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A61K 31/517 (2006.01)
A61K 31/519 (2006.01)
A61P 29/00 (2006.01)
A61P 19/02 (2006.01)
A61P 19/08 (2006.01)
A61P 21/00 (2006.01)

审查员 卢梦霞

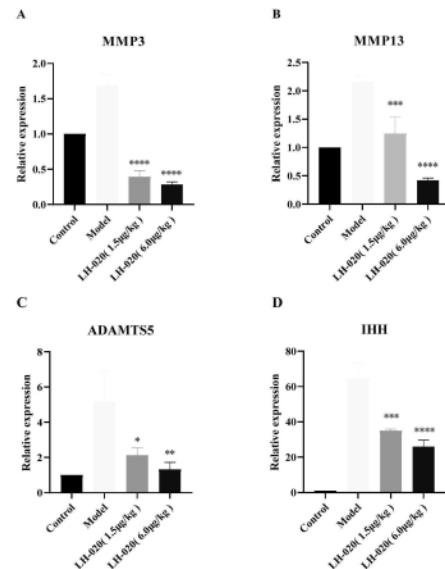
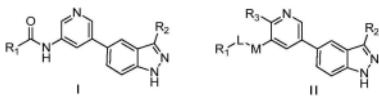
权利要求书5页 说明书29页 附图1页

(54) 发明名称

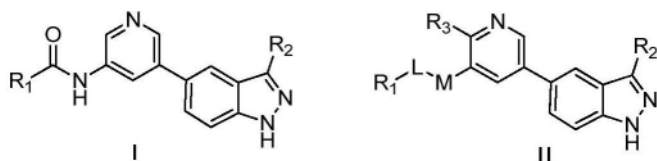
5-吡啶-1H-吡唑类化合物、药物组合物和应用

(57) 摘要

本发明公开了一种5-吡啶-1H-吡唑类化合物、药物组合物和应用。该化合物结构如式I或II,其还包含其异构体、药学上可接受的盐或它们的混合物。该类化合物及其药物组合物可有效抑制CLK2蛋白及DYRK1A蛋白活性,可制备为治疗骨关节炎的药物,在分子水平可以发挥药效,并且治疗效果更优异,最优可达到纳摩尔浓度水平。此外,化合物制备方法简便、易操作。



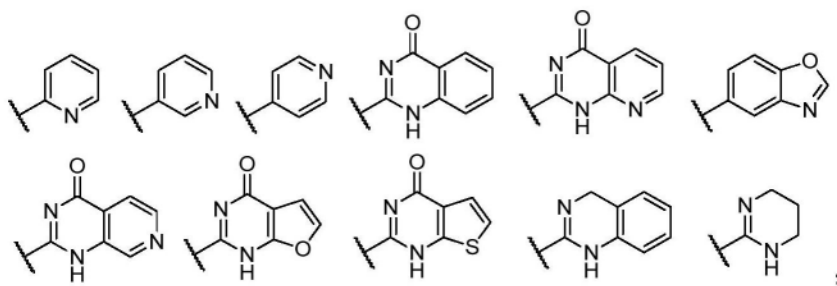
1. 一种5-吡啶-1H-咪唑类化合物,其特征在于,具有式I或II的结构,所述化合物还包含其异构体、药学上可接受的盐或它们的混合物:



其中, R_1 选自异丁基、环丙基甲基、环戊基、 α -氨基异戊基、3,3-二氟四氢吡咯基、氢、吗啉基、甲基、叔丁基、乙磺酰基或羟基;

L、M选自 $-\text{CH}_2-$ 、 $-\text{NH}-$ 、 $-\text{O}-$ 或化学键;

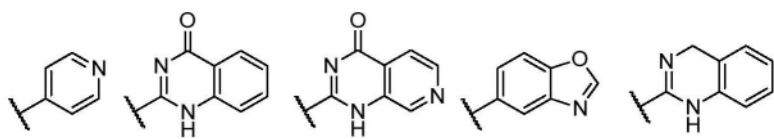
R_2 选自如下基团:



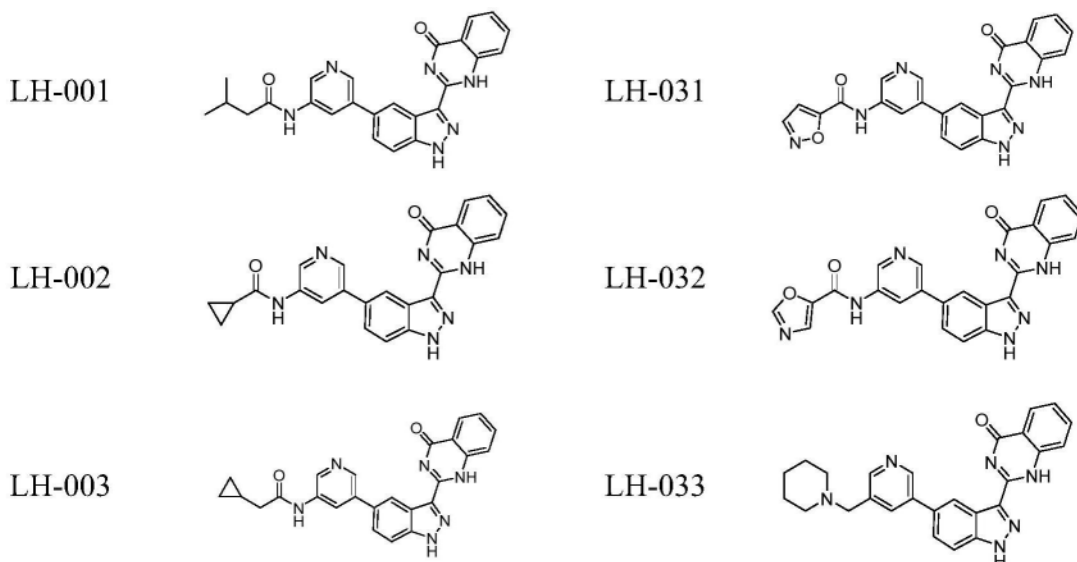
R_3 选自氢或 C_1-C_4 烷基。

2. 根据权利要求1所述的化合物,其特征在于,所述结构中:

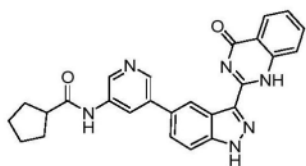
R_2 选自如下基团:



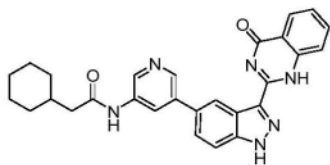
3. 一种5-吡啶-1H-咪唑类化合物,其特征在于,选自以下任一化合物:



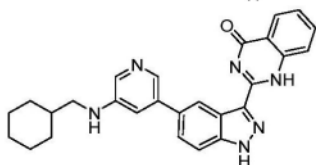
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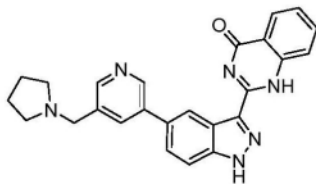
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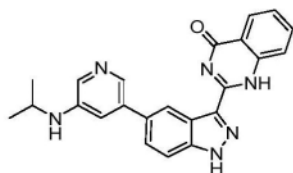
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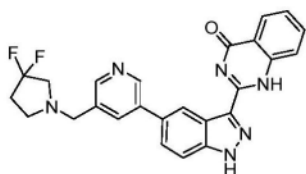
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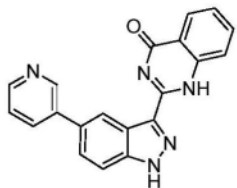
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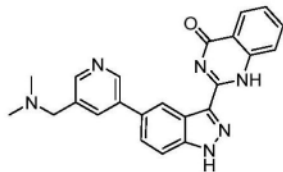
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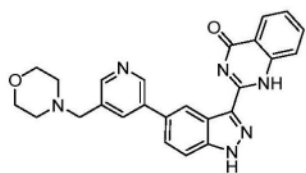
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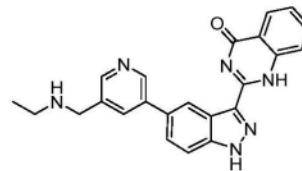
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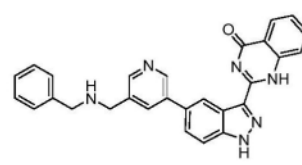
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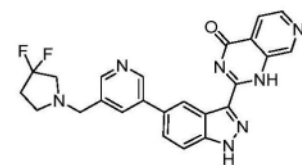
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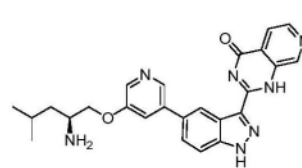
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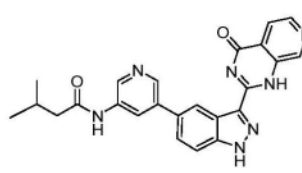
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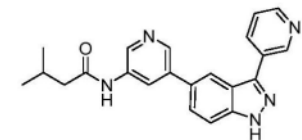
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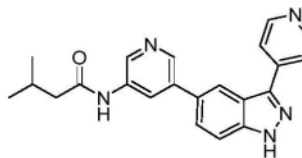
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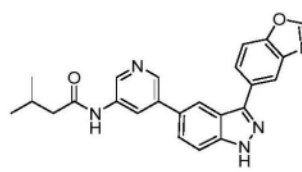
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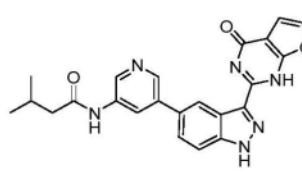
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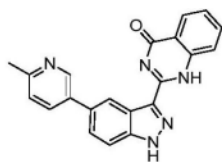
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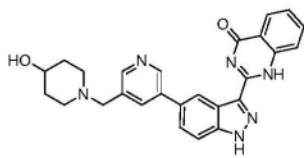
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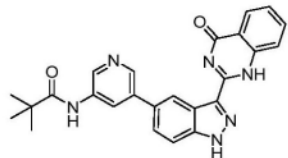
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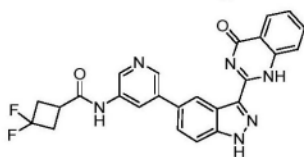
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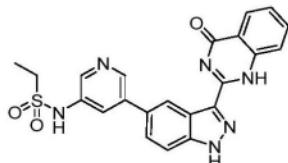
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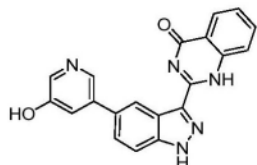
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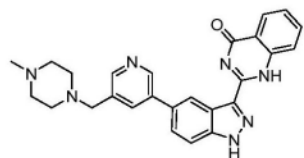
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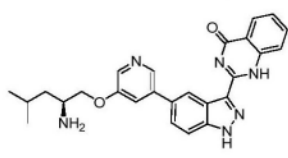
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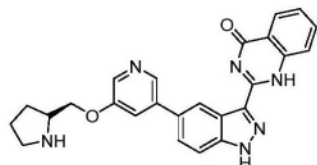
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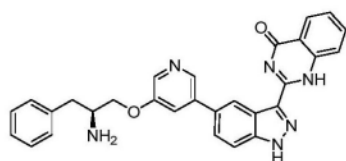
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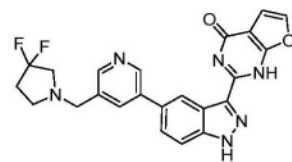
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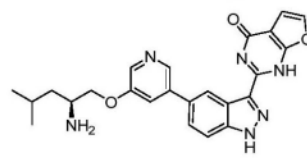
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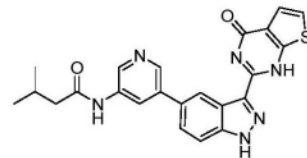
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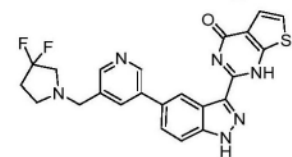
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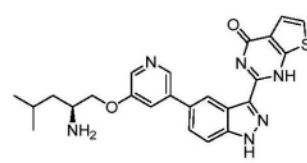
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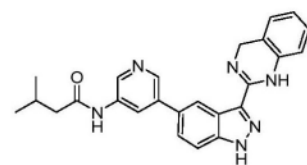
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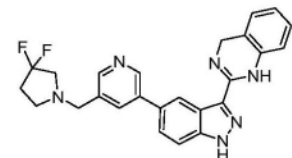
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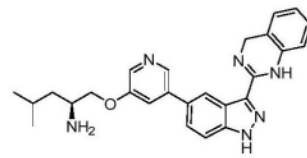
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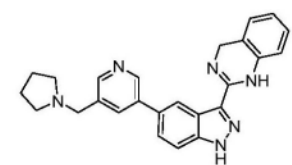
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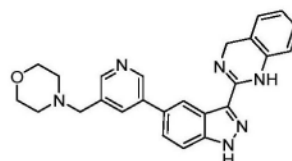
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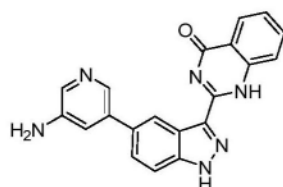
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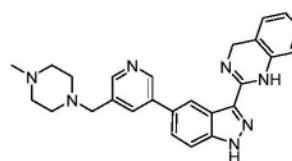
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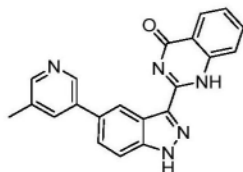
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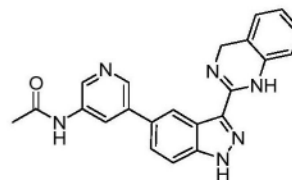
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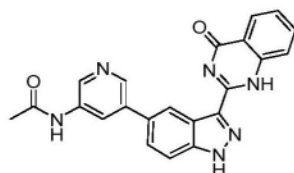
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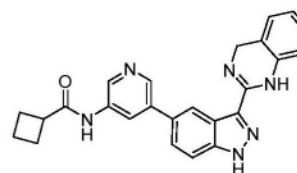
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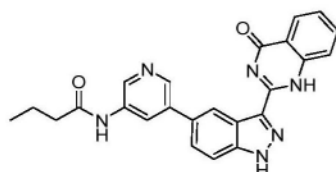
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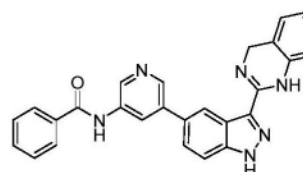
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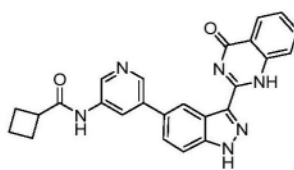
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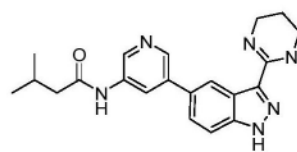
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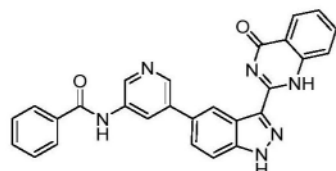
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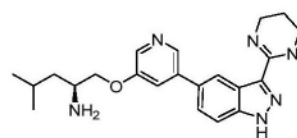
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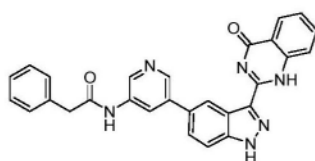
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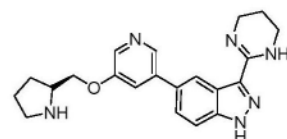
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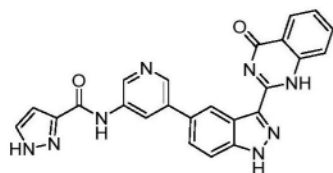
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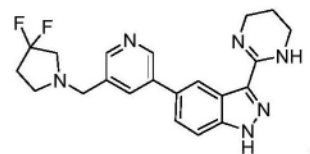
LH-059



LH-030



LH-060



4. 根据权利要求1所述的化合物,其特征在于,所述药学上可接受的盐为所述化合物与以下酸形成的盐:盐酸、硫酸、磷酸、碳酸、硝酸、氢溴酸、氢碘酸、马来酸、富马酸、酒石酸、柠檬酸、苹果酸、甲磺酸、对甲苯磺酸、萘磺酸、琥珀酸、乙酸、扁桃酸、异丁酸或丙二酸。

5. 一种药物组合物,其特征在于,包含权利要求1-4任一所述的化合物以及药学上可接受的载体。

6. 一种权利要求1-4任一所述的化合物或者权利要求5所述的药物组合物在制备CLK2

蛋白抑制剂药物中的应用。

7. 一种权利要求1-4任一所述的化合物或者权利要求5所述的药物组合物在制备DYRK1A蛋白抑制剂药物中的应用。

8. 根据权利要求6或7所述的应用,其特征在于,所述药物为治疗炎症的药物。

9. 根据权利要求8所述的应用,其特征在于,所述炎症为骨关节炎、肌腱病或类风湿性关节炎。

10. 根据权利要求9所述的应用,其特征在于,所述化合物具有软骨保护的作用。

5-吡啶-1H-吡唑类化合物、药物组合物和应用

技术领域

[0001] 本发明涉及一种5-吡啶-1H-吡唑类化合物、药物组合物和应用,尤其涉及一种可制备为能够有效抑制CLK2或DYRK1A蛋白活性的5-吡啶-1H-吡唑类化合物、药物组合物和应用。

背景技术

[0002] 骨关节炎(Osteoarthritis,OA)以滑膜炎症,软骨损失,软骨下骨重塑为特征。OA患者滑膜富含丰富的干细胞,关节软骨不能再生并不是由于干细胞供应不足,而是由干细胞分化不当所致。Wnt通路在器官发生、细胞分化和组织重塑中发挥着核心作用,Wnt信号通路的异常激活或抑制都会导致该疾病的发生。因此,Wnt信号通路是治疗骨关节炎的潜在靶点。

[0003] Wnt信号传导途径是由配体蛋白质Wnt和膜蛋白受体结合激发的一组多下游通道的信号传导途径。经此途径,细胞表面受体胞内的活化过程将细胞外信号传递到细胞内。在经典的Wnt通路中,当细胞膜表面无Wnt蛋白时,其下游的 β -Catenin蛋白在细胞质中会被糖原合酶3(GSK3)复合物分解,导致其不能进入细胞核启动相关Wnt基因的转录;而细胞膜表面存在Wnt蛋白时,其会抑制GSK3复合物,从而使 β -Catenin蛋白在细胞核内积聚,最终启动Wnt通路相关基因的转录。骨关节的稳态与Wnt通路存在着微妙的平衡,这一平衡被打破有可能导致OA的发生。

[0004] 蛋白激酶家族CLK(CDK-like kinase)是一种双特异性蛋白激酶,可通过酪氨酸、丝氨酸或苏氨酸残基底物蛋白磷酸化调控细胞内信号转导;其可分为四个亚型(CLK1,CLK2,CLK3和CLK4),此四个亚型编码的蛋白质C段都有一个高度保守的基因序列,并且具有同样一个结构类似的氨基酸序列。其中CLK2亚型存在于大部分真核生物中,其参与对SR(丝氨酸/精氨酸)蛋白结构域的磷酸化从而调节RNA的选择性剪切,在肝脏中糖异生和脂肪酸的氧化发挥着重要作用,同时也是肝癌、乳腺癌及阿尔茨海默病的治疗靶点,还是Wnt通路和骨关节炎的潜在治疗靶点。

[0005] 双特异性酪氨酸磷酸化调节激酶1A(Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A,DYRK1A)属于DYRK家族,其在进化上高度保守,在哺乳动物中,DYRK家族有五种不同的亚型,只有DYRK1A位于人21号染色体的DSCR区域。DYRK1A由dyrk1a基因表达,编码的成熟蛋白由763个氨基酸组成,包括一个蛋白激酶结构域以及其他特殊结构。许多重要蛋白可作为DYRK1A的底物,并受其调控而参与细胞中的多种生物学功能。例如神经发育、细胞增殖与分化、肿瘤发生以及神经退行性疾病等。

[0006] 目前尚无用于治疗骨关节炎的CLK2抑制剂药物成功上市,仅有一个小分子药物SM-04690进入临床III期,因此不能满足临床需求。SM04690虽然具有显著的CLK2抑制活性,但对CLK家族的选择性不足,这导致其有可能存在一定的副作用;此外其对DYRK1A靶点的抑制活性不足,水溶性不良,因此其成药性有待改善。

发明内容

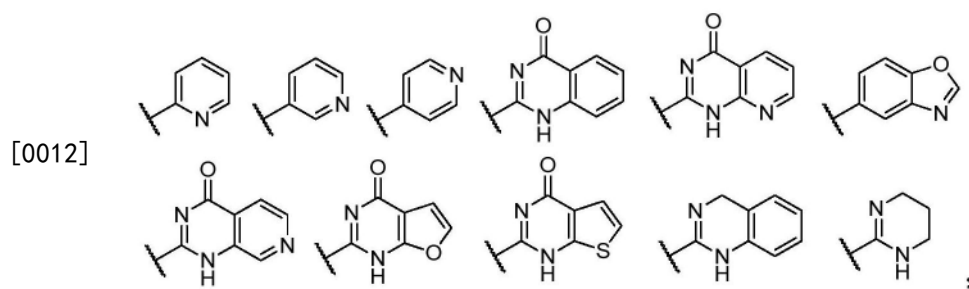
[0007] 发明目的：针对现有化合物存在的CLK家族选择性不足以及对DYRK1A靶点的抑制活性不足等问题，本发明旨在提供一种具有特异性抑制CLK2、DYRK1A蛋白活性的5-吡啶-1H-吡唑类化合物、药物组合物和应用。

[0008] 技术方案：作为本发明涉及的第一方面，本发明的5-吡啶-1H-吡唑类化合物具有式I或II的结构，还包含其异构体、药学上可接受的盐或它们的混合物：

[0009] 其中， R_1 选自被一个或多个氢、卤素、甲氧基、三氟甲基、硝基、羟基、氨基、叠氮基、磺酸基、3-6元环取代的如下基团：氢、直链或支链 C_1 - C_{10} 烷基、苯基、4-6元杂环或4-6元环；

[0010] L、M选自 $-CH_2-$ 、 $-NH-$ 、 $-O-$ 或化学键；

[0011] R_2 选自如下基团：



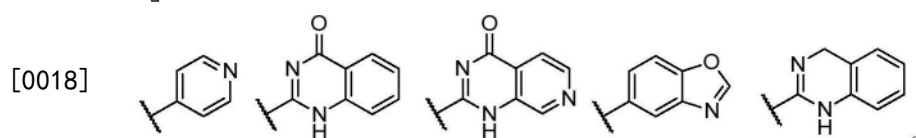
[0013] R_3 选自氢或 C_1 - C_4 烷基。

[0014] 本发明通过合理的药物设计，合成了系列衍生物，生物活性评价显示，所设计化合物具有显著的CLK2抑制活性，对CLK家族成员具有较好的选择性，同时具有显著的DYRK1A抑制活性。

[0015] 优选，上述化合物的结构中：

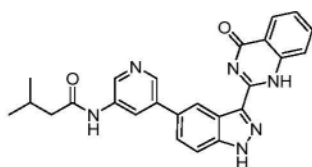
[0016] R_1 选自异丁基、环丙基甲基、环戊基、 α -氨基异戊基、3,3-二氟四氢吡咯基、氢、吗啉基、甲基、叔丁基、乙磺酰基或羟基；

[0017] R_2 选自如下基团：

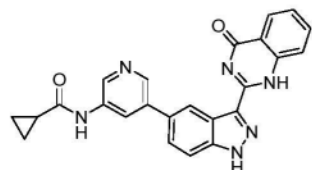


[0019] 更优选，上述化合物选自以下任一化合物：

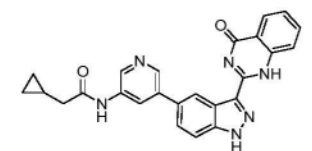
LH-001



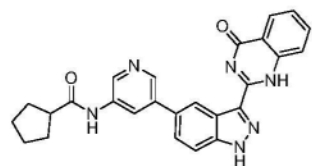
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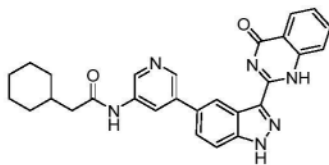
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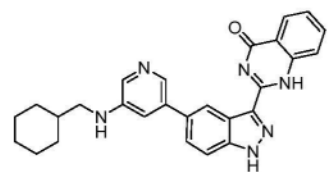
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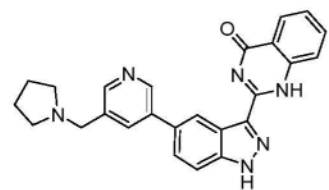
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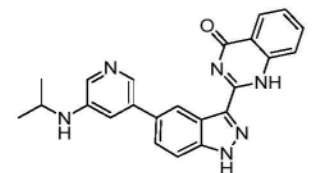
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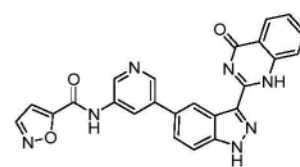
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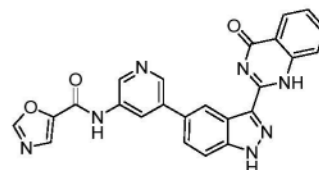
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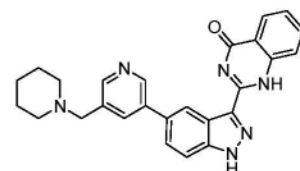
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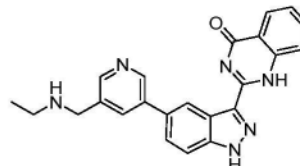
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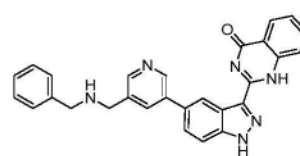
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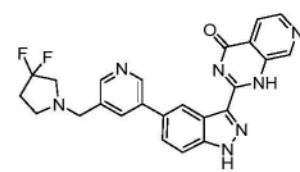
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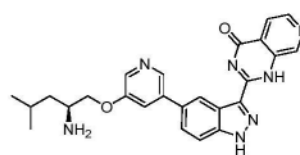
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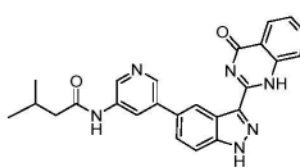
LH-036



LH-037

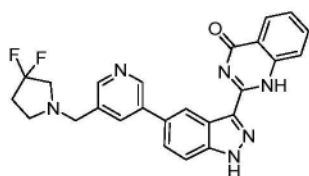


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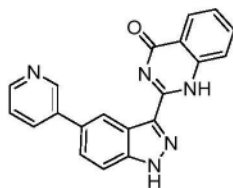


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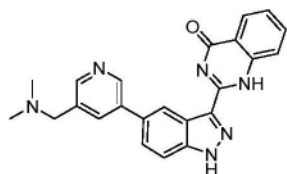
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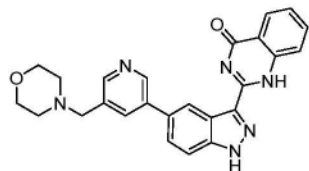
LH-010



LH-011

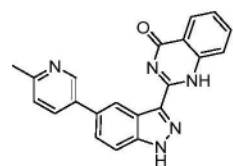


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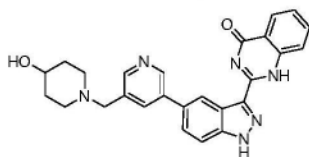


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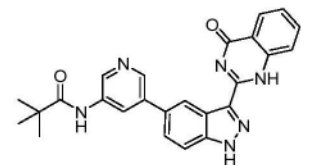
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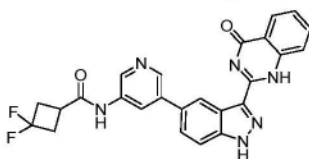
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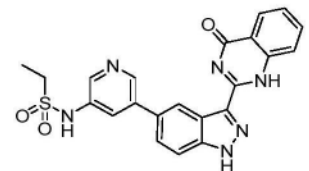
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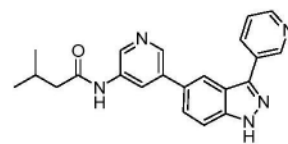
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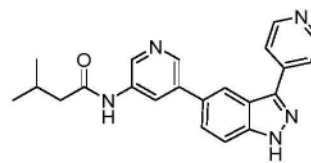
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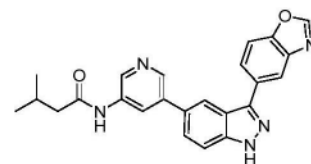
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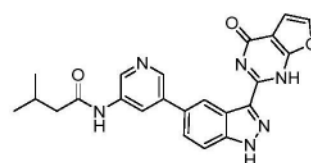
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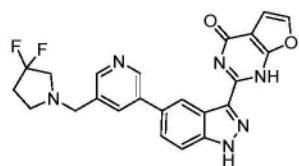
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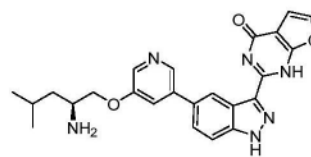
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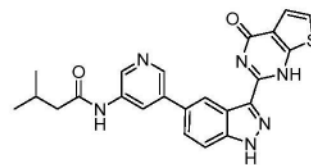
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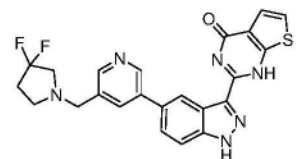
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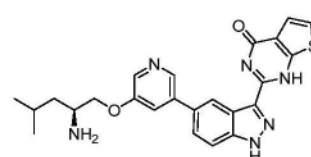
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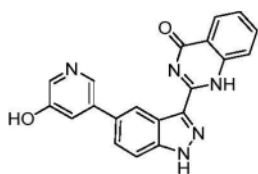
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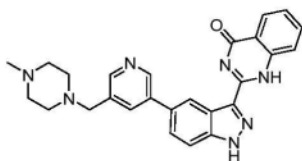
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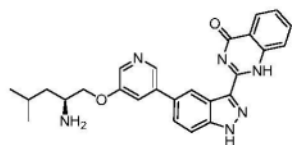
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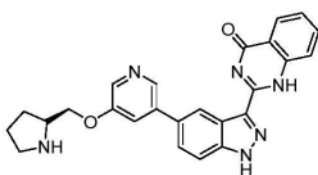
LH-019



LH-020

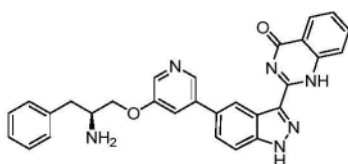


LH-021

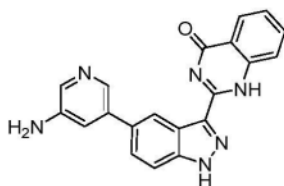


LH-022

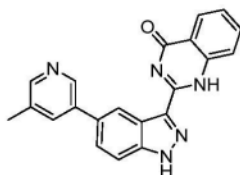
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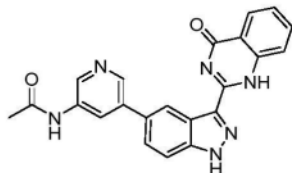
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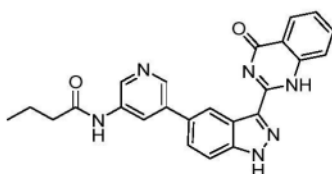
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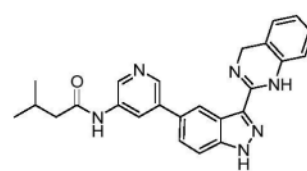
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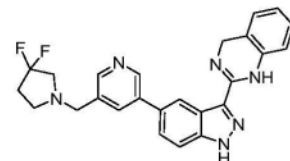
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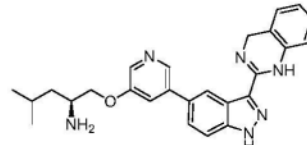
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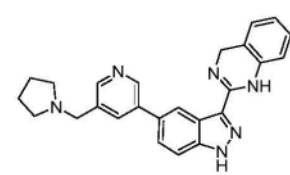
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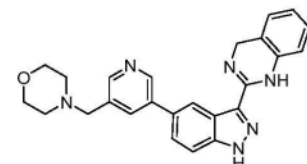
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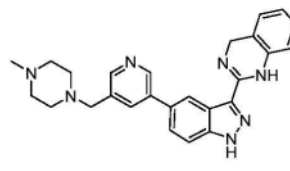
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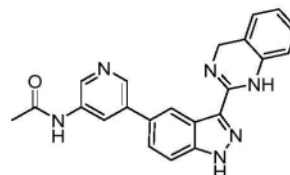
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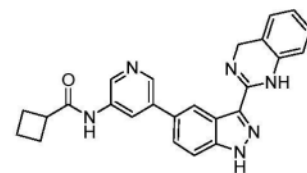
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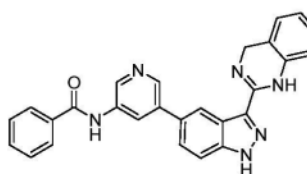
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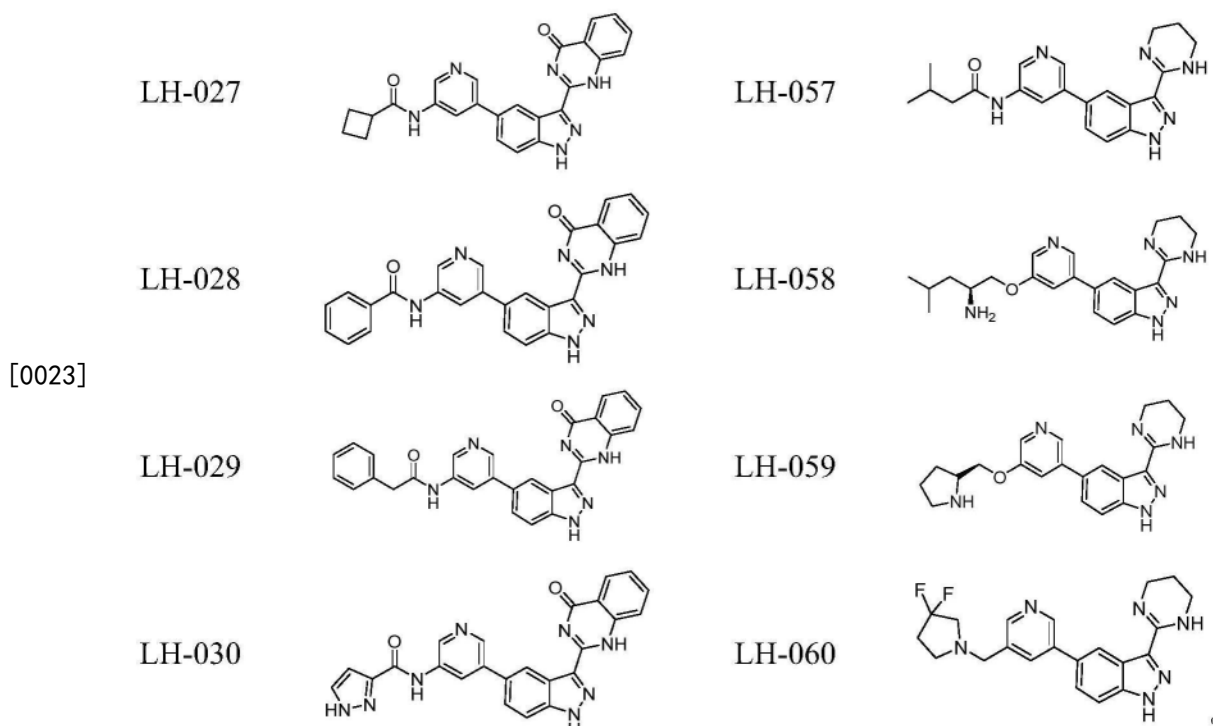


LH-055



LH-056





[0024] 上述化合物的药学上可接受的盐为上述化合物与以下酸形成的盐：盐酸、硫酸、磷酸、碳酸、硝酸、氢溴酸、氢碘酸、马来酸、富马酸、酒石酸、柠檬酸、苹果酸、甲磺酸、对甲苯磺酸、萘磺酸、琥珀酸、乙酸、扁桃酸、异丁酸或丙二酸。

[0025] 作为本发明涉及的第二方面，上述化合物以及药学上可接受的载体形成药物组合物，制成常见的药用制剂，如片剂、胶囊、糖浆、悬浮剂或注射剂，制剂可以加入香料、甜味剂、液体/固体填料、稀释剂等常用药用辅料。

[0026] 作为本发明涉及的第三方面，上述化合物或者其药物组合物可在制备为CLK2蛋白抑制剂药物，还可以制备为DYRK1A蛋白抑制剂药物，具体用于治疗炎症，包括骨关节炎、肌腱病或类风湿性关节炎，具有软骨保护的作用。

[0027] 有益效果：与现有技术相比，本发明具有如下显著优点：

[0028] (1) 该类化合物及其药物组合物可有效抑制CLK2蛋白及DYRK1A蛋白活性，酶水平抑制 IC_{50} 值均小于100nM，最优小于10nM；还可以显著下调炎症模型动物体内与软骨分解相关的蛋白酶的表达式，发挥软骨保护作用；

[0029] (2) 该类化合物及其药物组合物应用广泛，可制备为治疗骨关节炎的药物，在分子水平和动物水平可以发挥药效，并且治疗效果更优异，最优可达到纳摩尔浓度水平；

[0030] (3) 化合物制备方法简便、易操作。

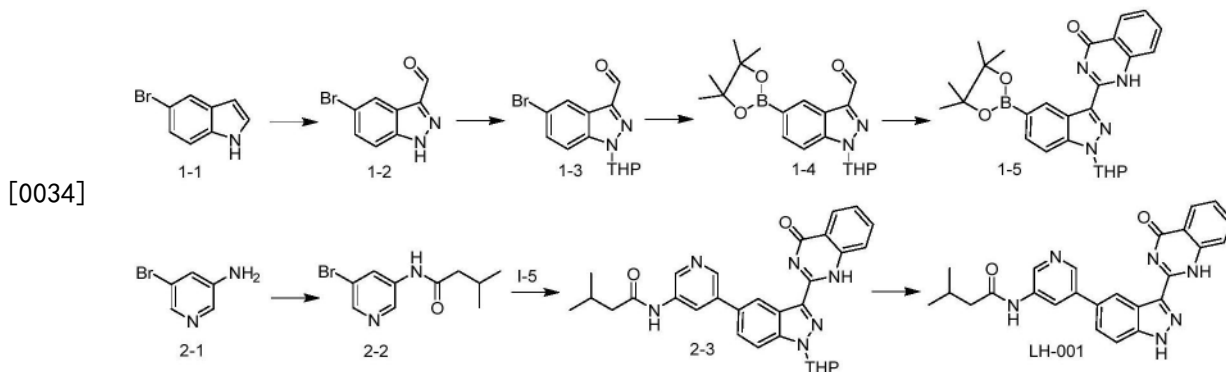
附图说明

[0031] 图1为ACLT模型大鼠第5周软骨RT-qPCR结果 (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$)。

具体实施方式

[0032] 下面结合实施例对本发明的技术方案作进一步说明。

[0033] 实施例1:LH-001的合成



[0035] 中间体1-2的合成:

[0036] 将原料1-1 (5-溴吲哚, 0.1mmol)、丙酮加入到三口瓶中, 冰盐浴降温至0℃下, 滴加亚硝酸钠水溶液 (0.8mmol), 保持温度不超过0℃。滴加完毕后, 继续滴加稀盐酸 (2N HCl)。随后升温至室温下反应4h。旋干、抽滤得粗品。粗品经二氯甲烷打浆得棕红色固体1-2。¹H NMR (300MHz, DMSO-d₆) δ 12.29 (s, 1H), 9.80 (s, 1H), 7.88 (d, J=1.2Hz, 1H), 7.44-7.31 (m, 2H) ppm. HR-MS (ESI): Calculated for C₈H₆BrN₂O [M+H]⁺: 224.9664, found 224.9657. 收率 67%。

[0037] 中间体1-3的合成:

[0038] 中间体1-2 (0.1mmol) 加入三口瓶中, 加入四氢呋喃 (80mL)、对甲基苯磺酸 (0.02mmol), 室温下滴加3,4-二氢吡喃 (0.2mmol), 随后升温至65℃下反应8h。TLC监测反应完全后, 减压蒸除溶剂, 经硅胶柱层析纯化得中间体I-3粗品, 粗品经石油醚打浆后得白色固体I-3。¹H NMR (300MHz, DMSO-d₆) δ 9.80 (s, 1H), 7.88 (s, 1H), 7.37 (d, J=1.0Hz, 2H), 6.27 (t, J=6.9Hz, 1H), 3.90 (dt, J=11.4, 7.0Hz, 1H), 3.77 (dt, J=11.5, 7.0Hz, 1H), 2.52-2.32 (m, 1H), 2.12-1.91 (m, 2H), 1.83-1.64 (m, 1H), 1.58-1.53 (m, 2H) ppm. HR-MS (ESI): Calculated for C₁₃H₁₄BrN₂O₂ [M+H]⁺: 309.0239, found 309.0239. 收率 74%。

[0039] 中间体I-4的合成:

[0040] 将中间体1-3 (0.1mmol) 加入到单口瓶中, 加入联硼酸频那醇酯 (0.12mmol)、乙酸钾 (0.3mmol)、Pd (dppf) Cl₂ (0.005mmol)、二氧六环 (26mL)。升温至回流反应6h, TLC监测反应完全后, 降温抽滤、旋干、经硅胶柱层析纯化的白色固体1-4。¹H NMR (300MHz, DMSO-d₆) δ 9.99 (s, 1H), 8.07 (d, J=1.6Hz, 1H), 7.46 (d, J=7.5Hz, 1H), 7.27 (dd, J=7.5, 1.6Hz, 1H), 6.25 (t, J=6.9Hz, 1H), 3.90 (dt, J=11.4, 7.0Hz, 1H), 3.77 (dt, J=11.4, 7.0Hz, 1H), 2.52-2.32 (m, 1H), 2.12-1.91 (m, 2H), 1.86-1.48 (m, 3H), 1.41 (d, J=15.1Hz, 12H) ppm. HR-MS (ESI): Calculated for C₁₉H₂₆BN₂O₄ [M+H]⁺: 357.1986, found 357.1983. 收率 89%。

[0041] 中间体I-5的合成:

[0042] 将中间体1-4 (0.1mmol) 加入到单口瓶中, 用乙醇:二氯甲烷 (5:1) 混合溶剂将其溶解, 然后加入邻氨基苯甲酰胺 (0.1mmol), 碘单质 (0.1mmol), 于80℃回流反应30min-60min, TLC反应完全后, 加入5%硫代硫酸钠淬灭反应, 然后用二氯与饱和食盐水萃取, 合并有机相, 干燥, 浓缩, 柱层析纯化得粗品, 粗品经 (石油醚:乙酸乙酯=1:1) 打浆得纯品I-5。¹H NMR (300MHz, DMSO-d₆) δ 12.35 (s, 1H), 8.13 (dd, J=7.6, 1.9Hz, 2H), 7.66 (dd, J=7.5, 2.1Hz, 1H), 7.56 (d, J=7.4Hz, 1H), 7.50-7.32 (m, 2H), 7.13 (td, J=7.5, 2.0Hz, 1H), 6.31

(t, J=6.9Hz, 1H), 3.90 (dt, J=11.4, 7.0Hz, 1H), 3.77 (dt, J=11.4, 7.0Hz, 1H), 2.52-2.32 (m, 1H), 2.12-1.91 (m, 2H), 1.86-1.48 (m, 3H), 1.42 (d, J=15.1Hz, 12H) ppm. HR-MS (ESI): Calculated for $C_{26}H_{30}BN_4O_4$ [M+H]⁺: 473.2360, found 473.2357. 收率68%。

[0043] 中间体2-2的合成:

[0044] 将异戊酸 (0.1mmol), HATU (0.3mmol) 溶解在40ml的四氢呋喃中, 然后加入DIPEA (0.3mmol), 室温搅约15分钟, 然后加入原料2-1 (0.1mmol), 室温搅拌反应1-3小时, 待TLC检测原料2-1反应完全, 停止反应, 柱层析分离纯化后可得中间体2-2。

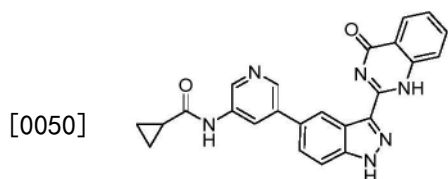
[0045] 中间体2-3的合成:

[0046] 将中间体2-2 (0.1mmol)、中间体1-5 (0.1mmol)、碳酸钠 (0.3mmol)、Pd (dppf) Cl₂ (0.005mmol)、二氧六环 (9mL)、水 (1.5mL) 加入到单口瓶中, 氮气置换空气4次, 升温至回流下反应8h。降温后抽滤、旋干、经硅胶柱层析纯化得到中间体2-3。

[0047] LH-001的合成:

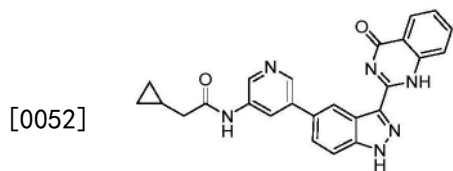
[0048] 将中间体2-3 (0.1mmol) 溶解于5ml二氯甲烷中, 于常温下加入三氟乙酸 (1mmol), 三乙基硅烷 (0.1mmol), 室温反应16-24小时, TLC检测反应完全后, 减压浓缩, 并用二氯甲烷与饱和碳酸氢钠萃取, 合并有机相, 干燥浓缩得终产LH-001。¹H NMR (300MHz, DMSO-d₆) δ 14.16 (s, 1H), 10.57 (s, 1H), 8.93 (t, J=12.4Hz, 3H), 8.67 (d, J=2.1Hz, 1H), 8.26-8.17 (m, 1H), 7.98-7.82 (m, 4H), 7.61-7.51 (m, 1H), 6.30 (s, 1H), 2.34 (d, J=7.1Hz, 2H), 2.17 (m, H), 1.03 (s, 3H), 1.01 (s, 3H) ppm. HR-MS (ESI): Calculated for $C_{25}H_{23}N_6O_2$ [M+H]⁺: 439.1882, found 439.1875。

[0049] 采用与实施例1相似的操作, 制得下列化合物:



LH-002

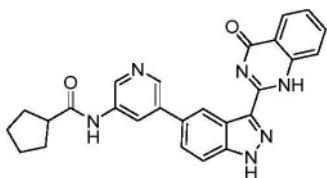
[0051] ¹H NMR (300MHz, DMSO-d₆) δ 14.25 (s, 1H), 12.27 (s, 1H), 10.69 (s, 1H), 8.91-8.82 (m, 2H), 8.66 (s, 1H), 8.42 (s, 1H), 8.14 (d, J=7.9Hz, 1H), 7.76 (d, J=8.4Hz, 3H), 7.62 (d, J=8.7Hz, 1H), 7.39 (t, J=7.2Hz, 1H), 1.90 (q, J=6.2Hz, 1H), 0.91 (q, J=7.9, 5.5Hz, 4H) ppm. HR-MS (ESI): Calculated for $C_{24}H_{19}N_6O_2$ [M+H]⁺: 423.1569, found 423.1562。



LH-003

[0053] ¹H NMR (300MHz, DMSO-d₆) δ 14.28 (s, 1H), 12.29 (s, 1H), 9.80 (s, 1H), 9.61 (d, J=1.3Hz, 1H), 8.83 (d, J=1.5Hz, 1H), 8.70 (d, J=1.3Hz, 1H), 8.13 (dd, J=7.4, 2.0Hz, 1H), 8.03 (t, J=1.3Hz, 1H), 7.71-7.55 (m, 3H), 7.45 (td, J=7.5, 2.0Hz, 1H), 7.14 (td, J=7.5, 2.0Hz, 1H), 2.10 (d, J=7.0Hz, 2H), 1.15 (m, 1H), 0.62-0.44 (m, 2H), 0.32-0.27 (m, 2H) ppm. HR-MS (ESI): Calculated for $C_{25}H_{21}N_6O_2$ [M+H]⁺: 437.1726, found 437.1722。

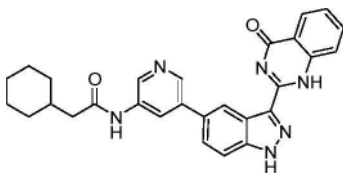
[0054]



LH-004

[0055] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.16 (s, 1H), 10.56 (s, 1H), 8.93 (s, 2H), 8.67 (s, 1H), 8.20 (d, $J=7.8\text{Hz}$, 1H), 7.95 (d, $J=8.1\text{Hz}$, 1H), 7.88 (d, $J=10.6\text{Hz}$, 3H), 7.56 (t, $J=7.4\text{Hz}$, 1H), 5.78 (s, 2H), 2.91 (d, $J=7.7\text{Hz}$, 1H), 1.99-1.57 (m, 8H) ppm. HR-MS (ESI): Calculated for $\text{C}_{26}\text{H}_{23}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 451.1882, found 451.1879.

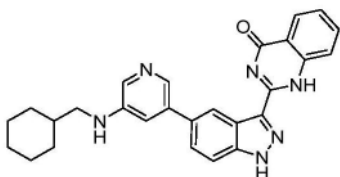
[0056]



LH-005

[0057] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.43 (s, 1H), 11.66 (s, 1H), 10.31 (s, 1H), 8.90 (s, 1H), 8.81 (s, 1H), 8.70 (s, 1H), 8.53 (s, 1H), 8.21 (d, $J=7.9\text{Hz}$, 1H), 7.88 (dd, $J=7.7, 8.3\text{Hz}$, 4H), 7.55 (t, $J=7.3\text{Hz}$, 1H), 2.32 (d, $J=6.9\text{Hz}$, 2H), 1.86-1.66 (m, 6H), 1.25 (q, $J=11.6, 10.8\text{Hz}$, 3H), 1.05 (q, $J=11.3\text{Hz}$, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{28}\text{H}_{27}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 479.2195, found 479.2191.

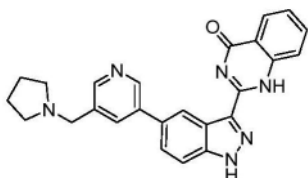
[0058]



LH-006

[0059] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.08 (s, 1H), 12.21 (s, 1H), 8.83 (s, 1H), 8.22 (d, $J=8.2\text{Hz}$, 2H), 8.04 (d, $J=2.4\text{Hz}$, 1H), 7.89 (d, $J=4.0\text{Hz}$, 2H), 7.82 (s, 2H), 7.57 (dt, $J=8.2, 4.1\text{Hz}$, 1H), 7.38 (s, 1H), 3.05 (d, $J=6.7\text{Hz}$, 2H), 1.88 (d, $J=12.6\text{Hz}$, 2H), 1.77-1.60 (m, 5H), 1.26 (t, $J=10.7\text{Hz}$, 3H), 1.02 (dt, $J=12.0, 6.0\text{Hz}$, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{27}\text{H}_{27}\text{N}_6\text{O}$ $[\text{M}+\text{H}]^+$: 451.2246, found 451.2241.

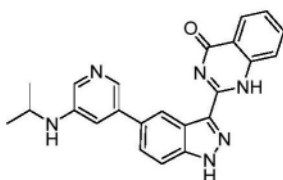
[0060]



LH-007

[0061] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.11 (s, 1H), 12.22 (s, 1H), 8.90 (d, $J=10.1\text{Hz}$, 2H), 8.58 (s, 1H), 8.21 (d, $J=7.9\text{Hz}$, 1H), 8.12 (s, 1H), 7.86 (dd, $J=14.2, 7.9\text{Hz}$, 4H), 7.57 (d, $J=7.2\text{Hz}$, 1H), 3.86 (s, 2H), 2.64 (s, 4H), 1.80 (d, $J=5.3\text{Hz}$, 4H) ppm. HR-MS (ESI): Calculated for $\text{C}_{25}\text{H}_{23}\text{N}_6\text{O}$ $[\text{M}+\text{H}]^+$: 423.1933, found 423.1928.

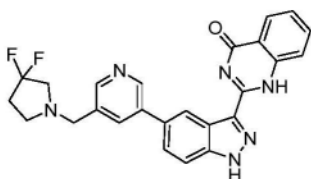
[0062]



LH-008

[0063] ^1H NMR (300MHz, DMSO- d_6) δ 14.15 (s, 1H), 12.63 (s, 1H), 8.81 (s, 1H), 8.21 (d, $J=7.9\text{Hz}$, 1H), 8.15 (s, 1H), 8.01 (d, $J=2.5\text{Hz}$, 1H), 7.87 (d, $J=3.6\text{Hz}$, 2H), 7.79 (d, $J=2.6\text{Hz}$, 2H), 7.55 (dq, $J=8.0, 3.8, 3.2\text{Hz}$, 1H), 7.23 (s, 1H), 5.88 (d, $J=7.9\text{Hz}$, 1H), 3.75 (d, $J=6.8\text{Hz}$, 1H), 1.25 (s, 3H), 1.23 (s, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{23}\text{H}_{21}\text{N}_6\text{O}[\text{M}+\text{H}]^+$: 397.1777, found 397.1769.

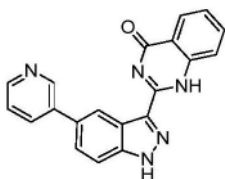
[0064]



LH-009

[0065] ^1H NMR (300MHz, DMSO- d_6) δ 14.14 (s, 1H), 12.21 (s, 1H), 8.96 (d, $J=2.2\text{Hz}$, 1H), 8.89 (s, 1H), 8.60 (s, 1H), 8.24-8.13 (m, 2H), 7.88-7.69 (m, 4H), 7.59-7.52 (m, 1H), 3.92 (s, 2H), 3.11 (t, $J=13.2\text{Hz}$, 2H), 2.94-2.88 (m, 2H), 2.43-2.36 (m, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{25}\text{H}_{21}\text{F}_2\text{N}_6\text{O}[\text{M}+\text{H}]^+$: 459.1745, found 459.1739.

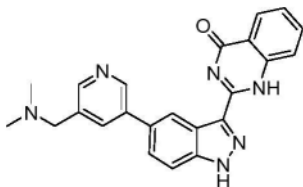
[0066]



LH-010

[0067] ^1H NMR (300MHz, DMSO- d_6) δ 14.27 (s, 1H), 12.28 (s, 1H), 9.00 (d, $J=2.3\text{Hz}$, 1H), 8.85 (s, 1H), 8.65-8.58 (m, 1H), 8.23-8.12 (m, 2H), 7.83 (dd, $J=9.9, 6.4\text{Hz}$, 3H), 7.73 (dd, $J=8.7, 1.8\text{Hz}$, 1H), 7.56 (dd, $J=8.0, 4.8\text{Hz}$, 1H), 7.49-7.42 (m, 1H) ppm. HR-MS (ESI): Calculated for $\text{C}_{20}\text{H}_{14}\text{N}_5\text{O}[\text{M}+\text{H}]^+$: 340.1198, found 340.1192.

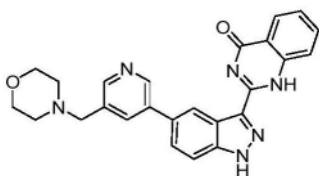
[0068]



LH-011

[0069] ^1H NMR (300MHz, DMSO- d_6) δ 14.28 (s, 1H), 12.14 (s, 1H), 8.89 (s, 2H), 8.52 (s, 1H), 8.17 (d, $J=7.9\text{Hz}$, 1H), 8.05 (s, 1H), 7.84-7.69 (m, 4H), 7.45 (d, $J=3.5\text{Hz}$, 1H), 3.58 (s, 2H), 2.25 (s, 6H) ppm. HR-MS (ESI): Calculated for $\text{C}_{23}\text{H}_{21}\text{N}_6\text{O}[\text{M}+\text{H}]^+$: 397.1777, found 397.1771.

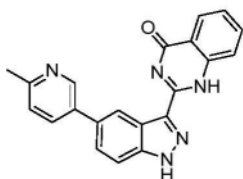
[0070]



LH-012

[0071] ^1H NMR (300MHz, DMSO- d_6) δ 14.09 (s, 1H), 12.25 (s, 1H), 8.91 (d, $J=2.2\text{Hz}$, 1H), 8.87 (s, 1H), 8.57 (d, $J=1.9\text{Hz}$, 1H), 8.21 (d, $J=7.9\text{Hz}$, 1H), 8.12 (d, $J=2.2\text{Hz}$, 1H), 7.93-7.81 (m, 6H), 7.57-7.51 (m, 1H), 3.72 (s, 2H), 3.64 (d, $J=4.7\text{Hz}$, 6H) ppm. HR-MS (ESI): Calculated for $\text{C}_{25}\text{H}_{23}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 439.1882, found 439.1879.

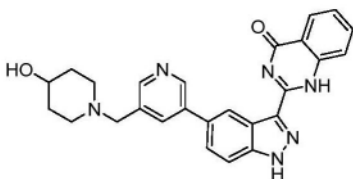
[0072]



LH-013

[0073] ^1H NMR (300MHz, DMSO- d_6) δ 14.11 (s, 1H), 12.22 (s, 1H), 8.61-8.47 (m, 3H), 8.19 (d, $J=7.9\text{Hz}$, 1H), 7.91-7.78 (m, 3H), 7.55 (dt, $J=11.2, 5.3\text{Hz}$, 2H), 7.44 (d, $J=5.0\text{Hz}$, 1H), 2.36 (s, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{21}\text{H}_{16}\text{N}_5\text{O}$ $[\text{M}+\text{H}]^+$: 354.1355, found 354.1348.

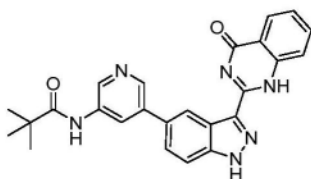
[0074]



LH-014

[0075] ^1H NMR (300MHz, DMSO- d_6) δ 14.11 (s, 1H), 12.29 (s, 1H), 8.92 (d, $J=13.6\text{Hz}$, 2H), 8.59 (s, 1H), 8.22 (d, $J=7.9\text{Hz}$, 2H), 7.91 (d, $J=7.4\text{Hz}$, 4H), 7.57 (ddd, $J=8.2, 6.0, 2.2\text{Hz}$, 1H), 3.77 (s, 1H), 3.65 (s, 2H), 1.81 (s, 2H), 1.53 (s, 3H), 1.25 (s, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{26}\text{H}_{25}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 453.2039, found 453.2041.

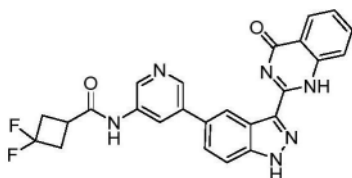
[0076]



LH-015

[0077] ^1H NMR (300MHz, DMSO- d_6) δ 14.14 (s, 1H), 12.20 (s, 1H), 9.68 (s, 1H), 8.98 (d, $J=11.2\text{Hz}$, 2H), 8.77 (s, 1H), 8.60 (s, 1H), 8.29 (t, $J=7.7\text{Hz}$, 1H), 8.04-7.89 (m, 4H), 7.63 (d, $J=7.7\text{Hz}$, 1H), 1.44-1.36 (m, 9H) ppm. HR-MS (ESI): Calculated for $\text{C}_{25}\text{H}_{23}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 439.1882, found 439.1878.

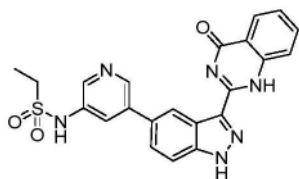
[0078]



LH-016

[0079] ^1H NMR (300MHz, DMSO- d_6) δ 14.21 (s, 1H), 12.29 (s, 1H), 10.85 (s, 1H), 8.89 (d, $J=8.1\text{Hz}$, 2H), 8.73 (s, 1H), 8.56 (s, 1H), 8.21 (d, $J=7.8\text{Hz}$, 1H), 7.91 (d, $J=7.2\text{Hz}$, 4H), 7.63-7.50 (m, 1H), 2.93-2.85 (m, 3H), 1.23 (s, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{25}\text{H}_{19}\text{F}_2\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 473.1538, found 473.1532.

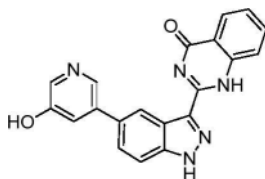
[0080]



LH-017

[0081] ^1H NMR (300MHz, DMSO- d_6) δ 14.14 (s, 1H), 12.31 (s, 1H), 10.34 (s, 1H), 8.92 (s, 1H), 8.79 (s, 1H), 8.52 (s, 1H), 8.22 (d, $J=7.6\text{Hz}$, 1H), 8.05 (s, 1H), 7.90 (d, $J=12.0\text{Hz}$, 4H), 7.58 (s, 1H), 3.31 (s, 2H), 1.25 (s, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{22}\text{H}_{19}\text{N}_6\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$: 447.1239, found 447.1231.

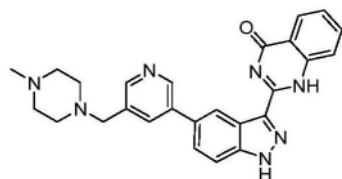
[0082]



LH-018

[0083] ^1H NMR (300MHz, DMSO- d_6) δ 14.73 (s, 1H), 12.25 (s, 1H), 8.84 (s, 1H), 8.39 (s, 1H), 8.14 (d, $J=8.9\text{Hz}$, 2H), 7.74 (d, $J=4.3\text{Hz}$, 3H), 7.63 (d, $J=8.4\text{Hz}$, 1H), 7.52 (s, 1H), 7.39 (d, $J=8.4\text{Hz}$, 1H) ppm. HR-MS (ESI): Calculated for $\text{C}_{20}\text{H}_{14}\text{N}_5\text{O}_2$ $[\text{M}+\text{H}]^+$: 356.1147, found 356.1141.

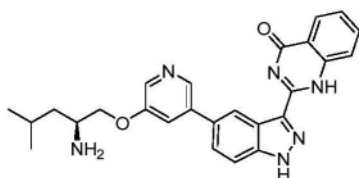
[0084]



[0085] LH-019

[0086] ^1H NMR (300MHz, DMSO- d_6) δ 14.22 (s, 1H), 12.32 (s, 1H), 9.03-8.92 (m, 2H), 8.65 (d, $J=1.9\text{Hz}$, 1H), 8.30 (d, $J=7.9\text{Hz}$, 1H), 8.18 (t, $J=2.1\text{Hz}$, 1H), 8.02-7.90 (m, 4H), 7.65-7.59 (m, 1H), 3.81 (s, 2H), 2.95 (s, 4H), 2.72 (s, 4H), 2.61 (s, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{26}\text{H}_{26}\text{N}_7\text{O}$ $[\text{M}+\text{H}]^+$: 452.2199, found 452.2203.

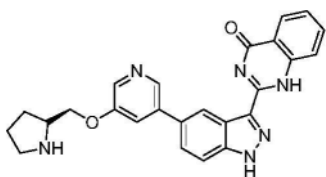
[0087]



LH-020

[0088] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.16 (s, 1H), 12.30 (s, 1H), 8.88 (s, 1H), 8.67 (d, $J=1.7\text{Hz}$, 1H), 8.43 (d, $J=2.6\text{Hz}$, 1H), 8.23-8.18 (m, 2H), 7.93-7.84 (m, 4H), 7.61-7.53 (m, 1H), 4.46 (dd, $J=10.7, 3.2\text{Hz}$, 1H), 4.25 (dd, $J=10.6, 6.7\text{Hz}$, 1H), 3.66 (s, 1H), 2.91 (s, 1H), 2.75 (s, 1H), 1.83-1.60 (m, 3H), 0.98 (d, $J=1.9\text{Hz}$, 3H), 0.96 (d, $J=2.0\text{Hz}$, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{26}\text{H}_{27}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 455.2195, found 455.2191.

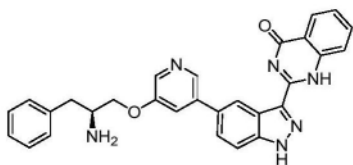
[0089]



LH-021

[0090] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.36 (s, 1H), 12.18 (s, 1H), 8.57 (d, $J=1.3\text{Hz}$, 1H), 8.22-8.08 (m, 2H), 7.72-7.52 (m, 4H), 7.45 (td, $J=7.5, 2.0\text{Hz}$, 1H), 7.14 (td, $J=7.5, 2.0\text{Hz}$, 1H), 4.33 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 4.16 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 3.25 (d, $J=7.0\text{Hz}$, 1H), 2.97 (dt, $J=9.4, 6.9\text{Hz}$, 1H), 2.79 (dt, $J=9.3, 6.9\text{Hz}$, 1H), 1.77-1.65 (m, 3H), 1.41 (ddd, $J=12.3, 7.6, 6.3\text{Hz}$, 1H), 1.19 (s, 1H) ppm. HR-MS (ESI): Calculated for $\text{C}_{25}\text{H}_{23}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 439.1882, found 439.1878.

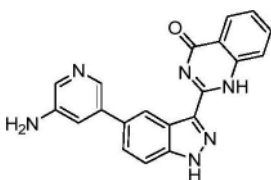
[0091]



LH-022

[0092] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.05 (s, 1H), 12.53 (s, 1H), 8.78-8.71 (m, 1H), 8.59 (d, $J=1.3\text{Hz}$, 1H), 8.18-8.05 (m, 2H), 7.71-7.51 (m, 4H), 7.44 (td, $J=7.5, 2.0\text{Hz}$, 1H), 7.35-7.22 (m, 2H), 7.22-7.07 (m, 4H), 4.26 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 4.07 (dd, $J=12.4, 6.9\text{Hz}$, 1H), 3.67-3.61 (m, 1H), 3.11-2.98 (m, 1H), 2.81-2.71 (m, 1H), 1.54 (s, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{29}\text{H}_{25}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$: 489.2039, found 489.2029.

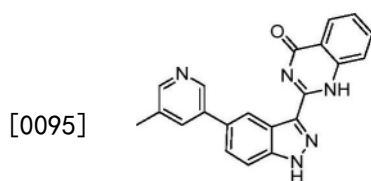
[0093]



LH-023

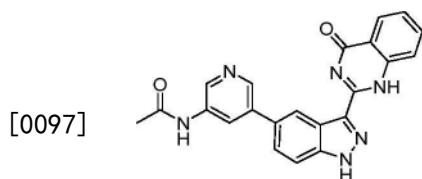
[0094] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.16 (s, 1H), 12.38 (s, 1H), 8.79 (d, $J=1.1\text{Hz}$, 1H), 8.52 (d, $J=1.3\text{Hz}$, 1H), 8.36 (d, $J=1.3\text{Hz}$, 1H), 8.13 (dd, $J=7.4, 2.0\text{Hz}$, 1H), 7.71-7.53 (m, 3H), 7.51-7.39 (m, 2H), 7.14 (td, $J=7.5, 2.0\text{Hz}$, 1H), 3.50 (s, 2H) ppm. HR-MS (ESI):

Calculated for $C_{20}H_{15}N_6O[M+H]^+$: 355.1307, found 355.1301.



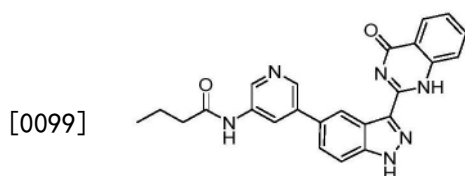
LH-024

[0096] ^1H NMR (300MHz, DMSO- d_6) δ 14.42 (s, 1H), 12.27 (s, 1H), 8.83-8.73 (m, 2H), 8.48 (d, $J=1.3$ Hz, 1H), 8.13 (dd, $J=7.4, 2.0$ Hz, 1H), 7.89 (t, $J=1.3$ Hz, 1H), 7.66 (dd, $J=7.5, 2.0$ Hz, 1H), 7.63-7.50 (m, 2H), 7.45 (td, $J=7.5, 2.0$ Hz, 1H), 7.14 (td, $J=7.5, 2.0$ Hz, 1H), 2.48 (s, 3H) ppm. HR-MS (ESI): Calculated for $C_{21}H_{16}N_5O[M+H]^+$: 354.1355, found 354.1350.



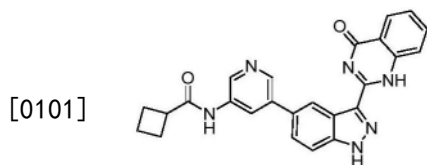
LH-025

[0098] ^1H NMR (300MHz, DMSO- d_6) δ 14.52 (s, 1H), 12.22 (s, 1H), 9.80 (s, 1H), 9.58 (d, $J=1.3$ Hz, 1H), 8.87-8.79 (m, 1H), 8.70 (d, $J=1.3$ Hz, 1H), 8.13 (dd, $J=7.5, 2.0$ Hz, 1H), 8.04 (t, $J=1.3$ Hz, 1H), 7.71-7.55 (m, 3H), 7.45 (td, $J=7.5, 2.0$ Hz, 1H), 7.14 (td, $J=7.5, 2.0$ Hz, 1H), 2.20 (s, 3H) ppm. HR-MS (ESI): Calculated for $C_{22}H_{17}N_6O_2[M+H]^+$: 397.1413, found 397.1410.



LH-026

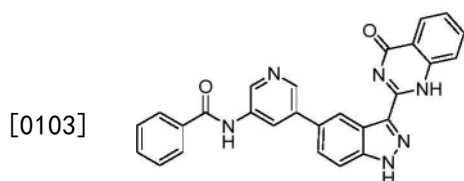
[0100] ^1H NMR (300MHz, DMSO- d_6) δ 14.62 (s, 1H), 12.12 (s, 1H), 9.80 (s, 1H), 9.60 (d, $J=1.3$ Hz, 1H), 8.76 (d, $J=1.6$ Hz, 1H), 8.68 (d, $J=1.3$ Hz, 1H), 8.13 (dd, $J=7.5, 2.1$ Hz, 1H), 7.91 (t, $J=1.3$ Hz, 1H), 7.66 (dd, $J=7.5, 2.0$ Hz, 1H), 7.65-7.49 (m, 2H), 7.45 (td, $J=7.5, 2.0$ Hz, 1H), 7.14 (td, $J=7.5, 2.0$ Hz, 1H), 2.37 (t, $J=8.1$ Hz, 2H), 1.69-1.55 (m, 2H), 0.98 (t, $J=8.0$ Hz, 3H) ppm. HR-MS (ESI): Calculated for $C_{24}H_{21}N_6O_2[M+H]^+$: 425.1726, found 425.1720.



LH-027

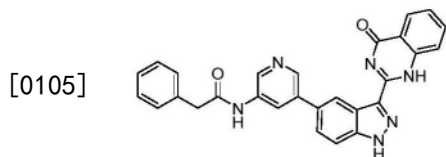
[0102] ^1H NMR (300MHz, DMSO- d_6) δ 14.55 (s, 1H), 12.14 (s, 1H), 9.80 (s, 1H), 9.62 (d, $J=1.4$ Hz, 1H), 8.76 (d, $J=1.5$ Hz, 1H), 8.68 (d, $J=1.3$ Hz, 1H), 8.13 (dd, $J=7.5, 2.0$ Hz, 1H),

7.91 (t, J=1.3Hz, 1H), 7.71-7.55 (m, 2H), 7.54 (dd, J=7.5, 1.5Hz, 1H), 7.45 (td, J=7.5, 2.0Hz, 1H), 7.14 (td, J=7.5, 2.0Hz, 1H), 3.22 (d, J=7.0Hz, 1H), 2.55-2.37 (m, 2H), 2.17-1.82 (m, 4H) ppm. HR-MS (ESI): Calculated for $C_{25}H_{21}N_6O_2 [M+H]^+$: 437.1726, found 437.1719.



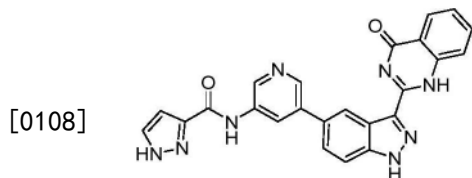
LH-028

[0104] ^1H NMR (300MHz, DMSO- d_6) δ 14.25 (s, 1H), 12.25 (s, 1H), 9.50 (d, J=1.3Hz, 1H), 8.86 (d, J=1.4Hz, 1H), 8.71 (d, J=1.3Hz, 1H), 8.14 (d, J=9.8Hz, 2H), 8.06 (d, J=1.2Hz, 1H), 8.02-7.90 (m, 2H), 7.71-7.40 (m, 6H), 7.31 (ddt, J=9.6, 7.2, 2.0Hz, 1H), 7.15 (td, J=7.5, 2.1Hz, 1H) ppm. HR-MS (ESI): Calculated for $C_{27}H_{19}N_6O_2 [M+H]^+$: 459.1569, found 459.1560.



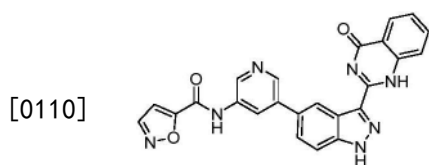
LH-029

[0107] ^1H NMR (300MHz, DMSO- d_6) δ 14.56 (s, 1H), 12.17 (s, 1H), 9.80 (s, 1H), 9.61 (d, J=1.2Hz, 1H), 8.71 (dd, J=14.1, 1.4Hz, 2H), 8.13 (dd, J=7.4, 2.0Hz, 1H), 7.81 (t, J=1.3Hz, 1H), 7.71-7.28 (m, 6H), 7.28-7.07 (m, 4H), 3.76 (s, 2H) ppm. HR-MS (ESI): Calculated for $C_{28}H_{21}N_6O_2 [M+H]^+$: 473.1726, found 473.1719.



LH-030

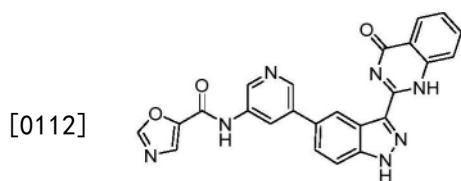
[0109] ^1H NMR (300MHz, DMSO- d_6) δ 14.16 (s, 1H), 12.12 (s, 1H), 12.35 (s, 1H), 9.53-9.42 (m, 2H), 8.83 (t, J=1.0Hz, 1H), 8.70 (d, J=1.3Hz, 1H), 8.19-8.02 (m, 2H), 7.71-7.55 (m, 3H), 7.45 (td, J=7.5, 2.0Hz, 1H), 7.35 (d, J=7.5Hz, 1H), 7.14 (td, J=7.5, 2.0Hz, 1H), 6.86 (d, J=7.5Hz, 1H) ppm. HR-MS (ESI): Calculated for $C_{24}H_{17}N_8O_2 [M+H]^+$: 449.1474, found 449.1470.



LH-031

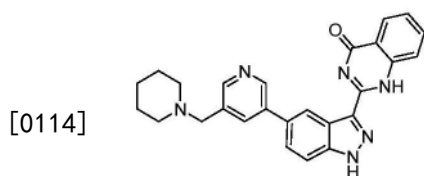
[0111] ^1H NMR (300MHz, DMSO- d_6) δ 14.11 (s, 1H), 12.27 (s, 1H), 9.48 (d, J=1.3Hz, 2H),

8.90 (s, 1H) , 8.80 (d, J=1.5Hz, 2H) , 8.71 (d, J=1.3Hz, 2H) , 8.13 (dd, J=7.4, 2.0Hz, 2H) , 7.96 (t, J=1.3Hz, 2H) , 7.67 (d, J=2.0Hz, 1H) , 7.45 (s, 1H) ppm. HR-MS (ESI) : Calculated for $C_{24}H_{16}N_7O_3 [M+H]^+$: 450.1315, found 450.1310.



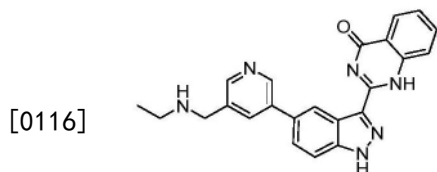
LH-032

[0113] ^1H NMR (300MHz, DMSO- d_6) δ 14.11 (s, 1H) , 12.27 (s, 1H) , 9.49 (d, J=1.3Hz, 1H) , 8.90 (s, 1H) , 8.79 (d, J=1.5Hz, 1H) , 8.70 (d, J=1.3Hz, 1H) , 8.13 (dd, J=7.5, 2.0Hz, 1H) , 8.06 (s, 1H) , 7.95 (t, J=1.3Hz, 1H) , 7.71-7.50 (m, 3H) , 7.45 (td, J=7.5, 2.0Hz, 1H) , 7.39 (s, 1H) , 7.14 (td, J=7.5, 2.0Hz, 1H) ppm. HR-MS (ESI) : Calculated for $C_{24}H_{16}N_7O_3 [M+H]^+$: 450.1315, found 450.1309.



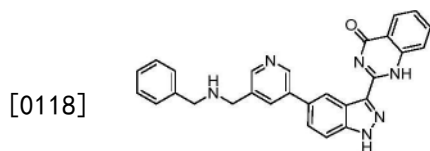
LH-033

[0115] ^1H NMR (300MHz, DMSO- d_6) δ 14.14 (s, 1H) , 12.25 (s, 1H) , 8.88-8.74 (m, 2H) , 8.71 (d, J=1.3Hz, 1H) , 8.23-8.13 (m, 2H) , 7.71-7.51 (m, 3H) , 7.45 (td, J=7.5, 2.0Hz, 1H) , 7.14 (td, J=7.5, 2.0Hz, 1H) , 3.69 (s, 2H) , 2.43 (t, J=5.3Hz, 4H) , 1.62-1.36 (m, 6H) ppm. HR-MS (ESI) : Calculated for $C_{26}H_{25}N_6O [M+H]^+$: 437.2090, found 437.2088.



LH-034

[0117] ^1H NMR (300MHz, DMSO- d_6) δ 14.63 (s, 1H) , 12.32 (s, 1H) , 8.86-8.70 (m, 3H) , 8.18-8.04 (m, 2H) , 7.71-7.51 (m, 3H) , 7.45 (td, J=7.5, 2.0Hz, 1H) , 7.14 (td, J=7.5, 2.0Hz, 1H) , 3.65 (s, 2H) , 2.59 (q, J=8.0Hz, 2H) , 1.37 (s, 1H) , 1.11 (t, J=8.0Hz, 3H) ppm. HR-MS (ESI) : Calculated for $C_{23}H_{21}N_6O [M+H]^+$: 397.1777, found 397.1770.

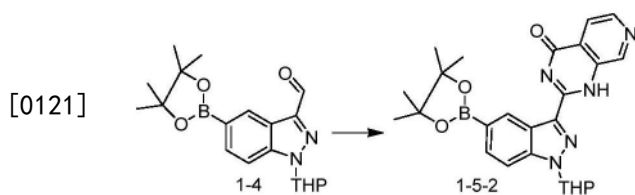


LH-035

[0119] ^1H NMR (300MHz, DMSO- d_6) δ 14.51 (s, 1H) , 12.31 (s, 1H) , 8.84-8.74 (m, 2H) , 8.65 (d, J=1.3Hz, 1H) , 8.20-8.09 (m, 2H) , 7.71-7.56 (m, 3H) , 7.51-7.20 (m, 6H) , 7.14 (td, J=7.5, 2.0Hz, 1H) , 3.65 (s, 4H) , 1.69 (s, 1H) ppm. HR-MS (ESI) : Calculated for $C_{28}H_{23}N_6O [M+H]^+$:

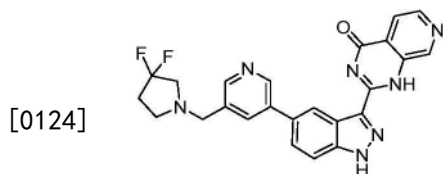
459.1933, found 459.1926。

[0120] 实施例2:LH-036的合成



[0122] 将中间体1-4(0.1mmol)加入到单口瓶中,用5mL二甲基乙酰胺将其溶解,然后加入3-氨基吡啶-4-甲酰胺(0.15mmol),对甲苯磺酸水合物(0.15mmol),亚硫酸氢钠(0.15mmol)于120℃反应6-8小时,TLC反应完全后,用乙酸乙酯与饱和食盐水萃取三次,合并有机相,干燥,浓缩,柱层析纯化得中间体1-5-2。¹H NMR(300MHz,DMSO-d₆) δ12.31(s,1H),9.47(s,1H),8.41-8.27(m,2H),8.13(d,J=1.6Hz,1H),7.56(d,J=7.5Hz,1H),7.37(dd,J=7.5,1.6Hz,1H),6.31(t,J=6.9Hz,1H),3.90(dt,J=11.4,7.0Hz,1H),3.77(dt,J=11.4,7.0Hz,1H),2.52-2.32(m,1H),2.12-1.91(m,2H),1.86-1.48(m,3H),1.42(d,J=14.9Hz,12H) ppm.HR-MS(ESI):Calculated for C₂₅H₂₉BN₅O₄[M+H]⁺:474.2313,found474.2310。收率75%。

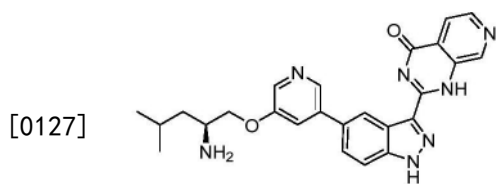
[0123] 其余步骤与实施例1相同,得到化合物LH-036。



LH-036

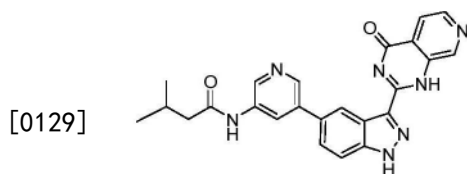
[0125] ¹H NMR(300MHz,DMSO-d₆) δ14.19(s,1H),12.66(s,1H),9.29(s,1H),8.97(s,1H),8.90(s,1H),8.69(d,J=5.1Hz,1H),8.61(s,1H),8.18(s,1H),8.02(d,J=5.1Hz,1H),7.88(q,J=8.7Hz,2H),3.95(s,2H),3.17-3.08(m,2H),2.93(t,J=6.9Hz,2H),2.38(dq,J=15.3,7.9,7.4Hz,2H) ppm.HR-MS(ESI):Calculated for C₂₄H₂₀F₂N₇O[M+H]⁺:460.1697,found 460.1690。

[0126] 采用与实施例2相似的操作,制得下列化合物:



LH-037

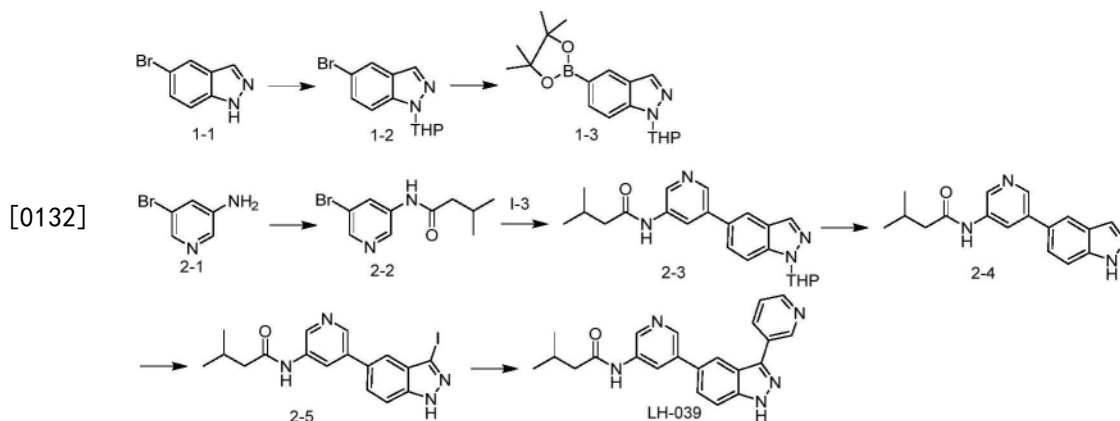
[0128] ¹H NMR(300MHz,DMSO-d₆) δ14.55(s,1H),12.33(s,1H),9.47(s,1H),8.76(t,J=1.0Hz,1H),8.65(d,J=1.3Hz,1H),8.40(d,J=5.0Hz,1H),8.31(d,J=5.0Hz,1H),8.22(d,J=1.4Hz,1H),7.61(dd,J=16.8,1.2Hz,3H),4.33(dd,J=12.4,7.0Hz,1H),4.08(dd,J=12.4,7.0Hz,1H),3.23-3.13(m,1H),1.73-1.58(m,2H),1.58-1.40(m,1H),1.34(s,2H),0.99-0.82(m,6H) ppm.HR-MS(ESI):Calculated for C₂₅H₂₆N₇O₂[M+H]⁺:456.2148,found 456.2140。



LH-038

[0130] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.34 (s, 1H), 12.21 (s, 1H), 9.80 (s, 1H), 9.64 (d, $J=1.4\text{Hz}$, 1H), 9.47 (s, 1H), 8.82-8.70 (m, 2H), 8.40 (d, $J=5.0\text{Hz}$, 1H), 8.31 (d, $J=5.0\text{Hz}$, 1H), 8.00 (s, 1H), 7.61 (d, $J=1.0\text{Hz}$, 2H), 2.22-2.05 (m, 3H), 1.08-0.93 (m, 6H) ppm. HR-MS (ESI): Calculated for $\text{C}_{24}\text{H}_{22}\text{N}_7\text{O}_2$ $[\text{M}+\text{H}]^+$: 440.1835, found 440.1835.

[0131] 实施例3: LH-039的合成



[0133] 中间体1-2的合成:

[0134] 中间体1-1 (0.1mmol) 加入三口瓶中, 加入四氢呋喃 (50mL)、对甲基苯磺酸 (0.02mmol), 室温下滴加3,4-二氢吡喃 (0.2mmol), 并在65°C下反应8h. TLC反应完全后, 减压蒸除溶剂, 经硅胶柱层析纯化得白色固体I-2. ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 7.97 (d, $J=1.5\text{Hz}$, 1H), 7.89 (s, 1H), 7.40-7.27 (m, 2H), 6.27-6.16 (m, 1H), 3.90 (dt, $J=11.4, 7.0\text{Hz}$, 1H), 3.77 (dt, $J=11.5, 7.0\text{Hz}$, 1H), 2.38-2.16 (m, 1H), 2.04-2.01 (m, 2H), 1.84-1.65 (m, 1H), 1.58-1.32 (m, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{12}\text{H}_{14}\text{BrN}_2\text{O}$ $[\text{M}+\text{H}]^+$: 281.0290, found 281.0292. 收率85%。

[0135] 中间体1-3的合成:

[0136] 将中间体1-2 (0.1mmol) 加入到单口瓶中, 加入联硼酸频那醇酯 (0.12mmol)、乙酸钾 (0.3mmol)、Pd(dppf) Cl_2 (0.005mmol)、二氧六环 (26mL)。升温至回流反应6h, TLC反应完全后, 降温抽滤、旋干、经硅胶柱层析纯化得白色固体1-3. ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 7.97 (d, $J=1.5\text{Hz}$, 1H), 7.82-7.74 (m, 1H), 7.39 (d, $J=7.5\text{Hz}$, 1H), 7.25 (dd, $J=7.5, 1.5\text{Hz}$, 1H), 6.21 (t, $J=6.9\text{Hz}$, 1H), 3.90 (dt, $J=11.4, 7.0\text{Hz}$, 1H), 3.77 (dt, $J=11.4, 7.0\text{Hz}$, 1H), 2.40-2.21 (m, 1H), 2.16-1.91 (m, 2H), 1.85-1.48 (m, 3H), 1.40 (d, $J=15.1\text{Hz}$, 12H) ppm. HR-MS (ESI): Calculated for $\text{C}_{18}\text{H}_{26}\text{BN}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 329.2036, found 329.2033. 收率89%。

[0137] 中间体2-2的合成:

[0138] 将原料2-1 (0.1mmol) 溶解于四氢呋喃中, 加入三乙胺 (0.15mmol), 异戊酸酐 (0.15mmol), 升温至回流反应6-8小时, TLC检测反应完全后, 减压浓缩除去溶剂, 将反应液倒入冰饱和氢氧化钠溶液中除去过量的酸酐, 用乙酸乙酯及饱和食盐水萃取三次, 合并有

机相,干燥,浓缩得黄色粉末状固体2-2。¹H NMR (300MHz, DMSO-d₆) δ 9.80 (s, 1H), 9.58 (d, J=1.3Hz, 1H), 8.49 (d, J=1.3Hz, 1H), 7.87 (t, J=1.3Hz, 1H), 2.24 (m, 1H), 2.10 (d, J=6.9Hz, 2H), 0.95 (d, J=6.7Hz, 6H) ppm. HR-MS (ESI): Calculated for C₁₀H₁₄BrN₂O [M+H]⁺: 257.0290, found 257.0286. 收率85%。

[0139] 中间体2-3的合成:

[0140] 将中间体2-2 (0.1mmol)、中间体1-3 (0.1mmol)、碳酸钠 (0.3mmol)、Pd (dppf) Cl₂ (0.005mmol)、二氧六环 (9mL)、水 (1.5mL) 加入到单口瓶中,氮气置换空气4次,升温至回流下反应8h。降温后抽滤、旋干、经硅胶柱层析纯化得到中间体2-3。¹H NMR (300MHz, DMSO-d₆) δ 9.80 (s, 1H), 9.63 (d, J=1.3Hz, 1H), 8.69 (d, J=1.3Hz, 1H), 8.19 (t, J=1.6Hz, 1H), 8.03 (d, J=1.5Hz, 1H), 7.90 (t, J=1.3Hz, 1H), 7.68 (d, J=7.5Hz, 1H), 7.51 (dd, J=7.5, 1.5Hz, 1H), 6.25 (t, J=6.9Hz, 1H), 3.90 (dt, J=11.5, 7.0Hz, 1H), 3.77 (dt, J=11.5, 7.0Hz, 1H), 2.64-2.57 (qd, J=12.4, 7.0Hz, 2H), 2.39-2.13 (m, 2H), 2.13-1.91 (m, 2H), 1.85-1.48 (m, 3H), 0.95 (dd, J=15.0, 6.8Hz, 6H) ppm. HR-MS (ESI): Calculated for C₂₂H₂₇N₄O₂ [M+H]⁺: 379.2134, found 379.2132. 收率78%。

[0141] 中间体2-4的合成:

[0142] 将中间体2-3 (0.1mmol) 溶解于10mL四氢呋喃中,于常温下加入对甲苯磺酸水合物 (1mmol),升温至回流反应16-24小时,TLC检测反应完全后,减压浓缩,并用二氯甲烷与饱和碳酸氢钠萃取,合并有机相,干燥浓缩得中间体2-4。¹H NMR (300MHz, DMSO-d₆) δ 12.75 (s, 1H), 9.80 (s, 1H), 9.63 (d, J=1.3Hz, 1H), 8.50 (d, J=1.3Hz, 1H), 8.06 (d, J=1.6Hz, 1H), 7.96 (t, J=1.5Hz, 1H), 7.61 (t, J=1.3Hz, 1H), 7.43-7.24 (m, 2H), 2.24-2.05 (m, 3H), 0.95 (d, J=6.3Hz, 6H) ppm. HR-MS (ESI): Calculated for C₁₇H₁₉N₄O [M+H]⁺: 295.1559, found 295.1554. 收率74%。

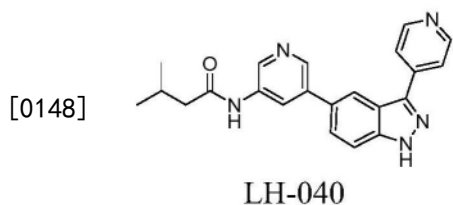
[0143] 中间体2-5的合成:

[0144] 将中间体2-3 (0.1mmol) 溶解于5mL二甲基甲酰胺中,加入氢氧化钾 (0.15mmol),碘单质 (0.15mmol),常温搅拌16-24小时,TLC检测反应完全后,用乙酸乙酯和饱和食盐水萃取三次,合并有机相后干燥,浓缩得中间体2-5。¹H NMR (300MHz, DMSO-d₆) δ 12.35 (s, 1H), 9.64 (s, 1H), 9.25 (d, J=1.3Hz, 1H), 8.57 (d, J=1.3Hz, 1H), 7.23 (t, J=1.5Hz, 1H), 7.4 (t, J=1.3Hz, 1H), 7.40-7.22 (m, 2H), 2.24-2.05 (m, 3H), 0.82 (d, J=6.3Hz, 6H) ppm. HR-MS (ESI): Calculated for C₁₇H₁₈IN₄O [M+H]⁺: 421.0525, found 421.0525. 收率65%。

[0145] LH-039的合成:

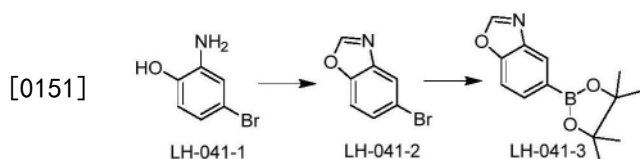
[0146] 将中间体2-5 (0.1mmol)、3-吡啶硼酸 (0.1mmol)、碳酸钠 (0.3mmol)、Pd (dppf) Cl₂ (0.005mmol)、二氧六环 (9mL)、水 (1.5mL) 加入到单口瓶中,氮气置换空气4次,升温至回流下反应8h。降温后抽滤、旋干、经硅胶柱层析纯化得到LH-039。¹H NMR (300MHz, DMSO-d₆) δ 13.62 (s, 1H), 10.23 (s, 1H), 9.30 (d, J=2.2Hz, 1H), 8.82 (d, J=2.3Hz, 1H), 8.74 (d, J=2.1Hz, 1H), 8.67 (dd, J=4.8, 1.6Hz, 1H), 8.49 (dt, J=7.9, 2.0Hz, 1H), 8.40-8.32 (m, 2H), 7.83-7.73 (m, 2H), 7.60 (dd, J=8.0, 4.8Hz, 1H), 2.29 (d, J=7.1Hz, 2H), 2.14 (dq, J=13.5, 6.6Hz, 1H), 1.00 (s, 3H), 0.98 (s, 3H) ppm. HR-MS (ESI): Calculated for C₂₂H₂₂N₅O [M+H]⁺: 372.1824, found 372.1818。

[0147] 采用与实施例3相似的操作,制得下列化合物:



[0149] ^1H NMR (300MHz, DMSO- d_6) δ 13.74 (s, 1H), 10.22 (s, 1H), 8.79 (t, $J=3.3\text{Hz}$, 1H), 8.70 (dd, $J=4.9, 1.8\text{Hz}$, 3H), 8.40-8.30 (m, 2H), 8.11-8.01 (m, 2H), 7.81-7.66 (m, 2H), 2.25 (d, $J=7.1\text{Hz}$, 2H), 2.11 (dt, $J=13.7, 6.8\text{Hz}$, 1H), 0.96 (s, 3H), 0.93 (d, $J=4.7\text{Hz}$, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{22}\text{H}_{22}\text{N}_5\text{O}[\text{M}+\text{H}]^+$: 372.1824, found 372.1819.

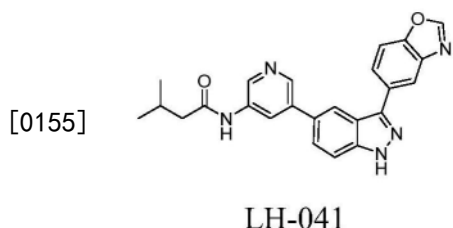
[0150] 实施例4: LH-041的合成



[0152] 将原料LH-041-1 (2-氨基-4-溴苯酚, 0.1mmol) 溶解在原甲酸三乙酯中回流过夜, 减压浓缩并用柱层析纯化得中间体LH-041-2. ^1H NMR (300MHz, DMSO- d_6) δ 8.25 (s, 1H), 7.58 (d, $J=1.5\text{Hz}$, 1H), 7.49 (dd, $J=7.5, 1.6\text{Hz}$, 1H), 7.25 (d, $J=7.5\text{Hz}$, 1H) ppm. HR-MS (ESI): Calculated for $\text{C}_7\text{H}_5\text{BrNO}[\text{M}+\text{H}]^+$: 197.9555, found 197.9523. 收率57%。

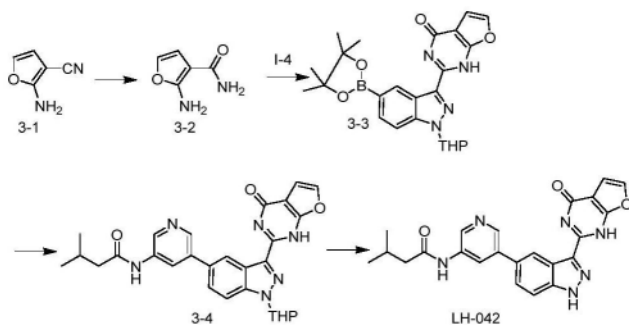
[0153] 将中间体LH-041-2 (0.1mmol) 加入到单口瓶中, 加入联硼酸频那醇酯 (0.12mmol)、乙酸钾 (0.3mmol)、Pd (dppf) Cl_2 (0.005mmol)、二氧六环 (25mL)。升温至回流反应6h, TLC反应完全后, 降温抽滤、旋干、经硅胶柱层析纯化得中间体LH-041-3. ^1H NMR (300MHz, DMSO- d_6) δ 8.25 (s, 1H), 7.75 (dd, $J=7.5, 1.5\text{Hz}$, 1H), 7.58 (d, $J=7.4\text{Hz}$, 1H), 7.48 (d, $J=1.5\text{Hz}$, 1H), 1.40 (s, 12H) ppm. HR-MS (ESI): Calculated for $\text{C}_{13}\text{H}_{17}\text{BN}_3\text{O}_3[\text{M}+\text{H}]^+$: 246.1301, found 246.1281. 收率88%。

[0154] 其余步骤与实施例3相同, 得到化合物LH-041。



[0156] ^1H NMR (300MHz, DMSO- d_6) δ 13.44 (s, 1H), 10.19 (s, 1H), 8.83 (s, 1H), 8.78 (d, $J=2.3\text{Hz}$, 1H), 8.70 (d, $J=2.1\text{Hz}$, 1H), 8.41 (d, $J=1.6\text{Hz}$, 1H), 8.34 (t, $J=2.2\text{Hz}$, 1H), 8.30 (d, $J=1.4\text{Hz}$, 1H), 8.14 (dd, $J=8.5, 1.7\text{Hz}$, 1H), 7.92 (dd, $J=8.5, 0.6\text{Hz}$, 1H), 7.78-7.67 (m, 1H), 6.54 (s, 1H), 2.25 (d, $J=7.1\text{Hz}$, 2H), 2.18-2.04 (m, 1H), 0.96 (s, 3H), 0.94 (s, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{24}\text{H}_{22}\text{N}_5\text{O}_2[\text{M}+\text{H}]^+$: 412.1773, found 412.1769.

[0157] 实施例5: LH-042的合成



[0158]

[0159] 中间体3-2的合成:

[0160] 将3-1 (0.1mmol) 加入到单颈瓶中, 然后加入过量的浓硫酸常温搅拌2h, 反应完毕后将反应体系降温至0℃, 将混合物用10%氢氧化钠溶液碱化, 然后用乙酸乙酯萃取。合并的萃取液用硫酸镁干燥, 真空除去溶剂, 然后从乙醇中重结晶得3-2。

[0161] 中间体3-3的合成:

[0162] 将中间体3-2 (0.1mmol) 加入到单口瓶中, 用乙醇将其溶解, 然后加入实施例1中间体1-4 (0.1mmol), 碘单质 (0.1mmol), 于80℃回流反应8h, TLC反应完全后, 加入5%硫代硫酸钠淬灭反应, 然后用二氯与饱和食盐水萃取, 合并有机相, 干燥, 浓缩, 柱层析纯化得中间体3-3。

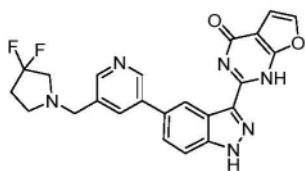
[0163] 中间体3-4的合成:

[0164] 将实施例1的中间体2-2 (0.1mmol)、中间体3-3 (0.1mmol)、碳酸钠 (0.3mmol)、Pd(dppf)Cl₂ (0.005mmol)、二氧六环 (9mL)、水 (1.5mL) 加入到单口瓶中, 氮气置换空气4次, 升温至回流下反应8h。降温后抽滤、旋干、经硅胶柱层析纯化得到中间体3-4。

[0165] LH-042的合成:

[0166] 将中间体3-4 (0.1mmol) 加入50mL单口瓶中, 用10mL二氯甲烷溶解后, 加入过量三氟乙酸 (1mmol), 常温搅拌过夜, TLC监控反应完全后, 真空浓缩除去溶剂和过量的三氟乙酸, 制砂后, 柱层析纯化得LH-042。¹H NMR (300MHz, DMSO-d₆) δ 14.26 (s, 1H), 12.20 (s, 1H), 10.12 (s, 1H), 9.80 (s, 1H), 9.59 (d, J=1.3Hz, 1H), 8.75 (d, J=1.5Hz, 1H), 8.68 (d, J=1.3Hz, 1H), 8.36 (s, 1H), 7.91 (t, J=1.2Hz, 1H), 7.64-7.49 (m, 2H), 6.88 (q, J=7.5Hz, 2H), 2.53-2.32 (m, 1H), 2.10 (d, J=7.0Hz, 2H), 0.96 (d, J=6.7Hz, 6H) ppm. HR-MS (ESI): Calculated for C₂₃H₂₁N₆O₃ [M+H]⁺: 429.1675, found 429.1670。

[0167] 采用与实施例5相似的操作, 制得下列化合物:

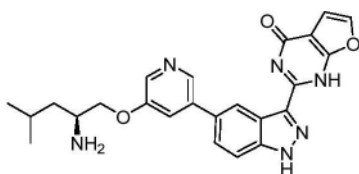


[0168]

LH-043

[0169] ¹H NMR (300MHz, DMSO-d₆) δ 12.64 (s, 1H), 10.34 (s, 1H), 8.83 (d, J=1.3Hz, 1H), 8.74 (dd, J=6.1, 1.5Hz, 2H), 8.08 (t, J=1.3Hz, 1H), 7.63-7.50 (m, 2H), 7.31 (d, J=7.5Hz, 1H), 6.98 (d, J=7.5Hz, 1H), 3.69 (s, 2H), 2.70-2.55 (m, 4H), 1.91-1.87 (m, 2H) ppm. HR-MS (ESI): Calculated for C₂₃H₁₉F₂N₆O₂ [M+H]⁺: 449.1538, found 449.1528。

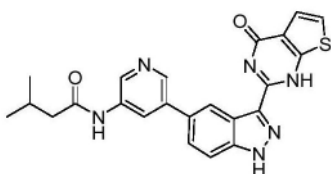
[0170]



LH-044

[0171] ^1H NMR (300MHz, DMSO- d_6) δ 12.12 (s, 1H), 10.15 (s, 1H), 8.79-8.71 (m, 1H), 8.60 (d, $J=1.3\text{Hz}$, 1H), 8.18 (d, $J=1.4\text{Hz}$, 1H), 7.60 (dt, $J=16.7, 1.4\text{Hz}$, 3H), 7.31 (d, $J=7.5\text{Hz}$, 1H), 6.98 (d, $J=7.6\text{Hz}$, 1H), 4.32 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 4.09 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 3.22-3.05 (m, 1H), 1.73-1.41 (m, 3H), 1.54 (s, 2H), 0.99-0.81 (m, 6H) ppm. HR-MS (ESI): Calculated for $\text{C}_{24}\text{H}_{25}\text{N}_6\text{O}_3$ $[\text{M}+\text{H}]^+$: 445.1988, found 445.1976.

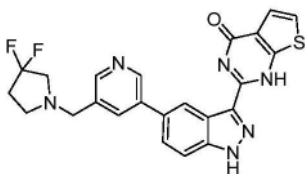
[0172]



LH-045

[0173] ^1H NMR (300MHz, DMSO- d_6) δ 13.24 (s, 1H), 10.31 (s, 1H), 9.80 (s, 1H), 9.60 (d, $J=1.3\text{Hz}$, 1H), 8.76 (d, $J=1.5\text{Hz}$, 1H), 8.68 (d, $J=1.3\text{Hz}$, 1H), 7.91 (t, $J=1.2\text{Hz}$, 1H), 7.65-7.49 (m, 2H), 7.36 (d, $J=7.6\text{Hz}$, 1H), 6.85 (d, $J=7.6\text{Hz}$, 1H), 2.32 (dt, $J=13.6, 6.8\text{Hz}$, 1H), 2.10 (d, $J=7.0\text{Hz}$, 2H), 0.96 (d, $J=6.8\text{Hz}$, 6H) ppm. HR-MS (ESI): Calculated for $\text{C}_{23}\text{H}_{21}\text{N}_6\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$: 445.1447, found 445.1438.

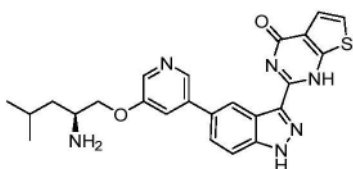
[0174]



LH-046

[0175] ^1H NMR (300MHz, DMSO- d_6) δ 13.26 (s, 1H), 10.18 (s, 1H), 8.84 (d, $J=1.4\text{Hz}$, 1H), 8.81-8.68 (m, 2H), 8.10 (t, $J=1.3\text{Hz}$, 1H), 7.64-7.50 (m, 2H), 7.36 (d, $J=7.5\text{Hz}$, 1H), 6.85 (d, $J=7.5\text{Hz}$, 1H), 3.69 (s, 2H), 2.70-2.55 (m, 4H), 1.91-1.75 (m, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{23}\text{H}_{19}\text{F}_2\text{N}_6\text{O}\text{S}$ $[\text{M}+\text{H}]^+$: 465.1309, found 465.1298.

[0176]



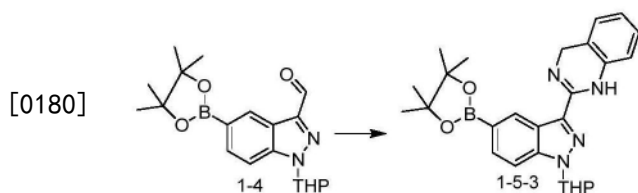
[0177]

LH-047

[0178] ^1H NMR (300MHz, DMSO- d_6) δ 12.24 (s, 1H), 10.12 (s, 1H), 8.77 (t, $J=1.0\text{Hz}$, 1H), 8.60 (d, $J=1.3\text{Hz}$, 1H), 8.18 (d, $J=1.3\text{Hz}$, 1H), 7.60 (dt, $J=15.1, 1.4\text{Hz}$, 3H), 7.36 (d, $J=7.6\text{Hz}$, 1H), 6.85 (d, $J=7.5\text{Hz}$, 1H), 4.31 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 4.09 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 3.13-2.92 (m, 1H), 1.75-1.41 (m, 3H), 1.52 (s, 2H), 0.90 (dd, $J=15.0, 6.5\text{Hz}$,

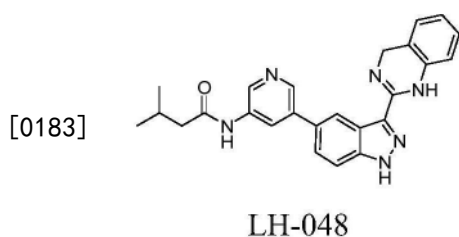
6H) ppm. HR-MS (ESI): Calculated for $C_{24}H_{25}N_6O_2S[M+H]^+$: 461.1760, found 461.1756.

[0179] 实施例6: LH-048的合成



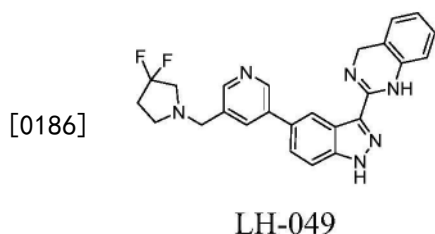
[0181] 将路线1中间体1-4 (0.1 mmol) 加入到单口瓶中, 用10 mL 二甲基乙酰胺将其溶解, 然后加入邻氨基卞氨 (0.15 mmol), 对甲苯磺酸水合物 (0.15 mmol), 亚硫酸氢钠 (0.15 mmol) 于120℃反应6-8小时, TLC反应完全后, 用乙酸乙酯与饱和食盐水萃取三次, 合并有机相, 干燥, 浓缩, 柱层析纯化得中间体1-5-3. ^1H NMR (300 MHz, DMSO- d_6) δ 12.24 (s, 1H), 7.99 (d, J = 1.5 Hz, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.34 (dd, J = 7.5, 1.5 Hz, 1H), 7.15-7.10 (m, 1H), 7.04 (td, J = 7.4, 2.0 Hz, 1H), 6.89 (td, J = 7.5, 2.1 Hz, 1H), 6.41 (dd, J = 7.4, 2.1 Hz, 1H), 6.26 (t, J = 6.9 Hz, 1H), 4.94 (d, J = 1.1 Hz, 2H), 3.90 (dt, J = 11.4, 7.0 Hz, 1H), 3.77 (dt, J = 11.5, 7.0 Hz, 1H), 2.52-2.32 (m, 1H), 2.12-1.91 (m, 2H), 1.86-1.48 (m, 3H), 1.41 (d, J = 14.9 Hz, 12H) ppm. HR-MS (ESI): Calculated for $C_{26}H_{32}BN_4O_3[M+H]^+$: 459.2567, found 459.2558. 收率78%。

[0182] 其余步骤与实施例1相同, 得到化合物LH-048。



[0184] ^1H NMR (300 MHz, DMSO- d_6) δ 12.36 (s, 1H), 10.28 (s, 1H), 9.80 (s, 1H), 9.51 (d, J = 1.3 Hz, 1H), 8.67 (d, J = 1.3 Hz, 1H), 8.47 (d, J = 1.5 Hz, 1H), 7.99 (t, J = 1.3 Hz, 1H), 7.62-7.47 (m, 2H), 7.15-7.11 (m, 1H), 7.04 (td, J = 7.5, 2.1 Hz, 1H), 6.90 (td, J = 7.5, 2.1 Hz, 1H), 6.41 (dd, J = 7.5, 2.1 Hz, 1H), 2.60 (d, J = 1.0 Hz, 2H), 2.29 (dd, J = 13.5, 6.8 Hz, 1H), 2.10 (d, J = 6.9 Hz, 2H), 0.96 (d, J = 6.7 Hz, 6H) ppm. HR-MS (ESI): Calculated for $C_{25}H_{25}N_6O[M+H]^+$: 425.2090, found 425.2088.

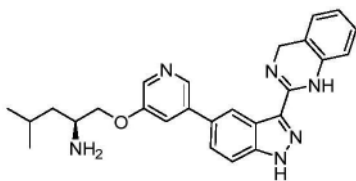
[0185] 采用与实施例6相似的操作, 制得下列化合物:



[0187] ^1H NMR (300 MHz, DMSO- d_6) δ 12.11 (s, 1H), 10.22 (s, 1H), 8.82 (d, J = 1.3 Hz, 1H), 8.71 (d, J = 1.3 Hz, 1H), 8.48 (d, J = 1.4 Hz, 1H), 8.08 (t, J = 1.3 Hz, 1H), 7.60-7.48 (m, 2H), 7.15-7.05 (m, 1H), 7.04 (td, J = 7.5, 2.0 Hz, 1H), 6.90 (td, J = 7.4, 2.0 Hz, 1H), 6.41 (dd, J = 7.5, 2.1 Hz, 1H), 3.69 (s, 2H), 2.70-2.55 (m, 2H), 2.60 (s, 3H), 1.91-1.75 (m, 2H) ppm. HR-MS

(ESI): Calculated for $C_{25}H_{23}F_2N_6[M+H]^+$: 445.1952, found 445.1949.

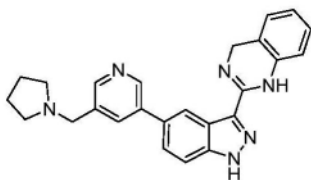
[0188]



LH-050

[0189] ^1H NMR (300MHz, DMSO- d_6) δ 14.21 (s, 1H), 12.31 (s, 1H), 8.56-8.42 (m, 2H), 8.15 (d, $J=1.3$ Hz, 1H), 7.57 (dd, $J=13.9, 1.2$ Hz, 3H), 7.15 (ddt, $J=7.4, 2.1, 1.1$ Hz, 1H), 7.04 (td, $J=7.5, 2.1$ Hz, 1H), 6.90 (td, $J=7.4, 2.0$ Hz, 1H), 6.41 (dd, $J=7.5, 2.1$ Hz, 1H), 4.95 (d, $J=1.2$ Hz, 2H), 4.33 (dd, $J=12.4, 7.0$ Hz, 1H), 4.08 (dd, $J=12.4, 7.0$ Hz, 1H), 3.13-3.07 (m, 1H), 1.74-1.39 (m, 3H), 1.56 (s, 2H), 0.90 (dd, $J=15.0, 6.4$ Hz, 6H) ppm. HR-MS (ESI): Calculated for $C_{26}H_{29}N_6O[M+H]^+$: 441.2403, found 441.2395.

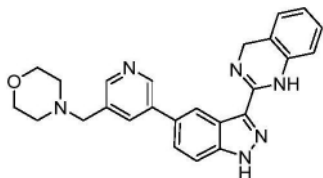
[0190]



LH-051

[0191] ^1H NMR (300MHz, DMSO- d_6) δ 12.32 (s, 1H), 10.21 (s, 1H), 8.82 (d, $J=1.2$ Hz, 1H), 8.69 (d, $J=1.3$ Hz, 1H), 8.49 (t, $J=1.0$ Hz, 1H), 8.10 (t, $J=1.3$ Hz, 1H), 7.55 (s, 1H), 7.15 (ddt, $J=7.4, 2.1, 1.0$ Hz, 1H), 7.04 (td, $J=7.5, 2.1$ Hz, 1H), 6.90 (td, $J=7.4, 2.0$ Hz, 1H), 6.41 (dd, $J=7.4, 2.1$ Hz, 1H), 3.69 (s, 2H), 2.68-2.51 (m, 3H), 2.60 (s, 4H), 1.91-1.74 (m, 4H) ppm. HR-MS (ESI): Calculated for $C_{25}H_{25}N_6[M+H]^+$: 409.2141, found 409.2098.

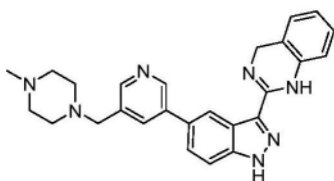
[0192]



LH-052

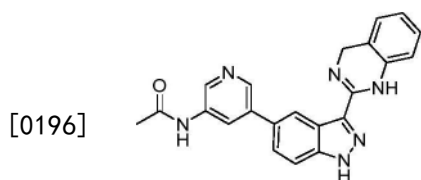
[0193] ^1H NMR (300MHz, DMSO- d_6) δ 12.30 (s, 1H), 10.29 (s, 1H), 8.82 (d, $J=1.3$ Hz, 1H), 8.70 (d, $J=1.3$ Hz, 1H), 8.49 (d, $J=1.9$ Hz, 1H), 8.09 (t, $J=1.3$ Hz, 1H), 7.60-7.48 (m, 2H), 7.15 (ddt, $J=7.5, 2.1, 1.0$ Hz, 1H), 7.04 (td, $J=7.5, 2.1$ Hz, 1H), 6.90 (td, $J=7.4, 2.0$ Hz, 1H), 6.41 (dd, $J=7.5, 2.1$ Hz, 1H), 3.69 (s, 2H), 3.58 (t, $J=4.7$ Hz, 4H), 2.60 (d, $J=1.0$ Hz, 2H), 2.39 (t, $J=4.7$ Hz, 4H) ppm. HR-MS (ESI): Calculated for $C_{25}H_{25}N_6O[M+H]^+$: 425.2090, found 425.2081.

[0194]



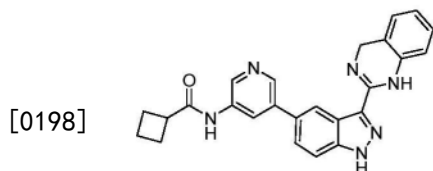
LH-053

[0195] ^1H NMR (300MHz, DMSO- d_6) δ 12.33 (s, 1H), 10.21 (s, 1H), 8.82 (d, $J=1.3\text{Hz}$, 1H), 8.69 (d, $J=1.3\text{Hz}$, 1H), 8.49 (t, $J=1.0\text{Hz}$, 1H), 8.10 (t, $J=1.3\text{Hz}$, 1H), 7.55 (s, 2H), 7.15 (ddt, $J=7.5, 2.2, 1.0\text{Hz}$, 1H), 7.04 (td, $J=7.5, 2.0\text{Hz}$, 1H), 6.90 (td, $J=7.5, 2.1\text{Hz}$, 1H), 6.41 (dd, $J=7.5, 2.1\text{Hz}$, 1H), 3.69 (s, 2H), 2.60 (d, $J=1.0\text{Hz}$, 2H), 2.48 (t, $J=5.1\text{Hz}$, 4H), 2.34 (t, $J=5.2\text{Hz}$, 4H), 2.24 (s, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{26}\text{H}_{28}\text{N}_7[\text{M}+\text{H}]^+$: 438.2406, found 438.2395.



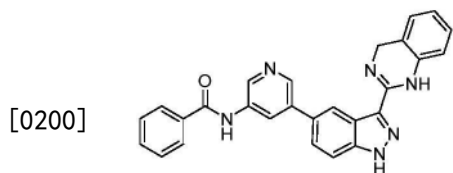
LH-054

[0197] ^1H NMR (300MHz, DMSO- d_6) δ 13.26 (s, 1H), 12.22 (s, 1H), 9.80 (s, 1H), 9.58 (d, $J=1.3\text{Hz}$, 1H), 8.67 (d, $J=1.3\text{Hz}$, 1H), 8.47 (d, $J=1.5\text{Hz}$, 1H), 7.91 (t, $J=1.2\text{Hz}$, 1H), 7.61-7.47 (m, 2H), 7.15 (ddt, $J=7.4, 2.1, 1.0\text{Hz}$, 1H), 7.04 (td, $J=7.5, 2.1\text{Hz}$, 1H), 6.90 (td, $J=7.4, 2.0\text{Hz}$, 1H), 6.41 (dd, $J=7.5, 2.1\text{Hz}$, 1H), 2.60 (d, $J=1.0\text{Hz}$, 2H), 2.20 (s, 3H) ppm. HR-MS (ESI): Calculated for $\text{C}_{22}\text{H}_{19}\text{N}_6\text{O}[\text{M}+\text{H}]^+$: 383.1620, found 383.1598.



LH-055

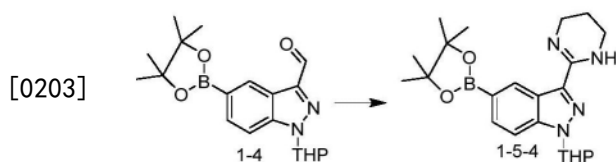
[0199] ^1H NMR (300MHz, DMSO- d_6) δ 13.26 (s, 1H), 12.22 (s, 1H), 9.80 (s, 1H), 9.61 (d, $J=1.3\text{Hz}$, 1H), 8.67 (d, $J=1.3\text{Hz}$, 1H), 8.47 (d, $J=1.4\text{Hz}$, 1H), 7.90 (t, $J=1.3\text{Hz}$, 1H), 7.61-7.47 (m, 2H), 7.15 (ddt, $J=7.4, 2.1, 1.0\text{Hz}$, 1H), 7.04 (td, $J=7.5, 2.1\text{Hz}$, 1H), 6.90 (td, $J=7.4, 2.0\text{Hz}$, 1H), 6.41 (dd, $J=7.5, 2.1\text{Hz}$, 1H), 3.21 (d, $J=6.9\text{Hz}$, 1H), 2.60 (d, $J=1.0\text{Hz}$, 2H), 2.48 (dt, $J=12.4, 6.8\text{Hz}$, 2H), 2.17-1.82 (m, 4H) ppm. HR-MS (ESI): Calculated for $\text{C}_{25}\text{H}_{23}\text{N}_6\text{O}[\text{M}+\text{H}]^+$: 423.1933, found 423.1912.



LH-056

[0201] ^1H NMR (300MHz, DMSO- d_6) δ 14.26 (s, 1H), 12.22 (s, 1H), 9.50 (d, $J=1.2\text{Hz}$, 1H), 8.68 (d, $J=1.2\text{Hz}$, 1H), 8.49 (d, $J=1.4\text{Hz}$, 1H), 8.04 (s, 1H), 8.00-7.88 (m, 3H), 7.62-7.47 (m, 4H), 7.31 (ddt, $J=9.6, 7.2, 2.0\text{Hz}$, 1H), 7.15 (ddt, $J=7.4, 2.2, 1.0\text{Hz}$, 1H), 7.04 (td, $J=7.5, 2.1\text{Hz}$, 1H), 6.90 (td, $J=7.5, 2.1\text{Hz}$, 1H), 6.41 (dd, $J=7.4, 2.1\text{Hz}$, 1H), 2.60 (d, $J=1.0\text{Hz}$, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{27}\text{H}_{21}\text{N}_6\text{O}[\text{M}+\text{H}]^+$: 445.1777, found 445.1765.

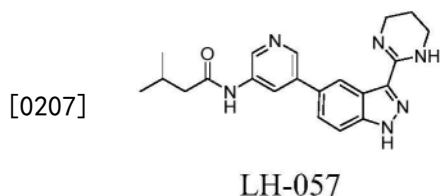
[0202] 实施例7: LH-057的合成



[0204] 将路线1中间体1-4 (0.1mmol) 加入到单口瓶中, 用5mL二甲基乙酰胺将其溶解, 然后加入丙二胺(0.15mmol), 对甲苯磺酸水合物(0.15mmol), 亚硫酸氢钠(0.15mmol) 于120℃反应6-8小时, TLC反应完全后, 用乙酸乙酯与饱和食盐水萃取三次, 合并有机相, 干燥, 浓缩, 柱层析纯化得中间体1-5-4。

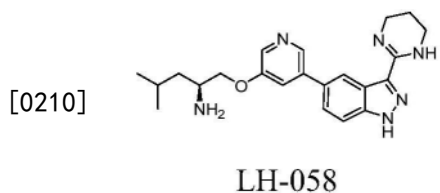
[0205] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 12.22 (s, 1H), 7.86 (d, $J=1.5\text{Hz}$, 1H), 7.47-7.33 (m, 2H), 7.25 (dd, $J=7.5, 1.6\text{Hz}$, 1H), 6.22 (t, $J=6.8\text{Hz}$, 1H), 3.98-3.83 (m, 3H), 3.77 (dt, $J=11.4, 7.0\text{Hz}$, 1H), 3.62 (ddd, $J=11.9, 4.4, 1.7\text{Hz}$, 2H), 2.52-2.32 (m, 1H), 2.12-1.91 (m, 2H), 1.86-1.52 (m, 3H), 1.40 (d, $J=15.1\text{Hz}$, 13H), 1.23-1.08 (m, 1H) ppm. HR-MS (ESI): Calculated for $\text{C}_{22}\text{H}_{32}\text{BN}_4\text{O}_3$ $[\text{M}+\text{H}]^+$: 411.2567, found 411.2566. 收率78%。

[0206] 其余步骤与实施例1相同, 得到化合物LH-057。

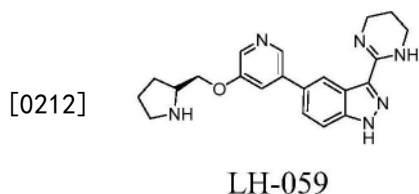


[0208] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.32 (s, 1H), 9.80 (s, 1H), 9.58 (d, $J=1.3\text{Hz}$, 1H), 8.65 (d, $J=1.3\text{Hz}$, 1H), 8.38 (d, $J=1.3\text{Hz}$, 1H), 7.92 (t, $J=1.3\text{Hz}$, 1H), 7.60-7.43 (m, 3H), 3.52 (dt, $J=9.2, 5.5\text{Hz}$, 4H), 2.30-2.10 (m, 2H), 2.09 (d, $J=1.1\text{Hz}$, 1H), 1.60 (d, $J=5.6\text{Hz}$, 2H), 0.97 (d, $J=6.6\text{Hz}$, 6H) ppm. HR-MS (ESI): Calculated for $\text{C}_{21}\text{H}_{25}\text{N}_6\text{O}$ $[\text{M}+\text{H}]^+$: 377.2090, found 377.1998。

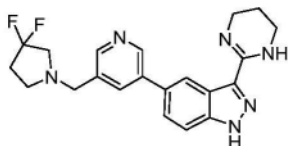
[0209] 采用与实施例7相似的操作, 制得下列化合物:



[0211] ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 14.35 (s, 1H), 8.56 (d, $J=1.3\text{Hz}$, 1H), 8.16 (d, $J=1.3\text{Hz}$, 1H), 7.61 (t, $J=1.3\text{Hz}$, 1H), 7.53 (d, $J=1.1\text{Hz}$, 2H), 7.44 (s, 1H), 4.32 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 4.08 (dd, $J=12.4, 7.0\text{Hz}$, 1H), 3.92 (td, $J=12.2, 3.1\text{Hz}$, 2H), 3.66 (ddd, $J=11.9, 4.4, 1.7\text{Hz}$, 2H), 3.20-3.04 (m, 1H), 1.74-1.33 (m, 6H), 1.25-1.12 (m, 1H), 0.90 (dd, $J=15.0, 6.4\text{Hz}$, 6H) ppm. HR-MS (ESI): Calculated for $\text{C}_{22}\text{H}_{29}\text{N}_6\text{O}$ $[\text{M}+\text{H}]^+$: 393.2403, found 393.2403。



[0213] ^1H NMR (300MHz, DMSO- d_6) 14.35 (s, 1H), 8.56 (d, $J=1.2\text{Hz}$, 1H), 8.38 (t, $J=1.0\text{Hz}$, 1H), 8.17 (d, $J=1.4\text{Hz}$, 1H), 7.57 (dd, $J=15.2, 1.2\text{Hz}$, 3H), 7.45 (s, 1H), 3.93 (td, $J=12.2, 3.1\text{Hz}$, 2H), 3.63 (ddd, $J=11.9, 4.4, 1.7\text{Hz}$, 2H), 3.21 (d, $J=6.9\text{Hz}$, 1H), 3.03-2.87 (m, 1H), 2.78 (dt, $J=9.6, 7.0\text{Hz}$, 1H), 1.89-1.18 (m, 7H) ppm. HR-MS (ESI): Calculated for $\text{C}_{21}\text{H}_{23}\text{N}_7\text{O}$ $[\text{M}+\text{H}]^+$: 389.1964, found 389.1971.



[0214]

LH-060

[0215] ^1H NMR (300MHz, DMSO- d_6) δ 14.36 (s, 1H), 8.79 (d, $J=1.3\text{Hz}$, 1H), 8.71 (d, $J=1.4\text{Hz}$, 1H), 8.37 (d, $J=1.3\text{Hz}$, 1H), 8.07 (t, $J=1.3\text{Hz}$, 1H), 7.59-7.42 (m, 3H), 3.69 (s, 2H), 3.52 (dt, $J=9.2, 5.5\text{Hz}$, 4H), 2.70-2.55 (m, 4H), 1.91 (tt, $J=15.9, 6.3\text{Hz}$, 2H), 1.60-1.55 (m, 2H) ppm. HR-MS (ESI): Calculated for $\text{C}_{21}\text{H}_{23}\text{F}_2\text{N}_6$ $[\text{M}+\text{H}]^+$: 397.1952, found 397.1946.

[0216] 实施例8: 本发明部分化合物对CLK家族及DYEK1A蛋白的体外抑制活性

[0217] 一、实验方法

[0218] 1、制备1X激酶反应缓冲液

名称	储液浓度	体积	终浓度
Tris	1M (25X)	240 μL	40mM
MgCl_2	1M (50X)	120 μL	20mM
BSA	7.5% (75X)	80 μL	0.1%
DTT	1M (500X)	3 μL	0.5mM
ddH ₂ O		5557 μL	

[0220] 2、酶活实验

[0221] (1) 2X激酶配制:

名称	储液浓度	终浓度
DYRK1A	200ng/ μL	0.2ng/ μL
CLK1	100ng/ μL	0.5ng/ μL
CLK2	100ng/ μL	0.5ng/ μL
CLK3	100ng/ μL	1ng/ μL
CLK4	100ng/ μL	0.5ng/ μL

[0223] (2) 4X底物混合物配制:

	ATP	Substrate	
名称	终浓度	储液浓度	终浓度
DYRK1A	30 μM	5 mg/mL	0.1 mg/mL
CLK1	50 μM	10 mg/mL	0.1 mg/mL
CLK2	50 μM	10 mg/mL	0.1 mg/mL
CLK3	30 μM	5 mg/mL	0.1 mg/mL
CLK4	50 μM	10 mg/mL	0.1 mg/mL

[0224]

[0225] ①在稀释板中用DMSO对阳性药进行4倍梯度稀释,化合物最终起始浓度为1&0.02&0.1μM。

[0226] ②将化合物50倍稀释到1X激酶反应缓冲液中,在振荡器上震荡20分钟。

[0227] ③用1X的酶反应缓冲液配制准备2X激酶。

[0228] ④向反应板中每孔加入2μL激酶。

[0229] ⑤向每孔加入1μL在缓冲液中稀释好的化合物,用封板膜封住板子1000rpm离心60秒,25℃孵育10分钟。

[0230] ⑥用1X的酶反应缓冲液配制4X ATP&sub混合液,向反应板中加入1μL 4X ATP&sub混合液。

[0231] ⑦用封板膜封住板子1000rpm离心60秒,25℃孵育60分钟。

[0232] ⑧转移4μL ADP-Glo到384反应板中1000rpm,离心1min,25℃孵育40min。

[0233] ⑨转移8μL Detection溶液到384反应板中1000rpm,离心1min,25℃孵育40min。

[0234] ⑩使用BMG多功能读板机读取RLU(Relative luminescence unit)信号,信号强度用于表征激酶的活性程度。

[0235] 3、数据分析

[0236] (1)计算每孔的比率;

[0237] (2)抑制率计算如下:

[0238] 化合物抑制率(% inh) = 100% - (化合物-阳性对照) / (阴性对照-阳性对照) × 100%

[0239] (3)计算IC₅₀并绘制化合物的抑制曲线:

[0240] 利用以下非线性拟合公式来得到化合物的IC₅₀(半数抑制浓度):用Graphpad7.0软件进行数据分析。

[0241] $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\log \text{IC}_{50} - X) \times \text{Hill Slope}))}$;

[0242] X:化合物浓度log值,Y:抑制率(% inhibition)。

[0243] 二、实验结果

[0244] 表1. 本发明中部分化合物的酶活性(IC₅₀)

ID	CLK1	CLK3	CLK4	CLK2	DYRK1A
LH-001	A	A	C	A	B
LH-003	A	A	D	A	C
LH-004	A	B	C	B	B
LH-005	C	C	B	B	B
LH-006	B	C	B	B	B
LH-009	A	A	B	A	A
LH-010	B	B	D	A	B

[0246]

LH-011	C	C	D	A	C
LH-015	B	B	A	A	A
LH-016	A	B	B	A	B
LH-017	A	D	C	A	A
LH-018	C	B	A	A	A
LH-019	A	D	C	A	B
LH-020	A	C	B	A	A
LH-037	A	A	B	B	A
LH-039	C	A	C	B	B
LH-040	C	B	A	A	A
LH-041	B	A	B	A	B
LH-048	A	A	B	A	A
LH-049	C	B	D	A	A

[0247] 注:A: <10nM, B: 10-50nM, C: 50-100nM, D: >100nM。

[0248] 由表1可见,本发明所述的化合物对CLK2、DYRK1A显示出有效的抑制活性。同时,本发明所述化合物如LH-020显示较优的CLK2抑制活性和CLK3选择性(对于CLK2、CLK3、DYRK1A的IC₅₀分别为2nM、81nM、3nM,对于CLK3的选择性指数为41.5),这为LH-020发挥其药理活性和避免可能的副作用提供了依据。

[0249] 实施例9:本发明代表化合物LH-020在骨关节炎大鼠体内药效(PD)结果

[0250] 一、实验方法

[0251] 1、将40只大鼠平均分为4组,分别为Control(正常大鼠关节腔内注射生理盐水)、Model(ACLT模型大鼠关节腔内注射生理盐水)、低剂量给药组(ACLT模型大鼠关节腔内注射LH020(1.5μg/kg))、高剂量给药组(ACLT模型大鼠关节腔内注射LH020(6μg/kg))。

[0252] 2、通过切除大鼠前交叉韧带致膝骨关节炎(ACLT模型)。

[0253] 3、术后第一周关节腔注射生理盐水或LH-020(1.5μg/kg, 6μg/kg)。

[0254] 4、给药后第5周取软骨并通过RT-qPCR检测软骨相关蛋白酶的表达(MMP3、MMP13、ADAMTS5、IHH等)。

[0255] 二、实验结果

[0256] 如图1可见,与Control组(关节腔注射生理盐水)相比,关节炎模型中MMP3、MMP13、ADAMTS5、IHH这些分解软骨的蛋白酶表达明显升高。此外,与模型组相比,LH-020的低、高剂量组均能显著下调MMP3、MMP13、ADAMTS5、IHH的表达,且高剂量组效果优于低剂量组,表示LH-020具有显著的软骨保护作用,这为其发挥治疗骨关节炎的药理作用提供了依据。

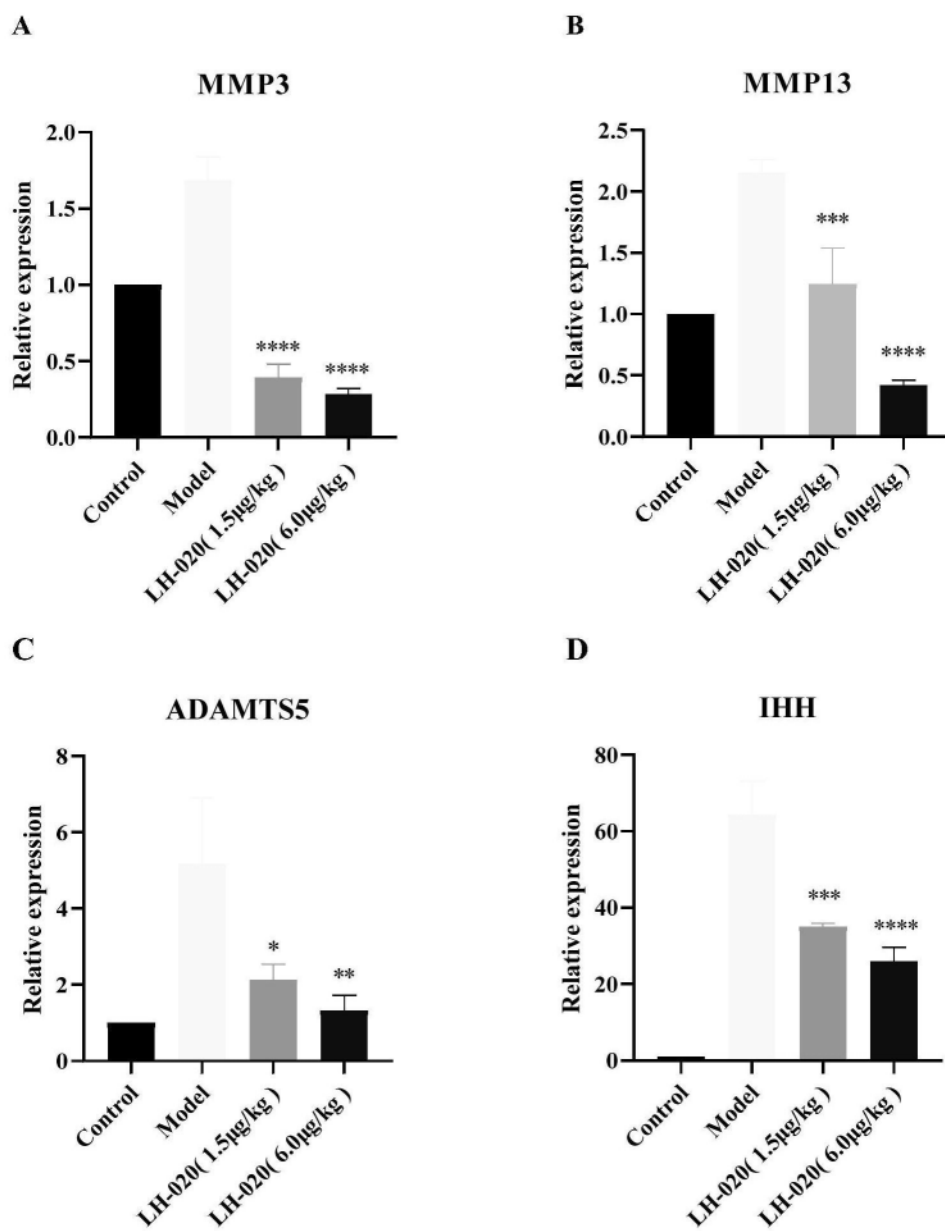


图1