[a]Compounds were made using Method A (Scheme 1).
[b]IC_{50} values were determined from the log concentration-inhibition curves (at least 6 points) and are mean values of at least 2 experiments.

Table 4. Biological data for phthalimides, succinimides and maleimides[a]

Phthalimides 41



Succinimides/Maleimides 41



Phthalimides:	IC_{50} δ (nM)[b]
41{1}	112
41{2}; W = N	24
41{3}; A = NO_2	48
41{4}; B = NO_2	496
41{5}; A = F	42
41{6}; A,D = F	16
41{7}; A,B,C,D = F	464
41{8}; B = Me	496
41{9}; A,D = Cl	1659
41{10}; B,C = Cl	6709
41{11}; B = Cl	244
41{12}; W,Z = N	164
41{13}; B = F	173
41{14}; A-B = CHCHCHCH	2691
41{15}; B-C = CHCHCHCH	1302
41{16}; W = N, B = Me	35

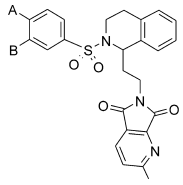
Succinimides:	IC_{50} δ (nM)[b]
41{17};	195
41{18}; A-C = $CH_2CHCHCH_2$ (*cis*)	249
41{19}; A-C = $CH_2CH_2CH_2CH_2$ (*cis*)	339
41{20}; A-C = $CH_2CH_2CH_2CH_2$ (*trans*)	202
41{21}; A = $PhCH_2OCONH$ (S)	517
41{22}; A-C = bicyclo[2.2.1]hept-5-ene (endo)	279
41{23}; A-C = bicyclo[2.2.2]oct-5-ene (endo)	2152
41{24}; A = HCONH (R)	627
41{25}; A-C = CH_2	86
41{26}; A,B = Me	165
41{27}; A = Ph	174
41{28}; A = Me	75
41{29}; A,C = Me	86
Maleimide:	
41{30}; A-C = $CH_2CH_2CH_2CH_2$	343

Unless stated otherwise A,B,C,D represent hydrogen and W,X,Y,Z represent carbon. A,B indicates both positions are substituted as shown. A,B indicates a bridging group between the two positions. R = 1-(2-ethyl)-2-(4-toluenesulphonyl)-1,2,3,4-tetrahydroisoquinoline.
[a]Compounds were made using Method C (Scheme 2).
[b]IC_{50} values were determined from the log concentration-inhibition curves (at least 6 points) and are mean values of at least 2 experiments.

Interestingly amide **19**, a side product obtained during the synthesis of **20**, was also active at the DOR (110 nM), with reasonable selectivity over μ and κ opioid receptors, >100-fold and 30-fold respectively. Significantly, whereas it was not possible to produce a salt form of **20** with either hydrochloric acid or sulphuric acid in order to improve aqueous solubility, the hydrosulphate salt of **19** could be obtained. Both the molecular weight and cLogP are lower for **19** than for **1**, which suggested that the new compound may have superior CNS penetration (Table 7).[12]

Having made some progress towards our goal of improving both the affinity and reducing the lipophilicity of **1**, we designed two follow-up libraries. In the first of these, the R^2 region was held constant as the pyridine-containing cyclic imide, found in **41{16}**, and the R^1 region was varied (Table 5; Scheme 2). The main driving force behind this library was an attempt to improve further the affinity of **41{16}** by introducing alternative substituents on the aryl sulphonamide ring. In the second of these libraries, we used a solution phase route to make analogues of the amide **19** and varied both the R^1 and R^2 regions combinatorially. The main emphasis for this library was to explore a wider range of other water-solubilising groups in the R^2 region, since we knew that this region was most likely to tolerate basic groups (Table 6; Scheme 4).

Table 5. Biological data for sulphonamide variations[a]



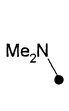
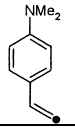
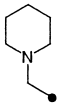
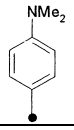
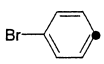
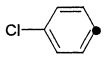
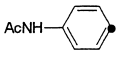
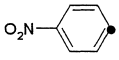
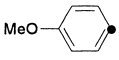
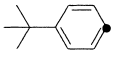
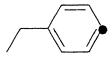
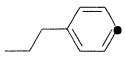
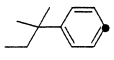
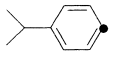
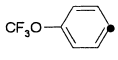
	A	B	IC_{50} δ (nM)[b]
42{1}	Me	H	52
42{2}	nBu	H	6
42{3}	Cl	H	1100
42{4}	Et	H	53
42{5}	nBuO	H	80
42{6}	Et_2NCH_2	H	4350
42{7}	H	Me	608
42{8}	CF_3O	H	800

[a]Compounds were made using Method D (Scheme 2).
[b]IC_{50} values were determined from the log concentration-inhibition curves (at least six points) and are mean values of at least two experiments.

Replacement of the tosyl group in **42{1}** [= **41{16}**] with a 4-(*n*-butylphenyl)-sulphonyl group, **42{2}** gave a 9-fold improvement in affinity. However, the 4-chloro **42{3}**, diethylaminomethyl **42{6}**, 3-methyl **42{7}** and 4-trifluoromethyl **42{8}** analogues were much less active than the parent. Two other analogues retained similar affinity to the tosyl compound, the 4-(ethylphenyl)-sulphonamide **42{4}** and the 4-(*n*-butoxyphenyl)-sulphonamide **42{5}**. This SAR suggests that binding of the butyl chain within a hydrophobic channel on the receptor may be responsible for the improved affinity of **42{2}**.

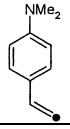
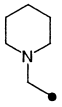
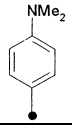
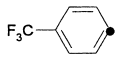
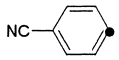
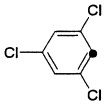
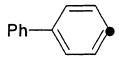
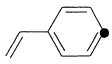
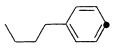
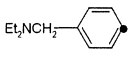
The % inhibition of ^{3}H-NTI binding for each of the 312 amides **22** in the DOR binding assay was determined at two concentrations: 100 nM and 1000 nM (Table 6). Although single concentration inhibition data of this type should be treated with caution, it did enable us to discern SAR trends which were consistent with those observed in the imide library above. As in the imide library, replacement of the tosyl group (sulphonamide No. 9) with a 4-(*n*-butylphenyl)-sulphonyl group, (sulphonamide No. 23),

Table 6. The upper and lower figures are the % inhibition of ^{3}H-Naltrindole binding to the DOR at 1000 nM and 100 nM respectively. If determined, the figure in bold is the IC_{50} (nM). Compounds made using *Method F*

		1	2	3	4	5	6	7	8	9	10	11	12
22													
1		41 9	49 25	46 22	9 25 **>10000**	17 9	12 44	29 22	35 21	32 17	7 26	16 20	30 16
2		46 15	52 24	46 23	23 27 **>10000**	13 18	20 25	29 26	31 13	35 25	13 33	21 20	37 25
3		50 17	48 19	59 30	37 −4 **3470**	27 18	42 21	29 28	32 22	34 28	31 28	23 24	46 27
4		55 18	52 16	60 25	34 15 **6975**	23 14	46 28	30 25	36 23	30 22	35 36	22 17	41 18
5		48 21	50 29	50 17	16 13 **>10000**	15 18	26 22	34 19	33 10	33 15	12 36	13 15	39 22
6		46 18	51 22	48 23	13 23 **>10000**	9 22	27 23	29 2	33 11	32 15	11 34	12 18	41 23
7		70 39	67 33	81 56 **225**	66 15 **676**	53 23	75 14	39 31	56 41	63 43	69 48	26 20	70 33
8		66 27	65 31	65 33	52 16 **292**	57 26	55 21	36 25	40 38	60 46	47 28	31 19	71 31
9		72 42	65 35	84 51 **155**	67 36 **283**	55 42	77 46	36 33	53 41	64 46	68 42	30 22	67 32
10		48 13	52 19	50 21	14 22 **>10000**	14 38	15 31	35 7	36 18	28 15	0 11	20 21	43 22
11		52 21	56 22	57 22	36 31 **5963**	36 31	41 39	37 8	39 30	38 35	16 13	21 21	48 25
12		73 34	71 32	86 50 **166**	66 47 **252**	62 15	78 31	40 39	66 45	66 49	58 35	42 18	69 33
13		78 33	77 43	84 56 **185**	61 57 **175**	72 18	76 20	51 41 **3950**	74 50 **435**	75 55 **443**	65 35	54 27	77 41
14		59 21	63 30	61 20	37 54 **355**	58 35	42 37	4326 35	45 35	55 43	31 26	42 21	64 34
15		72 30	68 43	77 41	61 37 **291**	55 43	64 41	35 27	58 41	64 49	78 44	40 21	73 51 **1191**
16		49 23	51 31	57 23	39 23 **7940**	22 32	40 28	36 18	30 21	30 19	24 24	20 15	52 24

(continued)

Table 6 (*continued*)

		1	2	3	4	5	6	7	8	9	10	11	12
22													
17		45 14	50 19	47 19	22 32 **7150**	15 40	25 37	38 15	31 17	28 12	21 20	181 16	492 28
18		49 15	50 18	53 27	24 20 **6450**	16 25	33 22	34 3	34 10	28 9	17 21	20 18	43 20
19		49 15	53 26	53 26	17 7 **2555**	27 20	24 22	39 17	37 22	32 31	18 23	26 17	52 22
20		54 22	59 22	53 28	36 12 **2252**	41 25	28 25	38 17	48 44	56 40	30 17	37 26	69 31
21		44 24	53 19	55 34	23 13 **1328**	19 20	52 35	35 31	37 37	40 36	54 29	43 26	59 22
22		58 18	51 23	61 35	39 20 **2850**	22 27	53 31	33 16	31 23	32 29	20 22	32 23	52 21
23		83 51 **122**	88 62 **127**	88 63 **44**	77 73 **22**	86 54 **318**	80 61 **103**	66 55 **718**	86 66 **240**	86 74 **103**	72 47 **257**	74 38 **992**	90 59 **225**
24		79 45	74 44	85 60 **233**	74 65 **213**	71 49	80 39	52 40	68 52 **430**	73 55 **529**	73 43	58 28	78 42
25		48 24	49 25	47 23	18 40 **7600**	16 44	23 36	38 20	32 20	30 10	9 22	20 12	50 48
26		52 25	46 26	50 27	24 43 **2200**	8 43	26 22	34 14	33 12	28 5	23 32	25 14	52 47

gave derivatives with much improved activity (Fig. 2). Generally sulphonamides bearing 4-alkyl substituents gave the highest activity, at both 100 and 1000 nM, in the order: 4-*n*Bu > 3-Cl, 4-Me > 4-*n*Pr > 4-iPr∼4-Me∼4-Et. With the exception of 4-OMe (sulphonamide No. 7), which gave reasonable inhibition, particularly at 1000 nM, more polar substituents in the 4-position were not well tolerated. Likewise, multiply-substituted aromatics produced low activity, e.g., 2,3,4,5,6-pentafluoro-, 2,4,6-trimethyl and 2,3,4,5,6-pentamethyl-phenylsulphonyl. The SAR for the 12 amine variants appeared to be flatter than that for the sulphonamides, particularly at 100 nM (Fig. 3). At both 100 nM and 1000 nM, amine No. 3 appeared to be the most active.

All compounds which produced >50% inhibition at 100 nM were selected for IC_{50} determination (Table 6; bold text). In order to obtain more SAR information, IC_{50}s were also determined for all derivatives containing either amine No. 4 or sulphonamide No. 23. The SAR trend observed from the crude data was essentially confirmed for the 26 different sulphonamides in combination with amine No. 4 (Fig. 2). Again the most active compound **22{23,4}** contained the 4-(*n*-butylphenyl)-sulphonyl group (22 nM), and the order of activity was 4-*n*Bu > 4-*n*Pr∼3-Cl, 4-Me∼4-iPr∼4-Me∼4-Et. In combination with sulphonamide No. 23, the amine variant which gave rise to the most active compound was the original (4-methyl)-3-pyridyl group, amine No. 4 (Fig. 3). The next most active contained a (3-pyridyl)methyl group (98 nM). Confirming a relatively flat SAR in this region, all 12 amines, in combination with sulphonamide No. 23, provided IC_{50}s of less than 1 μM.

Four compounds from the amide library were selected for resupply as hydrochloride salts for in vivo testing:

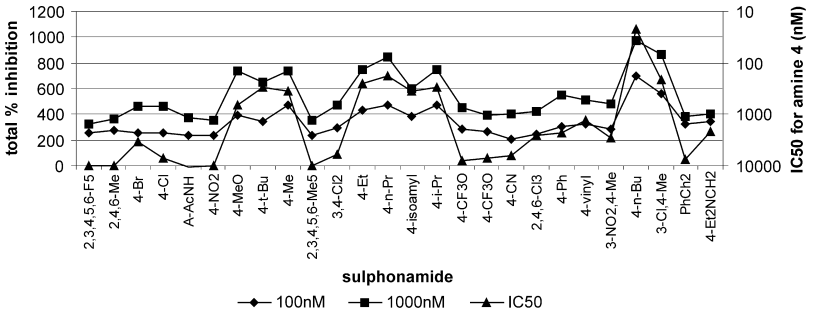
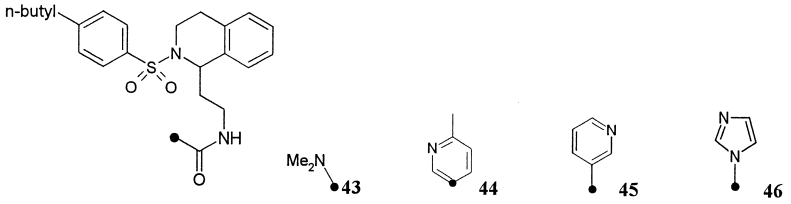


Figure 2. Trends in activity for the 26 sulphonamide variants in **22**. The total % inhibition data (100 nM and 1000 nM; left hand linear scale) represent the sum of the % inhibitions for the 12 different amines at these concentrations (shown in Table 6). The IC_{50} data (right hand logarithmic scale) were determined for amine No. 4 only.

Table 7. Opioid binding and in vivo data for reference μ and δ opioids, compound **1** and analogues[a]



	IC_{50} δ (nM)[a]	IC_{50} μ (nM)	IC_{50} κ (nM)	mouse FPT: Phase 1[bc] (dose in μmol/kg i.v.)	mouse FPT: Phase 2 [b,c] (dose in μmol/kg i.v.)	M.Wt.	clogP
SNC-80	2.2[d]	9600	8200	48 (3)	79 (3)	449.64	4.9
				6 (3)	37 (3)		
TAN-67	1.4[d]	1197	1160	61 (20)	52 (20)	344.46	3.8
Morphine	257	3.5	105	58 (10)	69 (10)	285.34	0.6
Fentanyl	254	4.1	485	97 (0.1)	79 (0.1)	336.48	3.6
1	98	> 10,000	> 10,000	nt[e]	nt[e]	460.55	5.5
19	110	> 10,000	3300	55 (10)	47 (10)	449.57	4.9
				62 (20)	73 (20)		
				36 (20)	*22 (20)*		
43	256 (127)	> 10,000	1010	57 (10)	47 (10)	457.63	5.3
44	37 (22)	> 10,000	1285	53 (20)	39 (20)	491.65	6.4
45	172 (98)	> 10,000	1680	68 (10)	58 (10)	491.65	5.1
46	265 (185)	> 10,000	2005	39 (10)	10 (10)	480.62	4.9

[a]Mean value of at least two experiments; in brackets IC_{50} for unpurified library compound for comparison.
[b]% Inhibition of paw licking behaviour.
[c]In italics, % inhibition in the presence of Naltrindole (10 mg/kg ip) administered 35 min before and immediately prior to compound.
[d]Affinity data consistent with published data.[15,16]
[e]nt = not tested due to low aqueous solubility of compound.

43, **44**, **45** and **46**, equivalent to **22{23,2}**, **22{23,4}**, **22{23,9}**, **22{23,12}** respectively (Table 7). All compounds appeared to have similar affinity to the equivalent unpurified library compounds, albeit slightly lower. Selectivity over the MOR was very good for all compounds, but KOR selectivity was somewhat lower. For **44**, μ selectivity was > 270-fold and κ selectivity was 35-fold. It would appear that the replacement of the tosyl group with a 4-(*n*-butylphenyl)-sulphonyl group, increases both κ and δ affinity.

In vivo pharmacology. Analgesic activity of test compounds was assessed in mice in the formalin paw test in mice (FPT), a well-validated model of inflammatory pain.[13,14] Whilst compound **1** was too insoluble to be tested, the more soluble compound **19** was found to possess analgesic activity in the FPT (Table 7). At a dose of 10 μmol/kg i.v., **19** inhibited paw licking behaviour by 58% in Phase 1 of the test and by 42% in Phase 2. The inhibitory effect is dose dependent, with higher activity being observed at a dose of 20 μmol/kg i.v.: 65% in Phase 1 and 71% in Phase 2. Unsurprisingly, considering its much lower affinity at

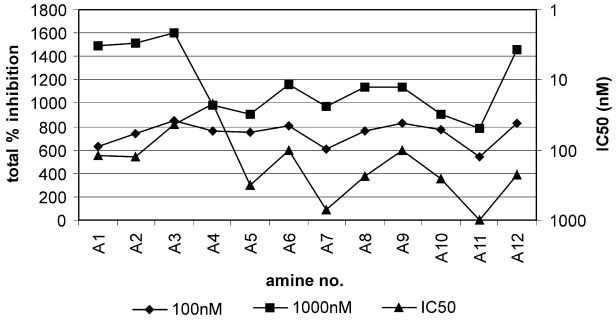


Figure 3. Trends in activity for the 12 amine variants in 22. The total % inhibition data (100 nM and 1000 nM; left hand linear scale) represent the sum of the % inhibitions for the 26 sulphonamides at these concentrations (shown in Table 6). The IC_{50} data (right hand log. scale) were determined for sulphonamide No. 23 only.

the DOR, **19** was less active than the literature δ opioid, SNC-80. SNC-80 produced 48% inhibition in Phase 1 and 79% inhibition in Phase 2 at a dose of 3 μmol/kg i.v. The analgesic activity of both **19** and SNC-80 was reduced significantly by the δ-selective antagonist, naltrindole (NTI). Encouragingly, this confirms that compound **19,** like SNC-80, produces analgesia which is mediated primarily via a DOR mechanism.

Compounds **43**, **44**, **45** and **46** were also tested in the FPT in mice and all showed some analgesic activity. The highest affinity ligand **44** showed weaker analgesic activity than **43** or **45** in both phases of the test. Only the imidazole-containing **46** was less active. It is tempting to speculate that the high lipophilicity of **44** (cLogP 6.4; Table 7) is a contributory factor, perhaps causing extensive plasma protein binding which is reducing CNS penetration.[12]

Conclusion

The tetrahydroisoquinoline sulphonamide-based ligands described in this paper represent a completely new structural class of δ opioid ligand. Whilst the compounds were attractive leads in terms of affinity, selectivity and synthetic accessibility, the main challenge in this series was to improve physicochemical properties, notably aqueous solubility. The most active analogue had an affinity of 6 nM for the cloned human DOR, representing a 15-fold improvement relative to the original hit **1**. However this analogue also had poor aqueous solubility. It was difficult to combine in a single molecule structural features which provided both improved affinity and solubility. Improved aqueous solubility was obtained by replacing the phthalimide group in the original hit by basic groups, allowing the synthesis of salt forms. The best compromise between affinity and solubility was found in compound **44** which had 3-fold better affinity than the original hit (37 nM) and a >10-fold improvement in aqueous solubility. Several analogues showed activity in an in vivo model of antinociception, the formalin paw test. In the case of compound **19**, this analgesic activity was shown to be mediated primarily via a δ opioid mechanism. The most active compound in vivo, **45**, showed similar potency in this test to morphine and the previously reported delta opioid ligands, TAN-67 and SNC-80.

Experimental

General chemical procedures

All reagents were either purchased from common commercial sources or synthesised according to literature references using commercial sources. Proton NMR (^{1}H NMR) were obtained on a Bruker DPX 400 spectrometer and are referenced to internal TMS. Mass spectra were recorded on a Shimadzu LC-8A (HPLC) PE Sciex API 150EX LC–MS. Analytical reversed-phase LC–MS analysis was carried out on LUNA C18 column (5 μm; 30×4.6 mm) under gradient conditions (90% water/0.1% formic acid to 90% acetonitrile/0.1% formic acid) at a flow rate of 4 mL/min. Analytical GC–MS (EI) analysis was carried out on a Hewlett Packard 5890 Series II gas chromatogram (temperature gradient 100 °C–310 °C, 25 °C/min; RTX1 column: 30 m×0.25 mm, DF 0.25 μm) and Hewlett Packard 5971 mass selective detector. Analytical GLC was recorded on Shimadzu GC-14A gas chromatograph (240 °C; RTX1 column: 30 m×0.25 mm; DF 0.25 μm). Preparative reversed-phase HPLC purification was carried out on a Gilson system using a LUNA C18 column (5 μm; 60×21.2 mm) under gradient conditions (10 min; 90 % water/0.1 % TFA to 90% acetonitrile/0.1% TFA) at a flow rate of 5 mL/min. Lipophilicity (clog P) was calculated using the CLOGP3, PCModels version 4.34 (Daylight Chemical Information Systems Inc. Irvine, CA, USA).

1-[2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)ethyl]-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (1) (Method A). A mixture of **3** (9.12 g, 33.1 mmol) and **5** (7.40 g, 36.4 mmol) in trifluoroacetic acid (70 mL) was stirred at reflux for 90 min and then allowed to cool to room temperature. The reaction mixture was added to 200 mL of ice and water and the product was extracted into CH_2Cl_2 (2×100 mL), and combined extracts were washed with saturated aqueous $KHCO_3$ and then dried (Na_2SO_4) and concentrated to give a gum which crystallised. The crude product was recrystallised from CH_2Cl_2/EtOH to give a powder which was further purified by column chromatography on silica gel using heptane/EtOAc (4:1) followed by CH_2Cl_2/CH_3OH (95:5) as eluent. The product was obtained as a crystalline solid (13.83 g, 90%). ^{1}H NMR ($CDCl_3$): δ 1.95–2.29 (m, 2H), 2.31 (s, 3H), 2.48–2.60 (m, 2H), 3.53–3.73 (m, 1H), 3.85–4.06 (m, 3H), 5.06–5.18 (m, 1H), 6.84 (d, 1H),7.00-7.18 (m, 5H),7.60 (d, 2H), 7.64–7.76 (m, 2H), 7.76–7.89 (m, 2H).

1-[2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)ethyl]-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (1) (Method B). To a solution of 1-[*N*-(2-phthalimidoethyl)]-1,2,3,4-tetrahydroisoquinoline (**10**) (3.0 g, 9.8 mmol) and diisopropylethylamine (1.29 g, 10.0 mmol) in CH_2Cl_2 (30 mL) was added *p*-toluenesulfonyl chloride (1.91 g, 10.0 mmol). Solution was stirred at room temperature for 2 h then washed with water, dried (Na_2SO_4) and solvent removed under reduced pressure. The residue was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH 95:5 as eluent to afford (**1**) as a crystalline solid (4.50 g, 99%).

4-methyl-*N*-(2-phenylethyl)-benzenesulfonamide (3). To a solution of 2-phenethylamine **2** (12.12 g, 0.1 mol) in dry pyridine (100 mL) strirring under N_2 in a cooling bath at −20 °C was added *p*-toluenesulfonyl chloride (25.0 g, 0.13 mol) over 10 min, keeping internal temperature below −20 °C during the addition. The resultant red solution was stirred for 18 h at room temperature, then quenched with water (400 mL). The mixture was extracted into CH_2Cl_2 (3×150 mL), and the extracts were combined and washed with 2 N HCl (3×200 mL), brine, and then dried (Na_2SO_4). Solvent was evaporated to obtain the product (27.23 g, 99%). ^{1}H NMR

($CDCl_3$): δ 2.43 (s, 3H), 2.76 (t, 2H), 3.21 (t, 2H), 4.33 (br t, 1H), 7.06 (d, 2H), 7.20–7.35 (m, 5H), 7.70 (d, 2H).

1,3-dihydro-1,3-dioxo-2H-isoindole-2-propanal, (5). To a stirred solution of *N*-(3-hydroxypropyl)phthalimide **4** (15.0 g, 73.1 mmol) in dry CH_2Cl_2 under N_2 at 0 °C was added pyridinium dichromate (17.4 g, 80.7 mmol) over 2 min. Cooling bath was removed and mixture was stirred at room temperature for 18 h. Reaction mixture was filtered through a dicalite pad and filtrate was collected. Solvent was evaporated under reduced pressure and the resulting residue was purified by column chromatography on silica gel using CH_2Cl_2/CH_3OH (99:1) as eluent to afford the desired product as a crystalline solid (8.88 g, 59%). ^1H NMR ($CDCl_3$): δ 2.89 (t, 2H), 4.04 (t, 2H), 7.65–7.78 (m, 2H), 7.78–7.93 (m, 2H), 9.83 (s, 1H).

1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-1-isoquinolineethanamine (11). To a stirring suspension of **1** (5.20 g, 11.3 mmol) in EtOH was added hydrazine monohydrate (10.1 g, 214 mmol) in one portion, and the resultant mixture was stirred at reflux for 18 h. The reaction was cooled, quenched with water (300 mL) and product was extracted into EtOAc (3×100 mL). Extracts were combined, washed with water (50 mL) and dried (Na_2SO_4). Solvent was evaporated to obtain the product (3.62 g, 97%). ^1H NMR (DMSO-d_6): δ 1.57–1.98 (m, 2H), 2.27 (s, 3H), 2.33–2.70 (br m, 4H), 3.34–3.54 (m, 1H), 3.65–3.82 (m, 1H), 4.95–5.10 (m, 1H), 6.89 (d, 1H), 6.98–7.17 (m, 3H), 7.22 (d, 2H), 7.60 (d, 2H).

1-(2-aminoethyl)-3,4-dihydro-2(1H)-isoquinolinecarboxylic acid-1,1-dimethylethyl ester (15). A mixture of **10**[10] (35.68 g, 0.116 mol), di-*tert*-butyl dicarbonate (25.23 g, 0.116 mol), and sodium hydrogen carbonate (28.81 g, 0.343 mol) in CH_3OH (600 mL) was placed in an ultrasonic bath for 1 h. Insoluble material was filtered off, and filtrate was evaporated under reduced pressure to afford the crude intermediate. This was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (98:2) followed by CH_2Cl_2/CH_3OH (97:3) as eluent (37.2 g, 78%). ^1H NMR ($CDCl_3$): δ 1.48 (s, 9H), 2.05–2.30 (br m, 2H), 2.64–3.12 (br m, 2H), 3.21–3.45 (br m, 1H), 3.64–4.36 (br m, 3H), 5.49–5.41 (m, 1H), 7.15 (m, 4H), 7.70 (m, 2H), 7.82 (m, 2H). The phthalimide (37.2 g, 0.091 mmol) was then dissolved in EtOH (400 mL) and hydrazine monohydrate (68.7 g, 1.37 mol) was added rapidly. The mixture was stirred at room temperature for 18 h. The resultant thick white precipitate was quenched with water (2 L) and product was extracted into EtOAc (3×500 mL). Extracts were combined, washed with brine (2×200 mL) and dried (Na_2SO_4). Solvent was evaporated to afford **15** (24.7 g, 98%). ^1H NMR ($CDCl_3$): δ 1.50 (s, 9H), 2.05–2.30 (br m, 2H), 2.67–2.77 (br m, 1H), 2.85–3.05 (br m, 1H), 3.22–3.42 (br m, 1H), 3.67–4.30 (br m, 3H), 5.13–5.35 (br m, 1H), 7.08–7.22 (br m, 4H), 7.68–7.73 (br m, 2H), 7.80–7.86 (br m, 2H).

6-methyl-3-[2-[1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-1-isoquinolinyl]ethyl]amino]carbonyl]-2-pyridinecarboxylic acid (17) and 6-methyl-2-[2-[1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-1-isoquinolinyl]ethyl]amino]-carbonyl]-3-pyridinecarboxylic acid (18). To a stirring suspension of 6-methyl-2,3-pyridinedicarboxylic acid (90 mg, 0.5 mmol) in dry THF (10 mL) was added triethylamine (102 mg, 1.0 mmol), forming a solution. Ethyl chloroformate (108 mg, 1.0 mmol) was added and the resultant suspension was stirred at room temperature for 30 min. A solution of **11** (165 mg, 0.5 mmol) in THF (1 mL) was added at a rapid dropwise rate and the reaction mixture was stirred at reflux for 2 h. Solvent was removed under reduced pressure and residue was partitioned between EtOAc (10 mL) and water (10 mL). Organic layer was washed with water (2×5 mL), dried (Na_2SO_4) and concentrated, then purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (95:5) as eluent, to give a mixture of the amides **17** and **18** (150 mg). ^1H NMR confirmed the product was a mixture. The product was used in the next step without any further purification.

1-[2-[[(6-methyl-3-pyridinyl)carbonyl]amino]ethyl]-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (19) and 1-[2-(5,7-dihydro-2-methyl-5,7-dioxo-6H-pyrrolo[3,4-b]pyridin-6-yl)ethyl]-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (20). The crude mixture of **17** and **18** (150 mg) was heated in a Pyrex test tube to 200 °C for 45 min. The resultant glassy solid was purified by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (99:1) as eluent, to give the amide **19** (38 mg, 17 %). ^1H NMR ($CDCl_3$): δ 1.90–2.02 (m, 2H), 2.30 (s, 3H), 2.32–2.50 (m, 2H), 2.63 (s, 3H), 3.34–3.52 (m, 2H) 3.95–4.05 (m, 1H), 4.05–4.15 (m, 1H), 5.05–5.12 (m, 1H), 6.81 (d, 1H), 6.92–7.13 (m, 5H), 7.26 (d, 1H), 7.56 (d, 2H), 7.66 (br t, 1H), 8.12 (d, 1H), 9.10 (s, 1H), MS 450.4 (MH+). The imide **20** (58 mg, 16%) was also isolated from the chromatography. ^1H NMR ($CDCl_3$): δ 2.10–2.28 (m, 2H), 2.31 (s, 3H), 2.47–2.58 (m, 2H), 2.76 (s, 3H), 3.50–3.68 (m, 1H), 3.85–4.05 (m, 3H), 5.05–5.18 (m, 1H), 6.86 (d, 1H), 7.00–7.30 (m, 6H), 7.44 (d, 1H), 7.59 (d, 2H), 8.03 (d, 1H), MS 475.9 (MH+).

General procedure for the synthesis of amides (21). A solution of carboxylic acid (4.0 mmol), 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (0.92 g, 4.80 mmol) and triethylamine (0.49 g, 4.80 mmol) was stirred at room temperature for 15 min (for acids 7, 8, and 11, a further 0.41 g, 4.0 mmol of triethylamine was added because the carboxylic acid starting material contained an amine hydrochloride). To this solution was added 1-hydroxybenzotriazole (0.54 g, 4.0 mmol) and **15** (1.11 g, 4.0 mmol) and stirring at room temperature was continued for 18 h. Reaction mixture was washed with water (2×10 mL) and brine (10 mL) then dried (Na_2SO_4) and concentrated. Purification by flash chromatography on silica gel using CH_2Cl_2/CH_3OH (95:5) or $CH_2Cl_2/CH_3OH/NH_4OH$ (90:10:0.5) as eluent afforded the desired amides which were characterised by ^1H NMR.

General procedure for BOC-deprotection of amides. The amides **21** were dissolved in CH_2Cl_2 (20 mL), trifluoroacetic acid (5 mL) was added and reaction was stirred at room temperature for 1 h. Reaction was evaporated to dryness and saturated K_2CO_3 solution

(20 mL) was added followed by brine (20 mL). Product was extracted into EtOAc (4×20 mL) then combined extracts were dried (Na_2SO_4) and solvent was evaporated under reduced pressure to give the secondary amine. All amines had structures confirmed by ^1H NMR and were used for sulfonamide formation without further purification.

General procedure for formation of sulfonamides (22). To solutions of the BOC-deprotected tetrahydroisoquinolines (0.07 mmol) in CH_2Cl_2 (3 mL) were added the sulfonyl chlorides (0.0875 mmol). PS resin-bound piperidine (2 mmol g^{-1} substitution, 100 mg, 0.28 mmol) was added to each reaction, then reactions were allowed to stand at room temperature (no agitation was required) for 48 h. Aminomethyl PS resin (1.39 mmol g^{-1} substitution, 50 mg, 0.07 mmol) was then added to each reaction, then reactions were allowed to stand at room temperature for a further 48 h. Resin was removed by filtration through a fritted tube, washing resin with CH_2Cl_2 (2×2 mL). Filtrates were concentrated to give the products.

Modified procedure for formation of *N,N*-diethybenzylamine sulfonamides (Table 6, sulfonamide No. 26). To a solution of each of the BOC-deprotected tetrahydroisoquinolines (0.07 mmol) in CH_2Cl_2 (3 mL) was added 4-bromomethylbenzenesulfonyl chloride (0.0875 mmol). PS resin-bound piperidine (2 mmol g^{-1} substitution, 100 mg, 0.28 mmol) was added to each reaction, then the reactions were allowed to stand at room temperature (no agitation was required) for 48 h. Diethylamine (0.35 g, 4.83 mmol) was added to each reaction vessel, then the reactions were allowed to stand at room temperature for 48 h. Resin was removed by filtration through a fritted tube, washing resin with CH_2Cl_2 (2×2 mL). Filtrates were concentrated to give the crude products, which were purified by SPE (500 mg silica, $CH_2Cl_2/CH_3OH/NH_4OH$ (90:10:0.5) as eluent).

1-[2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)ethyl]-1,2,3,4-tetrahydro-2-(phenylsulfonyl)-isoquinoline (23). To a solution of **10**[10] (90 mg, 0.29 mmol) in CH_2Cl_2 (10 mL) was added benzenesulfonyl chloride (78 mg, 0.44 mmol) followed by diisopropylethylamine (93 mg, 0.72 mmol). Solution was stirred at room temperature for 2 h then concentrated under reduced pressure. Residue was purified using flash chromatography on silica gel, using CH_2Cl_2/CH_3OH (98:2) as eluent to afford **23** (85 mg, 66%). ^1H NMR ($CDCl_3$): δ 2.03–2.26 (m, 2H), 2.50–2.61 (m, 2H), 2.59–2.68 (m, 1H), 3.85–4.04 (m, 3H), 5.10–5.17 (m, 1H), 6.81–6.87 (d, 1H), 6.98–7.15 (m, 3H), 7.25–7.34 (m, 2H), 7.38–7.44 (m, 2H), 7.68–7.76 (m, 4H), 7.80–7.86 (m, 2H). MS 447.2 (MH^+).

1-[2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)ethyl]-1,2,3,4-tetrahydro-2-(4-methylbenzoyl)-isoquinoline (24). To a solution of **10**[10] (100 mg, 0.33 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (41 mg, 0.41 mmol) and the solution was stirred at room temperature for 1min. *p*-toluoyl chloride (51 mg, 0.33 mmol) was added and the reaction was stirred for 1 h at room temperature then washed with 5% Na_2CO_3, dried (Na_2SO_4) and concentrated under reduced pressure. Residue was purified using flash chromatography on silica gel, using CH_2Cl_2/CH_3OH 98:2 as eluent to afford **24** (60 mg, 43%). ^1H NMR ($CDCl_3$): δ 2.03–2.35 (m, 2H), 2.41 (s, 3H), 2.62–2.77 (br m, 1H), 2.80–3.05 (m, 1H), 3.40–4.18 (m, 4H), 5.85–6.00 (m, 1H), 6.98–7.45 (m, 8H), 7.64–7.90 (m, 4H). MS 424.4 (MH^+).

1-[2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)ethyl]-1,2,3,4-tetrahydro-2-(phenylmethyl)-isoquinoline (25). A mixture of **10**[10] (100 mg, 0.33 mmol), benzaldehyde (35 mg, 0.33 mmol), sodium acetate (44 mg, 0.53 mmol) and glacial acetic acid (0.26 g, 4.30 mmol) was stirred in ethanol (2 mL) and cooled in an ice-water bath. Sodium borohydride (62 mg, 1.63 mmol) was added over 90 min, then cooling bath was removed and resultant suspension was stirred at room temperature for 30 min. Reaction mixture was made basic with 5% Na_2CO_3, and the product was extracted into CH_2Cl_2 (2×5 mL), dried (Na_2SO_4) and solvent removed under reduced pressure to afford the crude **25**. Purification using flash chromatography on silica gel, using toluene/EtOAc (9:1) as eluent afforded the desired product (10 mg, 8%). ^1H NMR ($CDCl_3$): δ 2.00–2.09 (m, 1H), 2.14–2.25 (m, 1H), 2.54–2.62 (m, 1H), 2.78–2.85 (m, 1H), 2.86–2.96 (m, 1H), 3.22–3.31 (m, 1H), 3.66–3.87 (m, 4H), 3.89–3.97 (m, 1H), 7.03–7.15 (m, 4H), 7.21–7.35 (m, 3H), 7.38–7.44 (m, 2H), 7.65–7.70 (m, 2H), 7.77–7.82 (m, 2H). MS 397.2 (MH^+).

1-[2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)ethyl]-1,2,3,4-tetrahydro-2-(methylsulfonyl)-isoquinoline (26). Prepared from **10**[10] (50 mg, 0.16 mmol) and methanesulfonyl chloride (37 mg, 0.32 mmol) using the procedure described for **23** (22 mg, 36%). ^1H NMR ($CDCl_3$): δ 2.08–2.26 (m, 2H), 2.76 (s, 3H), 2.77–2.84 (m, 1H), 3.02–3.13 (m, 1H), 3.59–3.69 (m, 1H), 3.87–3.96 (m, 2H), 4.02–4.10 (m, 1H), 4.89–4.95 (m, 1H), 7.10–7.21 (m, 4H), 7.66–7.72 (m, 2H), 7.80–7.86 (m, 2H). GLC $t_R = 1.06$ min (98.5%). MS 385.0 (MH+).

1-[2-(acetamido)ethyl]-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (28). To **11** (20 mg, 0.06 mmol) was added acetic anhydride (1 mL). The mixture was stirred at 20 °C for 5 h then evaporated to dryness, with the aid of a toluene azeotrope. The residue was taken up in DCM and evaporated once more. The product was pure according to TLC and NMR. ^1H NMR: ($CDCl_3$; 400 MHz) δ 7.57 (d, $J = 8.3$ Hz, 2H), 7.00–7.12 (m, 5H), 6.83 (d, $J = 7.5$ Hz, 1H), 6.70 (m, 1H), 5.05 (dd, 1H), 3.95 (m, 1H), 3.85 (m, 1H), 3.40 (m, 1H), 3.15 (m, 1H), 2.35–2.50 (m, 2H), 2.31 (s, 3H), 2.07 (s, 3H), 2.00–2.15 (m, 1H), 1.85 (m, 1H).

1-Methyl-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (29). Prepared by Method A from 4-methyl-*N*-(2-phenethyl)benzenesulfonamide and acetaldehyde. Obtained as a colourless gum in 76% yield. ^1H NMR ($CDCl_3$) δ 1.48 (d, 3H), 2.36 (s, 3H), 2.65 (m, 2H), 3.38 (m, 1H), 3.85 (m, 1H), 5.12 (q, 1H), 6.97 (d, 1H), 7.04 (d, 1H), 7.11 (m, 2H), 7.17 (d, 2H) and 7.67 (d, 2H). GLC $t_R = 6.27$ min (98.3%). GC–MS 301 (M^+).

1-[3-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)propyl]-1,2,3,4-tetrahydro-2-[(4-methylphenyl) sulfonyl]-isoquinoline (30). Prepared by Method A from 4-methyl-*N*-(2-phenethyl) benzenesulfonamide and 4-phthalimidobutyraldehyde. Obtained as a colourless gum in 61% yield. ^{1}H NMR ($CDCl_3$) δ 1.73 (m, 1H) and 1.87 (m, 3H), 2.27 (s, 3H), 2.5 (m, 2H), 3.44 (m, 1H), 3.74 (m, 1H), 3.85 (m, 2H), 5.07 (m, 1H), 6.84 (d, 1H), 7.06 (m, 5H), 7.58 (m, 2H), 7.73 (m, 2H) and 7.86 (m, 2H). GLC $t_R = 3.86$ min (99.7%). MS 475.2 (MH^+).

1-[(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)methyl]-1,2,3,4-tetrahydro-2-[(4-methylphenyl) sulfonyl]-isoquinoline (31). Prepared by Method A from 4-methyl-*N*-(2-phenethyl) benzenesulfonamide and 2-phthalimidoacetaldehyde. Obtained as a colourless solid in 81% yield. ^{1}H NMR ($CDCl_3$) δ 2.19 (s, 3H), 2.68 (m, 2H), 3.76 (m, 2H), 3.96 (m, 1H), 4.07 (m, 1H), 5.28 (dd, 1H), 6.93 (d, 2H), 7.03 (d, 1H), 7.18 (m, 2H), 7.27 (d, 2H), 7.71 (m, 2H), and 7.84 (m, 2H). GLC $t_R = 6.21$ min (98.8%). MS 447.4 (MH^+).

1-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)ethyl]-7-hydroxy-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (32). 4-Methyl benzensulphonyl chloride (2.77 g, 14.53 mmol) was added into a solution of tyramine (2.0 g, 14.58 mmol) in pyridine (40 mL) and the resulting mixture was stirred over 16 h at room temperature. The reaction mixture was diluted with water (50 mL) and the product extracted with DCM (2×50 mL). The organic layer was washed with 2 N HCl (150 mL), water (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic phase was dried with $MgSO_4$ and concentrated under reduced pressure to obtain crude 4-methyl-*N*-[2-(4-hydroxyphenyl)ethyl]benzenesulfonamide which was purified by recrystallization from hot ethanol (20 mL). Yield: 3.98 g (94%). ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 2.62 (t, 2H), 3.09 (t + bs, 3H), 6.74 (d, 2H), 6.92 (d, 2H), 7.31 (d, 2H) and 7.68 (d, 2H). MS 291 (MH^+). Compound **32** was prepared by Method A from 4-methyl-*N*-[2-(4-hydroxyphenyl)ethyl]benzenesulfonamide and 3-phthalimidopropionaldehyde. Obtained as a colourless gel in 61% yield. ^{1}H NMR ($CDCl_3$) δ 2.08 (m, 1H), 2.16 (m, 1H), 2.31 (s, 3H), 2.44 (m, 2H), 3.56 (m, 1H), 3.89 (m, 3H), 5.02 (dd, 1H), 5.36 (s, 1H), 6.55 (dd, 1H), 6.65 (d, 1H), 6.71 (d, 1H), 7.09 (d, 2H), 7.58 (d, 2H), 7.68 (m, 2 H), and 7.81 (m, 2H). LC–MS purity, 93.4%. MS 477.2 (MH^+).

1-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)ethyl]-5-methoxy-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (33). Prepared by Method A from 4-methyl-*N*-[2-(2-methoxyphenyl)ethyl]benzenesulfonamide and 3-phthalimidopropionaldehyde. Obtained as a colourless gel in 82% yield.^{1}H NMR ($CDCl_3$) δ 2.12 (m, 2H), 2.29 (s + m, 5H), 3.55 (m, 1H), 3.68 (s, 3H), 3.88 (m, 2H), 4.02 (m, 1H), 4.98 (m, 1H), 6.59 (dd, 1H), 6.74 (dd, 1H), 7.08 (m, 3H), 7.59 (d, 2H), 7.69 (m, 2H) and 7.79 (m, 2H). GLC $t_R = 16.43$min (88.9%). MS 491.4 (MH^+).

1-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)ethyl]-7-methoxy-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (34). Prepared by Method A from 4-methyl-*N*-[2-(4-methoxyphenyl)ethyl]benzenesulfonamide and 3-phthalimidopropionaldehyde. Obtained as a colourless gel in 83% yield. ^{1}H NMR ($CDCl_3$) δ 2.08 (m, 2H), 2.28 (s, 3H), 2.44 (m, 2H), 3.48 (m, 1H), 3.69 (s + m, 6H), 4.98 (t, 1H), 6.59 (dd, 1H), 6.81 (m, 2H), 7.17 (d, 2H), 7.56 (d, 2H) and 7.84 (m, 4H). GLC $t_R = 7.59$ min (98.2%). MS 491.4 (MH^+).

1-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)ethyl]-6-methoxy-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (35). Prepared by Method A from 4-methyl-*N*-[2-(3-methoxyphenyl)ethyl]benzenesulfonamide[3] and 3-phthalimidopropionaldehyde. Obtained as a colourless gel in 81% yield. ^{1}H NMR ($CDCl_3$) δ 2.06 (m, 1H), 2.17 (m, 1H), 2.31 (s, 3H), 2.52 (m, 2H), 3.57 (m, 1H), 3.69 (s, 3H), 3.88 (m, 3H), 5.07 (dd, 1H), 6.36 (d, 1H), 6.65 (dd, 1H), 7.02 (d, 1H), 7.09 (d, 2H), 7.58 (d, 2H), 7.69 (m, 2H) and 7.81 (m, 2H). GLC $t_R = 12.41$ min (94.1%). MS 491.4 (MH^+).

1-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)ethyl]-6,7-dihydroxy-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (36). An intermediate in synthesis of **27**, 1,2,3,4-tetrahydro-6,7-dimethoxy-2[(4-methylphenyl)-sulfonyl]-1-(2-phthalimidoethyl)isoquinoline, was prepared by Method A from 4-methyl-*N*-[2-(3,4-dimethoxyphenyl)ethyl]benzenesulfonamide and 3-phthalimidopropionaldehyde. Obtained as a colourless gel in 52% yield. ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 1H), 2.17 (m, 1H), 2.32 (s, 3H), 2.41 (m, 2H), 3.57 (m, 1H), 3.74 (s, 3H), 3.86 (s, 3H), 3.91 (m, 3H), 5.03 (dd, 1H), 6.31 (s, 1H), 6.62 (s, 1H), 7.09 (d, 2H), 7.59 (d, 2H), 7.71 (m, 2H) and 7.82 (m, 2H). MS 521.2 (MH^+). A solution of 1,2,3,4-tetrahydro-6,7-dimethoxy-2-[(4-methylphenyl)sulfonyl]-1-(2-phthalimidoethyl) isoquinoline (128 mg, 0.245 mmol) in DCM (5 mL) was added dropwise into a solution of BBr_3 (0.185 mL, 1.96 mmol) in DCM (10 mL) under nitrogen at −5 °C. After 2 h the reaction mixture was allowed to warm up and stirred for another 16 h at room temperature. The reaction was quenched with water (20 mL) and extracted with ether (3×150 mL). The combined organic layers were concentrated under reduced pressure and the crude product was purified by flash chromatography on silica gel eluting with EtOAc/heptane (1:1), to obtain **37** as a colourless gum. Yield: 14 mg (12%). ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 1H), 2.12 (m, 1H), 2.32 (s, 3H), 2.37 (m, 2H), 3.49 (m, 1H), 3.88 (m, 3H), 4.94 (dd, 1H), 5.58 (bs, 1H), 6.02 (bs, 1H), 6.37 (s, 1H), 6.64 (s, 1H), 7.1 (d, 2H), 7.58 (d, 2H), 7.68 (m, 2H) and 7.83 (m, 2H). MS 493.4 (MH^+).

1-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)ethyl]-7-methyl-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (37). Prepared by Method A from 4-methyl-*N*-[2-(4-methylphenyl)ethyl]benzenesulfonamide and 3-phthalimidopropionaldehyde. Obtained as a colourless solid in 85% yield. ^{1}H NMR ($CDCl_3$) δ 2.13 (m, 2H), 2.24 (s, 3H) 2.31 (s, 3H), 2.49 (m, 2H), 3.57 (m, 1H), 3.94 (m, 3H), 5.06 (dd, 1H), 6.76 (d, 1H), 6.86 (d, 1H), 6.93 (s, 1H), 7.09 (d, 2H), 7.61 (d, 2H), 7.69 (m, 2H) and 7.81 (m, 2H). GLC $t_R = 9.15$min (98.4%). MS 475.2 (MH^+).

1-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2yl)ethyl]-7-[(4-benzonitrile)oxymethyl]-1,2,3,4-tetrahydro-2-[(4-methylphenyl)sulfonyl]-isoquinoline (38). 4-Bromomethyl benzonitrile (106 mg, 0.54 mmol) and K_2CO_3 (76 mg, 0.55 mmol) were added into a solution of phenol **32** (258 mg, 0.54 mmol) in DMF (20 mL) and the resulting mixture heated at 80 °C for 1h. The reaction mixture was cooled to room temperature, diluted with toluene and the resulting solution concetrated under reduced pressure. The crude product was purified by flash chromatography on silica gel eluting with EtOAc/heptane (1:1), to obtain compound **38** as a colourless gum. Yield: 150 mg (47%). ^{1}H NMR ($CDCl_3$) δ 2.06 (m, 1H), 2.15 (m, 1H), 2.32 (s, 3H), 2.47 (m, 2H), 3.54 (m, 1H), 3.88 (m, 3H), 5.08 (s + m, 3H), 6.68 (dd, 1H), 6.76 (d, 1H), 6.79 (d, 1H), 7.07 (d, 2H), 7.53 (d, 2H), 7.59 (d, 2H), 7.72 (m, 4H) and 7.84 (m, 2H). GLC $t_R = 9.01$ min (98.9%). MS 592.4 (MH^+).

1-[(4-methylphenyl)sulfonyl]-2-(2-phthalimidoethyl)piperidine (39). 4-Methyl sulfonylchloride (4.42 g, 23.21 mmol) was added in small portions into a solution of 2-piperidineethanol (1 g, 7.73 mmol) and pyridine (2.5 mL, 30.9 mmol) in chloroform (40 mL) at 0 °C. The resulting solution was allowed to warm up to room temperature and stirred for 16 h. The reaction mixture was concentrated under reduced pressure and the residue reconstituted in diethyl ether (100 mL). The organic phase was washed with aqueous 2 N HCl (50 mL), 5% $NaHCO_3$ (50 mL) and water (50 mL). The organic phase was dried with $MgSO_4$ and the solvent removed under reduced pressure to obtain a brown oil, which after purification by flash chromatography on silica gel eluting with 3:2 Et_2O/heptane, afforded *N*,*O*-bis(4-methyl benzenesulfonyl)-2-piperidineethanol as a colourless solid. Yield: 1.64 g (48%). ^{1}H NMR ($CDCl_3$) δ 1.13, 1.41 and 1.74 (m, 6H), 1.78 (m, 1H), 2.07 (m, 1H), 2.42 (s, 3H), 2.46 (s, 3H), 2.92 (t, 1H), 3.78 (dd, 1H), 4.05 (m, 3H), 7.26 (d, 2H), 7.35 (d, 2H), 7.67 (d, 2H) and 7.80 (d, 2H). GLC $t_R = 8.35$ min (85%). Potassium phthalimide (1.74 g, 9.4 mmol) was added into a solution of *N*,*O*-bis(4-toluenesulfonyl)-2-piperidineethanol (3.16 g, 7.23 mmol) in anhydrous DMF (75 mL) under vigorous stirring, at room temperature. The reaction mixture was heated at 80 °C for 3 h, cooled down to room temperature and stirred over 16 h. The reaction was quenched with water (100 mL) and the product extracted with DCM (2×50 mL). The combined DCM extract was washed with brine (50 mL), 0.2N NaOH (50 mL) and brine again (50 mL). The organic phase was dried over $NaSO_4$ and concentrated under reduced pressure to obtain crude **39** which was purified by recrystallization from DCM/Et_2O (1:1). Yield: 2.6 g (87%). ^{1}H NMR ($CDCl_3$) δ 1.21 and 1.51 (m, 6H), 1.68 (m, 1H), 2.04 (m, 1H), 2.41 (s, 3H), 3.09 (t, 1H), 3.67 (m, 2H), 3.85 (dd, 1H), 4.12 (m, 1H), 7.26 (d, 2H), 7.72 (m, 4H) and 7.83 (m, 2H). GLC $t_R = 8.25$ min (99.7%). MS 413.2 (MH^+). GC–MS 238 (M^+-199 ($TsOCH_2CH_2$)).

4-[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)ethyl]-6-[(4-methylphenyl) sulphonyl] -4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine (40). Histamine (111 mg, 1 mmol) and **5** (244 mg, 1.2 mmol) were dissolved in a mixture of ethanol (1 mL) and toluene (9 mL). After heating at reflux for 3 h, the reaction mixture was stirred at 20 °C for 18 h, then evaporated to dryness under reduced pressure. After purification by SiO_2 flash chromatography (CH_3OH/DCM; (1:4)), the tetrahydroisoquinoline was obtained in 36% yield (130 mg). To a suspension of the tetrahydroisoquinoline (120 mg, 0.405 mmol) in anhydrous pyridine (1 mL), was added *p*-toluenesulphonyl chloride (77 mg, 0.405 mmol) in anhydrous pyridine (0.5 mL). The reaction mixture was stirred at 20 °C for 3 h, then at 80 °C for 18 h. Volatiles were removed under reduced pressure and the residue was chromatographed on flash SiO_2, eluting with 5% CH_3OH in DCM. The product **40** was obtained as a clear gum in 36% yield (59 mg). ^{1}H NMR: ($CDCl_3$; 400 MHz) δ 7.90 (m, 2H), 7.75 (m, 2H), 7.40–7.60 (m, 3H), 7.13 (d, $J = 7.4$Hz, 2H), 4.95 (m, 1H), 4.15 (m, 1H), 3.85 (m, 2H), 3.45 (m, 1H), 2.40 (m, 2H), 2.34 (s, 3H), 2.15 (m, 2H). MS (ES^+) *m*/*z* 452 $(M + H)^+$.

2-(dimethylamino)-*N*-[[[2-[1,2,3,4-tetrahydro-2-[(4-butylphenyl)sulfonyl] - 1 - isoquinolinyl]ethyl]amino]carbonyl] - acetamide (43). The crude library product **22{23,2}** (25 mg) was purified by SPE (1 g silica cartridge, CH_2Cl_2/CH_3OH 98:2 followed by CH_2Cl_2/CH_3OH 95:5 as eluent) to give (**43**) (20 mg, 0.044 mmol). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.20 (m, 2H), 1.47 (m, 2H), 1.95 (m, 1H), 2.07 (m, 1H), 2.36 (s, 6H), 2.45 (m, 2H), 2.55 (t, 2H), 3.01 (s, 2H), 3.30 (m, 1H), 3.45 (m, 1H), 3.72 (m, 1H), 3.95 (m, 1H), 5.00 (m, 1H), 6.80 (m, 1H), 7.00 (m, 2H), 7.09 (m, 3H), 7.59 (d, 2H), 7.93 (br t, 1H). HPLC $t_R = 3.78$ min (95.0%). MS 458.0 (MH+).

1-[2-[[(6-methyl-3-pyridinyl)carbonyl]amino]ethyl]-1,2,3,4-tetrahydro-2-[(4-butylphenyl)sulfonyl]-isoquinoline (44). The crude library product **22 {23,4}** (23 mg) was purified by SPE (1g silica cartridge, CH_2Cl_2/CH_3OH (98:2) followed by CH_2Cl_2/CH_3OH (95:5) as eluent) to give 44 (20 mg, 0.041 mmol). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.20 (m, 2H), 1.49 (m, 2H), 1.95 (m, 1H), 2.20 (m, 1H), 2.33–2.47 (m, 2H), 2.54 (m, 2H), 2.63 (s, 3H), 3.42 (m, 2H), 4.03 (m, 1H), 4.12 (m, 1H), 5.08 (m, 1H), 6.80 (m, 1H), 6.94–7.10 (m, 5H), 7.27 (m, 1H), 7.57 (m, 2H), 7.67 (br t, 1H), 8.14 (m, 1H), 9.10 (m, 1H). HPLC $t_R = 3.96$ min (93.6%). MS 492.5 (MH+).

***N*-[[[2-[1,2,3,4-tetrahydro-2-[(4-butylphenyl)sulfonyl]-1-isoquinolinyl]ethyl]amino]carbonyl] - 3 - pyridineacetamide (45).** The crude library product **22{23,9}** (19 mg) was purified by SPE (1g silica cartridge, CH_2Cl_2/CH_3OH (98:2) followed by CH_2Cl_2/CH_3OH (95:5) as eluent) to give **45** (14 mg, 0.028 mmol). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.20 (m, 2H), 1.48 (m, 2H), 1.85 (m, 1H), 1.98 (m, 1H), 2.28–2.45 (m, 2H), 2.55 (m, 2H), 3.20 (m, 1H), 3.33 (m, 1H), 3.63 (s, 2H), 3.83 (m, 1H), 3.93 (m, 1H), 4.79 (m, 1H), 6.75–6.90 (m, 3H), 6.98–7.12 (m, 4H), 7.32 (m, 1H), 7.49 (m, 2H), 7.80 (m, 1H), 8.55 (m, 1H), 8.66 (m, 1H). HPLC $t_R = 3.83$ min (94.0%). MS 492.5 (MH+).

***N*-[[[2-[1,2,3,4-tetrahydro-2-[(4-butylphenyl)sulfonyl]-1-isoquinolinyl]ethyl]amino]carbonyl]-1H-imidazole-1-acetamide (46).** The crude library product **22{23,12}** (21 mg) was purified by SPE (1g silica cartridge, CH_2Cl_2/

CH_3OH (98:2) followed by $CH_2Cl_2/CH_3OH/NH_4OH$ (90:10:0.5) as eluent) to give **46** (17mg, 0.035 mmol). ^1H NMR ($CDCl_3$): δ 0.87 (t, 3H), 1.20 (m, 2H), 1.48 (m, 2H), 1.73–2.02 (m, 2H), 2.30–2.45 (m, 2H), 2.55 (m, 2H), 3.20–3.40 (m, 2H), 3.82 (m, 1H), 3.92 (m, 1H), 4.71 (s, 2H), 4.78 (m, 1H), 6.80 (m, 2H), 6.91 (m, 1H), 7.02 (m, 1H), 7.10 (m, 4H), 7.20 (s, 1H), 7.52 (m, 2H), 7.64 (m, 1H). HPLC $t_R = 3.74$ min (95.0%). MS 481.5 (MH+).

Receptor binding assays

Membrane preparation. Rat brains with cerebellum removed, from male Wistar rats were homogenised in 5 vol (w/v) of ice-cold 50 mM Tris–HCl buffer (pH 7.4) using a Potter-Elvehejm homogeniser (six passes at 1000 rpm). The homogenate was centrifuged at 45,000 *g* for 15 min at 4 °C. The pellet was resuspended by homogenisation in a further 5 vol of ice-cold buffer. To remove endogenous opioids that may interfere with the radioligand binding assay, the membranes were incubated at 25 °C for 30 min in a shaking water bath, and re-centrifuged as described above. The membranes were resuspended in 10 vol of ice-cold buffer (based on the original weight of tissue), snap frozen and stored at −80 °C. For the preparation of guinea pig brain membranes, six guinea pig cortex were homogenised in 5 vol (w/v) in ice-cold 50mM Tris–HCl, pH 7.4. The homogenate was allowed to settle for 30 min on ice and the larger particles re-homogenised in 10 mL Tris–HCl. The combined homogenates were centrifuged at 45,000 *g* for 15 min at 4 °C. The supernatant was discarded and the pellet resuspended by homogenisation in 10 vol (w/v) Tris–HCl, pH 7.4. The homogenate was incubated at 25 °C for 30 min in a shaking waterbath and re-centrifuged, as above. The pellet was resuspended in a final volume of 130 mL ice-cold Tris–HCl, snap frozen in liquid nitrogen and stored as aliquots at −80 °C.

μ Opioid binding assay. Briefly, 300 μg rat brain membrane protein was incubated with 1.5 nM [^{3}H] DAMGO [Tyrosyl-3,5-^{3}H(N)], 50 mM Tris–HCl buffer (pH 7.4), 0.1mg mL^{-1} bacitracin in a final vol of 1 mL. Non-specific binding was determined in the presence of 10 μM naltrexone. Assays were initiated by the addition of the membrane preparation and incubated at 23 °C for 5 h. Assays were terminated by rapid filtration through Whatman GF/B, which had been pre-soaked in ice-cold 0.5%(v/v) polyethyleneimine for 1 h, using a Brandel M24R cell harvester (Brandel Gaithersburg, MD). Filters were washed three times with ice-cold Milli-Q water, dried and placed in 24-well plates with 1 mL of Ultima Gold scintillation fluid and counted for tritium using a Microbeta Scintillation Counter (Wallac).

κ Opioid binding assay. Briefly, 250 μg guinea pig brain membrane protein was incubated with 1.5 nM [^{3}H] U69593 [Phenyl-3,4-^{3}H], 50 mM Tris–HCl buffer (pH 7.4) in a final vol of 1 mL. Non-specific binding was determined in the presence of 10 μM naltrexone. Assays were initiated by the addition of the membrane preparation and incubated at 23 °C for 3 h. Assays were terminated by rapid filtration through Whatman GF/B, which had been pre-soaked in ice-cold 0.5%(v/v) polyethyleneimine for 1 h, using a Brandel M24R cell harvester (Brandel Gaithersburg, MD). Filters were washed three times with ice-cold Milli-Q water, dried and placed in 24-well plates with 1mL of Ultima Gold scintillation fluid and counted for tritium using a Microbeta (Wallac).

δ Opioid binding assays. Briefly, crude homogenates from 0.3×10^6 Chinese Hamster Ovary cells (CHO) cells stably expressing the human delta opioid receptor cDNA were incubated with 0.15 nM [^{3}H] Naltrindole (5′, 7′–^{3}H], 50 mM Tris–HCl buffer (pH 7.4), 5 mM $MgCl_2$ in a final volume of 1.5 mL. Non-specific binding was determined in the presence of 10 μM naltrexone. Assays were initiated by the addition of the membrane preparation and incubated at 25 °C for 3 h. Assays were terminated by rapid filtration through Whatman GF/B Packard 96-well plates which had been pre-soaked in ice-cold 0.5%(v/v) polyethyleneimine for 1 h, using a Packard cell harvester. Filters were washed three times with ice-cold 50 mM Tris–HCl (pH 7.4), dried. Following the addition of 50 μl of MicroScint to each well, the plates were sealed and counted for tritium using a Packard TopCount Scintillation Counter.

Data analysis. IC_{50} values from competitive binding data were determined by computer analysis using Prism, whereby a non-linear repression analysis using four-parameter logistic equation was utilised, assuming a single set of binding sites. A curve was fitted to a graph of binding (% total binding) values plotted against the log values of the corresponding drug concentrations.

In vivo assay

Formalin paw test (FPT). In this test, the injection of a dilute solution of formalin into one hind paw of the mouse induces an inflammatory response in the paw, which may provide a more valid model for clinical pain than acute threshold tests using a phasic, high intensity stimulus e.g., hot plate test. The response to sub-plantar formalin injection shows two distinct phases of nociceptive behaviour in several species.[13] The early phase (Phase 1) begins immediately after formalin injection and lasts for 4–5 min. This phase is followed by a period of 10–15 min of quiescent behaviour, after which a second, late phase (Phase 2) occurs. This phase starts ∼20 min post-formalin injection and continues for a further 20–30 min. In mice, recording manually the time spent licking or biting the injected paw is the most common method of behavioural assessment.[14] Agonists at μ, κ and δ opioid receptors inhibit both phases of the formalin response.

Male ICR mice (22–30 g) were habituated to their test environment by placing them, singly, into clear perspex observation boxes for ∼2 h on the day prior to the

experiment and for 1 h prior to drug administration on the day of the experiment. Formalin solution, 0.3% in sterile saline, was prepared as a fresh solution daily. Due to the limited aqueous solubility of some of the compounds, test compounds or vehicle were dissolved in 10% cyclodextrin in water and administered intravenously (iv), 10 mL kg^{-1}, 15 min prior to the subplantar injection into one hind paw of 20 μl of formalin solution. The total time in seconds that each animal spent licking its injected paw was recorded in 5-min epochs for 40 min, beginning immediately after formalin injection. Mean and SEM values were calculated at each time point for each treatment group. Area under the curve was calculated in seconds between 0 and 5 min after formalin injection for Phase 1 (early phase) and between 15 and 40 min after formalin injection for Phase 2 (late phase). Test compound-induced inhibition of licking was calculated as follows:

$$\%\ \text{Inhibition of Phase 1 (0}-\text{5 min after formalin)} = 100\% - \frac{\text{Mean time (s) spent licking in test compound-treated group}}{\text{Mean time (s) spent licking in control group}} \times 100\%$$

$$\%\ \text{Inhibition of Phase 2 (15}-\text{40 min after formalin)} = 100\% - \frac{\text{Mean time (s) spent licking in test compound-treated group}}{\text{Mean time (s) spent licking in control group}} \times 100\%$$

For tests in which Naltrindole was adminstered in addition to the test compound, Naltrindole (10 mg kg^{-1} i.p.) was given 35 min before administration of the test compound and again immediately prior to the test compound. Inhibition of licking behaviour induced by the mu agonist fentanyl (3 nmol kg-1; i.v.) in the FPT was shown not to be significantly antagonised by this dose of Naltrindole.

Acknowledgements

The authors thank the analytical department at Organon Newhouse for providing NMR, IR, MS and HPLC data in support of this work.

References

1. Rapaka, R. S.; Porreca, F. *Pharmacol. Res.* **1991**, *8*, 1.
2. Dondio, G.; Clarke, G.D.; Giardina, G.; Petrillo, P.; Petrone, G.; Ronzoni, S.; Visentin, L.; Vecchietti, V. *Analgesia,* **1995**, *1*, 394
3. Portoghese, P. S.; Sultana, M.; Takemori, A. E. *J. Med. Chem.* **1990**, *43*, 1714.
4. Salvadori, S.; Balboni, G.; Guervini, R.; Tomatis, R.; Bianchi, C.; Bryant, S. D.; Cooper, P. S.; Lazarus, L. H. *Med. Chem.* **1997**, *40*, 3100.
5. Suzuki, T.; Tsuji, M.; Mori, T.; Misawa, M.; Endoh, T.; Nagase, H. *Life Sci.* **1995**, *57*, 155.
6. Calderon, S. N.; Rothman, R. B.; Porreca, F.; Flippen-Anderson, J. L.; McNutt, R. W.; Xu, H.; Smith, L. E.; Bilsky, E. J.; Davis, P.; Rice, K. C. *J. Med. Chem.* **1994**, *37*, 2125.
7. Ronai, A. Z.; Botyanszki, J.; Hepp, J.; Medzihradszky, K. *Life Sciences* **1992**, *50*, 1371.
8. Barn, D. R.; Morphy, J. R. *J. Comb. Chem.* **1999**, *1*, 151.
9. Schiller, P. W.; Berezowska, I.; Nguyen, T. M.-D.; Schmidt, R.; Lemieux, C.; Chung, N. N.; Falcone-Hindley, M. L.; Yao, W.; Liu, J.; Iwama, S.; Smith, A. B.; Hirschmann, R. *J. Med. Chem.* **2000**, *43*, 551.
10. Hall, I. H.; Wong, O. T.; Futch, G.; Scovill, J. P. *Biomed. Biochim. Acta* **1990**, *49*, 103.
11. Barnes, R. A.; Godfrey, J. C. *J. Org. Chem.* **1957**, *22*, 10431.
12. The Lipinski rules for drug-like activity, which were published during the course of this work, suggest that good permeability is less likely if molecular weight is over 500 and that good solubility and permeability is less likely if LogP is over 5: Lipinski, C. A. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3
13. Dubuisson, D.; Dennis, G. *Pain* **1977**, *4*, 161.
14. Hunskaar, S.; Fasmer, O. B.; Hole, K. *J. Neurosci. Meth* **1985**, *14*, 69.
15. Knapp, R. J.; Santoro, G.; De leon, I. A.; Lee, K. B.; Edsall, S. A.; Waite, S.; Malatynska, E.; Calderon, S. N.; Rice, K. C.; Rothman, R. B.Porreca F; Roeske W. R.; Yamamura H. I. *J. Pharm. Expt. Ther.* **1996**, *277*, 1284.
16. Nagase, H.; Wakita, H.; Kawai, K.; Endoh, T.; Matsura, H.; Tanaka, C.; Takezawa, Y. *Jpn. J. Pharmacol.* **1994**, *64* (Suppl 1), 35.