

研究论文

新型SARS-CoV 3CL蛋白酶荧光多肽底物的设计、制备及其酶动力学研究

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摘要 以邻氨基苯甲酸(Abz)为荧光发射基团、2, 4-二硝基苯基乙二胺(Eddnp)为荧光猝灭基团, 设计合成了SARS-CoV 3CL蛋白酶的新型荧光多肽底物: H₂N-E(Eddnp)STLQSLK(Abz)-CONH₂。用液相色谱-质谱(LC-MS)联用技术进行了表征, 表明该多肽底物能被SARS-CoV 3CL蛋白酶识别, 并在QS之间被专一性酶解。另外, 利用该多肽底物的荧光共振能量转移(FRET)特性, 对SARS-CoV 3CL蛋白酶的酶解动力学性质进行了研究, 结果表明, 此荧光多肽底物可以作为荧光探针, 应用于SARS-CoV 3CL蛋白酶活性的测定及其抑制剂的筛选。

关键词 [荧光共振能量转移\(FRET\)](#) [SARS-CoV 3CL蛋白酶](#) [荧光探针](#) [液相色谱-质谱](#)

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Design and Synthesis of a Novel Fluorescent Peptide Substrate of SARS-CoV 3CL^{pro} and Studies on Its Enzymatic Kinetics

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Abstract A novel fluorescent peptide substrate, H₂N-E(Eddnp) STLQSLK(Abz)-CONH₂, based on fluorescence resonance energy transfer(FRET) technique, was designed and synthesized by solid-phase peptide synthesis. Abz/Eddnp was used as the fluorescence donor and quencher pair. The highly fluorescent Abz group was efficiently quenched by energy transfer to the Eddnp group. The fluorescent substrate was specifically hydrolyzed by SARS-CoV 3CL^{pro} between QS with 15-fold increase in fluorescence and the cleavage site was determined by HPLC-MS. The studies on its enzymatic kinetics($K_m = 525.5 \mu\text{mol/L}$ and $K_{cat} = 1.29 \text{ min}^{-1}$) elucidate that the synthetic fluorescent peptide substrate is suited to be used as a fluorescent probe in determination of the activity of SARS-CoV 3CL^{pro} and screening assays for its inhibitors.

Key words [Fluorescence resonance energy transfer\(FRET\)](#) [SARS-CoV 3CL^{pro}](#) [Fluorescent probe](#) [LC-MS](#)

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