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Benzoxazinones as potent positive allosteric AMPA receptor modulators: Part I

Rudolf Mueller*, Yong-Xin Li, Aidan Hampson, Sheng Zhong, Clayton Harris, Christopher Marrs, Stanislaw Rachwal, Jolanta Ulas, Lena Nielsson, Gary Rogers

Cortex Pharmaceuticals Inc., 15231 Barranca Parkway, Irvine, CA 92618, USA

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ABSTRACT

AMPA receptors (AMPARs) are an increasingly important therapeutic target in the CNS. Aniracetam, the first identified potentiator of AMPARs, led to the rigid and more potent **CX614**. This lead molecule was optimized in order to increase affinity towards the AMPA receptor. The substitution of the dioxine with a benzoxazinone ring system increased the activity and allowed further investigation of the sidechain SAR.

(L)-Glutamic acid is the main excitatory neurotransmitter in the CNS, with the subset of ionotropic glutamatergic postsynaptic receptors (iGluRs) consisting of *N*-methyl-D-aspartic acid (NMDA), kainic acid (KA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. Positive allosteric modulators of AMPARs (AMPAKINE®s) have been the subject of a number of studies and reviews.[1–7] Based on neural pathways, these compounds could be used to treat a range of psychiatric and neurologic disorders[8–13] such as Attention Deficit Hyperactivity Disorder (ADHD), mood disorders, Schizophrenia, Huntington's, Parkinson's and Alzheimer's diseases. A subset of AMPAR modulators have been shown to upregulate the production of brain derived neurotrophic factor (BDNF).[14–16] A structural subset of Ampakines and most other known potentiators, bind at an extracellular subunit interface, located near the agonist binding site. Most of these modulators increase the binding affinity of glutamate at the agonist site in a manner analogous to what is seen for the effect of benzodiazepines on the GABA receptor. In addition, various potentiators may alter the duration of channel opening, desensitisation and/or receptor rate constants for transmitter binding. It was shown, that these molecules facilitate the induction of long term potentiation (LTP), a key element in memory formation and learning, which could lead to the development of these molecules into cognition enhancers.[17–24]

Aniracetam binds at the dimer interface[5] of AMPARs, enhancing the action of glutamate for a brief period of time. However, it is weak and has a short in vivo half life.

The imide structure of aniracetam is hydrolytically labile, which explains the short in vivo duration of action. With this in mind, the conformationally rigid benzoxazinone **CX614** was synthesized, by linking the amide oxygen to the benzene ring, in order to transform the hydrolytically unstable imide into a chemically more stable molecule (Fig. 1). The methoxy group was also exchanged for the 1,4-dioxine moiety to provide a six-membered ring. Taken to-

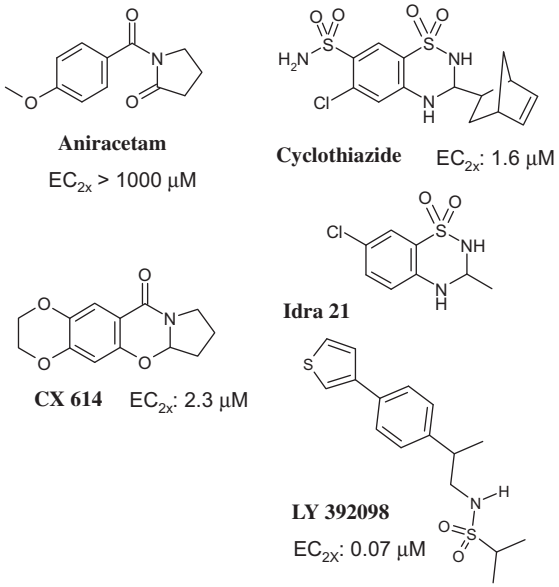

Figure 1. Allosteric AMPAR positive modulators.

* Corresponding author.
E-mail address: rudolfmueller@yahoo.com (R. Mueller).

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gether, these modifications translated into the significantly enhanced affinity and stability of **CX614**.

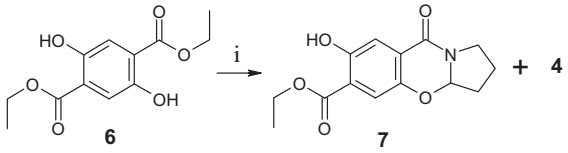
Several pharmacological scaffolds are now known to positively modulate AMPAR function,[25–44] among them are the intensively studied sulfonamides cyclothiazide, Idra21, LY392098 and their derivatives. The benzamide type AMPAKINE®s in our study are chemically distinct, however, it was shown that the binding sites of aniracetam, **CX614** and cyclothiazide overlap.[5]

In this Letter, we wish to report the optimization of our lead **CX614** into a series of structurally related derivatives. To study the role of the 1,4-dioxine ring when **CX614** binds to the receptor, the oxygen atoms of the dioxine ring were moved in **1** and **2**, or one oxygen was replaced with a methylene group to form the pyran derivative **3** (Fig. 2). Because the geometric shapes of **CX614**, **1**, **2** and **3** are nearly identical, we expexted to be able to determine the importance of the oxygen atoms in the ring system.

The effect of these molecules on AMPA induced currents was evaluated in cultured rat embryonic hippocampal neurons in a patch clamp electrophysiology assay. The readout was the EC_{2x}, the concentration of a compound, which when perfused onto those neurons, doubled the current flow.[45] The comparison to **CX614** (EC_{2x}: 2.3 μM) was used as the guideline to optimize the in vitro activity of the derivatives in this study. Compound **4** turned out to be a very potent Ampakine (EC_{2x}: 0.06 μM) and was, therefore, chosen to be radiolabeled (^3H) and used as a probe for in vitro receptor binding studies[46] since it also binds to the **CX614**/cyclothiazide binding site. The radioligand binding assay was performed using a cell membrane preparation from adult rat forebrain.

Besides investigating these modified **CX614** derivatives, we intentionally synthesized the bis-benzoxazinones **4** and **5** in order to investigate whether symmetrical molecules bind to the receptor as well.

The synthesis started with commercially available diethyl 2,5-dihydroxy-terephthalate **6**, which was heated (no solvent) together with 4-amino butyraldehyde diethyl acetal (Scheme 1). The crude amide cyclized under acidic workup conditions to yield the very versatile intermediate **7** and **4** (which consists of three isomers). The products of this reaction were separated easily using column chromatography, which enabled us to quickly isolate 50 g batches of **7** for further use. The original synthesis of **4**, using an excess of amine, resulted in an almost quantitative yield of **4**. The conditions in Scheme 1 were optimized in order to maximize the yield of **7**.



Scheme 1. Synthesis of intermediate **7** and benzoxazinone **4**. Reagents and conditions: (i) 1.0 equiv **6**, 1.6 equiv 4-amino butyraldehyde diethylacetal, 120 °C, 7 min; HCl, 20 °C, 60 min, 64–71% **7** and 10–20% **4**.

The reduction of **7** (Scheme 2) with $LiBH_4$ to the colorless alcohol **8** required prolonged heating of the reaction mixture. The yellow aldehyde **9** was isolated as the main product, when the reaction temperature was maintained at 20 °C.

Ring closure of **8**, using trioxane under acidic conditions yielded **1**, which turned out to be slightly more active (EC_{2x}: 1.1 μM) than **CX614**. This example shows, that the oxygen can be moved from position 4 into position 3, without having a significant impact on activity. Ring closure of **9** with triphenylvinylphosphonium bromide[47] yielded the chromene derivative, which was hydrogenated to **3** (EC_{2x}: 1.1 μM). Replacement of the oxygen with CH_2 in the 4-position barely affected the activity of the molecule, which demonstrates, that the oxygen in the 4-position of **CX614** is not essential and can be replaced by other functional groups or substituents, potentially leading to more potent molecules.

To explore, whether the hetero atom in the 1-position of **CX614** is important to receptor binding, we planned to synthesize **2** and **5** (Scheme 3). The Kolbe–Schmitt reaction, using **10** as the starting material, yielded the bis-acid, which was transformed into the bis-ester **11** in good overall yield. This material was treated with 4-amino butyraldehyde diethylacetal as before, to yield **5** and **12**. The bisbenzoxazinone **5** (EC_{2x}: 2.6 μM) is as active as **CX614**, but clearly less active than its isomer **4** (EC_{2x}: 0.06 μM). Reduction of **12**, followed by ring closure gave **2** (EC_{2x}: 22 μM), which is again clearly less active than its isomers **CX614** and **1**.

Based on these observations, it is clear, that the oxygen in position 1 of **CX614** is important for in vitro binding activity. We wanted to modify the remaining ring-system further, and the benzoxazinone ring appeared to be an ideal starting point, since increased activity was observed for **4** and this structure presented an interesting scaffold, to which side chains can be attached via the nitrogen atom. However, we had tested only six-membered derivatives and needed to investigate five- and seven-membered

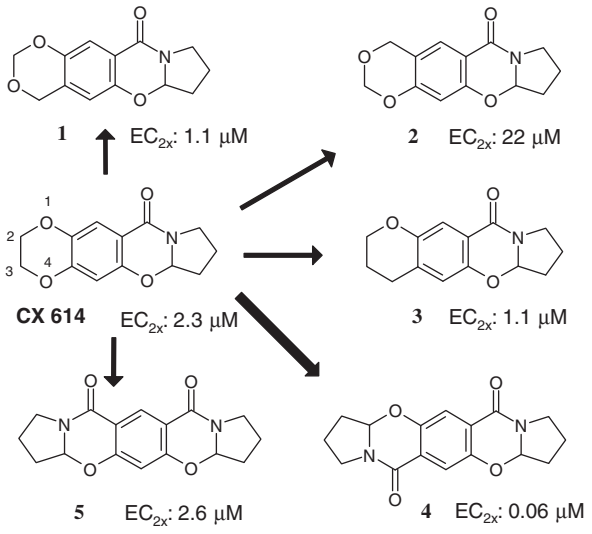
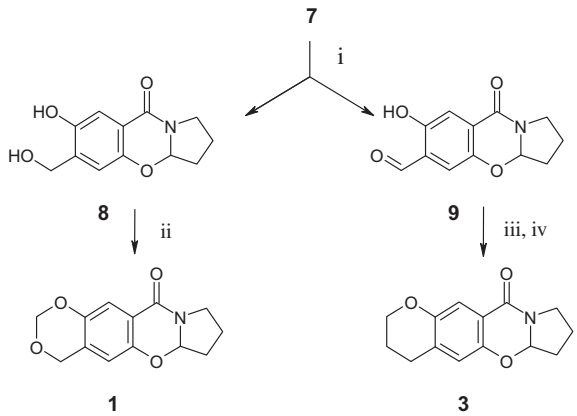
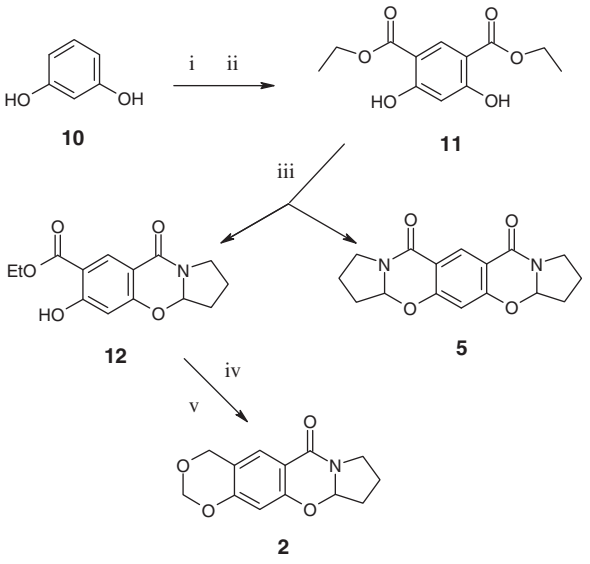


Figure 2. Modification of the lead **CX614**.

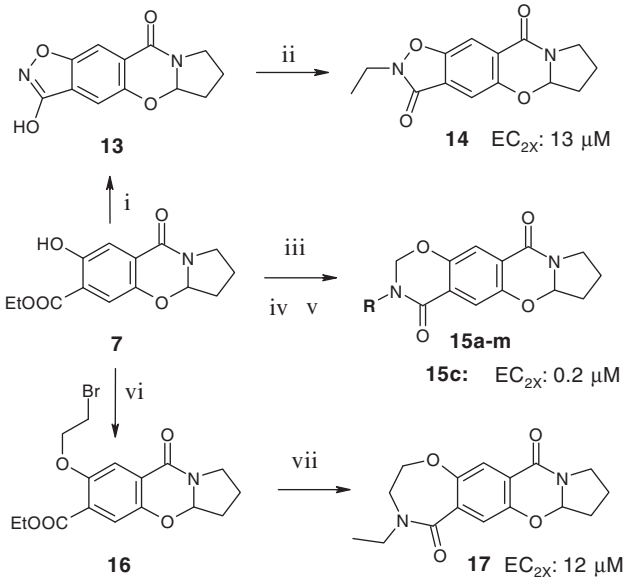


Scheme 2. Synthesis of 1,3-dioxine **1** and dihydrochromene **3**. Reagents and conditions: (i) 1.0 equiv **7**, 2 equiv $LiBH_4$, 20 °C, 2 h, 20–48% **8** and 27–42% **9**; (ii) dioxane, excess trioxane, $CuSO_4$, H_2SO_4, 20 °C, 18 h, 76% **1**; (iii) toluene/DMF 1.2 equiv NaH, 2 equiv CH_2CHPPh_3Br, 2 h, 110 °C, 54%; (iv) $Pd/C/H_2$, AcOEt, 2.5 h, 86% **3**.



Scheme 3. Synthesis of reversed benzoxazinone **5** and 1,3-dioxine **2**. Reagents and conditions: (i) 1.0 equiv **10**, 3 equiv $KHCO_3$, 800 psi CO_2, 230 °C, 6 h, 97%; (ii) EtOH, $SOCl_2$, 90 °C, 8 h, 69% **11**; (iii) 2.3 equiv 4-amino butyraldehyde dimethylacetal, 120 °C, 15 min, then HCl concd 10% **5** and 73% **12**; (iv) 3.8 equiv $LiBH_4$, 70 °C, 2.5 h, 65%; (v) dioxane, excess trioxane, cat. H_2SO_4, 18 h, 20 °C, 33% **2**.

rings in comparison. The synthetic route (Scheme 4) to these molecules started in each case with **7**, which was treated with hydroxylamine and thionylchloride to form the isoxazole derivative **13**. Alkylation occurred only on the nitrogen to form the five-membered ring in **14**. The benzoxazinones **15a**–**m** were synthesized[48] by hydrolyzing ester **7**, followed by amide formation using standard conditions. Ring closure with trioxane and an acid catalyst formed compound **15c** (R = Et). In order to synthesize the larger



Scheme 4. Synthesis of five-, six- and seven- membered rings. Reagents and conditions: (i) 1.0 equiv **7**, 1.6 equiv H_2NOH, 3 equiv NaOH, 2 h, 20 °C; 2.3 equiv $SOCl_2$, 15 min, 3 equiv NEt_3, 60 min, 20 °C, 73% **13**; (ii) 3.6 equiv K_2CO_3, 2.5 equiv EtI, 58 °C, 18 h, 50% **14**; (iii) 15 equiv KOH, MeOH/water, 1.5 h, 50 °C; (iv) 1.0 equiv DMAP, 1.0 equiv HOBT, 2.5 equiv NEt_3, 2.0 equiv EDCI, 1.3 equiv amine, DMF, 20 °C, 4 h; (v) $CHCl_3$, excess trioxane, cat. H_2SO_4, 2 h, 70 °C, 20–90% (three steps) **15a**–**m**. (vi) 2.5 equiv K_2CO_3, excess $BrCH_2CH_2Br$, 110 °C, four days, 76%; (vii) $EtNH_2$, 4 equiv KOH, 1 equiv HOBT, 1 equiv DMAP, 1 equiv NEt_3, 3 equiv EDCI, three days, 20 °C, 59% **17**.

Table 1
Influence of alkyl-substitution on AMPAR activity

Compound	Substituent	EC_{2x}[a] (μM)	Slice ampl.[b] % (μM)	K_i[c] (μM)
15a	H–	5.0	5 [30]	225
15b	Methyl–	0.1	19 [10]	18
15c	Ethyl–	0.2	17 [10]	20
15d	*n*Propyl–	2.5	0 [1]	70
15e	*i*Propyl–	0.9	3 [10]	100
15f	Cyclopropyl–	0.8	10 [10]	26
15g	Propargyl–	0.25	6 [3]	7
15h	Allyl–	0.5	—	18
15i	2-Methylpropyl–	41	—	—
15k	Cyclopentyl–	7.0	—	163
15l	FCH_2CH_2–	0.5	9 [10]	33
15m	CF_3CH_2–	8.8	0 [1]	330

[a] EC_{2x} values[45] are defined as the concentration of compound required to double the steady-state current induced by glutamate.
[b] In vitro slice assay:[49] percentage increases in the amplitude of fEPSP, recorded at indicated concentration (— = no data available).
[c] Displacement of tritiated AMPAKINE radioligand **4** in cell membrane preparations from adult rat forebrain.

ring, the phenol of **7** was first alkylated with 1,2-dibromoethane to form **16**, then the halogen was substituted with ethylamine and the seven-membered ring closed to form **17**.

The six-membered ring derivative **15c** is clearly superior to the 5- and 7-ring analogs **14** and **17** by comparison of the EC_{2x} values.[45] Consequently we focused our attention on a series of derivatives of **15c** (as shown in Table 1).

The derivatives **15a**–**m** were synthesized following the general procedure in Scheme 4. A small substituent on the nitrogen provides optimum affinity as demonstrated by **15b**, **15c** and **15g**, while the unsubstituted derivative **15a** loses activity both in the binding assay and the patch clamp electrophysiology assay. The larger substituents in **15i** and **15k** are clearly detrimental.

The fluorine in **15l** has only a minimal effect, however, the three fluorines in **15k** reduce the activity significantly, in addition to having a negative effect on solubility.

The slice data[49], reflecting activity in a neuronal network, support the results based on binding data and EC_{2x} (single cell recordings) that smaller alkyl substituents are preferable in comparison to larger ones (compare **15b**, **15c** and **15e**).

In summary, the oxygen in position 1 of **CX614** proved to be important for in vitro AMPAR activity, whereas the oxygen in position 4 can be replaced or moved into position 3 without activity change. A six-membered ring attached to the central benzene ring is favorable over five- or seven-membered rings. A second benzoxazinone system increases AMPAR activity up to 23-fold in combination with a small substituent and 38-fold when a second pyrrolo benzoxazinone system is attached. Radioligand binding data, EC_{2x} and slice data correlate for the most part when the alkyl derivatives (Table 1) are compared. This approach permits attachment of a wide variety of nitrogen bound substituents, which will be investigated further.

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45. *Patch clamp recording and EC_{2x} estimate*: On cultured hippocampal neurons (Sprague–Dawley rat cells removed at embryonic day 18, 4–7 days in culture), 500 μM Glutamate-induced currents with or without AMPA receptor modulators were measured under whole-cell recording with patch-clamp amplifiers (Axopatch 200B; MDS, Inc., Toronto, Canada) at room temperature. All compounds or saline were applied by DAD-12 or DAD-VC superfusion system (ALA Scientific Instruments Inc., New York). The 13–1 or 17–1 tip was placed approximately 100–150 μM from the cell. Cells (voltage-clamped at −80 mV) were pre-treated for 20 s with the saline/modulators before the application of glutamate/glutamate plus the modulator for 1 s. The mean value of plateau current between 600 to 900 ms after application of glutamate was measured and used as the parameter to evaluate the compound's effect. EC_{2x} value is defined as the concentration (in μM) of the compound required to double the steady-state current induced by glutamate.
46. In a test run with **4**, we were able to achieve 95% H/D exchange, using D_2 gas and Crabtree's catalyst. The active, tritiated enantiomer was synthesized accordingly, isolated by HPLC (Chiralpak IA) and used in binding studies.
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49. *In vitro slice assay*: Transverse 300–400 μM hippocampal slices were taken from adult male Sprague–Dawley rats (4–6 week old) and incubated on nylon net in a rapid-flow linear interface recording chamber. The chamber was perfused with oxygenated artificial cerebral spinal fluid (ACSF). Field EPSP (excitatory postsynaptic potential) recordings were made with glass electrodes filled with 2 M NaCl (1–5 megaohm), placed in the stratum radiatum or stratum oriens of CA1. Electrical stimuli were produced with twisted bipolar nichrome wire electrodes in the Schaffer-commissural fiber afferents. EPSP was induced by activating Schaffer-commissural fiber using pair pulse stimulation with a pulse interval at 200 ms delivered at 0.05 Hz. The amplitude, half-width and area were measured for each response and plotted against time. After a stable baseline was established (20–30 min), test compound was infused for 30 min to observe the effect. Then the solution was switched to control ACSF and continued to record until drug effect was washed back to the baseline. Most of the compounds were made into stock solution by dissolving in DMSO then adding into ACSF. If DMSO concentration has to be >0.05%, DMSO was also added into the control ACSF. Both Control ACSF and test compound were infused to the recording chamber bath by gravity infusion system.