

PHENYL TRIAZOLE MLL1-WDR5 PROTEIN-PROTEIN INTERACTION INHIBITOR

Technical field

[0001] The invention is in the medicinal chemistry field, more specifically, is for a class of phenyl triazole MLL1-WDR5 protein-protein interaction inhibitor, its preparation and pharmaceutical uses.

Technical background

[0002] Methylation of histones plays key roles in many biological processes and is the main content of study in epigenetic regulation field. Translocation and re-arrangement of the methyl transferase MLL1 gene for histone H3K4 can lead to mixed lineage leukemia (MLL1, acute myeloid leukemia and acute lymphoid leukemia). About 10% of leukemia patients have MLL1 gene rearrangement. After MLL1 gene arrangement, it fuses with other chaperone proteins to form fusion proteins, and the carcinogenic MLL fusion protein is expressed. The fusion protein can interact with RNA polymerase II (Pol II) related elongation factors to form the super elongation complex (SEC). The complex can lead to abnormal expression of the MLL1 regulated Hox gene, which causes series of serious consequences to induce MLL leukemia onset.

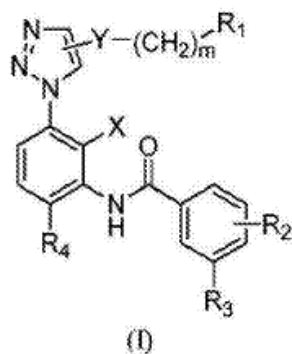
[0003] Rearrangement of the MLL1 gene occur on one allele and the wildtype MLL1 is still present. When the wildtype MLL1 allele is knocked out, the MLL fusion protein alone will not lead to leukemia, and the enzyme activity of the wildtype MLL1 is necessary for the MLL1 fusion protein to cause leukemia. Thus, specific inhibition of the wildtype MLL1 enzyme activity will cure leukemia.

[0004] Catalytic activity on H3K4 methylation by MLL1 alone is very weak and can only result in monomethylation; the catalytic activity improves greatly upon the formation of the MLL1 core catalytic complex, especially the catalytic activity on H3K4me2. The WIN motif on the C-terminus of the MLL binds to WDR5, RbBP5, Ash2L and DPY30 to form complexes. MLL1 interacts with WDR5 directly through the C-terminus WIN motif, to mediate the interaction between the catalytic domain of MLL1SET and other protein complexes. When WDR5 is knocked out, the level of H3K4me2/3 decreases and the Hox gene expression level decreases.

[0005] Thus, the use of small molecule to interfere the protein-protein interaction of MLL1-WDR5 is an effective method to inhibit MLL1 enzymatic activity and lower Hox and Meis-1 gene expression levels and to block the progression of leukemia.

Summary

[0005a] In one aspect, the present disclosure refers to a compound of the formula (I) or a pharmaceutically acceptable salt thereof:



wherein X is hydrogen, methyl, methoxy or halogen;

Y is $-\text{CH}_2-$, $-\text{O}-$, $-\text{S}-$, $-\text{CO}-$, $-\text{CH}_2\text{O}-$, $-\text{NR}_5-$, $-\text{CONR}_6-$ or $-\text{NR}_7\text{CO}-$, wherein R_5 , R_6 , or R_7 each independently is hydrogen, C_1 - C_4 alkyl, C_1 - C_4 haloalkyl, phenyl or substituted phenyl, the substituent is halogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, amino, hydroxyl, thiol, carboxyl, cyano, trifluoromethyl or imidazolyl;

m is 0 - 6;

R_1 is hydrogen, amino, hydroxyl, thiol, carboxyl, cyano, $-\text{CONH}_2$, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, phenyl, substituted phenyl, substituted or unsubstituted nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring, $-\text{NR}_8\text{COR}_9$, $-\text{CONR}_{10}\text{R}_{11}$ or $-\text{NR}_{10}\text{R}_{11}$, wherein R_8 is hydrogen, C_1 - C_4 alkyl, C_1 - C_4 haloalkyl, phenyl or substituted phenyl, R_9 is amino, hydroxyl, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, phenyl or substituted phenyl, substituted or unsubstituted nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring, R_{10} and R_{11} are independently hydrogen, C_1 - C_4 alkyl, phenyl or substituted phenyl, substituted or unsubstituted nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring, or R_{10} and R_{11} are bonded to form nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring, wherein the substituent is halogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, amino, hydroxyl, thiol, carboxyl, cyano, trifluoromethyl or imidazolyl;

R_2 represents two or three substituents selected from halogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, trifluoromethyl, nitro and cyano;

R_3 is amino, methylamino, aminomethyl, hydroxyl, hydroxymethyl, thiol or $-\text{CONH}_2$;

R_4 is N-methylpiperazine, 1,2-dimethyl piperazine or N-methylhomopiperazine.

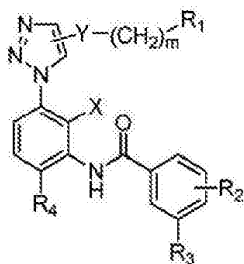
[0005b] In another aspect, the present disclosure refers to a pharmaceutical composition comprising a compound disclosed herein or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carrier.

[0005c] In yet another aspect, the present disclosure refers to a use of a compound disclosed herein or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of acute leukemia.

[0005d] In a further aspect, the present disclosure refers to a compound disclosed herein or a pharmaceutically acceptable salt thereof for use in treating acute leukemia.

Content of the invention

[0006] This invention publishes a small molecule compound that regulates MLL1-WDR5 protein-protein interactions, through interference of which, it inhibits the enzymatic activities of catalysis, down-regulates the methylation levels of H3K4, and gene expression levels of Hox and Meis-1 genes, which in turn to induce the apoptosis of leukemia cells for the applications of leukemia treatment. The structure of the compound in this invention is:



[0007]

(I)

[0008] Wherein, X is hydrogen, methyl, methoxy or halogen groups;

[0009] Y is $-\text{CH}_2-$, $-\text{O}-$, $-\text{S}-$, $-\text{CO}-$, $-\text{CH}_2\text{O}-$, $-\text{NR}_5-$, $-\text{CONR}_6-$ or $-\text{NR}_7\text{CO}-$, wherein, R_5 , R_6 and R_7 are independently hydrogen, C1-C4 alkyl, C1-C4 substituted alkyl, phenyl or substituted phenyl, substituted groups are halogen, C1-C4 alkyl, C1-C4 alkoxy, amino, hydroxy, decyl, carboxy, cyano, trifluoromethyl or imidazolyl groups;

[0010] m is 0~6;

[0011] R_1 is hydrogen, amino, hydroxy, decyl, carboxy, cyano, $-\text{CONH}_2$, C1-C4 alkyl, C1-C4 alkoxy, phenyl, substituted phenyl, substituted or unsubstituted nitrogen containing or oxygen containing 3~7 membered heterocyclic, $-\text{NR}_8\text{COR}_9$, $-\text{CONR}_{10}\text{R}_{11}$ or $-\text{NR}_{10}\text{R}_{11}$ groups, wherein R_8 is hydrogen, C1-C4 alkyl, C1-C4 haloalkyl, phenyl or substituted phenyl, R_9 is amino, hydroxyl, C1-C4 alkyl, C1-C4 alkoxy, phenyl or substituted phenyl, substituted or unsubstituted nitrogen containing or oxygen containing 3~7 membered heterocyclic, R_{10} and R_{11} independently represent hydrogen, C1-C4 alkyl, phenyl or substituted phenyl, substituted or unsubstituted nitrogen containing or oxygen containing 3~7 membered heterocyclic groups or nitrogen or oxygen containing 3~7 membered heterocyclic ring formed by linking R_{10} and R_{11} , the said substitution groups are halogen, C1-C4 alkyl, C1-C4 alkoxy, amino, hydroxy, decyl, carboxy, cyano, trifluoromethyl or imidazolyl groups;

[0012] R_2 represents disubstituted or trisubstituted halogen, C1-C4 alkyl, C1-C4 alkoxy, trifluoromethyl, nitro or cyano;

[0013] R_3 represents amino, methylamino, aminomethyl, hydroxy, hydroxymethyl, decyl or $-\text{CONH}_2$;

[0014] R_4 represents N-methylpiperazine, 1,2-dimethyl piperazine or N-methylhomopiperazine.

[0015] X is preferentially hydrogen, fluorine, chlorine or alkyl groups.

[0016] Y is preferentially $-\text{NR}_5-$, CONR_6- or $-\text{NR}_7\text{CO}-$; R_5 , R_6 and R_7 are independently hydrogen, alkyl, ethyl, propyl, cyclopropyl or isopropyl groups.

[0017] Y is also preferentially $-\text{NR}_5-$, $-\text{CONR}_6-$ or $-\text{NR}_7\text{CO}-$; R_5 , R_6 and R_7 are independently substituted phenyl group, wherein the substitutions are methyl, ethyl, isopropyl, tert-butyl, cyclopropyl, methoxy, cyano, halogen, trifluoromethyl or imidazolyl.

[0018] The said substituted or unsubstituted nitrogen containing or oxygen containing 3~7 membered heterocyclic groups are preferentially azacyclopropyl, azacycline, tetrahydropyrrole, piperidine, cycloheximide, lactam, tetrahydrofuran, tetrahydropyran, morpholine, 1,4-oxazinidine, hexahydropyridazine, imidazoline, imidazolium, piperazine, the substituent is halogen, methyl, ethyl, phenyl, hydroxy, amino, hydroxymethyl or aminomethyl.

[0019] R_1 preferentially represents $-\text{NR}_8\text{COR}_9$, $-\text{CONR}_{10}\text{R}_{11}$ or $-\text{NR}_{10}\text{R}_{11}$, while R_8 , R_9 , R_{10} and R_{11} represents C1-C4 alkyl groups.

[0020] R₂ preferentially represents trisubstitutions, where the substitutions are fluorine, chlorine, bromine, methyl, methoxy, nitro, trifluoromethyl or cyano groups.

[0021] The invention also includes pharmaceutically acceptable salts of compound (I) and its solvates, which all have the same pharmacological functions as that of compound (I).

- 5 [0022] The invention publishes a drug combination, which includes compound (I) or its pharmaceutically usable salts, or solvates, and one or more pharmaceutically usable carriers, diluents and excipients.

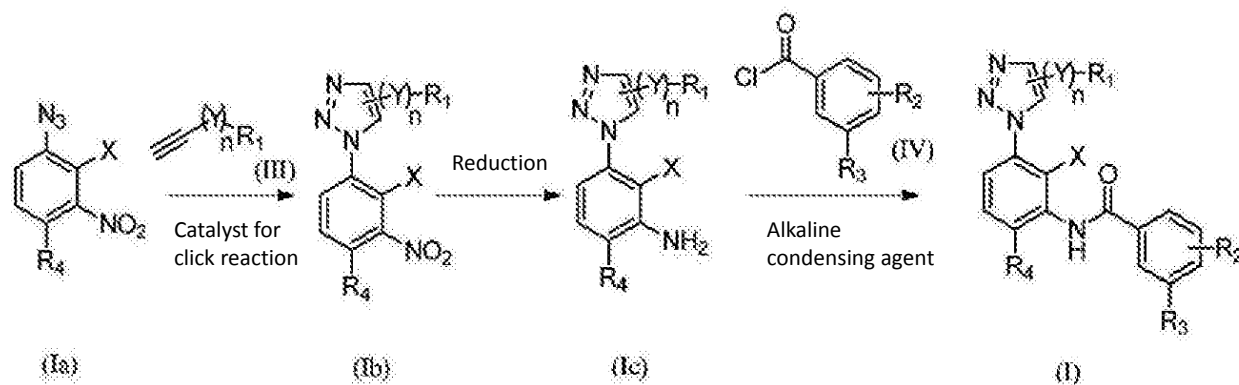
[0023] The invention also provides applications of using compound (I) or its pharmaceutically usable salts or solvates to prepare drugs to treat diseases mediated by the enzyme through inhibiting MLL1-WDR5 protein-protein interaction, the said diseases are such as MLL gene fusion type leukemia that can be treated through inhibition of MLL1 enzymatic activities.

[0024] Dosage of the compound in this invention used clinically is 0.01mg~1000mg/day, which can deviate from the range according to severity of diseases or different formulation.

15 [0025] In some of the preferred applications, compounds based on compound (I) can contain sufficient basic groups to form salts. Representative salts include inorganic acid salts, organic acid salts, hydrobromide and sulfuric acid salts; pharmaceutical use organic salts, including acetate, trifluoroacetate, lactate, succinate, fumarate, maleate, citrate, methanesulfonate, p-benzoate and p-toluenesulfonate salts.

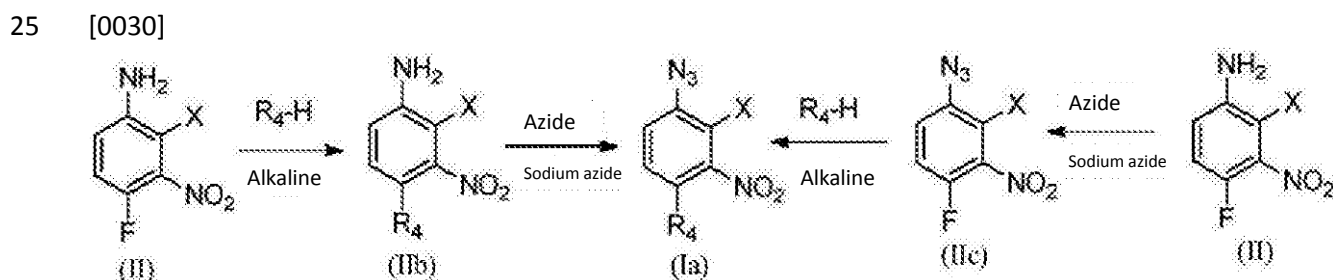
[0026] In the meantime, the invention also publishes the preparation methods of compounds related to compound (I), including the following steps:

[0027]



[0028] Wherein R₁, R₂, R₃, R₄, X, Y and n are as defined previously;

[0029] Wherein the intermediate Ia can be obtained through the following synthesis route,



[0031] The following are some of the pharmacodynamic tests and results of the compound in the invention:

[0032] MLL1 enzymatic activities are determined by MLL1 and WDR5 protein-protein interactions; MLL1 enzymatic activities affect the acetylation levels of H3K4. The H3K4 acetylation levels increase abnormally in MLL fusion type leukemia, while the downstream Hox and Meis-1 gene expression levels increase abnormally. When MLL1-WDR5 protein-protein interactions are inhibited, MLL1 catalytic activities decrease, H3K4 acetylation levels decrease, Hox and Meis-1 expression levels decrease, (which) inhibit leukemia cell proliferation.

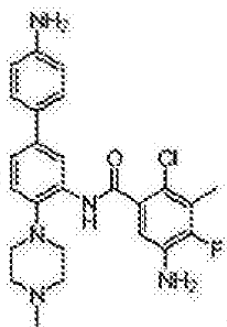
[0033] Biphenyl compound DDO-2084 was already reported to be able to inhibit MLL1-WDR5 protein-protein interactions, lower MLL1 catalytic activities, downregulate small molecule inhibitor expressions from Hox and Meis-1 genes (Eur. J. Med. Chem. 2016, 124, 480-489.), wherein DDO-2084 was the compound used as positive control.

[0034] Table 1 Inhibition activities of the compounds in this invention over MLL1-WDR5 protein-protein interactions and related biological activities

[0035]

Compound numbering in application examples ^a	MLL1-WDR5 PPI inhibition activity (nM)	Inhibition on H3K4 acetylation level	Downregulation of Hox and Meis-1 expression
1	<80	Yes	Yes
2	<50	Yes	Yes
3	<10	Yes	Yes
4	<10	Yes	Yes
5	<10	Yes	Yes
6	<10	Yes	Yes
7	<10	Yes	Yes
8	<10	Yes	Yes
9	<50	Yes	Yes
10	<10	Yes	Yes
12	<80	Yes	Yes
13	<80	Yes	Yes
14	<10	Yes	Yes
15	<10	Yes	Yes
16	<10	Yes	Yes
17	<50	Yes	Yes
18	<50	Yes	Yes
19	<50	Yes	Yes
21	<80	Yes	Yes
22	<10	Yes	Yes
23	<50	Yes	Yes
24	<50	Yes	Yes
25	<50	Yes	Yes
DDO-2084 ^b	88.7±4.9	Yes	Yes

[0036] ^a Refer to application examples for specific compound structures; ^b Structure of DDO-2084:

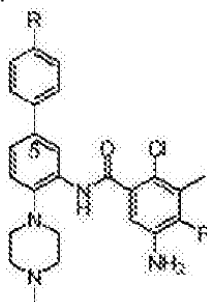


[0037] As shown in table 1, the compounds in this invention have relatively strong inhibitory activities on MLL1-WDR5 protein-protein interactions.

5 [0038] In the meantime, RT-PCR experiments at cellular level were conducted with some of the compounds in the invention, with results listed in table 1 on whether some of the compounds inhibited downstream Hox and Meis-1 gene expression levels. Results showed that all compounds in the invention with inhibitory activities on MLL1-WDR5 protein-protein interactions can all inhibit downstream Hox and Meis-1 gene expressions. Inhibition results at the cellular levels of some of the
10 compounds on the downstream Hox and Meis-1 expression levels were plotted in figure 1, wherein figure 1 showed, the inhibition level of the compound in application example 7 at 2.5 μ M reached the same level as that of the positive control DDO-2084 at 5 μ M, while the effects of that from the application example 7 were better than that of DDO-2084 at the same 5 μ M.

[0039] In addition, Western-blot experiments at cellular level were conducted with some of the
15 compounds in the invention, with results listed in table 1 on whether some of the compounds in the invention inhibited H3K4 acetylation levels. Results showed that all compounds in the invention with inhibitory activities on MLL1-WDR5 protein-protein interactions all downregulated H3K4 acetylation levels. The inhibition on MLL1 catalytic activities at the cellular level for some of the compounds was plotted in figure 2. As figure 2 shows, application example 7 can inhibit MLL1 catalytic activities in a
20 dose-dependent manner to reduce the expression levels of H3K4me1/2/3, and it is showed that the results from application example 7 was better than that of DDO-2084 at the same 10 μ M concentration.

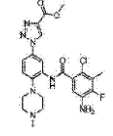
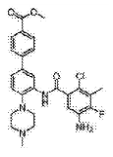
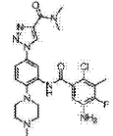
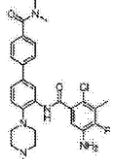
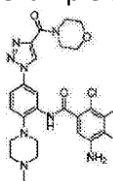
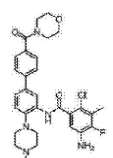
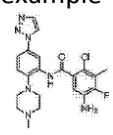
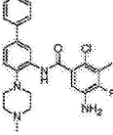
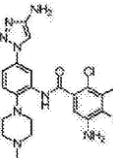
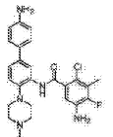
[0040] In addition, published paper (Eur. J. Med. Chem. 2016, 124, 480-489. Referred to as paper 1 below) has reported series of biphenyl inhibitors on MLL1-WDR5 protein-protein interactions, with



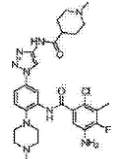
structures of: , while the compounds in this invention differ from them by changing
25 the structures on how the two benzene rings are linked, wherein position 5 is linked by triazole groups, such changes increased the water solubility significantly of the compounds in this invention, reduced the toxicity of the compounds but retained the original MLL1-WDR5 inhibitory activities. We have chosen some of the biphenyl compounds from paper 1 and some of the un-reported biphenyl compounds for solubility and toxicity tests using the same testing methods in this invention, and the
30 results are below:

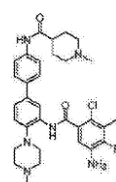
[0041] Table 2 Comparison of target activity and solubility of some of the compounds in this invention and some of the compounds in paper 1

[0042]

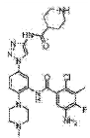
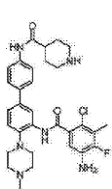
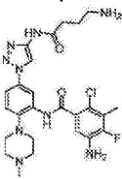
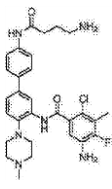
Compound number and structure of this invention	MLL1-WDR5 PP1 inhibition activity (nM)	Solubility (pH=7.4) $\mu\text{g/mL}$	Structures of some compounds from publication (Eur. J. Med. Chem. 2016, 124, 480-489.)	MLL1-WDR5 PP1 inhibition activity (nM)	Solubility (pH=7.4) $\mu\text{g/mL}$
 Application example 1	67.8	20		70.0	1.9
 Application example 3	33.9	170		73.0	21
 Application example 4	11.2	200		55.6	27
 Application example 11	65.7	30		47.9	3.1
 Application example 13	90.3	110		88.7	9.5

[0043]

 Application example 14	6.0	630
---	-----	-----



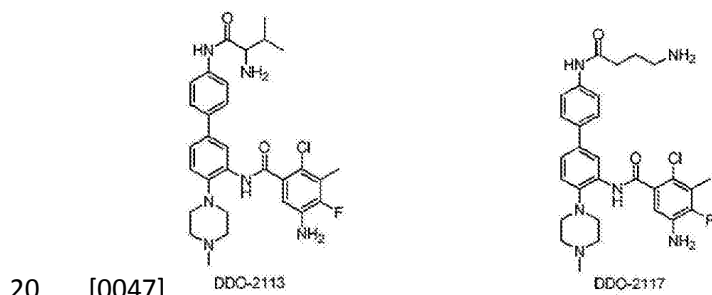
6.5 54

 Application example 15	12.3	1235		8.5	125
 Application example 16	6.7	1303		7.6	110

[0044] It is noted from the comparison of data in table 2 for the compounds that, when the other groups remained the same, replacement of the benzene rings by triazole groups in this invention retained targeting activity and improve water solubility significantly.

- 5 [0045] In the meantime, subacute toxicity experiments were conducted to evaluate the safety of some of the compounds in the invention in mouse. Some of the triazole compounds from this invention (application examples 4, 6, 7, 16 and 22) and compounds DDO-2113 and DDO-2117 from paper 1 were given by intraperitoneal injection at 80mg/kg to female balb/c mice, 6 mice per group, for 10 consecutive days to observe mouse survival and average body weight changes. As shown in figure 3,
- 10 there were no death after dosing for 10 days for some of the compounds from this invention (application examples 4, 6, 7, 16 and 22) with slight body weight increase, while after dosing with the compounds DDO-2113 and DDO-2117 from the paper (Eur. J. Med. Chem. 2016, 124, 480-489.), all mice died after dosing for 5 days with DDO-2113 at 80mg/kg and those given DDO-2117 had apparent body weight decrease. The comparison of post dosing survival and average body weight changes, that no
- 15 death after dosing for 10 days with the triazole compounds in this invention with slight body weight increase in contrast to that with deaths and body weight decreases after dosing with diphenyl series compounds of DDO-2113 and DDO-2117, indicates the very good dosing safety of the phenyltriazoles in this invention.

[0046] Wherein, DDO-2113 and DDO-2117 are compounds from paper 1 with the structures:



- [0048] Anti-proliferation experiments were conducted with leukemia cells with some of the compounds in the invention. Table 3 listed the results of evaluation of the anti-proliferation activities for some of the compounds from this invention conducted with acute leukemia cells, wherein MV4-11 is human acute leukemia monocyte, Molm-13 is human acute myeloid leukemia cells, and K562 is
- 25 human chronic myeloid leukemia cells. Table 3 indicated that the compounds in this invention have very good inhibition activity of different kinds of leukemia cells.

[0049] Table 3 Anti-proliferation activities of some of the compounds in this invention on leukemia cells

[0050]

Compound numbers in the application examples ^a	GI ₅₀ /μM (MV-411)	GI ₅₀ /μM (Molm-13)	GI ₅₀ /μM (K562)
1	8.9±0.3	11.8±0.9	ND
3	44.6±1.3	22.8±5.5	>100
4	10.5±2.0	ND ^b	34.4±0.9
5	15.1±1.2	13.0±0.6	27.5±2.8
6	10.9±0.2	8.2±0.6	10.4±0.7
7	8.0±1.2	9.9±1.9	12.9±0.4
8	17.8±3.6	11.9±1.5	34.1±1.2
9	27.4±2.1	ND ^b	35.8±2.7
10	13.5±1.2	13.5±1.2	13.5±1.2
11	13.5±1.5	13.5±1.5	13.5±1.5
12	23.2±3.2	ND ^b	ND ^b
13	10.1±1.3	9.2±1.4	10.5±2.0
14	9.5±1.0	11.0±2.0	14.1±1.3
15	14.3±1.7	18.5±1.9	12.9±0.2
16	15.1±0.9	ND ^b	15.0±0.8
17	10.4±1.1	7.3±0.8	21.3±2.4
18	11.2±1.3	15.4±2.2	ND ^b
19	13.7±1.4	16.8±2.0	13.5±1.2
20	8.6±0.6	10.1±1.3	10.5±2.0

[0051]

21	12.7±0.8	7.7±0.9	10.5±0.8
22	9.5±0.4	11.2±0.8	10.9±0.7
23	ND ^b	17.9±1.6	27.7±0.3
24	11.3±0.7	12.9±0.9	13.5±2.2
25	10.9±0.9	8.5±0.7	11.3±0.7
DDO-2084	17.7±2.3	ND ^b	50.5±5.5

[0052] ^a Refer to the application examples for the chemical structures; ^b ND indicates not tested;

- 5 [0053] The phenyl triazole compounds in this invention have relatively strong inhibition activity on MLL1-WDR5 protein-protein interactions, lowering the MLL1 catalytic activities of MLL1 at the cellular level, down regulating the gene expression levels of Hox and Meis-1 and induce apoptosis of leukemia cells, and that the phenyl triazole compounds in this invention have shown very good water solubility and pharmaceutical safety, and can be used for treating leukemia.

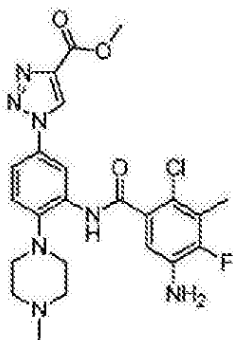
10 Notes for attached figures

[0054] Figure 1 is the RT-PCR experiment for application example 7 to show the lowered Hoxa9 and Meis-1 gene expressions in cells

[0055] Figure 2 is the Western Blot experiment for application example 7 to show the effects on MLL1 enzymatic activity in cells

- 15 [0056] Figure 3 is the toxicity comparison of the compounds in this invention to some of the compounds on article 1

[0057] Application example 1



[0058]

[0059] 1-(3-(5-amino-2-chloro-4-fluoro-3-methyl benzoylamino)-4-(4-methyl piperazine-1-group)phenyl)-1H-1,2,3-methyl triazole-4-carboxylate

[0060] Preparation of 4-(4-methylpiperazin-1-group)-3-nitroaniline (IIb):

[0061] Dissolve 4-fluoro-3-nitroaniline (II) (6g, 38.4mmol) in 50mL acetonitrile, add N-methylpiperazin (5.8 g, 6.3mL, 57.6mmol) and N, N-diisopropylethylamine (9.5mL, 57.6mmol), and heat and reflux for 12h. The crude is obtained after spin dry and purified by silica gel column chromatography (dichloromethane:methanol=20:1) to obtain red-brown solid (8.9g, 97.8%). ¹H NMR (300MHz, DMSO-d₆)δ7.06(d,J=8.6Hz, 1H), 6.76(s,1H), 6.69(d,J=8.5Hz,1H), 5.34(s,2H), 2.70(t,J=4.4Hz,4H), 2.27 (br s,4H), 2.09 (s,3H).m/z(EI-MS);259.1[M+Na]⁺.

[0062] Preparation of 1-(4-azido-2-nitrophenyl)-4-methylpiperazine (Ia):

[0063] Dissolve 4-(4-methylpiperazin-1-group)-3-nitroaniline (IIb)(4.0g, 17.0mmol) in 100mL 2M/HCl, reduce the temperature to 0°C, add 10mL of sodium nitrite (1.76g, 25.5mmol) water solution dropwise, stir for 30min at 0°C, add 10mL sodium azide (2.2g, 34.0mmol) water solution dropwise, stir 30min at 0°C and then stir 2h at room temperature. The product is precipitated with 2M/NaOH at pH=9~10, vacuum filter and heat dry to obtain red-brown solid (4.0g, 91.3%). ¹H NMR (300MHz, DMSO-d₆)δ7.48(d,J=2.2Hz, 1H), 7.34-7.20(m,2H), 2.85(t,J=4.7Hz,4H), 2.31(t,J=4.8Hz,4H), 2.11(s,3H).m/z(EI-MS);261.1 [M+H]⁺.

[0064] Preparation of 1-(4-(4-methylpiperazin-1-group)-3-nitrophenyl)-1H-1,2,3-triazole-4-methyl carboxylate (Ib):

[0065] Dissolve 1-(4-azido-nitrophenyl)-4-methylpiperazine (Ia) (1.0g, 3.8mmol) in 50mL methanol, add methyl propiolate (0.96g, 11.4mmol), cuprous iodide (0.07g, 0.38mmol), N,N-diisopropylethylamine (0.12mL, 0.76mmol), heat reflux for 48h, filter, concentrate, and beat up with ethyl acetate to obtain red-brown solid (0.8g, 61.5%). ¹H NMR (300MHz, DMSO-d₆)δ9.45(s,1H), 8.36(d,J=2.7Hz,1H), 8.11-8.01(m, 1H), 7.42(d,J=9.1 Hz, 1H), 3.80(s, 3H), 2.99 (t,J=5.4Hz,4H), 2.35 (t,J=5.2Hz,4H), 2.13(s, 3H).m/z(EI-MS);369.2[M+Na]⁺.

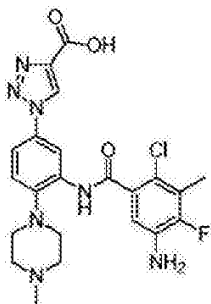
[0066] Preparation of 1-(3-amino-4-(4-methylpiperazin-1-group)phenyl)-1H-1,2,3-methyl triazole-4-methyl carboxylate (Ic):

[0067] Dissolve 1-(4-(4-methylpiperazin-1-group)-3-nitrophenyl)-1H-1,2,3-methyl triazole-4-methyl carboxylate (Ib) (3.8g, 12.0mmol) in 50mL methanol, add Pd/C of catalytic amount, pump in hydrogen, stir at room temperature for 7h, concentrate by vacuum filtering to obtain pink solid (3.0g, 78.9%). ¹H NMR (300MHz, DMSO-d₆)δ9.28(s,1H), 7.28(d,J=1.9Hz, 1H), 7.07 (d,J=1.9Hz, 2H), 5.15 (s,2H), 3.90 (s,3H), 2.87(t,J=4.5Hz,4H), 2.53 (br s, 4H), 2.26 (s,3H).m/z(ESI-MS);317.1763 [M+H]⁺.

[0068] Preparation of 1-(3-(5-Amino-2-chloro-4-fluoro-3-methylbenzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-1H-1,2,3-triazole-4-carboxylate (1):

[0069] Dissolve 1-(3-amino-4-(4-methylpiperazin-1-group)phenyl)-1H-1,2,3-triazole-4-methyl carboxylate (1c) (1.7g, 5.3mmol) in 100mL anhydrous dichloromethane, add pyridine (0.43mL, 5.3mmol),
 5 add 20mL dichloromethane solution of 2-chloro-3-methyl-4-fluoro-5-nitrobenzoyl chloride (1.6g, 6.4mmol) drop wise in ice-water bath, stir 2h at room temperature, filter under vacuum and heat dry to obtain light yellow solid; dissolve the light yellow (2.6g, 4.9mmol) in ethyl acetate, add stannous chloride (5.5g, 24.4mmol), heat and reflux for 3h before cooling down to room temperature, dilute with 100mL ethyl acetate, neutralize with saturated sodium bicarbonate till no additional white gel-like
 10 precipitating out, filter under vacuum, wash the filter cake with ethyl acetate till no ultraviolet absorption, extract the filtrate with ethyl acetate till no ultraviolet absorption, combine the organic phases, dry with anhydrous sodium sulfate, concentrate to obtain the crude product, beat up with ethyl acetate, filter under vacuum to obtain gray-white solid 1 (2.3g, 93.9%). ¹H NMR (300MHz, DMSO-d₆)δ9.52-9.45(m,2H), 8.69(s,1H), 7.73(dd,J=8.7,2.7Hz, 1H), 7.46(d,J=8.6Hz, 1H), 6.92(d,J=9.2Hz, 1H), 5.53
 15 (s,2H), 3.92 (s, 3H), 3.00-2.90 (m, 4H), 2.51 (br s, 4H), 2.28 (d, J=2.6Hz, 3H), 2.24 (s, 3H). (EI-MS):502.9[M+H]⁺.

[0070] Application example 2

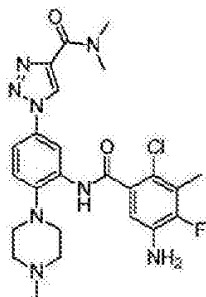


[0071]

[0072] Preparation of 1-(3-(5-Amino-2-chloro-3-methylbenzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-1H-1,2,3-Triazole-4-carboxylic acid (2)

[0073] Dissolve 1-(3-(5-Amino-2-chloro-3-methylbenzoylamino)-4-(4-methylpiperazin-1-yl)phenyl)-1H-1,2,3-triazole-4-Methyl formate (1) (2.3g, 4.6mmol) in THF, add lithium hydroxide solution (1M, 15mL), stir for 8h at room temperature, rotate dry to remove THF followed by acidification using 2M chloric acid to obtain white solid (1.7g, 80.4%). ¹H NMR (300MHz, DMSO-d₆)δ9.52-9.45(m,2H), 8.69(s,1H),
 25 7.73(dd,J=8.7,2.7Hz, 1H), 7.46(d,J=8.6Hz, 1H), 6.92(d,J=9.2Hz, 1H), 5.53 (s,2H), 3.92 (s, 3H), 3.00-2.90 (m, 4H), 2.50 (br s, 4H), 2.28 (d, J=2.6Hz, 3H), 2.24 (s, 3H). (EI-MS):488.9[M+H]⁺.

[0074] Application example 3



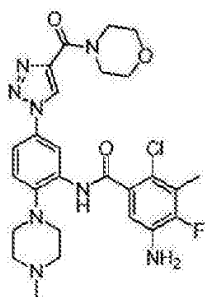
[0075]

[0076] Preparation of 1-(3-(5-Amino-2-chloro-4-fluoro-3-methylbenzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-N,N-dimethyl-1H-1,2,3-triazole-4-carboxamide (3):

30

[0077] Dissolve 1-(3-(5-Amino-2-chloro-4-fluoro-3-methylbenzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-1H-1,2,3-Triazole-4-carboxylic acid (2) (0.18g, 0.36mmol) in 10mL DMF, add BOP (0.32g, 0.72mmol), trimethylamine (0.10mL, 0.72mmol) and dimethylamino hydrochloride (58.7mg, 0.72mmol), stir for 4h at room temperature. Dilute the reaction mixture with 50mL ethyl acetate, remove DMF with saturated sodium chloride, dry the organic phase with anhydrous sodium sulphate, dry out the organic solvent with rotations to obtain raw product, isolate and purify with silica gel column chromatography (Dichloromethane:methanol=50:1) to obtain gray white solid. The yield is 78.4%. ¹H NMR (300MHz, DMSO-d₆)δ9.59(s,1H), 9.18(s,1H), 8.63(d, J=2.5Hz, 1H), 7.71(dd, J=8.6,2.6Hz, 1H), 7.42(d, J=8.6Hz, 1H), 6.83(d, J=9.2Hz, 1H), 5.50 (s,2H), 3.11 (br s, 10H), 3.02 (s, 3H), 2.70-2.69(m, 4H), 2.24 (d, J=2.5Hz, 3H). (EI-MS):515.9[M+H]⁺.

[0078] Application example 4

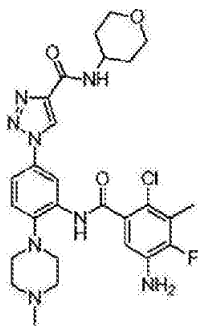


[0079]

[0080] Preparation of 5-amino-2-chloro-4-fluoro-3-methyl-N-(2-(4-methylpiperazin-1-group)-5-(4-((morpholine-4-carbonyl)-1H-1,2,3-triazol-1-yl)phenyl)benzamide (4):

[0081] Using the methods in application example 3, replace dimethylamino hydrochloride by morpholine to obtain gray white solid. The yield is 67.5%. ¹H NMR (300MHz, DMSO-d₆)δ9.61(s,1H), 9.24(s,1H), 8.64(d, J=2.5Hz, 1H), 7.73(dd, J=8.6,2.6Hz, 1H), 7.44(d, J=8.6Hz, 1H), 6.86(d, J=9.2Hz, 1H), 5.52 (s,2H), 4.06 (s,2H), 3.68 (s,6H), 3.12 (br s, 8H), 2.69 (s, 3H), 2.26 (d, J=2.6Hz, 3H). (EI-MS):558.9[M+H]⁺.

[0082] Application example 5

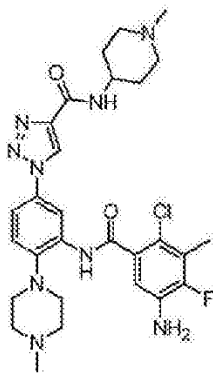


[0083]

[0084] Preparation of 1-(3-(5-Amino-2-chloro-4-fluoro-3-methylbenzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-N-(tetrahydro-2H-pyran-4-yl)-1H-1,2,3-triazole-4-carboxamide:

[0085] Using the methods in application example 3, replace dimethylamino hydrochloride with 4-aminotetrahydropyran to obtain gray white solid. The yield is 82.9%. ¹H NMR (300MHz, DMSO-d₆)δ9.52(s,1H), 9.26-9.24(m, 1H), 8.65(s,2H), 7.71(d, J=8.7Hz, 1H), 7.42(d, J=9.4Hz, 1H), 6.89(s,1H), 5.53(s, 2H), 4.06(s,1H), 3.9-3.86(m,2H), 2.99(s,6H), 2.71(s,4H), 2.38(s,3H), 2.25(s,3H), 1.71(s,4H). (EI-MS):572.0[M+H]⁺.

[0086] Application example 6



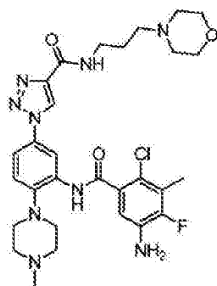
[0087]

[0088] Preparation of 1-(3-(5-Amino-2-chloro-4-fluoro-3-methylbenzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-N-(1-methylpiperidin-4-group)-1H-1,2,3-triazole-4-carboxamide:

- 5 [0089] Using the methods in application example 3, replace dimethylamino hydrochloride with 4-amino-1-methylpiperidin to obtain gray white solid. The yield is 49.9%. ^1H NMR (300MHz, DMSO- d_6) δ 9.48(s,1H), 9.22(s,1H), 8.65(d,J=2.6Hz,1H), 8.52(d,J=8.2Hz,1H), 7.70(dd,J=8.5,2.7Hz,1H), 7.42(d,J=8.6Hz,1H), 6.89(d,J=9.2Hz,1H), 5.54(s,2H), 3.78(s,1H), 2.92(t,J=4.6Hz,4H), 2.82-2.78(m,2H), 2.26(d,J=2.7Hz,3H), 2.22(s,3H), 2.19(s,3H), 2.07-1.86(m,4H), 1.75-1.66(m,4H). (EI-MS):585.0[M+H] $^+$.

10

[90] Application example 7

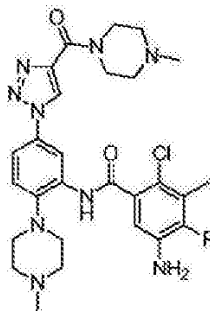


[0091]

[0092] Preparation of 1-(3-(5-amino-2-chloro-4-fluoro-3-ethyl benzoylamino) 4-(4-methylpiperazin-1-group)-N-(3-morpholinopropyl)-1H-1,2,3-triazole-4-carboxamide (7):

- 15 [0093] Using the methods in application example 3, replace dimethylamino hydrochloride with N-(3-aminopropyl)morpholine, and obtain gray-white solid. The yield is 94.2%. ^1H NMR (300MHz, DMSO- d_6) δ 9.51 (s, 1H), 9.22 (s, 1H), 8.85 (t, J=5.8Hz, 1H), 8.66 (s, 1H), 7.71 (dd, J=8.6, 2.7Hz, 1H), 7.43 (d, J=8.8Hz, 1H), 6.89 (d, J=9.2Hz, 1H), 5.53 (s, 2H), 3.62 (t, J=4.6Hz, 4H), 2.98-2.97 (m, 4H), 2.63 (s, 4H), 2.54 (s, 2H), 2.46-2.36 (m, 6H), 2.33 (s, 3H), 2.26 (d, J=2.6Hz, 3H), 1.74-1.70 (m, 2H). (ESI-
20 MS):615.1[M+H] $^+$.

[0094] Application example 8

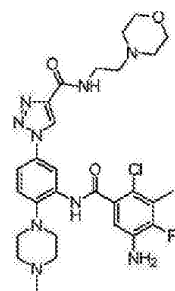


[0095]

[0096] Preparation of 5-amino-2-chloro-4-fluoro-3-ethyl-N-(2-(4-methylpiperazin-1-group)-5-(4-(methylpiperazin-1-Carbonyl)-1H-1,2,3-triazole-1-group) Phenyl)-carboxamide (8):

- 5 [0097] Using the methods in application example 3, replace dimethylamino hydrochloride with N-methylpiperazin to obtain gray-white solid. The yield is 94.2%. ^1H NMR (300MHz, DMSO-d_6) δ 9.51 (s, 1H), 9.22 (s, 1H), 8.85 (t, $J=5.8\text{Hz}$, 1H), 8.66 (s, 1H), 7.71 (dd, $J=8.6, 2.7\text{Hz}$, 1H), 7.43 (d, $J=8.8\text{Hz}$, 1H), 6.89 (d, $J=9.2\text{Hz}$, 1H), 5.53 (s, 2H), 3.62 (t, $J=4.6\text{Hz}$, 4H), 2.98-2.97 (m, 4H), 2.63 (s, 4H), 2.54 (s, 2H), 2.46-2.36 (m, 6H), 2.33 (s, 3H), 2.26 (d, $J=2.6\text{Hz}$, 3H), 1.74-1.70 (m, 2H). (ESI-MS):571.0[M+H] $^+$.

10 [0098] Application example 9

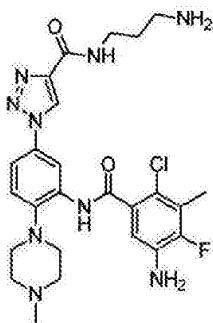


[0099]

[0100] Preparation of 1-(3-(5-amino-2-chloro-4-fluoro-3-ethyl benzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-N-(2-Morpholine ethyl)-1H-1,2,3-triazole-4-carboxamide (9):

- 15 [0101] Using the methods in application example 3, replace dimethylamino hydrochloride with N-Aminoethylmorpholine to obtain gray-white solid. The yield is 72.5%. ^1H NMR (300MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.66 (d, $J=2.4\text{Hz}$, 1H), 8.56 (t, $J=5.8\text{Hz}$, 1H), 7.71 (dd, $J=8.7, 2.6\text{Hz}$, 1H), 7.43 (d, $J=8.7\text{Hz}$, 1H), 6.89 (d, $J=9.2\text{Hz}$, 1H), 5.54 (s, 2H), 3.58 (t, $J=4.6\text{Hz}$, 4H), 3.44 (s, 2H), 2.96 (t, $J=4.8\text{Hz}$, 4H), 2.59 (s, 4H), 2.54 (s, 2H), 2.44 (s, 4H), 2.30 (s, 3H), 2.26 (d, $J=2.6\text{Hz}$, 3H). (ESI-MS):601.0[M+H] $^+$.

[0102] Application example 10

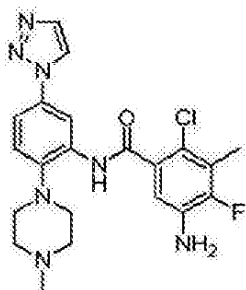


20 [0103]

[0104] Preparation of 1-(3-(5-amino-2-chloro-4-fluoro-3-ethyl benzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-N-(3-aminopropyl)-1H-1,2,3-triazole-4-carboxamide (10):

[0105] Using the methods in application example 3, replace dimethylamino hydrochloride with 1,3-propylene diamine to obtain gray-white solid. The yield is 64.5%. ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 8.85 (s, 1H), 8.70(s, 1H), 8.45(d, $J=2.1\text{Hz}$, 1H), 7.36(dd, $J=7.5, 2.0\text{Hz}$, 1H), 7.25(s, 1H), 6.84(d, $J=7.5\text{Hz}$, 1H), 6.78(d, $J=5.7\text{Hz}$, 1H), 4.13(s, 2H), 3.25-3.19(m, 6H), 2.98(t, $J=4.9\text{Hz}$, 4H), 2.67-2.58(m, 5H), 2.39(s, 3H), 2.19-2.13(m, 2H), 1.14(s, 2H). (ESI-MS):545.0[M+H] $^+$.

[0106] Application example 11

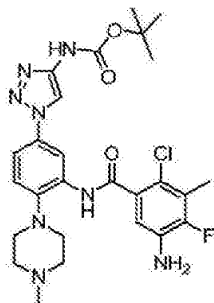


[0107]

[0108] Preparation of 5-amino-2-chloro-4-fluoro-3-methyl-N-(2-(4-methylpiperazin-1-yl)-5-(1H-1,2,3-triazole-1-yl)phenyl)benzamide (11):

10 [0109] Using the methods in application example 1, replace ethyl propiolate with Trimethyl ethynyl silicon to obtain gray-white solid after three reaction steps. The yield of the three steps is 23.8%. ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 8.76-8.68(m, 2H), 8.49(d, $J=2.1\text{Hz}$, 1H), 8.18(d, $J=7.5\text{Hz}$, 1H), 7.27(dd, $J=7.5, 2.0\text{Hz}$, 1H), 6.82(d, $J=7.5\text{Hz}$, 1H), 6.75(d, $J=5.7\text{Hz}$, 1H), 4.15(s, 2H), 3.20(t, $J=5.1\text{Hz}$, 4H), 2.98(t, $J=5.0\text{Hz}$, 4H), 2.60(s, 3H), 2.39(s, 3H). (EI-MS):444.9[M+Na] $^+$.

15 [0110] Application example 12

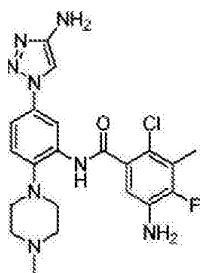


[0111]

[0112] Tert-butyl(1-(3-(5-amino-2-chloro-4-fluoro-3-ethyl benzoylamino)-4-(4-methylpiperazin-1-yl)phenyl)-1H-1,2,3-triazole-4-yl)aminomethyl ester (12)

20 [0113] Using the methods in application example 1, replace Methyl propiolate with tert-butyl ethynyl carbamate to obtain gray-white solid after three reaction steps. The yield of the three steps is 20.1%. ^1H NMR (300MHz, $\text{DMSO}-d_6$) δ 8.70(s, 1H), 8.59(d, $J=2.0\text{Hz}$, 1H), 8.08(s, 1H), 7.25(dd, $J=7.5, 2.0\text{Hz}$, 1H), 7.18(s, 1H), 6.80(dd, $J=19.7, 6.6\text{Hz}$, 2H), 4.17(s, 2H), 3.20(t, $J=5.1\text{Hz}$, 4H), 2.98(t, $J=5.1\text{Hz}$, 4H), 2.60(s, 3H), 2.39(s, 3H), 1.50(s, 9H). (EI-MS):560.0[M+Na] $^+$.

[0114] Application example 13

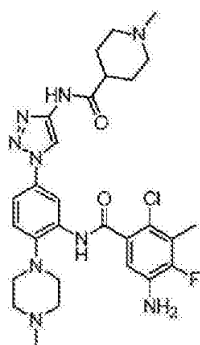


[0115]

[0116] 5-amino-N-(5-(4-amino-1H-1,2,3-triazole-1-yl)-2-(4-methylpiperazin-1-yl)phenyl)-2-chloro-4-fluoro-3-methylbenzamide (13)

[0117] Dissolve Tert-butyl(1-(3-(5-amino-2-chloro-4-fluoro-3-ethyl benzoylamino)-4-(4-methylpiperazin-1-yl)phenyl)-1H-1,2,3-triazole-4-yl) aminomethyl ester (1.0g, 2.2mmol) in 20mL dichloromethane, add 10mL trifluoroacetate, stir for 1h at room temperature, adjust pH=8~9 using saturated sodium bicarbonate, extract with dichloromethane, dry the organic phase with anhydrous sodium sulfate and dry with rotation to obtain grey solid. The yield is 87.3%. ¹H NMR (300MHz, DMSO-d₆) δ 8.70(s,1H), 8.49(d,J=2.0Hz,1H), 8.08(s,1H), 7.25(dd,J=7.5,2.0Hz,1H), 6.80(d,J=7.5Hz,1H), 6.70(d,J=5.7Hz,1H), 5.80(s,2H), 4.15(s,2H), 3.20(t,J=5.3Hz,4H), 2.98(t,J=5.2Hz,4H), 2.60(s,3H), 2.39(s,3H). (EI-MS):459.9[M+Na]⁺.

[0118] Application example 14

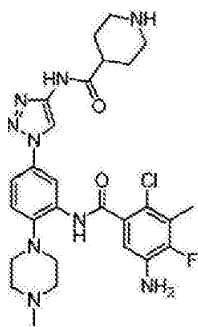


[0119]

[0120] N-(1-(3-(5-amino-2-chloro-4-fluoro-3-ethyl benzoylamino)-4-(4-methylpiperazin-1-yl)phenyl)-1H-1,2,3-triazole-4-yl)-1-methylpiperidine-4-formamide (14)

[0121] Dissolve 5-amino-N-(5-(4-amino-1H-1,2,3-triazole-1-yl)-2-(4-methylpiperazin-1-yl)phenyl)-2-chloro-4-fluoro-3-methylbenzamide (13) (0.2g, 0.34mmol) in 5mL DMF, add BOP (0.30g, 0.68 mmol), trimethylamine (0.09mL, 0.68mmol) and 1-Methylpiperidine-4-formic acid (97.3mg,0.68mmol), stir for 4h at room temperature. Dilute the reaction with 50mL ethyl acetate, remove DMF by washing with saturated sodium chloride, dry the organic phase with anhydrous sodium sulfate, dry out the organic solvent with rotation to obtain raw product, isolate and purify with chromatography on silica gel column (dichloromethane:methanol=20:1) to obtain grey white solid. The yield is 73.9%. ¹H NMR (300MHz, DMSO-d₆) δ 8.72-8.64(m,2H), 8.08(s,1H), 7.71(s,1H), 7.25(dd,J=7.5,2.0Hz,1H), 6.87(d,J=7.5Hz,1H), 6.69(d,J=5.7Hz,1H), 4.16(s,2H), 3.20(t,J=5.3Hz,4H), 3.05-2.95(m,6H), 2.60(s,3H), 2.50-2.45(m,1H), 2.39-2.37(m,6H), 2.14-2.09(m,2H), 2.05-1.98(m,2H), 1.81-1.70(m,2H). (EI-MS):485.1[M+Na]⁺.

[0122] Application example 15

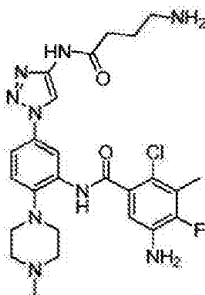


[0123]

[0124] N-(1-(3-(5-amino-2-chloro-4-fluoro-3-Methyl benzoylamino)-4-(4-methylpiperazin-1-group)phenyl)-1H-1,2,3-Triazopyridine-4-group) Piperidine-4-formamide (15)

5 [0125] Using the methods in application example 14, replace 1-Methylpiperidine-4-formic acid with 4-piperidinic acid, grey white solid is obtained. The yield is 88.7%. ¹H NMR (300MHz, DMSO-d₆) δ 8.70(s,1H), 8.55(d, J=2.0Hz, 1H), 8.08(s,1H), 7.71(s,1H), 7.26(dd, J=7.5, 2.0Hz, 1H), 6.80(dd, J=23.8, 6.6Hz, 2H), 4.15(s, 2H), 3.27-3.17(m, 6H), 2.98(t, J=5.0Hz, 4H), 2.82-2.65(m, 3H), 2.60(s, 3H), 2.39(s, 3H), 2.03-1.96(m, 2H), 1.74-1.69(m, 2H), 1.22(s, 1H). (EI-MS): 571.1 [M+Na]⁺.

[0126] Application example 16

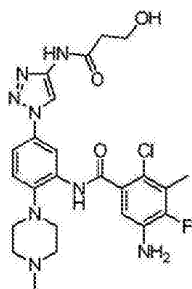


10 [0127]

[0128] 5-amino-N-(5-(4-(4-Aminobutyrylamino)-1H-1,2,3-triazol-1-group)-2-(4-methylpiperazin-1-group)phenyl)-2-chloro-4-fluoro-3-methyl benzoylamino (16)

15 [0129] Using the methods in application example 14, replace 1-Methylpiperidine-4-formic acid with γ-aminobutyric acid to obtain grey white solid. The yield is 88.7%. ¹H NMR (300MHz, DMSO-d₆) δ 8.70(s,1H), 8.08(s,1H), 7.71(s,1H), 7.25(dd, J=7.5, 2.0Hz, 1H), 6.80(dd, J=21.4, 6.6Hz, 2H), 4.17(s, 2H), 4.17(s, 2H), 3.20(t, J=5.1Hz, 4H), 3.08-3.04(m, 2H), 2.98(t, J=5.1Hz, 4H), 2.60(s, 3H), 2.50(t, J=8.2Hz, 2H), 2.39(s, 3H), 2.10-2.04(m, 2H), 1.19(s, 2H). (EI-MS): 571.1 [M+Na]⁺.

[0130] Application example 17



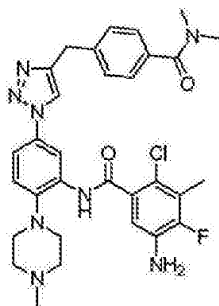
[0131]

20 [0132] 5-amino-2-chloro-4-fluoro-N-(5-(4-(3-Hydroxypropionylamino)-1H-1,2,3-triazol-1-group)-2-(4-methylpiperazin-1-group)phenyl)-3-methyl benzoylamino

[0133] Using the methods in application example 14, replace 1-Methylpiperidine-4-formic acid with 3-Hydroxypropionic acid to obtain grey white solid. The yield is 84.9%. ¹H NMR (300MHz, DMSO-

δ 8.70(s,1H), 8.57(d,J=2.0Hz,1H), 8.08(s,1H), 7.71(s,1H), 7.25(dd,J=7.5,2.0Hz,1H), 6.80(dd,J=21.8,6.6Hz,2H), 4.36(t,J=5.0Hz,1H), 4.17(s,2H), 3.83-3.79(m,2H), 3.20(t,J=5.1Hz,4H), 2.98(t,J=5.0Hz,4H), 2.60(s,3H), 2.39-2.35(m,5H). (EI-MS): 532.1[M+Na]⁺.

[0134] Application example 18



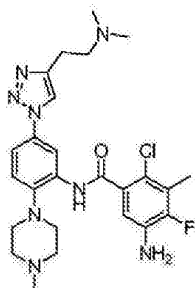
5 [0135]

[0136] 5-amino-2-chloro-N-(5-(4-(4-(dimethylaminomethyl)benzyl)-1H-1,2,3-triazol-1-yl)-2-(4-methylpiperazin-1-yl)phenyl)-4-fluoro-3-methylbenzoyl (18)

[0137] Using the methods in application example 1, replace methyl propiolate with N,N-Dimethyl-4-(prop-2-yn-1-yl) to obtain grey white solid in three steps. The yield of the three steps is 34.4%. ¹H NMR (300MHz, DMSO-d₆) δ 8.70(s,1H), 8.49-8.44(m,2H), 7.74-7.68(m,2H), 7.56-7.50(m,2H), 7.26(dd,J=7.4,1.9Hz,1H), 6.75(d,J=5.9Hz,1H), 4.16(s,2H), 3.88(d,J=1.2Hz,2H), 3.20(t,J=5.1Hz,4H), 3.0-2.95(m,10H), 2.60(s,3H), 2.38(s,3H). (EI-MS): 606.1[M+Na]⁺.

10

[0138] Application example 19



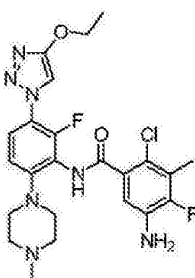
[0139]

15 [0140] 5-amino-2-chloro-N-(5-(4-(2-(Dimethylamino)ethyl)-1H-1,2,3-triazol-1-yl)-2-(4-methylpiperazin-1-yl)phenyl)-4-fluoro-3-methylbenzoyl (19)

[0141] Using the methods in application example 1, replace methyl propiolate with N,N-dimethyl-but-3-alkyne-1-amine to obtain grey white solid in three steps. The yield of the three steps is 31.6%. ¹H NMR (300MHz, DMSO-d₆) δ 8.70(s,1H), 8.48(d,J=2.0Hz,1H), 8.28(s,1H), 7.26(dd,J=7.5,2.0Hz,1H), 6.82(d,J=7.5Hz,1H), 6.74(d,J=5.7Hz,1H), 4.15(s,2H), 3.20(t,J=5.1Hz,4H), 2.98(t,J=5.0Hz,4H), 2.71-2.61(m,4H), 2.60(s,3H), 2.40-2.39(m,9H). (EI-MS): 606.1[M+Na]⁺.

20

[0142] Application example 20

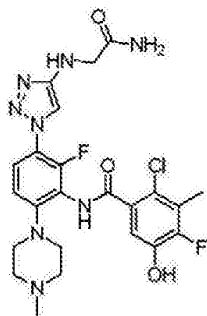


[0143]

[0144] 5-amino-2-fluoro-N-(3-(4-Ethoxy-1H-1,2,3-triazol-1-group)-2-fluoro-6-(4-methylpiperazin-1-group)phenyl)-4-fluoro-3- Methyl benzoyl (20)

[0145] Using the methods in application example 1, replace 4-fluoro-3-Nitroaniline with 2,4-difluoro-3-nitroaniline and methyl propiolate with ethyl ethynyl ether to obtain grey white solid in five steps. The yield of the five steps is 12.8%. ¹H NMR (300MHz, DMSO-d₆)δ8.70(s,1H), 7.98(s,1H), 7.30(dd,J=7.5,5.7Hz,1H), 6.74(d,J=5.7Hz,1H), 6.59(d,J=7.5Hz,1H), 4.76-4.72(m,2H), 4.15(s,2H), 3.20(t,J=5.1Hz,4H), 2.98(t,J=5.0Hz,4H), 2.60(s,3H), 2.39(s,3H), 1.56(t,J=8.0Hz,3H). (EI-MS): 507.1[M+Na]⁺.

[0146] Application example 21

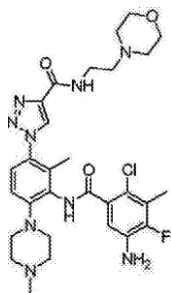


[0147]

[0148] N-(3-(4-((2-amino-2-oxoethyl)amino)-1H-1,2,3-triazol-1-group)-2-fluoro-6-(4-methylpiperazin-1-group)phenyl)-2-chloro-4-fluoro-5-hydroxy-3-methyl benzoyl (21)

[0149] Using the method in application example 1, replace 4-fluoro-3-Nitroaniline with 2,4-difluoro-3-nitroaniline, methyl propiolate with 2-(ethynylamino) Acetamide and 2-chloro-3-methyl-4-fluoro-5-Nitrobenzoyl chloride with 2-chloro-3-methyl-4-fluoro-5-hydroxybenzoyl chloride to obtain white solid in five reactions. The yield of the five reactions is 9.7%. ¹H NMR (300MHz, DMSO-d₆)δ8.70(s,1H), 8.07(d,J=10.4Hz,2H), 7.34-7.24(m,2H), 6.59(d,J=7.5Hz,1H), 5.98(s,2H), 5.93(s,1H), 4.03(s,2H), 3.20(t,J=5.1Hz,4H), 2.98(t,J=5.0Hz,4H), 2.60(s,3H), 2.38(s,3H). (EI-MS): 507.1[M+Na]⁺.

[0150] Application example 22

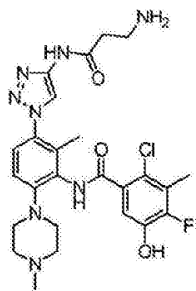


[0151]

[0152] 1-(3-(5-amino-2-chloro-4-fluoro-3-methyl benzoylamino)-2-methyl-4-(4-methylpiperazin-1-group)phenyl)-N-(2-Morpholine ethyl)-1H-1,2,3-triazol-4-formamide (22)

[0153] Using the methods in application example 3, replace 4-fluoro-3-Nitroaniline with 2-fluoro-4-methyl-3-Nitroaniline and Dimethylamino hydrochloride with N-(2-Aminoethyl) Morpholine to obtain grey white solid. The yield of the six steps is 8.8%. ¹H NMR (300MHz, DMSO-d₆)δ8.70(s,1H), 8.52(s,1H), 7.25(s,1H), 7.13(d,J=7.5Hz, 1H), 6.78 (d, J=5.7Hz, 1H) 6.72 (d,J=7.5Hz, 1H), 4.15 (s, 2H), 3.74(t, J=4.7Hz,4H), 3.55-3.53(m,2H), 3.20(t,J=5.1Hz, 4H), 2.98(t,J=5.1Hz, 4H), 2.61-2.59 (m, 5H), 2.51 (t, J=4.7Hz, 4H), 2.40-2.39(m,6H). (EI-MS): 615.1[M+Na]⁺.

[0154] Application example 23

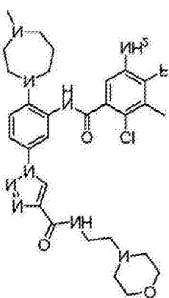


[0155]

[0156] N-(3-(4-(3-aminopropionylamino)-1H-1,2,3-triazol-1-yl)-2-methyl-6-(4-methylpiperazin-1-yl)phenyl)-2-chloro-4-fluoro-5-hydroxy-3-methylbenzoyl (23)

[0157] Using the methods in application example 14, replace 4-fluoro-3-nitrobenzene with 2-fluoro-4-methyl-3-nitrobenzene, 2-chloro-3-methyl-4-fluoro-5-nitrobenzoyl chloride with 2-chloro-3-methyl-4-fluoro-5-hydroxybenzoyl chloride, 1-methylpiperidine-4-carboxylic acid with β -alanine to obtain white solid in six steps. The yield of the six steps is 7.2%. ^1H NMR (300MHz, DMSO- d_6) δ 8.70(s, 1H), 8.08(s, 1H), 7.71(s, 1H), 7.19(d, J=7.5Hz, 1H), 7.06(d, J=5.7Hz, 1H), 6.72(d, J=7.5Hz, 1H), 5.93(s, 1H), 3.20(t, J=5.1Hz, 4H), 3.01-2.93(m, 4H), 2.93-2.91(m, 1H), 2.60(s, 3H), 2.45(s, 3H), 2.44-2.42(m, 2H), 2.38(s, 3H), 1.24(s, 2H). (EI-MS): 615.1 [M+Na] $^+$.

[0158] Application example 24

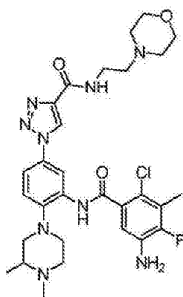


[0159]

[0160] 1-(3-(5-amino-2-chloro-4-fluoro-3-methylbenzoylamino)-4-(4-methyl-1,4-diazepane-1-yl)phenyl)-N-(2-morpholine ethyl)-1H-1,2,3-triazol-4-formamide (24)

[0161] Using the methods in application examples 3, replace N-methylpiperazine with N-methyl homopiperazine and dimethylamino hydrochloride with N-(2-aminoethyl)morpholine to obtain white solid. The yield of the six steps is 7.3%. ^1H NMR (300MHz, DMSO- d_6) δ 8.79(s, 1H), 8.70(s, 1H), 7.31(dd, J=7.5, 2.0Hz, 1H), 7.25(s, 1H), 6.83(d, J=7.5Hz, 1H), 6.70(d, J=5.7Hz, 1H), 4.16(s, 2H), 3.74(t, J=4.7Hz, 4H), 3.60(t, J=4.8Hz, 2H), 3.55-3.53(m, 2H), 3.46-3.44(m, 2H), 2.91-2.89(m, 2H), 2.61-2.58(m, 4H), 2.52-2.50(m, 4H), 2.39(s, 3H), 2.31(s, 3H), 1.64-1.60(m, 2H). (EI-MS): 615.1 [M+Na] $^+$.

[0162] Application example 25



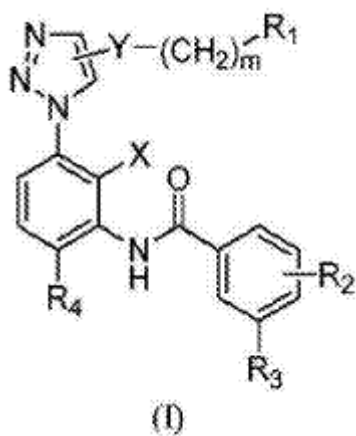
[0163]

[0164] 1-(3-(5-amino-2-chloro-4-fluoro-3-methylbenzoylamino)-4-(3,4-dimethylpiperazine-1-yl)phenyl)-N-(2-morpholine ethyl)-1H-1,2,3-triazol-4-formamide (24)

[0165] Using the methods in application example 3, replace N-Methylpiperazine with 1,2-Dimethylpiperazine and Dimethylamino hydrochloride with N-(2-aminoethyl)morpholine to obtain grey white solid. The yield of the six steps is 7.3%. ¹H NMR (300MHz, DMSO-d₆) δ 8.80(s,1H), 8.70 (s, 1H), 8.54 (d, J=2.0Hz, 1H), 7.31 (dd, J=7.5, 2.0Hz, 1H), 7.25 (s, 1H), 6.81 (d, J=7.5Hz, 1H), 6.70 (d, J=5.7Hz,1H), 4.18 4.04(m,7H), 3.66 3.53(m,2H), 3.35 3.31 (m,1H), 3.20 3.07 (m,4H), 2.93 2.87(m,1H), 2.73 2.58(m,2H), 2.51 2.37(m,7H), 2.34-2.20 (m, 3H), 1.16 (d, J=6.8Hz, 3H). (EI MS): 615.1[M+Na]⁺.

Claims:

1. A compound of the formula (I) or a pharmaceutically acceptable salt thereof:



wherein X is hydrogen, methyl, methoxy or halogen;

Y is $-\text{CH}_2-$, $-\text{O}-$, $-\text{S}-$, $-\text{CO}-$, $-\text{CH}_2\text{O}-$, $-\text{NR}_5-$, $-\text{CONR}_6-$ or $-\text{NR}_7\text{CO}-$, wherein R_5 , R_6 , or R_7 each independently is hydrogen, C_1 - C_4 alkyl, C_1 - C_4 haloalkyl, phenyl or substituted phenyl, the substituent is halogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, amino, hydroxyl, thiol, carboxyl, cyano, trifluoromethyl or imidazolyl;

m is 0 - 6;

R_1 is hydrogen, amino, hydroxyl, thiol, carboxyl, cyano, $-\text{CONH}_2$, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, phenyl, substituted phenyl, substituted or unsubstituted nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring, $-\text{NR}_8\text{COR}_9$, $-\text{CONR}_{10}\text{R}_{11}$ or $-\text{NR}_{10}\text{R}_{11}$, wherein R_8 is hydrogen, C_1 - C_4 alkyl, C_1 - C_4 haloalkyl, phenyl or substituted phenyl, R_9 is amino, hydroxyl, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, phenyl or substituted phenyl, substituted or unsubstituted nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring, R_{10} and R_{11} are independently hydrogen, C_1 - C_4 alkyl, phenyl or substituted phenyl, substituted or unsubstituted nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring, or R_{10} and R_{11} are bonded to form nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring, wherein the substituent is halogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, amino, hydroxyl, thiol, carboxyl, cyano, trifluoromethyl or imidazolyl;

R_2 represents two or three substituents selected from halogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, trifluoromethyl, nitro and cyano;

R_3 is amino, methylamino, aminomethyl, hydroxyl, hydroxymethyl, thiol or $-\text{CONH}_2$;

R_4 is N-methylpiperazine, 1,2-dimethyl piperazine or N-methylhomopiperazine.

2. The compound according to claim 1 or a pharmaceutically acceptable salt thereof, wherein X is hydrogen, fluorine, chlorine or methyl.

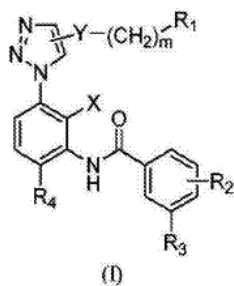
3. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein Y is $-\text{NR}_5-$, $-\text{CONR}_6-$ or $-\text{NR}_7\text{CO}-$; R_5 , R_6 or R_7 each independently is hydrogen, methyl, ethyl, propyl, cyclopropyl or isopropyl.
4. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein Y is $-\text{NR}_5-$, $-\text{CONR}_6-$ or $-\text{NR}_7\text{CO}-$; R_5 , R_6 or R_7 each independently is substituted phenyl, and the substituent is methyl, ethyl, isopropyl, t-butyl, cyclopropyl, methoxy, cyano, halogen, trifluoromethyl or imidazolyl.
5. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein said substituted or unsubstituted nitrogen- or oxygen-containing 3 to 7 membered heterocyclic ring is aziridine, azetidine, tetrahydrogen pyrrole, piperidine, cycloheximide, lactam, tetrahydrofuran, tetrahydropyran, morpholine, 1,4-oxazaxane, hexahydropyridazine, imidazoline, pyrazolidine, piperazine, the substituent is methyl, ethyl, phenyl, hydroxyl, amino, hydroxymethyl, or aminomethyl, the substituent is halogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, amino, hydroxyl, thiol, carboxyl, cyano, or trifluoromethyl.
6. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein R_1 is $-\text{NR}_8\text{COR}_9$, $-\text{CONR}_{10}\text{R}_{11}$ or $-\text{NR}_{10}\text{R}_{11}$, wherein R_8 , R_9 , R_{10} or R_{11} is C_1 - C_4 alkyl.
7. The compound of claim 1 or a pharmaceutically acceptable salt thereof, wherein R_2 represents three substituents selected from fluorine, chlorine, bromine, methyl, methoxy, nitro, trifluoromethyl and cyano.
8. A pharmaceutical composition comprising a compound of claim 1 or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carrier.
9. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of acute leukemia.
10. The use of claim 9, wherein the acute leukemia is an acute leukemia having MLL1 gene rearrangement.
11. A compound of claim 1 or a pharmaceutically acceptable salt thereof for use in treating acute leukemia.

PHENYL TRIAZOLE MLL1-WDR5 PROTEIN-PROTEIN INTERACTION INHIBITOR

Abstract

The invention relates to the field of medicinal chemistry, in particular to a phenyl triazole MLL1-WDR5 protein-protein interaction inhibitor (I) and a preparation method thereof, and

- 5 pharmacodynamics experiments prove that the compound of the invention has relatively strong MLL1-WDR5 protein-protein interaction inhibition activity.



[No Suitable Figure]