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Structure–activity relationship study of novel NR2B-selective antagonists with arylamides to avoid reactive metabolites formation

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Abstract—A novel potent NMDA-NR2B selective antagonist (**5b**) without the reactive metabolites formation issue was identified. Through this study, a close correlation between reactive metabolites formation and calculated HOMO energies of parent compounds was found.

Glutamate is an essential amino acid in the central nervous system (CNS) and plays an important role as the principal excitatory neurotransmitter. There are two major classes of receptors, ionotropic and metabotropic. Ionotropic receptors are classified into three major subclasses, *N*-methyl-aspratate (NMDA), 2-amino-3(methyl-3-hydroxyisoxazol-4-yl)propionic acid (AMPA), and kainate. Hyperalgesia and allodynia following peripheral tissue or nerve injury were reported to depend on NMDA receptor-mediated central changes in synaptic excitability.[1] In fact, non-selective NMDA receptor antagonists such as ketamine[2] and dextromethorphan[3] have been found to decrease both pain perception and sensitization in humans. However, many available NMDA receptor antagonists are prone to cause potentially serious CNS related side effects.[4,5] NMDA subunits are differentially distributed in the CNS. Especially, NR2B is believed to be restricted to the forebrain and laminas I and II of the dorsal horn. This more discrete distribution of NR2B subunit in the CNS may support a reduced side-effect profile of agents that act selectively at this site. For example, CP-101,606 (**1**, Fig. 1) is a highly selective NR2B NMDA antagonist with good in vivo potency in a variety of animal pain models, and its effect to suppress pain intensity in patients with spinal cord injury was confirmed without serious side effects in an experimental clinical study.[6] Therefore, there has been considerable interest in developing NR2B antagonists as analgesics.

Our efforts began with a high throughput screening (HTS) of Pfizer compound collection by measurement of Ca^{2+} influx in HEK (human embryonic kidney) 293 cells stably transfected with human NR1b/2B receptors. The HTS provided 876 compounds with an IC_{50} less than 10 μM. In our preceding paper, we reported that removal of aliphatic basic nitrogen in the center of a molecule is beneficial to reduce HERG current inhibitory activity.[7] Therefore, compound **2** without a basic amine center was selected among them, which showed moderate binding activity for rat NR2B receptor

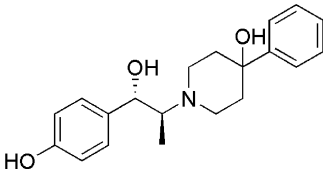

Figure 1. Structure of CP-101,606 (**1**).

Keywords: Reactive metabolite; Bioactivation; NR2B; NMDA; Metabolic stability.
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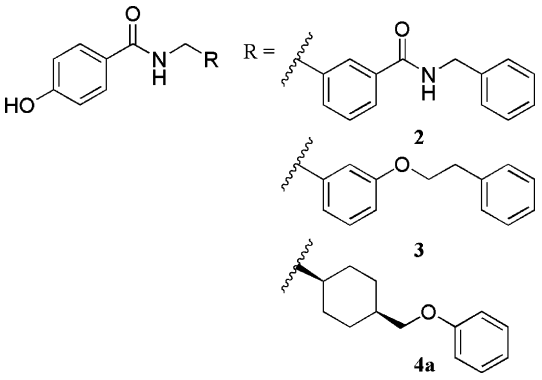
Table 1. Biological data of lead compound **2** and advanced leads (**3**, **4a**)



Compound	NR2B[a] K_i (nM)	Rat haloperidol catalepsy, *po* MED[b] (mg/kg)[c]
2	15	>30
3	5.6	10
4a	6.8	3

[a] Measured as the K_i value for displacement of tritiated racemic CP-101,606 from the rat forebrain P2 membrane.
[b] MED, Minimum Effective Dose.
[c] Determining the latency to remove both forepaws from the bar.[8]

(K_i = 15 nM) and very weak HERG binding affinity (IC_{50} > 30 μM). However, its in vivo activity was very weak due to low CNS penetration. A preliminary structure–activity relationship (SAR) study indicated that elimination of one of two amide groups was effective to increase CNS penetration, leading to compounds **3** and **4a**. (Table 1) Compound **4a** especially showed better in vivo activity to be selected as a lead compound. Herein, we wish to describe SAR around it.

Although efficacy of compound **4a** was sufficient, metabolic instability ($t_{1/2}$ = 2.3 min) and formation of reactive metabolites were major issues of this series. The reactive metabolites resulting from bioactivation of compounds by human liver microsomes (HLM) are electrophilic presumably to cause toxicity by covalently altering essential cellular macromolecules and can lead to undesirable events such as cell death and carcinogenesis. Furthermore, adverse events in human caused by reactive metabolites are not normally observed until phase III or post-launch due to their sporadic nature. The events generally lead to termination of development or withdrawal of drugs from market. Therefore, formation of reactive metabolites has to be avoided to reduce such risks.[9,10] The formation of reactive metabolites was

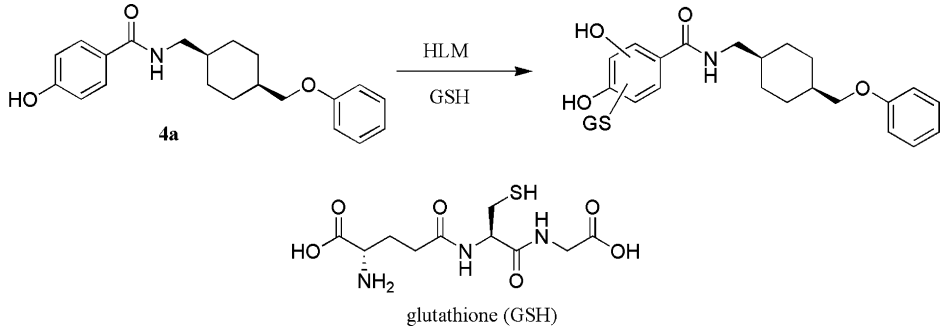
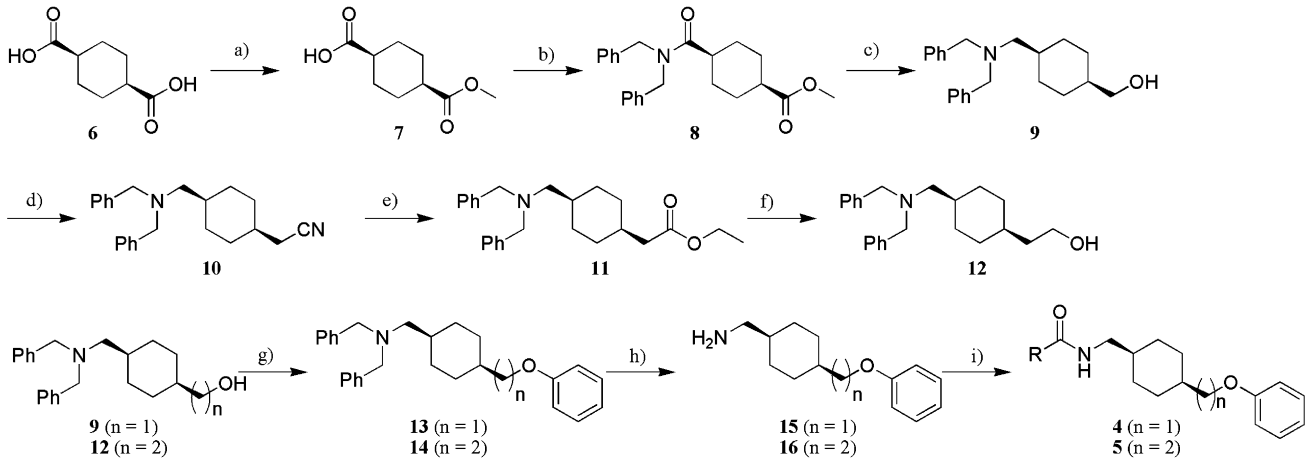
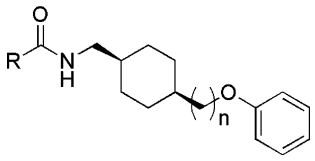


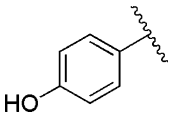
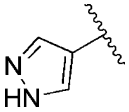
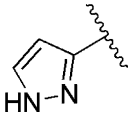
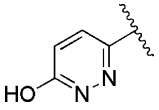
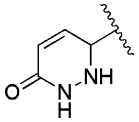
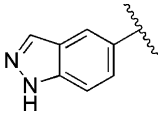
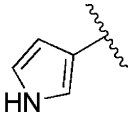
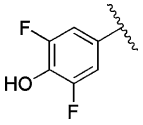
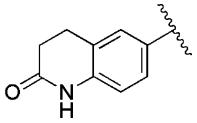
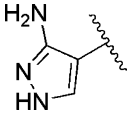
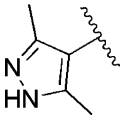
Figure 2. Trapping reactive metabolites by glutathione.



Scheme 1. Reagents and conditions: (a) 1—concd H_2SO_4, MeOH, reflux, 21 h; 2—$Ba(OH)_2{\cdot}8H_2O$, MeOH, H_2O, rt, 24 h, 75%; (b) Bn_2NH, EDCI, HOBt, Et_3N, DMF, rt, 72 h, 77%; (c) $LiAlH_4$, THF, reflux, 2 h; 89%; (d) 1—MsCl, Et_3N, CH_2Cl_2, 50 °C; 2—NaCN, 15-crown-5, DMSO, 60 °C, 19 h, 68%; (e) concd H_2SO_4, EtOH, reflux, 5 h, 63%; (f) $LiAlH_4$, THF, 0 °C, 1 h, 73%; (g) DIAD, PhOH, PPh_3, toluene, 0 °C-rt, 18 h, 93%, (h) HCO_2NH_4, 10%Pd/C, MeOH, reflux, 1 h, 60%; (i) RCOOH, EDCI, HOBt, Et_3N, DMF, rt.

Table 2. Biological data for phenol bioisosteres



Compound	R	*n*	NR2B K_i (nM)	HLM $t_{1/2}$ min	Reactive metabolites screening[a]
4a		1	6.8	2.3	Positive
5a		2	20		
4b		1	14	44	Negative
5b		2	4.2	44	Negative
4c		1	>100		
5c		2	8.9	36	Positive
4d		1	>100		
5d		2	5.6	20	Negative
4e		1	>100		
5e		2	11	26	Positive
5f		2	15	20	Negative
4g		1	84		
5g		2	6.8	33	Positive
4h		1	12	13	
5h		2	8.5	25	Positive
4i		1	18	16	
5i		2	5.9	8.4	
4j		1	>100		
5j		2	3.7	59	Positive
4k		1	>100		
5k		2	>100		

[a] This assay protocol is described in Ref. 11.

evaluated by trapping reactive metabolites using glutathione (or glutathione ethyl ester) as a nucleophile.[11] MS analysis of the conjugates with glutathione obtained from derivatives around compound **4a** revealed that a major activation site was the phenol ring (Fig. 2). Therefore optimization to avoid the formation of reactive metabolites was initiated by exploring phenol bioisosteres. Improvement of metabolic stability was also expected by this modification since the phenol moiety was the major metabolic site.

Analogs of **4a** with phenol bioisosteres were synthesized as shown in Scheme 1. Dimethyl ester of **6** was monohydrolyzed by barium hydroxide,[12] followed by amidation with dibenzylamine to afford compound **8**. The amide and ester of this compound were simultaneously reduced by lithium aluminum hydride to afford alcohol **9**. To extend the carbon chain, compound **9** was treated with methansulfonyl chloride and sodium cyanide subsequently to afford compound **10**. After esterification using concentrated sulfuric acid and ethanol, reduction by lithium aluminum hydride led to the alcohol **12**. Mitsunobu reaction with the alcohols (**9**, **12**) yielded compounds **13** and **14**, followed by deprotection to afford the key intermediates (**15**, **16**) which were converted to amides (**4**, **5**) by a parallel synthesis method.

The results of an SAR study for representative phenol bioisosteres are summarized in Table 2. Most of the analogs were found to retain potent NR2B antagonist activity. The NR2B activity was generally improved by one-carbon prolongation of the spacer ($n = 2$) between the cyclohexane and the terminal benzene ring. Replacements with pyrazole (**4b**, **5b**), pyridazine **5d**, and indazole **5f** suppressed reactive metabolite formation. However, metabolic stabilities of compound, **5d** and **5f** were insufficient. A regioisomer **(5c)** of **5b**, other heterocycles (**5e**, **5g**), and a compound with phenol substituted by a difluoro group (**5h**) were positive in reactive metabolites screening (RMS). Although 3,4-dihydro-2(1*H*)-quinolinone derivatives (**4i**, **5i**) were potent, they were metabolized rapidly by HLM. Compounds **4b** and **5b** were modified further with substituents on the pyrazole moiety. However, all attempts resulted in loss of potency (**4j**, **4k**, **5k**) or induction of reactive metabolites formation (**5j**).

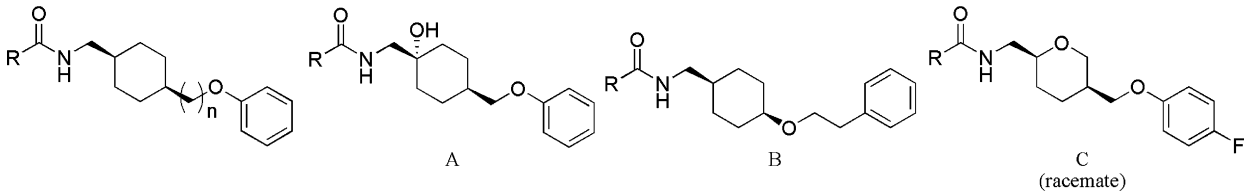
A pharmacological profile of compound **5b**,[13] the most favorable pyrazole derivative, is summarized in Table 3. Compound **5b** exhibited potent analgesic activity in the mouse partial sciatic nerve legation (PSL) model

Table 3. Pharmacological profile of compound **5b**

NR2B binding (K_i)	4.2 nM
HERG binding (IC_{50})	>30 μM
HERG current inhibition at 10 μM	36%
$t_{1/2}$ in human liver microsomes	44 min
Reactive metabolites screening	Negative
Bioavailability in rats	43%
Mouse in vivo model (PSL)[a]	3 mg/kg
Minimum Effective Dose (*sc*)	

[a] The mouse partial sciatic nerve ligation (PSL) model.[8]

Table 4. Results of RMS and calculated HOMO energies for phenol bioisosteres



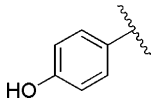
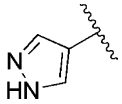
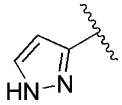
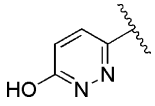
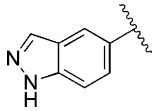
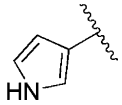
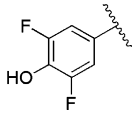
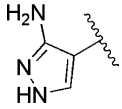
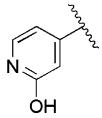
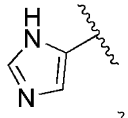
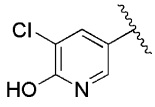
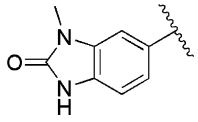
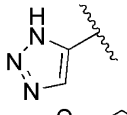
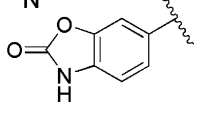
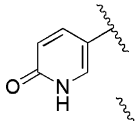
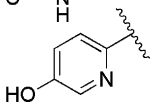
Compound	R	Amine (*n*)	Reactive metabolites screening	Calculated HOMO energy (eV)
4a		1	Positive	−9.37
5b		2	Negative	−9.43
5c		2	Positive	−9.35
5d		2	Negative	−9.66
5f		2	Negative	−9.17
5g		2	Positive	−9.20
5h		2	Positive	−9.72 (−3.84)[b]
5j		2	Positive	−8.68
5l		2	Negative	−9.70
5m		2	Negative	−9.58
5n		2	Negative	−9.63
5o		2	Positive	−8.92
5p		2	Negative	−9.95
17[a]		A	Positive	−9.33

Table 4 (*continued*)

Compound	R	Amine (*n*)	Reactive metabolites screening	Calculated HOMO energy (eV)
18[a]		B	Positive	−9.17
19[a]		C	Positive	−9.27

[a] The syntheses of these compounds are described in Ref. 8.
[b] An anion form.

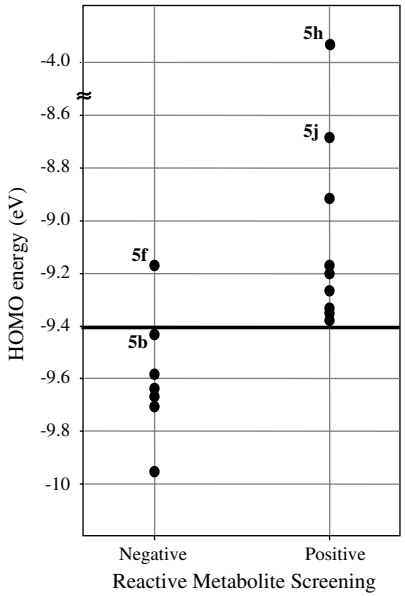


Figure 3. Correlation between HOMO energy and RMS.

(Minimum Effective Dose = 3 mg/kg, *sc*) and showed lower HERG current inhibitory activity (36% at 10 μM). In addition, this compound possesses a good pharmacokinetic profile, high metabolic stability (44 min), and bioavailability (43%).

The SAR for formation of reactive metabolites was analyzed using 16 different phenol bioisosteres (aromatic rings) summarized in Table 4. From the result of the structure of glutathione conjugate of reactive metabolite (Fig. 2), a reaction between the heme oxygen in metabolic enzyme(s) and HOMO (Highest Occupied Molecular Orbital) of the compound is speculated to be the first step for production of the reactive metabolite.[14] The correlation between reactive metabolite formation and calculated HOMO values is shown in Figure 3. Compounds with low HOMO energy (<−9.4 eV) tend to be negative in RMS presumably due to low reactivity to the oxygen of HEME. In fact, all compounds were negative. On the other hand, 9 out of 10 compounds with high HOMO energy (>−9.4 eV) were positive. As for compound **5h**, the proton of the phenol was predicted to an anion form in this assay condition (pH = 7.4), because its calculated pK_a value is about 6.05. Therefore, its HOMO value (−3.84 eV) is calculated based on the anion form. (the phenol form; −9.73 eV) Introduction of an amino group to the pyrazole (**5j**) enhanced its HOMO energy, which turned out to be positive in RMS. Despite an exception (**5f**), the results of RMS are significantly correlated with calculated HOMO energies. Specifically compounds with high HOMO energy are prone to formation of reactive metabolites. The tendency would be applicable to other chemotypes.

In conclusion, we have explored bioisosteres for phenol of the lead series to avoid reactive metabolite formation and to improve metabolic stability. Replacement of phenol with pyrazole was effective to prevent formation of reactive metabolites, and pyrazolecarboxiamide **5b** possessing analgesic activity and a good pharmacokinetic profile was identified. The good correlation between the formation of reactive metabolites and HOMO energies of parent compounds found in this study may be applicable to other chemotypes.

Acknowledgment

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References and notes

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