Bioorganic & Medicinal Chemistry Letters 20 (2010) 2680–2683

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Conformationally-restricted amino acid analogues bearing a distal sulfonic acid show selective inhibition of system x_c^- over the vesicular glutamate transporter

Jean-Louis G. Etoga [a], S. Kaleem Ahmed [b], Sarjubhai Patel [b], Richard J. Bridges [b], Charles M. Thompson [b,c,*]

[a] Department of Chemistry and Biochemistry, The University of Montana, Missoula, MT 59812, USA
[b] COBRE Center for Structural and Functional Neuroscience, Department of Biomedical and Pharmaceutical Sciences, The University of Montana, Missoula, MT 59812, USA
[c] ATERIS Technologies LLC, 901 N Orange Street, Missoula, MT 59802, USA

ARTICLE INFO

Article history:
Received 1 September 2009
Revised 5 October 2009
Accepted 5 October 2009
Available online 12 October 2009

Keywords:
Glutamate
Amino acid analog
Inhibitor
VGLUT
System x_c^-
Sulfonic acid

ABSTRACT

A panel of amino acid analogs and conformationally-restricted amino acids bearing a sulfonic acid were synthesized and tested for their ability to preferentially inhibit the obligate cysteine–glutamate transporter system x_c^- versus the vesicular glutamate transporter (VGLUT). Several promising candidate molecules were identified: *R*/*S*-4-[4′-carboxyphenyl]-phenylglycine, a biphenyl substituted analog of 4-carboxyphenylglycine and 2-thiopheneglycine-5-sulfonic acid both of which reduced glutamate uptake at system x_c^- by 70–75% while having modest to no effect on glutamate uptake at VGLUT.

L-Glutamate (**1**) is a key neurotransmitter responsible for the vast majority of the fast excitatory synaptic communication in the mammalian CNS. L-Glutamate acts at ionotropic glutamate receptors to mediate ligand gated ion channels and at metabotropic glutamate receptors to couple intracellular second messenger systems via G-proteins.[1–5] The importance of L-glutamate as a contributor to higher order processing required in development, plasticity, learning, and memory is well established.[4,6,7] However, glutamatergic excitotoxicity can result when an excess of L-glutamate occurs and continually activates glutamate receptors.[2,5,8] To maintain the proper titer of L-glutamate there is a network of strategically positioned transporters that shuttle L-glutamate in and out of cells and organelles. Most notable among these transporters are the excitatory amino acid transporters (EAATs) that facilitate the uptake of L-glutamate into neurons.[8]

In addition to EAATs, other transporters maintain intra- and extra-cellular levels of glutamate including; system x_c^-, a chloride-dependent, sodium-independent obligate exchanger that couples the export of intracellular L-glutamate with the import of extra-cellular L-cystine[9–12] and the vesicular glutamate transporter (VGLUT) that mediates the uptake of intracellular glutamate into synaptic vesicles.[6,13,14]

System x_c^- and VGLUT are structurally and functionally distinct from the EAATs and also from each other. Although system x_c^- and VGLUT differ pharmacologically,[5,8,15,16] both transporters remove L-glutamate from the cytosol. In principle, therefore, intracellular L-glutamate levels could be regulated by modulating one or both of these transporters.[16] As such, the development of inhibitors that selectively block system x_c^- and/or VGLUT represents an interesting pharmacologic challenge. Some inhibitors[9,16,17] have been reported for system x_c^- and for VGLUT[6,15,18–26] (Fig. 1). Two interesting features common to several system x_c^- and VGLUT inhibitors are the use of aromatic rings to conformationally lock[27] the acid groups (e.g., CPG) and sulfonic acid isosteres[24,25,28] in place of a carboxylic acid. Seeing these as opportunities to explore similarities and differences in the specificities of system x_c^- and VGLUT, we prepared a number of conformationally-restricted glutamate analogs bearing a sulfonic acid group in place of the distal (γ) carboxylic acid of glutamate.

The target compounds were synthesized as shown in Schemes 1 and 2. Simple amino acid analogs (**3a–e**) of phenylglycine were synthesized via hydrolysis of the corresponding hydantoin intermediates (**2a–e**).[29–32] The preparation of sulfonic acid analogs **5a–i** was carried out by reaction of commercially available amino acids with fuming sulfuric acid to afford monosulfonic acid analogs.[33] To explore the relative contribution of the amino acid center to inhibition two additional targets, compounds **7a–b**, were

* Corresponding author. Tel.: +1 406 243 4643.
E-mail address: charles.thompson@umontana.edu (C.M. Thompson).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.10.020

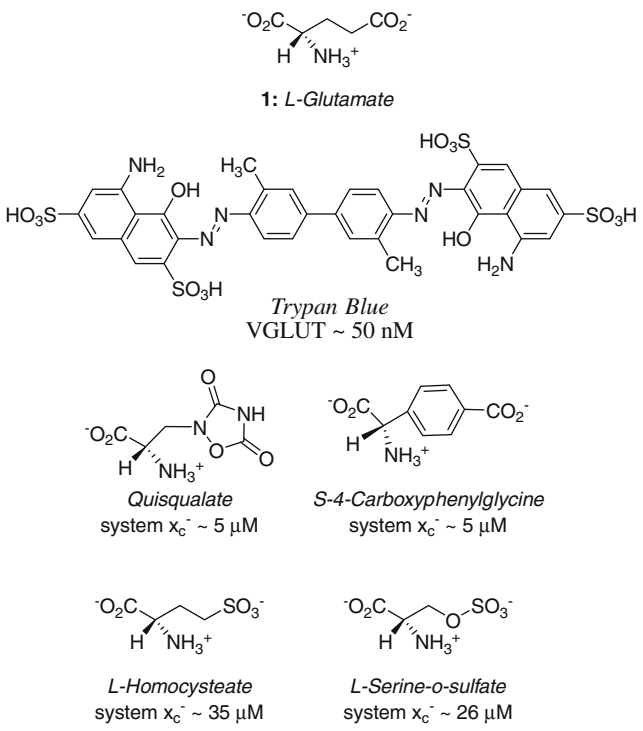
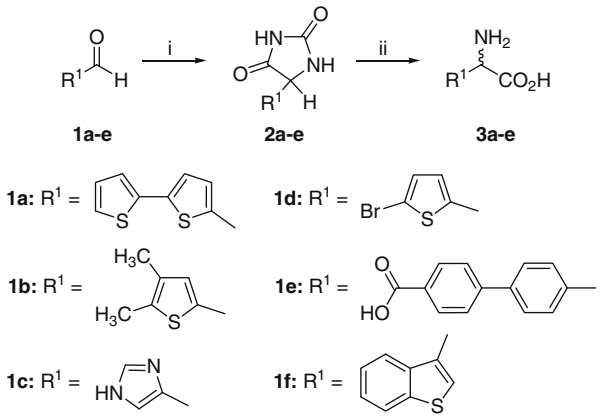
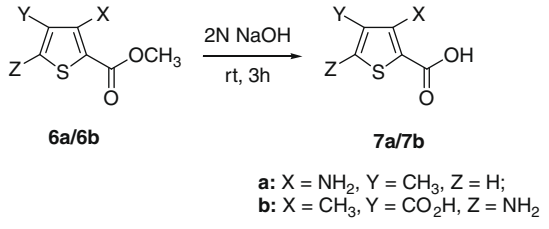


Figure 1. Structures of glutamate, VGLUT and system x_c^- inhibitors and their corresponding IC_{50} values.



Scheme 1. Synthesis of amino acid analogs **3a**–**f**. Reagents and conditions: (i) $(NH_4)_2CO_3$, KCN, 1:1 MeOH, H_2O, 50–60 °C, 3 h; (ii) $Ba(CO_3)_2$, H_2O, 100 °C, 72 h.



Scheme 2. Preparation of thiophene analogs **7a**/**7b**.

synthesized by hydrolysis of the commercially available structures **6a–b** using 2 N NaOH. Each synthesized compound was characterized by ^1H NMR, IR, and mass spectral analysis[34] prior to testing at the two transporter systems (Table 1). Activity was assessed by quantifying the ability of the compounds to inhibit the specific uptake of ^3H-L-gluamate by either system x_c^- or VGLUT. System x_c^- mediated uptake of L-glutamate (100 μM) was measured in SNB19 glioma cells under Na-free conditions, corrected for non-specific uptake, and normalized to protein content.[9] VGLUT mediated uptake of L-glutamate (250 μM) was measured in synaptic vesicles isolated from rat brain, corrected for non-specific uptake, and normalized to protein content [20].The rationale for testing compounds **3a–f** was based on the fact that a thienylglycine heterocycle contains an embedded cysteine. The imidazole structure was prepared as a control analog. Structure **3e** was built as a chain extended homologue of 4-CPG, which was found to be a good inhibitor of system x_c^- but a poor inhibitor of VGLUT. The activity of **3e** also suggests the likelihood that the compounds are interacting with lipophilic domains associated with the transporter, as has also been shown to occur with EAAT inhibitors.[12] Interestingly, all the thiophene-containing structures showed inhibition of system x_c^- and VGLUT with the 5-bromo thienylglycine **3d** and benzothienylglycine **3f** blocking about 60% and 70% of VGLUT uptake, respectively (Table 1). The imidazo analog **3c** was completely inactive indicating the importance of the thiophene ring and/or possibly contribution by the sulfur atom. Most surprising in this initial screen was the finding that the biphenyl analog of CPG **3e** blocked 73% of glutamate uptake at system x_c^- but was a poor inhibitor of VGLUT.

Sulfonic acid analogs of the amino acids phenylglycine, phenylalanine and thienylglycine **5a–i** were prepared to determine the role of stereochemistry, isostere contribution and limitations of the γ-carboxylic acid group. We rationalized that CPG and cysteate are inhibitors of system x_c^- and therefore, CPG analogs bearing a γ-sulfonic acid would be more potent, and potentially highly selective inhibitors when compared as inhibitors of VGLUT. Neither D- or L-4-sulfophenylalanine **5ab** nor α-methyl 4-sulfophenylalanine **5d** blocked glutamate uptake at either transporter (Table 1). Sulfonation of 4-bromophenylalanine was conducted to afford the phenylalanine-2-sulfonic acid analog **5c** to reduce the distance between the two acid groups, and render it a conformationally-restricted analog of homocysteate.

However, compound **5c** was a poor inhibitor of both transporters. (*R*)-4-Sulfo-phenylglycine **5e** did not block glutamate uptake at either transporter, yet **5f** was a selective inhibitor of system x_c^-. We attribute this selectivity to the fact that system x_c^- generally requires an *S*-configured amino acid center for inhibitors whereas VGLUT shows no need for this center and, in fact, does not require a basic amine.

Using (*S*)-4-sulfophenylglycine as a new lead, we prepared the thiophene analogs that position the sulfonic acid and amino acid groups at a distance midway between 4-sulfophenylglycine and homocysteic acid. Both (*R*)-**5h** and (*S*)-4-sulfothienylglycine **5g** blocked uptake of glutamate at system x_c^-, 45% and 70%, respectively (Table 1). The latter compound proved as potent as the endogenous substrate L-cystine. Unlike system x_c^- both were less potent at VGLUT.

The last set of analogs we prepared to test specificity differences between system x_c^- and VGLUT were aminothiophenecarboxylic acids **7ab**. Since the thiophene scaffold showed promise in system x_c^- inhibitors, we next queried whether or not replacement of the -amino acid group with an aniline-type amine and carboxylic acid would preferentially block VGLUT. In both instances, glutamate uptake was blocked at system x_c^- and not VGLUT indicating that the presence of an α-amino acid group is not a requirement for system x_c^- inhibitor structure. This is also consistent with the activity of sulfazaline, an inhibitor of system x_c^-, which lacks the free α-amino acid head group that typifies the majority of known inhibitors. Sulfasalazine is of particular interest because it suggests that system x_c^- may represent a viable point of therapeutic intervention in the treatment of glial brain tumors.[35]