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Synthesis and SAR development of novel mGluR1 antagonists for the treatment of chronic pain

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ABSTRACT

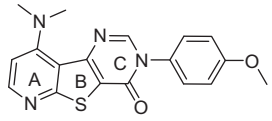
High throughput screening identified the pyridothienopyrimidinone **1** as a ligand for the metabotropic glutamate receptor 1 (mGluR1 = 10 nM). Compound **1** has an excellent in vivo profile; however, it displays unfavorable pharmacokinetic issues and metabolic stability. Therefore, using **1** as a template, novel analogues (**10i**) were prepared. These analogues displayed improved oral exposure and activity in the Spinal Nerve Ligation (SNL) pain model.

Glutamate is the principal excitatory neurotransmitter in mammalian brains. Glutamate receptors can be divided into two subtypes: ionotropic and metabotropic. Ionotropic receptors have been associated with the opening of cationic channels,[1] and are responsible for rapid neuronal excitation of glutamate transmission.[1b] Metabotropic G-protein receptors (mGluR) indirectly regulate electrical signaling by influencing intracellular metabolic processes via G-proteins.[1c] Eight metabotropic G-protein coupled receptors have been reported[1] which have been divided into three groups: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR7 and mGluR8).[1] It has been reported that mGluR1 is essential for motor coordination,[2] perception of pain,[3] and may play an important role in seizures and related disorders.[4] mGluR1 knock out mice exhibit lower pain sensitivity and are also more receptive to morphine than the wild types.[5] Thus an mGluR1 receptor antagonist would be beneficial for the treatment of neuropathic pain.[3] Our goal for this project was to develop a compound that would have a potency of less than 10 nM in our binding assay, oral exposure[6] at six hours and activity in the Spinal Nerve Ligation (SNL) animal model [7].

The SNL[7] animal model is done by ligating the L-5 spinal nerve in rats and then observing the sensitivity of the hind paw of the animal to mechanical stimulation with von Frey filaments. As a point of comparison there is a second group of rats that undergo a similar surgery but not the ligation of the spinal nerve. This is done to rule out the effects of surgery in the neuropathic pain model. Our goal was to obtain an inhibitor that would equal the response to stimulus with the von Frey filaments of the rats that had undergone the surgery but not the ligation.

The commercially available compound **1** exhibited potent binding at mGluR1.[8] However, further testing showed that the oral exposure for this compound was relatively poor. (AUC = 679 ngh/mL, Fig. 1) Compound **1** did show activity in the SNL disease model with an 87% reversal of tactile allodynia at 10 mg/kg. This compound also was prone to rapid demethylation of the *N,N*-dimethyl amine moiety which contributed to the activity in the SNL animal model. Two of the possible metabolites were then tested in the binding and pharmacokinetic assays (Fig. 2). These two potential metabolites were potent at mGluR1, but showed similar oral exposure to the parent.

The goal was therefore to improve the oral exposure and maintain the mGluR selectivity of analogue **1** and develop molecules with improved metabolic stability**.** It was our hope that by replacing the A ring with a pyrimidine moiety, we would improve the



1

hmGluR1 IC_{50} = 10 nM
hmGluR5 IC_{50} = inactive at 10μM
hmGluR2 IC_{50} = inactive at 10μM
RR $AUC_{0\text{-}6h}$[6] @ 10 mpk 679 ngh/mL

Figure 1. Initial lead from high throughput screening.

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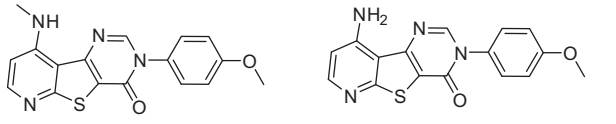
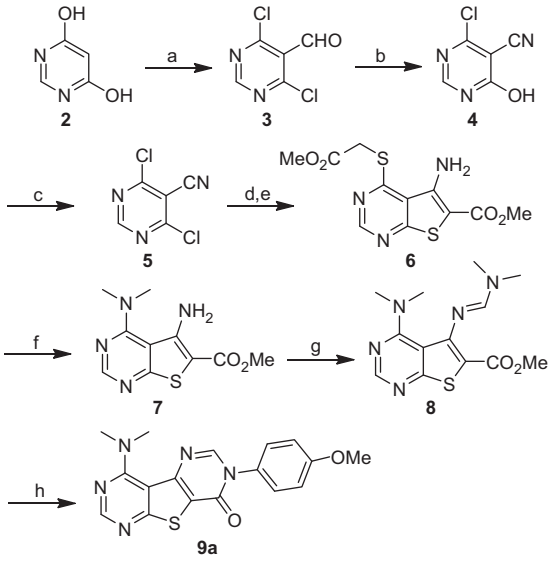


Figure 2. Metabolites of Compound 1.

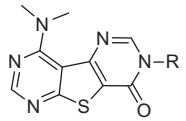
metabolic stability of the core. Modifications to the peripheral functional groups of the tricyclic core would also be investigated.

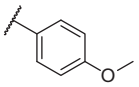
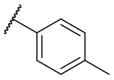
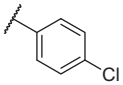
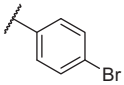
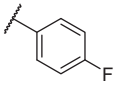
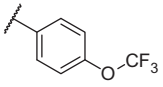
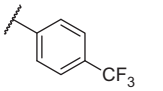
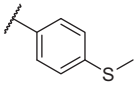
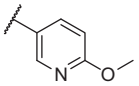
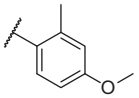
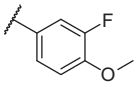
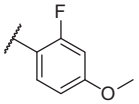
To access these analogues, a versatile synthetic route was developed[8e], exemplified by the preparation of analogue **9a** (Scheme 1) These modifications not only generated potent, selective compounds but provided analogues with improved pharmacokinetic profiles relative to **1**. Starting with readily available 4,6-dihydroxy pyrimidine (**2**), Vilsmeier conditions were used to effect two transformations: installation of a formyl handle at the pyrimidine 5-position and transformation of both hydroxyl moieties into chlorides. Exposure of the formyl group to hydroxyl amine hydrochloride under acidic aqueous conditions followed by thionyl chloride yielded the nitrile **4**. This step installs the expected oxime and dehydrates to the corresponding nitrile; however, unwanted monohydroxylation also occurs. Resubjecting **4** to refluxing phosphorus oxychloride in the presence of a small amount of triethylamine yields the requisite dichloropyrimidine. Treatment of **5** with two equivalents of methyl thioglycolate followed by exposure to refluxing triethylamine provided bicycle **6**. This moiety proved to be a versatile intermediate in that the modification to the C-4 thioether allows for the installation of a variety of functional groups to facilitate SAR investigation. In this instance, displacement of the methyl thioglycolate using a solution of dimethylamine in THF provided **7**.



Scheme 1. Reagents and conditions: (a) $POCl_3$, DMF, reflux, 24 h; (b) H_2NOH, AcOH, reflux, 12 h. Then $SOCl_2$ reflux, 12 h; (c) $POCl_3$, reflux, 3 h. (d) $HSCH_2CO_2Me$, TEA, THF, RT, 1 h; (e) TEA, toluene, reflux 24 h; (f) $HNMe_2$, THF, reflux, 12 h; (g) $(MeO)_2CHNMe_2$, reflux, 5 h R_3NH_2, HOAc, toluene, reflux, 12 h.

Table 1
In vitro data for pyrimidinone N-1 modifications

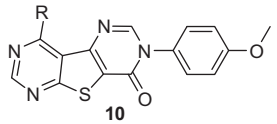


Entry	R	hmGluR1 IC_{50} (nM)	hmGluR5 IC_{50} (nM)	RR AuC_{0-6h} (nM h)
9a		2	1000	1313
9b		1	10,000	0
9c		1	ND	0
9d		1	ND	0
9e		4	ND	0
9f		14	10,000	0
9g		16	10,000	166
9h		8	10,000	950
9i		7	200	ND
9j		204	ND	ND
9k		20	10,000	45
9l		15	ND	1380
9m		5	ND	0
9n		7	10,000	15,431

The IC_{50} data is an average of at least three measurements, performed on human mGluR1/5. The standard error was 10%, and variability was less than twofold from assay to assay.

Reaction of **7** with dimethyl amine dimethyl acetal generated intermediate **8**. Exposure of **8** to a variety of amines under acidic

Table 2
Modification of the 4-position of the pyrimidine A-ring



10

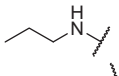
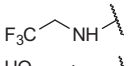
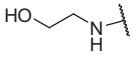
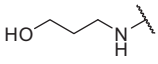
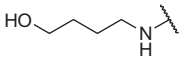
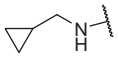
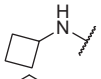
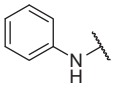
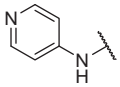
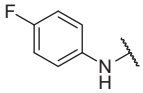
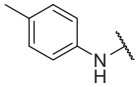
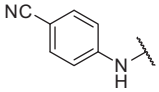
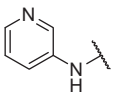
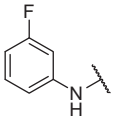
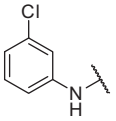
Entry	R	hmGluR1 IC_{50} (nM)	hmGluR5 IC_{50} (nM)	RR AuC_{0-6h} (nM h)
10a		5	10,000	778
10b		7	10,000	4018
10c		3	10,000	934
10d		19	ND	1905
10e		24	ND	5405
10f		86	ND	ND
10g		12	ND	1174
10h		9	ND	1973
10i		6	10,000	9614
10j		154	ND	ND
10k		5	ND	443
10l		60	ND	ND
10m		277	ND	ND
10n		>1000	ND	ND
10o		>1000	ND	ND
10p		>1000	ND	ND
10q		>1000	ND	ND
10r		>1000	ND	ND
10s		192	ND	ND

Table 2 (*continued*)

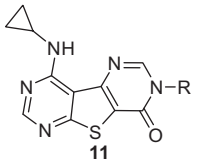
Entry	R	hmGluR1 IC_{50} (nM)	hmGluR5 IC_{50} (nM)	RR AuC_{0-6h} (nM h)
10t		>1000	ND	ND

The IC_{50} data is an average of at least three measurements, performed on human mGluR1/5. The standard error was 10%, and variability was less than twofold from assay to assay.

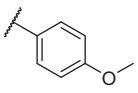
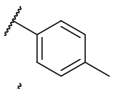
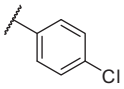
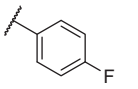
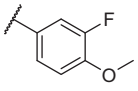
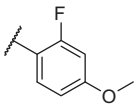
conditions proceeded smoothly to give the target compounds (**9a**–**n**, Table 1).

From the data shown in Table 1 it can be seen that most modifications at the pyrimidinone N-1 position were successful in yielding compounds that were both potent and selective mGluR1 inhibitors. Modifications to the *para* position of the pendant aromatic ring were well tolerated (entries **9a**–**9h**), while replacement of the phenyl ring with a 3-pyridine substituent (entry **9j**) led to a significant decrease in potency. This activity could be restored upon the addition of a suitably disposed methoxy. Disubstitution

Table 3
Pyrimidinone N-1 modifications of **10i**



11

Entry	R	hmGluR1 IC_{50} (nM)	hmGluR5 IC_{50} (nM)	RR AuC_{0-6h} (nM h)
10i		6	10,000	9614
11a		9	10,000	890
11b		3	10,000	4540
11c		82	ND	ND
11d		70	ND	ND
11e		19	ND	8599
11f		30	ND	948

The IC_{50} data is an average of at least three measurements, performed on human mGluR1/5. The standard error was 10%, and variability was less than twofold from assay to assay.

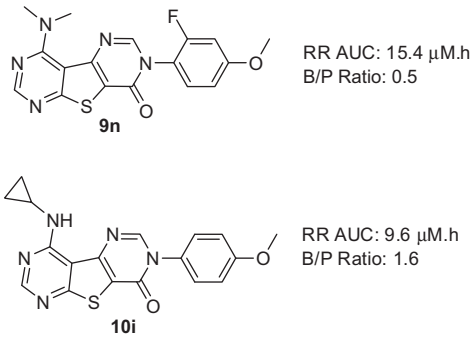


Figure 3. Pharmacokinetic comparisons of two potent mGluR1 antagonists.

of the aromatic ring also appeared to be well tolerated. (entries **9l**–**9n**) The 2-fluoro-4-methoxy substitution pattern (**9n**) on the right hand side aromatic ring yielded not only a potent, selective inhibitor of mGluR1 but also gave an analogue with oral exposure that was superior to any of the other compounds examined. Compound **9n** exhibited a rat K_i of 13.4 nM and turned out to be fairly efficacious in the rat SNL model. At 10 mg/kg dose there was almost a complete reversal of allodynia with an ED_{50} of 3.8 mg/kg at the 2 h time point.

Subsequent metabolite studies showed that both **9n** and **1** were readily demethylated at the *N*,*N*-dimethylamino moiety. In order to further improve the oral exposure of **9n** and thus lower the ED_{50}, a second round of investigations focused on identifying a suitable replacement for the *N*,*N*-dimethyl substituent (Table 2).

Amino substituents possessing small alkyl groups generally gave rise to analogues with slightly improved potency relative to **9n** (entries **10a**–**10d**). Several of these analogues had reasonable levels of oral exposure; curiously, none offered any improvement compared to **9n**. Likewise, analogues bearing hydroxyl alkyl substituted amines were also tolerated (entries **10f**–**h)** Hydroxypropyl (**10g**) and hydroxybutyl (**10h)** analogues exhibited comparable mGluR1 inhibitory activity to **9n**. Unfortunately the pharmacokinetic profiles for all three of these compounds were modest. Analogues possessing aminocycloalkyl substituents were generally equipotent to the dimethylamino analogue **9n** (entries **10i**–**10l**). Notably, the cyclopropylamino species **10i** exhibited exposure levels similar to **9n**. Arylamino analogues were all significantly less potent that **9n** (entries **10m**–**10t**).

The SAR of the pyrimidinone ring substituent was then reinvestigated, using a fixed cyclopropylamino group appended to the pyrimidine (Table 3). It was anticipated that this group would provide analogues with improved pharmacokinetic profiles relative to the initial lead **9a**. This was not the case. Although analogues possessing a 4-methylphenyl (**11a**) and 4-chloro substituent (**11c**) were equipotent to 4-methoxyphenyl species **10i**, their corresponding rat AUC values were approximately 10 fold and twofold less, respectively. Replacing the chloro substituent in compound **11b** with a fluoro group led to 30-fold decrease in activity (**11c**). Likewise, an analogue in which the phenyl ring had been replaced with a cyclohexyl group was not potent enough to warrant further investigation.

The addition of a fluoro substituent to the pendant aromatic ring, as in entries **11e** and **11f**, gave no improvement in oral exposure when compared to their non-fluoro counterpart **10i**. This stands in contrast to what had been observed in the dimethylamino series outlined in Table 1.

Compound **10i**, when compared to **9n,** has a similar pharmacokinetic profile, but carries the advantage of a more metabolically stable cyclopropylamino substituent. Further, **10i** exhibits a threefold increase in brain:plasma ratio (Fig. 3).

The favorable pharmacokinetic profile of **10i** translates into an orally efficacious compound in the rat SNL model for the treatment of tactile allodynia. **10i** exhibited a rat K_i of 14.5 nM and at 3 mg/kg there is almost a complete reversal of allodynia with an ED_{50} of 2.0 mg/kg at the 2 h time point.

In conclusion, modifications to the peripheral functional groups of the tricyclic core of **9a** led to the identification of potent, selective mGluR1 inhibitors with good oral exposure. The two highlighted compounds (**9n**, **10i**) also exhibited desirable pharmacological properties, as well as efficacy in the rat SNL model.

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