Bioorganic & Medicinal Chemistry Letters 19 (2009) 1666–1669

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Fluorinated 9*H*-xanthene-9-carboxylic acid oxazol-2-yl-amides as potent, orally available mGlu1 receptor enhancers

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ARTICLE INFO

Article history:
Received 18 December 2008
Revised 30 January 2009
Available online 6 February 2009

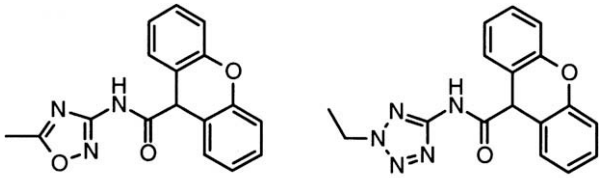
Keywords:
Metabotropic glutamate receptor 1
mGluR1
GPCR
Positive allosteric modulator
PAM
Enhancer

ABSTRACT

Small molecule mGluR1 enhancers, which are 9*H*-xanthene-9-carboxylic acid [1,2,4]oxadiazol-3-yl- and (2*H*-tetrazol-5-yl)-amides, have been previously reported. Fluorinated 9*H*-xanthene-9-carboxylic acid oxazol-2-yl-amides with improved pharmacokinetic properties have been 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 class of positive allosteric modulators of mGlu1 receptors will be discussed in detail.

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 (GPCRs), 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, leading to mobilization of intracellular calcium stores and increase of the intracellular calcium concentration. 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 proposed in physiological processes including pain perception, learning and memory, as well as in certain psychiatric and neurological disorders.[4] Positive allosteric modulators (PAMs) of the mGluR5 receptor have gained recent interest due to their possible use for the treatment of schizophrenia.[5–7] Although both mGluR1 and mGluR5 belong to group 1 mGluR's, the expression patterns of these receptors in the brain are quite different, mGluR1 expression being for example much higher in cerebellar Purkinje cells.[8] Thus mGluR1 PAMs could be of interest in indications where reduced mGluR1 function in this brain area leads to motor impairment (cerebellar ataxia).[9–12] Another interesting finding is the in vitro reversal of synaptic plasticity in mouse dopaminergic (DA) neurons induced by cocaine with the mGluR1 enhancer **Ro 67-7476**,[13,14] which could find potential use in the field of drug addiction.

Recently, we described a series of carbamates that behave as selective positive allosteric modulators (enhancers) of mGlu1 receptors.[15] Potent orally available mGluR1 enhancers **1** and **2** (Fig. 1) have also been reported in a preceding paper.[16] The compounds were initially screened using cells having recombinant mGlu1 receptors transiently expressed at very high levels. In this system, the constitutive activity of the receptor is such that the compound elicits an agonist response in the absence of glutamate site ligands. However, in recombinant systems with a lower level of receptor expression, the compound potentiated the agonist-stimulated response without any detectable intrinsic activity.



1 1,2,4-oxadiazole derivative **2** tetrazole derivative

Figure 1. Structures of oxadiazole- and tetrazole derivatives **1** and **2**.

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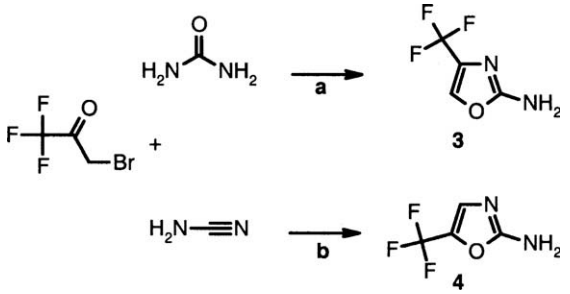
doi:10.1016/j.bmcl.2009.01.108

Although these lead compounds were orally available, improvement of pharmacokinetic (PK) properties, for example, clearance, half-life, and bioavailability, was desirable. Replacement of the oxadiazole or tetrazole moiety with the oxazole ring system led to compounds with similar activities. Metabolism of compound **1** was found to take place on the methyl substituent of the oxadiazole ring as well as on positions 2 and 7 of the xanthene moiety. To reduce metabolism at these positions, the synthesis of fluorinated analogues was envisaged.

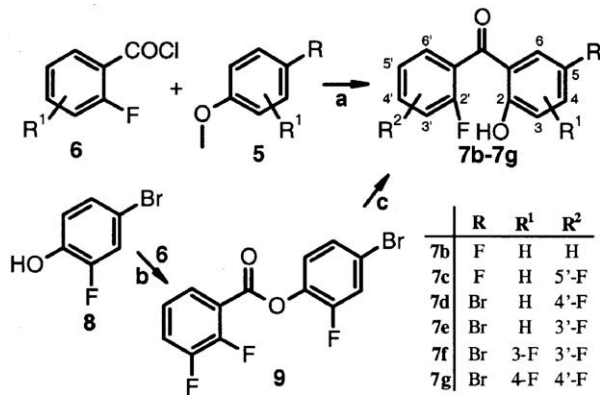
Syntheses of the trifluoromethylated 2-amino-oxazoles **3** and **4** were realized by reacting commercially available trifluorobromoacetone with 5 equiv of urea[17] or cyanamide[18] in refluxing tert-butanol to afford the corresponding 4- and 5-trifluoromethyl-2-amino-oxazoles in 70% and 14% yields, respectively, (Scheme 1). The synthesis of selectively fluorinated xanthene-9-carboxylic acid derivatives is described in Schemes 2 and 3.[18] Friedel–Crafts acylation of appropriately substituted *para*-substituted anisoles **(5)** with 2-fluorobenzoic acid chlorides **(6)** leads selectively to *ortho*-acylation products with concomitant deprotection of the methoxy group, to form the corresponding 2-hydroxy-benzophenones **7b–e** and **7g** in good yields. In the case of compound **7f** this reaction did not proceed as desired. It was necessary to proceed by esterification of phenol **8** to yield ester **9** which was transformed into ketone **7f** via Fries rearrangement. Cyclisation of ketones **7b–g** proceeded smoothly to yield the corresponding xanthones **10b–g**.[19] Borane-dimethylsulfide reduction in tetrahydrofuran (THF) of the xanthones to the corresponding xanthenes **11b–g** followed by deprotonation using lithium diisopropylamide (LDA) and carboxylation with carbon dioxide afforded the corresponding carboxylic acids **12b–g** in acceptable yields. For compounds **12d–g**, the bromine atom which was used to block the *para* position of anisole **5** during the Friedel–Crafts acylation or the Fries rearrangement procedure was removed via hydrogenation. One obtains the desired acids **13d–g** in nearly quantitative yields. Reaction of the aminooxadiazoles **3** and **4** with xanthene-9-carboxylic acid chloride afforded amides **14a** and **15a** in 69% and 75% yields, respectively. Reaction of the aminooxadiazoles **3** and **4** with the xanthene-9-carboxylic acid derivatives **12b–c** and **13d–g** in presence of the coupling reagent CBMIT (1,1′-carbonylbis (3-methylimidazolium) triflate) in nitromethane[20] yielded the carboxamides **14b–g** and **15b–g** in acceptable yields. The asymmetrical compounds (entries **b**, **d**, and **e**) have been tested as racemic mixtures.

Since the activation of mGluR1 leads to an increase of intracellular calcium concentration, the functional activities of the compounds at rat mGlu1 receptors were assessed using a fluorimetric intracellular Ca^{2+} ($[Ca^{2+}]I$, FLIPR) assay.[21,22] The compounds showing agonistic activity in the standard screening assay were selected for further evaluation of their enhancing activity in systems with receptor expression corresponding to physiological levels in neurons (electrophysiology). The values show the effect of the tested compounds on glutamate-activated inward K^+ currents in a CHO cell stably expressing GIRKs[24] and transiently transfected with the rat mGlu1a receptor cDNA. In this setup, glutamate (3 μM) induced a current that was potentiated by an mGluR1 enhancer.[13] The effect of the compounds was reversible and greater than that obtained with a saturating concentration of glutamate (100 μM). No effect was observed in the absence of glutamate. The current amplitudes were normalized to the control responses (3 μM glutamate) and fitted individually for each cell ($n = 3$). The EC_{50} values [nM] as well as the efficacies (effect expressed in percentage of control response) are shown in Table 1.

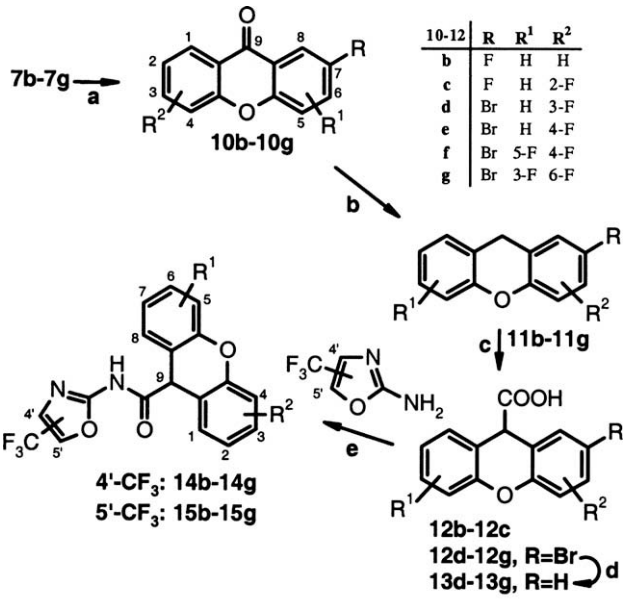
Using various functional models it was found that, in analogy to compounds **1** and **2**,[16] **14a** was devoid of any enhancing effect at rat mGlu2, mGlu4, mGlu5 and mGlu8 and human GABA-B receptors. In addition, **14a** had no activity in radioligand binding assays at major adenosine, adrenergic, GABA-A, glycine, histamine, muscarinic, nicotinic, opiate, purinergic and 5-HT receptors or at adenosine, norepinephrine, GABA and 5-HT uptake sites. Similarly to the reference compound **1**, most of the synthesized compounds



Scheme 1. Synthesis of trifluoromethylated oxazoles **3** and **4**. Reagents and conditions: (a) *t*BuOH, 8 h, reflux, 70%; (b) *t*BuOH, 5 h, rt, 14%.



Scheme 2. Synthesis of benzophenones **7b–g**. Reagents and conditions: (a) $AlCl_3$, 1,2-dichloromethane, 50 °C,50–95%; (b) Et_3N, CH_2Cl_2, rt, 92%; (c) $AlCl_3$, 150 °C, 30%.



Scheme 3. Synthesis of carboxamides **14a–g** and **15a–g**. Reagents and conditions: (a) K_2CO_3, 2-butanone, reflux 99%; (b) $BH_3.Me_2S$, THF, 2 h, 45 °C, quant.; (c) 1. $(iPr)_2NLi$, THF, −70 °C; 2. CO_2, 45–87%; (d) H_2, 5% Pd/C, Et_3N, THF, 93–100%; (e) 1. carbonyl diimidazole, MeOTf, CH_3NO_2, 10 °C, 10 min; 2. **12b–g**, 10 °C- rt, 30 min; 3. **3** or **4**, rt, 15 h, 30–80%.

Table 1
Activities (rat) of oxazoles **14a**–**g**, **15a**–**g** (n.m. = not measured)

Compd	R^1, R^2	FLIPR assay		Electrophysiology		In vitro clear. (rat microsomes)
		mGluR1 EC_{50} (nM)	Effect (%)	mGluR1 EC_{50} (nM)	Effect (%)	Cl int. (μl/min/mg prot.)
1	Ref.1	52	100	220	n.m.	42
14a	H, H	56	86	202	1128	108
rac-14b	H, 2-F	40	63	n.m.[23]	n.m.[23]	61
14c	2,7-F	44	48	90	300	9
rac-14d	H, 3-F	40	50	260	570	34
rac-14e	H, 4-F	19	84	102	1191	33
14f	4,5-F	47	51	161	707	18
14g	3,6-F	Inactive	n.m.	n.m.	n.m.	38
15°	H, H	38	104	394	3055	50
rac-15b	H, 2-F	20	57	67	1219	40
15c	2,7-F	124	205	140	629	26
rac-15d	H, 3-F	Inactive	n.m.	n.m.	n.m.	28
rac-15e	H, 4-F	21	50	131	910	32
15f	4,5-F	25	36	162	568	12
15g	3,6-F	Inactive	n.m.	n.m.	n.m.	n.m.

Table 2
Pharmacokinetics of selected compounds in Wistar rats

Compd	Dose (iv/po) (mg/kg)	Cl (ml/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	Brain/plasma ratio	C_{max} (ng/ml)
1	10/10	60	1.7	0.32	1.5	1330
14a	10/10	19.6	0.67	0.91	0.8	1330
14c	3/6	7.4	0.9	2.46	0.5	1810
rac-14e	5/10	3.3	0.99	3.7	n.m.	1440
14f	1/10	3.79	0.43	3.9	0.2	2070

show affinities in the nanomolar range and good efficacies against rat recombinant mGluR1 in the standard screening assay. The lower potencies obtained in the electrophysiological experiments may be due to the lower receptor expression levels in this model. With the exception of **14d**, substitution at position 3 of the xanthene moiety is generally not well tolerated. The compounds with the best overall activity profile were further characterized with respect to their metabolic stability in vitro as well as their PK properties in Wistar rats. Brain to plasma ratios were determined by iv-infusion experiments.[25]

Although the in vitro clearance in rat microsomes of the trifluoromethyloxazole **14a** is higher than for the methyl substituted oxadiazole **1**, the rat in vivo clearance of **14a** is much lower, leading to a three fold increase in half-life compared to **1** (Table 2). Bioavailabilities of these compounds are in the acceptable range (15–100%), with plasma levels ranging between 1300 and 2100 ng/ml after po administration. Intermediate volumes of distribution as well as variable brain penetration are observed. In comparison to the unsubstituted xanthene derivative **14a**, additional fluorination of the xanthene moiety leads in most cases to further reduction of the in vitro as well as the in vivo clearance values. The half-life of these compounds is also markedly increased. The volume of distribution is in the intermediate range, while brain penetration seems to decrease for the fluoroxanthene derivatives.

Based on the potent and selective positive allosteric modulator **1**, two series of trifluoromethyloxazole derivatives with improved metabolic stability and longer half-life have been discovered. Several derivatives with good activities at rat mGlu1 receptors have been prepared and the compounds with the most promising in vitro profiles were selected for further PK evaluation.

Recently, acute oral administration of compound **14a** consistently improved motor performance in a rat model of experimental autoimmune encephalomyelitis (EAE).[26] With respect to their PK profile and brain penetrating properties, several of these compounds could serve as suitable tools to further study the role of positive allosteric modulation of mGlu1 receptors in vivo.

Acknowledgments

The skillful synthetic work of Nadia Bitter,[§] Martin Kuratli,[§] and Patrick Scheifele[¶] is gratefully acknowledged.

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25. The iv and po and infusion experiments are performed using two animals per group. For the iv and po experiments, blood sampling and iv bolus of compounds were realized via implanted jugular vein catheters. Blood samples were taken at 0.3, 0.5, 1, 2, 4, 8, and 24 h after administration. For the infusion experiments (1 mg/kg/h, via catheter), blood samples were taken sublingual at 1, 3 and 5 h post administration. Brain tissue samples were obtained by decapitation of the animals following isofluran anesthesia. Sample analysis of blood samples or brain tissue homogenate was performed using standard LC–MS methodology.
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