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2-Substituted *N*γ-glutamylanilides as novel probes of ASCT2 with improved potency

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ABSTRACT

Herein, we report the discovery and structure–activity relationships (SAR) of 2-substituted glutamylanilides as novel probes of the steric environment comprising the amino acid binding domain of alanine–serine–cysteine transporter subtype 2 (ASCT2). Focused library development led to three novel, highly potent ASCT2 inhibitors, with *N*-(2-(morpholinomethyl)phenyl)-L-glutamine exhibiting the greatest potency in a live-cell glutamine uptake assay. This level of potency represents a three-fold improvement over the most potent, previously reported inhibitor in this series, GPNA. Furthermore, this and other compounds in the series exhibit tractable chemical properties for further development as potential therapeutic leads.

Emerging evidence implicates oncogenic signaling pathways with nutrient uptake in cancer cells. The natural amino acid glutamine is essential for cell growth and proliferation. In addition to glucose, cancer cells utilize glutamine as a carbon source for ATP production and biosynthesis. Mammalian cells can internalize glutamine through an evolutionary redundant repertoire of cell surface transporters, though a primary sodium-dependent transporter of glutamine, ASCT2 (gene symbol *SLC1A5*), stands out as a promising target for probe development. In cancer cells, *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] Demonstrating that ASCT2/SCL1A5 activity might be 'actionable' in variety of settings in oncology, Fuchs and co-workers first 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 the potential fruitfulness of developing small molecules capable of inhibiting ASCT2 activity as precision cancer medicines.

To date, few pharmacological inhibitors of ASCT2 have been reported. Grewer and Grabsch described a series of serine and cysteine derivatives as inhibitors of ASCT2. The benzyl analogs of serine and cysteine were reported to have K_i values equal to 0.9 mM and 0.78 mM, respectively.[10] Further elaboration within this series led to serine biphenyl-4-carboxylate which inhibits ASCT2 function with an apparent affinity of 30 μM.[11] As an early entrant to the field, in 2004, Esslinger et al. described L-γ-glutamyl-*p*-nitroanilide (GPNA), a glutamine analog, as a commercially available probe of the ASCT2 amino acid binding site.[12] While this work illustrated that GPNA could inhibit glutamine uptake in cells at millimolar levels and ascribes certain potential electronic requirements possessed by GPNA and similar analogues from that series, this work did not address the steric requirements for binding to ASCT2 within this class of glutamine analogs.

To discover ASCT2 inhibitors with greater potency and to elucidate SAR around this target, we merged structure-based design

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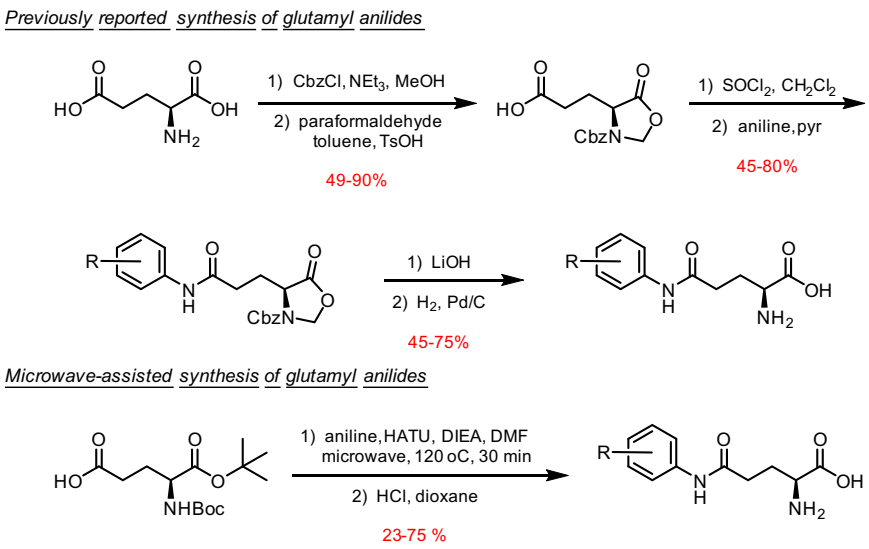
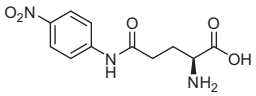
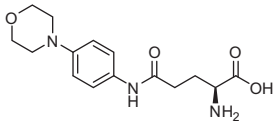
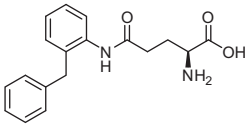
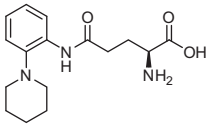
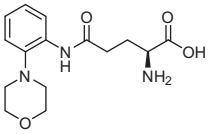
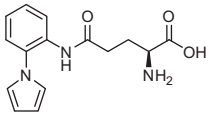
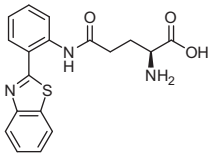
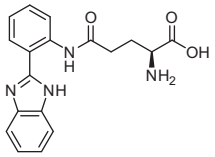
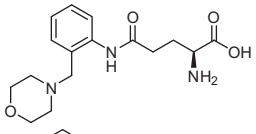
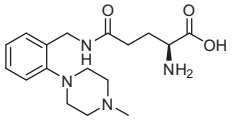
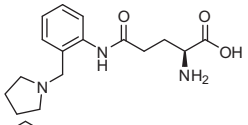
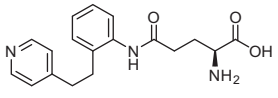
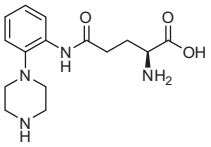
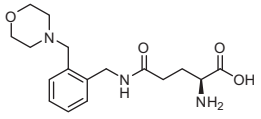
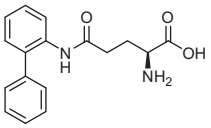
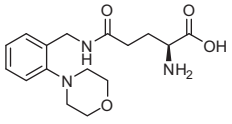


Figure 1. Synthetic route towards 2-substituted *N*γ-glutamylanilides.

Table 1
SAR of *N*γ-glutamylanilides

Compound		IC_{50}	Compound		IC_{50}
1		954 μM	**11**		Inactive
2		Inactive	**12**		776 μM
3		664 μM	**13**		Inactive
4		436 μM	**14**		Inactive
5		312 μM	**15**		Inactive
6		Inactive	**16**		832 μM
7		Inactive	**17**		Inactive
8		Inactive	**18**		Inactive