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9*H*-Xanthene-9-carboxylic acid [1,2,4]oxadiazol-3-yl- and (2*H*-tetrazol-5-yl)-amides as potent, orally available mGlu1 receptor enhancers☆

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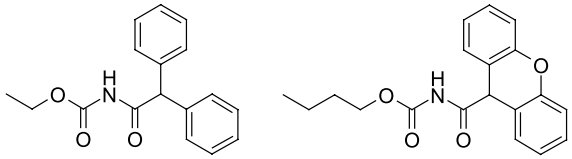
Abstract—Small molecule mGluR1 enhancers based on the lead compound (9*H*-xanthene-9-carbonyl)-carbamic acid butyl ester derived from random-screening hit diphenylacetyl-carbamic acid ethyl ester were designed and synthesized as useful pharmacological tools for the study of the physiological roles mediated by mGlu1 receptors. The synthesis and the structure–activity relationship of this new class of positive allosteric modulators of mGlu1 receptors will be discussed in detail.

1. Introduction

L-Glutamate, the major excitatory amino acid neurotransmitter in the central nervous system, binds to and activates several classes of receptors which are divided into two groups termed ionotropic (iGluR) and metabotropic glutamate receptors (mGluR)[1]. The latter family comprises eight subtypes of G-protein coupled receptors, grouped according to pharmacology and coupling to second messengers.[2] The primary transduction mechanism of group I mGlu receptors (mGluR1 and mGluR5) is the stimulation of phosphoinositide (PI) hydrolysis, whereas group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) receptors inhibit forskolin-stimulated cyclic AMP accumulation.[3] A role for group I mGlu receptor activation has been implicated in physiological processes including pain perception, learning, and memory, as well as in certain psychiatric and neurological disorders.[4] Recently, we described a series of carbamates like the screening hit **1**, which behave as selective positive allosteric modulators (enhancers) of mGlu1 receptors (Fig. 1).[5,6] Recent reports of allosteric modulators of mGlu2[7,8] and mGlu5[9,10] receptors have raised considerable interest.[4] Here, we report on the development of heterocyclic derivatives—based on the xanthyl-carbamate **2**—which are suitable for in vivo use. The random-screening hit **1** was identified initially by using recombinant mGlu1 receptors expressed at very high levels. The constitutive activity of the receptor is such that the compound elicits a response in the absence of glutamate site ligands. However, in physiologically more relevant recombinant systems with a lower level of receptor expression, the compound potentiated the agonist-stimulated response without any detectable intrinsic activity. Using this screening hit as a starting point, we have discovered **2** as a potent and selective positive allosteric modulator of mGlu1 receptors.[5,6] To eluci-



1 [EC_{50} = 130 nM] **2** [EC_{50} = 9 nM]

Figure 1. Representative mGluR1 enhancers: screening hit **1** and lead structure **2**.

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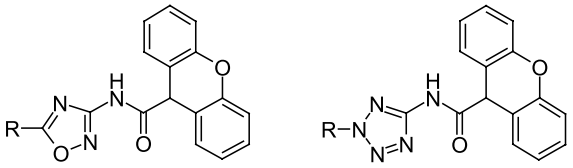


Figure 2. Structures oxadiazoles **3** and tetrazoles **4**.

date the putative binding site of these compounds, chimeras and point mutants of rmGlu1a and rmGlu5a were prepared. The compounds enhanced the glutamate-induced current in all dimeric receptors containing the TM region of rmGlu1a which is required for the enhancing effect.[5] Hydrolysis and decarboxylation of the carbamate moiety of **2** by esterases in blood plasma leads to formation of inactive amides. To overcome this problem, the corresponding ester bioisosteres 1,2,4-oxadiazoles **3** and tetrazoles **4** have been prepared (Fig. 2).

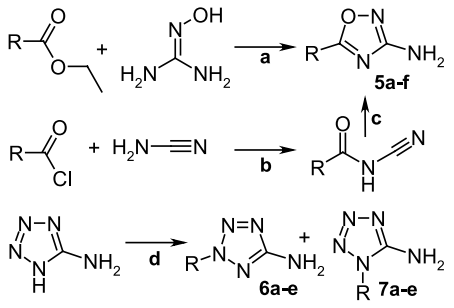
2. Chemistry

Syntheses of the amino-oxadiazoles **5a–f** and aminotetrazoles **6a–e** were realized using methods described in the literature[11–13] (Scheme 1).

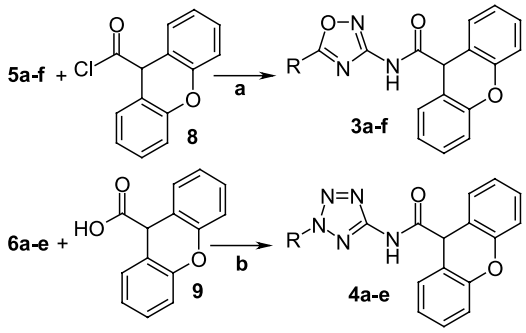
Reaction of the aminooxadiazoles **5a–f** with xanthene-9-carboxylic acid chloride **8** yielded, after conventional workup and purification, the desired compounds **3a–f** in good yields.[14] The aminotetrazoles **6a–e** and **7a–e** were obtained by alkylation of 2-aminotetrazole and separation of the regioisomers by column chromatography. Reaction of **6a–e** with xanthene-9-carboxylic acid **9** using carbonyl diimidazole (CDI) as coupling reagent formed the adducts **4a–e** in acceptable yields[15] (Scheme 2).

3. Structure–activity relationship

In both the series, small alkyl and cycloalkyl substituents on the heterocyclic moiety led to compounds with higher activities, whereas larger groups were less tolerated. The best overall properties in terms of potency, efficacy, and metabolic stability were observed for the methyl- and ethyl-substituted derivatives (Table 1).



Scheme 1. Synthesis of oxadiazoles **5a–f** and tetrazoles **6a–e**. Reagents: (a) NaOH, EtOH, 5–20%; (b) NaOH, H_2O, acetone, quant.; (c) $NH_2OH{\cdot}HCl$, Py, EtOH, 8–55%; (d) R–X, K_2CO_3, EtOH, 17–25%.



Scheme 2. Synthesis of oxadiazoles **3a–f** and tetrazoles **4a–e**. Reagents and conditions: (a) Py, 0 °C to rt, 50–90%; (b) CDI, 0 °C to rt, 25–80%.

The methyl-substituted 1,2,4-oxadiazole **3a** and the ethyl-substituted tetrazole **4b** showing the best overall profiles were selected for further evaluation regarding pharmacology and in vivo PK analysis.

4. Pharmacology

The activities of the compounds at rat mGlu1 receptors were assessed using intracellular Ca^{2+} measurements on rat mGlu1a transiently transfected HEK-293 cells expressing recombinant mGlu1 receptors at very high levels. The constitutive activity of the receptor is such that the compounds elicit an agonist response in the absence of glutamate site ligands. $[Ca^{2+}]i$ measurements were performed after incubation of the cells with Fluo-3 AM (Molecular Probes, Eugene, OR, USA) for 1 h and four washes with assay buffer (DMEM supplemented with Hank's salt and 20 mM HEPES). $[Ca^{2+}]i$ measurements were done using a fluorometric imaging plate reader (FLIPR, Molecular Devices Corporation, La Jolla, CA, USA). Fluorescence ratio values were calculated as described.[16] EC_{50} values for the enhancers are the mean of separate values from at least three individual experiments. The agonist effect is normalized to the maximum response induced by 10 μM of glutamate (Table 1).

5. Selectivity

Using various functional models, it was found that **3a** was devoid of any enhancing effect at rat mGlu2, mGlu4, mGlu5, and mGlu8, and human GABA-B receptors. In addition, **3a** had no activity in radioligand binding assays at major adenosine; adrenergic; GABA-A; glycine; histamine; muscarinic; nicotinic; opiate; purinergic and 5-HT receptors, and adenosine; norepinephrine; GABA and 5-HT uptake sites.

6. Electrophysiology

Figure 3 shows the effect of **3a** on glutamate-activated inward current in a CHO cell stably expressing

Table 1. Structure–activity relationship of oxadiazoles **3a**–**3f** and tetrazoles **4a**–**4e**

	R	mGluR1 EC_{50}[a] (nM)	Agonist effect (%)[a]	In vitro metabolism (rat microsomes) (μl/min/mg protein)	In vitro metabolism category	Solubility (pH 6.5) (μg/ml)
3a	Me	52	100	42	Intermediate	12
3b	Et	6	57	78	Intermediate	0.056
3c	*n*-Pr	25	90	97	High	<1
3d	*i*-Pr	22	80	130	High	4
3e	Cyprop	23	80	227	High	<1
3f	*i*-Bu	10	54	17	Low	7
4a	Me	180	65	16	Low	35
4b	Et	65	80	41	Intermediate	21
4c	*n*-Pr	29	80	92	High	4
4d	*i*-Pr	45	70	63	Intermediate	4
4e	*i*-Bu	34	28	229	High	n.m.

[a] See details in pharmacology. n.m. indicates not measured.

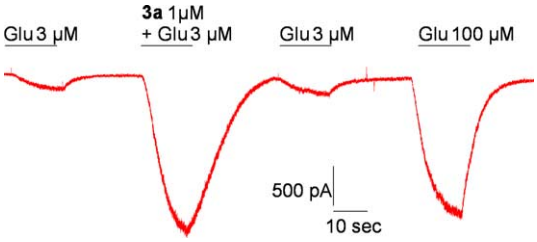


Figure 3.

GIRKs and transiently transfected with the rat mGlu1a receptor cDNA. Glutamate (3 μM) induced a current that was potentiated 10-fold by **3a**. The effect of **3a** was reversible and greater than a saturating concentration of glutamate (100 μM). Compound **3a** had no effect when applied alone. This effect was similar to that observed for screening hit **1**.[5] Figure 4 shows the concentration–response curve for glutamate-induced current potentiation by **3a**. The current amplitudes were normalized to the control responses (3 μM glutamate) and fitted individually for each cell ($n = 3$). The EC_{50} was 220 nM.

7. Pharmacokinetics

Both the methyl-substituted 1,2,4-oxadiazole **3a** and the ethyl-substituted tetrazole **4b** have an intermediate to high plasma clearance (Cl) and an intermediate volume of distribution (V_{ss}) (Table 2).

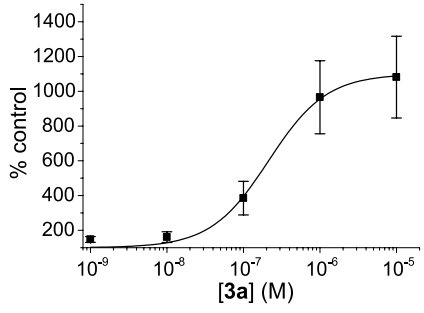


Figure 4.

Table 2. Pharmacokinetics of selected compounds **3a** and **4b** (data are mean values, $n = 2$)

Dose route of administration	DMPK parameters	**3a**	**4b**
10 (mg/kg) iv bolus	Half-life (h)	0.32	0.49
	Cl (ml/min/kg)	60.0	49.2
	V_{ss} (L/kg)	1.82	2.09
10 (mg/kg) po bolus	C_{max} (ng/ml)	1174	1130
	T_{max}(h)	1.5	1.5
	Brain/plasma	1.5	
	CSF/plasma	0.08	
15 mg/kg/h iv infusion	Brain/plasma		0.16
	CSF/plasma		0.06

Oral bioavailability at low doses was up to 100% for both compounds. In the case of compound **3a**, a mean maximum plasma concentration (C_{max}) of 1174 ng/ml, a mean brain level of 1761 ng/ml, and a CSF concentration of 94 ng/ml were achieved after 1.5 h (T_{max}) at an oral dose of 10 mg/kg (Table 2).

Although similar maximal plasma concentrations were observed upon oral dosing of the less lipophilic compound **4b**, an infusion experiment of this compound at a dose of 15 mg/kg/h showed very low steady-state brain tissue concentrations indicative of poor brain penetration. Several compounds of both the series were evaluated in a functional P-glycoprotein assay and did not stimulate P-gp ATPase activity.

8. Discussion

On the basis of the potent and selective positive allosteric modulator **2**, two series of heterocyclic derivatives have been discovered: 1,2,4-oxadiazoles **3** and tetrazoles **4**. In both the series, several derivatives with high activities at rat mGlu1 receptors have been prepared. In each series, one compound with the most promising in vitro profile was selected for further evaluation. With respect to the PK profile, the methyl-substituted 1,2,4-oxadiaz-

ole **3a** could serve as a suitable tool to further study the role of positive allosteric modulation of mGlu1 receptors in vivo.

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