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Alkyl diphenylacetyl, 9*H*-xanthene- and 9*H*-thioxanthene-carbonyl carbamates as positive allosteric modulators of mGlu1 receptors

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Abstract

Starting from the random-screening hit **1a**, a series of alkyl diphenylacetyl, 9*H*-xanthene- and 9*H*-thioxanthene-carbonyl carbamates **1** has been prepared. These derivatives turned out to be selective positive allosteric modulators of mGlu1 receptors. These compounds do not directly activate mGlu1 receptors but markedly potentiate agonist stimulated responses, increasing potency and maximum efficacy.

Keywords: Metabotropic glutamate receptors; Positive allosteric modulators; Carbamates

1. Introduction

Excitatory amino acid (EAA) receptors mediate synaptic excitation in the mammalian central nervous system [1]. These receptors have been classified into two major classes, the ion channel type, or ionotropic glutamate (iGlu) receptors and the G-protein coupled, or metabotropic glutamate (mGlu) receptors. The former are further subdivided into *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and kainic acid (KA) receptors according to the selective actions of these agonists [2].

After the independent discovery of the first mGlu receptor by Sladeczeck et al. in 1985 [3] and by Nicoletti et al. in 1986 [4], the multiplicity of this class has been disclosed by further homology cloning [5]. Currently, eight mGlu receptors (and several splice variants) have been isolated and subdivided in three groups according to sequence homology, signal transduction and pharmacology: the first group includes mGlu1 and mGlu5 receptors which are coupled to IP_3/Ca^{2+} signal transduction pathway via activation of phospholipase C, whereas the Group 2 (mGlu2, mGlu3) as well as Group 3 members (mGlu4, mGlu6, mGlu7, mGlu8) are negatively coupled to adenylate cyclase.

In recent years, there has been a growing interest in the mGlu receptor family due to their implication in a variety of fundamental neuronal functions and they have been claimed to represent therapeutic targets for several brain diseases and disorders [6,7]. In particular, Group I mGlu subtypes have long been proposed to be involved in the developmental processes [8] or in certain physiopathological states like pain, epilepsy or ischemia [9]. However, although for mGlu5 receptors in vivo active antagonists with high affinity and specificity like MPEP [10] recently appeared, there is still a need for selective and in vivo active mGlu1 receptor ligands to clarify the physiological roles mediated by this mGlu receptor subtype.

As part of our ongoing effort to discover non-amino acid subtype selective mGlu receptor ligands [11,12], we have identified, by random screening, the alkyl diphenylacetyl, 9*H*-xanthene- and 9*H*-thioxanthene-carbonyl carbamates **1** which behave as selective positive allosteric modulators (enhancers) of the rat and human mGlu1 receptor [13]. In this paper, we present the structure–activity relationships of this series as well as the properties of these compounds to enhance agonist-stimulated response.

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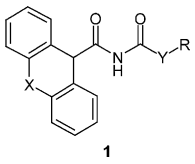

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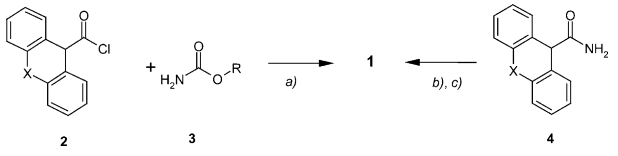
2. Chemistry

The alkyl diphenylacetyl, 9*H*-xanthene- and 9*H*-thioxanthene-carbonyl carbamates **1a**–**r** were prepared according to Scheme 1. There are two simple routes to synthesize these derivatives, either by the reaction of acid chloride **2** with carbamate **3** or by the condensation of an alcohol with an acylisocyanate prepared from the corresponding amide **4**. The diphenylacetyl urea **1s** was prepared from diphenylacetyl isocyanate and ethylamine. For further details see Ref. [14].



2 3 4

Scheme 1. (a) Pyridine, DMAP, CH_2Cl_2, RT; (b) oxalylchloride, dichloroethane, reflux; (c) RYH, CH_2Cl_2, RT.

3. Pharmacology

The activities of the compounds at rat mGlu1 receptors were assessed using intracellular Ca^{2+} measurements. $[Ca^{2+}]$i measurements were performed on rat mGlu1a-transiently transfected HEK-293 cells 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). A cooled charge-coupled device camera (CH-250, Photometrics, Tuscon, AZ) was used to acquire image pairs at 340 and 380 nm excitation wavelengths (with dark correction) to computer. Exposure times were 400 ms. The intrinsic fluorescence in cells not dye-loaded was less than 5% and did not contribute a significant error to the measurements. Fluorescence ratio values were calculated as described [15]. EC_{50} values for the enhancers are the mean of separate values from at least three individual experiments (Table 1).

The *n*-alkyl diphenylacetyl as well as the 9*H*-xanthene-carbonyl carbamates show good activities with EC_{50} values below 0.15 μM, however, the latter are somewhat more active. In both series, a maximum of activity is achieved with the *n*-butyl side-chain (**1c** and **1i**). Replacement of the 9*H*-xanthene by a 9*H*-thioxanthene leads to a 10-fold decrease in activity (**1j** and **1k**). Also, the thio-carbamate **1l** is 10-fold less active than the corresponding carbamate **1c**. The ethyl diphenylacetyl urea **1s** turned out to be inactive indicating that a carbamate moiety is necessary for activity. Sterically more demanding substituents like isopropyl (**1e**), *tert*-butyl (**1f**) or benzyl (**1r**) lead to a reduced activity compared to the *n*-alkyl derivatives. The cyclopropylmethyl derivative **1n** shows an $EC_{50} = 0.03$ μM comparable to that of the corresponding ethyl analogue **1g**, whereas the trifluoroethyl derivative **1m** was found to be 10-fold less active. The most active compounds in this series are the *n*-propyl and *n*-butyl 9*H*-xanthene-carbonyl carbamates **1h**–**i** with an EC_{50} value of 0.01 μM.

Table 1
EC_{50} values obtained using $[Ca^{2+}]$i measurements with Fluo-3 labeled rat mGlu1 receptor-transiently transfected cells

Comp.	X	Y	R	EC_{50} (μM)
1a	2H	O	ethyl	0.13
1b	2H	O	propyl	0.03
1c	2H	O	butyl	0.03
1d	2H	O	pentyl	0.05
1e	2H	O	isopropyl	0.94
1f	2H	O	*tert*-butyl	0.72
1g	O	O	ethyl	0.03
1h	O	O	propyl	0.01
1i	O	O	butyl	0.01
1j	S	O	ethyl	0.20
1k	S	O	butyl	0.14
1l	2H	S	butyl	0.37
1m	2H	O	trifluoroethyl	0.28
1n	O	O	cyclopropylmethyl	0.03
1o	2H	O	1-pentin-5-yl	0.14
1p	2H	O	cyclopropylmethyl	0.22
1q	2H	O	allyl	0.51
1r	2H	O	benzyl	0.95
1s	2H	NH	ethyl	inactive

Compound **1i** is devoid of any enhancing effect at recombinant rat mGlu2, mGlu4, and mGlu8 and human $GABA_B$ receptors, however, a small enhancing effect was observed with this compound (10 μM) at the rat mGlu5a receptor. In addition, no modulatory effects were observed at the muscarinic M1 receptor, which is expressed endogenously in HEK-293 cells, or at recombinant dopamine D2 receptors. The compound did not directly block the GIRK channel and exhibited no activity in radioligand binding assays at adenosine A1, A2; adrenergic α1, α2, β2; $GABA_A$; glycine, histamine H1; muscarinic M1, M2, M3; nicotinic; opiate, purinergic P2x and serotonine receptors as well as adenosine, norephinephrine, GABA and serotonine uptake sites.

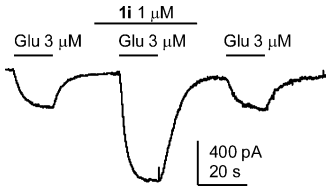


Fig. 1. Effect of compound **1i** on glutamate-induced currents in CHO cells expressing rat mGlu1 receptors and GIRKs. The first trace represents a K^+ current induced by a glutamate application which duration is indicated by the bar. Application of **1i** alone did not induce a current. However, when glutamate was applied simultaneously with **1i**, the current was markedly potentiated (middle trace). The last trace represents the effect of glutamate alone after washout of **1i** (last trace).

4. Electrophysiology

To assess the electrophysiological properties of compound **1i**, we performed whole-cell recordings of voltage-clamped Chinese hamster ovary (CHO) cells expressing rat mGlu1 receptors and G Protein-coupled, inwardly-rectifying potassium channels (GIRKs). Application of glutamate (3 μM) induced a current which was markedly potentiated when glutamate was applied in the presence of 1 μM of compound **1i**. However, application of 1 μM of compound **1i** alone had no effect (Fig. 1).

5. Conclusion

The random-screening hit **1a** was identified initially by using recombinant mGlu1 receptors expressed at very high levels. In this system, 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 a series of selective and potent positive allosteric modulators of mGlu1 receptors. Although allosteric modulation of G-protein coupled receptors has been reported [16–20], such a robust effect as observed here has not been described. Positive allosteric modulation is an attractive mechanism for enhancing appropriate physiological receptor activation. Thus, the discovery of selective mGlu1 receptor enhancers opens the possibility for therapeutically relevant positive modulation of family three G-protein coupled receptors.

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