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2-Amino-4-bis(aryloxybenzyl)aminobutanoic acids: A novel scaffold for inhibition of ASCT2-mediated glutamine transport

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Dedicated to the memory of Eric S. Dawson, Ph.D

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ABSTRACT

Herein, we report the discovery of 2-amino-4-bis(aryloxybenzyl)aminobutanoic acids as novel inhibitors of ASCT2(SLC1A5)-mediated glutamine accumulation in mammalian cells. Focused library development led to two novel ASCT2 inhibitors that exhibit significantly improved potency compared with prior art in C6 (rat) and HEK293 (human) cells. The potency of leads reported here represents a 40-fold improvement over our most potent, previously reported inhibitor and represents, to our knowledge, the most potent pharmacological inhibitors of ASCT2-mediated glutamine accumulation in live cells. These and other compounds in this novel series exhibit tractable chemical properties for further development as potential therapeutic leads.

Cancer cells exhibit an altered metabolic profile compared to normal cells. In addition to increased utilization of glucose, they can become highly dependent on the amino acid glutamine. Mammalian cells can regulate glutamine import and export through an evolutionarily redundant series of cell surface transporters. A sodium-dependent transporter of glutamine, ASCT2 (gene symbol SLC1A5), stands out as a major transporter for glutamine uptake making it a promising target for probe development. In addition to being the primary glutamine transporter in cancer, SLC1A5 expression is associated with oncogenic MYC[1,2] and KRAS,[3,4] suggesting its relevance in many clinically important tumors, including those of the lung, colon, and pancreas.[5–7] Fuchs and co-workers demonstrated that SLC1A5 antisense RNA triggered apoptosis in human hepatocellular carcinoma cells.[8] Furthermore, Hassanein et al. more recently reported that SLC1A5 was expressed in 95% of squamous cell carcinomas (SCC), 74% of adenocarcinomas (ADC), and 50% of neuroendocrine tumors. In those studies, siRNA down-regulation of ASCT2 in lung cancer cells resulted in significant growth inhibition.[9] Collectively, these studies suggest development of small molecules capable of inhibiting ASCT2 activity could be promising as precision cancer medicines.

To date, few compounds that target ASCT2 have been reported and most are derivatives of endogenous ASCT2 substrates. As an early entrant to the field, in 2004 Esslinger and co-workers described a series of glutamine analogs that explored pK_a effects on the amide NH bond to probe the ASCT2 amino acid binding site through the addition of electron-donating and electron-withdrawing aryl groups to the terminal amide of glutamine. The best compound, L-γ-glutamyl-p-nitroanilide (GPNA, compound **1**), exhibited modest potency in the low millimolar range and no observations were made regarding steric requirements for binding to ASCT2.[10] Our group was able to expand upon this class of

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inhibitors by exploring the steric requirements for binding to ASCT2 and found that while SAR was flat, 2-substituted glutamylanilides were preferred. We described *N*-(2-(morpholinomethyl) phenyl)-L-glutamine (compound **2**) as a novel glutamyl-anilide with three-fold improved activity against ASCT2 compared to GPNA.[11] In 2011, Albers et al. described a series of ASCT2 inhibitors based on the ASCT2 substrate serine. Using an in silico approach along with experimental validation, they found that side chain aromaticity was required for high-affinity interaction. This lead to the discovery of *O*-(4-phenylbenzoyl)-L-serine (compound **3**), an inhibitor of ASCT2 with an apparent affinity of 30 μM.[12] In 2012, Oppedisano et al. identified the first small molecule lacking an amino acid that blocked glutamine uptake in ASCT2 reconstituted in proteoliposomes (compound **4**). This series of 1,2,3-dithiazoles likely inhibited uptake of glutamine non-competitively through formation of mixed sulfides at Cys-207 or Cys-210 with the most potent compound exhibiting an IC_{50} of 3.7 μM (Fig. 1).[13]

To continue our efforts toward novel ASCT2 inhibitors, we focused our work around elaborating the glutamylanilide scaffold, particularly the amide linker. After screening multiple iterations of this scaffold, we arrived at a series of substituted 2,4-diaminobutanoic acids. While there was no SAR within a library of 4-*N*-mono-substituted derivatives, the resultant library, generated via reductive amination, afforded small amounts of 4-*N*-disubstituted products. We were able to cleanly isolate these byproducts and test their ability to block ASCT2-mediated ^{3}H-glutamine uptake. Surprisingly, these compounds demonstrated up 10 fold better activity against ASCT2 compared with GPNA and had tractable structural elements for further development. While there were many areas on this new scaffold where SAR could be investigated, we chose to focus on the distal aromatic rings and the linker region connecting it (Fig. 2).

We developed a facile synthetic scheme to yield target 2-amino-4-bis(aryloxybenzyl)aminobutanoic acids. (Fig. 3). This synthesis required just two steps starting from *N*-Boc-L-2,4-diaminobutyric acid *tert*-butyl ester hydrochloride and afforded products in overall yields ranging from 52% to 75%. Similar to the initial lead compound, libraries focused on 2-amino-4-bis(aryloxybenzyl)aminobutanoic acids with 2 identical functional groups on the 4-*N* position.

Screening of the synthesized amino acids 5–11 (Table 1), in both C6 (rat) and HEK293 (human) cell lines revealed that compounds **6**, **7**, and **11** displayed roughly equal potencies in both cells lines.[14] Interestingly, other compounds in this series, (Table 1, compounds **8** and **10**) demonstrated some preference for blocking

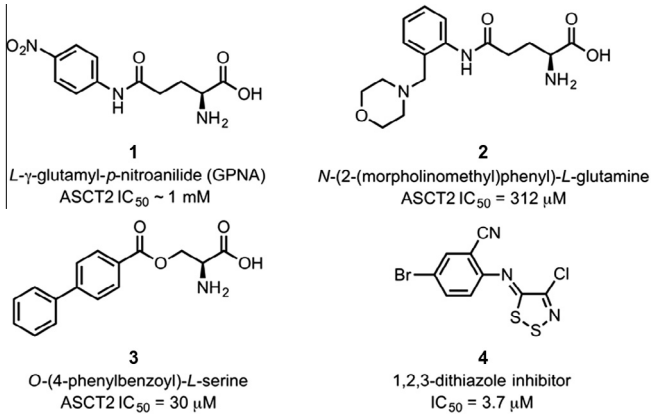


Figure 1. Structures of previously reported ASCT2 inhibitors: GPNA (**1**) and an improved glutamylanilde (**2**), A potent inhibitor derived from the ASCT2 substrate serine (**3**), and a small molecule inhibitor of ASCT2 lacking an amino acid moiety (**4**).

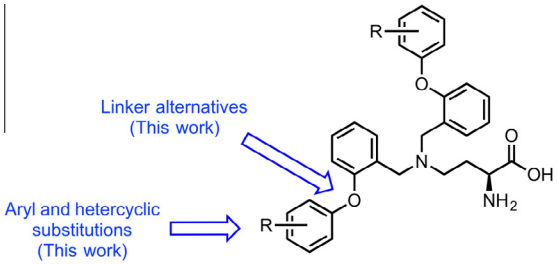


Figure 2. Chemical optimization plan for hit compound (*S*)-2-amino-4-(bis(2-(4-fluorophenoxy)benzyl)amino)butanoic acid via iterative parallel synthesis.

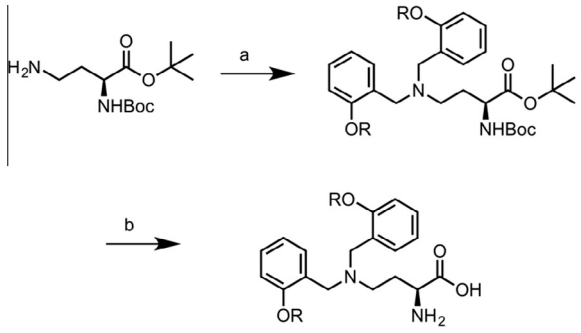
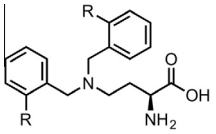
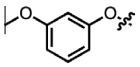
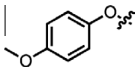
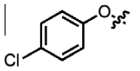
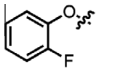
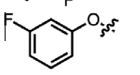
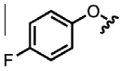
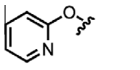


Figure 3. Two-step synthesis of 2-amino-4-bis(aryloxybenzyl)-aminobutanoic acids. Reagents and conditions: (a) RCHO, $NaBH(OAc)_3$, CH_2Cl_2, rt, 12 h. (b) HCl, dioxane, 40 °C, 5 h.

Table 1
Structures and activities of phenoxybenzyl analogs



Compound	R=	IC_{50} (rat) (μM)	SEM (μM)	IC_{50} (human) (μM)	SEM (μM)
5		1.3	±0.7	57.2	±20.8
6		8.7	±0.5	11.9	±0.4
7		24.3	±2.8	26.0	±4.0
8		1.8	±0.6	33.8	±7.2
9		36.3	±10.9	11.5	±2.2
10		10.5	±2.7	141.7	±14.2
11		68.2	±10.3	59.6	±4.9

All IC_{50} values are reported as the mean of at least 3 biological replicates.

glutamine transport in the rat cell line. Our most potent compound in the rat cell line (Table 1, compound **5**) also exhibited this preference and blocked ASCT2-mediated glutamine uptake in C6 cells with an IC_{50} of 1.3 μM.