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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6811–6815
Bioorganic & Medicinal Chemistry Letters

Synthesis and characterization of 8-ethynyl-1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives: New potent non-competitive metabotropic glutamate receptor 2/3 antagonists. Part 1

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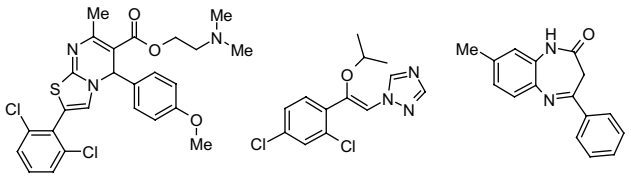
Received 30 August 2007; revised 10 October 2007; accepted 10 October 2007
Available online 17 October 2007

Abstract—A series of 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives was evaluated as non-competitive mGluR2/3 antagonists. Attachment of an 8-(2-aryl)-ethynyl-moiety 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.

L-Glutamic acid (L-Glu) is an excitatory neurotransmitter in the mammalian central nervous system (CNS) and activates several classes of glutamate receptors. These are divided into two major groups termed ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors.[1] The family of mGluRs consists of at least eight subtypes, grouped according to their sequence homology, pharmacology, and intracellular 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 adenyl cyclase. In recent years the mGluRs were recognized as valuable therapeutic targets.[2] In particular mGluR2/3 agonists show antipsychotic properties and mGluR2/3 antagonists may be useful as antidepressants and cognitive enhancers as demonstrated in different animal models.[3,4]

Our program focused initially on non-amino acid mGluR2/3 ligands, including non-competitive ones, with the aim to increase receptor selectivity and improve pharmacokinetic properties. Random screening was performed using a forskolin-stimulated cAMP production in mGluR2-transfected CHO cells. Compounds with antagonistic properties were further characterized using the inhibition of (1*S*,3*R*)-ACPD (10 μM)-stimulated [^{35}S]GTPγS binding using membranes from rat mGluR2-transfected CHO cells.[5] This study revealed two promising structural classes (**1** and **2**, Fig. 1), which have been evaluated until moderately potent and selective compounds were identified in both classes.[6,7] Interestingly when compounds of type **2** were tested in



1 2 3

Figure 1. Random screening hits. **1**, 1-[2-(2,6-Dichloro-phenyl)-7-methyl-5*H*-thiazolo[3,2-*a*]pyrimidin-6-yl]-ethanone IC_{50} 30 μM; **2**, *E*-1-[2-(2,4-Dichlorophenyl)-2-2isobutoxyvinyl]-1*H*-[1,2,4]-triazole IC_{50} 6 μM [^{35}S]GTPγS binding assay.

Keywords: Metabotrophic glutamate receptors; mGluR2; LY354740; 1,3-Dihydro-benzo[*b*][1,4]diazepin-2-one; mGluR2/3 antagonists; Non-competitive antagonists.
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doi:10.1016/j.bmcl.2007.10.026

displacement studies they were able to inhibit concentration dependently both [^{3}H]-DCG IV and [^{3}H]-LY354740 binding to rat mGluR2[5,7,8] and appeared to be selective versus AMPA and NMDA ionotropic receptors. Therefore, a second random screening was carried out using the [^{3}H]-LY354740 binding assay which discovered among other hits the compound **3** (Fig. 1). Herein we report on the characterization of compounds obtained from the screening hit **3**—a 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one—with an inhibition of [^{3}H]-LY354740 binding to rat mGlu2 receptors with IC_{50} values ranging from 6.4 μM to low nanomolar affinity. Selectivity and specificity data are also provided.

We were attracted to compounds of type **3** due to the high levels of chemical tractability through their assembly via condensation reactions of 1,2-phenylenediamines and β-ketoesters (Scheme 1). The central building block for the variation of the phenylenediamine part was prepared by iodination[9] of 5-chloro-2-nitroaniline **4** furnishing 5-chloro-4-iodo-2-nitroaniline **5**. Mono Boc protection was most conveniently achieved by a two-step procedure: first bis-protection with Boc_2O/cat. DMAP and second selective removal of one Boc group by treatment with TFA. The chlorine was replaced by *O*- (in compound **5**) and *N*-nucleophiles (in compound **6**) in an S_NAr fashion, whereas the iodine in compounds of type **8** was amenable for palladium-catalyzed cross couplings, for example, Sonogashira-type reactions with terminal alkynes to give compounds of type **9**. Finally very mild reduction of the nitro group was performed with $SnCl_2{\cdot}2H_2O$ in pyridine/DMF at ambient temperature to produce the mono Boc protected 1,2-phenylenediamine building blocks **10**. The regioselective synthesis was achieved by simple condensation of mono Boc protected 1,2-phenylenediamines **10** and β-ketoesters **11** or [1,3]-dioxinones **12** in refluxing toluene to the corresponding β-ketoamides **13**, which were deprotected and concomitantly cyclized by treatment with TFA yielding the unsymmetrically 7,8-substituted 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones **14**.[10]

The concentration-dependent displacement of the [^{3}H]-LY354740 binding to rat mGluR2 in the experimental conditions described by Schweizer et al.[8] was then used to determine the structure activity relationship (SAR) of compounds obtained after the screening hit **3** (>95% purity). Compound **14m** and derivatives partially inhibit [^{3}H]-LY354740 binding to mGluR2 with a residual of 25% specific bound (Fig. 2b). Considering the non-competitive nature of this inhibition (Fig. 2a) IC_{50} values are reported (Table 1) as relative measure of affinity.[8]

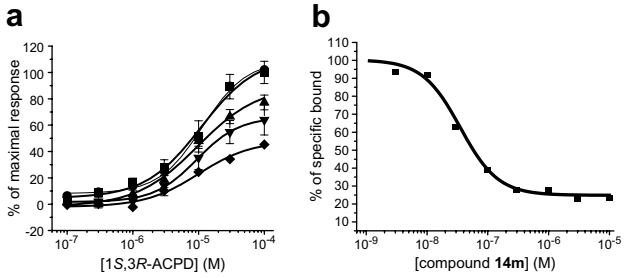
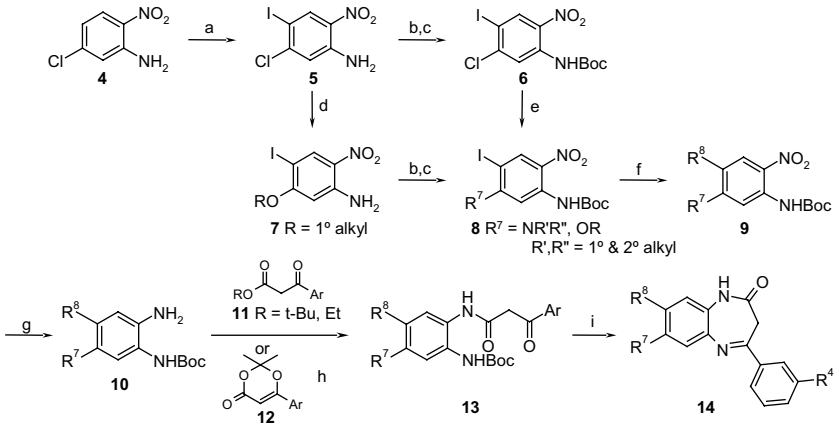
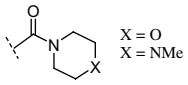


Figure 2. (a) Effect of the non-competitive antagonist **14m** on the functional responses induced by increasing concentrations of the mGluR2 agonist (1*S*,3*R*)-ACPD in the [^{35}S]GTPγ S assay. Increasing concentrations of **14m** reduce the maximal response to the agonist with no changes in EC_{50} values. EC_{50} values: (1*S*,3*R*)-ACPD 12.1 μM, + **14m** (10 nM) 11.8 μM, + **14m** (30 nM) 12.6 μM. [■, (1*S*,3*R*)-ACPD ($n = 6$); ●, **14m** 3 nM ($n = 4$); ▲, **14m** 10 nM ($n = 3$); ▼, **14m** 30 nM ($n = 4$); ♦, **14m** 100 nM ($n = 3$)]. (also cf. Ref. 12). (b) Indirect measurement of the affinity of **14m** for the recombinant rat mGluR2 expressed in CHO cells. Inhibition curve is normalized as % of the maximum specific bound. Compound **14m** partially inhibits the radioligand binding to mGluR2 with a residual 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.



Scheme 1. General synthesis of unsymmetrically 7,8-substituted 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones **14**. Reagents and conditions: (a) ICl, NaOAc, AcOH, 23–80 °C, 95%; (b) Boc_2O, DMAP, THF, rt, 99%; (c) TFA, DCM, 0 °C, 99%; (d) ROH, KOH, DMSO, rt, 75–95%; (e) R′R″NH, DMSO, rt, 80–98%; (f) alkyne, $PdCl_2(PPh_3)_2$, PPh_3, CuI, Et_3N, THF, 50–65 °C, 50–95%; (g) $SnCl_2{\cdot}2H_2O$, pyridine, DMF, 50–90%; (h) toluene, reflux, 40–95%; (i) TFA [optional anisole], DCM, rt.

Table 1. Activities of the 1,3-dihydro-benzo[*b*][1,4]diazepin-2-ones **14** (R^4, R^7, and R^8 refer to the positions of Rs in Scheme 1)

Compound	R^4	R^8	R^7	Rat mGluR2 [^{3}H]-LY354740 binding[a] IC_{50} (μM)
14a	MeO	Me	H	6.58
14b	Cl	Me	H	2.82
14c	CF_3	Me	H	1.53
14d	CN	Me	H	2.90
14e	H	Ph-C≡C–	H	0.026
14f	MeO	Ph-C≡C–	H	0.962
14g	Cl	Ph-C≡C–	H	0.080
14h	CF_3	Ph-C≡C–	H	0.268
14i	I	Ph-C≡C–	H	0.154
14j	$CONH_2$	Ph-C≡C–	H	0.358
14k	 X = O X = NMe	Ph-C≡C–	H	0.360
14l				1.04
14m	CN	Ph-C≡C–	H	0.034
14n	CN	2-Cl–C_6H_4-C≡C–	H	0.076
14o	CN	4-Me-C_6H_4-C≡C–	H	0.064
14p	CN	4-MeO-C_6H_4-C≡C–	H	0.068
14q	CN	4-F-C_6H_4-C≡C–	H	0.034
14r	CN	2-F-C_6H_4-C≡C–	H	0.030
14s	CN	2,4-Di-F-C_6H_3-C≡C–	H	0.480
14t	CN	2-Thiophenyl-C≡C–	H	0.032
14u	CN	2-Thiazolyl-C≡C–	H	0.300
14v	CN	2-Pyridyl-C≡C–	H	0.870
14w	CN	$HO(Me)_2C$-C≡C–	H	26
14x	CN	H_2C=C(Me)-C≡C–	H	0.400
14y	CN	Ph-C≡C–	NMe_2	0.060
14z	CN	Ph-C≡C–	X = O	0.378
14aa	CN	Ph-C≡C–	X = NMe	0.278
14ab	CN	Ph-C≡C–	X = S	0.040
14ac	CN	Ph-C≡C– 	X = SO	0.446
14ad	CN	Ph-C≡C–	$X = SO_2$	0.160
14ae	CN	Ph-C≡C–	X = CHOMe	0.082
14af	CN	Ph-C≡C–	OMe	0.092
14ag	CN	Ph-C≡C–	OCH_2CH_2OMe	0.028
14ah	CN	Ph-C≡C–	OCH_2CO_2H	0.600
14ai	CN	Ph-C≡C–	OCH_2CONH_2	2.4
14aj	CN	Ph-C≡C–	$OCH_2CONHBu^t$	0.026
14ak	CN	Ph-C≡C–	OCH_2CN	0.018

[a] Values are means of three experiments.

In the R^4 position a clear preference for meta-substitution at the phenyl ring was quickly established (**14a–d**). With the introduction of a phenylacetylene moiety in the 8-position we found an enhanced affinity compared to **3** (**14e**) and more thoroughly investigated the structure activity relationship (SAR) at the phenyl in 4-position. The functional group tolerance proved to be wide, ranging from simple halides (**14g** and **14i**), methoxy (**14f**), trifluoromethyl (**14h**) to carboxamides (**14j–l**), but among the substituted compounds the cyano group showed the highest affinity (**14m**).

Keeping this nitrile constant we explored the variation of the alkyne in the 8-position. Substituted phenyl residues produced very active molecules, for example, a 2-chloro- (**14n**), a 4-methyl- (**14o**) or a 4-methoxy-phenyl (**14p**) were almost equal in affinity. Fluoro substitution in 2- and 4-position of the phenyl group led to compounds with equal properties (**14q** and **14r**) like **14m**, as well as the 2-thiophenyl-group (**14t**). 2,4-Di-fluoro substitution however led to the less favored **14s**. The introduction of a nitrogen containing heterocycle, for example, 2-thiazolyl (**14u**) or 2-pyridyl (**14v**), resulted in an approximately 10- and 30-fold drop in affinity. Using a smaller non-aromatic substituent with a tertiary alcohol as in **14w** produced an almost inactive compound, but elimination of water from **14w** generated the olefin **14x** and a regain in potency was observed. As a conclusion, the acetylenic groups in 8-position were preferably chosen from lipophilic aromatic residues like phenyl-, 2- or 4-F-phenylacetylene (**14m**, **14q** and **14r**). To complete the overall SAR, we decorated **14m** with a MeO group in 6- or with a Cl in 9-position, which led to completely inactive compounds (data not shown).

The addition of more polar substituents in 7-position was made to reduce the high lipophilicity of the compounds (**14m** *c*log*P* 4.44; calculated $\log(c_{octanol}/c_{water})$). As described in Scheme 1, *N*-nucleophiles were introduced in the 7-position by S_NAr reaction. The simple lipophilic dimethylamino group (**14y** *c*log*P* 4.62) was well tolerated, but the larger and more polar morpho-

lino group (**14z** *c*log*P* 4.23) and the more basic *N*-methylpiperazinyl group (**14aa** *c*log*P* 4.20) resulted in loss of affinity. For the substituents in the 7-position not the size but more likely the polarity seemed to be the dominant factor for affinity since the even larger but most lipophilic **14ae** (*c*log*P* 5.14) proved to be a very potent compound.

The restricted scope in the 7-position was also nicely demonstrated by the examination of the thiomorpholino compound and its oxide derivatives (**14ab**–**ad**). The most lipophilic **14ab** (*c*log*P* 5.08) was most active, the most polar sulfoxide **14ac** (*c*log*P* 2.94) the least active, whereas the sulfone **14ad** with intermediate polarity (*c*log*P* 3.06) again showed intermediate properties.

As for the oxygen-linked moieties the small simple methoxy group (**14af**, *c*log*P* 4.52) and the larger 2-(methoxy)ethoxy group (**14ag**, *c*log*P* 4.25) are both well tolerated. The attachment of a carboxylic acid group *via* an oxygen linker as in **14ah** (*c*log*P* 3.78) produced a moderately active compound.

A correlation between polarity of groups in the 7-position with the in vitro activity was obtained in the corresponding amide series. The primary amide (**14ai** *c*log*P* 3.01) is about 100-fold less active than the less polar *N*-*tert*-butylamide (**14aj**, *c*log*P* 4.84) and the corresponding nitrile (**14ak**, *c*log*P* 3.54), therefore exhibiting the same trend of activities as observed for the thiomorpholino compound and its oxides (**14ab**–**ad**).

We further assessed the selectivity of **14m** (among other compounds) and this compound neither activated nor inhibited glutamate-stimulated rat mGlu1a and mGlu5a receptors (using a Ca^{2+} mobilization functional assay, when tested at 30 μM final concentration) nor the binding of [^{3}H]-L-AP4 to rat mGlu8a receptor. Compound **14m** is also devoid of any affinity at the NMDA and $GABA_A$ receptors (data not shown).

The non-competitive nature of **14m** was exemplified by measuring the effect on the binding of [^{35}S]GTPγS induced by (1*S*,3*R*)-ACPD (Fig. 2a),[11,12] which partially inhibits [^{3}H]-LY354740 binding to mGluR2 with a residual of 25% specific bound (Fig. 2b). The antagonist properties of **14m** were also evaluated electrophysiologically in CHO cells stably expressing the human Kir3.1 and Kir3.2c GIRK subunits and transiently transfected with either rat mGlu2 or rat mGlu3 receptors (Fig. 3). In these cells, glutamate induced an inward K^+ current that was reversible and dependent on the glutamate concentration, which was concentration-dependently inhibited by **14m**.

In summary, we have explored the SAR of a novel series of 1,3-dihydro-benzo[*b*][1,4]diazepin-2-one derivatives which are selective, non-competitive antagonists at mGluR2/3. Attachment of an 8-(2-aryl)-ethynyl moiety produced compounds inhibiting the binding of [^{3}H]-LY354740 to rat mGluR2 with low nanomolar affinity. These compounds represent a very promising structural class of non-amino acid antagonists with very high affinity to group II mGlu receptors.

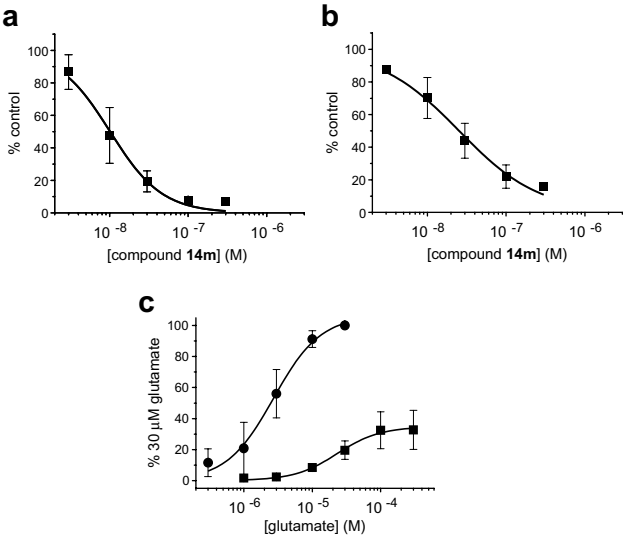


Figure 3. Concentration–response curves for the inhibition of GIRK currents by **14m** in CHO cells stably expressing GIRKs and transiently transfected with: (a) rat mGluR2 (Glutamate 10 μM) and (b) rat mGluR3 (glutamate 1 μM). (c) Glutamate concentration–response curves in CHO cells expressing GIRKs and rat mGluR2 generated in the absence (●, control) and presence of **14m** (■, 100 nM) showing the non-competitive nature of block of the compound. This effect was reversible.

Acknowledgments

We cordially thank J. Beck, N. Benedetti, D. Buchy, M. Burn, S. Chaboz, M. Dellenbach, A. Maier, A. Nilly, H. Schär, and W. Vifian for their skillful technical assistance.

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