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Synthesis and characterization of 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives: Part 3. New potent non-competitive metabotropic glutamate receptor 2/3 antagonists

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Abstract—A series of 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives was evaluated as non-competitive mGluR2/3 antagonists. Replacement of the (2-aryl)-ethynyl-moiety in 8-position with smaller less lipophilic substituents produced compounds inhibiting the binding of [3H]-LY354740 to rat mGluR2 with low nanomolar affinity and consistent functional effect at both mGluR2 and mGluR3. These compounds were able to reverse LY354740-mediated inhibition of field excitatory postsynaptic potentials in the rat dentate gyrus and in vivo activity could be demonstrated by reversal of the LY354740-induced hypoactivity in mice after oral administration.

The family of metabotropic glutamate receptors consists of eight subtypes, classified into three groups according to their sequence homology, pharmacology, and second messenger coupling.[1] Group I receptors (mGluR 1 and 5) are positively coupled to phospholipase C, whereas group II (mGluR2 and 3) and group III (mGluR4, 6, 7 and 8) receptors are negatively coupled to the activity of adenyl cyclase. Ligands for the different mGluR's are under development for the treatment of different CNS disorders, such as Parkinson's disease, anxiety and schizophrenia.[2] It was recently shown that while mGluR2/3 agonists exhibit anxiolytic and antipsychotic properties, mGluR2/3 antagonists may be useful as antidepressants, cognitive enhancers and to inhibit the growth of malignant gliomas.[3,4]

We recently reported the discovery of the random screening hit (8-methyl-4-phenyl-1,3-dihydro-benzo[*b*][1,4]diazepin-2-one), its characterization as a non-competitive antagonist at both mGluR2 and mGluR3 and the lead optimization program to obtain compounds like **3**, with low nanomolar affinity assessed by displacement of [^{3}H]-LY354740 binding to rat mGluR2 and ability to reverse LY354740-mediated inhibition of field excitatory postsynaptic potentials in the rat dentate gyrus in vitro (Fig. 1).[5] The present study describes the optimization of orally active, brain penetrating, in vivo active compounds, and their pharmacological profiling.

The regioselective synthesis of unsymmetrically 7,8-substituted 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones has already been described.[6,7] Simple condensation of mono Boc protected 1,2-phenylenediamines **4** and *tert*-butyl β-ketoesters **5** in refluxing toluene led to the corre-

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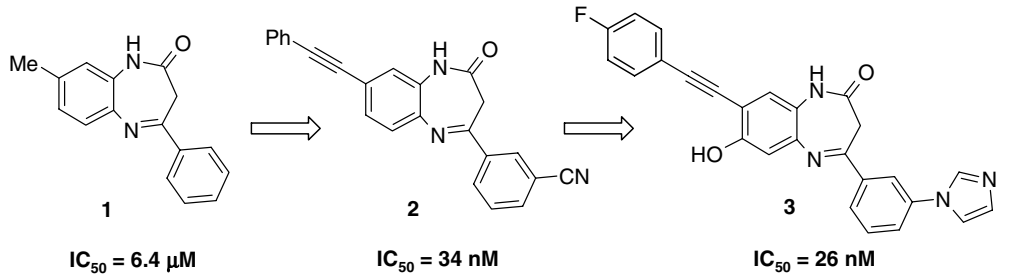
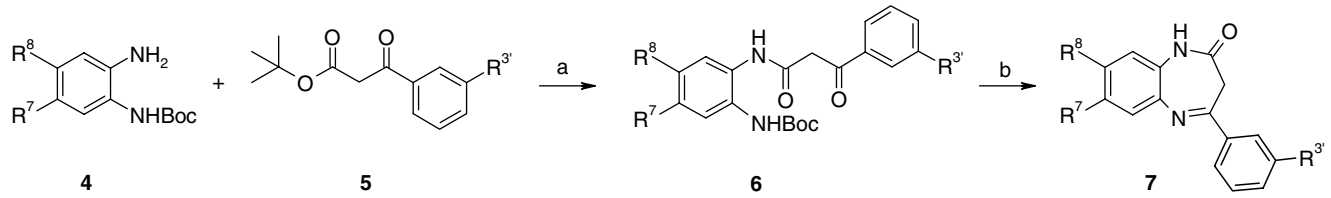


Figure 1. Development of the random screening hit **1** into compounds, like, for example, **2** and **3**, with low nanomolar affinity in inhibition of [^{3}H]-LY354740 binding to rat mGluR2.[5]



Scheme 1. Regioselective synthesis of unsymmetrically 7,8-substituted 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones **7**. Reagents and conditions: (a) toluene, reflux; (b) TFA [optional anisole], DCM, rt.

Table 1. Structure–activity-relationship of the new 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones **7** reported in this study

Compound	$R^{3'}$	R^8	R^7	*c*log *P*	Rat mGluR2 [^{3}H]-LY354740 binding[a], IC_{50} (μM)	Rat mGluR2 (1*S*,3*R*)-ACPD inhibition of Forskolin stimulated cAMP[b], IC_{50} (μM)
3	1-Imidazolyl	4-F—C_6H_4—C≡C—	HO	3.85	0.026	0.011
7a	1-Imidazolyl	Ph	H	4.43	0.039	0.047
7b	1-Imidazolyl	4-F—C_6H_4—	H	4.62	0.017	0.024
7c	1-Imidazolyl	2-F—C_6H_4—	H	4.62	0.012	0.027
7d	1-Imidazolyl	3-F—C_6H_4—	H	4.62	0.025	nt
7e	1-Imidazolyl	2,5-di-F—C_6H_3—	H	4.78	0.007	0.017
7f	1-Imidazolyl	2-F—C_6H_4—	HO	3.48	0.022	nt
7g	1-Imidazolyl	*cyclo*-Propyl	H	3.48	0.210	nt
7h	1-Imidazolyl	Br	H	3.66	0.072	nt
7i	1-Imidazolyl	F_3C—	H	3.87	0.043	nt
7j	1-Imidazolyl	F_3C—	Me_2N	4.13	0.009	0.052
7k	1-Imidazolyl	F_3C—	*iso*-ButylNH	5.04	0.019	0.073
7l	1-Imidazolyl	F_3C—	Cl	4.48	0.017	0.015
7m	1-Imidazolyl	F_3C—	Me	4.37	0.012	0.019
7n	1-Imidazolyl	F_3C—	Et	4.90	0.004	0.010
7o	1,2,3-Triazolyl	4-F—C_6H_4—	H	4.61	0.031	0.036
7p	1,2,3-Triazolyl	2-F—C_6H_4—	H	4.61	0.016	0.037
7q	1,2,3-Triazolyl	2,5-di-F—C_6H_3—	H	4.77	0.017	0.018
7r	1,2,3-Triazolyl	*iso*-Propyl	H	3.96	0.466	nt
7s	1,2,3-Triazolyl	Br	H	3.65	0.130	nt
7t	1,2,3-Triazolyl	Cl	H	3.50	0.060	nt
7u	1,2,3-Triazolyl	F_3C—	H	3.86	0.092	nt
7v	1,2,3-Triazolyl	F_2CH—	H	2.73	0.608	nt
7w	1,2,3-Triazolyl	F_3CCH_2O—	H	3.89	0.070	nt
7x	1,2,3-Triazolyl	F_3C—	Me_2N	4.12	0.038	0.184
7y	1,2,3-Triazolyl	F_3C—	*iso*-ButylNH	5.03	0.042	0.268
7z	1,2,3-Triazolyl	F_3C—	*iso*-ButylN(Me)	5.58	0.011	0.014
7aa	1,2,3-Triazolyl	F_3C—	Cl	4.47	0.027	0.116
7ab	1,2,3-Triazolyl	F_3C—	Me	4.36	0.009	0.039
7ac	1,2,3-Triazolyl	F_3C—	MeO	3.91	0.029	0.074
7ad	1,2,3-Triazolyl	F_3C—	EtO	4.44	0.007	0.034

$R^{3'}$, R^7 and R^8 refer to the positions of R's in Scheme 1.
nt, not tested.
[a] Values are means of at least 2 independent experiments.
[b] Values are means of 3 independent experiments.

sponding β-ketoamides **6**. We found that the *tert*-butyl β-ketoester was superior to the corresponding ethyl ester in this condensation reaction, probably due to its ability of reacting through a ketene mechanism. Deprotection and concomitant cyclization were achieved by treatment with TFA yielding the unsymmetrically 7,8-substituted 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones **7** (Scheme 1).

We reported that 5-membered heterocycles were suitable replacements for the cyano group, especially the employment of 1-imidazoles and 1,2,3-triazoles lead to very potent mGluR2/3 antagonists.[5b] Although these modifications improved the physico-chemical properties of the compounds they remained quite lipophilic (**2**: *c*log*P* 4.44; calculated $\log(c_{octanol}/c_{water})$; **3**: *c*log*P* 3.85). Therefore we sought for further alteration of the substituents enabling the in vivo evaluation of compounds from this class in an mGlu2 receptor-mediated behavioral test. Our main focus was the search for a suitable—preferably smaller—replacement for the (2-aryl)-ethynyl-moiety in 8-position.

Omitting the acetylene linker and using fluorinated phenyl groups produced compounds **7a**–**f**, which proved to be equipotent to **3** (Table 1). In the cases of simple halides, Br (**7h** and **7s**) appeared to be less effective than Cl (**7t**). Employing alkyl groups like cyclopropyl (**7g**) and isopropyl (**7r**) resulted in ~10- to 20-fold loss of affinity. Interestingly, introducing fluorinated alkyl groups a remarkable ~7-fold advantage of trifluoromethyl over difluoromethyl was observed (**7u** versus **7v**). The 2,2,2-trifluoroethoxy group was also tolerated (**7w**). In order to increase the potency of the compounds while maintaining a small molecule size we kept the 8-(trifluoromethyl) group and attached additional small alkyl, alkoxy, and alkylamino groups as well as Cl in the adjacent 7-position. Overall, a significant enhancement in activity was observed (~2- to 9-fold for **7j**–**n** versus **7i** and ~2- to 13-fold for **7x**–**ad** versus **7u**).

Table 2. IC_{50} values for GIRK current inhibition by selected compounds in CHO cells expressing mGluR2 and mGluR3 receptors

Compound	Rat mGluR2 GIRK inhibition [a], IC_{50} (μM)	Rat mGluR3 GIRK inhibition[a], IC_{50} (μM)
3	0.024	nt
7b	0.299	0.017
7c	0.011	0.033
7f	0.035	0.122
7p	0.022	0.042

[a] Values are means of 3–5 independent experiments, (nt = not tested).

In conclusion the (2-aryl)-ethynyl-moiety in the 8-position could most effectively be replaced by a 2-fluorophenyl group or the combination of a trifluoromethyl group in 8-position together with a small alkyl or alkoxy group in 7-position.

The closest derivative to **3** and most polar compound **7f** (*c*log*P* 3.48) was not suitable for in vivo evaluation, since it revealed high clearance in rat microsomes (~100 mL/min/kg, MAB ~20%), whereas **7c** and **7p** are metabolically stable compounds (MAB 93% and 100%). Therefore only the reduction in size could be achieved but not in lipophilicity (**7c**: *c*log*P* 4.62; **7p**: *c*log*P* 4.61; **7n**: *c*log*P* 4.90; **7ab**: *c*log*P* 4.36; **7ad**: *c*log*P* 4.44).

The pharmacological properties of these compounds were consistent with a mechanism of non-competitive antagonism at both mGluR2 and mGluR3 as previously described demonstrated by partial inhibition of the binding of the selective agonist [^{3}H]-LY354740 to rat mGluR2, full blockade of the effect of LY354740, (1*S*,3*R*)-ACPD and L-glutamate in both GTPγS and cAMP assays and inhibition of the glutamate-induced GIRK current. Table 1 summarizes the IC_{50} values calculated for each compound in affinity studies (partial displacement of 10 nM [^{3}H]-LY354740) and concentration dependent blockade of (1*S*,3*R*)-ACPD (10 μM-EC_{90}) induced inhibition on intracellular cAMP levels (Forskolin 10 μM, n:3 for each value) in the experimental conditions described earlier.[5] In this chemical series the effect of all the tested compounds does not seem to vary depending on the mGluR2 agonist under study.

For representative compounds we have determined the IC_{50}s for the GIRK inhibition at both rat mGluR2 and mGluR3 (Table 2). The concentration response curves of the antagonistic properties of **7c** (**a**) and **7p** (**b**) are shown in Figure 2. All compounds were potent

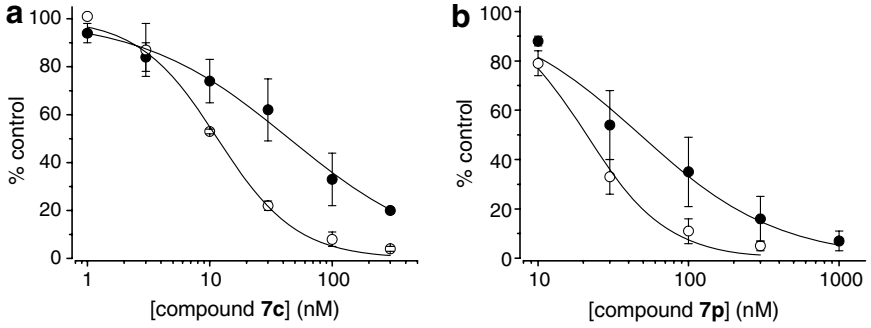


Figure 2. Concentration-response curves for the inhibition of GIRK currents by **7c** and **7p** in CHO cells stably expressing GIRKs and transiently transfected with rat mGluR2 (○) and rat mGluR3 (●) and stimulated with L-glutamate (10 μM). (**a**) **7c**: mGluR2 IC_{50} = 11 nM, mGluR3 IC_{50} = 33 nM. (**b**) **7p**: mGluR2 IC_{50} = 22 nM, mGluR3 IC_{50} = 42 nM.

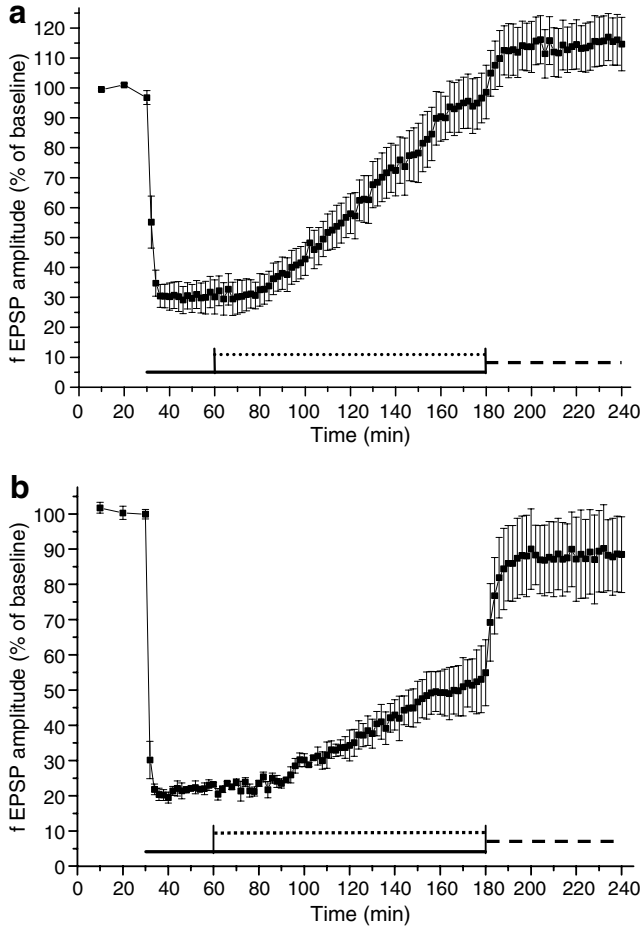


Figure 3. Reversal of mGluR2-mediated inhibition of synaptic transmission (fEPSP) evoked by stimulation of the medial perforant path input to the dentate gyrus mid-moleculare by **7c** (0.1 μM) and by **7p** (0.1 μM) in presence of the mGluR2/3 agonist LY354740 (1 μM). (a) ...., **7c** (0.1 μM), —— LY354740 (1 μM), – – wash-out period; mean ± SE, $n = 6$. (b) .... **7p** (0.1 μM), —— LY354740 (1 μM), – – wash-out period; mean ± SE, $n = 3$.

group II mGluR antagonists, with a slight preference for the mGluR2.

We also assessed in vitro the selectivity of **7p** on group I and group III mGluRs, respectively: rat mGluR1a, mGluR5a (using a Ca^{2+} mobilization functional assay) and rat mGluR8a (displacement studies with [^{3}H]-L-AP4) (with **7p** tested at 30 μM final concentration). In addition **7p** was devoid of any affinity at ionotropic glutamate receptors and $GABA_A$ receptors (data not shown).

Using compounds **7c** and **7p** we could also demonstrate antagonism at native group II metabotropic glutamate receptors by reversal of LY354740-mediated inhibition of fEPSPs in the dentate gyrus in vitro (Fig. 3).[8] Complete reversal was observed in the case of **7c** while only partial reversal could be measured in the case of **7p**. This is possibly related to the poor solubility of **7p** in the experimental conditions used for this study and it does not correspond to the observations in other functional assays where it always exhibited complete blockade of the effects of the mGluR2/3 agonists.

To evaluate the drug-drug-interaction (DDI) potential we measured the binding to cytochrome P450 3A4 isoenzyme. Not surprisingly **7c** and **7f**—bearing the well-known 1-imidazolyl pharmacophore for CYP450 3A4—were strong inhibitors with an $IC_{50} \sim 1$ nM, whereas **7p** showed only weak activity ($IC_{50} = 4.3$ μM).

For compounds **7c** and **7p** a single dose pharmacokinetic (SDPK) assessment in rats at 10 mg/kg p.o. was performed and plasma and brain concentration measurements after 1.5 h revealed for **7c** 2656 ng/mL and 1344 ng/mL (brain/plasma 0.5) and for **7p** 1423 and 1060 ng/mL, respectively (brain/plasma 0.9, CSF/plasma 0.5%) (4 animals/group).[9]

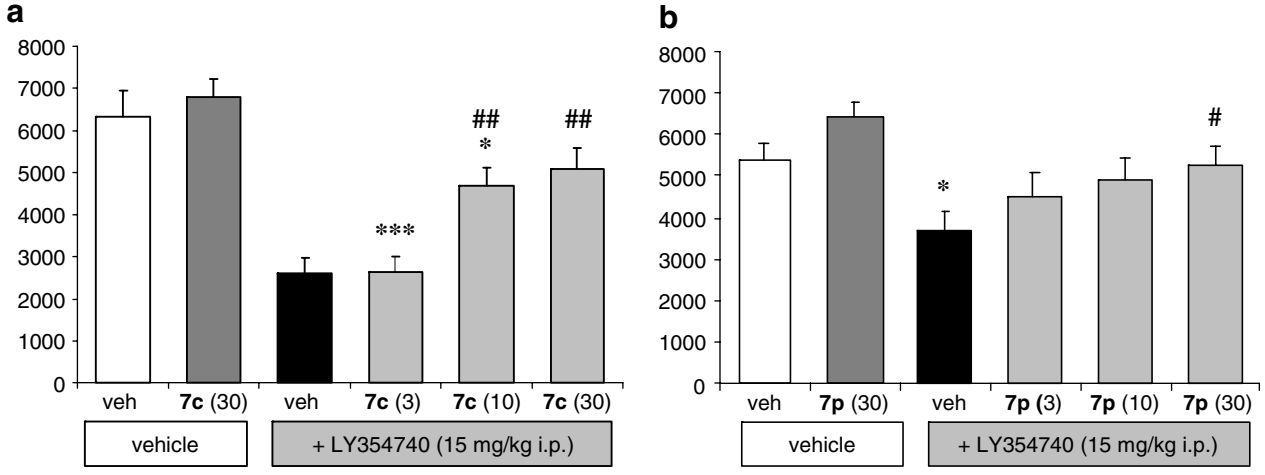


Figure 4. Spontaneous locomotor activity in C57BL/6J mice (total horizontal activity counts in 30 min): reversal of LY354740-induced hypoactivity by compounds **7c** and **7p**. Dose–response measurements of **7c** (a) and **7p** (b) suspension prepared in 0.3% Tween 80 v/v saline (at 3, 10 and 30 mg/kg p.o.) revealed for **7c** (a) an $ED_{50} = 12.5$ mg/kg p.o. ($n = 8$ mice/group; ANOVA $p < 0.0001$; $^{*}p < 0.05$, $^{***}p < 0.001$ versus veh. + veh.; $^{\#\#}p < 0.01$ versus veh. + LY354740) and for **7p** (b) an $ED_{50} = 3.3$ mg/kg p.o. ($n = 8$ mice/group; ANOVA $p < 0.01$; $^{*}p < 0.05$ versus veh. + veh.; $^{\#}p < 0.05$ versus veh. + LY354740).

Consistent with the in vivo exposure and the brain penetration data are the in vivo antagonistic properties of both **7c** and **7p** when administered per os by gavage in mice (Fig. 4).[9] The mGluR2/3 agonist LY354740 produces a dose-dependent decrease of the horizontal activity after intraperitoneal administration (1–30 mg/kg, 30 min prior to testing) in mice. The experimental conditions of this test have been validated for the contribution of mGluR2 using mGluR2 null mutant mice.[3,10] The p.o. administration of either **7c** or **7p** 1 h prior to the mGluR2/3 agonist (15 mg/kg i.p.) was able to block completely the hypoactivity caused by the administration of LY354740. It should be noted that neither **7c** nor **7p** caused a significant increase in locomotor activity when administered alone.

In summary, by replacement of the (2-aryl)-ethynyl-moiety in 8-position with smaller less lipophilic substituents we were able to develop a series of in vivo active 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives. These compounds are selective, non-competitive antagonists at recombinant (inhibiting the binding of [^{3}H]-LY354740 to rat mGluR2 with low nanomolar affinity) and native (reversal of LY354740-mediated inhibition of fEPSPs in the dentate gyrus) group II metabotropic glutamate receptors, they are orally active and brain penetrating and also exhibit in vivo activity by reversal of the LY354740-induced hypolocomotion in mice.

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