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2,4-Diaminopyridine δ-opioid receptor agonists and their associated hERG pharmacology

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ABSTRACT

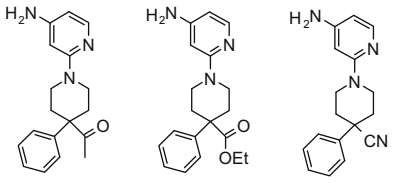
A number of libraries were produced to explore the potential of 2,4-diaminopyridine lead 1. The resulting diaminopyridines proved to be potent and selective δ-opioid receptor agonists. Several rounds of lead optimisation using library chemistry identified compound 17 which went on to show efficacy in an electromyography model of neuropathic pain. The structure–activity relationship of the series against the hERG ion channel proved to be a key selectivity hurdle for the series.

For a number of years considerable effort has been made to discover and develop selective δ-opioid receptor (DOR) agonists for the treatment of chronic pain. This is largely because DOR agonists have the potential to exhibit lower abuse liability than μ-opioid receptor agonists as well as reduced gastrointestinal, respiratory and cognitive effects.[1,2] DOR agonists demonstrate effective antinociceptive activity in preclinical models of inflammatory,[3,4] neuropathic[3] and cancer pain[5] but have little effect on acute nociception models.[3] Furthermore translocation of delta opioid receptors from intracellular compartments to neuronal plasma membranes may account for their effectiveness in chronic pain states.[6] Convulsive effects related to DOR agonism have been studied in rats with certain chemical classes of non-peptidic agonists. The conclusions drawn suggest that the potential remains to further develop DOR agonists.[7]

In addition to Pain, DOR agonists have recently received interest in other indications such as depression,[8] cardioprotection[9] and overactive bladder.[10] A highly potent, peripherally selective, zwitterionic series from these laboratories, initially designed to target irritable bowel syndrome, has also been disclosed.[11] In our continued search for both peripherally selective and central nervous system penetrant δ-opioid agonists, we sought further chemical series from targeted file screening. The identification and exploration of a new chemical series based on 2,4-diaminopyridines is described herein.

Compound **1** (Fig. 1) was identified as a singleton hit using a human recombinant cell line δ-opioid receptor functional assay[12] (DOR EC_{50}) during targeted file screening. All compounds within this Letter proved to be full δ-agonists using this assay format. These compounds were also cross-screened in equivalent μ and κ-opioid functional assays. Unless otherwise stated, all compounds were at least 50-fold selective over these opioid sub-types.

Despite the low number of hits from those compounds screened, we were attracted by the chemical possibilities of a hit to lead programme based on **1** with its encouraging potency (DOR EC_{50} 263 nM, E_{max} 100%[12]), in vitro metabolic stability in human liver microsomes[13] (HLM, Cl_{int} < 7 μl/min/mg) and particularly its selectivity over the μ-opioid receptor (μ-opioid EC_{50} > 10 μM).



1 2 3

Figure 1. Screening hit and prototypes.

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Singleton follow up compounds were designed to increase confidence in structure activity relationships (SAR) and the synthetic viability of the series. These prototypes proved to be active (**2** DOR EC_{50} 64 nM and **3** DOR EC_{50} 61 nM, Fig. 1).

Singleton synthesis was characterised by an amine displacement (generally a piperidine) at the 2-position of 2-chloro-4-nitropyridine-*N*-oxide. Reduction to the 2,4-diaminopyridine using iron in acetic acid yielded the desired final compounds (Scheme 1). The potential for library synthesis was also apparent. Two templates were prepared presenting either a carboxylic acid or amine, ready for the parallel synthesis of amides. (Scheme 1).

The selection of monomers for each library was achieved through computationally designed properties for the final target compounds. Limits were set for molecular weight, polar surface area, lipophilicity[14] and on the presence of reactive or vulnerable functional groups. This resulted in libraries of ~300 target compounds being designed each time.

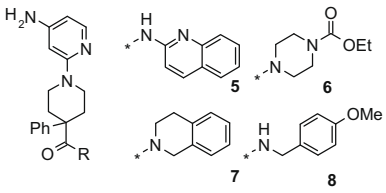
Two amide libraries were initially prepared using the differing cores to explore the scope of the chemical series based on the activity of the ester **2** and nitrile **3**. Using the acid template **4** (X = Ph, R = CO_2H) as the core (Library 1), amine monomers that contained aryl groups produced the majority of the active compounds (Fig. 2). For each library described, four selected compounds are chosen to illustrate the nature of the SAR for DOR agonist pharmacology, in vitro human liver microsomal stability (HLM) and hERG ion channel activity.[15]

Library 2 was based on the amino version of template **4** (X = Ph, R = NH_2). With the amide linkage now reversed, aryl acid derived substituents featured in most, but not all of the active compounds (Fig. 3).

Given the compact nature of the lead **1**, greater increases in DOR potency were expected for the addition of such lipophilic aryl groups. In addition, any gains in potency were offset by the in vitro metabolic instability (as measured by HLM) and the considerable hERG liability that were now characteristic of these larger compounds.[16]

Alongside the 4,4-disubstituted piperidine approach of Scheme 1, other templates were considered that could offer alternate synthetic possibilities and SAR. Piperidine based templates, known from other opioid receptor sub-types, were attached to the 2,4-diaminopyridine group to explore the effect on delta activity and opioid sub-type selectivity. Early efforts with this activity/selectivity hypothesis proved promising. In an example taken from μ agonists, the phenethyl group of a fentanyl[17]-related compound was replaced with the 2,4-diaminopyridine to give **13** (Fig. 4).

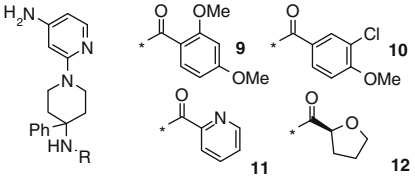
The spirocyclic template known from opioid receptor like-1 (ORL-1) pharmacology[18] was also combined with the 2,4-diaminopyridine to give **14**. Each compound was prepared by the singleton method of Scheme 1. In both cases moderately active δ-opioid agonists were identified (**13** DOR EC_{50} 207 nM, and **14** DOR EC_{50} 308 nM, Fig. 4). These two compounds were greater than 12-fold selective over any other opioid sub-type pharmacology. Off-setting the basic centre from the piperidine using the 2,4-diaminopyridine



R/ Compound	DOR EC_{50} (nM)	HLM Cl_{int} ul/min/mg	hERG K_i (nM)
5	5	22	17
6	24*	62	169
7	13	46	35
8	83	40	201

* 17-fold selective over μ-opioid receptor

Figure 2. Library 1 SAR.



R/ Compound	DOR EC_{50} (nM)	HLM Cl_{int} ul/min/mg	hERG K_i (nM)
9	14	43	82
10	59	69	343
11	60	14	548
12	171	13	>10000

Figure 3. Library 2 SAR.

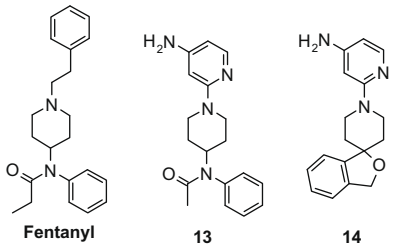
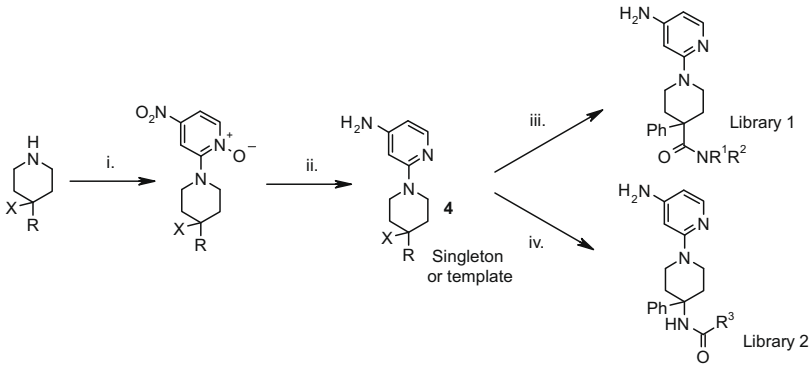


Figure 4. μ to δ opioid selectivity switch mediated by 2,4-diaminopyridine.



Scheme 1. Reagents: (i) 2-chloro-4-nitropyridine-*N*-oxide, $NaHCO_3$, *t*-amyl alcohol, 50 °C; (ii) Fe, AcOH; (iii) X = Ph, R = CO_2Et; (a) 6 N HCl, reflux; (b) HNR^1R^2, HBTU, Hunig's base, NMP; (iv) X = Ph, R = NHBOC; (a) TFA, DCM; (b) R^3CO_2H, HBTU, Hunig's Base, NMP.

appeared to be a useful strategy in conferring δ-opioid selectivity on piperidine templates originally identified for other opioid pharmacologies (μ, ORL-1).

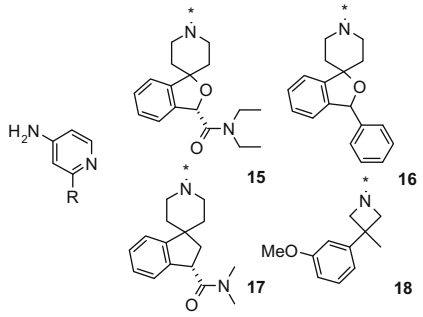
The observations around **13** and **14** prompted further library chemistry efforts. The spirocyclic **14** triggered a library varying the cyclic amine at the 2-position of the 2,4-diaminopyridine. Of the piperidines selected from the company file, those containing a spirocyclic ring at the 4-position were prioritised in light of compound **14**.[19] These compounds were prepared by a parallel equivalent of the singleton method in Scheme 1 where amine displacement of 2-chloro-4-nitropyridine-*N*-oxide was followed by reduction of the whole library. The reduction conditions were changed to a transfer hydrogenation method for convenience in the library protocol.

From the data generated, piperidines were confirmed as the best ring size for the core, as opposed to azetidines and pyrrolidines. Spirocyclic systems at the piperidine 4-position performed favourably. The spirocyclic indane **17** proved to be very active and had some encouraging in vitro properties (Fig. 5).

The discovery of compound **13** (Fig. 4) offered greater synthetic scope for SAR exploration. In moving away from 4,4-disubstituted piperidines and spirocyclic systems, compound design was now based on a simpler 4-aminopiperidine core. Synthetically, two substituents could be varied easily, enabling molecular properties (cLogP, molecular weight) to be better kept in balance during compound design.

In order to replace the aniline in **13**, a 2-pyridyl group was investigated as a phenyl isostere off the 4-amino position of the piperidine core. This isostere proved to be active (**19**, Fig. 6). This did however trigger a change in the synthetic strategy for library production. The 2-aminopyridine intermediate **20** (Scheme 2) was not sufficiently reactive under standard amide coupling conditions with our desired set of carboxylic acid monomers. In response to this, acid chlorides were synthesised in situ using Ghosez's conditions.[20] This method proved to be particularly effective when operating in a 96-well synthesis block format on a 10 mg scale. The library protocol now featured three synthetic steps—acid chloride generation, amide bond formation and nitro/*N*-oxide reduction. Despite having three chemically orthogonal, linear steps in a parallel synthesis format, success rates were regularly above 80% for this optimised protocol (Scheme 2).

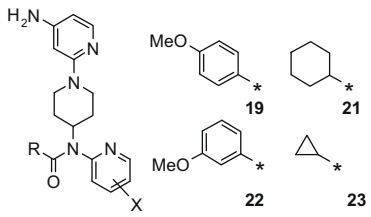
Compound **19** was a rare example of a sub-10 nM DOR agonist with ~500-fold selectivity over hERG binding. It also showed some of the best in vitro metabolic stability in HLM thus far. Smaller acid-derived substituents such as cyclopropyl compound **23** showed low hERG affinity and excellent in vitro metabolic stability.



R/ Compound	DOR EC_{50} (nM)	HLM Cl_{int} ul/min/mg	hERG K_i (nM)
15	12	13	>10000
16	59	10	18
17	27	<7	4710
18	846*	-	1840

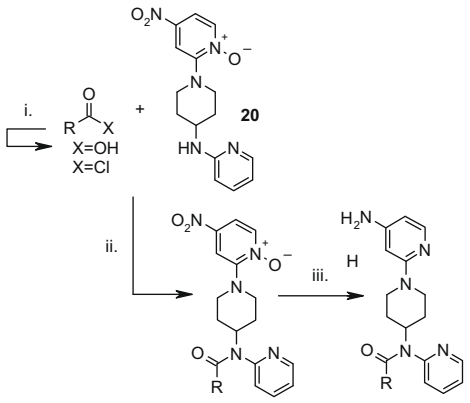
* 2-fold selective over μ-opioid receptor

Figure 5. Cyclic amine library SAR.



R/ Compound	DOR EC_{50} (nM)	HLM Cl_{int} ul/min/mg	hERG K_i (nM)
19 X=4-Me	9	12	5030
21 X=H	30	80	>10000
22 X=H	52	17	9690
23 X=H	108	<7	>10000

Figure 6. 2-Aminopyridine amide library SAR.



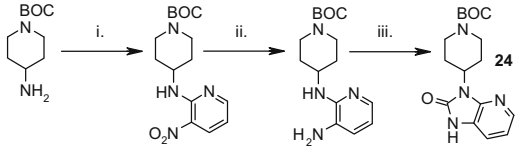
Scheme 2. Reagents: (i) 1-chloro-*N*,*N*-2-trimethylpropenylamine, DCM; (ii) Et_3N, DCM; (iii) 10%Pd/Al_2O_3, NH_4HCO_2, MeOH.

However, this was at a 10-fold cost in DOR activity when compared to **19** (Fig. 6).

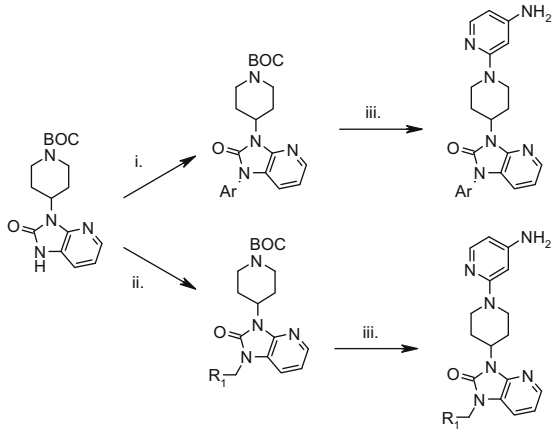
Having investigated SAR in several templates using mainly library chemistry, the 4-aminopiperidine amides were selected for further optimisation through cyclisation strategies. The versatility of the starting 4-aminopiperidine template enabled variation of ring systems and substituents at the 4-position of the piperidine core.

Azabenzimidazolone template **24** was prepared (Scheme 3) such that both arylation[21] and alkylation chemistries could be funded from the same intermediate. Once these divergent functionalisations had been performed (Scheme 4), the installation of the 2,4-diaminopyridine was carried out by the now standard method.

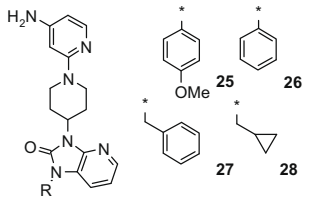
This potent sub-series identified some of the most active compounds yet, optimizing potency through a cyclisation strategy. However, minimizing rotatable bonds did little for metabolic stability in this case with the compounds showing tangible turnover in the HLM assay. Most significant was their pronounced activity in the hERG binding assay (Fig. 7). This clearly ruled out further progression of the azabenzimidazolone sub-series.



Scheme 3. Reagents: (i) 2-chloro-3-nitropyridine, Et_3N, *n*-BuOH, 80 °C; (ii) $Pd(OH)_2$, NH_4HCO_2, EtOH, reflux; (iii) Triphosgene, Et_3N, THF.



Scheme 4. Reagents: (i) $ArB(OR)_2$, $Cu(OAc)_2$, Et_3N, DCE, 60 °C; (ii) R^1CH_2Br, K_2CO_3, DMF, 50 °C; (iii) (a) TFA, DCM; (b) 2-chloro-4-nitropyridine-*N*-oxide, $NaHCO_3$, *t*-amyl alcohol, 50 °C; (c) 10%Pd/Al_2O_3, NH_4HCO_2, MeOH.



R/ Compound	DOR EC_{50} (nM)	HLM Cl_{int} ul/min/mg	hERG K_i (nM)
25	3	23	865
26	16	19	903
27	28	20	518
28	41	13	746

Figure 7. Azabenzimidazolone SAR.

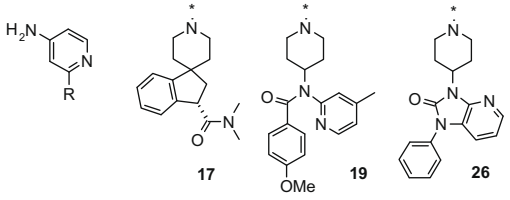
With a selection of potent δ-opioid agonists identified from the series, pain efficacy was assessed in vivo. Compound **17** was selected as it displayed the best overall in vitro properties with respect to primary pharmacology, selectivity and metabolic stability. Despite having in vitro HLM stability, **17** had a clearance of >70 ml/min/kg in rat making it unsuitable for oral dosing. Using an infusion protocol to achieve suitable plasma levels, **17** was tested in an electromyography (EMG) model of pain in rat (Fig. 8).[22]

Compound **17** showed maximal efficacy at a free plasma concentration of 23–76 nM in the EMG model. This reflected 1–3× the DOR EC_{50} from the primary functional screen. An analgesic, but non-sedative 3 mg/kg iv dose of ketamine, was used as a standard for the in vivo model to guage the relative efficacy of a delta opioid agonist. This dose of ketamine produced a 60% inhibition relative to control animals—the same effect as the top dose of DOR agonist **17**.

The binding affinity for the hERG ion channel was closely tracked throughout this 2,4-diaminopyridine series. Analysis of all compounds screened in the dofetilide binding assay from this

Infusion protocol	% inh. of. control	Mean nM free in plasma
76μg/kg + 72μg/kg/hr	0	1.6
240μg/kg + 290μg/kg/hr	48	23
660μg/kg + 920μg/kg/hr	61	76

Figure 8. EMG wind up efficacy of **17**.



R/ Compound	hERG K_i (nM)	hERG patch (nM)	*In vivo* QTC (nM)
17	4710	1420	291
19	5030	2020	-
26	903	54	-

Figure 9. Selected cardiovascular safety parameters.

series (>500 compounds), against their calculated lipophilicity, showed a stringent cut off for the compound to be devoid of hERG channel activity. As a general trend, a cLogP of >1 for the compound meant an 80% chance of obtaining a binding K_i below 10 μM in the hERG assay.

This selectivity issue was studied further by looking at selected compounds in the more physiologically relevant hERG patch clamp assay. Compounds showed the rare property of being up to ten fold *more* potent in the patch clamp assay than the hERG binding assay, thus reducing any selectivity window even further (Fig. 9). In vivo studies in dog[23] showed an even greater sensitivity to QTC prolongation with small but significant effects observed at free plasma concentrations as low as 291 nM for compound **17** (Fig. 9).

In summary, a singleton hit from targeted file screening was rapidly explored using both new and established multi-step library protocols. This Letter summarises >1500 analogues from the 2,4-diaminopyridine series, mainly synthesised in libraries. The use of the 2,4-diaminopyrdine group conferred excellent δ-opioid selectivity over other opioid sub-types on piperidine-derived cores. However, the same heterocycle also seemed to bring a significant hERG liability to the entire series, even in compounds of seemingly moderate lipohilicity. Given that exceptionally potent compounds were not identified (<1 nM), the series was deemed to have insufficient selectivity over this key cardiovascular safety parameter for further progression.

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12. All pharmacology values in the tables are geometric means of at least three experiments. Differences of <2-fold should not be considered significant. Delta opioid agonist and antagonist activities were measured using a functional cell based ALPHAscreen assay. ALPHAscreen is a bead-based, non-radioactive **A**mplified **L**uminescent **P**roximity **H**omogeneous **A**ssay. Binding of molecules captured on beads leads to an energy transfer from one bead to the other producing a luminescent signal. The ALPHAscreen method measured the

inhibition of cAMP produced via adenylate cyclase, following direct activation of the enzyme by forskolin. The assay was based on competition between endogenous cAMP and exogenously added biotin-cAMP. Donor beads were streptavidin-coated and acceptor beads were coated with an anti-cAMP antibody. The assay used CHO cells stably transfected with a CHO-Flp In delta opioid receptor construct. The opioid receptor is Gi coupled to adenylate cyclase, therefore agonists inhibited forskolin-stimulated cAMP production. Delta opioid cells were cultured in T225 flasks in DMEM/nutrient mix F12 supplemented with 2 mM L-glutamine, 500 μg/ml hygromycin and 10% heat inactivated foetal bovine serum. For assay the cells were harvested by removing the media, washing twice with PBS and adding cell dissociation solution. A viable cells count was performed and cells were resuspended at 1.6×10^6/ml (8000 cells/well in the assay) in stimulation buffer (5 mM HEPES pH 7.4, HBSS, 0.1% BSA, 10 mM $MgCl_2 \cdot 6H_2O$ and 0.5 mM IBMX). Compound, cells and acceptor bead mix were added to the plate before addition of forskolin at a final assay concentration of 20 μM. Following a 30 min incubation period the streptavidin donor beads were added and the plate incubated overnight before reading on a Fusion reader. Percentage agonist activity was calculated and EC50 values generated. All compounds screened appeared as full agonists (E_{max} 100%). Known non-peptidic delta opioid agonist BW-373U86 was a 3.2 nM full agonist in the assay.

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