Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5203–5206
Bioorganic & Medicinal Chemistry Letters

A novel class of positive allosteric modulators of AMPA receptors: Design, synthesis, and structure–activity relationships of 3-biphenyl-4-yl-4-cyano-5-ethyl-1-methyl-1*H*-pyrrole-2-carboxylic acid, LY2059346

Hamideh Zarrinmayeh,[a,*] Eric Tromiczak,[a] Dennis M. Zimmerman,[a] Nancy Rankl,[a] Ken H. Ho,[a] Esteban Dominguez,[b] Ana Castaño,[b] Ana Escribano,[b] Carmen Fernandez,[b] Alma Jimenez,[b] William J. Hornback[a] and Eric S. Nisenbaum[a]

[a]*Eli Lilly and Co, Lilly Research Laboratories, Indianapolis, IN 46285, USA*
[b]*Eli Lilly and Co, Lilly S.A., Avenida de la Industria, 30, 28108 Alcobendas, Madrid, Spain*

Received 26 May 2006; revised 23 June 2006; accepted 5 July 2006
Available online 25 July 2006

Abstract—The synthesis and structure–activity relationship (SAR) of novel and highly potent positive allosteric modulators of AMPA receptors, 3-biphenyl-4-yl-4-cyano-5-ethyl-1-methyl-1*H*-pyrrole-2-carboxylic acid, are described. These studies indicated that higher potency was achieved with ortho substitution of the distal (D) phenyl of the 3-biphenyl ring and resulted in the discovery of a potent pyrrole LY2059346 (**23q**), that was selected for further evaluation in in vitro native tissue assays and in vivo experiments.

L-Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) acting on metabotropic and ionotropic glutamate receptors. On the basis of differences in their molecular sequences and sensitivity to selective agonists, three subtypes of ionotropic receptors have been identified, including AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid), NMDA (*N*-methyl-L-aspartate), and kainate receptors.[1] Of these subtypes, AMPA receptors mediated the majority of rapid glutamatergic signaling in the CNS. Functional AMPA receptors are proposed to be tetrameric structures, generated by the assembly of one or more distinct subunits GluR1–4, yielding either homomeric or heteromeric configurations.[2] Further diversity among native AMPA receptors is conferred by alternative splicing in an extracellular domain of each subunit, giving rise to two isoforms named flip (i) and flop (o).[3] Activation of AMPA receptors is initiated by binding of glutamate (or agonist) to each of the subunits which directs a conformational change in the ion channel gate, permitting ion flux through the channel pore.[4] Termination of current flow can occur upon removal of glutamate and unbinding of agonist (deactivation) or in the continued presence of glutamate, uncoupling of agonist binding from the channel gate, permitting channel closure (desensitization).[4]

An accumulating body of evidence has indicated that dysfunction of glutamatergic signaling in the CNS may contribute to the deficits associated with a variety of neurological and psychiatric disorders.[5] Clinical and experimental data have suggested that positive modulation of AMPA receptors may be a novel therapeutic approach in the treatment of such disorders.[6] Positive allosteric modulators of AMPA receptors exert their effects only in the presence of agonist binding and act to enhance ion flux through the channel either by attenuating the deactivation or desensitization process(es).[4] Several classes of positive allosteric modulators have been identified and reported, including benzothiadiazides (cyclothiazide, **1**);[5] pyrrolidinones (aniracetam, **2a**, and piracetam, **2b**)[6]; and benzamides (CX516, **3**)[7] (Fig. 1). However, the value of such tools for understanding AMPA receptor pharmacology and physiology has been limited by their relatively week potency. We previously reported on a class of biarylpropylsulfonamide modulators **4** that possess significantly higher potencies than

Keywords: Positive allosteric modulators; AMPA receptors.
* Corresponding author. Tel.: +1 317 276 5964; e-mail: Zarrinmayeh_Hamideh@lilly.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.07.012

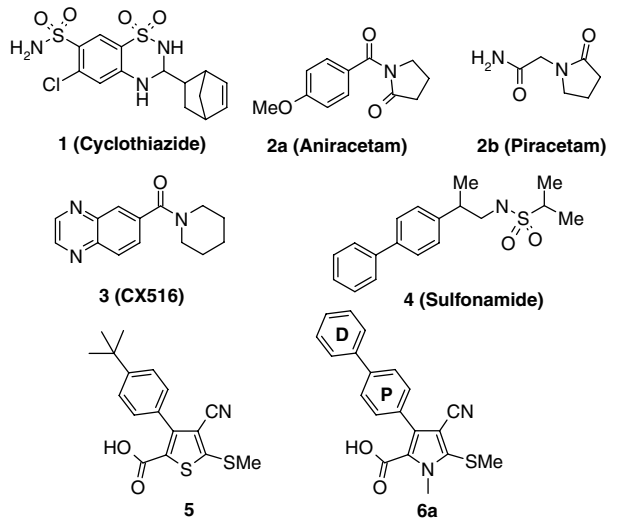
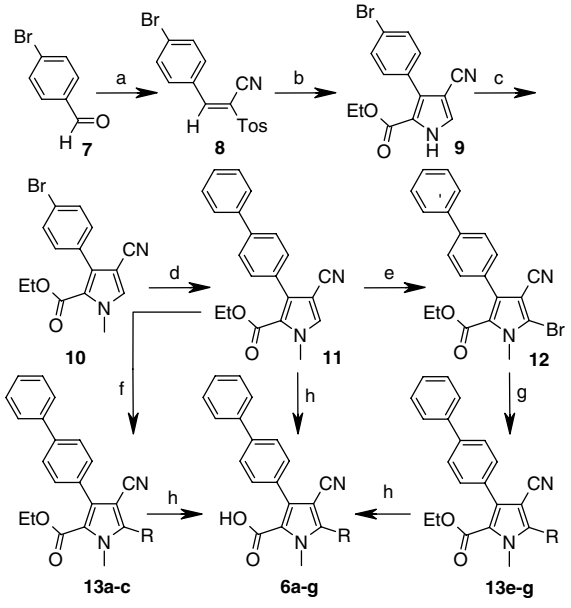


Figure 1. Structures of positive allosteric modulators of AMPA receptors.

those of **1**–**3**.[8,9] A subsequent high-throughput screen of Lilly compound collections resulted in the discovery of 2-thiophene carboxylic acid **5** as the lead candidate. Preliminary study of the core heterocyclic ring led to the discovery of *N*-methylpyrrole 2-carboxylic acid **6a**. In this communication, we describe the SAR of these pyrrole 2-carboxylic acid compounds as novel class of allosteric modulators of AMPA receptors.

Synthesis of pyrrole **6a**–**g** is shown in Scheme 1.

Commercially available 4-bromo benzaldehyde **7** was converted to acrylonitrile **8** upon refluxing with *p*-toluenesulfonylacetonitrile. This intermediate was subsequently converted to pyrrole **9** when reacted with ethylisocyanoacetate in the presence of DBU. N-methylation of **9** was followed by Suzuki cross coupling of **10** with phenylboronic acid using palladium tetrakis to yield product **11**. Lithiation of **11** at −78 °C in THF followed by treatment with dialkyl disulfide yielded analogs **13a**–**c**. Pyrrole **11** was also converted to the brominated analog **12** that was converted to the corresponding products **13f**,**g** upon coupling reaction with tetramethyl tin or diethyl zinc, respectively.[10] Hydrolysis of the esters with lithium hydroxide yielded *N*-methylpyrrole-2-carboxylic acids **6a**–**g**.

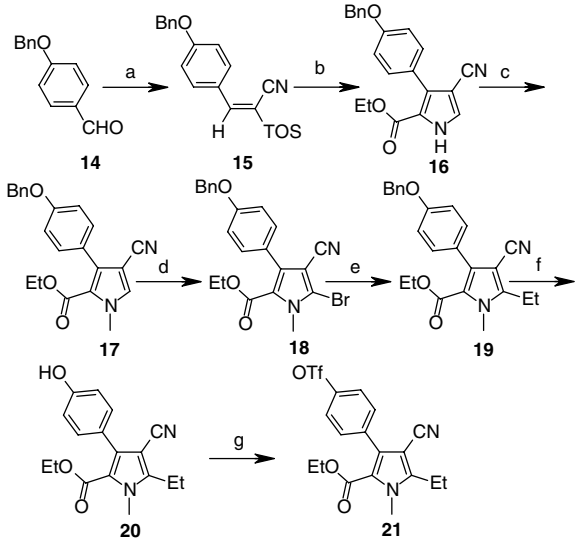


Scheme 1. Synthesis of compounds **6a**–**g**. Reagents and conditions: (a) *p*-toluenesulfonylacetonitrile, piperidine, AcOH, toluene, reflux, 92%; (b) ethylisocyanoacetate, DBU, THF, rt, 99%; (c) MeI, K_2CO_3, DMSO, 77%; (d) $PhB(OH)_2$, $Pd(PPh_3)_4$, dioxane, 80 °C, 95%. (e), NBS, CCl_4, rt, 82%. (f) i—LHMS, THF, −78 °C; ii—$(R–S)_2$, −78 °C to rt, 72–80%; (g) Me_4Sn, $Pd(PPh_3)_4$, for **13f** 62%, Et_2Zn, Hartwig's ligand, for **13g** 95%; (h) LiOH, THF/MeOH/H_2O (3:2:1), 50 °C, 90–95%.

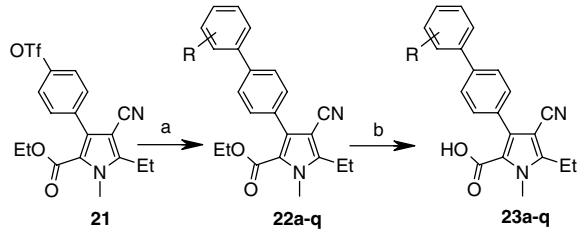
As shown is Scheme 2, *N*-methylpyrrole-2-carboxylic acid ethyl ester **21** was prepared in seven steps from commercially available 4-benzyloxybenzaldehyde **14** which was converted to acrylonitrile **15** when treated with *p*-toluenesulfonylacetonitrile under refluxing condition. Subsequent reaction of **15** with ethylisocyanoacetate resulted in the formation of pyrrole **16** which was methylated to **17**. Bromination of **17** to **18** followed by reaction of **18** with diethyl zinc using Hartwig's ligand under a mild condition resulted in the formation of 5-ethylpyrrole **19**. Deprotection of **19** and subsequent triflation of the phenol **20** resulted in the synthesis of intermediate pyrrole **21**.

Intermediate **21** was converted to pyrrole **22a**–**q** via Suzuki cross coupling reaction with phenyl boronic acids using palladium catalyst. Hydrolysis of the esters by lithium hydroxide resulted in the formation of the acids **23a**–**q** as shown in Scheme 3.

The activity of allosteric modulators was evaluated using HEK293 cells stably transfected with recombinant human homomeric GluR2i, GluR2o, GluR4i, and



Scheme 2. Synthesis of compound **21**. Reagents and conditions: (a) *p*-toluenesulfonylacetonitrile, piperidine, AcOH, toluene, reflux, 95%; (b) ethylisocyanoacetate, DBU, THF, rt, 91%; (c) MeI, K_2CO_3, DMSO, 77%; (d) NBS, THF, 60%; (e) Et_2Zn, Hartwig's ligand, THF, rt, 1 h, 95%; (f) H_2, $Pd(OH)_2$/C, EtOH/THF (1:2), 50 psi, rt, 97%; (g) Tf_2O, Et_3N, CH_2Cl_2, rt, 93%.



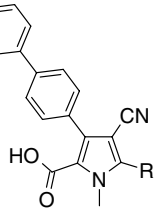
Scheme 3. Synthesis of compounds **23a**–**q**. Reagents and conditions: (a) $ArB(OH)_2$, Pd catalyst, 30–90%; (b) LiOH, THF/MeOH/H_2O (3:2:1), 50 °C, 80–95%.

GluR4o receptors. Changes in intracellular calcium ion (Ca^{2+}) were measured in the presence of glutamate alone and in response to co-application with the modulators at concentrations of 0.1–3 M using a FLIPR technology. Responses were normalized to those produced by co-application of 5 and 100 μM glutamate (maximum response) and 100 μM glutamate alone (minimum response). The in vitro potency of the compounds was reported as EC_{50} values in nanomolars as shown in Tables 1 and 2.

In this study, the 2-carboxylic acid, 3-biaryl, and 4-cyano groups substitutions on the pyrrole ring were kept constant since preliminary studies revealed the necessity of such functionalities for minimum activity. We first focused on the evaluation of the 5-thiomethyl functionality, its significance and possible replacement with more neutral and metabolically stable groups.

As shown in Table 1, parent pyrrole **6a** exhibited reasonable activity with EC_{50} values of 394, 1301 nM at GluR4i/o and 174 nM, 473 nM at GluR2i/o, respectively. Flip splice variant selectivity was observed for both subunits with higher potency at GluR2i. Larger thio-

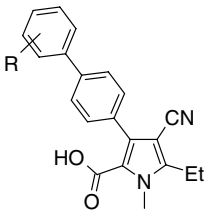
Table 1. EC_{50} values of selected novel AMPA receptor's positive allosteric modulators



Compound	R	EC_{50} (nM)			
		iGluR4i	iGluR4o	iGluR2i	iGluR2o
5[a]	—	214	1559	97	309
6a[b]	S-Me	394	1301	174	473
6b[b]	S-Et	619	NT	NT	NT
6c[b]	S-*i*-Pr	>3 μM	>3 μM	>3 μM	>3 μM
6d[b]	H	>3 μM	>3 μM	>3 μM	>3 μM
6e[c]	Br	679	1689	>3 μM	>3 μM
6f[b]	Me	532	2388	308	391
6g[c]	Et	213	1766	103	398

NT, not tested.
[a] EC_{50} value ($n \geqslant 100$).
[b] EC_{50} value ($n \geqslant 3$).
[c] EC_{50} value ($n \geqslant 10$).

Table 2. EC_{50} values of selected novel AMPA receptor positive alosteric modulators



Compound	R	EC_{50} (nM)			
		iGluR4i	iGluR4o	iGluR2i	iGluR2o
6g[a]	H	213	1766	103	398
23a[b]	*p*-Me	1143	>3000	412	1647
23b[b]	*m*-Me	2085	>3000	965	2133
23c[b]	*o*-Me	790	1181	270	990
23d[b]	*p*-OMe	738	>3000	423	370
23e[b]	*m*-OMe	1051	>3000	391	924
23f[b]	*o*-OMe	673	386	280	902
23g[b]	*o*-OEt	158	146	83	36
23h[b]	*o*-SMe	105	77	57	32
23i[b]	*p*-F	242	2947	345	1035
23j[b]	*m*-F	223	>3000	426	1490
23k[b]	*o*-F	80	185	111	143
23l[b]	*p*-Cl	910	>3000	142	984
23m[b]	*m*-Cl	1082	>3000	186	900
23n[b]	*o*-Cl	225	157	42	55
23o[b]	*p*-CN	373	>3000	423	370
23p[b]	*m*-CN	631	>3000	395	974
23q[c]	*o*-CN	56	53	82	73
LY2059346					

[a] EC_{50} value ($n \geqslant 10$).
[b] EC_{50} value ($n \geqslant 3$).
[c] EC_{50} value ($n \geqslant 20$).

alkyl groups were not tolerated as shown in **6b**,**c**. Lack of activity of 5-proto pyrrole **6d** demonstrated the need for substitution of 5 position with groups larger than proton. Alkyl-substituted analogs **6f**,**g** were prepared and tested. Even though the activity of 5-methyl analog **6f** was modest, the 5-ethyl analog **6g** showed a desired improvement in activity compared to that of the parent **6a**, especially at the flip isoform with EC_{50} values of 213 and 103 nM at GluR4i and GluR2i receptors, respectively. In addition, **6g** was the first pyrrole analog to possess similar activity to that of the potent lead thiophene **5**. Ethyl functionality was also superior to other groups that were studied and not presented in Table 1. As a result, ethyl was selected as the desired functionality to substitute 5 position of the pyrrole for subsequent SAR studies.

To optimize substituents and the pattern of substitution on the distal phenyl ring D of the 3-biaryl, we prepared analogs **23a**–**q**. As shown in Table 2, methyl substitution of the phenyl ring provided pyrrole **23a**–**c** with only modest activities. Methoxy analogs **23d**–**f** also demonstrated similar potencies. However, some pattern of substitution was observed in that higher activity resided in *ortho*-substituted analogs. Therefore, analogs **23g**,**h** were prepared and displayed an increase in activity at all receptors. In addition, thiomethyl anolog **23h** possessed

significant potency and selectivity for the flop isoform. More *ortho* preference for substitution of the phenyl D ring was observed with fluorinated analogs **23i**–**k** where ortho fluoro anaolog **23k** was the most potent of the three regioisomers. The same pattern of substitution was also observed with chlorinated analogs **23l**–**n**, where *ortho* chloro analog **23n** was significantly more potent than the other regioisomers **23l**,**m**. Within the cyano analog series **23o**–**q**, of the three regioisomers, *ortho* cyano analog **23q** showed the highest activities at GluR4i/o and desired activities at GluR2i/o. A unique property of **23q** was its equally high potency at all receptor subtypes. This broad range of activity has prompted further evaluation of **23q**, LY2059346,[11] in in vivo assays.

In summary, we have designed, synthesized, and evaluated a novel class of positive allosteric modulators of AMPA receptors. Initial SAR studies on the *N*-methylpyrrole **6a** resulted in replacement of thiomethyl with ethyl functionality at position 5. Further SAR on the distal phenyl ring D of the biphenyl ring system at 3 position resulted in analogs where their higher potencies came from not only the substituents, but also from the pattern of substitution. *ortho* Substitution of ring D with ethoxy, thiomethoxy, fluoro, and cyano functionalities, independent of the electronic features of the substituents, resulted in significant increase of potency. Isoform selectivity was not predominant in this SAR and only a mode preference for GluR2 versus GluR4 receptors was observed. The most potent pyrrole **23q**, LY2059346, exhibited equally high potency at all four homomeric receptors and has been selected for further in vitro and in vivo biological studies.

References and notes

1. (a) Hollmann, M.; Heinemann, S. *Ann. Rev. Neurosci.* **1994**, *17*, 31; (b) Schoepp, D. D.; Conn, P. J. *Pharmacol. Biochem. Behav.* **2002**, *74*, 255.
2. Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. *Pharmacol. Rev.* **1999**, *51*, 7.
3. Seeburg, P. H.; Higuchi, M.; Sprengel, R. *Brain Res. Rev.* **1998**, *26*, 217.
4. Sun, Y.; Olson, R.; Horning, M.; Armstrong, N.; Mayer, M.; Gouaux, E. *Nature* **2002**, *417*, 245; Jin, R.; Clark, S.; Weeks, A. M.; Dudman, J. T.; Gouaux, E.; Partin, K. M. *J. Neurosci.* **2005**, *25*, 9027.
5. (a) Franciosi, S. *Cell. Mol. Life Sci.* **2001**, *58*, 921; (b) Yamada, K. A. *Neurobiol. Dis.* **1998**, *5*, 67.
6. Dimond, S. J.; Scammell, R. E.; Pryce, I. C.; Huws, D.; Gray, C. *Psychopharmacology* **1979**, *64*, 341; Ingvar, M.; Ambros-Ingerson, J.; Davis, M., et al. *Exp. Neurol.* **1997**, *146*, 553; Staubli, U.; Perez, Y.; Xu, F. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 777; O'Neill, M. J.; Murray, T. K.; Clay, M. P.; Lindstrom, T.; Yang, C. R.; Nisenbaum, E. S. *CNS Drug Rev.* **2005**, *11*, 77; Alt, A.; Nisenbaum, E. S.; Bleakman, D.; Witkin, J. M. *Biochem. Pharmacol.* **2006**, *71*, 1273.
7. Ito, I.; Tanabe, S.; Kohda, A.; Sugiyama, H. *J. Physiol.* **1990**, *424*, 533; Bertolino, M.; Braldi, M.; Parenti, C.; Braghioli, D.; DiBella, M.; Vicini, S.; Costa, E. *Receptors and Channels* **1993**, *1*(4), 267; Arai, A.; Kessler, M.; Xiao, P.; Ambros-Ingerson, J.; Rogers, G.; Lynch, G. *Brain Res.* **1994**, *638*, 343.
8. Ornstein, P. L.; Zimmerman, D. M.; Arnold, M. B.; Bleisch, T. J.; Cantrell, B.; Simon, R.; Zarrinmayeh, H.; Baker, S. R.; Gates, M.; Tizzano, J. P.; Mandelzys, A.; Jarvie, K.; Ho, K.; Deverill, M.; Kamboj, R.; Bleakman, D. *J. Med. Chem.* **2000**, *43*, 4354.
9. Zarrinmayeh, H.; Bleakman, D.; Gates, M. R.; Yu, H.; Zimmerman, D. M.; Ornstein, P. L.; McKennon, T.; Arnold, M. B.; Wheeler, W. J.; Skolnick, P. *J. Med. Chem.* **2001**, *44*, 302.
10. Uno, H.; Tanaka, M.; Inoue, T.; Ono, N. *Synthesis* **1999**, *3*, 471.
11. ^{1}H NMR (DMSO-d_6, 500.0 MHz): δ 1.26 (t,3H), 2.87 (q, 2H), 3.88 (s, 3H), 7.52 (d, 2H), 7.62 (t, 1H), 7.65 (d, , 2H), 7.71 (d, 1H), 7.83 (t,1H), 7.99 (d,1H), 12.85 (s, 1H); IR (KBr): 2938, 2225, 1654, 1476, 1440, 1356, 1280, 1252, 1166, 763^{cm-1}. PCT Int. Appl. WO 2005040110.