Small Molecule Ligands for Autophagy Proteins LC3B and GABARAP

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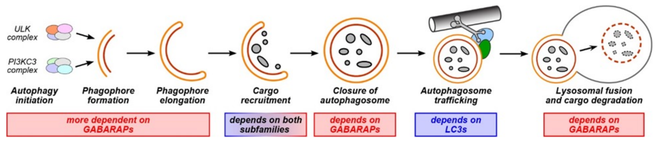
The Kritzer Laboratory

What are LC3B and GABARAP?

Autophagy is a cellular degradation process. Evidence in the literature shows that inhibition of autophagy could be a promising therapeutic strategy for late-stage cancers.[1] LC3B and GABARAP are members of the autophagy protein family and are involved in key protein-protein interactions at several steps during the autophagic process.

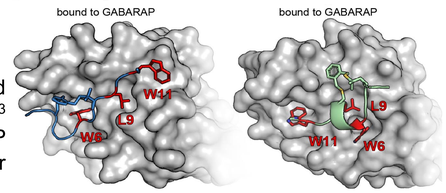
Genetic knockdowns and knockouts of LC3/GABARAP family proteins block autophagy selectively and have demonstrated that this family of autophagy proteins are highly promising targets.[2] GABARAP proteins are close paralogs of the more well-studied LC3 proteins, but it is unknown which human paralogs are more relevant for autophagy inhibition and AUTAC function.

(1) Kocak, M.; Erdi, S. E.; Jorba, G.; Maestro, I.; Farres, J.; Kirkin, V.; Martinez, A.; Pless. O., *Autophagy*. **2022**, 18:3, 473-495.
(2) Karsli-Uzunbas, G.; Guo, J. Y.; Price, S.; Teng, X.; Laddha, S. V.; Khor, S.; Kalaany, N. Y.; Jacks, T.; Chan, C. S.; Rabinowitz, J. D.; White, E., *Cancer Discovery* **2014**, *4*, 914-927.



(3) Brown, H.; Chung, M.; Üffing, A.; Batistatou, N.; Tsang, T.; Doskocil, S.; Mao, W.; Willbold, D.; Bast, R. C. Jr.; Lu, Z.; Weiergräber, O. H.; Kritzer, J. A. *J. Am. Chem. Soc.* **2022**, *144*, 14687-14697.

Stapled peptide	Sequence	Staple	GABARAP K_d (nM)	LC3B K_d (nM)
1	DAnYTWECLAWP	*ortho*	14 ± 2	1590 ± 80
2	DACYTWEnLAWP	*ortho*	12 ± 2	33 ± 5
3	DA4YTWECLAWP	*meta*	≤ 5	≥ 2500
4	DA4YTWACLAWP	*meta*	≤ 5	≥ 16000

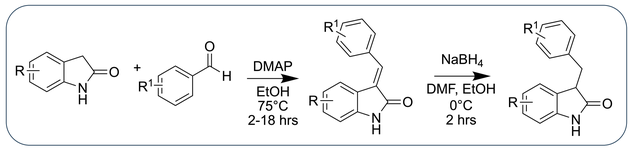


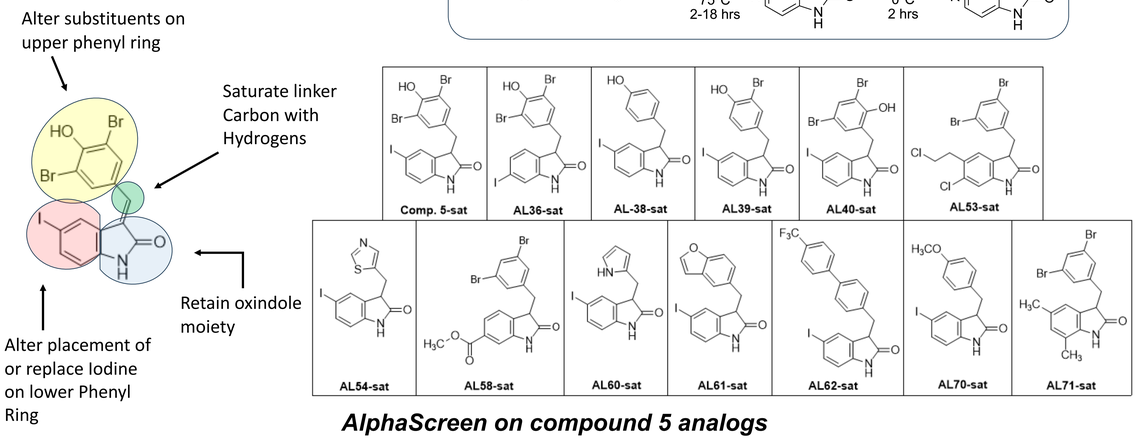
The Kritzer lab recently reported stapled peptides that bind LC3B and GABARAP with low-nanomolar affinity that successfully inhibit autophagy.[3] The crystal structures of these peptides binding to LC3B and GABARAP revealed two independent binding modes, providing critical information for drugging these proteins

SAR on Existing Lead Compound 5

Compound 5 is one of the more promising compounds which was reported to bind LC3B, however much more research on this compound must be done.[4] For example, it was found in a very limited small-molecule screen, has not undergone any optimization or structure-activity relationship studies, there are conflicting reports in the literature about its binding affinity, its binding to GABARAP has not been explored, and no crystal structures exist which demonstrate its binding mode to LC3B.

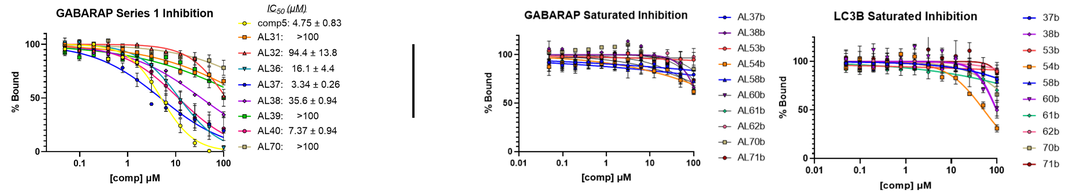
(4) Li, Z.; Wang, C.; Wang, Z.; Zhu, C.; Li, J.; Sha, T.; Ma, L.; Gao, C.; Yang, Y.; Sun, Y.; Wang, J.; Sun, X.; Lu, C.; Difiglia, M.; Mei, Y.; Ding, C.; Luo, S.; Dang, Y.; Ding, Y.; Fei, Y.; Lu, B. *Nature* **2019**, *575* (7781), 203-209.





AlphaScreen on compound 5 analogs

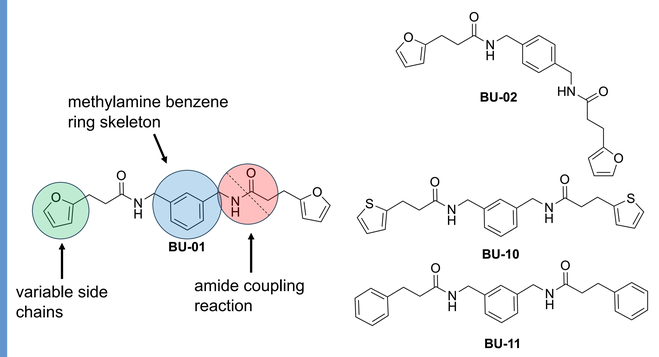
An AlphaScreen assay measured the displacement of a peptide ligand known to bind to these proteins. While some unsaturated compounds performed well, the saturated ones did not. However, this data will inform future SAR studies.



High-Throughput Screen for Small-Molecule Autophagy Inhibitors

Our lab has obtained a 10,569-member library of small molecules that were carefully curated as potential protein-protein interaction inhibitors, while filtering out toxic and nonspecific compounds.[5] We have also obtained another 3,840-member library of small molecules from the Center for Molecular Diversity, an NIGMS-supported program at Boston University.[6] These libraries were then tested for binding to LC3B and GABARAP using a high-throughput fluorescence polarization screen. Putative hits were validated using the orthogonal AlphaScreen assay.

(5) Bosc, N.; Muller, C.; Hoffer, L.; Lagorce, D.; Bourg, S.; Derviaux, C.; Gourdel, M.-E.; Rain, J.-C.; Miller, T. W.; Villoutreix, B. O.; Miteva, M. A.; Bonnet, P.; Morelli, X.; Sperandio, O.; Roche, P. *ACS Chem Biol.* **2020**, *15* (6), 1566-1574.
(6) https://www.bu.edu/cmd/



Design and SAR of BU Compound

Several hits were obtained through the AlphaScreen and fluorescence polarization assays. Of these, one compound (BU-01) stood out for its specific binding, one-step synthesis, and potential for differentiation. An amide coupling reaction was designed to link carboxylic acid-labeled chains with a methylaminated benzene ring to create several analogs of BU-01, accounting for chemical availability.[7]

(7) Au, R.; Jennings, M. C.; Puddephatt, R. J. *Organometallics* **2009** *28* (13), 3734-3743.

Note: On 8/9/2023, analysis of the CMD's original BU-01 sample through mass spectrometry revealed the given structure was not present. Upon further FP testing, this was confirmed. At this time, other hits are being considered for further investigation.

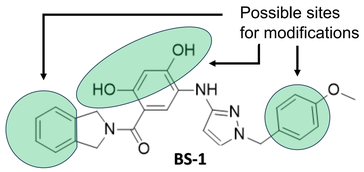
Impact: Fighting Cisplatin Resistance in Chemotherapy

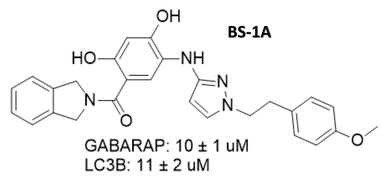
Today, many ovarian, pancreatic, lung, cervical, and colorectal cancers involve platinum-based therapy such as cisplatin. These treatments tend to show a very positive response initially, but many patients eventually relapse with cisplatin-resistant tumors.[8] In some cases, drug resistance causes treatment failure and death in over 90% of patients with metastatic disease.[9] Many of these cancers upregulate autophagy to successfully defend against cisplatin and accompanying treatments, but inhibiting this process could provide current treatments with enough competitive edge to permanently eliminate the disease.[10,11]

(8) Shen, D. W.; Pouliot, L. M.; Hall, M. D.; Gottesman, M. M., Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. *Pharmacol. Rev.*, **2012**, *64*, 706- 721.
(9) Agarwal, R., & Kaye, S. B. (2003). Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nature Reviews Cancer*, *3*(7), 502–516.
(10) Amaravadi, R. K.; Kimmelman, A. C.; Debnath, J., Targeting autophagy in cancer: recent advances and future directions. *Cancer Discovery.* **2019**, *9* (9), 1167-1181.
(11) Selvakumaran, M.; Amaravadi, R. K.; Vasilevskaya, I. A.; O'Dwyer, P. J., Autophagy Inhibition Sensitizes Colon Cancer Cells to Antiangiogenic and Cytotoxic Therapy. *Clinical Cancer Research*, **2013**, *19* (11), 2995-3007.

Future Direction

Two further compounds (BU-1 and 2A) from the high-throughput fluorescence polarization screen have been identified as reasonably synthesizable and have promising IC50 binding levels for both LC3B and GABARAP. Syntheses for these two similar compounds will be prepared and tested, followed by the syntheses of analogs to attempt to find compounds with better LC3B/GABARAP binding. Areas on the molecule that could be potentially beneficial to modify are highlighted on the BU-1 molecule below and include the three benzene groups.





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