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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4936–4940

Correlation between brain/plasma ratios and efficacy in neuropathic pain models of selective metabotropic glutamate receptor 1 antagonists

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Received 3 May 2006; revised 14 June 2006; accepted 14 June 2006
Available online 30 June 2006

Abstract—We have discovered a novel, potent, and selective triazafluorenone series of metabotropic glutamate receptor 1 (mGluR1) antagonists with efficacy in various rat pain models. Pharmacokinetic and pharmacodynamic profiles of these triazafluorenone analogs revealed that brain/plasma ratios of these mGluR1 antagonists were important to achieve efficacy in neuropathic pain models. This correlation could be used to guide our in vivo SAR (structure–activity relationship) modification. For example, compound **4a** has a brain/plasma ratio of 0.34, demonstrating only moderate efficacy in neuropathic pain models. On the other hand, antagonist **4b** with a brain/plasma ratio of 2.70 was fully efficacious in neuropathic pain models.

Glutamate is the most prominent excitatory neurotransmitter in the central nervous system. Glutamate receptors are divided into two major categories: ionotropic and metabotropic. Ionotropic glutamate receptors are responsible for fast neurotransmission. Metabotropic glutamate receptors play a slower, modulatory role through interactions with a variety of other intracellular signaling systems. There are three different mGluR groups, group I, II, and III. A total of eight distinct subtypes, mGluR1 to mGluR8, based on their primary sequence similarity, signal transduction linkages, and pharmacological profile has been identified.[1] Group I mGluRs, mGluR1 and mGluR5, play key roles in the central sensitization of pain, in addition to a variety of functions with potential implications in neurological and psychiatric disorders.[2] Glutamate and other excitatory amino acids are released from nerve endings in the periphery under inflammatory conditions. Glutamate or group I mGluR agonists induce hyperalgesia when administered peripherally.[3] MGluRs modulate pain transmission in the spinal cord, most likely via sensitization of dorsal horn neurons to sustain high intensity C-fiber input.[1c] Normalization of glutamatergic neurotransmission in the spinal cord and nociceptive afferents via inhibition of the group I mGluRs is manifested in the attenuation of pain.[4]

We are interested in mGluR1 antagonists as a therapy for the treatment of pain.[5] The role of mGluR1 in the treatment of pain has not been validated, due to lack of potent, selective, and systemically active mGluR1 antagonists. Earlier efforts to evaluate amino acid antagonists, competing with the glutamate binding site, were not successful due to poor selectivity, weak antagonism, and lack of CNS availability.[2] Literature reported, non-amino acid-like mGluR1 antagonists, such as CPCCOEt [7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester],[6] BAY 36-7620,[7] R214127,[8] dicarboxypyrroles,[9] and JNJ16259685,[10] showed limited efficacy in various pain models, especially in neuropathic pain models.

Triazafluorenone derivatives (Fig. 1) were identified as potent group I mGluR antagonists from high-throughput screening (HTS). Our early SAR of this series has

Keywords: Metabotropic; Glutamate; Receptor; Antagonist; mGluR1; Neuropathic pain.
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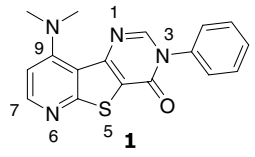
doi:10.1016/j.bmcl.2006.06.053



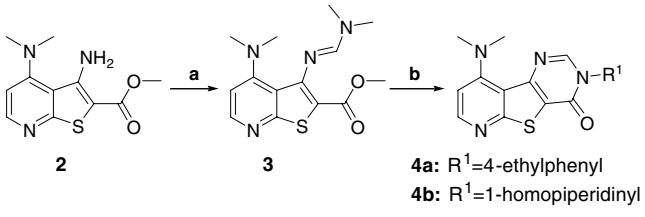
*h*mGluR1 IC_{50} = 20 nM

Figure 1. Triazafluorenone analog identified from HTS.

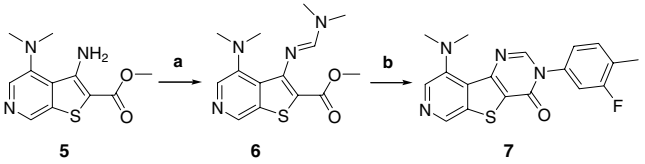
been reported.[5] The early leads of the series demonstrated full efficacy in various pain models, except neuropathic pain models. While we thought that CNS penetration, mainly brain concentration, was important to predict efficacy of these mGluR1 antagonists in neuropathic pain models, we were puzzled by the fact that the brain concentration of these antagonists did not correlate well with their efficacy in neuropathic pain models. Apparently, brain distribution, not the total concentration, played a more important role for these compounds to demonstrate efficacy in neuropathic pain models. With an ongoing effort to correlate local brain concentrations of these compounds to the efficacy in neuropathic pain models, we were also actively looking for any other indications/parameters to guide our in vivo SAR study, and to improve in vivo potency of these compounds in neuropathic pain models. We describe here that we had observed a correlation between brain/plasma ratios of these triazafluorenone analogs and their in vivo efficacy in neuropathic pain models.

We have established several synthetic methods to access these triazafluorenone analogs to accommodate variations at the different parts of the triazafluorenone pharmacophore.[5] The *para*-dimethylaminopyridine analogs **4a**,**b** were synthesized from intermediate **2** in 2 steps. Intermediate **2** was made with known procedures.[5,11] The formamidine derivative **3** was obtained in quantitative yield after condensation of intermediate **2** with *N*,*N*-dimethylformamide dimethyl acetal. Formamidine **3** was then treated with primary amines, 4-ethylaniline or 1-amino-1-homopiperidine, in the presence of *para*-toluenesulfonic acid to form the desired triazafluorenone products **4a** and **4b**, respectively (Scheme 1).

The *meta*-dimethylaminopyridine analog **7** was prepared as described in Scheme 2. Intermediate **5** was made with known procedures.[5,12] The aminoester **5** was then heated to reflux with *N*,*N*-dimethylformamide dimethyl acetal to generate formamidine intermediate **6** quantitatively. The intermediate **6** was then reacted with



Scheme 1. Reagents and conditions: (a) $Me_2NCH(OMe)_2$, reflux, 5 h (99%); (b) R^1NH_2, toluene, *p*-TsOH, **4a** (73%), **4b** (45%).

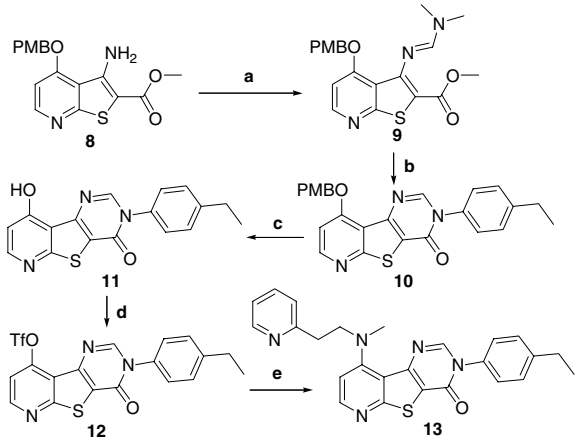


Scheme 2. Reagents and conditions: (a) $Me_2NCH(OMe)_2$, EtOH, reflux 16 h, 90%; (b) 4-methyl-3-fluoroaniline, toluene, *p*-TsOH (cat.), reflux 16 h, 58%.

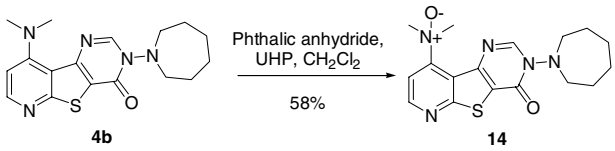
3-fluoro-4-methylaniline in the presence of *para*-toluenesulfonic acid to form the final triazafluorenone product **7** (Scheme 2).

Triazafluorenone analog **13** was made from triflate intermediate **12** (Scheme 3). Triflate **12** was generated from intermediate **8** as described.[13] Compound **9**, formed from **8** in the presence of *N*,*N*-dimethylformamide dimethyl acetal, was treated with 4-ethylaniline to produce tricyclic compound **10**. The protecting group PMB (*para*-methoxybenzyl) of **10** was then removed with trifluoroacetic acid (TFA) to give **11**.[14] The hydroxy group of **11** was then converted to a triflate group in the presence of *N*,*N*-bis(trifluoromethanesulfonyl)phenylamide.[15] The final triazafluorenone analog **13** was obtained by treating triflate **12** with corresponding amine (Scheme 3).

The *N*-oxide analog **14** was obtained by treating **4b** with phthalic anhydride and hydrogen peroxide-urea complex (58% yield) (Scheme 4).[16]



Scheme 3. Reagents and conditions: (a) $Me_2NCH(OMe)_2$, reflux 10 h, 100%; (b) *p*-ethylaniline, toluene, *p*-TsOH (cat.), microwave 160 °C, 1 h, 39%; (c) TFA, 0 °C, 1.2 h, 100%; (d) Tf_2NPh, $EtN(i\text{-}Pr)_2$, 80%; (e) 2-(2-pyridyl)ethylmethyl-amine, 23 °C, 89%.



Scheme 4.

We have thus established several synthetic routes to accommodate variations of these triazafluorenone analogs for SAR studies, and to modify their ADME profiles. These triazafluorenone analogs are non-amino acid-like and non-competitive mGluR1 antagonists which bind at 7-TMD region of the receptor.[6] Compound **4a** was a selective mGluR1 antagonist (mGluR1 $IC_{50} = 3 \pm 0.9$ nM). It was also active in mGluR5 ($IC_{50} = 442 \pm 93$ nM) with a ratio of mGluR5/mGluR1 around 147. Compound **4a** was inactive in mGluR2, mGluR4, and mGluR7 ($IC_{50} > 10$ μM). MGluR1 antagonist **4a** was able to achieve full efficacy in various animal pain models, including CFA (complete Freund's adjuvant-induced thermal hyperalgesia, $ED_{50} = 15$ μmol/kg, ip), Carrageenan ($ED_{50} = 11$ μmol/kg, ip), formalin ($ED_{50} = 19$ μmol/kg, ip), skin incision ($ED_{50} = 47$ μmol/kg, ip), and OA (osteoarthritic pain, $ED_{50} = 29$ μmol/kg, ip). Pharmacokinetics analysis revealed that compound **4a** had moderate bioavailability ($F_{ip} = 45\%$, $F_{oral} = 12\%$). There was no locomotor side effect caused by this mGluR1 antagonist ($ED_{50} > 100$ μmol/kg, ip). This compound was also capable to penetrate BBB (blood–brain barrier) with a brain/plasma ratio of 0.34 (30 μmol/kg, ip) (Table 1).

While we were encouraged by the efficacy demonstrated by compound **4a** in the above-mentioned pain models, we were puzzled by its weak and partial efficacy in neuropathic pain models, such as Chung (Spinal Nerve (L5/L6) Ligation Model, $ED_{50} > 100$ μmol/kg, ip).[17]

The site of action for this target is not very clear. It was known that mGluR1 has expression and distribution in both peripheral and CNS system.[18] Compound **4a**, with distribution in both peripheral and central compartments, achieved full efficacy in various animal pain models listed in Table 1, but not the Chung model of neuropathic pain. We thought that total brain concentration might be able to correlate efficacy in neuropathic pain models of these compounds, and could be used to guide our in vivo SAR study. We quickly realized that this was not case for these mGluR1 antagonists. Compound **7** had much lower total brain concentration (total brain concentration: 125 ± 41 ng/g at 30 μmol/kg, ip; protein binding: 99.9%, free brain concentration: 0.13 ± 0.04 ng/g) than **4a** (total brain concentration: 1180 ± 258 ng/g at 30 μmol/kg, ip; 97.8% protein binding, free brain concentration: 25.5 ± 5.7 ng/g). Yet this compound **7** was more efficacious (Chung $ED_{50} = 55$ μmol/kg, ip) than the latter antagonist **4a** (Chung $ED_{50} > 100$ μmol/kg, ip) in neuropathic pain models. Apparently, neither total brain concentration, nor free brain concentration of these compounds correlated well their in vivo efficacy in neuropathic pain models. The assumption made from these results was that the local distribution of mGluR1 antagonist in the brain might play a more crucial role in the neuropathic pain models.

It is certainly a technical challenge to first identify the actual location of the action site in the brain, and then determine the local concentration of these mGluR1 antagonists. We were looking for some type of parameter(s) we could use to guide our in vivo SAR study to improve efficacy in neuropathic pain models. We did find that there was a correlation between brain/plasma ratio of these mGluR1 antagonists and their efficacy in neuropathic pain models. We used this correlation to guide our in vivo SAR study and identified compound with full efficacy in neuropathic pain models.

Compound **13**, made according to Scheme 3 in an effort to modify solubility and metabolic profiles of the dimethylamino group in parent analog **4a**, was a relatively more polar compound with its 9-pyridylethylmethylamino group. This compound **13** had a lower brain/plasma ratio (0.16) than its parent compound **4a** (brain/plasma ratio = 0.34), and was inactive in Chung model (11% at 100 μmol/kg, ip Table 2).

Incorporating a dimethylamino *N*-oxide group at the C9 position of the triazafluorenone pharmacophore generated a very polar mGluR1 antagonist **14**. This *N*-oxide analog was still quite potent and selective mGluR1 antagonist ($IC_{50} = 33 \pm 17$ nM). Compound **14** had a very low brain/plasma ratio (0.05). Again, this compound showed no efficacy in Chung model (12% at 100 μmol/kg, ip Table 2).

It seemed clear that mGluR1 antagonists with high brain/plasma ratio might be advantageous in terms of their in vivo efficacy in neuropathic pain models. Compound **7**, as discussed earlier, had very low total brain concentration compared to the parent compound **4a**. Yet this compound **7** was more efficacious (72% at 100 μmol/kg, ip) than **4a** (41% at 100 μmol/kg, ip). Compound **7** did have a higher brain/plasma ratio of 0.51, compared to compound **4a** (brain/plasma ratio = 0.34).

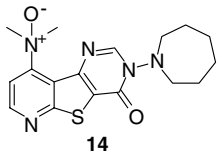
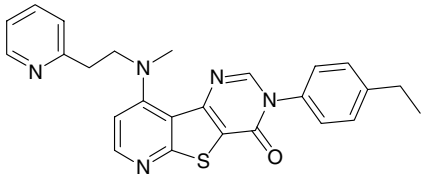
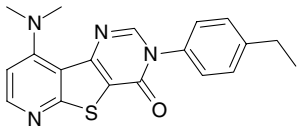
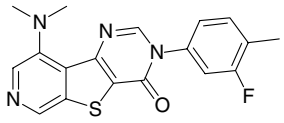
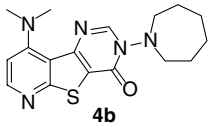
Table 1. Selectivity, efficacy, and side effects of triazafluorenone mGluRl antagonist **4a**

	mGluRl (nM)	mGluR2 (nM)	mGluR4 (nM)	mGluR5 (nM)	mGluR7 (nM)
In vitro[a]	3 (±0.9)	>10,000	>10,000	442 (±93)	>10,000
	Model (rat)		ED_{50} (μmol/kg, ip)		
In vivo[b]	CFA		15		
	Carrageenan		11		
	Formalin		19		
	Skin incision		47		
	OA		29		
	Chung		>100		
	Model (rat)		ED_{50} (μmol/kg, ip)		
Side effect	Locomotor		>100		
	Rotorod		>300		

[a] 1321N1 cells expressing human mGluRs, mean of multiple results with standard error of mean.

[b] Tests performed 30 min after intraperitoneal administration of compound in rats (6 rats per group). Vehicle was 10% DMSO/PEG (5 mL/kg).

Table 2. Correlation between brain/plasma ratios and efficacy in neuropathic pain model of triazafluorenone mGluR1 antagonists

Structure	mGluR1[a] IC_{50} (nM)	mGluR5[a] IC_{50} (nM)	$c\log P$[b]	ratio[c] (brain/plasma)	Chung[d] % effect at 100 μmol/kg
 **14**	33 (±17)	>10,000	1.26	0.05 (±0.02)	12%
 **13**	52 (±16)	>10,000	4.84	0.16 (±0.06)	11%
 **4a**	5 (±3)	1330 (±490)	4.24	0.34 (±0.08)	41%[e]
 **7**	21 (±15)	>10,000	3.85	0.51 (±0.1)	72%[f]
 **4b**	1 (±05)	>10,000	2.80	2.70 (±0.4)	100%[f]

[a] 1321N1 cells expressing either rat mGluR1 or mGluR5, mean of multiple results with standard error of mean.
[b] Calculated from SPARK ACD program.
[c] Determined from the corresponding in vivo experiments at 30 μlmol/kg ip (see Footnote d). Mean of multiple results with standard error of mean.
[d] Tests performed 30 min after intraperitoneal administration of compound in rats (6 rats per group). Vehicle was 10% DMSO/PEG (5 mL/kg).
[e] $P < 0.05$ versus vehicle group.
[f] $P < 0.01$ versus vehicle group.

Compound **4b**, a potent and selective mGluR1 antagonist ($IC_{50} = 1 \pm 0.5$ nM), was able to penetrate BBB better than compound **7**, based on their brain/plasma ratios (2.70 and 0.51, respectively). With the highest brain/plasma ratio of 2.70 (total brain concentration = 851 ng/g at 30 μmol/kg, ip) among this series of mGluR1 antagonists, compound **4b** now achieved full efficacy at 100 μmol/kg ($ED_{50} = 22$ μmol/kg, ip) in the Chung model of neuropathic pain.

Combined with $c\log P$ values of these compounds (Table 2), the use of brain/plasma ratio to guide our SAR study is now even more practical.

In summary, we have observed a correlation between brain/plasma ratios and in vivo efficacy in neuropathic pain model of our triazafluorenone mGluR1 antagonists. Potent and selective mGluR1 antagonists with low brain/plasma ratios demonstrated little or no efficacy in neuropathic pain model. When an mGluR1 antagonist, such as compound **4b**, achieved a favorable high brain/plasma ratio (2.70), it demonstrated full efficacy in neuropathic pain model.

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