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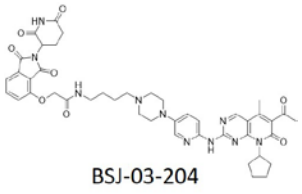
Characterization of a CDK4/6-targeted protein degrader - Binding affinity, Inhibitory activity and Selectivity -

CDK6 overexpression is involved in the acquired resistance to CDK4/6 inhibitors.

Cyclin-dependent kinases 4 and 6 (CDK4/6) are important regulators of the cell cycle. In hormone-receptor-positive /HER2-negative (HR+/HER2-) advanced or metastatic breast cancer, several selective CDK4/6 small molecule inhibitors have been approved for the treatment, but resistance remains an issue with >20% of patients exhibiting intrinsic resistance and up to 70% of patients developing acquired resistance within three years[1]. CDK6 overexpression was identified as a mechanism of this acquired resistance across multiple studies. For example, it is reported that cell lines with CDK6 overexpression show impaired sensitivity to CDK4/6 inhibitors[2][3]. Additionally, a genomic analysis of estrogen receptor-positive (ER+)/HER2- breast cancers treated with CDK4/6 inhibitors suggested that loss-of-function mutations in the *FAT1* tumor suppressor was implicated in the process by promoting elevation of CDK6 expression, which leads to resistance to CDK4/6 inhibitors[4][5].

As a strategy to overcome resistance to CDK4/6 inhibitors, targeted protein degraders capable of degrading CDK6 have garnered significant interest, and a clinical trial of BTX-9341 is currently in progress[6]. Degraders can offer advantages over inhibitors acting via occupancy-driven mechanisms in terms of improved selectivity, efficacy at lower doses, and prolonged duration of action[7]. The development of degraders is anticipated to contribute to overcoming drug resistance as well as improving the safety profile of CDK4/6-targeted therapeutics.



BSJ-03-204

Carna evaluated the binding affinity, inhibitory activity and selectivity of **BSJ-03-204**, a CDK4/6 degrader, using Carna products and services.

If you have any questions regarding these data, or inquiries about these protein products and services, please feel welcome to contact us at info@sb.carnabio.com.

Binding affinity & inhibitory activity

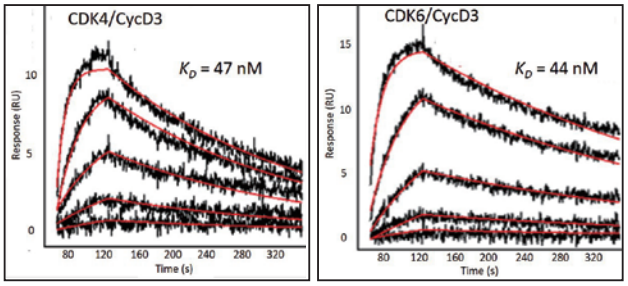


Fig.1. BTN-CDK4/CycD3 and BTN-CDK6/CycD3 can be used to measure direct affinity. The above SPR sensorgrams of BSJ-03-204 show binding to BTN-CDK4/CycD3 (Cat.No. 04-405-20N) and BTN-CDK6/CycD3 (Cat.No. 04-407-20N). The binding experiments were performed using single-cycle kinetics on the Carterra Ultra.

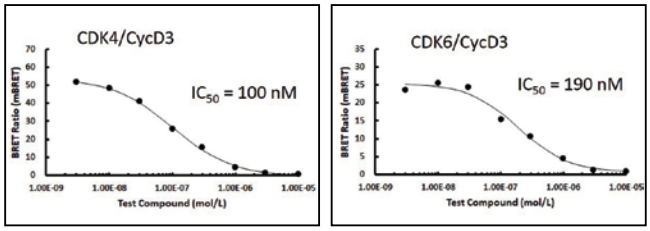


Fig.2. Degrader cellular engagement can be measured by NanoBRET technology. The cellular target engagement profile of BSJ-03-204 against CDK4/CycD3 and CDK6/CycD3 is shown. Promega's NanoBRET™ TE Intracellular Kinase Assay was used to obtain the cellular IC_{50}s of interaction.

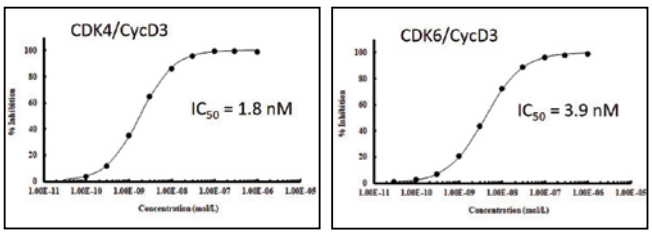


Fig.3. In vitro degrader binding can be assessed by enzymatic inhibition. The inhibitory activity of BSJ-03-204 against the kinase activity of CDK4/CycD3 and CDK6/CycD3 is shown. Mobility shift assay (MSA) was used to obtain the IC_{50}s in the presence of Km ATP in

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an enzymatic biochemical assay.

Selectivity

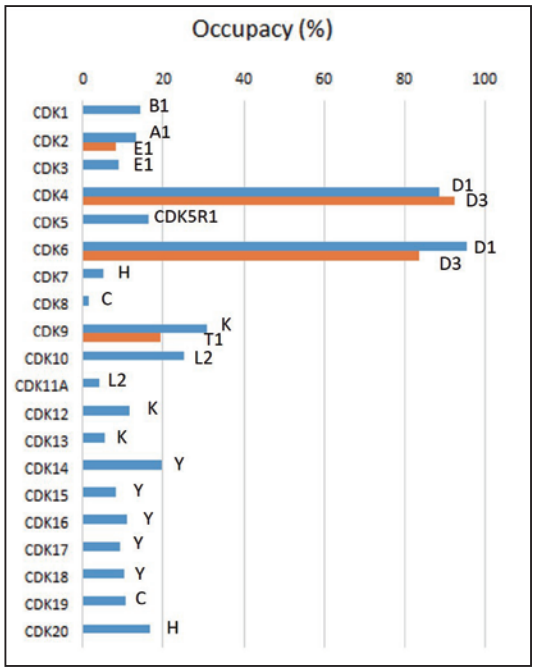

Fig.4. CDK family cellular selectivity of the degrader can be measured using Carna's NanoBRET Panel. Selectivity of BSJ-03-204 against CDKs is shown. NanoBRET™ TE Intracellular Kinase Assay was used to obtain the occupancy of 1μM BSJ-03-204. The respective cyclin types are indicated to the right of each bar graph.

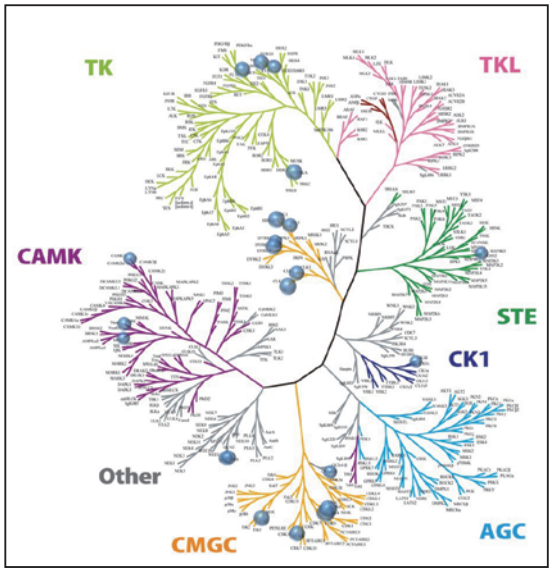

Fig.5. Degrader kinase selectivity can be measured by enzymatic inhibition assays. Selectivity of BSJ-03-204 against 259 wild type kinases is shown.MSA and IMAP™ assays were used to measure %inhibition of 1μM BSJ-03-204 in the presence of Km ATP. Kinases with more than 50% inhibition are shown.

References：

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4) Cancer Cell. 2018; 34(6): 893-905.e8. Li Z.
5) Nat Cancer. 2025; 6(1): 24-40. Asciolla JJ.
6) Biotheryx announces, Nov. 2025, “Biotheryx Announces Completion of Enrollment in Phase 1a Clinical Trial of BTX-9341 for the Treatment of HR+/HER2- Breast Cancer”
7) Poster. AACR 2024. Abstract ID: 6068. Biotheryx. Majeski H.