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(56) 对比文件

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黄昊哲 等. Janus 激酶及其抑制剂的研究进展.《药学进展》. 2019, 第43卷(第1期), 42-50.
Chieyeon Chough, et al.. Design, synthesis and evaluation of (R)-3-(7-(methyl (7H-pyrrolo[2,3-d]pyrimidin-4-yl) amino)-5-azaspiro[2.4]heptan-5-yl)-3-oxopropanenitrile as a JAK1-selective inhibitor.《Med. Chem. Commun.》. 2018, 第9卷 477-489.

审查员 韦丹青

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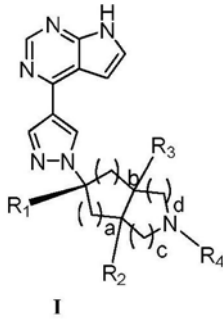
(54) 发明名称
作为选择性JAK2抑制剂的吡咯并嘧啶类化合物、其合成方法及用途

(57) 摘要

本发明属于生物医药技术领域, 具体涉及一种作为选择性JAK2抑制剂的吡咯并嘧啶类化合物、其合成方法及用途。与现有技术相比, 本发明提供的吡咯并嘧啶类化合物、其立体异构体及其药学上可接受的盐具有更好的两面神激酶抑制活性, 且其对JAK2抑制靶点选择性显著优于现有化合物, 且本发明的优选化合物表现出良好的药代动力学性质, 具有开发成为选择性JAK2抑制剂

的潜力。

1. 一种如式I所示的吡咯并嘧啶类化合物或其药学上可接受的盐，



其中：

R₁为C_{1~6}烷基或C_{1~6}烷基氰基；所述C_{1~6}烷基氰基指C_{1~6}烷基上的任意氢被一个氰基取代；

R₂和R₃各自独立的为氢或C_{1~6}烷基；

R₄为C_{1~6}烷基、-C(O)R₅、-C(O)NR₆R₇、-S(O)₂R₈或-S(O)₂NR₉R₁₀；

a为0、1或2；

b为0、1或2；

c为0、1或2；

d为0、1或2；

R₅为C_{1~6}烷基、C_{2~6}烯基、C_{3~6}环烷基或C_{1~6}烷基氰基，所述C_{1~6}烷基氰基指C_{1~6}烷基上的任意氢被一个氰基取代；

R₆和R₇各自独立的为氢、C_{1~6}烷基、C_{3~6}环烷基或R₆、R₇与相连的N原子共同形成3~6元环；

R₈为C_{1~6}烷基或C_{3~6}环烷基；

R₉和R₁₀各自独立的为氢、C_{1~6}烷基、C_{3~6}环烷基或R₉、R₁₀与相连的N原子共同形成3~6元环。

2. 如权利要求1所述的吡咯并嘧啶类化合物或其药学上可接受的盐，其特征在于，所述：

R₁为C_{1~4}烷基或C_{1~4}烷基氰基；所述C_{1~4}烷基氰基指C_{1~4}烷基上的任意氢被一个氰基取代；

R₂和R₃各自独立的为氢、甲基或乙基；

R₄为C_{1~6}烷基、-C(O)R₅、-C(O)NR₆R₇、-S(O)₂R₈或-S(O)₂NR₉R₁₀；

a为0、1或2；

b为0或1；

c为0、1或2；

d为1或2；


R₅为C_{1~4}烷基、C_{2~6}烯基、C_{3~6}环烷基或C_{1~4}烷基氰基，所述C_{1~4}烷基氰基指C_{1~4}烷基上的任意氢被一个氰基取代；

R₆和R₇各自独立的为氢、C_{1~4}烷基、C_{3~6}环烷基或R₆、R₇与相连的N原子共同形成3~6元脂肪环；

R₈为C_{1~5}烷基或C_{3~6}环烷基；

R₉和R₁₀各自独立的为氢、C_{1~6}烷基、C_{3~6}环烷基或R₉、R₁₀与相连的N原子共同形成3~6元脂肪环。

3. 如权利要求1所述的吡咯并嘧啶类化合物或其药学上可接受的盐,其特征在于,所述:

R₁为甲基、乙基或CN;

R₂和R₃为氢;



R₄为C_{1~3}烷基、-C(O)R₅、-C(O)NR₆R₇、-S(O)₂R₈或-S(O)₂NR₉R₁₀;

a为0、1或2;

b为0或1;

c为1或2;

d为1;

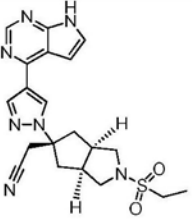
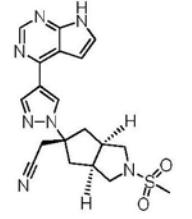
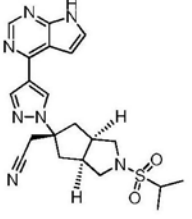
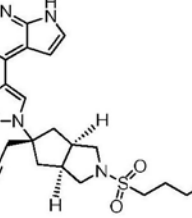
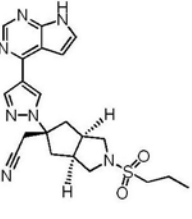
R₅为C_{1~3}烷基、C_{3~6}环烷基、或CN;

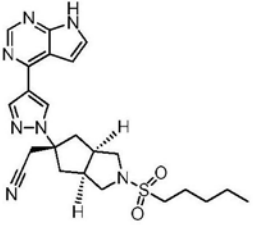
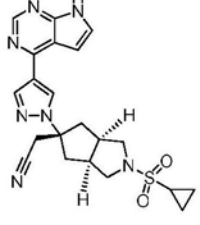
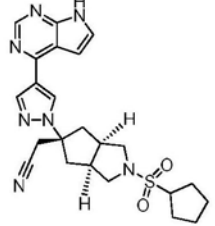
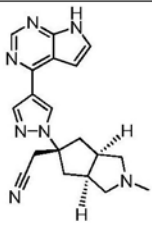
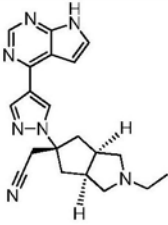
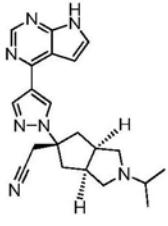
R₆和R₇各自独立的为氢、C_{1~3}烷基、C_{3~6}环烷基或R₆、R₇与相连的N原子共同形成5~6元脂肪环;

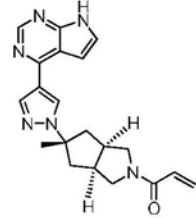
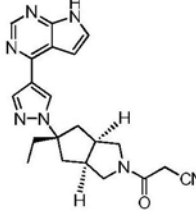
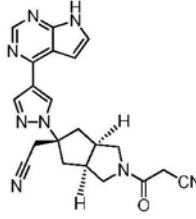
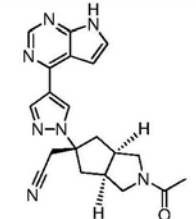
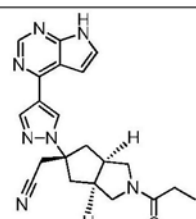
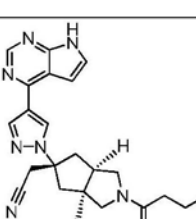
R₈为C_{1~5}烷基或C_{4~5}环烷基;

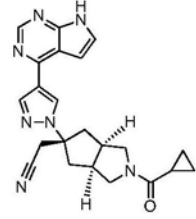
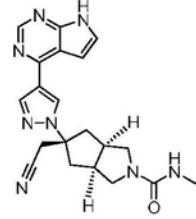
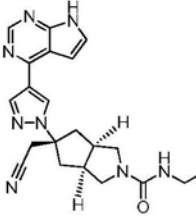
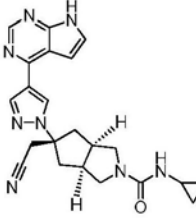
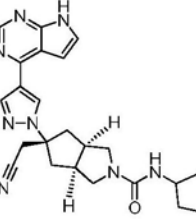
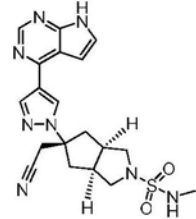
R₉和R₁₀各自独立的为氢、C_{1~3}烷基、C_{3~5}环烷基或R₉、R₁₀与相连的N原子共同形成5~6元脂肪环。

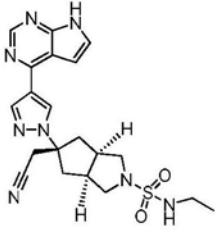
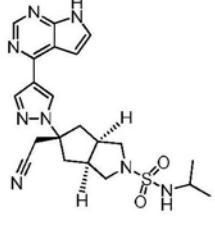
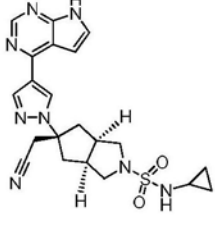
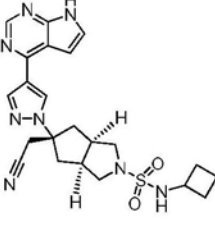
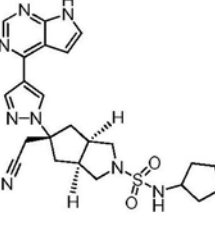
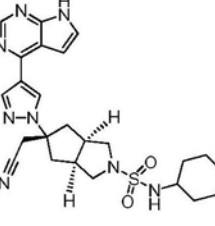
4. 如权利要求1所述的吡咯并嘧啶类化合物或其药学上可接受的盐,其特征在于,选自如下化合物:

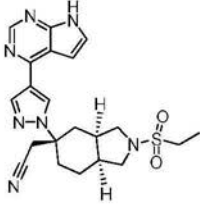
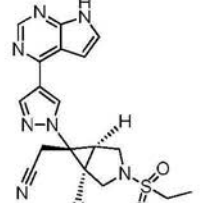
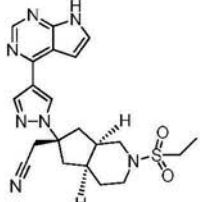
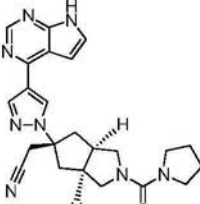
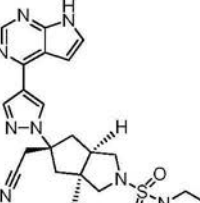
化合物	结构式
I-2	
I-4	
I-6	
I-8	
I-10	

I-12	
I-14	
I-16	
I-18	
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I-44	
I-46	

I-48	
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I-52	
I-54	
I-56	
I-58	

I-60	
I-62	
I-64	
I-66	
I-68	

5. 如权利要求1~4任一项所述的吡咯并嘧啶类化合物或其药学上可接受的盐在制备预防或治疗与JAK相关疾病的药物中的用途。

6. 如权利要求5所述的用途,其特征在于所述与JAK相关疾病包括器官移植排斥、狼疮、多发性硬化、类风湿性关节炎、青少年关节炎、银屑病、溃疡性结肠炎、克罗恩氏病、自体免疫性甲状腺疾病、牛皮癣、皮炎、特应性皮炎、哮喘、鼻炎、乙型肝炎、丙型肝炎、水痘-带状疱疹病毒、I型糖尿病与糖尿病并发症、阿尔茨海默病、干眼病、骨髓纤维化、血小板增多症、红细胞增多症、白血病、多发性骨髓瘤、前列腺癌、肾癌、肝癌、膜腺癌、胃癌、乳腺癌、肺癌、头颈部癌、甲状腺癌、胶质母细胞瘤、黑素瘤或淋巴瘤。

7. 一种组合物,所述组合物包括治疗有效量的如权利要求1~4任一项所述的吡咯并嘧啶类化合物或其药学上可接受的盐和药学上可接受的载体。

作为选择性JAK2抑制剂的吡咯并嘧啶类化合物、其合成方法及用途

技术领域

[0001] 本发明属于生物医药技术领域,具体涉及一种作为选择性JAK2抑制剂的吡咯并嘧啶类化合物、其合成方法及用途。

背景技术

[0002] JAK即Janus Kinase (两面神激酶),是一种非受体型酪氨酸蛋白激酶(PTK)。JAK-STAT通路主要由四个部分组成:(1)胞外信号因子;(2)受体;(3)JAK激酶;(4)信号转导及转录激活蛋白(STAT)。JAK-STAT是除了第二信使系统外最重要的信号途径。JAK激酶通过结合受体感受胞外的信号,如干扰素、白细胞介素、生长因子等,并将信息传送到STATs。磷酸化的STATs能够从胞内转移到细胞核。而每种不同的STAT结合到各不相同的启动子DNA序列上。启动子会控制其DNA序列表达,引起DNA转录与活性水平发生改变,进而影响细胞生长、分化及死亡等基本细胞功能。

[0003] JAK激酶家族的蛋白共有4个,包括JAK1、JAK2、JAK3、TYK2。从功能获得性表达或突变分析来看,JAK1、JAK3更多的与免疫调节有关,JAK2则与红细胞和血小板的生成直接相关。从功能缺失分析来看,JAK1、JAK2功能缺失会造成老鼠胚胎致死,在人体中尚未发现JAK1、JAK2功能缺失相关的疾病,也许间接表明了JAK1/2生理功能的重要性。JAK3功能缺失会造成严重的综合免疫缺陷,这也是后面提到的靶向JAK3,从而调节自身免疫相关疾病的依据。TYK2的功能研究较少,有报道其会引起与内在免疫相关的缺陷。

[0004] JAK2V617F突变在骨髓增生性肿瘤(MPN)中的发现大大促进了JAK2抑制剂的开发。MPN是一组以骨髓中异常造血祖细胞增殖为特征的慢性疾病。MPN包括骨髓纤维化(myelofibrosis, MF)、真性红细胞增多症(Polycythemia vera, PV)、原发性血小板增多症(Essential Thrombocythemia, ET)和慢性髓细胞白血病(Chronic Myelogenous Leukemia, CML)。大约95%的PV患者和50-60%的MF和ET患者都发现有JAK2V617F单氨基酸突变,引起了JAK2构象改变,造成了不依赖胞外细胞因子信号的激酶区域的持续激活,进而引起细胞增生和血液癌症。

[0005] W02007070514A报道的Ruxolitinib最初由Incyte开发,是一个JAK1/JAK2小分子激酶抑制剂。于2011年11月获得FDA批准,用于治疗中、高危的骨髓纤维化MF。2014年进一步获批用于真性红细胞增多症。Ruxolitinib能够达到缓解JAK2V617F突变引起的脾脏增大,减轻患者虚弱的症状。

[0006] Ruxolitinib不能够减少变异血癌细胞的JAK2V617F突变负荷,所以Ruxolitinib几乎不能带来治愈效果。另外由于Ruxolitinib的JAK2靶点选择性不高,副作用明显,Ruxolitinib的毒副作用主要包括贫血、血小板减少症、中性粒细胞减少症和腹泻等。

[0007] 早期的报道显示Ruxolitinib停药后出现明显的、预后较差的炎症综合症,在随后3年的随访中,没有观察到持续的类似的不良反应,提示此类反应可能为停用Ruxolitinib所致的严重的戒断性炎症综合征,应密切监测脾脏的大小,如果在Ruxolitinib治疗期间脾

脏仍有长大,停药后MF的相关症状有可能回归到基线水平甚至继续进展。因此当考虑中断Ruxolitinib治疗时,应逐渐减少剂量或合并使用皮质激素治疗。

[0008] 新一代的MPN药物开发重点集中在JAK2选择性的抑制剂,期望能够降低由于靶向JAK1引起的过多副作用的同时增加疗效。

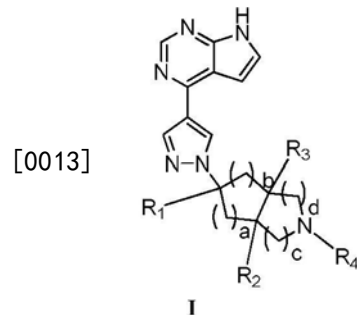
[0009] 目前,已经公开了一系列JAK抑制剂的专利申请,如W09965909A、W02013173720A、W02010039939A、W02018019222A、W02018087202A等。尽管已公开了一系列JAK抑制剂,但仍需开发新的具有更好药效、更低副作用的JAK抑制剂类化合物,特别是JAK2选择性抑制剂。

发明内容

[0010] 为了克服现有技术中所存在的问题,本发明的目的在于提供一种JAK2选择性抑制剂。

[0011] 为了实现上述目的以及其他相关目的,本发明采用如下技术方案:

[0012] 一种如式I所示的吡咯并嘧啶类化合物、其立体异构体、其药学上可接受的盐:



[0014] 其中:

[0015] R_1 为 $C_{1\sim6}$ 烷基或 $C_{1\sim6}$ 烷基氰基;所述 $C_{1\sim6}$ 烷基氰基指 $C_{1\sim6}$ 烷基上的任意氢被一个氰基取代;

[0016] R_2 和 R_3 各自独立的为氢或 $C_{1\sim6}$ 烷基;

[0017] R_4 为 $C_{1\sim6}$ 烷基、 $-C(O)R_5$ 、 $-C(O)NR_6R_7$ 、 $-S(O)_2R_8$ 或 $-S(O)_2NR_9R_{10}$;

[0018] a为0、1或2;

[0019] b为0、1或2;

[0020] c为0、1或2;

[0021] d为0、1或2;

[0022] R_5 为 $C_{1\sim6}$ 烷基、 $C_{2\sim6}$ 烯基、 $C_{3\sim6}$ 环烷基或 $C_{1\sim6}$ 烷基氰基,所述 $C_{1\sim6}$ 烷基氰基指 $C_{1\sim6}$ 烷基上的任意氢被一个氰基取代;


[0023] R_6 和 R_7 各自独立的为氢、 $C_{1\sim6}$ 烷基、 $C_{3\sim6}$ 环烷基或 R_6 、 R_7 与相连的N原子共同形成3-6元环;

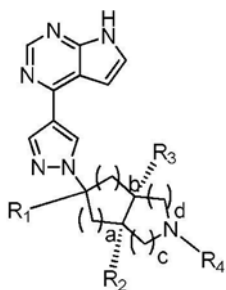
[0024] R_8 为 $C_{1\sim6}$ 烷基或 $C_{3\sim6}$ 环烷基;

[0025] R_9 和 R_{10} 各自独立的为氢、 $C_{1\sim6}$ 烷基、 $C_{3\sim6}$ 环烷基或 R_9 、 R_{10} 与相连的N原子共同形成3-6元环。

[0026] 优选的,所述 R_1 为 $C_{1\sim4}$ 烷基或 $C_{1\sim4}$ 烷基氰基;所述 $C_{1\sim4}$ 烷基氰基指 $C_{1\sim4}$ 烷基上的任意氢被一个氰基取代;

[0027] R_2 和 R_3 各自独立的为氢、甲基或乙基;

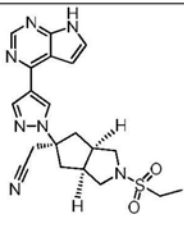
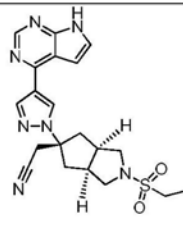
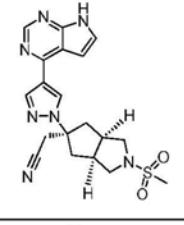
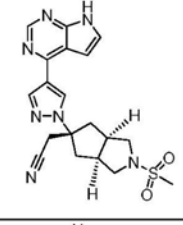
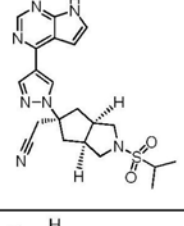
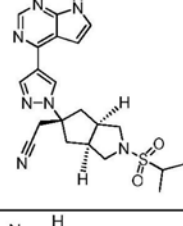
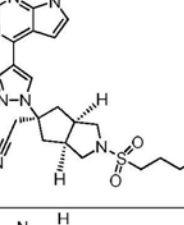
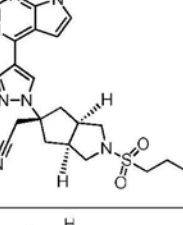
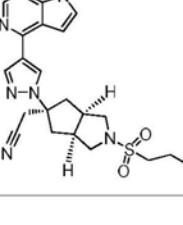
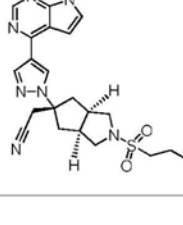
- [0028] R₄为C_{1~6}烷基、-C(O)R₅、-C(O)NR₆R₇、-S(O)₂R₈或-S(O)₂NR₉R₁₀;
- [0029] a为0、1或2;
- [0030] b为0或1;
- [0031] c为0、1或2;
- [0032] d为1或2;
- [0033] R₅为C_{1~4}烷基、C_{2~6}烯基、C_{3~6}环烷基或C_{1~4}烷基氰基,所述C_{1~4}烷基氰基指C_{1~4}烷基上的任意氢被一个氰基取代;
- [0034] R₆和R₇各自独立的为氢、C_{1~4}烷基、C_{3~6}环烷基或R₆、R₇与相连的N原子共同形成3-6元脂肪环;
- [0035] R₈为C_{1~5}烷基或C_{3~6}环烷基;
- [0036] R₉和R₁₀各自独立的为氢、C_{1~6}烷基、C_{3~6}环烷基或R₉、R₁₀与相连的N原子共同形成3-6元脂肪环。
- [0037] 更加优选的,所述:
- [0038] R₁为甲基、乙基或CN;
- [0039] R₂和R₃为氢;
- [0040] R₄为C_{1~3}烷基、-C(O)R₅、-C(O)NR₆R₇、-S(O)₂R₈或-S(O)₂NR₉R₁₀;
- [0041] a为0、1或2;
- [0042] b为0或1;
- [0043] c为1或2;
- [0044] d为1;
- [0045] R₅为C_{1~3}烷基、C_{3~6}环烷基、或CN ;
- [0046] R₆和R₇各自独立的为氢、C_{1~3}烷基、C_{3~6}环烷基或R₆、R₇与相连的N原子共同形成5-6元脂肪环;具体的所述5-6元脂肪环为四氢吡咯或六氢吡啶;
- [0047] R₈为C_{1~5}烷基或C_{4~5}环烷基;
- [0048] R₉和R₁₀各自独立的为氢、C_{1~3}烷基、C_{3~5}环烷基或R₉、R₁₀与相连的N原子共同形成5-6元脂肪环;具体的所述5-6元脂肪环为四氢吡咯或六氢吡啶。
- [0049] 更进一步的,所述立体异构体为与R₁直接相连的C原子形成的立体异构体。
- [0050] 更进一步的,如式I所示的吡咯并嘧啶类化合物、其立体异构体、其药学上可接受的盐:

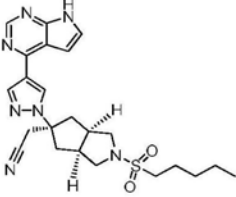
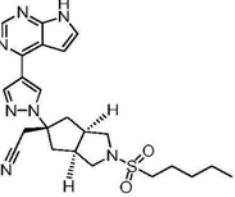
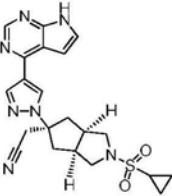
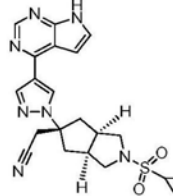
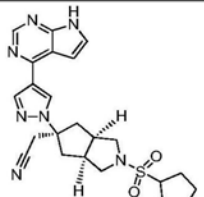
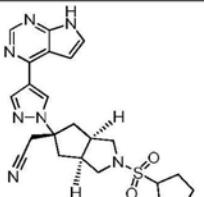
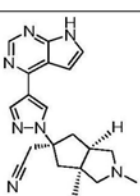
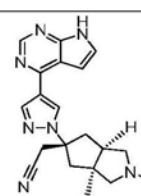
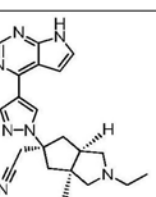
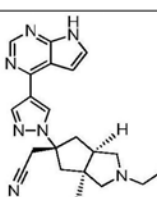
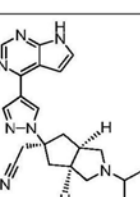
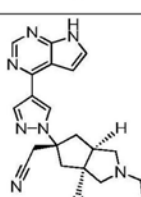
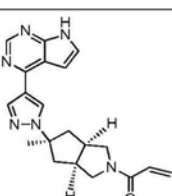
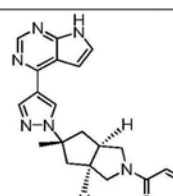
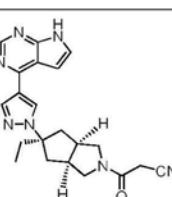
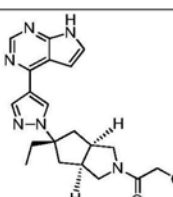


II

- [0052] 具体的,本发明提供的吡咯并嘧啶类化合物如下表所示:

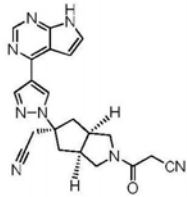
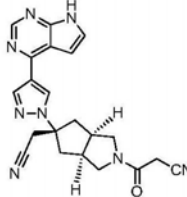
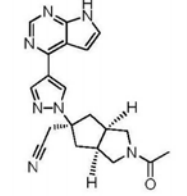
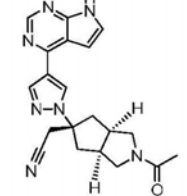
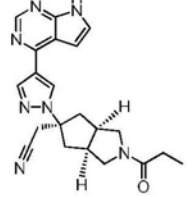
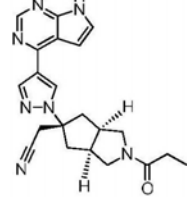
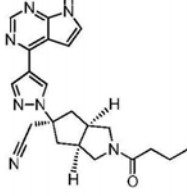
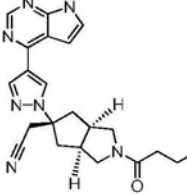
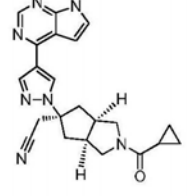
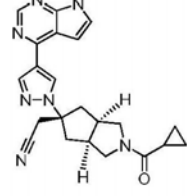
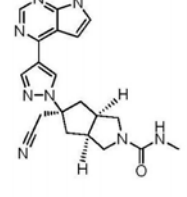
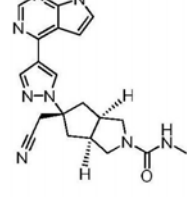
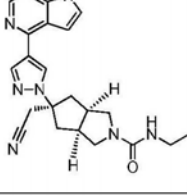
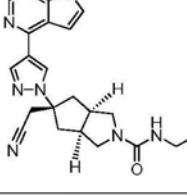
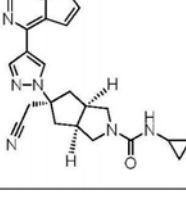
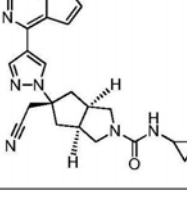
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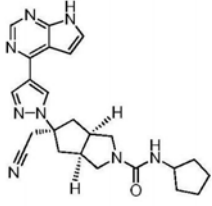
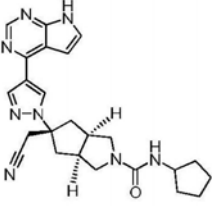
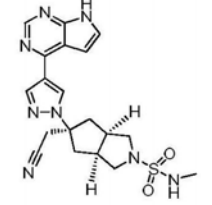
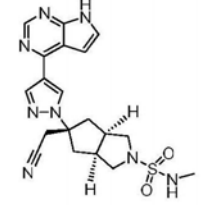
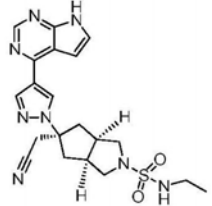
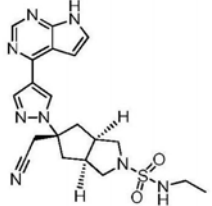
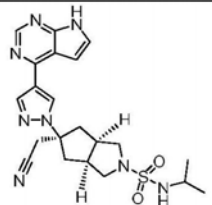
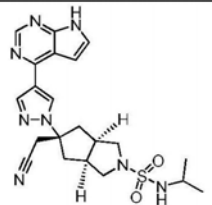
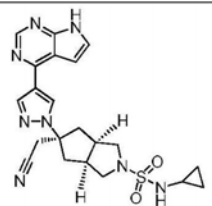
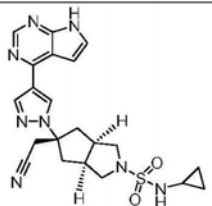
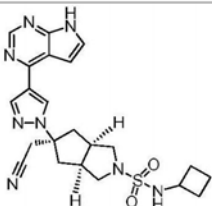
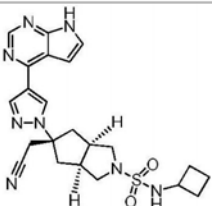
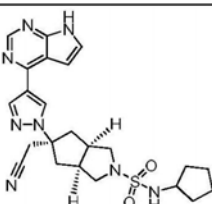
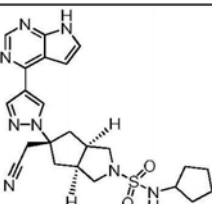
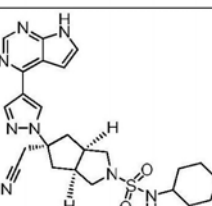
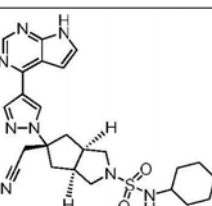
化合物	结构式	化合物	结构式
I-1		I-2	
I-3		I-4	
I-5		I-6	
I-7		I-8	
I-9		I-10	

I-11		I-12	
I-13		I-14	
I-15		I-16	
I-17		I-18	
I-19		I-20	
I-21		I-22	
I-23		I-24	
I-25		I-26	

[0054]

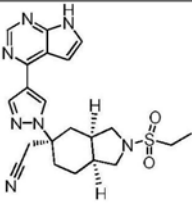
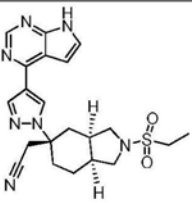
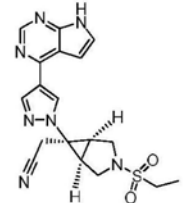
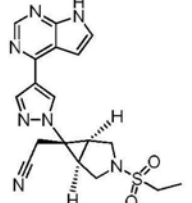
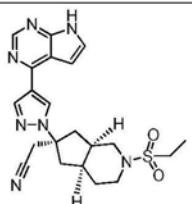
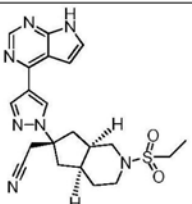
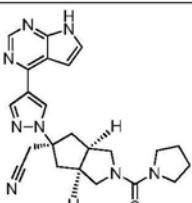
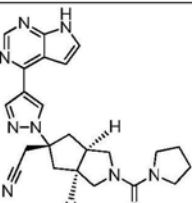
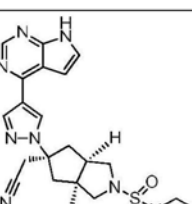
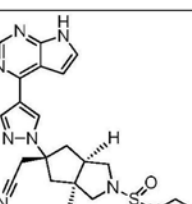
[0055]

I-27		I-28	
I-29		I-30	
I-31		I-32	
I-33		I-34	
I-35		I-36	
I-37		I-38	
I-39		I-40	
I-41		I-42	

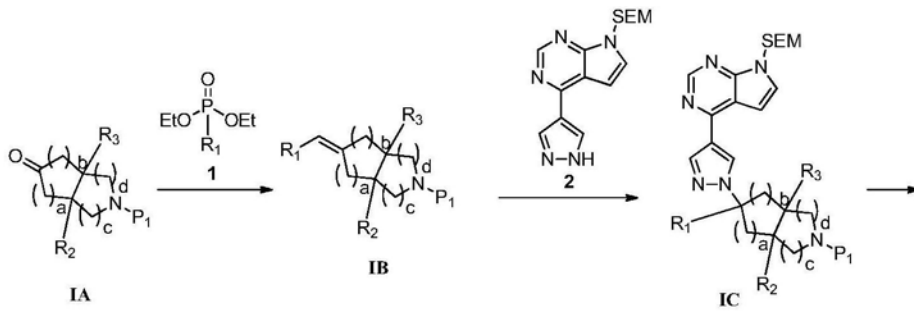
I-43		I-44	
I-45		I-46	
I-47		I-48	
I-49		I-50	
I-51		I-52	
I-53		I-54	
I-55		I-56	
I-57		I-58	

[0056]

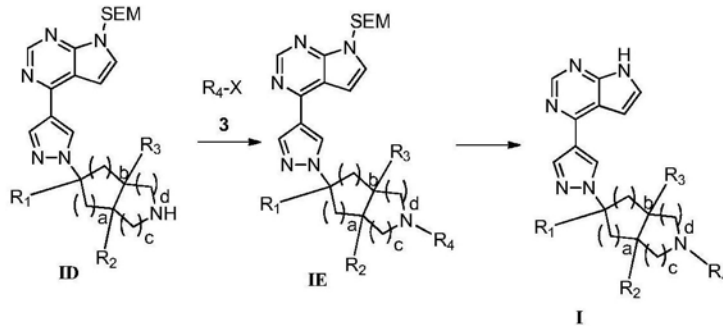
[0057]

I-59		I-60	
I-61		I-62	
I-63		I-64	
I-65		I-66	
I-67		I-68	

[0058] 本发明的第二目的在于提供了上述化合物的合成方法：



[0059]



[0060] (1) 通式IA化合物经Wittig反应制得通式IB化合物；

[0061] (2) IB与商购化合物2经催化偶联反应制得通式化合物IC；

[0062] (3) IC经脱保护、缩合反应制得IE；

[0063] (4) 化合物IE再经脱保护制得终产物I；上述反应式中P1基团为N的保护基团；X基团为活化基团；

[0064] 所述P1基团为C₁₋₆的烷氧基羰基，在本申请一些实施例中为叔丁氧基羰基；具体的，所述X基团选自卤素、C₁₋₃烷氧基或C₁₋₃烷磺酸基，在本申请一些实施例中X基团为卤素。

[0065] 本发明的第三目的在于提供上述化合物作为新型JAK抑制剂在制备预防或治疗与JAK相关疾病的药物中的用途，具体的主要是指预防或治疗下列疾病：免疫系统的疾病，包括器官移植排斥（如异体抑制排斥和移植物抗宿主疾病）；自身免疫性疾病，包括例如狼疮、多发性硬化、类风湿性关节炎、青少年关节炎、银屑病、溃疡性结肠炎、克罗恩氏病、自体免疫性甲状腺疾病等；皮肤病，包括例如牛皮癣、皮痒、特应性皮炎等；变应性病症，包括例如哮喘、鼻炎等；病毒性疾病，包括例如乙型肝炎、丙型肝炎、水痘-带状疱疹病毒等；I型糖尿病与糖尿病并发症；阿尔茨海默病、干眼病、骨髓纤维化、血小板增多症、红细胞增多症或白血病、多发性骨髓瘤；癌症，包括例如实体瘤（如前列腺癌、肾癌、肝癌、膜腺癌、胃癌、乳腺癌、肺癌、头颈部癌、甲状腺癌、胶质母细胞瘤、黑素瘤等）、皮肤癌（如皮肤T细胞淋巴瘤、皮肤仔细胞淋巴瘤）等。

[0066] 本发明的衍生物在实施疾病治疗过程中，可以组合物的形成通过口服、注射等方式，用于治疗相关癌症及其他疾病。用于口服时，可将其制备成常规的固体制剂如片剂、粉剂或胶囊等；用于注射时，可将其制备成注射液。

[0067] 本发明的第四目的在于提供一种组合物，所述组合物包括治疗有效量的上述吡咯并嘧啶类化合物、其立体异构体、其药学上可接受的盐和药学上可接受的载体。

[0068] 药学上可接受的盐，例如，可以提及金属盐、接盐、与有机碱形成的盐、与无机酸形成的盐、与有机酸形成的盐、与碱性或者酸性氨基酸形成的盐等。金属盐的非限制性实例包括但不限于碱金属的盐，例如钠盐、钾盐等；碱土金属的盐，例如钙盐、镁盐、钡盐、铝盐等。

与无机酸形成的盐的非限制性实例包括但不限于与盐酸、氢溴酸、硝酸、硫酸、磷酸等形成的盐。与有机酸形成的盐的非限制性实例包括但不限于与甲酸、乙酸、三氟乙酸、富马酸、草酸、苹果酸、马来酸、酒石酸、柠檬酸、琥珀酸、甲磺酸、苯磺酸、对甲基苯磺酸等形成的盐。

[0069] 所述及的载体是指药学领域常规的载体,如:稀释剂、赋形剂如水等;粘合剂如纤维素衍生物、明胶、聚乙烯吡咯烷酮等;填充剂如淀粉等;崩裂剂如碳酸钙、碳酸氢钠;另外,还可以在组合物中加入其他辅助剂如香味剂和甜味剂。

[0070] 本发明的组合物的各种剂型可以采用医学领域常规的方法进行制备,其中活性成分的含量为0.1%~99.5%(重量比)。

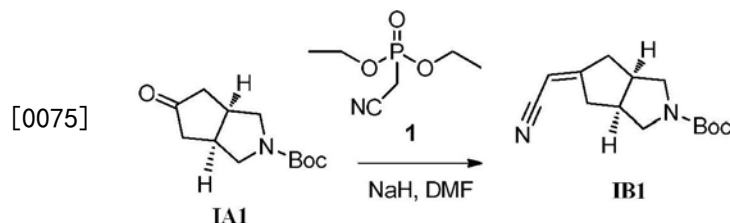
[0071] 本发明的施用量可根据用药途径、患者的年龄、体重、所治疗的疾病的类型和严重程度等进行变化,其日剂量为0.001-30mg/kg体重(口服)或0.005-30mg/kg体重(注射)。

[0072] 与现有技术相比,本发明提供的吡咯并咪啶类化合物、其立体异构体及其药学上可接受的盐具有更好的两面神激酶抑制活性,且其对JAK2抑制靶点选择性显著优于现有化合物,且本发明的优选化合物表现出良好的药代动力学性质,具有开发成为选择性JAK2抑制剂的潜力。

具体实施方式

[0073] 下面将对本发明实施例中的技术方案进行清楚、完整地描述,显然,所描述的实施例仅是本发明的一部分实施例,而不是全部的实施例。基于本发明中的实施例,本领域普通技术人员在没有做出创造性劳动前提下所获得的所有其它实施例,都属于本发明保护的范围。

[0074] 参考例1:片段IB1的合成路线。

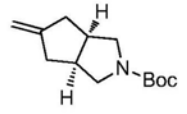
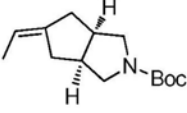
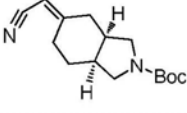
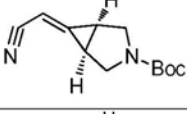
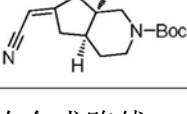


[0076] 操作步骤:

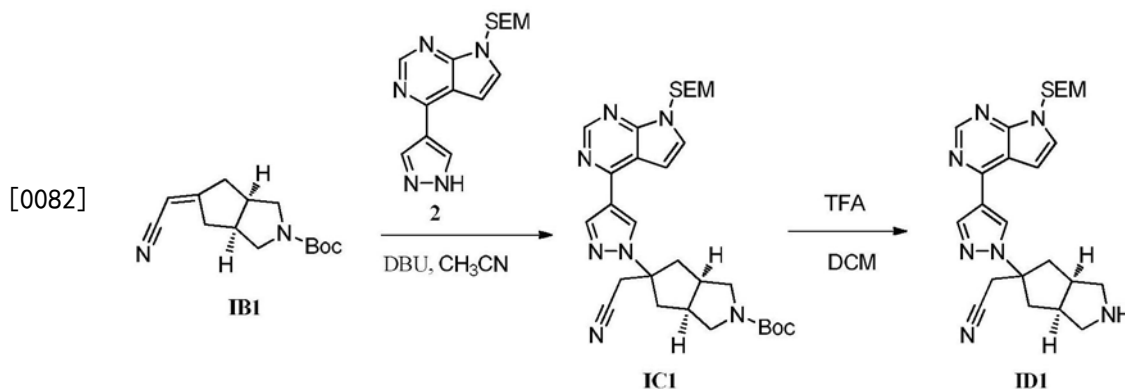
[0077] NaH (3.55g, 2eq, 60%) 加入DMF (150mL) 中,冷却至0℃,将氰甲基亚磷酸二乙酯 (15g, 2eq) 滴入以上反应液中,搅拌1小时,缓慢升至室温,滴入IA1 (10g, 44.4mol) 的DMF (50mL) 溶液,继续搅拌12小时。反应液倒入水中,加乙酸乙酯萃取,无水硫酸钠干燥、过滤、浓缩,得到粗产物。石油醚/乙酸乙酯 (10/1) 柱层析,得到无色油状产物IB1 9g,收率76%。MS: 249.0 [M+H]⁺, ¹H NMR (400MHz, CDCl₃) δ: 5.26 (s, 1H), 3.64-3.47 (m, 2H), 3.21-3.00 (m, 2H), 2.98-2.63 (m, 4H), 2.56 (d, J=16.0Hz, 1H), 2.41 (d, J=16.0Hz, 1H), 1.44 (s, 9H)。

[0078] 参考例2-6:片段IB2~IB6。

[0079] 参照参考例1中操作步骤的合成方法,合成下表2中各参考例:

参考例	片段	结构	核磁& MS
参考例 2	IB2		MS: 224.3 [M+H] ⁺ , ¹ H NMR (400 MHz, CDCl ₃) δ: 5.11(s, 1H), 4.92(s, 1H), 3.63-3.48 (m, 2H), 3.20-3.01(m, 2H), 2.89-2.63 (m, 4H), 2.53 (d, J = 16.0 Hz, 1H), 2.43 (d, J = 16.0 Hz, 1H), 1.45 (s, 9H).
参考例 3	IB3		MS: 238.3 [M+H] ⁺ , ¹ H NMR (400 MHz, CDCl ₃) δ: 5.21(s, 1H), 3.65-3.52 (m, 2H), 3.23-3.05 (m, 2H), 2.89-2.65 (m, 7H), 2.57 (d, J = 16.0 Hz, 1H), 2.45 (d, J = 16.0 Hz, 1H), 1.47 (s, 9H).
[0080] 参考例 4	IB4		MS: 263.2 [M+H] ⁺ , ¹ H NMR (400 MHz, CDCl ₃) δ: 5.02 (s, 1H), 3.57 (m, 2H), 3.20 (m, 2H), 2.04-1.97 (m, 4H), 1.62 (m, 2H), 1.45 (s, 9H), 1.41 (m, 1H), 1.11 (m, 1H).
参考例 5	IB5		MS: 221.2 [M+H] ⁺ , ¹ H NMR (400 MHz, CDCl ₃) δ: 5.21(s, 1H), 3.48 (m, 2H), 3.23(m, 2H), 2.32 (m, 2H), 1.41 (s, 9H).
参考例 6	IB6		MS: 263.2 [M+H] ⁺ , ¹ H NMR (400 MHz, CDCl ₃) δ: 5.05 (s, 1H), 3.42-3.17 (m, 4H), 2.04-1.81 (m, 4H), 1.59-1.52 (m, 3H), 1.43 (s, 9H), 1.35 (m, 1H).

[0081] 参考例7:片段IC1的合成路线。



[0083] 操作步骤:步骤1,IC1的合成。

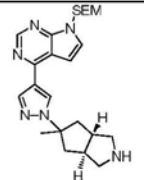
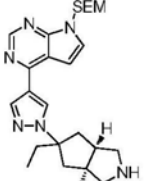
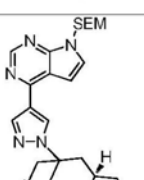
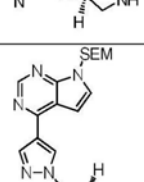
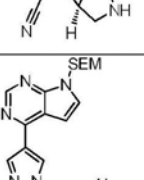
[0084] 室温下,将化合物IB1 (9.0g, 36.2mol) 与2 (9.0g, 28.5mol) 加入乙腈 (150mL) 中。DBU (5.6g, 37.1mol) 缓慢滴入该反应体系中,升温至65℃,搅拌反应24h。反应液加水淬灭,乙酸乙酯萃取、分液、干燥、过滤、浓缩,得油状物粗品。该油状物粗品经二氯甲烷/甲醇 (20/1) 柱层析,得到无色油状产物IC1 (9.5g, 收率59%)。MS: 564.8 [M+H]⁺, ¹H NMR (400MHz, DMSO-d₆) δ: 8.78 (s, 0.55H), 8.73 (d, J = 3.2Hz, 1H), 8.72 (s, 0.57H), 8.38 (d, J = 7.2Hz, 1H), 7.77-7.71 (m, 1H), 7.15 (dd, J = 7.2, 3.7Hz, 1H), 5.61 (s, 2H), 4.43 (s, 2H), 3.58-3.44 (m, 2H), 3.40-3.33 (m, 2H), 3.27-3.09 (m, 2H), 3.05-2.95 (m, 1H), 2.95-2.88 (m, 1H), 2.67-2.55 (m, 1H), 2.48-2.42 (m, 1H), 2.37-2.25 (m, 1H), 1.84 (dd, J = 13.6, 8.0Hz, 1H), 1.38 (s, 9H), 0.88-0.71 (m, 2H), -0.13 (s, 9H)。

[0085] 步骤2, ID1的合成。

[0086] 化合物IC1 (9.0g, 16.0mol) 加入20%的TFA二氯甲烷溶液 (90mL) 中,室温下搅拌3小时。降温至0℃,加入碳酸氢钾饱和溶液,调节pH值为8。二氯甲烷萃取、干燥、浓缩得到油状物粗品。该油状物粗品经二氯甲烷/甲醇 (20/1) 柱层析,得到产物ID1 (4.5g, 收率61%)。MS: 464.6 [M+H]⁺, ¹H NMR (400MHz, DMSO-d₆) δ: 8.89 (s, 1H), 8.72 (br, 1H), 8.61 (s, 1H), 8.31

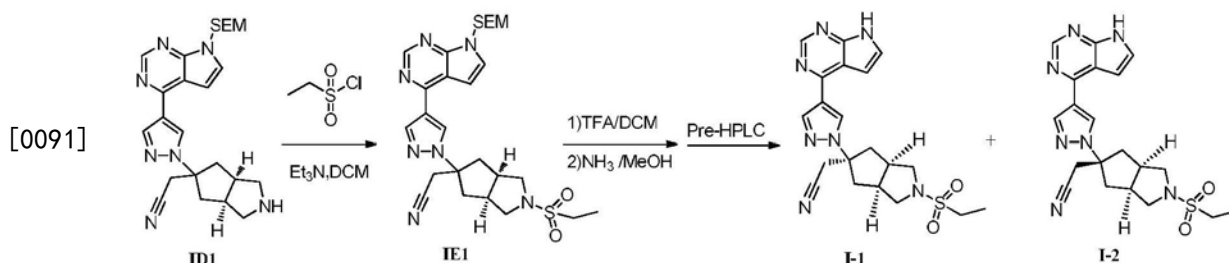
(s, 1H), 7.45 (d, $J=4\text{Hz}$, 1H), 6.86 (d, $J=4\text{Hz}$, 1H), 5.69 (s, 2H), 3.54 (t, $J=12\text{Hz}$, 2H), 2.60-2.30 (m, 6H), 2.30-2.10 (m, 6H), 0.79 (t, $J=12\text{Hz}$, 2H), -0.06 (s, 9H)。参考例8-12: 片段ID2~ID6。

[0087] 参照参考例7步骤1和步骤2的合成方法, 合成下表3中各参考例:

参考例	片段	结构	核磁& MS	所用片段
参考例 8	ID2		MS: 439.6 $[M+H]^+$, ^1H NMR (400 MHz, DMSO- d_6) δ : 9.31 (s, 1H), 8.71 (br, 1H), 8.57 (s, 1H), 8.30 (s, 1H), 7.38 (d, $J=4\text{Hz}$, 1H), 6.56 (d, $J=4\text{Hz}$, 1H), 5.75 (s, 2H), 3.55 (t, $J=12\text{Hz}$, 2H), 2.78-2.60 (m, 4H), 2.30-2.12 (m, 6H), 1.45 (s, 3H), 0.78 (t, $J=12\text{Hz}$, 2H), -0.03 (s, 9H)。	IB2
参考例 9	ID3		MS: 453.4 $[M+H]^+$, ^1H NMR (400 MHz, DMSO- d_6) δ : 9.33 (s, 1H), 8.78 (br, 1H), 8.55 (s, 1H), 8.27 (s, 1H), 7.35 (d, $J=4\text{Hz}$, 1H), 6.54 (d, $J=4\text{Hz}$, 1H), 5.73 (s, 2H), 3.56 (t, $J=12\text{Hz}$, 2H), 2.75-2.59 (m, 4H), 2.30-2.00 (m, 8H), 0.78-0.85 (m, 5H), -0.05 (s, 9H)。	IB3
[0088] 参考例 10	ID4		MS: 478.2 $[M+H]^+$, ^1H NMR (400 MHz, DMSO- d_6) δ : 8.97 (s, 1H), 8.68 (br, 1H), 8.63 (s, 1H), 8.27 (s, 1H), 7.47 (d, $J=4\text{Hz}$, 1H), 6.81 (d, $J=4\text{Hz}$, 1H), 5.59 (s, 2H), 3.59 (t, $J=12\text{Hz}$, 2H), 2.61-2.32 (m, 6H), 2.30-2.05 (m, 8H), 0.75 (t, $J=12\text{Hz}$, 2H), -0.01 (s, 9H)。	IB4
参考例 11	ID5		MS: 436.5 $[M+H]^+$, ^1H NMR (400 MHz, DMSO- d_6) δ : 8.99 (s, 1H), 8.73 (br, 1H), 8.61 (s, 1H), 8.28 (s, 1H), 7.45 (d, $J=4\text{Hz}$, 1H), 6.78 (d, $J=4\text{Hz}$, 1H), 5.57 (s, 2H), 3.55 (t, $J=12\text{Hz}$, 2H), 2.68-2.59 (m, 6H), 0.79 (t, $J=12\text{Hz}$, 2H), 0.69 (m, 2H), -0.05 (s, 9H)。	IB5
参考例 12	ID6		MS: 478.3 $[M+H]^+$, ^1H NMR (400 MHz, DMSO- d_6) δ : 8.99 (s, 1H), 8.72 (br, 1H), 8.61 (s, 1H), 8.25 (s, 1H), 7.45 (d, $J=4\text{Hz}$, 1H), 6.78 (d, $J=4\text{Hz}$, 1H), 5.57 (s, 2H), 3.55 (t, $J=12\text{Hz}$, 2H), 2.69-2.57 (m, 6H), 2.25-2.00 (m, 8H), 0.77 (t, $J=12\text{Hz}$, 2H), -0.06 (s, 9H)。	IB6

[0089] 实施例1: I-1和I-2

[0090] 合成路线:



[0092] 操作步骤:

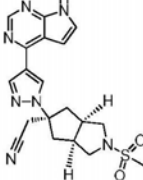

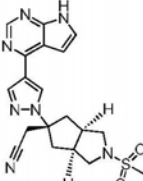
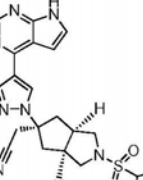
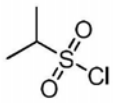
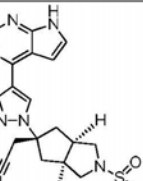
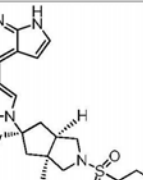
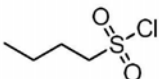
[0093] 化合物ID1 (2.5g, 5.4mol)、三乙胺 (2.3mL, 2eq) 依次溶解于二氯甲烷 (50mL), 降温至0℃, 搅拌下缓慢滴加乙基磺酰氯 (1.38g, 10.7mol), 自然升至室温, 搅拌反应2小时。反应

液水洗 (30mL)、饱和食盐水洗 (30mL)、分液、无水硫酸钠干燥、过滤、浓缩,得到中间体IE1粗产物。将该中间体加入25%的TFA/二氯甲烷溶液 (16mL) 中,室温下搅拌15小时以上,减压浓缩除去溶剂与TFA,加入15%的氨水-甲醇溶液 (40mL),室温搅拌3h,浓缩至干。剩余物加二氯甲烷 (50mL)/水 (30mL) 萃取、有机层再饱和食盐水洗 (30mL)、分液、无水硫酸钠干燥、过滤、浓缩,得I-1和I-2混合物粗品。该混合物经Pre-HPLC制备分离得I-1 (550mg,收率24%), I-2 (300mg,收率13%)。

[0094] I-1:MS:426.5[M+H]⁺。¹H NMR (400MHz, DMSO-d₆) δ:12.12 (br, 1H), 8.76 (s, 1H), 8.69 (s, 1H), 8.39 (s, 1H), 7.60 (d, J=3.6Hz, 1H), 7.05 (d, J=3.6Hz, 1H), 3.17 (q, J=9.6Hz, 2H), 3.10-3.00 (m, 8H), 2.63 (m, 2H), 1.79 (m, 2H), 1.10 (t, J=9.2Hz, 3H)。

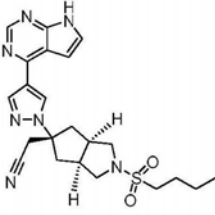
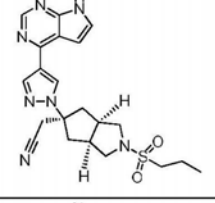
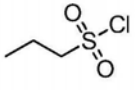
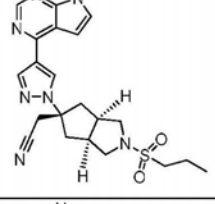
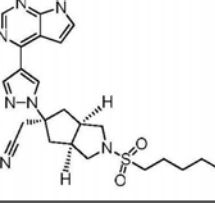
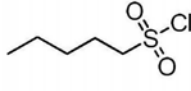
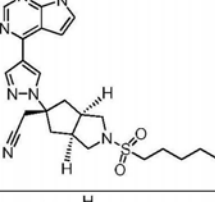
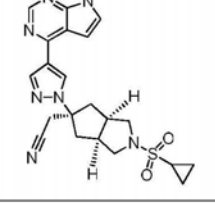
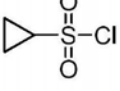
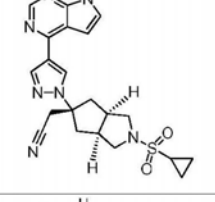
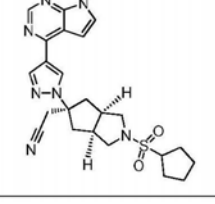
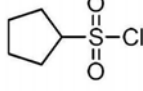
[0095] I-2:MS:426.5[M+H]⁺。¹H NMR (400MHz, DMSO-d₆) δ:12.11 (br, 1H), 8.91 (s, 1H), 8.68 (s, 1H), 8.41 (s, 1H), 7.61 (d, J=3.6Hz, 1H), 7.07 (d, J=3.6Hz, 1H), 3.24 (q, J=9.2Hz, 2H), 3.12-3.00 (m, 8H), 2.61 (m, 2H), 1.81 (m, 2H), 1.15 (t, J=9.2Hz, 3H)。

[0096] 参照实施例1中的合成方法,合成下表4中各实施例化合物:

实施例	化合物编号	结构式	所用片段
2	I-3		ID1 
	I-4		
3	I-5		ID1 
	I-6		
4	I-7		ID1 

[0097]

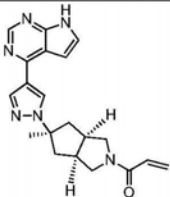
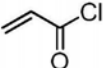
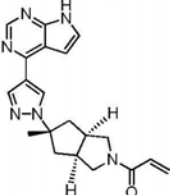
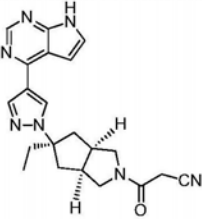
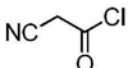
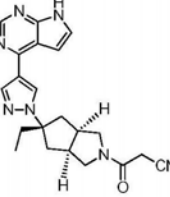
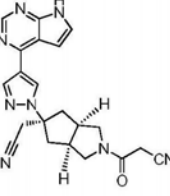
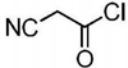
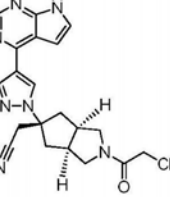
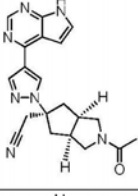

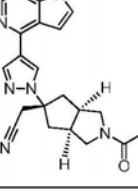
[0098]

	I-8			
5	I-9		ID1	
	I-10			
6	I-11		ID1	
	I-12			
7	I-13		ID1	
	I-14			
8	I-15		ID1	

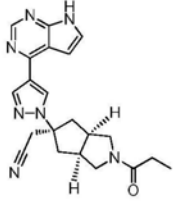
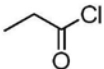
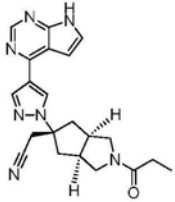
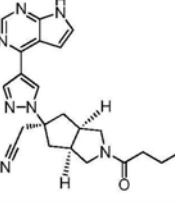
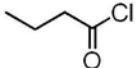
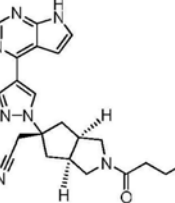
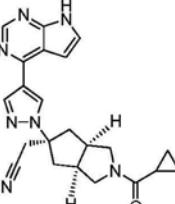
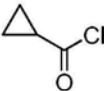
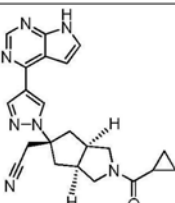
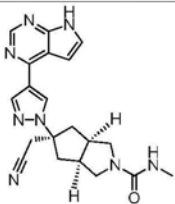
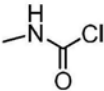
[0099]

	I-16			
9	I-17		ID1	—I
	I-18			
10	I-19		ID1	
	I-20			
11	I-21		ID1	
	I-22			

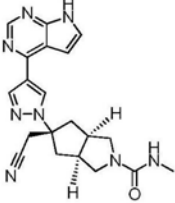
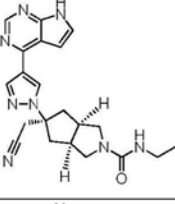
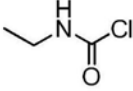
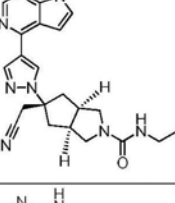
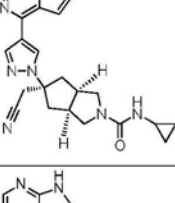
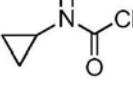
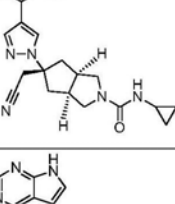
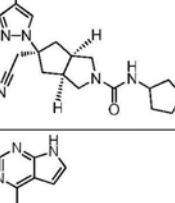
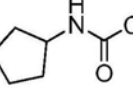
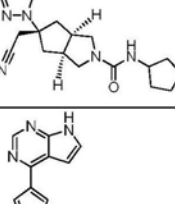
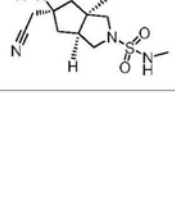
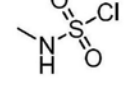
[0100]

12	I-23		ID2	
	I-24			
13	I-25		ID3	
	I-26			
14	I-27		ID1	
	I-28			
15	I-29		ID1	
	I-30			

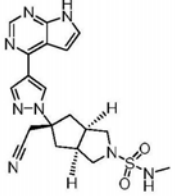
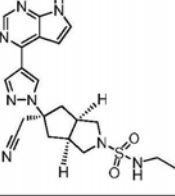
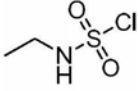
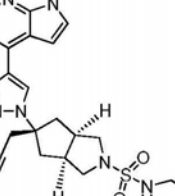
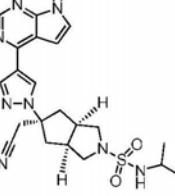
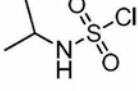
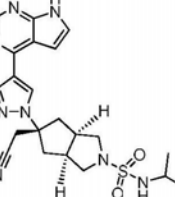
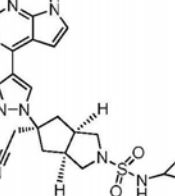
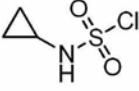
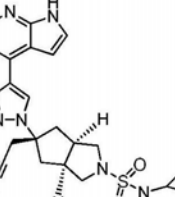
[0101]

16	I-31		ID1	
	I-32			
17	I-33		ID1	
	I-34			
18	I-35		ID1	
	I-36			
19	I-37		ID1	

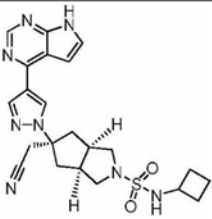
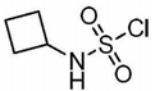
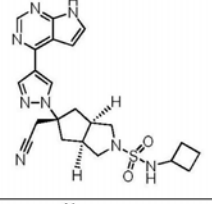
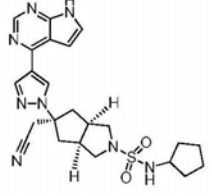
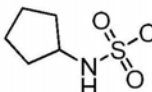
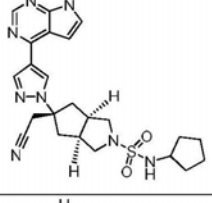
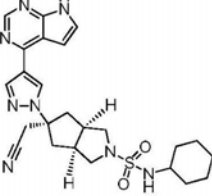
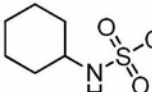
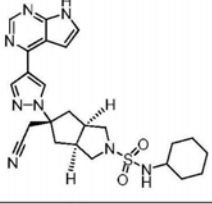
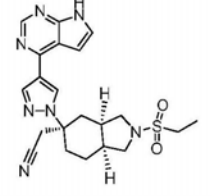
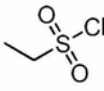
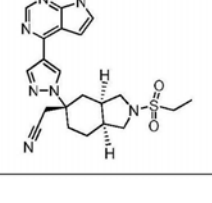
[0102]

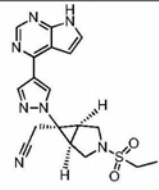
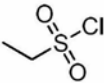
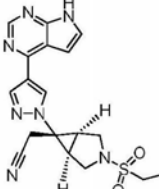
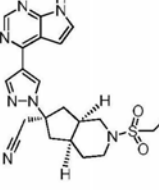
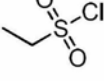
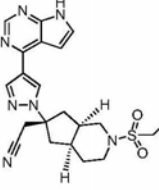
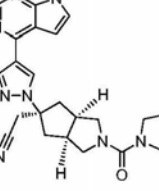
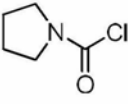
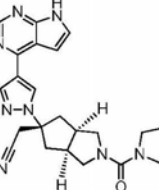
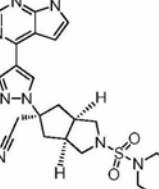
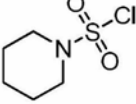
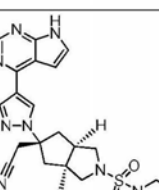
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	I-42			
22	I-43		ID1	
	I-44			
23	I-45		ID1	

[0103]

	I-46			
24	I-47		ID1	
	I-48			
25	I-49		ID1	
	I-50			
26	I-51		ID1	
	I-52			

[0104]

27	I-53		ID1	
	I-54			
28	I-55		ID1	
	I-56			
29	I-57		ID1	
	I-58			
30	I-59		ID4	
	I-60			

31	I-61		ID5	
	I-62			
32	I-63		ID6	
	I-64			
33	I-65		ID1	
	I-66			
34	I-67		ID1	
	I-68			

[0105] 各实施例化合物的NMR和MS数据汇总见下表5所示：

化合物	NMR	MS [M+H] ⁺
I-1	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.76 (s, 1H), 8.69 (s, 1H), 8.39 (s, 1H), 7.60 (d, <i>J</i> = 3.6 Hz, 1H), 7.05 (d, <i>J</i> = 3.6 Hz, 1H), 3.17 (q, <i>J</i> = 9.6 Hz, 2H), 3.10-3.00 (m, 8H), 2.63 (m, 2H), 1.79 (m, 2H), 1.10 (t, <i>J</i> = 9.2Hz, 3H)。	426.5
I-2	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.91(s,1H), 8.68 (s, 1H), 8.41 (s, 1H), 7.61 (d, <i>J</i> = 3.6 Hz, 1H), 7.07 (d, <i>J</i> = 3.6 Hz, 1H), 3.24 (q, <i>J</i> = 9.2 Hz, 2H), 3.12-3.00 (m, 8H), 2.61 (m, 2H), 1.81 (m, 2H), 1.15 (t, <i>J</i> = 9.2Hz, 3H)。	426.5
I-3	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.69 (s, 1H), 8.65 (s, 1H), 8.38 (s, 1H), 7.61 (d, <i>J</i> = 4 Hz, 1H), 7.04 (d, <i>J</i> = 4 Hz, 1H), 2.98 (s, 3H), 2.89-2.65 (m, 8H), 2.59 (m, 2H), 1.75 (m, 2H)。	412.4
I-4	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.89 (s, 1H), 8.67 (s, 1H), 8.34 (s, 1H), 7.64 (d, <i>J</i> = 4 Hz, 1H), 7.12 (d, <i>J</i> = 4 Hz, 1H), 2.99 (s, 3H), 2.88-2.69 (m, 8H), 2.62 (m, 2H), 1.73 (m, 2H)。	412.4
I-5	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.95 (s, 1H), 8.71 (s, 1H), 8.39 (s, 1H), 7.63 (m, 1H), 7.12 (m, 1H), 3.41 (m, 1H), 2.91-2.67 (m, 8H), 2.12 (m, 2H), 1.85 (m, 2H), 1.35 (m, 6H)。	440.5
I-6	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.93 (s, 1H), 8.70 (s, 1H), 8.38 (s, 1H), 7.62 (m, 1H), 7.05 (m, 1H), 3.40 (m, 1H), 2.91-2.69 (m, 8H), 2.07 (m, 2H), 1.88 (m, 2H), 1.33 (m, 6H)。	440.5
I-7	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.10 (br, 1H), 8.92 (s, 1H), 8.68 (s, 1H), 8.37 (s, 1H), 7.62 (d, <i>J</i> = 4 Hz, 1H), 7.09 (d, <i>J</i> = 4 Hz, 1H), 3.25 (m, 2H), 2.89-2.69 (m, 8H), 2.22-2.01 (m, 8H), 0.93 (m, 3H)。	454.3
I-8	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.93 (s, 1H), 8.69 (s, 1H), 8.39 (s, 1H), 7.61 (d, <i>J</i> = 4 Hz, 1H), 7.05 (d, <i>J</i> = 4 Hz, 1H), 3.29 (m, 2H), 2.95-2.71 (m, 8H), 2.23-2.01 (m, 8H), 0.95 (m, 3H)。	454.3
I-9	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.15 (br, 1H), 8.90 (s, 1H), 8.69 (s, 1H), 8.38 (s, 1H), 7.61 (d, <i>J</i> = 4 Hz, 1H), 7.05 (d, <i>J</i> = 4 Hz, 1H), 3.27 (m, 2H), 2.88-2.67 (m, 8H), 2.20-2.01 (m, 6H), 0.95 (m, 3H)。	440.4
I-10	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.95 (s, 1H), 8.71 (s, 1H), 8.41 (s, 1H), 7.59 (d, <i>J</i> = 4 Hz, 1H), 7.09 (d, <i>J</i> = 4 Hz, 1H), 3.29 (m, 2H), 2.89-2.71 (m, 8H), 2.21-2.00 (m, 6H), 0.94 (t, <i>J</i> = 7.2 Hz, 3H)。	440.4
I-11	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.91 (s, 1H), 8.65 (s, 1H), 8.39 (s, 1H), 7.60 (m, 1H), 7.12 (d, <i>J</i> = 4 Hz, 1H), 3.27 (t, <i>J</i> = 7.2 Hz, 2H), 2.88-2.68 (m, 8H), 2.10-1.97 (m, 6H), 1.30 (m, 4H), 0.90(m, 3H)。	468.6
I-12	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.93 (s, 1H), 8.72 (s, 1H), 8.38 (s, 1H), 7.63 (d, <i>J</i> = 4 Hz, 1H), 7.07 (d, <i>J</i> = 4 Hz, 1H), 3.29 (m, 2H), 2.91-2.71 (m, 8H), 2.21-1.98 (m, 6H), 1.32 (m, 4H), 0.93(m, 3H)。	468.6
I-13	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.88 (s, 1H), 8.67 (s, 1H), 8.38 (s, 1H), 7.61 (m, 1H), 7.14 (d, <i>J</i> = 4 Hz, 1H), 2.79-2.61 (m, 8H), 2.12-1.87 (m, 5H), 0.82-0.68 (m, 4H)。	438.2
I-14	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.07 (br, 1H), 8.95 (s, 1H), 8.68 (s, 1H), 8.37 (s, 1H), 7.62 (m, 1H), 7.16 (d, <i>J</i> = 4 Hz, 1H), 2.83-2.62 (m, 8H), 2.05-1.88 (m, 5H), 0.79-0.69 (m, 4H)。	438.2
I-15	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.98 (s, 1H), 8.72 (s, 1H), 8.37 (s, 1H), 7.60 (m, 1H), 7.19 (d, <i>J</i> = 4 Hz, 1H), 2.85-2.62 (m, 9H), 2.15-1.78 (m, 12H)。	466.4
I-16	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.16 (br, 1H), 8.97 (s, 1H), 8.75 (s, 1H), 8.39 (s, 1H), 7.62 (m, 1H), 7.21 (d, <i>J</i> = 4 Hz, 1H), 2.86-2.65 (m, 9H), 2.12-1.77 (m, 12H)。	466.4
I-17	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.85 (s, 1H), 8.63 (s, 1H), 8.39 (s, 1H), 7.63 (d, <i>J</i> = 4 Hz, 1H), 7.06 (d, <i>J</i> = 4 Hz, 1H), 2.81 (s, 2H), 2.73 (m, 2H), 2.34 (m, 2H), 2.26 (s, 3H), 2.05 (m, 4H), 1.75 (m, 2H)。	348.3
I-18	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.87 (s, 1H), 8.67 (s, 1H), 8.37 (s, 1H), 7.61 (d, <i>J</i> = 4.4 Hz, 1H), 7.10 (d, <i>J</i> = 4 Hz, 1H), 2.85(s, 2H), 2.76 (m, 2H), 2.34-2.27 (m, 5H), 2.07 (m, 4H), 1.78 (m, 2H)。	348.3
I-19	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.95 (s, 1H), 8.67 (s, 1H), 8.35 (s, 1H), 7.61 (m, 1H), 7.08 (m, 1H), 2.82 (s, 2H), 2.75 (m, 2H), 2.35-2.10 (m, 6H), 2.05-1.85 (m, 4H), 1.02 (t, <i>J</i> = 4 Hz, 3H)。	362.3
I-20	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.08 (br, 1H), 8.97 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 7.63 (m, 1H), 7.12 (m, 1H), 2.81-2.78 (m, 4H), 2.31-2.10 (m, 6H), 2.03-1.87 (m, 4H), 1.05 (t, <i>J</i> = 4 Hz, 3H)。	362.3
I-21	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.02 (br, 1H), 8.99 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 7.62 (m, 1H), 7.07 (m, 1H), 2.83-2.75 (m, 5H), 2.33-2.02 (m, 6H), 1.75 (m, 2H), 1.07 (s, 6H)。	376.5
I-22	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.89 (s, 1H), 8.65 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.10 (m, 1H), 2.85-2.77 (m, 5H), 2.35-2.07 (m, 6H), 1.77 (m, 2H), 1.05 (s, 6H)。	376.5
I-23	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.10 (br, 1H), 8.95 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 7.59 (d, <i>J</i> = 4.0 Hz, 1H), 7.10 (d, <i>J</i> = 4.0 Hz, 1H), 6.65 (m, 1H), 5.18 (d, <i>J</i> = 12.0 Hz, 2H), 3.54 (m, 2H), 3.19 (m, 2H), 2.87 (m, 2H), 2.01-1.67 (m, 4H), 1.45 (s, 3H)。	363.3
I-24	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.97 (s, 1H), 8.68 (s, 1H), 8.39 (s, 1H), 7.57 (d, <i>J</i> = 4.0 Hz, 1H), 7.05 (d, <i>J</i> = 4.0 Hz, 1H), 6.63 (m, 1H), 5.17 (d, <i>J</i> = 12.0 Hz, 2H), 3.57 (m, 2H), 3.21 (m, 2H), 2.88 (m, 2H), 2.00-1.67 (m, 4H), 1.44 (s, 3H)。	363.3

[0107]

I-25	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.99 (s, 1H), 8.71 (s, 1H), 8.39 (s, 1H), 7.65 (d, <i>J</i> = 4.0 Hz, 1H), 7.13 (d, <i>J</i> = 4.0 Hz, 1H), 4.04-3.75 (m, 2H), 3.65-3.32 (m, 4H), 2.85 (m, 2H), 2.01-1.75 (m, 6H), 0.95 (t, <i>J</i> = 6.0 Hz, 3H)。	390.4
I-26	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.96 (s, 1H), 8.73 (s, 1H), 8.37 (s, 1H), 7.69 (d, <i>J</i> = 4.0 Hz, 1H), 7.11 (d, <i>J</i> = 4.0 Hz, 1H), 4.01-3.77 (m, 2H), 3.65-3.39 (m, 4H), 2.87 (m, 2H), 2.01-1.77 (m, 6H), 0.97 (t, <i>J</i> = 7.2 Hz, 3H)。	390.4
I-27	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.95 (s, 1H), 8.73 (s, 1H), 8.38 (s, 1H), 7.67 (d, <i>J</i> = 4.0 Hz, 1H), 7.15 (d, <i>J</i> = 4.0 Hz, 1H), 3.64-3.51 (m, 6H), 2.87 (m, 2H), 2.81 (s, 2H), 2.01-1.85 (m, 4H)。	401.2
I-28	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.10 (br, 1H), 8.96 (s, 1H), 8.75 (s, 1H), 8.39 (s, 1H), 7.69 (d, <i>J</i> = 4.0 Hz, 1H), 7.11 (d, <i>J</i> = 4.0 Hz, 1H), 3.63-3.57 (m, 6H), 2.88 (m, 2H), 2.82 (s, 2H), 1.99-1.86 (m, 4H)。	401.2
I-29	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.93 (s, 1H), 8.71 (s, 1H), 8.39 (s, 1H), 7.62 (d, <i>J</i> = 4.0 Hz, 1H), 7.03 (m, 1H), 3.54 (m, 2H), 3.27 (m, 2H), 2.89 (m, 2H), 2.82 (s, 2H), 2.32 (s, 3H), 2.00-1.76 (m, 4H)。	376.4
I-30	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.95 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 7.65 (d, <i>J</i> = 4.0 Hz, 1H), 7.01 (m, 1H), 3.55 (m, 2H), 3.29 (m, 2H), 2.91 (m, 2H), 2.81 (s, 2H), 2.33 (s, 3H), 1.95-1.76 (m, 4H)。	376.4
I-31	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.10 (br, 1H), 8.92 (s, 1H), 8.70 (s, 1H), 8.41 (s, 1H), 7.60 (d, <i>J</i> = 4.0 Hz, 1H), 7.01 (d, <i>J</i> = 4.0 Hz, 1H), 3.55-3.37 (m, 4H), 2.85 (m, 2H), 2.79 (s, 2H), 2.27 (q, <i>J</i> = 6.0 Hz, 2H), 1.99-1.77 (m, 4H), 1.01 (t, <i>J</i> = 6.0 Hz, 3H)。	390.3
I-32	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.08 (br, 1H), 8.91 (s, 1H), 8.69 (s, 1H), 8.38 (s, 1H), 7.61 (d, <i>J</i> = 4.0 Hz, 1H), 7.00 (d, <i>J</i> = 4.0 Hz, 1H), 3.51-3.35 (m, 4H), 2.89 (m, 2H), 2.79 (s, 2H), 2.29 (q, <i>J</i> = 6.0 Hz, 2H), 2.02-1.77 (m, 4H), 0.98 (t, <i>J</i> = 6.0 Hz, 3H)。	390.3
I-33	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.08 (br, 1H), 8.95 (s, 1H), 8.69 (s, 1H), 8.40 (s, 1H), 7.62 (m, 1H), 7.03 (d, <i>J</i> = 8 Hz, 1H), 3.57-3.37 (m, 4H), 2.89 (m, 2H), 2.83 (s, 2H), 2.34 (t, <i>J</i> = 4.0 Hz, 2H), 2.00-1.71 (m, 6H), 0.95 (t, <i>J</i> = 6.0 Hz, 3H)。	404.1
I-34	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.07 (br, 1H), 8.99 (s, 1H), 8.69 (s, 1H), 8.38 (s, 1H), 7.61 (m, 1H), 7.01 (m, 1H), 3.55-3.01 (m, 6H), 2.81 (s, 2H), 2.35 (t, <i>J</i> = 8.0 Hz, 2H), 2.01-1.70 (m, 6H), 0.93 (t, <i>J</i> = 6.0 Hz, 3H)。	404.1
I-35	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.97 (s, 1H), 8.71 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.01 (d, <i>J</i> = 8.0 Hz, 1H), 3.58-3.35 (m, 4H), 2.88 (m, 2H), 2.81 (s, 2H), 2.01-1.73 (m, 4H), 1.15 (m, 1H), 0.80-0.65 (m, 4H)。	402.3
I-36	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.98 (s, 1H), 8.70 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.01 (m, 1H), 3.51-3.10 (m, 6H), 2.81 (s, 2H), 2.00-1.75 (m, 4H), 1.16 (m, 1H), 0.81-0.67 (m, 4H)。	402.3
I-37	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.81 (s, 1H), 8.69 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.22 (m, 1H), 7.09 (br, 1H), 3.39 (m, 2H), 3.10-2.99 (m, 8H), 2.71 (s, 3H), 2.69 (s, 2H)。	391.2
I-38	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.79 (s, 1H), 8.69 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.21 (m, 1H), 7.10 (br, 1H), 3.39 (m, 2H), 3.10-2.97 (m, 8H), 2.73 (s, 3H), 2.65 (s, 2H)。	391.2
I-39	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.77 (s, 1H), 8.68 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.21 (m, 1H), 7.08 (br, 1H), 3.37 (m, 2H), 3.09-2.97 (m, 8H), 2.63 (m, 2H), 1.85-1.79 (q, <i>J</i> = 8.0 Hz, 2H), 1.10-1.06 (t, <i>J</i> = 8.0 Hz, 3H)。	405.3
I-40	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.79 (s, 1H), 8.67 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.20 (m, 1H), 7.09 (br, 1H), 3.38 (m, 2H), 3.10-2.97 (m, 8H), 2.65 (m, 2H), 1.85-1.79 (q, <i>J</i> = 8.0 Hz, 2H), 1.11-1.07 (t, <i>J</i> = 8.0 Hz, 3H)。	405.3
I-41	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.79 (s, 1H), 8.69 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.20 (m, 1H), 7.11 (br, 1H), 3.39 (m, 2H), 3.08-2.91 (m, 9H), 2.65 (s, 2H), 0.82-0.70 (m, 4H)。	417.5
I-42	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.79 (s, 1H), 8.67 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.21 (m, 1H), 7.12 (br, 1H), 3.42 (m, 2H), 3.09-2.91 (m, 9H), 2.67 (s, 2H), 0.83-0.72 (m, 4H)。	417.5
I-43	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.77 (s, 1H), 8.65 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.21 (m, 1H), 7.07 (br, 1H), 3.61 (m, 1H), 3.42 (m, 2H), 3.10-2.89 (m, 8H), 2.67 (s, 2H), 1.85-1.60 (m, 8H)。	445.2
I-44	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.78 (s, 1H), 8.66 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.22 (m, 1H), 7.09 (br, 1H), 3.63 (m, 1H), 3.45 (m, 2H), 3.11-2.89 (m, 8H), 2.67 (s, 2H), 1.84-1.61 (m, 8H)。	445.2
I-45	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.82 (s, 1H), 8.69 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.21 (m, 1H), 7.08 (br, 1H), 3.42 (m, 2H), 3.29-3.10 (m, 6H), 2.95 (m, 2H), 2.75 (s, 3H), 2.65 (s, 2H)。	427.5
I-46	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.81 (s, 1H), 8.69 (s, 1H), 8.41 (s, 1H), 7.60 (m, 1H), 7.22 (m, 1H), 7.08 (br, 1H), 3.45 (m, 2H), 3.28-3.09 (m, 6H), 2.99 (m, 2H), 2.73 (s, 3H), 2.63 (s, 2H)。	427.5
I-47	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.10 (br, 1H), 8.81 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 7.60 (m, 1H), 7.20 (m, 1H), 7.09 (br, 1H), 3.44 (m, 2H), 3.28-3.08 (m, 8H), 2.71 (s, 2H), 1.87-1.81 (q, <i>J</i> = 8.0 Hz, 2H), 1.10-1.06 (t, <i>J</i> = 8.0 Hz, 3H)。	441.2
I-48	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.80 (s, 1H), 8.71 (s, 1H), 8.38 (s, 1H), 7.61 (m, 1H), 7.20 (m, 1H), 7.08 (br, 1H), 3.45 (m, 2H), 3.27-3.08 (m, 8H), 2.70 (s, 2H), 1.85 (m, 2H), 1.11-1.07 (t, <i>J</i> = 8.0 Hz, 3H)。	441.2
I-49	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.08 (br, 1H), 8.79 (s, 1H), 8.69 (s, 1H), 8.39 (s, 1H), 7.60 (m, 1H), 7.21 (m, 1H), 7.11 (br, 1H), 3.43 (m, 3H), 3.25-3.00 (m, 8H), 2.68 (s, 2H), 1.07 (s, 6H)。	455.4
I-50	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.79 (s, 1H), 8.68 (s, 1H), 8.37 (s, 1H), 7.61 (m, 1H),	455.4

[0108]

[0109]

	7.21 (m, 1H), 7.12 (br, 1H), 3.44 (m, 3H), 3.24-3.00 (m, 8H), 2.69 (s, 2H), 1.05 (s, 6H)。	
I-51	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.81 (s, 1H), 8.68 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.23 (m, 1H), 7.10 (br, 1H), 3.41 (m, 2H), 3.25-2.98 (m, 8H), 2.68 (s, 2H), 1.37 (m, 1H), 0.80-0.65 (m, 4H)。	453.2
I-52	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.80 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 7.61 (m, 1H), 7.21 (m, 1H), 7.13 (br, 1H), 3.44 (m, 2H), 3.26-2.98 (m, 8H), 2.67 (s, 2H), 1.38 (m, 1H), 0.81-0.65 (m, 4H)。	453.2
I-53	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.83 (s, 1H), 8.66 (s, 1H), 8.36 (s, 1H), 7.58 (m, 1H), 7.18 (m, 1H), 7.09 (br, 1H), 3.44 (m, 2H), 3.23-2.96 (m, 9H), 2.67 (m, 2H), 2.01-1.87 (m, 6H)。	467.3
I-54	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.10 (br, 1H), 8.81 (s, 1H), 8.68 (s, 1H), 8.37 (s, 1H), 7.59 (m, 1H), 7.21 (m, 1H), 7.09 (br, 1H), 3.43 (m, 2H), 3.27-2.99 (m, 9H), 2.73 (s, 2H), 2.00-1.87 (m, 6H)。	467.3
I-55	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.81(s, 1H), 8.69 (s, 1H), 8.38 (s, 1H), 7.65 (m, 1H), 7.23 (m, 1H), 7.12 (br, 1H), 3.45 (m, 2H), 3.22-2.97 (m, 8H), 2.65 (m, 3H), 1.87-1.59 (m, 8H)。	481.6
I-56	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.80(s, 1H), 8.68 (s, 1H), 8.39 (s, 1H), 7.67 (m, 1H), 7.24 (m, 1H), 7.10 (br, 1H), 3.40 (m, 2H), 3.21-2.97 (m, 8H), 2.66 (m, 3H), 1.88-1.59 (m, 8H)。	481.6
I-57	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.80(s, 1H), 8.69 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.20 (m, 1H), 7.10 (br, 1H), 3.44 (m, 2H), 3.20-2.97 (m, 9H), 2.66 (s, 2H), 1.47-1.29 (m, 10H)。	495.6
I-58	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.80(s, 1H), 8.68 (s, 1H), 8.36 (s, 1H), 7.60 (m, 1H), 7.18 (m, 1H), 7.08 (br, 1H), 3.45 (m, 2H), 3.190-2.95 (m, 9H), 2.65 (s, 2H), 1.50-1.29 (m, 10H)。	495.6
I-59	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.90 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 7.61 (m, 1H), 7.05 (m, 1H), 3.45 (q, <i>J</i> = 9.6 Hz, 2H), 3.10-2.89 (m, 8H), 2.00-1.79 (m, 6H), 1.21 (t, <i>J</i> = 9.2Hz, 3H)。	440.4
I-60	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.91 (s, 1H), 8.71 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.09 (m, 1H), 3.46 (q, <i>J</i> = 9.6 Hz, 2H), 3.10-2.95 (m, 8H), 2.00-1.82 (m, 6H), 1.22 (t, <i>J</i> = 9.2Hz, 3H)。	440.4
I-61	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.92 (s, 1H), 8.67 (s, 1H), 8.39 (s, 1H), 7.60 (m, 1H), 7.08 (m, 1H), 3.47 (q, <i>J</i> = 8.0 Hz, 2H), 3.11-2.88 (m, 8H), 1.23 (t, <i>J</i> = 8.0Hz, 3H)。	398.4
I-62	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.14 (br, 1H), 8.90 (s, 1H), 8.69 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.09 (m, 1H), 3.46 (q, <i>J</i> = 8.0 Hz, 2H), 3.10-2.88 (m, 8H), 1.24 (t, <i>J</i> = 8.0Hz, 3H)。	398.4
I-63	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.93 (s, 1H), 8.69 (s, 1H), 8.35 (s, 1H), 7.60 (m, 1H), 7.09 (m, 1H), 3.47 (q, <i>J</i> = 8.0 Hz, 2H), 3.08-2.81 (m, 8H), 2.02-1.79 (m, 6H), 1.24 (t, <i>J</i> = 8.0Hz, 3H)。	440.4
I-64	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.11 (br, 1H), 8.91 (s, 1H), 8.68 (s, 1H), 8.37 (s, 1H), 7.60 (m, 1H), 7.12 (m, 1H), 3.46 (q, <i>J</i> = 8.0 Hz, 2H), 3.07-2.85 (m, 8H), 2.03-1.81 (m, 6H), 1.25 (t, <i>J</i> = 8.0Hz, 3H)。	440.4
I-65	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.12 (br, 1H), 8.79 (s, 1H), 8.66 (s, 1H), 8.37 (s, 1H), 7.60 (m, 1H), 7.09 (m, 1H), 3.45 (m, 2H), 3.21-3.11 (m, 8H), 2.69 (s, 2H), 2.10-1.89 (m, 8H)。	431.5
I-66	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.13 (br, 1H), 8.81 (s, 1H), 8.67 (s, 1H), 8.38 (s, 1H), 7.60 (m, 1H), 7.12 (m, 1H), 3.47 (m, 2H), 3.20-3.11 (m, 8H), 2.68 (s, 2H), 2.08-1.85 (m, 8H)。	431.5
I-67	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.10 (br, 1H), 8.86 (s, 1H), 8.68 (s, 1H), 8.39 (s, 1H), 7.61 (m, 1H), 7.12 (m, 1H), 3.25-3.07 (m, 10H), 2.69 (s, 2H), 2.03-1.81 (m, 10H)。	481.6
I-68	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ: 12.09 (br, 1H), 8.85 (s, 1H), 8.69 (s, 1H), 8.37 (s, 1H), 7.60 (m, 1H), 7.09 (m, 1H), 3.24-3.07 (m, 10H), 2.68 (s, 2H), 2.01-1.80 (m, 10H)。	481.6

[0110] 生物测试

[0111] 测试例1、JAK1、JAK2、JAK3、TYK2活性测试

[0112] 化合物配制:

[0113] 化合物溶解在100%DMSO中,配制成10mM储存液,-20℃冻存。

[0114] 激酶反应过程:

[0115] (1) 配制1×Kinase buffer。

[0116] (2) 化合物浓度梯度的配制:受试化合物起始浓度为500nM,在384source板中稀释成100倍终浓度的100%DMSO溶液,用Precision 3倍稀释化合物,12个浓度。使用分液器Echo 550向目的板OptiPlate-384F转移250nL 100倍终浓度的化合物。

[0117] (3) 用1×Kinase buffer配制2.5倍终浓度的激酶溶液。

[0118] (4) 在化合物孔和阳性对照孔分别加10μL的2.5倍终浓度的激酶溶液;在阴性对照孔中加10μL的1×Kinase buffer。

[0119] (5) 1000rpm离心30秒,反应板振荡混匀后室温孵育10分钟。

[0120] (6) 用1×Kinase buffer配制5/3倍终浓度的ATP和Kinase substrate的混合溶液。

[0121] (7) 加入15μL的5/3倍终浓度的ATP和底物的混合溶液,起始反应。

[0122] (8) 将384孔板1000rpm离心30秒,振荡混匀后室温孵育相应的时间。

[0123] (9) 加入30μL终止检测液停止激酶反应,1000rpm离心30秒,振荡混匀。

[0124] (10) 用Caliper EZ Reader读取转化率。

[0125] 数据分析:

[0126] 计算公式:

$$[0127] \quad \% \text{ Inhibition} = \frac{\text{Conversion\%}_{\text{max}} - \text{Conversion\%}_{\text{sample}}}{\text{Conversion\%}_{\text{max}} - \text{Conversion\%}_{\text{min}}} \times 100$$

[0128] 其中:Conversion%_sample是样品的转化率读数;Conversion%_min:阴性对照孔均值,代表没有酶活孔的转化率读数;Conversion%_max:阳性对照孔比值均值,代表没有化合物抑制孔的转化率读数。

[0129] 拟合量效曲线:

[0130] 以浓度的log值作为X轴,百分比抑制率为Y轴,采用分析软件GraphPad Prism 5的log(inhibitor) vs. response-Variable slope拟合量效曲线,从而得出各个化合物对酶活性的IC50值,计算公式:

$$[0131] \quad Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogIC50} - X) * \text{HillSlope})})$$

[0132] 上述实验结果如表6所示。

[0133] 表6、化合物酶实验测试结果:

[0134]

化合物	JAK1 (nM)	JAK2 (nM)	JAK3 (nM)	TYK2 (nM)	JAK1/JAK2	JAK3/JAK2	TYK2/JAK2
Baricitinib	6.2	5.9	10	12	1.1	1.7	2.0
Ruxolitinib	2.8	2.5	7	5.6	1.1	2.8	2.2
I-1	4.8	0.6	13	15	8	21.7	25
I-2	0.25	0.03	0.84	5.5	8.3	28	183
I-3	5.5	0.7	13	25	7.9	18.6	35.7
I-4	0.26	0.04	0.76	1.5	6.5	19	37.5
I-5	5.3	1.3	23	35	4.1	17.7	26.9
I-6	0.56	0.03	1.2	1.5	18.6	40	50
I-7	7.7	1.7	27	39	4.5	15.9	22.9
I-8	1.3	0.18	2.5	5.7	7.2	13.9	31.7
I-9	6.0	1.3	15	29	4.6	11.5	22.3
I-10	1.5	0.2	2.9	4	7.5	14.5	20
I-11	6.5	1.3	19	27	5	14.6	20.8
I-12	0.8	0.1	1.9	2.0	8	19	20
I-13	5.7	1.2	12	18	4.75	10	15
I-14	0.6	0.1	1.6	2.1	6	16	21
I-15	8.9	2.1	12	23	4.2	5.7	10.9
I-16	4.5	1.0	10	12	4.5	10	12
I-17	3.5	0.7	10	17	5	14.3	24.3
I-18	0.7	0.05	0.8	1.2	14	16	24
I-19	5.6	0.7	10	12	8	14.3	17.1
I-20	1	0.1	1.9	3.4	10	19	34
I-21	7.2	1.8	12	24	4	6.7	13.3
I-22	1.5	0.3	2.9	4.0	5	9.7	13.3
I-23	10.7	2.7	25	49	4	9.3	18.1
I-24	4.6	1.0	12	9	4.6	12	9
I-25	4.6	1	12	33	4.6	12	33
I-26	2	0.5	5	6	4	10	12
I-27	7	1.5	12	34	4.7	8	22.7
I-28	1.4	0.3	3	4.1	4.7	10	13.7
I-29	4.8	1.2	8	9.8	4	6.7	8.2
I-30	0.8	0.1	1	2	8	10	20
I-31	9.3	2.1	12	29	4.4	5.7	13.8
I-32	2.3	0.5	3	7	4.6	6	14
I-33	5.6	1.3	17	26	4.3	13.1	20
I-34	1.0	0.2	2.1	3.1	5	10.5	15.5
I-35	6.5	1.5	15	18	4.3	10	12
I-36	1.6	0.3	3.1	5.7	5.3	10.3	19
I-37	3.7	0.8	11	33	4.6	13.8	41.3
I-38	0.5	0.03	0.8	1.0	16.7	26.7	33.3
I-39	4.6	1.1	9.8	13.6	14.8	8.9	12.4
I-40	0.8	0.1	1.3	4.6	8	13	46
I-41	6.1	1.5	12.7	36.9	4.1	8.5	24.6
I-42	1.0	0.2	2.3	3.6	5	11.5	18

I-43	4.7	1.1	9.7	7.9	4.3	8.8	7.2
I-44	0.8	0.07	1.5	1.7	11.4	21.4	24.3
I-45	6.7	1.3	32	69	5.2	24.6	53
I-46	0.9	0.09	49	103	10	544	1144
I-47	4.0	0.9	12	31	4.4	13.3	34.4
I-48	0.87	0.1	1.9	3.1	8.7	19	31
I-49	5.5	1.3	12.7	35.9	4.2	9.8	27.6
I-50	0.9	0.1	1.9	2.7	9	19	27
I-51	7.4	1.5	21	41	4.9	14	27.3
I-52	1.8	0.2	2.5	20	9	12.5	100
I-53	4.6	1.0	15.3	26.9	4.6	15.3	26.9
I-54	0.7	0.05	1.2	3.9	14	24	78
I-55	10.3	2.1	19.5	35.9	4.9	9.3	17.1
I-56	4.6	1.0	8.3	12.1	4.6	8.3	12.1
I-57	5.7	1.2	13	43	4.8	10.8	35.8
I-58	0.9	0.1	1.4	1.3	9	14	13
I-59	5.8	1.0	15	25	5.8	15	25
I-60	0.3	0.05	0.89	2.5	6	17.8	50
I-61	4.8	0.9	12.5	19.7	5.3	13.9	21.9
I-62	0.35	0.06	0.97	1.5	5.8	16.2	25
I-63	4.5	1.1	15.6	21.3	4.1	14.2	19.4
I-64	0.29	0.04	1.1	2.0	7.3	27.5	50
I-65	12.5	2.1	13	9.7	6	6.2	4.6
I-66	0.9	0.2	4.5	3.7	4.5	22.5	18.5
I-67	13	2.5	15.2	9.9	5.2	6.1	4
I-68	1.2	0.3	1.9	1.3	4	6.3	4.3

[0136] 注：以上对照均品、本发明化合物均为同一实验条件实测值。

[0137] 结论：本发明化合物对JAK2抑制活性及靶点选择性优于阳性对照Baricitinib、Ruxolitinib。

[0138] 测试例2、细胞增殖实验

[0139] HEL92.1.7细胞增殖实验

[0140] 实验步骤：

[0141] (1) 铺板

[0142] a. 将细胞消化重悬，使用自动细胞计数器计数。

[0143] b. 将细胞悬浮液稀释至所需密度。

[0144] c. 每个孔铺100u1细胞，37℃培养过夜。

[0145] (2) 化合物配制

[0146] a. 将化合物配成终浓度200倍的稀释溶液。

[0147] b. 用培养基稀释化合物，配成终浓度3倍的化合物。每孔加50u1化合物，以加入同体积的DMSO的孔作为对照，37℃，5%CO₂培养72小时。

[0148] (3) 检测

[0149] a. 将细胞板平衡到室温。

[0150] b. 每孔加40μL Cell Titer-Glo®试剂，振2分钟，静置10分钟，用EnVision检测。

[0151] 数据分析：

[0152] (1) 使用GraphPad Prism 5计算IC₅₀。

[0153] (2) %Inh = (Max signal - Compound signal) / (Max signal - Min signal) x 100。

[0154] (3) Max signal为阳性对照孔，只有和化合物同等体积的DMSO。

[0155] (4) Min signal为阴性对照孔，只有培养基。

[0156] TF-1细胞增殖实验

[0157] (1) 细胞铺板

- [0158] a. 配制完全培养基。
- [0159] b. 复苏细胞, 培养细胞。
- [0160] c. 细胞离心, 重悬, 计数, 铺板, 将培养板放置于CO₂培养箱中过夜。
- [0161] (2) 化合物的准备和添加
- [0162] a. 用DMSO将化合物配制成10mM的stock储存液, 将10mM稀释成工作浓度, 逐步倍数稀释, 得到多个浓度梯度的化合物。
- [0163] b. 从相应的化合物板中移取0.5ul加入过夜培养的细胞培养板中。
- [0164] c. 在37°C培养箱中孵育72小时。
- [0165] (3) 检测及分析
- [0166] a. 配制CellTiter Glo assay检测试剂。
- [0167] b. 将检测试剂加入培养板中, 混匀, 静置, 读板。
- [0168] 抑制率公式为 $(1 - (\text{对应孔的数值} - \text{BLANK的平均值}) / (\text{DMSO对照的平均值} - \text{BLANK的平均值})) * 100\%$
- [0169] 曲线拟合工具 (XL fit) 公式为 Data Analysis: (XLfit software: Fit model: Dose response one site/
 $f(x) = 205 [fit = (A + ((B - A) / (1 + ((C/x)^D)))]$)
- [0170] 上述实验结果如表7所示。
- [0171] 表7、细胞增殖实验测试结果:

[0172]

化合物	HEL92.1.7 (μM)	TF-1 (μM)	化合物	HEL92.1.7 (μM)	TF-1 (μM)
Baricitinib	1.25	4.75	Ruxolitinib	0.35	3.25
I-1	0.13	1.76	I-2	0.05	0.93
I-3	0.12	1.57	I-4	0.06	0.89
I-5	0.25	2.73	I-6	0.13	1.62
I-7	0.26	1.97	I-8	0.18	1.17
I-9	0.24	2.13	I-10	0.20	1.54
I-11	0.18	2.76	I-12	0.10	1.99
I-13	0.27	1.32	I-14	0.09	0.98
I-15	0.22	1.68	I-16	0.10	1.01
I-17	0.15	1.67	I-18	0.08	0.99
I-19	0.23	1.25	I-20	0.05	0.89
I-21	0.30	2.89	I-22	0.17	1.50
I-23	0.32	2.89	I-24	0.20	2.13
I-25	0.33	3.02	I-26	0.22	2.45
I-27	0.29	2.19	I-28	0.15	1.23
I-29	0.28	1.94	I-30	0.15	1.23
I-31	0.19	2.03	I-32	0.13	1.05
I-33	0.34	2.97	I-34	0.15	1.32
I-35	0.30	2.09	I-36	0.12	1.12
I-37	0.28	1.59	I-38	0.09	1.01
I-39	0.22	1.29	I-40	0.10	0.98
I-41	0.26	1.79	I-42	0.08	0.89
I-43	0.31	2.67	I-44	0.21	1.25
I-45	0.17	1.76	I-46	0.08	0.97
I-47	0.12	2.03	I-48	0.06	1.02
I-49	0.21	2.58	I-50	0.10	1.23
I-51	0.20	1.42	I-52	0.08	0.89
I-53	0.32	2.09	I-54	0.15	1.05
I-55	0.28	2.25	I-56	0.07	0.78
I-57	0.31	2.89	I-58	0.25	1.78
I-59	0.15	1.42	I-60	0.05	0.87
I-61	0.12	1.25	I-62	0.06	0.79
I-63	0.18	1.21	I-64	0.07	1.00

[0173]

I-65	0.29	2.13	I-66	0.15	1.45
I-67	0.32	3.15	I-68	0.16	1.52

[0174] 注:以上对照均品、本发明化合物均为同一实验条件实测值。

[0175] 结论:本发明化合物对HEL92.1.7、TF-1具有明显的增殖抑制活性,抑制活性优于Baricitinib、Ruxolitinib。

[0176] 测试例3、本发明化合物药代动力学测试

[0177] 以SD大鼠为受试动物,采用LC/MS/MS法测定大鼠灌胃给予Baricitinib、Ruxolitinib和本发明优选实施例化合物后,测定其不同时刻血浆中的药物浓度,研究本发

明化合物在大鼠体内药代动力学特征。

[0178] SD大鼠来源:北京维通利华实验动物技术有限公司

[0179] 给药方式:单次灌胃给药

[0180] 给药剂量及浓度:25mg/kg;1mg/mL

[0181] 制剂处方:0.5% methylcellulose

[0182] 取样点:5min,15min,30min,1h,2h,4h,8h,24h.

[0183] 标准曲线和质控样本配制处理:取适量储备液用50%乙腈水稀释成0.04、0.10、0.20、0.40、1.00、2.00、4.00 $\mu\text{g}/\text{mL}$ 的标准工作液,0.10、1.00、3.00 $\mu\text{g}/\text{mL}$ 的质控工作液。分别取47.5 μL 空白大鼠血浆中加入2.50 μL 的标准曲线工作液和质控工作液,配置成含待测物浓度为2.00、5.00、10.00、20.00、50.00、100.00、200.00 ng/mL 的标曲和浓度为5.00、50.00和150.00 ng/mL 的质控样本,分别加入200 μL 的乙腈(含内标氯雷他定5 ng/mL),涡旋振荡3min后,15000rpm,4 $^{\circ}\text{C}$ 离心15min,取上清液100L进行LC-MS/MS分析。采用WinNonlin $\text{\textcircled{R}}$ 8.0计算实验结果。

[0184] 本发明优选化合物药代动力学参数如表8所示。

[0185] 表8:优选化合物药代动力学参数

化合物	药代实验 (25mg/kg)				
	达峰时间	血药浓度	曲线面积	半衰期	滞留时间
	T_{\max} (h)	C_{\max} (ng/mL)	AUC_{last} (h*ng/mL)	$t_{1/2}$ (h)	MRT (h)
Baricitinib	0.5	550	559	4.8	1.8
Ruxolitinib	0.25	569	462	4.5	1.5
I-1	0.25	798	910	5.6	1.4
I-2	0.25	1090	1233	6.8	1.3
I-3	0.5	890	940	6.3	1.5
I-4	0.25	850	945	6.9	1.4
I-13	0.25	769	955	7.2	1.6
I-14	0.5	1023	1053	5.9	1.7
I-19	0.5	735	923	6.5	1.2
I-20	0.5	893	1025	6.4	1.4
I-37	0.25	798	997	7.0	1.3
I-38	0.25	801	1000	7.2	1.9
I-45	0.5	1020	1237	6.3	1.3
I-46	0.25	933	1040	5.8	1.7
I-47	0.25	799	923	6.0	1.6
I-48	0.25	823	1024	6.1	1.5
I-59	0.5	1022	1235	5.7	1.4
I-60	0.5	1079	1268	5.6	1.3

[0186] [0187] 结论:本发明实施例化合物表现出良好的药代动力学性质,与Baricitinib、Ruxolitinib相比,具有明显的药代动力学优势。

[0188] 以上所述仅为本发明的较佳实施例而已,并不用以限制本发明,凡在本发明的精神和原则之内,所作的任何修改、等同替换、改进等,均应包含在本发明的保护范围之内。