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Design, synthesis, and biological activity of novel triazole amino acids used to probe binding interactions between ligand and neutral amino acid transport protein SN1

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Abstract—Novel triazole amino acids were synthesized as probes to investigate ligand–protein binding interactions of the neutral amino acid transporter SN1. The bonding hypothesis to be tested was that the side chains of endogenous substrates are acting as H-bond acceptors. Although limited inhibition of ^{3}H-L-glutamine uptake by SN1 expressing oocytes was observed, the synthetic compounds show a trend that suggests a hydrogen bond interaction just outside the endogenous ligand binding pocket.

L-Glutamine is the most abundant free amino acid in mammalian blood plasma and cerebral spinal fluid.[1] As such an abundant bio-molecule, L-glutamine is involved in a variety of metabolic processes.[2] L-Glutamine is a major component of muscle,[3,4] and in times of post-surgical conditions has been classified as a 'conditionally essential' amino acid because of the increased metabolic requirements.[5] As a precursor to TCA cycle intermediates, glutamine is an important source of energy,[6,7] as well as being an upstream precursor to the natural reducing agent glutathione, and is thus closely related to the redox status of the cell.[8] Glutamine acts as a vehicle for both carbon and nitrogen shuttling.[9] This systemic glutamine shuttling, referred to as the glutamine cycle,[3] serves as an excretion route for toxic ammonia[10] through complimentary mechanisms balanced for proper pH buffering of the blood.[11] Glutamine also acts in a signaling role for a number of biological pathways,[12] such as low glutamine concentrations associated with a more facile initiation of the apoptotic cascade.[13] Glutamine is also accepted as being a major metabolic precursor for the excitatory neurotransmitter L-glutamate[14] and the inhibitory neurotransmitter GABA.[15] In order for glutamine to participate in all of these roles, this small polar molecule must move throughout the body. It is this glutamine movement in which we are interested, and particularly transporter protein facilitated movement.

Neutral amino acid transporter proteins are responsible for facilitating movement of these highly polar solutes across the lipophilic cellular membrane. A number of neutral amino acid transport systems have been identified, the main transport systems being the sodium dependent systems A, ASC, N, and the sodium-independent system L.[16–18] As these transport systems are responsible for the translocation of the neutral amino acids (16 of the 20 common mammalian amino acids), one may suspect there is considerable overlap of substrate specificity. One common thread of these transport systems is that they all transport L-glutamine.

Our interest involves the system N transport protein SN1. This neutral amino acid transporter is found in hepatocytes, muscle, and brain.[19] In the brain, the SN1 transporter is located on astrocytes and is believed to be responsible for glutamine efflux from astrocytes as part of the glutamine/glutamate cycle for recycling the neurotransmitter glutamate.[20] Although much has been discovered about the SN1 transport protein, such as ionic requirements,[21] electrogenic behavior,[22] and reversibility,[23] little is known about ligand–protein binding interactions. It is our goal to elucidate these ligand–protein binding interactions and develop an accurate

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binding-model pharmacophore (a model mapping out in three dimensions the locations of attractive and repulsive ligand–protein interactions). Once established, an accurate binding-model pharmacophore can be used to develop pharmacological tools for the SN1 transporter protein. Currently there are no selective inhibitors for the SN1 transporter.

The SN1 transport protein has three natural substrates: L-glutamine, L-asparagine, and L-histidine (Fig. 1).[19] One possible alignment for these three substrates, explaining how they interact with the transport protein, incorporates the side chain acting as a hydrogen-bond acceptor within the ligand binding site. This proposed pharmacophore is illustrated in Figure 2, showing the amide carbonyl oxygen of both L-glutamine and L-asparagine as well as the non-protonated nitrogen of L-histidine occupying a similar three-dimensional space while acting as the hydrogen-bond acceptor.

In order to test this hydrogen-bond acceptor hypothesis, the triazole functionality was proposed. The 1,2,3-triazole functionality offers a hydrolysis-stable amide replacement containing a hydrogen-bond acceptor fitting the alignment above. By incorporating the click methodology in the synthesis of the 1,2,3-triazoles, the 5-membered ring can easily be substituted at the 4-position with a variety of substituents that may offer additional insight into the binding pocket properties.[24] The panel of target compounds designed is shown below (Fig. 3.)

Starting with L-serine, the amino and acid functionalities are protected as the *N*-Boc benzylester, respectively. The benzylating agent **7** (IDC-benzyl alcohol adduct) was used for the ester formation[25] rather than more standard methods such as acidic alcohol or acid chloride intermediate in order to minimize serine lactone formation. Conversion of the hydroxyl to the azide under Mitsunobu conditions[26] affords the common azide intermediate for the click triazole formation. Reacting the chiral azide with the desired acetylene in the presence of Cu(II) and sodium ascorbate in water/*tert*-butanol solvent yielded the corresponding 1,2,3-triazoles in high yields.[27] Subsequent deprotection via refluxing 6 N HCl or hydrogenation followed by TFA treatment with subsequent HCl/TFA exchange gave the target compounds as the HCl salt (Scheme 1).[27]

The compounds were tested as inhibitors of L-glutamine uptake by the rat SN1 transporter by competition assays with ^{3}H-L-glutamine using *Xenopus* oocytes functionally

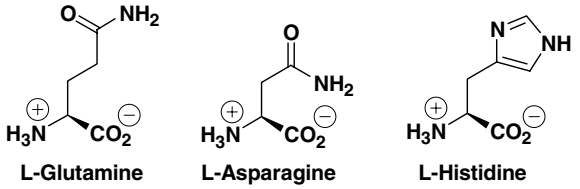


Figure 1. Endogenous substrates of the neutral amino acid transporter SN1.

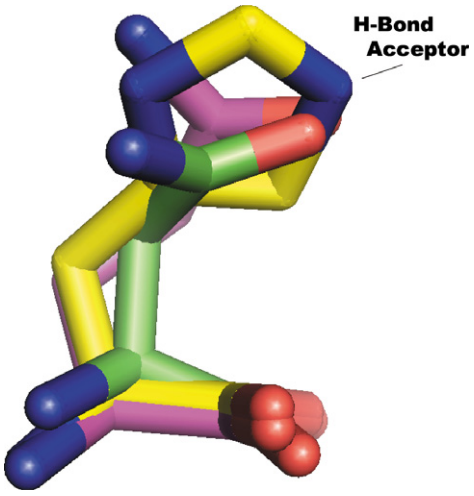


Figure 2. Overlay of energy-minimized structures of L-glutamine (pink), L-asparagine (green), and L-histidine (yellow) with amide carbonyl (red) or imidazole nitrogen (blue) in similar 3-D space able to act as a hydrogen-bond acceptor.

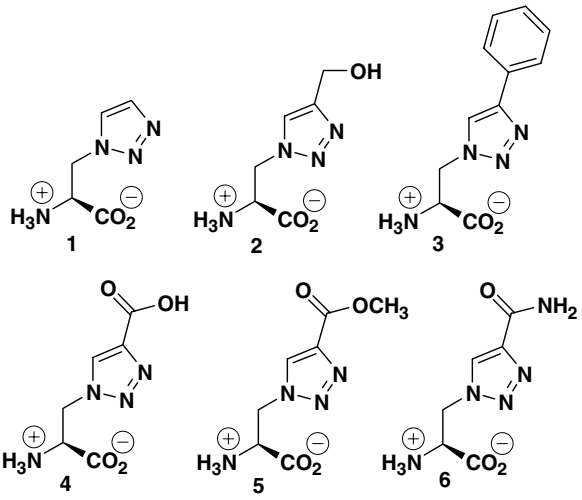
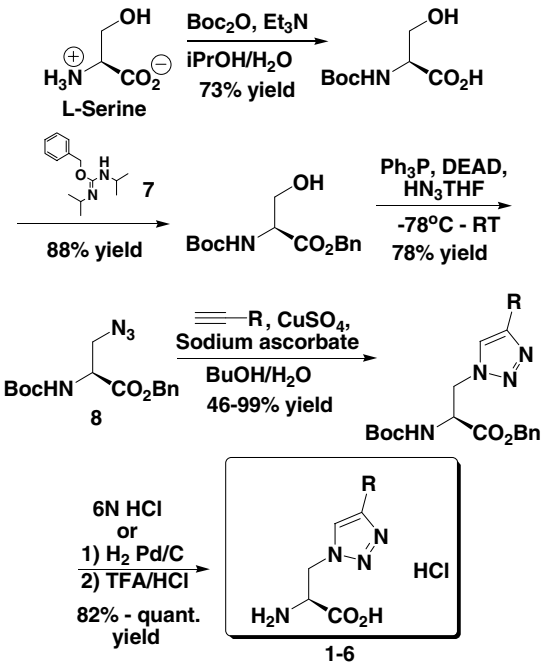


Figure 3. Panel of target triazole amino acids for testing as inhibitors of ^{3}H-L-glutamine uptake by the neutral amino acid transporter SN1.

expressing the transporter. Functional expression was achieved by injecting mRNA encoding for the rSN1 transporter (GenBank Accession No. NM_145776, *Rattus norvegicus* SLC38A3) into oocytes, followed by a four-day period to allow expression. Synthetic compounds were tested at 1 mM (^{3}H-L-glutamine at 100 μM) at a buffer pH of 8.0 and compared to control uptake containing no compound. Background levels were subtracted (identical conditions using un-injected oocytes) for each data point. For confirmation that the assay was functioning properly, both the negative control, L-alanine, and the positive control, L-histidine, were included in all assays.

As illustrated in Figure 4 below, the panel of triazole amino acids exhibited limited inhibition of ^{3}H-L-glutamine uptake into SN1 expressing oocytes. Uptake in the presence of compounds **1**–**6** was not significantly dif-



Scheme 1. Synthesis of substituted triazoles starting with L-serine. R-groups are shown in Figure 3.

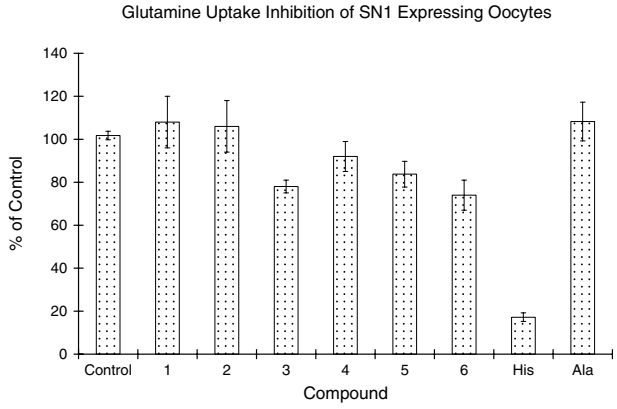


Figure 4. Inhibition of ^{3}H-L-glutamine uptake of substituted triazoles using mSN1 expressing oocytes. All [compounds] = 1 mM, [^{3}H-L-glutamine] = 100μM in Nd96 buffer at pH 8.0, incubation time = 10 -min. Background using un-injected oocytes under same conditions was subtracted. Each data point (n) is the average of five oocytes, $n \geqslant 3$, reported as % of control ± SEM. Assay details are given in the Supplementary material.

ferent from control values using one-way ANOVA. The figure does show a trend by the phenyl substituted triazole **3**, the ester substituted triazole **5**, and the amide substituted triazole **6** that there may be a hydrogen-bond occurring with these groups. These functional groups, although too far removed to be glutamine amide mimics, can all act as hydrogen bond acceptors.

The results of this study suggest the binding of ligand to the neutral amino acid transport protein SN1 requires interactions other than the ligand acting only as a hydrogen-bond acceptor on the amino acid side chain functionality within the ligand binding site. We may have discovered a hydrogen-bond interaction with protein or protein-bound water just outside the endogenous ligand binding site that may offer future possibilities.

Although the panel of triazole amino acids is essentially inactive at the SN1 transporter protein, there are a number of other glutamine utilizing proteins with which these compounds may interact significantly. Our laboratories are currently continuing to explore the binding requirements for the SN1 transporter protein and pursuing active hydrolysis-stable amide mimics.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.061.

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27. General procedure for Click chemistry Azide **8** 480 mg (1.5 mmol) and 1 equivalent of alkyne were dissolved in a mixture of 1 ml *t*-BuOH and 0.5 ml water. With vigorous stirring, sodium ascorbate (20 mg, 20 mol%) was added followed by copper sulfate (8 mg, 10 mol%) solution in 0.5 ml water. The mixtures were stirred at rt for 10 min–24 h. The solvents were then evaporated to dryness and the residue taken up in 50 ml AcOEt. The suspension was washed 2–3 times with 0.5 N HCl until the color was mostly gone (copper removal), and then with 50 ml water. Drying with Na_2SO_4, evaporation of the solvent, and recrystallization from either Et_2O/hexane or AcOEt/hexane yielded the pure compounds. The triazole was then refluxed in 6 N HCl for 1–48 h to yield targets **1**–**4**, or hydrogenated (Pd/C/H_2 (1 atm) in MeOH) followed by treatment with TFA (50% in CH_2Cl_2, 15 min.) with subsequent counterion exchange (0.1 N HCl, 24 h) and evaporated to yield targets **5** and **6**.

Compound **1**: 1H NMR (400 MHz, deuterium oxide) δ ppm 8.10 (1H, s), 7.90 (1H, s), 5.01 (2H, dd, $J = 9.5$, 5.9 Hz), 4.61 (1H, dd, $J = 9.5$, 3.7 Hz). 13C NMR (100 MHz, CD3OD), δ ppm 167.27 (C), 134.97 (CH), 123.35 (CH), 52.19 (CH_2), 50.42 (CH). HR-MS calculated for $C_5H_9N_4O_2$: 157.0726, found: 157.0715 (6.7 ppm). $[\alpha]_D^{23}$ -10^0 (0.070, H_2O).

Compound **2**: 1H NMR (400 MHz, DMSO-d_6) delta ppm 8.81 (1H, br. s), 8.02 (1H, s), 7.52, 7.39, 7.26 (1H, 3 × s), 4.90 (2H, m), 4.51 (1H, m), 4.49 (2H, s). 13C NMR (100 MHz, DMSO-d_6) δ ppm 168.89 (C), 148.72 (C), 124.77 (CH), 55.63 (CH_2), 52.48 (CH_2), 48.93 (CH). HR-MS calculated for $C_6H_{11}N_4O_3$: 187.0831, found: 187.0840 (4.7 ppm). $[\alpha]_D^{23}$ -6^0 (0.050, H_2O).

Compound **3**: 1H NMR (400 MHz, CD_3OD) δ ppm 8.25 (1H, s), 7.59–7.69 (2H, m), 7.21–7.29 (2H, m), 7.14–7.20 (1H, m), 4.90 (2H, d, $J = 5.1$ Hz), 4.55 (1H, t, $J = 5.1$ Hz). 13C NMR (100 MHz, DMSO-d_6) δ ppm 167.48 (C), 147.97 (C), 129.95 (CH), 128.89 (2 × CH), 128.54 (CH), 125.64 (2 × CH), 122.60 (C), 52.54 (CH_2), 49.02 (CH). HR-MS calculated for $C_{11}H_{13}N_4O_2$: 233.1039, found: 233.1049 (4.5 ppm).$[\alpha]_D^{23}$ 20^0 (*c* 0.090, TFA).

Compound **4**: 1H NMR (400 MHz, deuterium oxide) δ ppm 8.54, 8.43, 8.31 (1H, 3 × s; rotamers), 3.93–4.15 (1H, m), 3.20–3.47 (2H, m). 13C NMR (100 MHz, DMSO-d_6) δ ppm 168.77 (C), 162.22 (C), 140.34 (C), 131.09 (CH), 52.37 (CH_2), 50.78 (CH). HR-MS calculated for $C_6H_9N_4O_4$: 201.0624, found: 201.0623 (0.4 ppm). $[\alpha]_D^{23}$ 10^0 (0.100, H_2O).

Compound **5**: 1H NMR (400 MHz, CD_3OD) δ ppm 8.43 (1H, s), 4.89 (2H, d, $J = 4.4$ Hz), 4.53 (1H, t, $J = 4.4$ Hz), 3.68 (3H, s). 13C NMR (101 MHz, DMSO-d_6) δ ppm 167.33 (C), 161.04 (C), 139.64 (C), 129.98 (CH), 52.45 (CH_2), 51.60 (CH), 49.16 (CH3). HR-MS calculated for $C_7H_{11}N_4O_4$: 215.0780, found: 215.0783 (1.3 ppm). $[\alpha]_D^{23}$ 15^0 (0.095, MeOH).

Compound **6**: 1H NMR (400 MHz, DMSO-d_6) δ ppm 8.88 (3H, br s $NH3^+$), 8.57 (1H, s), 7.90 (1H, s, NH2a), 7.50 (1H, s, NH2b), 4.99 (2H, ddd, $J = 22.3$, 15.0, 5.1 Hz), 4.54 (1H, dd, $J = 5.1$ Hz) 13C NMR (101 MHz, DMSO-d_6) δ ppm 168.74 (C), 162.07 (C), 143.48 (C), 128.76 (CH), 52.39 (CH_2), 49.25 (CH) HR-MS calculated for $C_6H_{10}N_5O_3$: 200.0784, found: 200.0776 (3.8 ppm). $[\alpha]_D^{23}$ 27^0 (*c* 0.055, DMSO).