Optimizing the Aryl Substitutions of cjoc42 for Enhanced Gankyrin Binding and Anti-Cancer Activity

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INTRODUCTION

Gankyrin has emerged as a key oncoprotein which regulates numerous cellular pathways through its various protein-protein interactions (PPIs). Its overexpression has been identified in a variety of cancers and is typically associated with poor prognosis. These findings have highlighted gankyrin as an intriguing therapeutic target for the treatment of certain cancers^{1,2}. The first small molecule binder of gankyrin (cjoc42) was discovered in 2017 and it was found to disrupt its interaction with the S6 ATPase of the 26S proteasome³. Cjoc42 has also displayed anti-cancer activity against hepatocellular carcinoma while increasing key TSP levels (e.g., p53 and Rb)⁴. Recently, we identified a cjoc42 derivative (**AFM-1-2**, **Fig. 1**) which is more potent than cjoc42 both in its gankyrin binding ability and its anti-cancer properties⁵. Moving forward, our work has focused on further optimizing the cjoc42 scaffold as highlighted in **Fig. 1** to enhance its gankyrin binding affinity and anti-proliferative activity against hepatocellular carcinomas, lung cancer, and breast cancer.

PROPOSED CJOC42 DERIVATIVES

Figure 1. Molecular modeling of cjoc42 and its derivative AFM-1-2. (A) Cjoc42 (**1**) docked to its proposed gankyrin binding site (PDB: 1QYM). (B) AFM-1-2 (**2**) docked to its proposed gankyrin binding site (PDB: 1QYM). (C) Structure of cjoc42 (**1**), AFM-1-2 (**2**) and targeted cjoc42 derivatives **9-11**.

SYNTHESIS

Scheme: 1^a

^a**Reagents:** (a) NaNO₃, NaN₃, 6 M HCl; (b) 4-pentyn-1-ol, sodium ascorbate, CuSO₄, THF/BuOH/H₂O; (c) *p*-toluenesulfonyl chloride, TEA, DMAP, CH₂Cl₂

GANKYRIN BINDING EVALUATION OF 9a-9i

Figure 2. Evaluation of cjoc42 derivatives (**9a-9i**) for gankyrin-binding. Compounds (300 μM) and gankyrin (20 μM) were incubated together and subjected to a protein thermal shift assay. Absolute ΔT_m values are plotted.

GANKYRIN BINDING EVALUATION OF 10a-10g

Figure 3. Evaluation of cjoc42 derivatives (**10a-10g**) for gankyrin-binding. Compounds (300 μM) and gankyrin (20 μM) were incubated together and subjected to a protein thermal shift assay. Absolute ΔT_m values are plotted.

GANKYRIN BINDING EVALUATION OF 11a-11h

Figure 4. Evaluation of cjoc42 derivatives (**11a-11h**) for gankyrin-binding. Compounds (300 μM) and gankyrin (20 μM) were incubated together and subjected to a protein thermal shift assay. Absolute ΔT_m values are plotted.

ANTI PROLIFERATIVE EVALUATION

Compound	Hep3B IC ₅₀ (μM)	HepG2 IC ₅₀ (μM)	A549 IC ₅₀ (μM)	MDA-MB-231 IC ₅₀ (μM)
cjoc42	>50	>50	>50	>50
9a	>50	>50	79.9 (±4.7)	36.6 (±5.5)
9e	42.7 (±5.8)	42.6 (±6.5)	>50	>50
9f	>50	>50	74.6 (±4.4)	72.8 (±7.1)
10c	82.1 (±16.0)	61.8 (±12.1)	>50	>50
10g	44.9 (±8.5)	>50 ^e	>50	>50

SAR SUMMARY AND CONCLUSION

In summary, a novel series of cjoc42 derivatives modified at the R position (**Fig. 1**) were designed, synthesized, and evaluated for their ability to bind gankyrin and inhibit liver, lung and breast cancer cell proliferation. Modifications to the aryl triazole moiety resulted in 3 new derivatives of cjoc42 (**9f**, **10g**, and **11e**) with superior anti-cancer activity in Hep3B, HepG2, A549, and MDA-MB-231 as compared to cjoc42. In terms of gankyrin binding, an extensive SAR revealed that electron donating substituents were favored at the R = 4-position while at the R = 3-position the electron withdrawing CN group was favored, and at the R = 2-position the electron withdrawing CF₃ group was preferred. This marks a significant advance over cjoc42 in terms of both gankyrin binding as well as anti-cancer activity in liver, lung, and breast cancers.

REFERENCES

- Dawson, S.; Higashitsuji, H.; Wilkinson, A. J.; Fujita, J.; Mayer, R. J. Gankyrin: A New Oncoprotein and Regulator of PRb and P53. *Trends in Cell Biology* 2006, 16 (5), 229–233
- Higashitsuji, H.; Higashitsuji, H.; Itoh, K.; Sakurai, T.; Nagao, T.; Sumitomo, H.; Masuda, T.; Dawson, S.; Shimada, Y.; Mayer, R. J.; et al. The Oncoprotein Gankyrin Binds to MDM2/HDM2, Enhancing Ubiquitylation and Degradation of P53. *Cancer Cell* 2005, 8 (1), 75–87.
- Chattopadhyay, A.; O'Connor, C. J.; Zhang, F.; Galvagnion, C.; Galloway, W. R. J. D.; Tan, Y. S.; Stokes, J. E.; Rahman, T.; Verma, C.; Spring, D. R.; et al. Discovery of a Small-Molecule Binder of the Oncoprotein Gankyrin That Modulates Gankyrin Activity in the Cell. *Sci Rep* 2016
- D'Souza, A. M.; Jiang, Y.; Cast, A.; Valanejad, L.; Wright, M.; Lewis, K.; Kumbaji, M.; Shah, S.; Smithrud, D.; Karns, R.; et al. Gankyrin Promotes Tumor-Suppressor Protein Degradation to Drive Hepatocyte Proliferation. *Cell Mol Gastroenterol Hepatol* 2018, 6 (3), 239–255
- Kanabar, D.; Farrales, P.; Gnanamony, M.; Almasri, J.; Abo-Ali, E. M.; Otmankel, Y.; Shah, H.; Nguyen, D.; El Menyewi, M.; Dukhande, V. V.; et al. Structural Modification of the Aryl Sulfonate Ester of Cjoc42 for Enhanced Gankyrin Binding and Anti-Cancer Activity. *Bioorg. Med. Chem. Lett.* 2020, 30