Dual-Arm Fatty Acid Conjugated Peptides: Structural Optimization and Advantage Exploration

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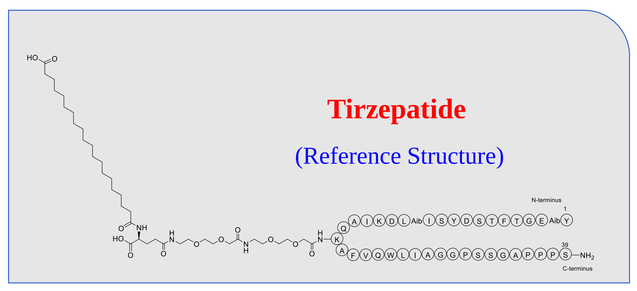
Abstract:

INTRODUCTION: Tirzepatide is a novel once-a-week dual GIP and GLP-1 receptor agonist with excellent efficacy in glycemic control and weight reduction. However, further extension of its half-life remains a clinical need. Inspired by studies demonstrating that dual-arm fatty acid conjugation could enhance albumin binding and prolong pharmacokinetics, we synthesized novel Tirzepatide analogs JK-1246R-011 and JK-1246R-012 via conjugation of dual-arm fatty acid side chains to its peptide backbone. We sought to evaluate these analogs for improved stability, bioactivity, and *in vivo* efficacy.

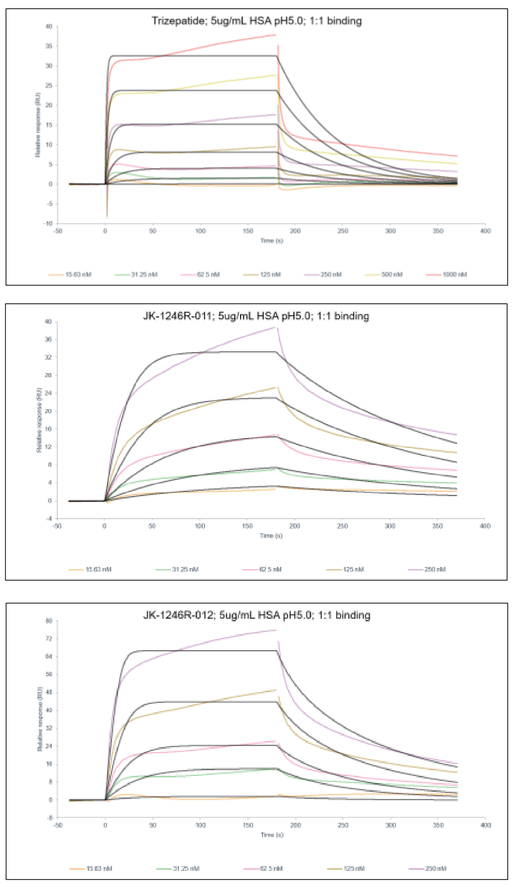
RESULTS: Mass spectrometry confirmed the successful conjugation of dual-arm fatty acids to the Tirzepatide peptide backbone, with molecular weights of 5395.32 Da (JK-1246R-011) and 5339.21 Da (JK-1246R-012). HPLC analysis showed purities of 98.33% and 97.82%, respectively. *In vitro* activity assays revealed that JK-1246R-011 exhibited ~1.5-fold lower GIPR and ~3.6-fold lower GLP-1R activation than Tirzepatide, while JK-1246R-012 maintained equivalent GIPR activity and ~2.1-fold lower GLP-1R activity. Surface plasmon resonance (SPR) analysis showed JK-1246R-012 had a stronger albumin binding affinity (KD = 1.98 × 10^{-8} M) than Tirzepatide (2.18 × 10^{-8} M). *In vivo* pharmacodynamic studies in C57BL/6J mice demonstrated superior glucose tolerance at 72 hours post-dose for both analogs-42% (JK-1246R-011) and 44% (JK-1246R-012) improvements over Tirzepatide (AUC, n = 6). JK-1246R-012 retained an 18% advantage even at 144 hours post-administration.

CONCLUSION: Dual-arm fatty acid conjugation significantly improves the pharmacokinetic and pharmacodynamic profiles of Tirzepatide analogs. JK-1246R-011 and JK-1246R-012 demonstrate enhanced albumin binding and sustained glucose-lowering effects, supporting their potential as next-generation long-acting GLP-1 therapeutics. Future research will explore the application of this conjugation strategy to other peptide drug candidates for prolonged action.

A Structures of Compounds



B Albumin Binding Affinity



C Albumin Binding Affinity Data

Compound	General Kinetics model	Channel	Immobilized ligand	Quality Kinetics Chi²(RU²)	1:1 binding ka (1/Ms)	kd(1/s)	KD(M)
Tirzepatide	1:1 binding	2	50ug/ml HAS pH5.0	5.65e0	7.58e+5	1.65e-2	2.18e-8
JK-1246R-011	1:1 binding	3	50ug/ml HAS pH5.0	1.65e0	2.43e+5	6.56e-3	2.07e-8
JK-1246R-012	1:1 binding	4	50ug/ml HAS pH5.0	8.52e0	1.02e+6	2.02e-3	1.98e-8

Figure 1: Compound design and Albumin Binding Affinity. A) Structures of Tirzepatide, JK-1246R-011 and JK-1246R-012. B and C): Albumin Binding Affinity: JK-1246R-012 exhibited stronger human serum albumin binding with a KD of 1.98 × 10^{-8} M, compared to Tirzepatide's 2.18 × 10^{-8} M.

In Vitro Receptor Activity

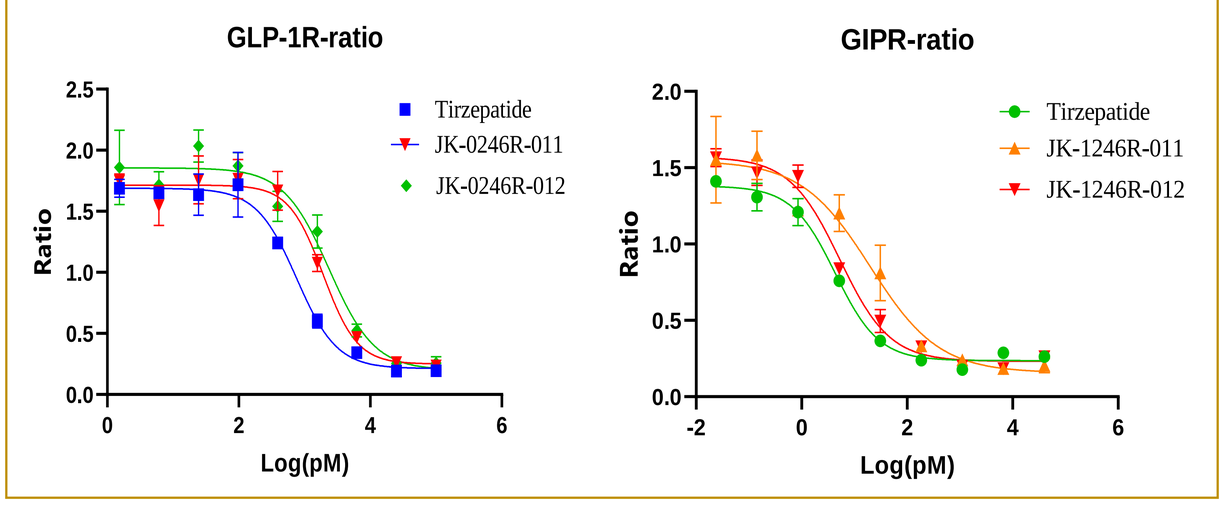
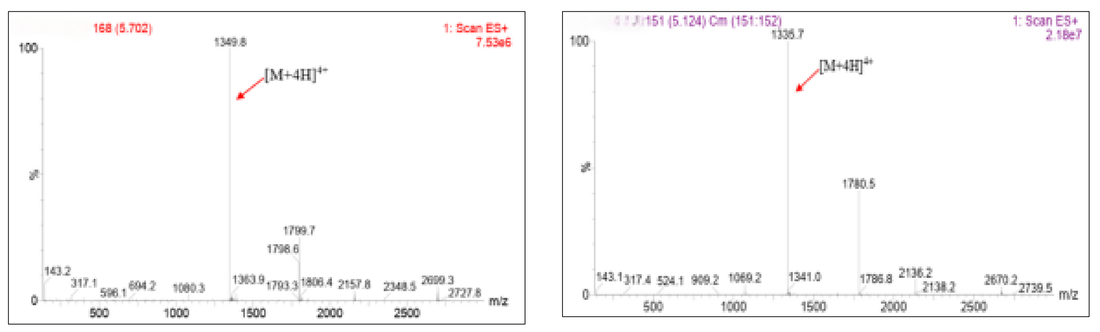


Figure 2: *In Vitro* Receptor Activity. JK-1246R-011: GIP receptor (GIPR) activity: ~1.5-fold weaker than Tirzepatide and GLP-1 receptor (GLP-1R) activity: ~3.6-fold weaker than Tirzepatide. JK-1246R-012: GIPR activity: comparable to Tirzepatide and GLP-1R activity: ~2.1-fold weaker than Tirzepatide.

A Mass Spectrometry and Purity Analysis



B HPLC Analysis Demonstrating High Purity

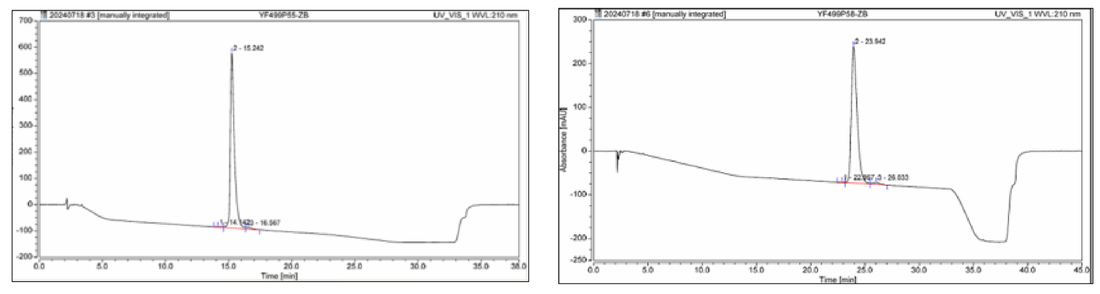


Figure 3A) Mass Spectrometry and Purity Analysis. Mass spectrometry confirmed the successful conjugation of dual-arm fatty acid chains to the amino acid backbone of Tirzepatide. Molecular weight of JK-1246R-011: 5395.32 Da. Molecular weight of JK-1246R-012: 5339.21 Da. 3B) : HPLC analysis demonstrated high purity - JK-1246R-011: 98.33%, JK-1246R-012: 97.82%. Both exceeded the 95% threshold required for further biological evaluation.

In Vivo Pharmacodynamics

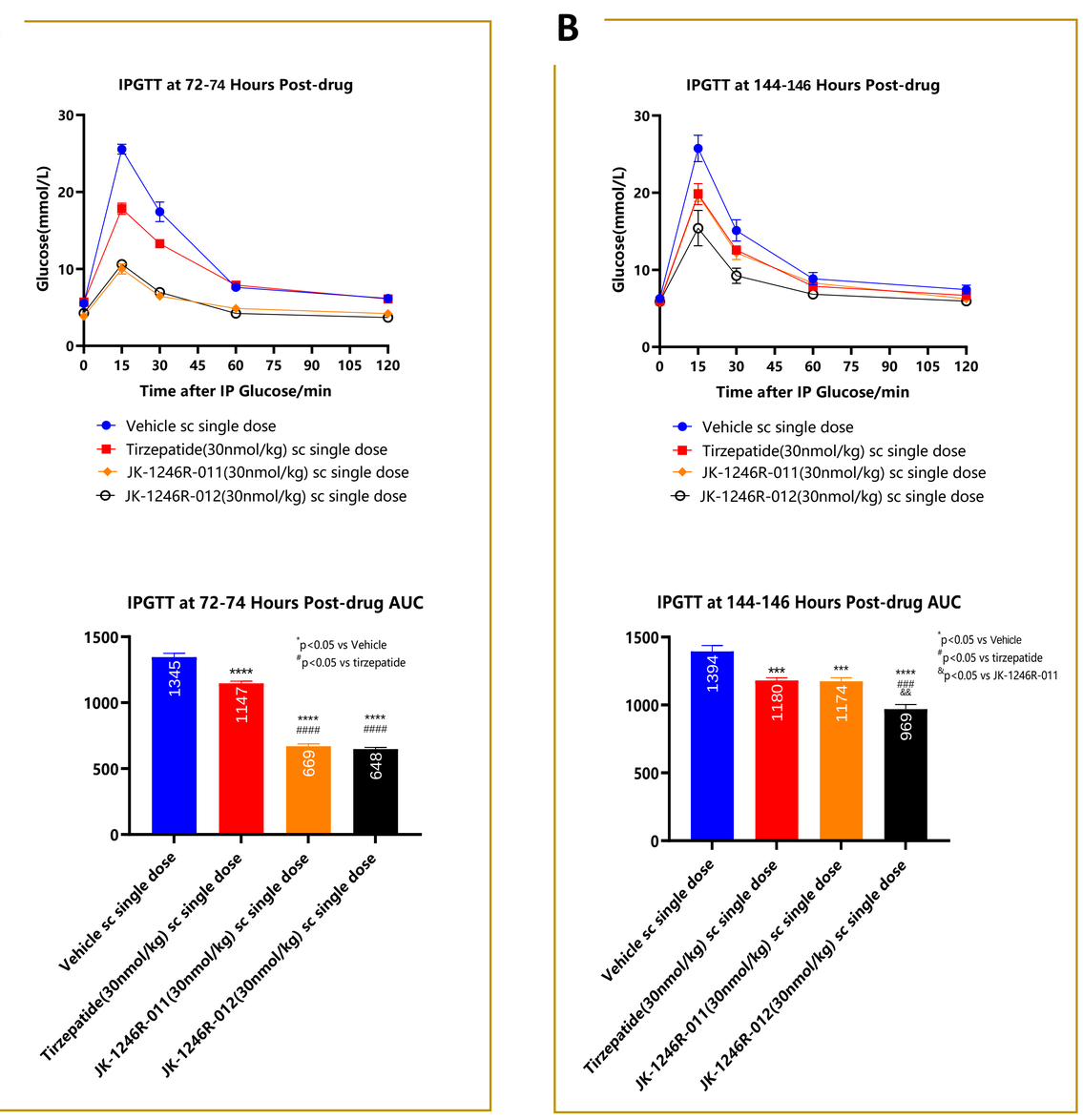


Figure 4: *In Vivo* Pharmacodynamics in Mice. A) At 72 hours post-administration, JK-1246R-011 and JK-1246R-012 improved glucose tolerance by 42% and 44%, respectively, over Tirzepatide (based on AUC, n = 6). B) At 144 hours, JK-1246R-012 maintained an 18% improvement in glucose tolerance versus Tirzepatide (based on AUC, n = 6)