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激酶抑制剂筛选新方法及钯催化芳肼的 Suzuki 偶联反应研究

The Novel Method for Screening Inhibitors of Protein Kinase and Palladium-catalyzed Suzuki Cross-coupling of Arylhydrazines

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摘 要

在药物研发中对具有生物活性的物质进行快速筛选是先导化合物开发的关键技术之一。蛋白激酶、G 蛋白偶联受体和离子通道并称药物设计的三大靶点，蛋白激酶抑制剂作为潜在的抗癌、抗肿瘤治疗药物而成为研究的热点。目前的蛋白激酶活性物质筛选方法如稳定同位素法、酶联免疫法、毛细管电泳法和荧光多肽法等都具有一定的局限性。本论文拟发展一种基于 γ-[$^{18}O_4$]-ATP 稳定同位素结合 MALDI-TOF 质谱技术的蛋白激酶抑制剂筛选的新方法, 该方法具有上述四种筛选法的所有优点：灵敏度高、分析速度快、经济实惠、适用于大规模的药物筛选。

实验以 PKA 为模型，Kemptide 为底物模拟肽，采用非放射性 ^{18}O 稳定同位素标记 ATP 的 γ-磷酸根，并与非标记的 ATP 进行平行测试，证明了稳定性同位素标记的 γ-P-$^{18}O_4$-ATP 与正常 ATP 的等效性、在激酶反应体系中的稳定性以及采用已知蛋白激酶 GSK3β 抑制剂测试方法的精确性，所测的 IC_{50} 值基本与文献符合，建立了 GSK3β 激酶抑制剂筛选新方法。

联苯类化合物作为有机分子的重要骨架，存在于各种天然产物中。同时也是一类极其重要的有机合成中间体，广泛应用于药物、染料、有机材料等领域。目前合成联苯类化合物有大量方法，包括电化学合成法、分子内偶联法、有机金属偶联法等，其中通过有机金属交叉偶联反应合成联苯类化合物在现代有机合成中占据举足轻重的地位。有机金属交叉偶联反应主要包括 Stille 偶联反应、Hiyama 偶联反应、Negishi 偶联反应、Suzuki 偶联反应等。这些偶联反应不断向开发新型偶联试剂方向发展。本论文发展了一种新型合成联苯类化合物的钯催化交叉偶联反应，将苯肼第一次用于经典的 Suzuki 交叉偶联反应中。苯肼作为一种新型偶联试剂具有稳定、易得等特点。该反应以醋酸钯为催化剂，反应条件温和，底物适应性好，合成了 23 个化合物，所有化合物都经过 ^{1}H NMR、^{13}C NMR、GC-MS 和 IR 等表征。

关键词：蛋白激酶；抑制剂；稳定同位素；MALDI-TOF-MS; 联苯；钯催化；交叉偶联；苯肼

Abstract

The high-throughput screening of bioactive compounds is the key technique for the discovery of lead compound in medicinal chemistry. Protein kinases, G protein-couple receptors and Ion channels, are the three targets of drug design. Protein kinase inhibitors become focus of research owe to the potential in anti-cancer and anti-tumor. Recently, the methodologies for screening of protein kinase inhibitors including Stable-isotope Method, Enzyme-Linked ImmunoSorbent Assay, Capillary Electrophoresis and Fluorescence-peptide Method have its disadvantages and limitations. This paper intends to develop a novel method was applied for screening inhibitors of protein kinase based on γ-[$^{18}O_4$]-ATP and MALDI-TOF-MS, which offers many advantages, such as high sensitivity, inexpensive cost, high-throughput etc.

Compared to the naturally existing γ-P-$^{16}O_4$-ATP (light-ATP), the foμr ^{16}O atoms attached to the γ-Phosphate are replaced by the stable isotope ^{18}O atoms. The biological equivalence of heavy-ATP to light-ATP was validated by using GST-ATF2, a substrate of protein kinase p38α, which can be randomly phosphorylated. Further experiments confirmed that heavy-ATP labeled GST-ATF2 can also be recognized by the phosphor-antibody without bias. Two parallel protein kinase assays were carried out with heavy-ATP in $H_2{}^{16}O$ and $H_2{}^{18}O$, respectively. No obivious difference was observed in comparison, which confirmed stability of heavy-ATP and the resulting phosphor-peptide and laid a robust foundation for the later quantitative measurements. Parallel kinase reactions were performed using light ATP or heavy ATP, in the absence or presence of an inhibitor, respectively. After the reactions, equal amounts of the reaction solution were mixed and measured directly by MS. Different resulting phosphor-products generated by corresponding kinases were separated based on their m/z. The effectiveness of a putative inhibitor could be readily determined by the suppression of the signal of the heavy phosphor-peptide peak and IC_{50} determined by

plotting inhibition curves based on the analysis of reactions using a series of concentrations of inhibitors.

Biaryls is most important intermediates in organic synthesis, applied widely in medicine, dyestuff, organic materials. It can be synthesized by many methods involving electrochemical synthesis, intramolecular coupling and organometallic coupling. Among these methodologies, synthesis of biaryls via pallidium-catalyzed cross-coupling reaction occupied an extremely important position, such as Stille cross-coupling reaction, Hiyama cross-coupling reaction, Negishi cross-coupling reaction, Suzuki cross-coupling reaction etc. Herein, a novel and efficient protocol for the synthesis of biaryls has been developed, which is the first Pd-catalyzed Suzuki cross-coμpling of readily available arylhydrazines with arylboronic acids. This method employed $Pd(OAc)_2$ as catalyst under mild condition and obtained good to excellent yield. 23 kinds of compounds was synthesized, and all of them has been confirmed by ^{1}H NMR, ^{13}C NMR, GC-MS and IR spectra.

Keyword: Protein Kinase; Inhibitors; Stable-isotopic lable; MALDI-TOF-MS; Biaryls; Palladium-catalyzed; Cross-coupling; Arylhydrazines

符号缩写表

符号	英文含义	中文含义
PKA	Protein kinase A	蛋白激酶 A
MALDI	matrix assisted laser desorption ionization	基质辅助激光解析电离
TOF	time of flight	飞行时间
MS	mass spectrometry	质谱
ESI	electrospray ionization	电喷雾电离
ATP	adenosine triphosphate	腺嘌呤核苷三磷酸
Ser	Serine	丝氨酸
Thr	Threonine	苏氨酸
cAMP	Cyclic adenosine monophosphate	环磷酸腺苷
TMB	3,3',5,5'-Tetramethylbenzidine	3, 3', 5, 5'-四甲基联苯胺
IC_{50}	Half-maximal inhibitory concentration	半数抑制浓度
Ar	Aryl	芳基
Cat.	Catalyst	催化剂
Pd	palladium	钯
Ph	Phenyl	苯基
PPh_3	Triphenylphosphine	三苯基膦
PivOH	Trimethylacetic acid	三甲基乙酸
BPY	2, 2'-Bipyridine	2, 2’−联吡啶
1,10-Phen	1, 10-Phenanthroline monohydrate	1, 10−菲啰啉
NMP	2-Pyrrolidone	*N*-甲基-2-吡咯烷酮
DMF	*N*, *N*-Dimethylformamide	*N*, *N*-二甲基甲酰胺
DMA	*N*, *N*-Dimethylacetamide	*N*, *N*-二甲基乙酰胺
DMSO	Dimethyl sulfoxide	二甲基亚砜
TFA	Trifluoroacetic acid	三氟乙酸
n-BuOH	Butyl alcohol	正丁醇

DPPP	1, 3-Bis(diphenylphosphino) propane	1, 3-双(二苯基膦)丙烷
DPPE	1, 2-Bis(diphenylphosphino)ethane	1, 2-双(二苯基膦)乙烷
DPPB	1, 4-bis(diphenylphosphino)butane	1, 4-双(二苯基膦)丁烷
TLC	Thin layer chromatography	薄层色谱
NMR	Nuclear magnetic resonance	核磁共振仪
GC-MS	Gas chromatograph-mass spectrometer	气相色谱-质谱联用仪
IR	Infrared spectrometry	红外光谱仪
r.t.	Room temperature	室温
n.d.	No detected	未检测到
h	Hour	小时

目 录