

Serotonin Analogues as Inhibitors of Breast Cancer Cell Growth

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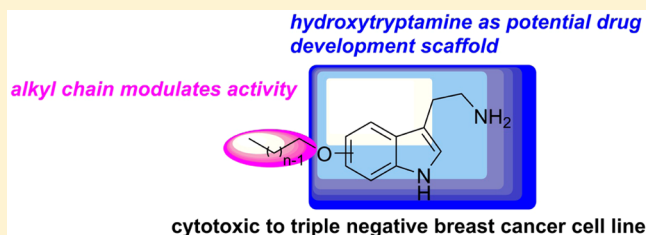
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S Supporting Information

ABSTRACT: Serotonin (5-hydroxytryptamine, 5-HT) is a critical local regulator of epithelial homeostasis in the breast and exerts its actions through a number of receptors. Dysregulation of serotonin signaling is reported to contribute to breast cancer pathophysiology by enhancing cell proliferation and promoting resistance to apoptosis. Preliminary analyses indicated that the potent 5-HT_{1B/1D} serotonin receptor agonist 5-nonyloxytryptamine (5-NT), a triptan-like molecule, induced cell death in breast cancer cell lines. Thus, we synthesized a series of novel alkyloxytryptamine analogues, several of which decreased the viability of various human cancer cell lines. Proteomic and metabolomic analyses showed that compounds **6** and **10** induced apoptosis and interfered with signaling pathways that regulate protein translation and survival, such as the Akt/mTOR pathway, in triple-negative breast cancer cells.

KEYWORDS: Breast cancer, tryptamine, serotonin receptor



Serotonin (5-hydroxytryptamine, 5-HT), a monoamine neurotransmitter, is a critical local regulator of epithelial homeostasis in the breast.¹ Serotonin exerts its actions through a repertoire of receptor proteins belonging to seven discrete families, six of which are G-protein-coupled receptors, including G_i, 5-HT₁; G_s, 5-HT_{4,6,7}; and G_{q/11}, 5-HT_{2,5}. Studies have suggested that serotonin plays a mitogenic role in cancer cells² and functions within the autocrine loops of growth factors thereby contributing to cell proliferation in aggressive cancers, such as small cell lung carcinoma,³ prostate cancer,⁴ bladder cancer,⁵ and breast cancer.⁶ Recently, it was demonstrated that in the normal mammary gland, serotonin acts as a physiological regulator of lactation and involution, in part by favoring growth arrest and cell death.⁷ This tightly regulated system becomes dysregulated in human breast cancers. For example, tyrosine hydroxylase (the rate-limiting enzyme in the serotonin biosynthetic pathway) is overexpressed during malignant

progression, and the expression patterns of the serotonin receptors are often severely altered.⁷ Interestingly, the expression of some isoforms increase, while others decrease, suggestive of significant alterations in downstream signaling, which may underlie, resulting resistance to 5-HT-induced apoptosis and to the promotion of proliferation.⁷

Here, we report the synthesis and characterization of a series of 5-alkoxy tryptamine analogues, which inhibit proliferation of breast cancer cell lines. Two compounds, **6** and **10**, were studied in detail and were shown to induce apoptosis in human breast TNBC cell lines in a dose-dependent manner. Both compounds inhibit the mTOR signaling pathway, as well as ribosomal S6 phosphorylation, suggesting that they inhibit

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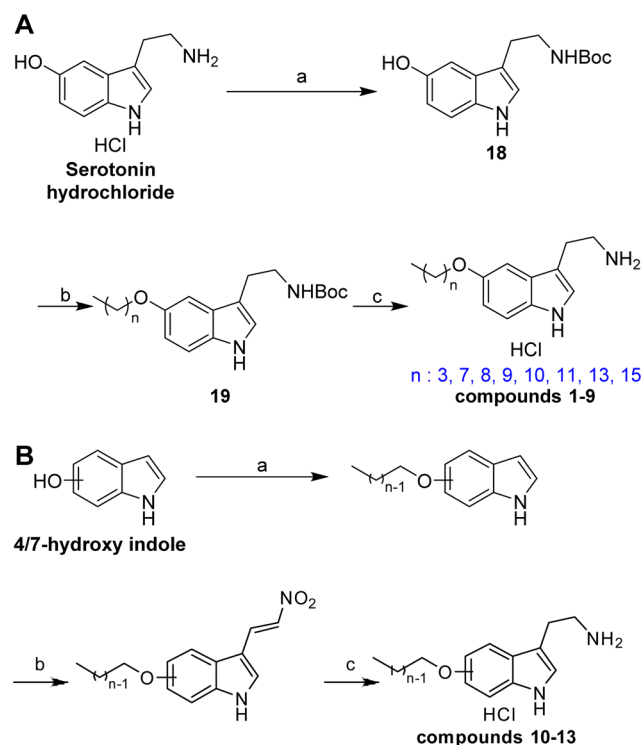
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protein synthesis, a notion supported by an observed elevation of free amino acids induced in MDA-MB-231 cells treated with **6**.

The 5-alkoxy tryptamine analogues were synthesized from serotonin (5-hydroxytryptamine) following a reported literature procedure.⁸ The serotonin primary amine was protected using a *t*-BOC group to afford intermediate **18**, which was alkylated with alkyl bromides of varying chain length to obtain **19**. Facile deprotection of the *t*-BOC group using HCl afforded compounds **1–9** in 59–90% yield (Scheme 1A).

Scheme 1. General Schemes for the Syntheses of 3-(2-Aminoethyl)-5-(alkoxy)indole Hydrochloride (Compound **6, $n = 11$) and 4/7-Alkoxy Tryptamine (Compound **10**, $n = 8$)^a**



^a(A) Reagents and conditions: (a) $(\text{Boc})_2\text{O}$, K_2CO_3 , H_2O , 25 °C, 24 h, 90%; (b) alkyl bromide, K_2CO_3 , CH_3CN , 80 °C, 24 h, 52–74%; (c) 3 M HCl/EtOAc, 25 °C, 2 h, 59–90%. (B) Reagents and conditions: (a) alkyl bromide, K_2CO_3 , CH_3CN , 25 °C, 24 h, 87–88% ($n=8$), 56–59% ($n=11$); (b) N,N -dimethyl-2-nitroethylethylamine, TFA, CH_2Cl_2 , 25 °C, 1 h, 32–37% ($n=8$), 59–60% ($n=11$); (c) (i) LiAlH_4 , THF, reflux, 6 h; (ii) 3 M HCl/EtOAc, 25 °C, 48–58% ($n=8$), 46–55% ($n=11$).

The 4- and 7-alkoxy tryptamine compounds **10–13** were synthesized as shown in Scheme 1B. Alkylation of the 4- or 7-hydroxy indole with the appropriate alkyl bromide afforded the alkoxy indole intermediates **20**. Michael addition with N,N -dimethyl-2-nitroethylethylamine gave the corresponding nitro vinyl intermediates **21**, which was subjected to LAH reduction to obtain the desired analogues in (46–58%) yields. Synthetic details of the less potent compounds **14–17** are provided in the Supporting Information.

Breast cancer cell lines MDA-MB-231 and MCF-7 were treated with increasing concentrations of the various serotonin derivatives. Viability was assayed using MTS, and IC_{50} values were generated. Table 1 shows IC_{50} values for each compound

Table 1. Median Effects of Compounds on Viability in Cancer Cell Lines

tryptamine	IC_{50} (μM)	
	MDA-MB-231 (breast)	MCF-7 (breast)
	>50	>50
1	48 \pm 2.5	45 \pm 5
2	7.3 \pm 0.6	13 \pm 1.0
3	4.7 \pm 0.4	8.8 \pm 0.1
4	4.4 \pm 0.2	9.0 \pm 0.6
5	4.4 \pm 0.0	6.1 \pm 1.1
6	4.6 \pm 0.1	5.1 \pm 0.9
7	8.2 \pm 0.4	15 \pm 1.3
8	4.8 \pm 0.1	8.4 \pm 0.6
9	7.5 \pm 0.2	13 \pm 1.5
10	4.2 \pm 0.5	4.9 \pm 0.1
11	4.1 \pm 0.4	5.0 \pm 0.2
12	8.0 \pm 0.3	15 \pm 1.0
13	4.6 \pm 0.2	8.6 \pm 0.7
14	10 \pm 0.3	17 \pm 0.8
15	>50	>50
16	20 \pm 0.0	24 \pm 3.2
17	18 \pm 2.1	22 \pm 0.8

in the aforementioned cell lines using full serum media. Structures of all synthesized compounds are shown in Table S1 in the Supporting Information. Trends are immediately apparent where those compounds containing a tryptamine with a long linear alkoxy chain (chain length 8–14) at the 4, 5, or 7 position exhibit the best activity (compounds **2–6**, **8**, **10–13**). Tryptamine itself does not reduce cell viability, and even the addition of a short *n*-butyl group (compound **1**) has little effect. A nonlinear alkoxy analogue of 5-hydroxytryptamine (compound **7**) showed a 2-fold drop in activity. Compound **9**, which has a linear chain length of 16 carbon atoms, also showed a reduction in activity similar to compound **7**. The addition of a bromide at the end of the linear alkyl chain seems to retain some activity (compound **14**), whereas substituting nitrile for a bromide resulted in significant loss of activity (compound **15**). Various serotonin dimers, which act as bivalent ligands, have been reported as selective 5-HT_{1B}/1D agonists.⁹ Therefore, we synthesized two homodimeric molecules, **16** and **17**, which showed similar but reduced activity compared to other compounds. Since we observe a plateau in potency for compounds with increasing chain length, it suggests that the tryptamine scaffold may be the mediator of compound activity and that the lipophilic hydrocarbon chain enables more efficient entry into the cell (possibly bypassing SERT-mediated entry), or it may target the compounds to receptors wholly different from the serotonin receptors.

Compounds **6** and **10** were then chosen for further analysis. Interestingly, both **10** (Figure 1a) and **6** (not shown) were found to exhibit more potency following incubation with cells for 48 or 72 h compared to 24 h, suggesting a cumulative effect of the treatment with time. However, the efficacy of **10**, but not **6**, was dramatically increased in serum-free media, possibly indicating a greater binding of **10** to serum compared to **6** (Figure 1b). This assertion is supported by the observation that the addition of BSA to serum-free media suppresses the inhibitory activity of **10** (Figure 1c). Compounds **10** (Figure 1d) and **6** (not shown) induce a dose-dependent increase in apoptosis after treatment, as evidenced by a luciferase-based caspase 3/7 assay, as well as Western blots showing poly-ADP

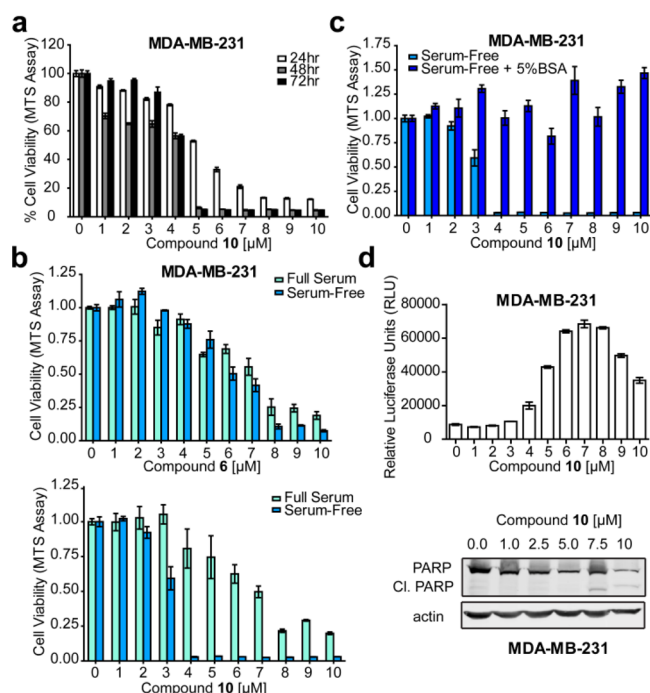


Figure 1. Compounds **6** and **10** decrease viability and increase apoptosis of TNBC cell lines.

ribose polymerase (PARP) cleavage. A similar effect is observed in the MDA-MB-157 triple negative cell line (Figure S1).

To explore possible signaling mechanisms by which **6** and **10** might inhibit breast cancer cell growth, MDA-MB-231 (triple-negative breast cancer) and MCF-7 (luminal A breast cancer) cells were treated with vehicle, **6**, or **10** (20 μ M) for 24 h, and samples were subjected to a reverse phase protein array (RPPA) analysis, which revealed similar overall changes in signaling the following treatment with either **6** or **10**. Select targets are represented as a heatmap in Figure S2 in the Supporting Information. Differences between cell lines including increased phosphorylation of AMPK and ACC in MCF7 cells over MDA-MB-231 cells may indicate signaling differences between the two breast cancer subtypes as they relate to treatment with these compounds. Notably, phosphorylation of proteins involved in downstream signaling of the mTOR pathway, but not total protein, are significantly decreased in MDA-MB-231 cells treated with **6** or **10**. Targets include p70S6K (threonine 389) and its substrate ribosomal protein S6 (serine 235, 236, 240, 244). To explore whether these effects are specific to the MDA-MB-231 cells or whether they might represent a mechanism effective against triple negative breast cancer in general, we confirmed these targets by Western blotting in both the MDA-MB-231 and MDA-MB-157 triple negative cell lines (Figure 2). While phosphorylation of p70S6K on T389 is strongly inhibited by compound **6** and **10**, the activation of the kinase responsible for this phosphorylation, mTOR Signaling Complex 1 (mTORC1),^{10,11} as judged by the PI3K/AKT-mediated phosphorylation of serine 2448,¹² is unchanged (Figures 2 and S2). Thus, both **6** and **10** potentially inhibit the activation of p70S6K/S6 by mTORC1.

Nutrient sensing and therefore control of metabolism occurs largely through mTOR signaling.¹³ Therefore, as **6** and **10** suppressed downstream signaling of mTOR to p70S6K/ribosomal protein S6, it was of interest to assess the metabolic changes induced by the treatment of MDA-MB-231 cells with

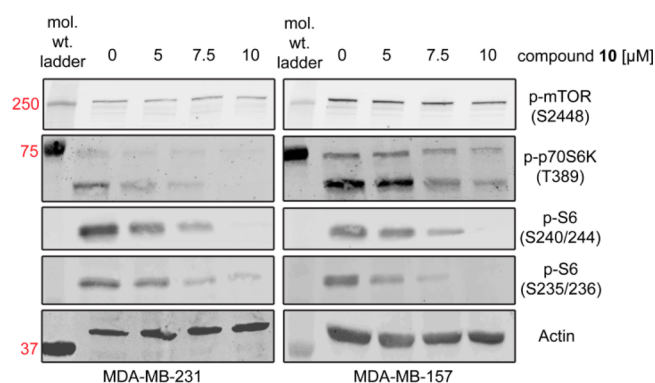


Figure 2. Compound **10** represses activation of translation associated proteins.

compound **6**. NMR was performed to analyze levels of amino acids and other metabolites in treated versus untreated cells. Data sets were analyzed using unsupervised multivariate statistical analysis (principal component analysis, PCA). The score plots (Figure S3) obtained from the PCA analysis clearly indicate very good grouping and separation of untreated and treated cells. Notably, the separation between untreated and compound **6**-treated groups increased with the longer treatment period (24 h versus 7 h). Direct metabolic modulation by a compound is expected to occur shortly after treatment, and those changes should intensify as well as occur with further modulations at later time-points. These additional late time-point modulations may be due to secondary or tertiary effects as well as cellular adaptive response to the treatment. After the initial PCA, we performed an in-depth analysis of the specific metabolic changes induced by treatment at 24 h, indicating the overall metabolic pathways affected by **6** and providing key mechanistic information.

Notably, all the detected amino acids, including the branched amino acids, alanine, aspartate, glutamine, proline, glycine, phenylalanine, and tyrosine accumulated following treatment (Figure 3). This observation could indicate that treatment with **6** hinders protein synthesis rates, possibly due to mTORC1 inhibition, or increases protein degradation. The activation of autophagy after mTORC1 inhibition may also explain the increased availability of amino acids.¹⁴ Similarly, glycans, such as uridine diphosphate (UDP) glucose and UDP *N*-acetylglucosamine, accumulated intracellularly at much higher levels in the MDA-MB-231 cells treated with **6** for 24 h. These results could be linked to a compound **6**-induced endoplasmic reticulum (ER) stress and unfolded protein response (UPR). In fact, O-linked β -*N*-acetylglucosamine (O-GlcNAc) signaling is upregulated in response to ER stress to prevent cardiomyocyte death.¹⁵ In addition, the intracellular levels of both myo-inositol and lactate were reduced, which may indicate a switch from glycolysis followed by lactic acid fermentation to glycolysis followed by the TCA cycle. As cancer cells rely heavily on the former method of metabolism to maintain their rate of proliferation (termed the Warburg effect¹⁶), these data suggest that **6** may exert an antiproliferative effect through interruption of this important mode of cancer cell survival.

A recent study of "metabolic landscapes" from human breast tumors revealed that a high degree of serotonin production was a prominent feature in tumors with poor prognosis, many of which are triple-negative.¹⁷ Here, we report that novel 5-HT analogues induce apoptosis in human breast cancer cell lines by diminishing signaling to nutrient sensing pathways resulting in

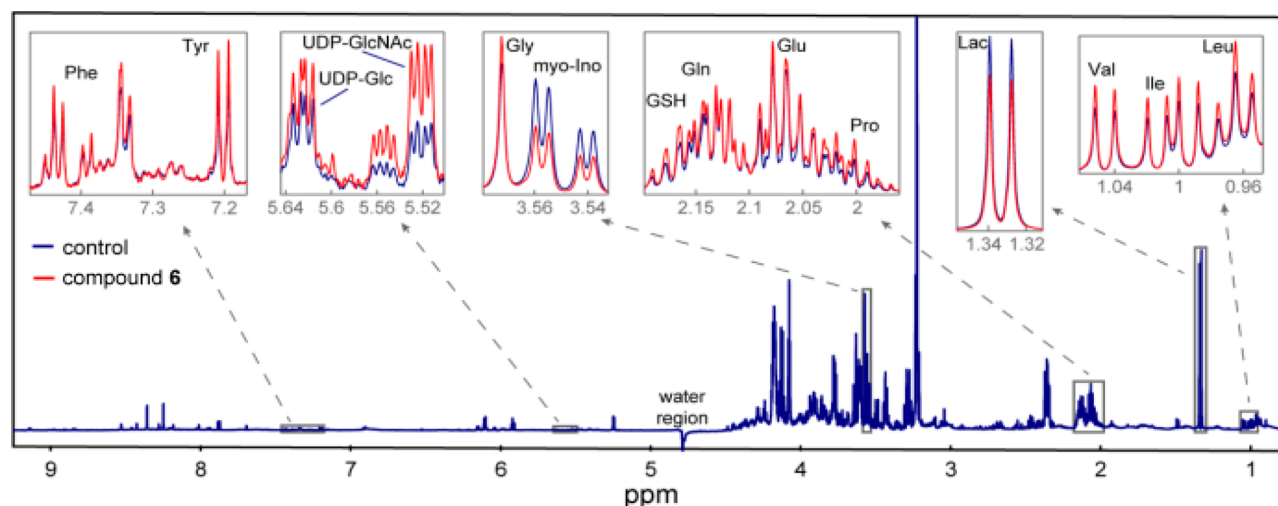


Figure 3. Compound 6 decreases amino acid abundance and increases glycans.

reduced protein synthesis, increased autophagy, and cell death. These effects may be due to the abrogation of cancer cell-specific functions of 5-HT receptors or distinct targeting of these compounds to other molecules, possibly kinases involved in the PI3K/Akt/mTOR signaling pathway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmedchemlett.7b00282](https://doi.org/10.1021/acsmedchemlett.7b00282).

Materials and methods, and supplemental Tables and Figures (PDF)

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Author Contributions

○These authors contributed equally to this work. J.J., C.D.J.T., N.D.E. and K.N.D. conceived the project and designed the research strategy and experimental approach; J.J. synthesized the compounds, with assistance from R.E.; C.D.J.T. and N.D.E. performed the experiments, with assistance from X.X., A.K.D. and T.S.K.; A.L. performed the metabolomic analysis; The data was analyzed by J.J., C.D.J.T., N.D.E., A.L. and K.N.D.; The manuscript was written by J.J., C.D.J.T., N.D.E. and K.N.D., with inputs from A.L., X.X. and C.B.; The studies were

supervised by C.L.V., S.T., E.V.A., C.B., and K.N.D. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

mTOR, mammalian target of rapamycin; TCA, tricarboxylic acid cycle; AMPK, 5' AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; MAPK, mitogen activated protein kinase

■ REFERENCES

- (1) Berger, M.; Gray, J. A.; Roth, B. L. The expanded biology of serotonin. *Annu. Rev. Med.* **2009**, *60*, 355–66.
- (2) Vicaut, E.; Laemmel, E.; Stucker, O. Impact of serotonin on tumour growth. *Ann. Med.* **2000**, *32* (3), 187–94.
- (3) Vicentini, L. M.; Cattaneo, M. G.; Fesce, R. Evidence for receptor subtype cross-talk in the mitogenic action of serotonin on human small-cell lung carcinoma cells. *Eur. J. Pharmacol.* **1996**, *318* (2–3), 497–504.
- (4) Siddiqui, E. J.; Shabbir, M.; Mikhailidis, D. P.; Thompson, C. S.; Mumtaz, F. H. The role of serotonin (5-hydroxytryptamine 1A and 1B) receptors in prostate cancer cell proliferation. *J. Urol.* **2006**, *176* (4), 1648–53.
- (5) Siddiqui, E. J.; Shabbir, M. A.; Mikhailidis, D. P.; Mumtaz, F. H.; Thompson, C. S. The effect of serotonin and serotonin antagonists on bladder cancer cell proliferation. *BJU Int.* **2006**, *97* (3), 634–9.
- (6) Sonier, B.; Arseneault, M.; Lavigne, C.; Ouellette, R. J.; Vaillancourt, C. The 5-HT_{2A} serotonergic receptor is expressed in the MCF-7 human breast cancer cell line and reveals a mitogenic effect of serotonin. *Biochem. Biophys. Res. Commun.* **2006**, *343* (4), 1053–9.
- (7) Pai, V. P.; Marshall, A. M.; Hernandez, L. L.; Buckley, A. R.; Horseman, N. D. Altered serotonin physiology in human breast cancers favors paradoxical growth and cell survival. *Breast Cancer Res.* **2009**, *11* (6), R81.
- (8) Glennon, R. A.; Hong, S. S.; Bondarev, M.; Law, H.; Dukat, M.; Rakhit, S.; Power, P.; Fan, E.; Kinnean, D.; Kamboj, R.; Teitler, M.; Herrick-Davis, K.; Smith, C. Binding of O-alkyl derivatives of

serotonin at human 5-HT1D beta receptors. *J. Med. Chem.* **1996**, 39 (1), 314–22.

(9) Halazy, S.; Perez, M.; Fourrier, C.; Pallard, I.; Pauwels, P. J.; Palmier, C.; John, G. W.; Valentin, J. P.; Bonnafous, R.; Martinez, J. Serotonin dimers: application of the bivalent ligand approach to the design of new potent and selective 5-HT(1B/1D) agonists. *J. Med. Chem.* **1996**, 39 (25), 4920–7.

(10) Dennis, P. B.; Pullen, N.; Kozma, S. C.; Thomas, G. The principal rapamycin-sensitive p70(s6k) phosphorylation sites, T-229 and T-389, are differentially regulated by rapamycin-insensitive kinase kinases. *Mol. Cell. Biol.* **1996**, 16, 6242–51.

(11) Holz, M. K.; Ballif, B. A.; Gygi, S. P.; Blenis, J. mTOR and S6K1 Mediate Assembly of the Translation Preinitiation Complex through Dynamic Protein Interchange and Ordered Phosphorylation Events. *Cell* **2005**, 123 (4), 569–580.

(12) Sekulić, A.; Hudson, C. C.; Homme, J. L.; Yin, P.; Otterness, D. M.; Karnitz, L. M.; Abraham, R. T. A Direct Linkage between the Phosphoinositide 3-Kinase-AKT Signaling Pathway and the Mammalian Target of Rapamycin in Mitogen-stimulated and Transformed Cells. *Cancer research* **2000**, 60 (13), 3504–3513.

(13) Howell, J. J.; Manning, B. D. mTOR couples cellular nutrient sensing to organismal metabolic homeostasis. *Trends Endocrinol. Metab.* **2011**, 22 (3), 94–102.

(14) Chen, R.; Zou, Y.; Mao, D.; Sun, D.; Gao, G.; Shi, J.; Liu, X.; Zhu, C.; Yang, M.; Ye, W.; Hao, Q.; Li, R.; Yu, L. The general amino acid control pathway regulates mTOR and autophagy during serum/glutamine starvation. *J. Cell Biol.* **2014**, 206 (2), 173–182.

(15) Ngoh, G. A.; Hamid, T.; Prabhu, S. D.; Jones, S. P. O-GlcNAc signaling attenuates ER stress-induced cardiomyocyte death. *American Journal of Physiology - Heart and Circulatory Physiology* **2009**, 297 (5), H1711–H1719.

(16) Young, C. D.; Anderson, S. M. Sugar and fat - that's where it's at: metabolic changes in tumors. *Breast Cancer Res.* **2008**, 10 (1), 202.

(17) Leoncik, V.; Wu, H.; Ward, L. T.; Kierzek, A. M.; Plant, N. J. Generation of 2,000 breast cancer metabolic landscapes reveals a poor prognosis group with active serotonin production. *Sci. Rep.* **2016**, 6, 19771.