

论文

流感病毒神经氨酸酶抑制剂筛选模型的建立和应用

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摘要:

目的建立适用于高通量筛选的流感病毒神经氨酸酶(neuraminidase,NA)抑制剂筛选模型。方法从甲型及乙型流感病毒中制备神经氨酸酶,以2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid(MUNANA)作为底物,建立检测神经氨酸酶活性的荧光测定法及其抑制剂体外筛选方法,用高通量筛选系统对1 200个化合物与提取物进行初筛。结果神经氨酸酶促反应以pH 3.5,二价阳离子浓度为2~6 mmol·L<sup>-1</sup>及37℃孵育时酶活性最佳;甲、乙型流感病毒不同株神经氨酸酶的米氏常数(K<sub>m</sub>)的范围为(4.89~5.94) μmol·L<sup>-1</sup>;初筛发现12个化合物对流感病毒神经氨酸酶有可重复的抑制活性。结论优化了神经氨酸酶反应体系,建立的体外模型可用于抗甲、乙型流感病毒药物的高通量筛选及酶抑制动力学研究。

关键词: 神经氨酸酶 甲、乙型流感病毒 筛选模型

ESTABLISHMENT AND APPLICATION OF HIGH THROUGHPUT SCREENING MODEL FOR INFLUENZA VIRUS NEURAMINIDASE INHIBITORS *IN VITRO*

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Abstract:

AIMIn order to find novel inhibitors of influenza virus neuraminidase (NA), an assay method of neuraminidase activity was established for high throughput screening. METHODSThe strain A (Yuefang 72-243 A and Jifang 90-15 A) and B (Sichuan 2000-38 B) influenza viruses were used as source of neuraminidase and the activity of neuraminidase was measured by fluorometric method. The reaction system of neuraminidase with substrate, 2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) was optimized by changing the conditions such as concentrations of neuraminidase, substrate, Ca<sup>2+</sup>, reactive system pH and temperature. At last, the inhibitory effects of 1 200 samples (including compounds and extracts from plants) were screened by the fluorometric assay. RESULTS The optimized neuraminidase reaction system contains enzyme, MUNANA 20 μmol·L<sup>-1</sup>, Ca<sup>2+</sup> 4 mmol·L<sup>-1</sup>, at pH 3.5 and 37℃. The Michaelis and Menten constants (K<sub>m</sub>) of influenza virus A (Yuefang 72-243 and Jifang 90-15) and B (Sichuan 2000-38) are (5.9±1.9), (5.2±0.5) and (4.9±1.2) μmol·L<sup>-1</sup> respectively. Among 1 200 samples, there were about 1% showed potential inhibitory effects on influenza virus neuraminidase. CONCLUSION The temperature, pH and concentrations of substrate and ions are very important factors affecting the influenza virus neuraminidase activity *in vitro*. The fluorometric assay of neuraminidase was performed by automatic laboratory station to screen inhibitors of influenza virus neuraminidase *in vitro*. It can also be used to study the enzyme inhibition kinetics.

Keywords: influenza A and B screening model neuraminidase

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