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Discovery of (−)-6-[2-[4-(3-fluorophenyl)-4-hydroxy-1-piperidinyl]-1-hydroxyethyl]-3,4-dihydro-2(1*H*)-quinolinone—A potent NR2B-selective *N*-methyl D-aspartate (NMDA) antagonist for the treatment of pain

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Abstract—(−)-6-[2-[4-(3-Fluorophenyl)-4-hydroxy-1-piperidinyl]-1-hydroxyethyl]-3,4-dihydro-2(1*H*)-quinolinone was identified as an orally active NR2B-subunit selective *N*-methyl-D-aspartate (NMDA) receptor antagonist. It has very high selectivity for NR2B subunits containing NMDA receptors versus the HERG-channel inhibition (therapeutic index = 4200 vs NR2B binding IC_{50}). This compound has improved pharmacokinetic properties compared to the prototype CP-101,606.

The *N*-methyl-D-aspartate (NMDA) receptor is a glutamate-gated ion channel playing important roles in a variety of neuronal processes including learning, memory, and pain transmission.[1] For treatment of pain, there is considerable preclinical evidence that hyperalgesia and allodynia following peripheral tissue or nerve injury are not only due to an increase in activity of primary afferent nociceptors at the site of injury but also depend on NMDA receptor-mediated central changes in synaptic excitability.[2–5] In humans, NMDA receptor antagonists, such as ketamine and dextramethorphan, have also been found to decrease both pain perception and sensitization.[6–13] However, while NMDA receptor antagonists may have therapeutic utility for the treatment of pain, there are significant hurdles to a widespread clinical use. Notably, new approaches need to be explored to overcome the potentially serious side effects such as psychotomimetic effects and cognitive disruption.[7,10,11]

One approach to developing novel NMDA antagonists is to target specific receptor subtypes. NMDA receptors are heteromers consisting of NR1 and NR2A, NR2B, NR2C, and NR2D subunits. Among them, the NMDA receptors with NR2B subunits are distributed predominantly in forebrain and laminas I and II of the dorsal horn, which are responsible for pain transmission. The more discrete distribution of NR2B subunits in the central nerve system (CNS) may support a reduced side-effect profile of agents that act selectively at this site. For example, lack of expression in the cerebellum may suggest a reduced propensity for causing ataxia. In fact, Boyce et al. reported that CP-101,606 (**1**) (Fig. 1), an NR2B-selective NMDA antagonist, demonstrated a wider safety profile than existing nonselective NMDA antagonists.[14] More significantly, it was recently reported that **1** significantly suppressed pain intensity in patients with spinal cord injury and monoradiculopathy compared to placebo with an acceptable tolerance.[15]

Keywords: NMDA; NR2B antagonist; PK variability; CYP2D6; HERG.
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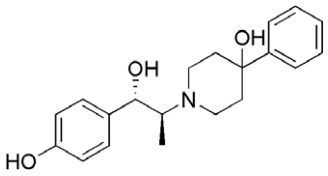
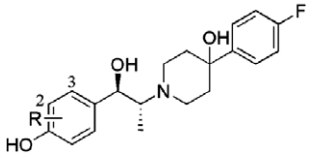


Figure 1. Structure of CP-101,606 (**1**).

Compound **1** is a highly selective NR2B NMDA antagonist discovered by Pfizer [16] and is being developed for stroke in the clinic. However, a chronic and safe use of compound **1** for nonlife-threatening indications such as pain is hampered by (1) its significant Pharmacokinetic (PK) variability and (2) QT prolongation issues.[20] The cause of this PK variability is predominant metabolism by cytochrome P450 (CYP) 2D6.[17–19] This enzyme is a polymorphic member of the CYP superfamily and is absent in 7% (poor metabolizers: PM) of the Caucasian population.[17–19] Thus, variation in expression of CYP2D6 results in variability in the metabolic inactivation of **1** in human studies. For this reason, it is essential to improve the metabolic profile of **1** for a chronic use. QT prolongation induced by CP-101,606 increases the likelihood of a polymorphous ventricular arrhythmia (torsades de pointes, TdP), which may cause syncope and degenerate into ventricular fibrillation and sudden death.[20] The QT prolongation is attributed to human ether-a-go-go-related gene (HERG) K^+ channel current inhibitory activity of compound **1**. Therefore, the HERG current inhibitory activity needed to be minimized to increase safety.

To identify a safer NR2B-selective NMDA antagonist for the chronic pain therapy, further modification of CP-101,606 was carried out with focus on alleviating those two issues. First, improvement of the metabolic profile of CP-101,606 by structural modification was performed. Most of the drugs metabolized by CYP2D6 contain a basic nitrogen atom and a flat hydrophobic region coplanar to the oxidation site which is either 5 or 7 angstroms away from the basic nitrogen atom.[21,22] In

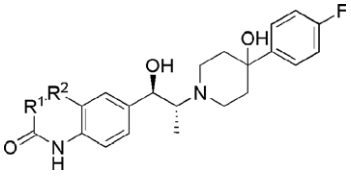
Table 1. NR2B IC_{50}, CYP2D6 contribution (±CYP2D6 ratio), and CYP2D6 inhibition of substituted phenols



Compound[a]	R	NR2B IC_{50}[b] (nM)	±CYP2D6 $t_{1/2}$ ratio[c]	2D6 inhibition IC_{50}[d] (μM)
1	CP-101606	14	>10	>10
2	2-Me	26	2.1	<0.3
3	3-Me	16	>4.5	3.9
4	2-Cl	5.6	1.6	3.5
5	2-^{t}Bu	>5000	NT[e]	NT[e]
6	2-Ph	570	NT[e]	8.4

[a] All compounds are racemate. The synthetic schemes for them are showed in Supplementary materials.
[b] Measured as the IC_{50} value for displacement of tritiated racemic CP-101,606 from the rat forebrain P2 membrane.
[c] ±CYP2D6 $t_{1/2}$ ratio = $t_{1/2}$ (HHM0168 (1 mg) + control vector (0.3 mg))/$t_{1/2}$ (HHM0168 (1 mg) + CYP2D6 (0.3 mg)): HHM0168 = PM-human liver microsomes.
[d] Measured as the IC_{50} value for displacement of tritiated bufuralol from CYP2D6 supersomes® (Gentest, 0.1 mg/ml) added NADPH-regenerating cofactor.
[e] NT, not tested.

Table 2. NR2B IC_{50}, CYP2D6 contribution (±CYP2D6 $t_{1/2}$ ratio), CYP2D6 inhibition, and HERG affinity of aniline derivatives

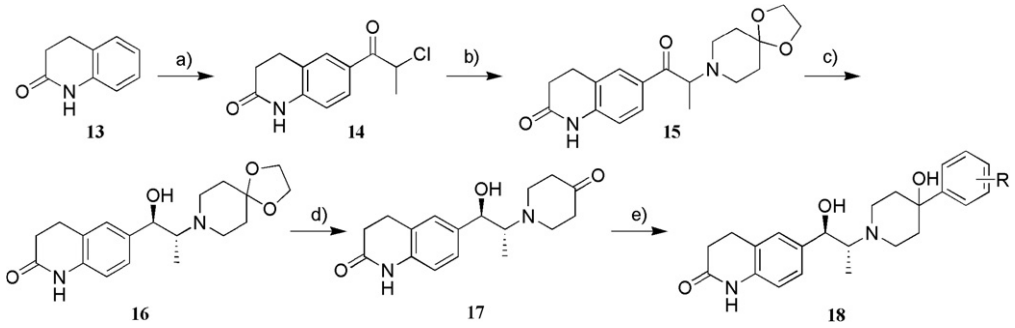


Compound[a]	R^1	R^2	NR2B IC_{50} (nM)	±CYP2D6 ratio	2D6 inhibition IC_{50} (μM)	HERG IC_{50}[b] (μM)	HERG/NR2B ratio
7	O		41	1	11	2.6	63
8	O	CH_2	18	1	>30	2.4	133
9	CH_2	O	8.7	1	>30	1.6	184
10	NH	CH_2	9.1	1	>30	7.4	813
11	NMe	CH_2	14	1	>30	3.1	221
12	CH_2	CH_2	11	1	>30	8.6	782

[a] All compounds are racemate. The synthetic scheme for them is showed in Supplementary materials.
[b] Measured as the IC_{50} value for displacement of tritiated dofetilide from human HERG channel expressed in HEK293 cells.

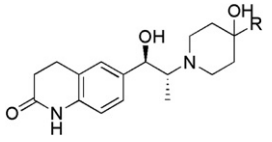
fact, MS analysis of the metabolites of CP-101,606 indicated that the *ortho* position of the phenol ring and the *para* position of the right benzene ring were mainly oxidized by human liver microsomes (HLM).[23] Thus, efforts were put into introducing substituents at the sites on the two aromatic rings, which are located around 5–7 angstroms away from the basic nitrogen on the piperidine ring. Introduction of a metabolically stable substituent at the *ortho* position of the phenol ring, however, unexpectedly resulted in increase of CYP2D6 inhibition (Table 1), although this modification was effective to reduce contribution of CYP2D6 metabolism.

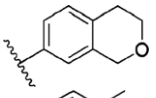
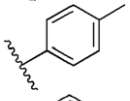
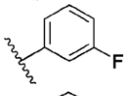
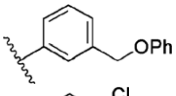
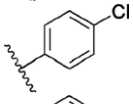
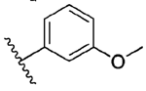
Based on the result, attempts were made to replace the phenol moiety with its isosteres to diminish the CYP2D6 metabolism and inhibition. Replacement with carboxanilides dramatically reduced contribution of CYP2D6 metabolism with weak CYP2D6 inhibition (Table 2). Especially, 3,4-dihydroquinolinon-2(1*H*)-one derivatives, represented by **12,** were active in the haloperidol-induced rat catalepsy model (MED = 10 mg/kg, po), with which in vivo NR2B antagonism of compounds in the CNS was evaluated. A 3,4-dihydroquinazolinon-2(1*H*)-one derivative (**10**) was the best in terms of weak HERG current inhibition, but not active in vivo probably due



Scheme 1. Synthetic route of 18. Reagents and conditions: (a) $AlCl_3$, 2-chloropropionylchloride, CS_2, 2 h, 50 °C, quant.; (b) 1,4-dioxa-8-azaspiro[4.5]decane, Et_3N, ethanol, reflux, 82%; (c) $NaBH_4$, ethanol; 43%; (d) 6 N HCl aq, acetone, reflux, 57%; (e) ArBr, *n*-BuLi, THF (the procedure was shown in Supplementary materials).

Table 3. NR2B IC_{50}, HERG affinity, potency in rat haloperidol catalepsy model, and clog *P* of analogs



Compound[a]	R	NR2B IC_{50} (nM)	HERG IC_{50} (μM)	HERG/NR2B ratio	Rat halodol catalepsy[b], po MED[c] (mg/kg)	clog *P*
18a		15	16	1067	>30	1.09
18b		9.1	3.3	323	NT[d]	2.12
18c		10	10	1000	10	1.76
18d		6.3	<1.5	<238	NT[d]	3.31
18e		6.6	<1	<151	10	2.33
18f		8.3	11	1325	30	1.41

[a] All compounds are racemate.
[b] This assay is described in Supplementary materials.
[c] MED, minimum effective dose.
[d] NT, not tested.

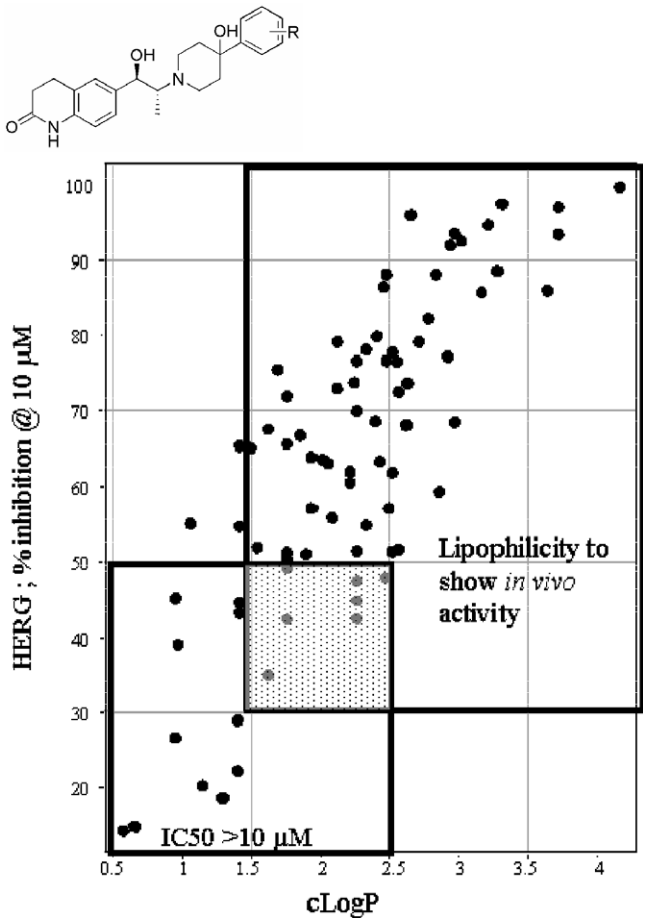


Figure 2. Correlation between HERG binding and clog *P*.

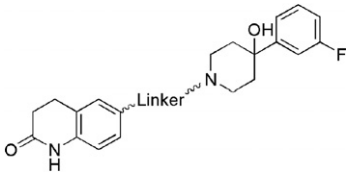
to its low CNS penetration. Therefore, further optimization around the right aromatic portion for minimizing the HERG current inhibitory activity was conducted with a 3,4-dihydroquinolinon-2(1*H*)-one series.

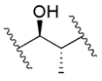
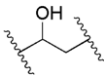
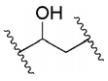
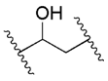
A library of 4-aryl-4-hydroxypiperidines with physicochemical diversity was built in order to conduct an SAR study of this series on HERG current inhibitory activity. Instead of evaluating HERG current inhibitory activity, a binding assay using [^{3}H]dofetilide, a potent HERG current inhibitor, was employed, because of its high assay throughput and a sufficient correlation between the HERG current inhibitory activity and the binding activity. Target compounds were prepared according to Scheme 1.

Friedel–Crafts reaction[24] of 3,4-dihydroquinolin-2(1*H*)-one **13** with 2-chloropropionyl chloride yielded α-chloroketone **14**, which was subsequently aminated to aminoketone **15**. After reduction of **15** by sodium borohydride, *threo*-alcohol **16** was isolated by crystallization. The key intermediate **17** was synthesized by deprotection with aq HCl. Treatment with various aryl lithiums (3 equiv) led to a compound library of 4-aryl-4-hydroxypiperidines **18** (see Table 3).

Analysis of the HERG binding data of the compound library revealed that there was a good correlation between HERG binding and lipophilicity as shown in Figure 2. Especially, compounds with clog *P* of 1.5–2.5 are likely to keep potent in vivo activity (MED $\leqslant$ 10 mg/kg, po) with reduced HERG binding (<50% inhibition at 10 μM). The weak in vivo activity of less lipophilic (clog *P* < 1.5) compounds was attributed to poor CNS

Table 4. NR2B IC_{50}, HERG affinity, and clog *P* of analogs



Compound[a]	Linker	NR2B IC_{50} (nM)	HERG IC_{50} (μM)	HERG/NR2B ratio	clog *P*	*i*HERG[b] IC_{20} (μM)
(±)-**18c**		10	10	1000	1.77	
(±)-**19**		4.5	13	2889	1.47	
(+)-**19**		6.8	9.8	1441	1.47	
(−)-**19**		5	21	4200	1.47	1.1
(+)-**1**	CP-101,606	14	10	714	1.80	0.15

[a] The synthetic scheme for them is showed in Supplementary materials.

[b] Measured as the degree of blockade by compounds on HERG potassium channels stably expressed in HEK-293 cells by an electrophysiological method.

penetration according to PK studies. Several individual data are shown in Table 2. Compounds with clog *P* of >2.5 in this table showed potent HERG binding activity (**18d**, HERG IC_{50} < 1.5 μM). On the other hand, compounds with clog *P* of <1.5 showed weaker HERG binding (HERG IC_{50} = **18f**: 11 μM, **18a**: <16 μM) but weaker in vivo activity (MED ⩾ 30 mg/kg, po). Among the synthesized compounds, **18c** had the best profile although the safety index between NR2B activity and HERG activity was still insufficient.

After thorough investigation of the SAR study around the two aromatic rings, our attention was turned to the linker moiety of **18c** connecting the two aromatic rings (Table 4). Removal of a methyl group from **18c** afforded **19** with reduced clog *P* and HERG affinity (IC_{50} = 13 μM) as well as a slight enhancement of NR2B affinity (IC_{50} = 4.5 nM). Compound (−)-**19** turned out to exhibit the better profile than the antipode (+)-**19**[25] and exhibited lower current inhibitory activity to HERG channel compared to CP-101,606.

In summary, replacement of the phenol group by 3,4-dihydroquinolinon-2(1*H*)-one solved the PK variability issue of CP-101,606 (**1**). Finding a correlation between HERG activity and clog *P* provided us with a clue to reducing the HERG activity, leading to (−)-6-[2-[4-(3-fluorophenyl)-4-hydroxy-1-piperidinyl]-1-hydroxyethyl]-3, 4-dihydro-2(1*H*)-quinolinone (−)-**19**[26] as a potential development candidate for the pain therapy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.08.014.

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26. Chemical data of (−)-19 (mesylate): ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.14 (s, 1H), 9.23 (s, 1H) , 7.490–7.40 (m, 1H), 7.32–7.18 (m, 4H), 7.15–7.07 (m, 1H), 6.87 (d, *J* = 8.1 Hz, 1H), 6.22 (s, 1H), 5.63 (s 1H), 5.03 (d, *J* = 10.4 Hz, 1H), 3.66–3.58 (m, 1H), 3.52–3.14 (m,7H), 2.93–2.84 (m, 2H), 2.32 (s, 3H), 2.53–2.21, (m, 2H), 1.92–1.70 (m, 2H); MS (ESI) *m*/*z* 385.15 ($M+H^+$), 383.20 ($M-H^+$); IR (KBr) 3261, 3050, 2737, 1655 cm^{-1}; $[\alpha]_D^{24}$ −29.46 (*c* 0.1154, methanol); mp 180–182 °C; Anal. Calcd for $C_{22}H_{25}N_2O_3F$, CH_4O_3S: C, 54.49; H, 6.08; N, 5.83. Found: C, 57.32; H, 6.24; N, 5.74.