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N-(6,7-Dichloro-2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl)-*N*-alkylsulfonamides as peripherally restricted *N*-methyl-D-aspartate receptor antagonists for the treatment of pain

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Abstract—It has been hypothesized that peripherally restricted NMDA receptor antagonists may be effective analgesics for osteoarthritis pain. A class of novel quinoxalinedione atropisomers, first discovered for an NMDA receptor antagonist program for the treatment of stroke, was evaluated and further optimized with the goal of finding peripherally restricted NMDA receptor antagonists.

The discovery and development of effective treatments for osteoarthritis (OA) pain remains a high priority for researchers in the pharmaceutical industry. Variations in effectiveness within the patient population as well as tolerability and side-effect issues continue to drive the search for better pain targets and therapies. The *N*-methyl-D-aspartate (NMDA) receptor is an ion-channel receptor distributed widely throughout the brain and the spinal cord, and studies indicate this receptor may play a crucial role in the transmission and maintenance of chronic pain.[1] Ionotropic glutamate receptors, such as NMDA, are also expressed in peripheral nerve tissue, and peripheral NMDA receptors may play a role in the transmission and maintenance of peripheral pain.[2–5] Data generated in our laboratories (not shown) reveal that NMDA receptors are also expressed in human and rat joint tissue.[6] These data suggest that NMDA receptors may play a role in the transmission and maintenance of chronic joint pain.

The challenge of exploiting the NMDA receptors as targets for controlling pain is precisely related to their ubiquity in the CNS and the potential for CNS-mediated adverse events. Within the central nervous system (CNS), the NMDA receptors are believed to play a role in learning, memory, and other cognitive tasks, and centrally penetrant NMDA receptor antagonists have been associated with memory deficits, ataxia, sedation, nausea, visual disturbances, and hallucinations.[7] However, NMDA receptor antagonists that do not penetrate the blood–brain barrier (BBB) could potentially block pain signals in the peripheral nerve fibers alone without the risk of CNS-mediated adverse events.[8]

The NMDA receptor is divided into up to four subunits: NR1, NR2A-D, and NR3A or NR3B.[9] The presence of NR1 is required, and throughout the rest of this article, the term NMDAr will be used to refer to the NMDA NR1 receptor.

The previously reported NMDAr glycine site antagonist, UK-240455 (**1**), was a quinoxalinedione developed as a potential treatment for stroke (Fig. 1). Envisioned

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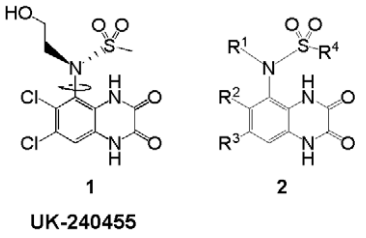
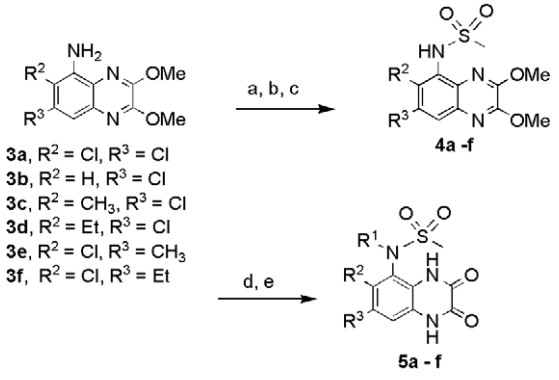


Figure 1. UK-240455 exhibited an NMDAr IC_{50} = 1.5 nM; %*F* = 17%. AUC (oral at 10 mg/kg) = 1790 ng h/mL.

as a drug that would be dosed by iv bolus injection after a stroke, UK-240455 was optimized for potency, aqueous solubility, rapid C_{max}, rapid CNS penetration, and rapid clearance. It exhibited low oral bioavailability in rats, a feature not relevant for a drug delivered intravenously.[10] However, this profile was not suitable for a drug intended as a potential OA pain therapeutic. Our program sought a potent, *peripherally restricted* NMDA receptor antagonist ($IC_{50} \leqslant 30$ nM) with oral bioavailability greater than 30% and a suitable plasma exposure level (>1000 ng h/mL).

It was envisioned that modifications at the R^1, R^2, and R^3 positions of the quinoxalinedione core **2** (Fig. 1) would be explored with the goal of decreasing the capacity of the analogs to cross the BBB by modification of the physicochemical properties of the lead compound. UK-240455 existed as a single, stable atropisomer due to restricted rotation around the aryl ring and the exocyclic nitrogen. We anticipated initially synthesizing and testing analogs as racemic mixtures. Promising leads would be separated into pairs of single atropisomers for further evaluation.

The analogs were generally prepared as shown in Scheme 1 from quinoxalines **3a**–**f**.[11,12] These quinoxalines were converted to the bis-sulfonamide upon treatment with methane sulfonic anhydride in refluxing acetonitrile. Treatment with 50% sodium hydroxide followed by acidification provided the mono-sulfonamide **4a**–**f**. The sulfonamide moiety of **4a**–**f** was readily alkylated with alkyl halides in the presence of potassium carbonate. Treatment with acid cleaved the methyl ethers and revealed the quinoxalinedione moiety in the final compounds **5a**–**f**.



Scheme 1. Reagents and conditions: (a) Ms_2O, pyridine, CH_3CN, reflux, 1 h; (b) 50% NaOH aq, rt; (c) concd HCl, rt; (d) R^1Br, K_2CO_3, CH_3CN, reflux 5 h; (e) 3 M HCl, reflux, 5 h.

While UK-240455 itself was centrally penetrant, several potent analogs were synthesized that were peripherally restricted as determined by their evaluation in the in vivo maximal electric shock (MES) model.[13] NMDAr antagonists are known neuroprotective agents that prevent seizures in the rat MES model. Potent NMDAr antagonists that provided protection against seizures in this model, when dosed intravenously (iv) or intraperitoneally (ip), were identified as brain penetrant. Conversely, *potent* NMDAr antagonists that did not provide protection against seizures were considered peripherally restricted.

Because compounds with polar surface areas (PSAs) >70–80 $Å^2$ typically do not cross the blood–brain barrier, substituents at the R^1 position were initially chosen to maintain or increase the rather high PSA of UK-240455 (PSA = 132 $Å^2$).[14] Unexpectedly, the analogs with the highest PSAs (compounds **6**–**8**) gave the greatest evidence of brain penetration (Table 1). These three compounds bore carboxylic acid moieties at the R^1 position and exhibited PSAs of 149 $Å^2$; however, they all penetrated the brain as indicated by the MES assay. These data suggested a transporter was involved in carrying such analogs across the blood brain barrier. Some hint of promise was seen in compounds **9** and **10** which carried a methyl substituent at the R^2 or R^3 position, respectively. While **9** and **10** retained their affinity for NMDAr (IC_{50} = 14 and 4 nM, respectively), they showed no evidence of brain penetration. Unfortunately, they had low oral bioavailability.[15]

The compounds listed in Table 1 showed low oral bioavailability; however, all the compounds were Lipinski Rule of 5 compliant and displayed good metabolic stability in rat and human liver microsome assays (data not shown).[16] It was apparent that the high PSAs of these compounds did not prevent brain penetration, and, indeed, the more polar nature of these compounds was likely contributing to the low oral bioavailability observed.

The next series of analogs, based on the quinoxalinedione template, was designed to explore the effect PSAs <132 $Å^2$ on both the oral bioavailability in rat and the in vitro affinity for NMDAr. Variations at the R^1 position were explored. While benzyl substituents at the R^1 position could give compounds with lower PSAs (**14** and **15**), these analogs exhibited low oral bioavailability and low plasma exposures in the rat (Table 2).

Cyclic and linear ethers at the R^1 position provided compounds **16** through **22** with PSAs around 121 $Å^2$. These analogs displayed higher oral bioavailability and variable plasma exposure levels in the rat. Significantly, compound **20** achieved our initial goals by exhibiting an NMDAr IC_{50} = 24 nM, an oral bioavailability of 39%, and an oral exposure of 6420 ng h/mL. The affinity of the analogs for NMDAr was sensitive to the bulk of

Table 1. NMDAr binding, brain penetration, and bioavailability data for analogs with polar surface areas $\geqslant 132$ Å^2

Compound	R^1	R^2	R^3	R^4	NMDAr IC_{50}[a] (nM)	MES[b] (%)	%*F*	$AUC_{(po)}$[c] ng h/mL	Solubility (μg/mL)	PSA (Å^2)
6	CH_2COOH	Cl	Cl	CH_3	2	100	—	393	76	149
7	CH_2COOH	CH_3	Cl	CH_3	2	87.5	7	1500	—	149
8	CH_2COOH	Cl	CH_3	CH_3	1	62.5	5	155	72	149
9	CH_2CH_2OH	CH_3	Cl	CH_3	14	0	4	605	70	132
10	CH_2CH_2OH	Cl	CH_3	CH_3	4	0	9	791	70	132
11	CH_2CH_2OH	Et	Cl	CH_3	58	0	1	183	72	132
12	$(CH_2)_3OH$	Cl	Cl	CH_3	41	—	1	136	76	132
13	$CH_2CH(CH_3)OH$	Cl	Cl	CH_3	40	—	1	680	76	132

[a] IC_{50} means were obtained in most cases by $n = 3$ or $n = 4$ determinations.
[b] Percent protected in the MES, maximal electroshock assay. Typically, 6-week-old Swiss Webster rats were tested in groups of 8–10 and dosed either iv or ip at 100 mg/kg. The percent protected was interpreted only as 'brain penetrant' or 'brain non-penetrant' and was not taken to indicate the degree to which compounds crossed the BBB.
[c] Dosed orally at 5 mg/kg in SD rats. po formulation: 5% PEG-200/95% (0.5%) methyl cellulose.

Table 2. NMDAr binding, bioavailability, and permeability data for analogs with PSAs generally <132 Å^2

Compound	R^1	R^2	R^3	R^4	NMDAr[a] IC_{50} (nM)	%*F*	$AUC_{(po)}$[b] ng h/mL	CaCO-AB[c] (10^{-6} cm/s)	CaCO-2 BA/AB[d]	Solubility (μg/mL)	PSA (Å^2)
14	*m*-Methoxybenzyl	Cl	Cl	CH_3	12	8	152	3.43	4.67	40	121
15	*m*-Fluorobenzyl	Cl	Cl	CH_3	45	10	589	1.75	4.07	23	112
16	Methyl-2-tetrahydropyran	Cl	Cl	CH_3	39	53	1490	<0.01	∞	25	121
17	Methyl-2-tetrahydrofuran	Cl	Cl	CH_3	59	31	922	1.52	7.55	16	121
18	$(CH_2)_2O(CH_2)_2OEt$	Cl	Cl	CH_3	166	90	840	0.97	12.4	88	130
19	$(CH_2)_2OCH_3$	Cl	Cl	CH_3	38	33	1080	0.01	—	76	121
20	$(CH_2)_3OCH_3$	Cl	Cl	CH_3	24	39	6420	2.06	6.05	79	121
21	$(CH_2)_2OEt$	Cl	Cl	CH_3	209	78	2260	10	1.38	79	121
22	$(CH_2)_3OEt$	Cl	Cl	CH_3	19	33	1270	0.44	28.4	82	121
23	$(CH_2)_2CF_3$	Cl	Cl	CH_3	18	36	1260	0.41	15.0	56	112
24	$(CH_2)_3OCH_3$	H	Cl	CH_3	728	—	—	0.01	0.1	72	121
25	$(CH_2)_2OCH_3$	Cl	CH_3	CH_3	77	57	1190	0.14	30.6	72	121
26	$(CH_2)_2OCH_3$	CH_3	Cl	CH_3	74	100	2620	0.01	∞	72	121
27	$(CH_2)_3OCH_3$	Cl	Et	CH_3	49	47	632	0.01	∞	78	121

[a] IC_{50} means were obtained in most cases by $n = 3$ or $n = 4$ determinations.
[b] Dosed orally at 5 mg/kg in SD rats. po formulation: 5% PEG-200/95% (0.5%) methyl cellulose.
[c] The apical to basolateral permeability as measured in CaCO-2 cells.
[d] The BA/AB ratio was monitored for efflux potential.

R^1 as well as the position of the ether oxygen in this side chain.

The trifluoromethylpropyl analog **23** was also promising exhibiting an NMDAr $IC_{50} = 18$ nM, oral bioavailability of 36%, and an oral exposure of 1260 ng h/mL.

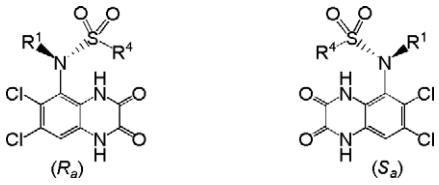
Generally, as shown in Table 2, our most potent antagonists carried chlorine substituents at the R^2 and R^3 positions; however, variations at these positions were also investigated. Compound **24**, an analog with a hydrogen at the R^2 position, showed a significant loss in potency with an $IC_{50} = 728$ nM. Placing a methyl group at the R^2 or R^3 position instead of a chlorine atom resulted in a general drop in potency. See **25** and **26** which gave IC_{50}'s = 77 and 74 nM, respectively. Placing an ethyl group at the R^3 position provided compound **27** which was less potent than its dichloro-analog **20** and had a considerably lower plasma exposure (NMDAr $IC_{50} = 49$ nM; AUC = 632 ng h/mL). Placing substituents other than chlorines at the R^2 and R^3 positions also resulted in less permeable compounds as measured in CaCO-2 cells.

Separation of racemic analogs into their constituent atropisomers impacted potency and oral bioavailability. The most promising racemic analog out of this study was compound **20**, due to its potency, oral bioavailability, and high plasma exposure.

Separation of **20** by chiral chromatography provided the pure atropisomers (S_a) **28** and (R_a) **29** with NMDAr IC_{50}'s of 282 and 8 nM, respectively (Table 3).[17,18]

The divergent potencies could be explained by modeling studies which showed that (R_a) atropisomer **29** directed its methyl sulfonamide moiety into the binding pocket where the sulfonamide oxygens hydrogen bonded with Ser-A180 and Val-A181 (Fig. 2). Conversely, the (S_a)

Table 3. NMDAr binding and bioavailability data for racemates and single atropisomers



Compound	R^1	R^4	Stereochemistry	NMDAr IC_{50}[a] (nM)	%*F*	$AUC_{(po)}$[b] (ng h/mL)
20	$(CH_2)_3OCH_3$	CH_3	(±)	24	39	6420
28	$(CH_2)_3OCH_3$	CH_3	(S_a)	282	43	6900
29	$(CH_2)_3OCH_3$	CH_3	(R_a)	8	41	771
23	$(CH_2)_2CF_3$	CH_3	(±)	18	36	1260
30	$(CH_2)_2CF_3$	CH_3	(S_a)	11	26	3150
31	$(CH_2)_2CF_3$	CH_3	(R_a)	22	2	168
32	$(CH_2)_2CF_3$	Et	(±)	27	—	—
33	$(CH_2)_2CF_3$	Et	(S_a)	20	32	3920
34	$(CH_2)_2CF_3$	Et	(R_a)	109	—	—

[a] IC_{50} means were obtained in most cases by $n = 3$ or $n = 4$ determinations.
[b] Dosed orally at 5 mg/kg in SD rats. po formulation, 5% PEG-200/95% (0.5%) methyl cellulose.

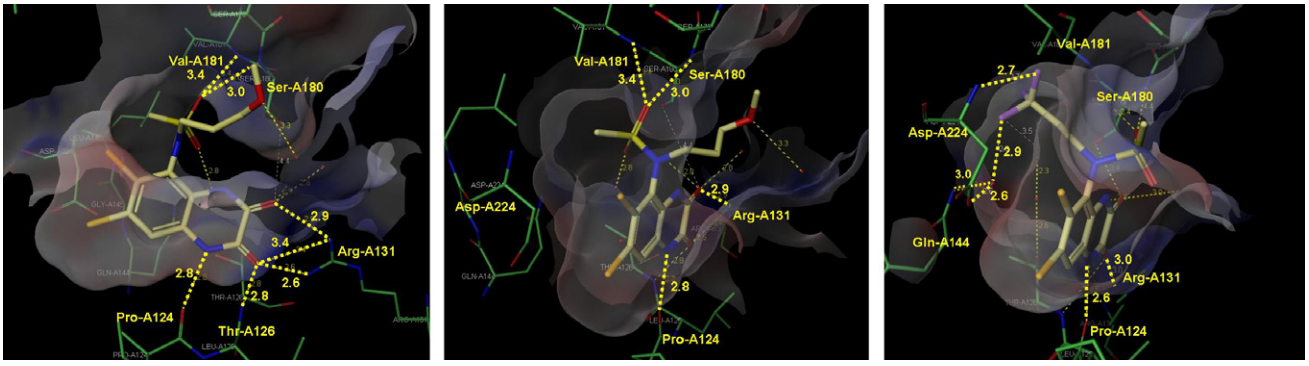


Figure 2. (Left panel) Compound **29** modeled in the glycine binding site of the NMDA NR1 receptor as pictured from the 'side' of the molecule. (Center panel) Compound **29** modeled in the binding pocket as seen from the 'edge view' of the molecule. Key hydrogen bonds between the sulfonamide and Val-A181 and Ser-A180 are evident. (Right panel) Compound **30** modeled in the glycine binding site as seen from the 'edge view' of the molecule. The key hydrogen bond between the trifluoromethyl group and Asp-A224 at the back of the binding pocket is evident. PDB Accession code: 1PBQ.

atropisomer **28** was unable to pick up these favorable interactions.[19]

Disappointingly, the desirable rat plasma exposures of the racemic **20** appeared to reside *predominately* with the less potent (S_a) atropisomer. The less potent **28** showed high exposure levels with AUC = 6900 ng h/mL when dosed orally at 5 mg/kg, and the more potent **29** showed only a low oral exposure with AUC = 771 ng h/mL.

A path forward was revealed when the trifluoropropyl compound **23** with an IC_{50} = 18 nM was separated into its constituent atropisomers (S_a) **30** and (R_a) **31** with IC_{50}'s *similar* to one another: 11 and 22 nM, respectively (Table 3). The absolute stereochemistry of **30** was verified by small molecule single crystal X-ray crystallography, and this was the first example of a potent atropisomer in which the methyl sulfonamide substituent was likely directed out of the binding pocket. Molecular modeling of **30** in the NMDAr glycine binding site showed that the trifluoromethyl group likely formed a hydrogen bond with Asp-A224 thus boosting the affinity this atropisomer had for the binding site (Fig. 2). As predicted, the greater plasma exposure level in rat plasma was found for **30**, the atropisomer with the (S_a) stereochemistry (AUC = 3150 ng h/mL).[20]

Following this lead, the ethyl sulfonamide analog **32** was prepared and separated into each atropisomer: (S_a) **33** and (R_a) **34**. In this instance, the binding pocket cannot accommodate the larger ethyl sulfonamide moiety, and the (R_a) atropisomer **34** exhibited an NMDAr IC_{50} = 109 nM.[21] Conversely, the (S_a) atropisomer **33** remained potent with an IC_{50} = 20 nM. Compound **33** had an oral bioavailability in rat of 32% and good plasma exposure: AUC = 3920 ng h/mL, slightly higher than the methyl sulfonamide **30**.

These lead compounds were evaluated in vivo to assess brain penetration and analgesic properties. Compounds **29**, **30**, and **33** were evaluated in the MES seizure model

to determine brain penetration. When challenged with a high dose of 300 mg/kg ip, **29** showed 20% protection in the MES model, indicating brain penetration. In contrast, compounds **30** and **33** gave 0% protection, suggesting no brain penetration at this dose. When dosed at 50 mg/kg ip, **30** showed a 40% reduction in pain response in the Rat Formalin Pain model,[22] while **33** showed a 60% reduction in pain response. When dosed orally at 50 mg/kg, **30** showed no effect in the pain model, whereas **33** showed a 30% reduction in pain response.

In summary, starting from lead compound **1** (UK-240455), a potent NMDAr glycine site antagonist with low oral bioavailability (%F = 17%) and the capacity to cross the blood–brain barrier, new analogs were designed to improve the oral PK properties and decrease CNS penetration. By altering physicochemical characteristics, analogs with improved PK profiles were produced while still retaining affinity for the NMDA receptor. A divergence of potency and desirable PK properties became apparent upon separation of atropisomers of active compounds. The subsequent discovery of nearly equipotent atropisomers bearing a trifluoromethylpropyl side chain on the sulfonamide nitrogen provided a path to a potent antagonist **33** with reasonable oral bioavailability (%F = 32%) and plasma exposure (AUC_{po} = 3920 ng h/mL at 5 mg/kg) sufficiently high to provide oral activity in in vivo models of pain without giving evidence of penetrating the CNS as measured by the MES assay.

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