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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5057–5061
Bioorganic & Medicinal Chemistry Letters

A novel class of AMPA receptor allosteric modulators. Part 1: Design, synthesis, and SAR of 3-aryl-4-cyano-5-substituted-heteroaryl-2-carboxylic acid derivatives

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Received 11 May 2006; revised 12 July 2006; accepted 13 July 2006
Available online 1 August 2006

Abstract—The synthesis and initial SAR studies of novel, highly potent positive allosteric modulators of AMPA receptors based on 3-(4-*tert*-butylphenyl)-4-cyano-5-methylsulfanyl-thiophene-2-carboxylic acid (**6a**) are described. SAR studies at the thioether moiety indicated that substitution at this position was mandatory and better potency was achieved with small groups.

L-Glutamate is a major excitatory neurotransmitter in mammalian central nervous system (CNS). Three ionotropic glutamate receptor subtypes have been identified based on differences in their molecular biology and sensitivity to the selective agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate (KA).[1] The AMPA receptor family includes four different subunits named GluR1-4. Functional AMPA receptors are most likely tetrameric ion channels formed by the assembly of one or more of the GluR subunits, yielding homomeric or heteromeric receptors.[2] Additional complexity among AMPA receptors is conferred by alternative splicing of RNA for each subunit giving rise to flip and flop variants.[3] Glutamate binding to AMPA receptors initiates conformational changes in the channel gate that lead to increase conductance of sodium and calcium ions into cells and subsequent membrane depolarization.[4]

Evidence indicates that decreases in glutamatergic neurotransmission in the CNS may contribute to cognitive deficits associated with a variety of neurological and psychiatric disorders.[5]

Clinical and experimental data suggest that positive modulation of AMPA receptors may be therapeutically effective in the treatment of cognitive deficits.[6] Several studies have identified compounds that enhance ion influx through AMPA receptors by positive allosteric modulation. Some examples are pyrrolidones such as aniracetam[7a–c] (**1**, Fig. 1), benzothiadiazides such as cyclothiazide (**2**),[7d,e] benzamides such as CX-516 (**3**), or 4-[2-(phenylsulfonylamino)thio]-difluoro-phenoxy-acetamide (**4**),[7f] and a family of sulfonamides (**5a** and **5b**) previously reported by our group.[7g]

Electrophysiological experiments have demonstrated that these biarylsulfonamides are potent modulators of native AMPA receptors enhancing glutamate evoked currents through AMPA channels of acutely isolated pyramidal neurons.[8a] Moreover, in vivo tests on spinal and hippocampal neurons in rats showed that **5a** and **5b** cross the blood–brain barrier and modulate the sensitivity of central AMPA receptors enhancing synaptic excitation.[8b]

Toward the goal of developing new AMPA potentiators, a high throughput screen of compounds was

Keywords: AMPA receptor allosteric modulators; Heteroaryl carboxylic acids.
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doi:10.1016/j.bmcl.2006.07.035

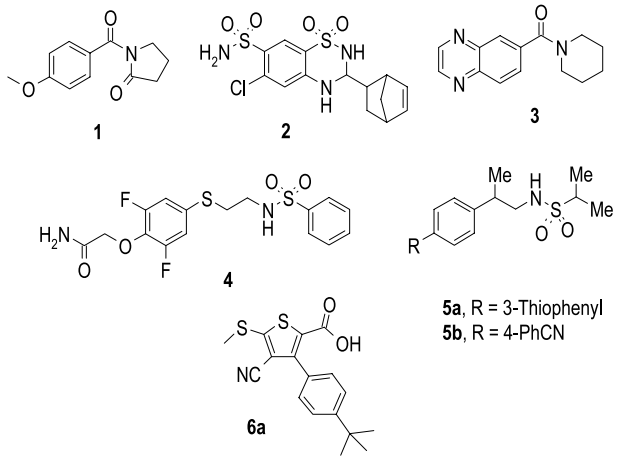
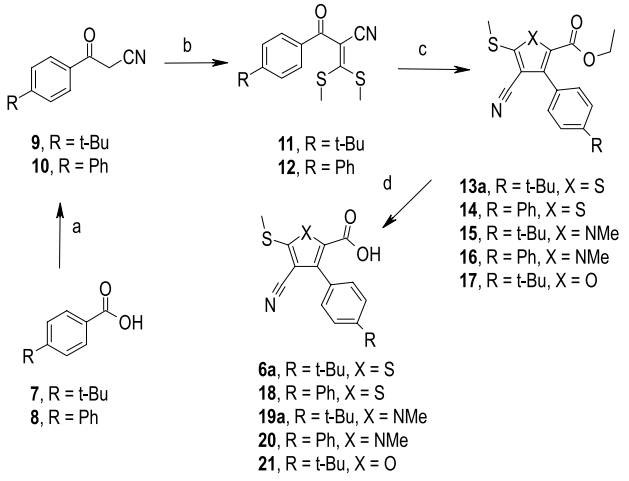


Figure 1. Structures of AMPA receptor allosteric modulators.

conducted using human cloned homomeric AMPA receptors. The screen identified thiophene **6a**[9] (Fig. 1) as a novel AMPA receptor modulator lead. Further structure–activity-relationship (SAR) studies led to the discovery of a new family of AMPA potentiators. In the present communication, we describe part of these SAR studies, focusing our attention on the identification of the optimal core ring and the substitution at thiomethyl moiety in thiophene ring.

The synthetic pathway for preparing compounds **6a**, **18**–**21** is shown in Scheme 1.

Methylsulfanyl acrylonitriles **11** and **12** were obtained by deprotonation of the corresponding propionitriles, previously prepared under standard conditions, followed by condensation with carbon disulfide and subsequent quenching with methyl iodide.[10] These intermediates were further converted to the thiophenyl (**13a** and **14**),[11] *N*-Me pyrrole (**15** and **16**), and furan (**17**) ethyl carboxylates by cyclocondensation with ethylthioglycolate, sarcosine ethyl ester hydrochloride and bromoethyl acetate, respectively, under basic conditions. Carboxylic acids **6a**, **18**–**21** were prepared from esters **13**–**17** by hydrolysis under alkaline conditions.



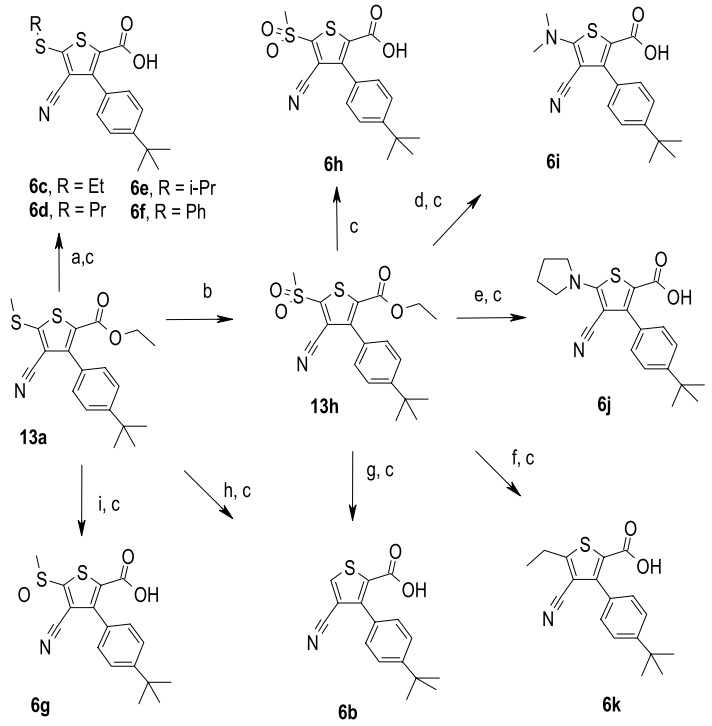
Scheme 1. Synthesis of compounds **6a**, **18**–**21**. Reagents and conditions: (a) i—$SOCl_2$, 50 °C, 1 h; ii—$CNCH_2CO_2H$, *n*-BuLi, THF, −78 °C to rt, 2h (50–64%); (b) i—CS_2, DMSO, 15 °C to rt, 2h; ii—MeI, rt, 1 h (42–56%); (c) ethylthioglycolate, Et_3N, EtOH, reflux, 15 min (X = S); $CH_3NHCH_2CO_2Et{\cdot}HCl$, Et_3N, EtOH, 0.5–2 h (X = NMe); Bromoethyl acetate, LHMDS, THF, −78 °C to rt, 6 h (X = O) (50–73%); (d) 2 M NaOH/EtOH 1:1 or 2.5 M LiOH/THF/MeOH 3:2:1, rt, overnight, then 1 M HCl (30–95%).

Intermediate **13a** was further elaborated to give a wide range of derivatives summarized in Scheme 2. Thiophenes **6c**–**f** were obtained by coupling of primary thiols with **13a** in toluene, using palladium chemistry. Oxidation of methylthio group with MCPBA followed by ester hydrolysis afforded the sulfoxide **6g**, while excess of the same oxidant yielded the sulfone **13h**. This intermediate was used to prepare the amino ester precursors of **6i**–**j** by nucleophilic substitution of the corresponding amines. Moreover, **13h** reacted with diethylzinc in dry dichloromethane to provide the ethylthiophenyl carboxylic ester intermediate that after ester hydrolysis afforded **6k**. Compound **6b** was obtained by reduction of **13 h** with Na–Hg amalgam in presence of base followed by hydrolysis. Alternatively, this compound could be prepared by reaction of **13a** with excess of sodium borohydride in ethanol and subsequent ester hydrolysis.

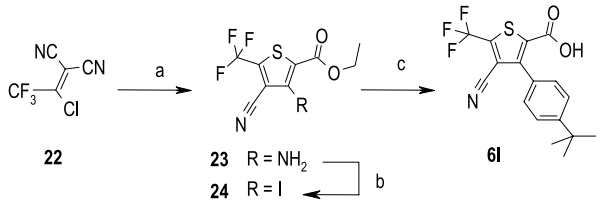
Compound **6l** was prepared using the synthetic approach described in Scheme 3. Condensation of (trifluoromethyl)ethylene **22**[12] with ethyl 2-mercaptoacetate in ethanol afforded the aminothiophene **23**. This intermediate reacted with isoamylnitrite and diiodomethane to provide the iodo derivative **24**. Then, Suzuki coupling of **24** with *tert*-butylphenyl boronic acid under standard conditions and subsequent ester hydrolysis provided thiophene derivative **6l**.

All compounds were dissolved in DMSO and tested for activity in calcium-imaging assay using stably transfected HEK-293 cells expressing homomeric GluR2 and GluR4 receptors in both flip and flop splice variant forms to provide information on the relative potency and splice variant selectivity. Changes in intracellular calcium concentration produced by application of glutamate (100 μM) alone and in response to co-application with compounds (0.1–3 μM) were measured using a Fluorometric Imagin Plate Reader (FLIPR) technology.[13] The activity of compounds at various concentrations was expressed as a percentage of the response evoked by a saturating concentration (100 μM) of cyclothiazide (**2**), and EC_{50} values were calculated. These data are shown in Tables 1 and 2.

The lead compound **6a** (Fig. 1) showed potentiator activity with EC_{50} values of 214 nM and 1558 nM at GluR4 flip and GluR4 flop receptors, and 97 nM and 309 nM at GluR2 flip and GluR2 flop receptors, respectively. Flip splice variant preferences were observed in both GluR2 and GluR4 receptors, although higher potency was exhibited in GluR2 receptors. Comparing thiophene **6a** with the previously reported sulfonamides **5a** and **5b** in GluR4 flip receptors; thiophene **6a** was equipotent with **5b** and 2-fold more potent than **5a**. Moreover, cyclothiazide **2** was 17-fold less active than **6a**.



Scheme 2. Synthesis of compounds **6b–k**. Reagents and conditions: (a) RSH, *t*-BuOK, (*R*)-(+)-BINAP, $Pd(PPh_3)_4$, toluene, 90 °C, (75–90%); (b) MCPBA (2.0 equiv), DCM, 0 °C to rt (47%); (c) 2 M NaOH/EtOH 1:1, rt, overnight, then 1 M HCl (35–80%); (d) Me_2NH (2 N) in THF, rt (55%); (e) pyrrolidine, rt (90%); (f) Et_2Zn, DCM, rt, 5 days; (g) Na–Hg, Na_2HPO_4, MeOH, rt, 48 h; (h) $NaBH_4$, EtOH, 0 °C (30%); (i) MCPBA (1.0 equiv), DCM, 0 °C to rt (53%).



Scheme 3. Synthesis of compound **6l**. Reagents and conditions: (a) ethyl 2-mercaptoacetate, KOAc, EtOH, 60–70 °C, 30 min (31%); (b) CH_2I_2, CH_3CN, isoamylnitrite, 35–65 °C, 10 min (81%); (c) i—*tert*-butyl phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, ethyleneglycol, 80 °C, overnight; ii—2 M NaOH/EtOH 1:1, rt, overnight, then 1 M HCl (25%, two steps).

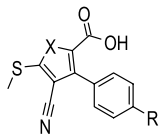
We next explored the effect of the heterocycle as modulator of AMPA receptors. For these studies, the carboxylic acid, cyano and aryl groups were kept intact while the thiophene moiety was replaced by furan and *N*-methypyrrole. As shown in Table 1, *N*-methylpyrrole **19a** was slightly less potent than **6a** at both flip and flop isoforms of GluR4 receptors but 7-fold less potent at GluR2 flip receptors. However, replacement of sulfur by oxygen (compound **21**) was not well tolerated reducing the potency 9-fold in GluR4 flip. Based on these results, we chose the thiophene and pyrrole cores and replaced the *tert*-butyl by a phenyl group (compounds **18** and **20** respectively) in order to avoid the potential metabolism issues related to *tert*-butyl group. In this case, phenyl derivative **20** increased the potency, slightly in case of GluR4 and 4-fold in GluR2 flip receptors compared to the *tert*-butyl analogue **19a**. However, this effect was not observed in thiophene derivative **18**.

Further details of SAR results in this region will be reported in further communications.

Once we confirmed that thiophene was one of the most potent five-membered heterocycles, we turned our attention to evaluating the effects of thioether replacements by other substituents. We started varying the alkyl group in thioether moiety (**6a**, **6c–f**, Table 2). All modifications in this region maintained flip selectivity in GluR4 receptor, but potency was more dependent on the steric hindrance of the substituents. In this way, activity decreased as larger groups were introduced (compounds **6d–f**). However, substitution at this position is mandatory since the unsubstituted thiophene **6b** did not show activity in any receptor at tested concentrations.

We also investigated whether groups with opposite electronic or polar features could replace thiomethyl moiety. Starting from the readily accessible oxidation products derived from **6a**, sulfoxide **6g** and sulfone **6h**, we observed that in both cases, these electron-withdrawing substituents decreased the activity at GluR4 flip receptors and did not show activity in GluR4 flop. However, trifluoromethyl derivative **6l** shown similar potency to **6a** in GluR4 (EC_{50} = 273 nM) and GluR2 (EC_{50} = 78 nM) flip receptors, but was 3-fold more potent in GluR4 flop (EC_{50} = 488 nM). The replacement of thiomethyl by ethyl group (entry **6k**) was well tolerated and exhibited similar potency and selectivity to **6a**. However, amino substitution (entries **6i** and **6j**) reduced the activity in tested receptors following the

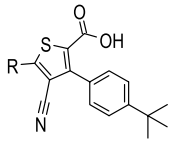
Table 1. EC_{50} values of selected novel AMPA receptor allosteric modulators



Compound	X	R	EC_{50} (nM)			
			GluR4-flip	GluR4-flop	GluR2-flip	GluR2-flop
2[a]	—	—	3800	NT[b]	NT	NT
5a[a]	—	—	660	NT	NT	NT
5b[a]	—	—	420	1061	559	1905
6a	S	*t*-Bu	214	1558	97	309
18	S	Ph	795	>3000	NT	NT
19a	NMe	*t*-Bu	554	1423	684	663
20	NMe	Ph	394	1301	174	474
21	O	*t*-Bu	1979	>3000	NT	NT

[a] See Figure 1 for structures of **2**, **5a**, and **5b**.
[b] NT, not tested.

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



Compound	R	EC_{50} (nM)			
		GluR4-flip	GluR4-flop	GluR2-flip	GluR2-flop
6a	MeS	214	1558	97	309
6b	H	>3000	>3000	>3000	>3000
6c	EtS	440	>3000	NT[a]	NT
6d	*n*-PrS	2892	>3000	NT	NT
6e	*i*-PrS	1873	>3000	NT	NT
6f	PhS	>3000	>3000	NT	NT
6g	SOMe	489	>3000	NT	NT
6h	SO_2Me	629	>3000	333	1808
6i	NMe_2	474	>3000	176	2854
6j		1009	>3000	1137	2433
6k	Et	183	1221	47	216
6l	CF_3	273	488	78	231

[a] NT, not tested.

same trend as thioether derivatives (**6a**, **6c**–**f**). The more hindered 5-pyrrolidinyl thiophene **6j** became less potent than the dimethyl amino analogue **6i** in both GluR2 and GluR4 receptors.

We also observed that carboxylic acid played a crucial role in terms of activity. While **6a** was very potent in both GluR2 and GluR4 receptors (Table 2), the ester precursor **13a** did not show activity in any receptor (EC_{50} > 3000 nM in GluR4 and GluR2 flip and flop) at tested concentrations. This result suggested that carboxylic acid could be establishing a hydrogen bond interaction with the receptor. In order to test this hypothesis, we explored more thoroughly this domain. Those results will be reported in further communications.

In summary, we have designed, synthesized, and evaluated a novel class of AMPA potentiators. Initial SAR studies demonstrated that the *N*-methylpyrrole derivative **19a** showed similar potency to the thiophene lead **6a** at GluR4 receptors and was only slightly less active at GluR2 receptors. SAR trends at the thioether moiety were achieved and better potency was found with small groups independently of the electronic features. However, substitution at this position was mandatory. Flip splice variant selectivity was also predominant in both series.

Acknowledgments

The authors gratefully acknowledge J. L. Roldán and J. C. de la Rosa and Prf. J. C. Carretero from Universidad Autónoma de Madrid for their contributions to this paper and to the Analytical Technologies group at Lilly for the analytical work.

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13. Typical protocol for this assay: 96-Well plates containing confluent monolayers of HEK-293 cell stably expressing human AMPA receptors were prepared. Cells were incubated in buffer solution (5.5 mM glucose, 136.9 mM NaCl, 0.8 mM $MgSO_4{\cdot}7H_2O$, 5.4 mM KCl, 5 mM $CaCl_2{\cdot}2H_2O$, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.34 mM $Na_2HPO_4{\cdot}7H_2O$, and 20 mM HEPES) containing 8 μM Fluo3-AM dye (obtained from Molecular Probes Inc., Eugene, OR) for 60 min. Measurements were made using a FLIPR that indicates changes in fluorescence upon influx of calcium into cells upon stimulation by glutamate (100 μM) in the presence of cyclothiazide (100 μM) or compound. Each compound was run in duplicate 10 point curves and each concentration and replicate were in a single well on the same plate on the same cells.