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Multiparameter exploration of piperazine derivatives as δ-opioid receptor agonists for CNS indications

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ABSTRACT

A novel series of piperazine derivatives exhibits sub-nanomolar binding and enhanced subtype selectivity as δ-opioid agonists. The synthesis and SAR are described as well as the application of computational models to improve in vitro ADME and safety properties suitable for CNS indications, specifically microsomal clearance, permeability, and hERG channel inhibition.

The delta opioid receptor (δOR) is a target for several disease areas with significant unmet medical need, including pain and mood disorders.[1] Delta, as well as the two other major opioid receptor subtypes, mu (μ) and kappa (κ), belongs to the G-protein coupled receptor (GPCR) family class. While the endogenous selective ligands for the δ-opioid receptor are the peptide agonists Met- and Leu-enkephalin, morphine is well known as a powerful μ agonist that also binds to the δ and κ subtypes.

Although efficacious, morphine's side effects such as respiratory depression and addiction leave ample room for improved therapeutics. Compounds that selectively target the δ-opioid receptor may have benefits without some of the drawbacks of non-selective drugs, although other mechanisms such as binding to heteroreceptors and receptor internalization have been postulated to account for additional side effects.[2] Prior research in this area has identified selective δ-opioid agonists, including derivatives of the endogenous peptides, morphine-derived structures, as well as diverse small molecule scaffolds beginning with SNC-80 (Fig. 1).[3]

We have expanded previous work on a series of potent piperazine derivatives (**1**, Fig. 2).[4] The activity and selectivity of this series remain attractive, and the synthesis, SAR, and computational modeling toward compounds with suitable CNS profiles are described.

Drug candidates were prepared by the application of a synthetic scheme that enables diversity at R^1, R^2, and R^3 via a common intermediate (Scheme 1). The addition of an aryl Grignard reagent **2** to an aromatic aldehyde **3** gives a secondary alcohol **4** which is converted to bromide **5**. Subsequent displacement by piperazine gives the racemic amine derivative **6**. This amine can be resolved classically through the selective crystallization of chiral tartrate salt

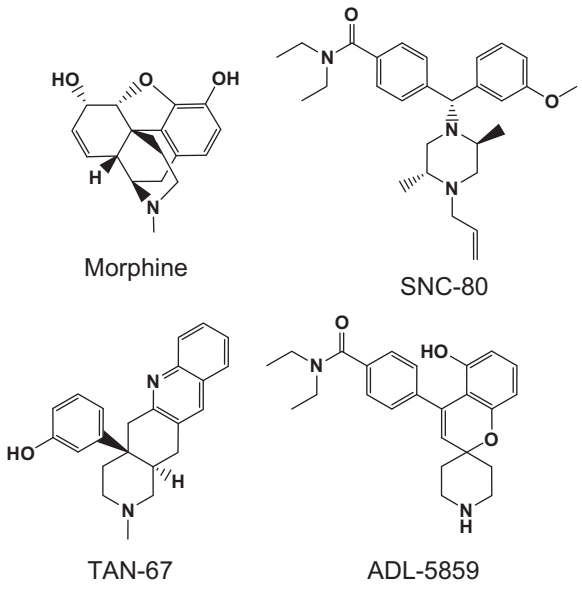

Figure 1. Morphine and subtype-selective δ-opioid agonists.

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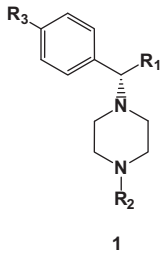
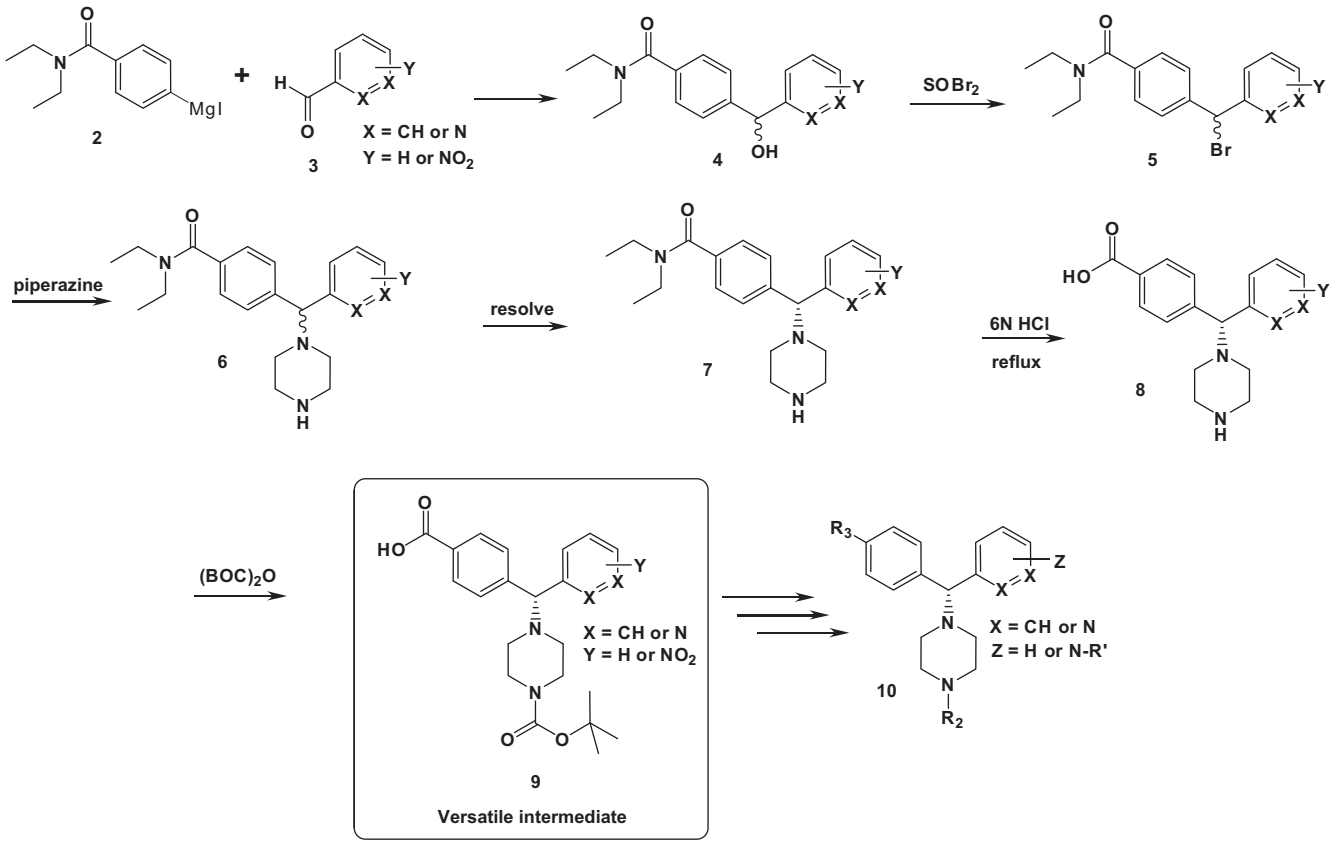


Figure 2. Generic structure of the piperazine series **1**.

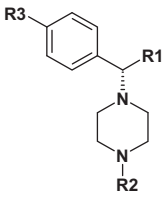
derivatives. Alternatively, preparative chiral stationary phase liquid or supercritical fluid chromatography can be performed at this (or a later) stage to give the single enantiomer intermediates **7**. Treatment with HCl yields carboxylic acid **8**, and BOC protection affords the versatile intermediate **9**. Selective protection and deprotection schemes are applied to give a series of compounds **10** that possess systematically varied substitutions at three different regions on the drug molecule (R^1, R^2, and R^3).

Either stereoisomer can exhibit potent binding at δOR. In this particular series, when both were potent binders, the *R* enantiomer typically showed slightly greater potency than its *S* enantiomer.



Scheme 1. Synthesis of chiral intermediate **9** which enables sequential installation of diverse R^1, R^2, and R^3 groups.

Table 1
δOR binding affinity, μOR selectivity, microsomal clearance, permeability through MDCK–MDR1 (Madin–Darby canine kidney cells transfected with human MDR1 gene) cell monolayers, and hERG inhibition for piperazines **11**–**26**



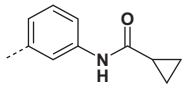
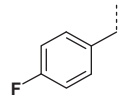
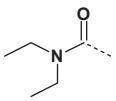
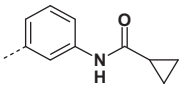
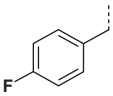
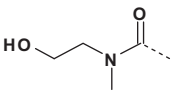
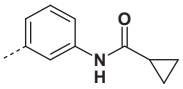
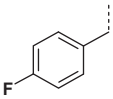
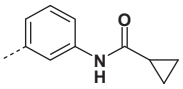
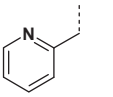
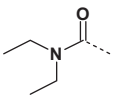
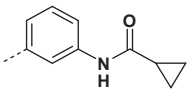
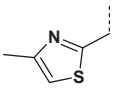
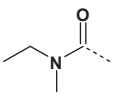
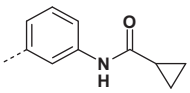
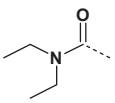
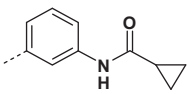
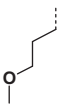
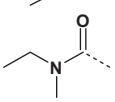
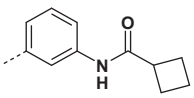
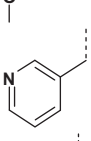
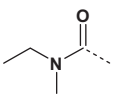
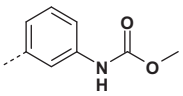
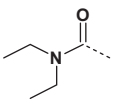
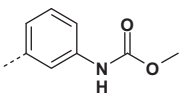
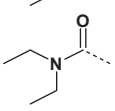
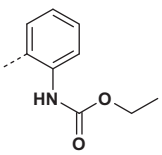
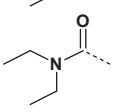
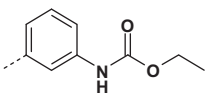
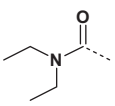
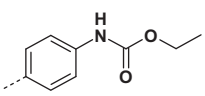
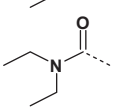
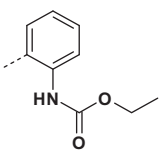
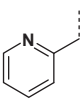
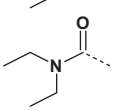
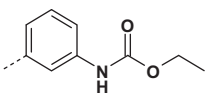
Cmpd	R^1	R^2	R^3	δOR[a] IC_{50} (nM)	μ/δ[a]	hCLint[b] (μl/min/mg)	Papp[c] (nm/s)	hERG[d] IC_{50} (μM)
11				0.29	5200	150	<1	11

Table 1 (*continued*)

Cmpd	R^1	R^2	R^3	δOR[a] IC_{50} (nM)	μ/δ[a]	hCLint[b] (μl/min/mg)	Papp[c] (nm/s)	hERG[d] IC_{50} (μM)
12				0.27	11000	36	<1	15
13				0.90	480	49	<1	3.6
14				0.068	>120000	260	NT	20
15				>100	<100	160	NT	18
16				1.1	>9400	26	<1	>30
17				23	33	<4	NT	13
18				6.6	>1500	85	<1	28
19				0.35	>5800	100	2.2	14
20				0.68	5100	110	3.0	11
21				0.78	11000	430	<1	8.7
22				0.071	6200	210	<1	5.4
23				1.3	2700	21	1.3	7.3
24				1.4	>6000	650	4.3	9.9
25				0.26	14000	17	<1	>33
26				0.70	>12000	<4	<1	>33

[a] The δOR and μOR binding assays employed iodinated Deltorphin II and FK33-824, respectively, as the radioligand and membranes were prepared from HEK 293 cells over-expressing either δOR or μOR. Quantification of the receptor-associated radioligand was done with a filtration format and an SPA format.

[b] Intrinsic clearance of compound in human liver microsomes.

[c] The permeability through MDCK–MDR1 cell monolayers as measured from the apical side to the basolateral side in a transport assay format.

[d] Voltage-dependent potassium channel encoded by the human ether-a-go-go related gene.

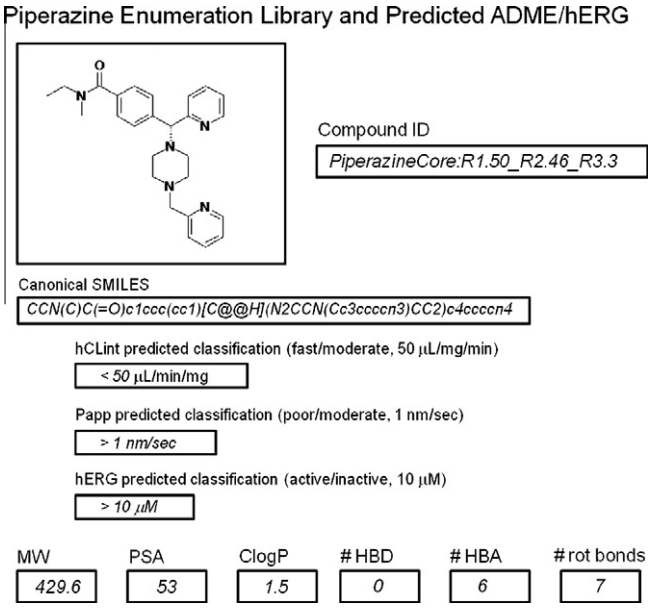


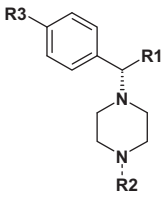
Figure 3. Schematic of structure–searchable database. Prioritized target **31** from the enumeration library is shown with ADME and hERG predictions as well as computed physical properties.

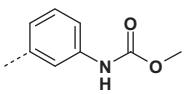
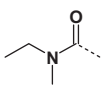
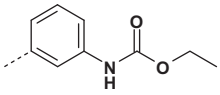
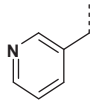
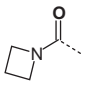
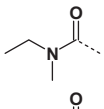
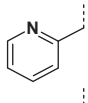
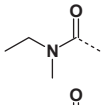
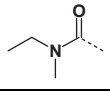
More significantly, the binding potency of the *R* enantiomer tended to be reflected in functional agonism, whereas the agonism was lost for the *S* enantiomer in many instances. This more consistent display of functional agonism led to a focus on the set of *R* enantiomers shown in Table 1.

In addition, ADME predictive models were built and applied as filters (Fig. 3). The ADME models utilized a binary QSAR methodology with six calculated properties (molecular weight, polar surface area, Clog*P*, number of hydrogen bond donors, number of hydrogen bond acceptors and number of rotatable bonds) using the MOE software package.[5] The models provided binary (pass/fail) predictions for passive permeability and microsomal clearance. For the hERG model, a support vector machine (SVM) approach yielded better pass/fail predictions for test sets and was used for predictions. Whereas models for microsomal clearance and hERG were built from robust distributions of experimental data, the model for passive permeability was of weaker predictive value due to a narrower range and distribution of experimental data within the series.

When considered together with physical properties, synthetic feasibility, and novelty, a total of 37 new compounds were prioritized to be made from the set of ca. 300,000 virtual compounds. Several newly synthesized and tested compounds simultaneously met hERG and multiple ADME criteria. Thirty six of the compounds obtained measured CLint and hERG, and the predicted classifications were correct for 34/36 (94%), and 30/36 (83%), respectively. Although all the compounds chosen for synthesis were also predicted to have measurable passive permeability (>1 nm/s), only 5 of the 16 tested in this assay were indeed >1 nm/s. Furthermore, δOR potency was sacrificed for many compounds, particularly for the newly explored substituents outside the training sets. The five

Table 2
δOR binding affinity, μOR selectivity, microsomal clearance, permeability through MDCK–MDR1 (Madin–Darby canine kidney cells transfected with human MDR1 gene) cell monolayers, and hERG inhibition for piperazines **27**–**31**. Predicted values using classification models for clearance, permeability, and hERG are shown in italics. Predictions consistent with later experimental results are highlighted in green and those inconsistent with experimental results are in red and labeled



Cmpd	R^1	R^2	R^3	δOR[a] IC_{50} (nM)	μ/δ[a]	Predicted hCLint[b] (μl/min/mg)	hCLint[b] (μl/min/mg)	Predicted Papp[c] (nm/s)	Papp[c] (nm/s)	Predicted hERG[d] IC_{50} (μM)	hERG[d] IC_{50} (μM)
27				0.094	15000	*>50*[e]	38	*>1*	1.2	*>10*	17
28				4.1	>2200	*>50*	53	*>1*	1.8	*>10*	11
29				4.7	>2100	*<50*	40	*>1*[e]	<1	*>10*	15
30				2.0	>5100	*<50*	33	*>1*	1.2	*>10*	13
31				5.2	>1900	*<50*	20	*>1*	2.5	*>10*	>33

[a] The δOR and μOR binding assays employed iodinated Deltorphin II and FK33-824, respectively, as the radioligand and membranes were prepared from HEK 293 cells over-expressing either δOR or μOR. Quantification of the receptor-associated radioligand was done with a filtration format and an SPA format.
[b] Intrinsic clearance of compound in human liver microsomes.
[c] The permeability through MDCK–MDR1 cell monolayers as measured from the apical side to the basolateral side in a transport assay format.[6]
[d] Voltage-dependent potassium channel encoded by the human ether-a-go-go related gene.
[e] Incorrectly classified by the predictive model.

compounds that met potency and most property criteria are shown in Table 2. Compound **27** (IC_{50} = 0.094 nM) is one of the most potent compounds in the series, while compound **31** is notable for its weak activity opposite the hERG channel (IC_{50} >33 μM). The R^2 pyridyls (**30**–**31**) surfaced as having the best compromise of overall properties. Although permeability was generally improved with this second set of compounds, the magnitude of the enhancement was modest. Furthermore, compounds that exhibited moderate permeability were measured to be P-glycoprotein efflux substrates[6] and are unlikely to sustain sufficient brain levels for efficacy. Thus, none of these compounds met our full panel of in vitro criteria for progression as a CNS drug, with permeability through MDCK–MDR1 cell monolayers most often as a primary liability.

This series of piperazines exhibits excellent δOR binding and subtype selectivity, as well as a range of microsomal clearance and hERG inhibition. The piperazine moiety itself remains a challenge for achieving permeability through the MDCK–MDR1 cell monolayer while retaining favorable potency and ADME characteristics. Modifications to the core scaffold that fundamentally alter the physical properties (e.g., pK_a and polar surface area) offer additional scope for CNS indications and are described in the following companion paper.[7]

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References and notes

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