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Design, synthesis, and biological evaluation of a scaffold for iGluR ligands based on the structure of (−)-dysiherbaine

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Abstract—The design and synthesis of four 2,2-disubstituted dihydrobenzofurans that are structurally related to several glutamate-containing natural products, including (−)-dysiherbaine, is described. Biological evaluation of these analogs shows that one is a KA receptor antagonist and another is an NMDA receptor agonist.
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1. Introduction

Over the past 5 years, significant attention has been devoted to the chemical synthesis of natural product-like libraries.[1] As Nature has already identified natural products as lead structures of biological relevance, libraries based on specific substructures found across a class of natural products are, to an extent, biologically validated.[2] Accordingly, libraries generated from core scaffolds derived from natural products are proving to be a valuable source of biologically active compounds that provide new probes for molecular targets.[3,4]

Our interest in natural product-based libraries was motivated by our ongoing research efforts to discover novel ligands for the ionotropic glutamate receptors (iGluRs).[5] Characterizing the iGluRs is a top priority not only because of their function in normal CNS processes such as learning and memory, but also due to their role in causing damage in neurodegenerative disorders including stroke, epilepsy, and Alzheimer's disease.[6] Attempts to delineate the properties of the iGluR subtypes, however, have been impeded by a lack of selective ligands. Given the successes of natural product-based libraries in the discovery of new ligands for biological targets,[1a,7,8] we envisioned application of this concept to biologically active natural products known to interact with glutamate receptors. In this Letter, we outline the first essential steps in this plan, the design and synthesis of a natural product-based library scaffold candidate, along with several substituted variants, and report their activity as iGluR ligands.

Naturally occurring γ,γ-disubstituted glutamates such as the kainate receptor agonists (−)-dysiherbaine[9] (**1**) and (−)-neodysiherbaine[10] (**2**), isolated from the Micronesian marine sponge *Dysidea herbacea*, and (*S*)-(+)-lycoperdic acid[11] (**3**), a non-proteinogenic α-amino acid isolated from the mushroom *Lycoperdon perlatum*, have attracted wide attention among the chemical and biological communities due to their distinctive structures (Fig. 1) and unique biological profiles.[12–14] These structurally similar glutamate-containing natural products were therefore selected as viable, biologically validated starting points for library design. Inspired by the current interest in using specific substructures found within a class of natural products as library scaffolds,[4,7] a glutamate-appended oxolane (tetrahydrofuran) ring—the core structural motif shared by **1**, **2**, and **3**—was selected as the template for library synthesis. In order to provide increased opportunities for structural diversity, a phenyl ring was introduced as a framework upon which to display diverse functional groups. Thus, the γ,γ-disubstituted glutamate-appended dihydrobenzofuran **4** evolved as the targeted scaffold for the synthesis of the next generation of glutamate analogs.

Clearly, synthetic accessibility and retention of reasonable levels of iGluR activity in the core scaffold **4** are important prerequisites for the success of this plan. In addition, information concerning which positions on

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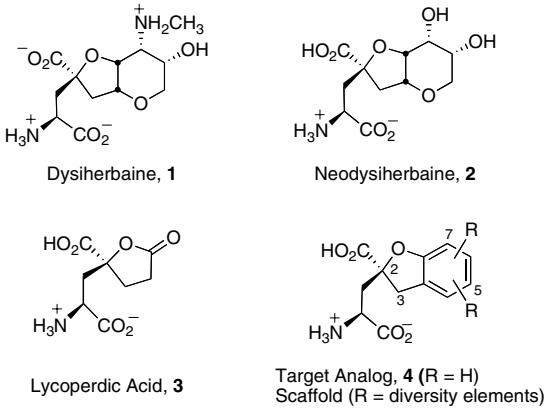


Figure 1. Glutamate-containing natural products and simplified analog.

the phenyl ring might best tolerate substituents would be useful from a library design perspective. Accordingly, we conducted preliminary modeling studies in order to assess computationally whether the proposed core scaffold **4** might be a reasonable ligand for AMPA/KA iGluR receptors.[15] Specifically, **4** was docked into the glutamate binding cleft of Gouaux's X-ray structure of a kainate–iGluR2 construct complex, which at the time was the most reliable structure available for AMPA/KA receptor binding sites.[16,17] The resultant calculated structure (Fig. 2) indicated that the aryl ring is well accommodated in the binding site (contact residues are shown in lighter teal). The α-amino and α-carboxyl groups (not visible in the view shown) make contacts analogous to the corresponding residues in the Gouaux kainic acid complex upon which this structure was based, while the methylene group of the dihydrobenzofuran is favorably wedged against the face of the phenolic ring of Tyr-450, as shown. In addition, two of the four C–H bonds of the scaffold's aryl ring—in the 4- and 5-positions—are directed toward the mouth of the binding cleft; in contrast, the corresponding bonds at the 6- and 7-positions are buried.

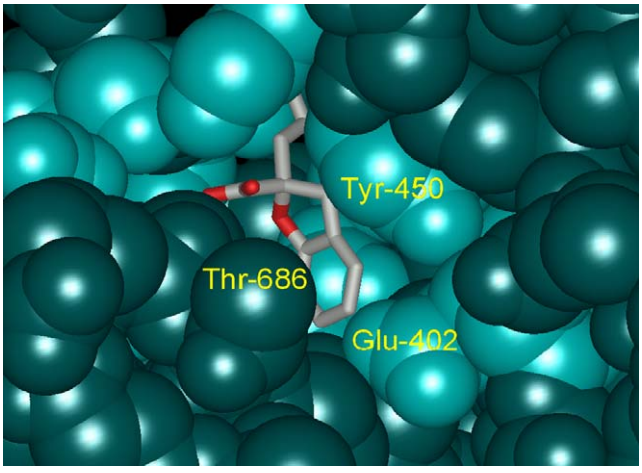


Figure 2. Docking of **4** into Gouaux's iGluR2 construct (α-carboxyl and amino groups are obscured; see text for the significance of labeled contacts).

This model provided some reassurance that **4** might indeed act as an acceptable surrogate for **1**–**3** as a library scaffold, and further, it suggested that substitutents would be better tolerated at C-4 and/or C-5 of the aryl ring than at C-6 or C-7. As an initial experimental test of this model, three analogs were envisioned in addition to the parent compound **4**: the C-5 and C-7 methyl derivatives, and the C-2 epimer (which in effect interchanges the oxygen and methylene substituents in the tetrahydrofuran ring without changing the overall orientation of binding). The calculated complex suggests not only that a C-5 methyl analog of **4** should be a better ligand than its C-7 methyl counterpart, but also that binding of the C-2 epimer would be unfavorable because the ring oxygen lone pairs, rather than the methylene group of the parent structure, would be in contact with the electron-rich face of the tyrosine-450 phenyl ring (assuming the overall orientation of the ligand does not change). To investigate these computational predictions, the analogs in question were synthesized and submitted to biological evaluation.

The development of a stereoselective and scalable synthesis of **4** is paramount to the success of this library plan. The major synthetic challenge in the preparation of **4** and related analogs is establishing the stereochemistry of the tetrasubstituted carbon center in the glutamate-appended oxolane ring. A synthesis plan that appeared most appealing in its generality, simplicity, and potential diastereoselectivity was a three-step pyroglutamate α-annulation consisting of: (1) α-benzylation of a suitably protected pyroglutamate derivative with an electrophile carrying a latent phenoxide nucleophile, (2) diastereoselective α-halogenation, and (3) stereospecific S_N2 intramolecular displacement of the halide by the phenoxide nucleophile.

Accordingly, the synthesis of scaffold **4** commenced with α-benzylation of the protected pyroglutamate **5**[18] using an aldol/elimination/reduction sequence[19] to afford the 3,5-disubstituted pyrrolidinone **6** in 85% yield over three steps. The second step of this synthesis plan required the diastereoselective halogenation of this pyrrolidinone, which after an exhaustive screening of various halogenation procedures was realized by the sequential treatment of **6** with Et_3N, TMSOTf, and *N*-bromosuccinimide to afford a separable mixture of the diastereomeric bromides **7** and **8** in 87% yield. The ratio of diastereomers was 5:1 as determined by ^1H NMR analysis of the crude reaction mixture, and the stereochemistry of the major isomer, **7**, was established by NOESY studies on the corresponding demethylated derivative **9**. The third step of the glutamate ring annulation, an S_N2 cyclization of brominated phenol **9**, was achieved by treatment with DBU at room temperature to furnish the dihydrobenzofuran **10** in 91% yield and establish the stereochemistry at the tetrasubstituted center (vide infra).

With all of the stereogenic centers of the target molecule secured, sequential *N*-Boc protection and desilylation produced pyroglutaminol **11** in 82% yield over two steps. At that point, the stereochemistry of the tetrasubstituted α-center was verified by NOESY experiments,

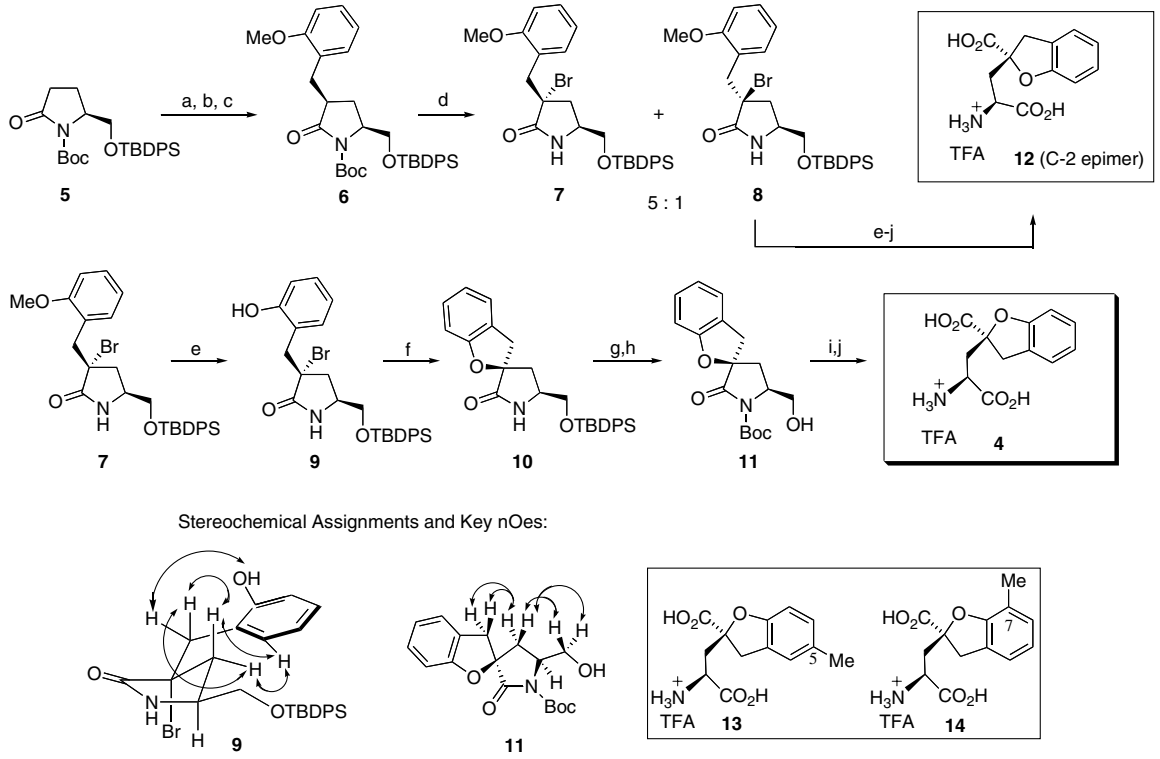
indicating that the S_N2 cyclization to form **11** had proceeded stereospecifically with inversion, as expected, to give the desired stereoisomer. Completion of the synthesis proceeded with Jones oxidation of pyroglutaminol **12** to the corresponding carboxylic acid followed by imide hydrolysis and *N*-Boc deprotection in refluxing 6M HCl to provide crude **4** as a white solid.[20] Purification by reverse-phase HPLC afforded the pure parent scaffold **4** as the TFA salt. The C-2 epimer **12** was similarly prepared from the minor bromination diastereomer **8** (Scheme 1), and the C-5 and C-7 methylated analogs, **13** and **14**, respectively, were likewise synthesized in an identical sequence by substituting the appropriate methyl-substituted anisaldehydes for the *o*-anisaldehyde employed in the initial benzylation of **5**. Overall, this sequence highlights an efficient and reasonably diastereoselective method for the annulation of a substituted oxolane ring onto the γ-position of glutamic acid, and it provided in this case ample quantities for biological evaluation.

The activities of the parent compound **4** and its analogs (**13**, **14**, and **12**) were screened in oocytes injected with native mRNA from rat cerebral cortex.[21] Potential KA receptor agonist activity was assayed based on the membrane current generated at a concentration of 100 μM for each analog against 100 μM of kainate as control. The same compounds were also screened for antagonist activity by measurements of 100 μM kainate-induced membrane currents inhibited by the presence of each analog (100 μM). Experiments were quite reproducible in multiple oocyte preparations ($N = 2–7$), generally with a standard error of only a few percent.

In the agonist activity assays (Fig. 3), there was no current observed for the parent scaffold **4** or for its 5-methyl analog **13** at concentrations of 100 μM, while the control agonist kainate induced currents at concentrations as low as 10 μM; the C-2 epimer **12** also showed no activity in this assay (data not shown). On the other hand, at 100 μM the corresponding 7-methyl analog **14** alone did elicit a weak current that was 6 ± 1% of the kainate control at the same concentration. Significantly, this signal was approximately doubled, to 13 ± 4% of control, in the presence of a 10 μM concentration of the AMPA-specific potentiator cyclothiazide (CTZ). These observations suggest that **14**, but not **4**, **12**, or **13**, is acting (at least in part) as an agonist—albeit a rather weak one—at AMPA receptors.

Similar experiments were conducted on the analogs in the presence of the NMDA-specific co-agonist glycine. Once again, the parent scaffold **4** showed no activity under these conditions, nor did **14**; however, the 5-methyl analog **13** elicited a significant current at 100 μM, 44 ± 11% of control, suggesting that it is an NMDA agonist.

In direct contrast to the complete inactivity of the parent scaffold **4** in the agonist assays, it proved to be a reasonably potent antagonist that reduces kainate control currents by approximately one-half (to 55 ± 13% of control) at a concentration of 100 μM (Fig. 4).



Scheme 1. Synthesis of parent core scaffold **4** and analogs **12**, **13**, and **14**. Reagents and Conditions: (a)—(i) LiHMDS, THF, −78 °C (ii) *o*-anisaldehyde, $BF_3 \cdot OEt_2$, 99%; (b) Ph_3P, I_2, imidazole, CH_2Cl_2, 0 °C to rt, 92%; (c) H_2, Pd/C, EtOAc, 93%; (d) TMSOTf, Et_3N, NBS, CH_2Cl_2, 0 °C, 87%; (e) BBr_3, CH_2Cl_2, 0 °C to rt, 63%; (f) DBU, CH_2Cl_2, 0 °C, 91%; (g) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 90%; (h) TBAF, AcOH, THF, 91%; (i) Jones reagent, acetone, 0 °C, 56%; (j) 6M HCl, reflux, then HPLC, 58%.

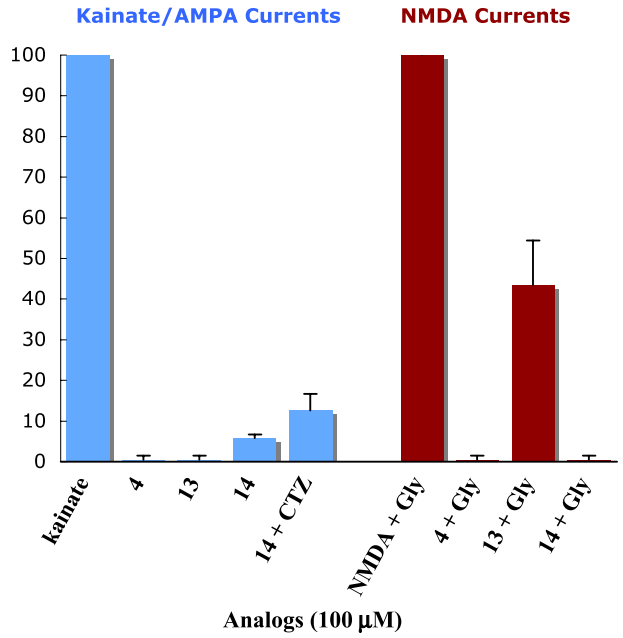


Figure 3. Agonist activity. Comparison of parent dysiherbaine analog **4** to its C-2 epimer **12**, and the methylated analogs **13** and **14**. Agonist activity assays were conducted in oocytes injected with native mRNA from rat cerebral cortex, and the results (blue bars) are expressed as the percentage of current induced by 100 μM kainate (with and without the AMPA receptor desensitization inhibitor cyclothiazide (CTZ, 10 μM). NMDA currents (red bars) were measured for each analog in the presence of NMDA plus the NMDA co-agonist glycine (both at 100 μM), with NMDA plus glycine (both at 100 μM) as the positive control.

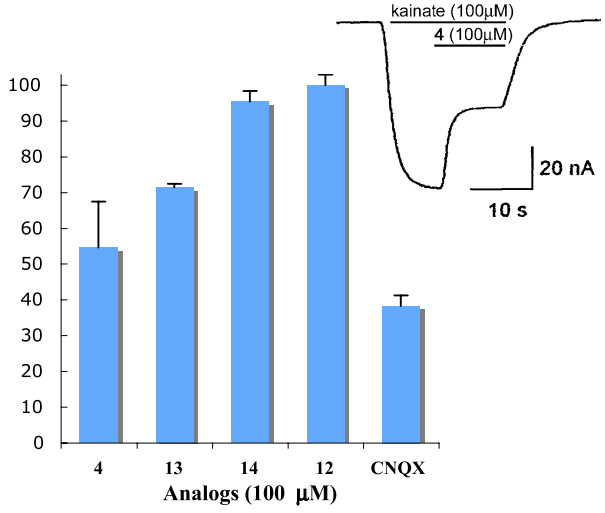


Figure 4. Antagonist Activity. Comparison of parent dysiherbaine analog **4** to its methylated counterparts **13** and **14**, its C-2 epimer **12**, and the AMPA/KA antagonist CNQX. Antagonist activity assays were conducted in oocytes injected with native mRNA from rat cerebral cortex, and the results are expressed as the percentage of current induced by kainate (100 μM) in the presence of equimolar amounts of analogs (100 μM; no analog = 100%). In this comparison, the very potent but non-selective CNQX was present at 1 μM. Inset shows a typical example of a recording for this measurement (analog **4** in this case), with current on the *y*-axis and time on the *x*-axis.

Significantly from the perspective of the binding model, neither the 7-methyl derivative **14** (95 ± 3% of control) nor the C-2 epimer **12** (100 ± 3% of control) at 100 μM showed significant activity in antagonizing 100 μM kainate currents. Conversely, the C-5 methyl analog **13** did block the kainate control currents, albeit somewhat less potently than the parent compound, to 72 ± 1% of control. For comparison, the very potent antagonist CNQX was also assayed in this system, blocking 38 ± 3% of the current at a concentration of 1 μM. None of the analogs (at 100 μM) antagonized 100 μM NMDA + 100 μM glycine currents (data not shown).

The potency of dysiherbaine as an AMPA/KA receptor agonist led to the expectation that analogs such as **4** with similar core structures and steric characteristics might also act as agonists at these same iGluRs; however, the assays demonstrated that, instead, one (**13**) is an NMDA receptor agonist and another (**4**) is an AMPA/KA receptor antagonist. Specifically, none of the four analogs **4**, **12**, **13**, and **14** by itself elicited strong currents relative to kainate. Indeed only **14** showed any detectable agonist activity, by itself, under the assay conditions. The fact that the weak current observed for **14** is doubled in the presence of the AMPA-specific potentiator CTZ suggests that it is a weak AMPA receptor agonist. More interestingly, the 5-methyl analog **13** (but not **4** or **14**) did elicit a significant current, but only in the presence of the NMDA receptor co-agonist glycine. This result suggests that **13** is a selective NMDA receptor agonist with a potency somewhat lower than that of NMDA itself (a detailed characterization of the potency and efficacy of this compound is underway). Thus, while **14** is a weak AMPA/KA receptor agonist and **13** is a moderately active (and selective) NMDA receptor agonist, the parent scaffold **4** shows no detectable iGluR agonist activity.

While this result was briefly disappointing in terms of our original expectations, it assumed favorable significance when the antagonist activity assays revealed that an equimolar concentration **4** reduced kainate-induced currents by approximately 50%, suggesting that the parent analog **4** is an AMPA/KA receptor antagonist that is comparable in potency to that of kainate itself.[22] The 7-methylated analog **14** and the C-2 epimer **12** were both essentially inactive, and the 5-methyl derivative was intermediate in potency, so that the order of potencies determined in the antagonist assays is **4** > **13** ≫ **14**, **12**. The observation that NMDA/glycine currents are not diminished by any of the analogs demonstrates that the antagonist activity of **4** and **13** is selective for AMPA/KA receptors. Further, while **13** is also an NMDA receptor agonist, **4**, in contrast, appears to be strictly an AMPA/KA receptor antagonist.

While the antagonist activity of **4** and **13** was unanticipated based on the agonist characteristics of the dysiherbaine core around which the analogs were designed, it is certainly not unusual to observe such differences in behavior for closely related compounds.[12h] In fact, we recently reported an example in which analogs based

on the structure of the agonist kainate proved to be antagonists.[5] In that case, and in that of **4**, we rationalize the antagonist behavior to be the result of the phenyl ring of the respective ligands interfering with the formation of a critical inter-domain Glu402–Thr686 interaction (residues labeled in yellow, Fig. 2) that develops during the conformational change leading to channel opening, as proposed by Gouaux for the known antagonist DNQX. Regardless of its mechanistic origins, this unexpected activity, although serendipitous, provides an exciting new lead for the development of new, selective iGluR antagonists.

While the number of analogs admittedly is small, it was gratifying to note that the relative potencies of the four compounds were consistent with the predictions of the modeled complex, notwithstanding their unanticipated antagonist behavior. The docked structure of **4** suggested that (a) the 4- and 5-positions of the aromatic ring can tolerate additional steric bulk and may be amenable to elaboration, (b) the 6- and 7-positions cannot be further functionalized because they are buried, and (c) the C-2 stereochemistry cannot be inverted because the result would be an unfavorable interaction between the tetrahydrofuran ring oxygen lone pairs and the electron-rich face of the tyrosine-450 aryl ring. The assay results for **4**, **13**, **14**, and **12** are consistent with these predictions. Both **4** and **13** are in harmony with all three criteria and show good activity, while **14** and **12** are incongruent with (b) and (c), and are essentially inactive. Thus supported experimentally, this calculated structure, in conjunction with the validated synthetic route described in this Letter, provides a reasonable paradigm for the design of focused libraries. Furthermore, the prototype analog **4** is significant in its own right as a selective AMPA/KA receptor antagonist, as is the new NMDA agonist **13**. These results provide a significant new lead for the development of iGluR antagonists and agonists, and further motivate the generation of a library based on **4**, which will be reported in due course.

Acknowledgments

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