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Synthesis and characterization of 8-ethynyl-1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives: Part 2. 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 a cyano group by a five-membered heterocycle produced compounds inhibiting the binding of [^{3}H]-LY354740 to rat mGluR2 with low nanomolar affinity and consistent functional effect at both mGluR2 and mGluR3. Further modification to improve the physicochemical properties led eventually to compounds with the ability to reverse LY354740-mediated inhibition of field excitatory postsynaptic potentials in the rat dentate gyrus.

The glutamate receptors are divided into two major groups: ionotropic (iGluR: ligand-gated ion channels) and metabotropic (mGluR: G-protein coupled receptors) glutamate receptors.[1] From mGluRs eight subtypes were identified and grouped into three groups according to their sequence homology, agonist pharmacology, and second messenger coupling. Group I receptors (mGluR1 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. Targeting mGluRs has been recognized as a valuable approach for the treatment of CNS disorders, such as depression, anxiety, and schizophrenia.[2] It was shown that mGluR2/3 agonists exhibit antipsychotic properties and mGluR2/3 antagonists may be useful as antidepressants and cognitive enhancers as demonstrated in different animal models.[3,4]

Recently we reported about the development of the random screening hit **1**—a compound from the chemical class of 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones—into compounds with low nanomolar affinity (Fig. 1) assayed by partial inhibition of [^{3}H]-LY354740 binding to rat mGluR2[5] (Fig. 2a).[6] The in vitro binding characteristics of this selective mGluR2/3 radioligand with agonist properties were described by Schweitzer et al.[5a] The specific binding of [^{3}H]-LY354740 was dependent on the addition of divalent cations, it showed the presence of a single binding site, and was partially inhibited by GTPγS. Saturation isothermal curves on [^{3}H]-LY354740 binding to rat mGluR2 and functional studies carried out with **1** and derivatives on (1*S*,3*R*)-ACPD

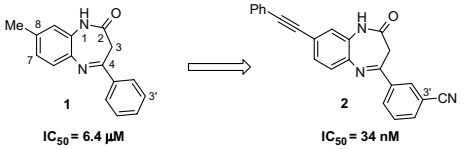

Figure 1. Development of the random screening hit **1** into compounds, like, for example, **2**, with low nanomolar affinity in inhibition of [^{3}H]-LY354740 binding to rat mGluR2.[6]

Keywords: Metabotropic glutamate receptors; mGluR2; LY354740; Non-competitive antagonists; 1,3-Dihydro-benzo[*b*][1,4]diazepin-2-ones; Excitatory postsynaptic potentials.
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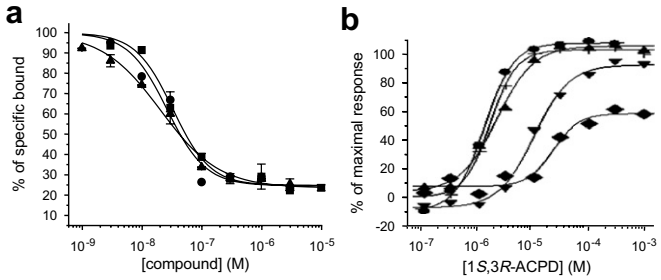


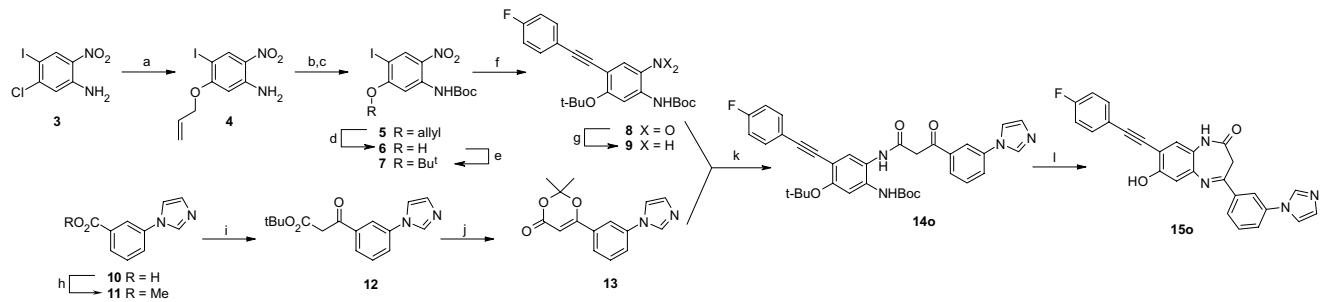
Figure 2. (a) **2** (■), **15o** (●), and **15q** (▲) inhibition of specific [^{3}H]-LY354740 binding to the recombinant rat mGluR2 permanently expressed in CHO cells. Inhibition curves are normalized as % of the maximum specific bound. All compounds partially inhibit the radioligand binding to mGluR2 with a residual of about 25% of specific bound ([^{3}H]-LY354740, 10 nM). The binding of this radioligand to mGluR2 is GTPγS sensitive and it is blocked by pertussin toxin. (b) (1*S*,3*R*)-ACPD-induced inhibition of cAMP production: the non-competitive mGluR2 antagonist **15b** reduces maximal response and causes rightward shift of dose–response curve of (1*S*,3*R*)-ACPD [+ (1*S*,3*R*)-ACPD (n = 5); (●) **15b** 1 nM (n = 2); (▲) **15b** 3 nM (n = 2); (▼) **15b** 10 nM (n = 6); (♦) **15b** 30 nM (n = 3)] (also cf. Ref. 7).

inhibition of forskolin stimulated cAMP production (Fig. 2b), on the ^{35}S-GTPγS binding induced by (1*S*,3*R*)-ACPD, and on the glutamate induced GIRK currents, respectively, showed the presence of a non-competitive antagonist activity. The conclusion that these mGluR2 antagonists do not interact with the orthosteric site of the receptor is also in agreement with the observation that they do not displace [^{3}H]-LY341495, a competitive antagonist to mGluR2.[7] The capacity of **1** and derivatives to partially displace [^{3}H]-LY354740 is most likely due to an effect on coupling efficiency of mGluR2. This property, in the described experimental conditions, was used in association to functional studies to optimize the structure–activity relationship for this class of compounds.

The extension of the methyl group in 8-position to a phenylacetylene residue as well as the attachment of a cyano group in meta-position on the phenyl in 4-position resulted in the very potent compound **2**. Nevertheless these modifications led to very lipophilic molecules (**2**: c$\log P$ 4.44; calculated $\log(c_{\text{octanol}}/c_{\text{water}})$) and therefore we sought for further alteration of the substituents enabling the evaluation of compounds from this class in different models. Our main focus was the search for a suitable nitrile replacement as well as the exploration of the 7-position.

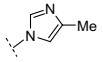
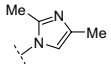
The regioselective synthesis of 1,3-dihydro-benzo[*b*]-[1,4]diazepin-2-ones has already been described[6] and is here exemplified by the preparation of compound **15o** (Scheme 1). The *o*-phenylenediamine part utilized 5-chloro-4-iodo-2-nitroaniline **3**[8] and the β-ketoester part used 3-imidazol-1-yl-benzoic acid **10**[9] as starting materials. Nucleophilic displacement of the Cl in **3** with allyl alcohol gave **4** wherein—after introduction of the Boc-group—the allyl group was switched to *tert*-butyl by Pd-catalyzed deprotection with morpholine and introduction of the *t*-Bu group by the method of Widmer[10] to give intermediate **7**. Sonogashira-type coupling with 4-fluorophenylacetylene was followed by reduction of the nitro group and yielded the mono-Boc protected *o*-phenylenediamine building block **9**. For the β-ketoester part the known 3-imidazol-1-yl-benzoic acid **10**[9] was converted via its methyl ester **11** into the *t*-Bu β-ketoester **12** following a protocol of Ohta.[11] To increase the reactivity of the *t*-Bu β-ketoester **12** for the condensation with the *o*-phenylenediamine building block **9**, it was transformed into the 2,2-dimethyl-[1,3]dioxin-4-one **13** by treatment with TFAA/TFA in acetone as described by Winkler.[12] Both pieces **9** and **13** were then condensed in refluxing toluene to the corresponding β-ketoamide **14o**, which was doubly deprotected and concomitantly cyclized by treatment with TFA yielding the unsymmetrically 7,8-substituted 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one **15o**.

We found that five-membered heterocycles were suitable replacements for the cyano group, especially the employment of imidazoles (**15a**–**c**) and 1,2,3-triazoles



Scheme 1. Synthesis of compound **15o**. Reagents and conditions: (a) allyl alcohol, KOH, DMSO, rt, 95%; (b) Boc_2O, DMAP, THF, rt, 99%; (c) TFA, DCM, 0 °C, 75%; (d) morpholine, $Pd(PPh_3)_4$, THF, rt, 98%; (e) $Me_2NCH(OBu^t)_2$, toluene, 80 °C, 73%; (f) 4-F–$C_6H_4C{\equiv}CH$, $PdCl_2(PPh_3)_2$, PPh_3, CuI, Et_3N, THF, 60 °C, 38%; (g) $SnCl_2{\cdot}2H_2O$, pyridine, DMF, rt, 68%; (h) concd H_2SO_4, MeOH, reflux, 98%; (i) *t*-BuOAc, LDA, $LiOBu^t$, THF, −78 °C, 95%; (j) TFAA, TFA, acetone, −78 to 23 °C, 65%; (k) toluene, reflux, 68%; (l) TFA, DCM, rt, 65%.

Table 1. Activities of the 8-ethynyl-1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones **15**

Compound	$R^{3'}$	R^8	R^7	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)
2	CN	Ph–C≡C–	H	0.034	0.017
15a		Ph–C≡C–	H	0.013	0.010
15b		4-F–C_6H_4–C≡C–	H	0.009	0.009
15c		2-F–C_6H_4–C≡C–	H	0.020	0.016
15d		4-F–C_6H_4–C≡C–	H	0.098	nt
15e		Ph–C≡C–	H	0.170	nt
15f		Ph–C≡C–	H	0.730	nt
15g		4-F–C_6H_4–C≡C–	H	0.098	nt
15h		4-F–C_6H_4–C≡C–	H	0.006	0.012
15i		4-F–C_6H_4–C≡C–	H	0.072	nt
15j		Ph–C≡C–	OCH_2CH_2OMe	0.023	0.015
15k		Ph–C≡C–	OCH_2CH_2OH	0.064	nt
15l		4-F–C_6H_4–C≡C–	OCH_2CH_2OH	0.046	nt
15m		Ph–C≡C–	OCH_2CN	0.018	0.013
15n		4-F–C_6H_4–C≡C–	$N(Me)CH_2CH_2OH$	0.140	nt
15o		4-F–C_6H_4–C≡C–	OH	0.026	0.011
15p		Ph–C≡C–	NMe_2	0.036	nt
15q		4-F–C_6H_4–C≡C–	OH	0.020	0.017
15r		4-F–C_6H_4–C≡C–	OH	0.378	nt

$R^{3'}$, R^7, and R^8 refer to the positions of Rs in Figure 1.

nt, not tested.

[a] Values are means of at least two independent experiments.

[b] Values are means of three independent experiments.

(**15h**,**p**,**q**) led to very potent mGluR2/3 antagonists (Table 1). Interestingly the *N*-linked 1-imidazoles (e.g., **15a** or **15e**) were far more active than its *C*-linked 2-isomer (**15f**). With regard to the methyl substitution on the 1-imidazoles it appeared only to some extent to be tolerated resulting a ca. 10-fold drop in affinity (**15a** vs **15e**; **15b** vs **15d**). The order of activity depending on the heterocycles incorporated was determined as follows: 1-imidazole ~ 1,2,3-triazole > 1-tetrazole ~ 1,2,4-triazole. Overall, the unsubstituted 1-imidazole and 1,2,3-triazole proved to be most favorable (e.g., **15a**, **15b**, **15c**, and **15h** all exhibit $IC_{50} \leqslant 20$ nM).

The structural class of the 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones offered in addition to the (2-aryl)-ethynyl-moiety in 8-position and the meta-attached heterocycle on the phenyl group in 4-position a third exit vector—the 7-position—where the introduction of further substituents was allowed as previously described.[6] The most successful ones in altering physicochemical properties to reduce the overall lipophilicity without loss of mGluR2 affinity were small oxygen- or nitrogen-linked C2 units bearing polar head groups like OH (**15k**,**l**,**n**), OMe (**15j**) or CN (**15m**). It was found that a 2-hydroxyethyl residue is more active when *O*-linked (**15l**) rather than *N*-linked (**15n**) to the core structure, but in general even the relatively small 2-hydroxyethyl group with the free OH (**15k**) resulted in loss of activity (ca. 3-fold) in comparison with the less polar OMe (**15j**) and CN (**15m**). Replacing the 2-hydroxyethyl group in **15n** by a simple methyl group produced the rather non-polar **15p** (bearing the NMe_2 group) but was accompanied with a regain in affinity. In analogy cutting off the 2-hydroxyethyl group in **15l** yielded the free phenol **15o**—a very potent imidazole compound. The corresponding 1,2,3-triazole compound **15q** proved to be equipotent.

Further increase in polarity by introduction of hydroxymethyl group in the 5-position on the 1,2,3-triazole in **15q** (clog*P* 3.80) resulted in **15r** (clog*P* 2.88) and ca. 20-fold loss of affinity.

In conclusion, only limited allowance for the introduction of more polar groups was found—the 1-imidazole or 1,2,3-triazole in combination with free phenol in 7-position appeared to be the maximum in order to achieve highly potent compounds.

As described earlier **15o** and **15q** partially inhibit [^{3}H]-LY354740 binding to mGluR2 (Fig. 2a).[13] To demonstrate non-competitive nature of the antagonism at mGluR2 by the newly generated compounds, we show hereby the effect of increasing concentrations of **15b** on the (1*S*,3*R*)-ACPD-induced inhibition of forskolin stimulated cAMP production (Fig. 2b).[7,13]

The antagonist properties of **15o** and **15q** were also evaluated electrophysiologically in CHO cells stably expressing the human Kir3.1 and Kir3.2c GIRK subunits and transiently transfected with either rat mGluR2 or rat mGluR3 (Fig. 3).[13] In these cells, glutamate concentration-dependently induced a reversible inward K^+ current, which was inhibited by both **15o** and **15q**.

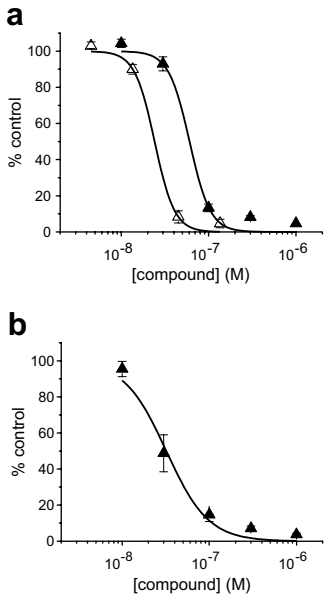


Figure 3. Concentration–response curves for the inhibition of GIRK currents by **15o** (△) and **15q** (▲) in CHO cells stably expressing GIRKs and transiently transfected with: (a) rat mGluR2 (glutamate 10 μM) and (b) rat mGluR3 (glutamate 1 μM).

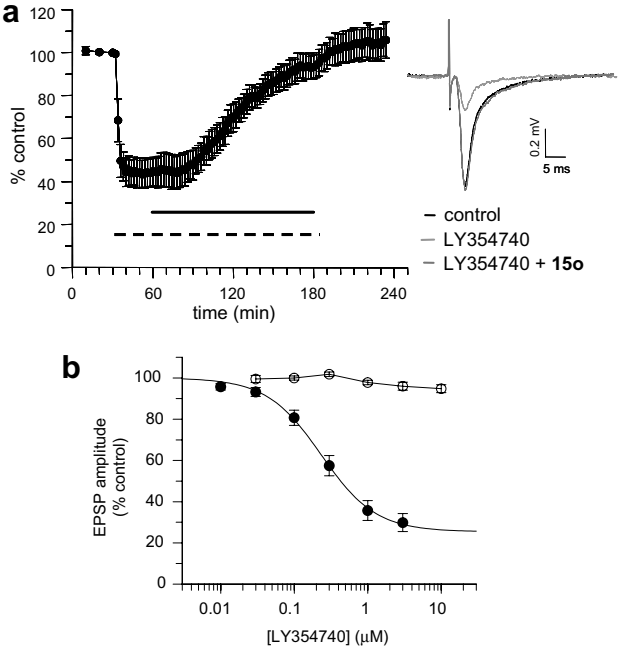


Figure 4. Antagonist activity of compound **15o** on the LY354740-mediated inhibition of synaptic transmission in the rat hippocampus: (a) Compound **15o** reverses the LY354740-mediated inhibition of field excitatory postsynaptic potentials (fEPSPs) evoked by stimulation of the medial perforant path and recorded in the dentate gyrus (— compound **15o** (1 μM), ---- LY354740 (1 μM); mean ± SE, $n = 4$). The inset shows representative fEPSPs under control conditions, in the presence of 1 μM LY354740, and in the presence of 1 μM LY354740 and 1 μM **15o** at 180 min. (b) The concentration-dependent inhibition of fEPSPs by LY354740 ($IC_{50} = 0.23$ μM, slope = 1.2) was completely blocked following 3-h pre-incubation of hippocampal slices with **15o** [• control ($n = 7$); ○ +30 nM **15o** ($n = 4$)].

We assessed the selectivity of **15o** and **15q** and these compounds neither activated nor inhibited glutamate-stimulated rat mGluR1a and mGluR5a (using a Ca^{2+} mobilization functional assay, when tested at 30 μM final concentration). Compound **15o** was also inactive in the binding of [^{3}H]-L-AP4 to rat mGluR8a, while **15q** showed an IC_{50} of 5 μM. In addition both compounds were devoid of any affinity at NMDA and $GABA_A$ receptors (data not shown).

Using compound **15o** we could also demonstrate antagonism at native group II metabotropic glutamate receptors by reversal of LY354740-mediated inhibition of fEPSPs in the dentate gyrus (Fig. 4).[14]

In summary, by replacement of a nitrile with a five-membered heterocycle we were able to develop a series of 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives which are selective and potent non-competitive antagonists at recombinant group II metabotropic glutamate receptors. Further optimization of physicochemical properties is required before approaching in vivo studies.

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