Discovery of Indolinone-based Multi-kinase Inhibitors as Potential Therapeutics for Idiopathic Pulmonary Fibrosis

Zhenhua Huang[†], Heran Li[†], Qian Zhang[‡], Fangzheng Lu[†], Mei Hong[‡], Zhigang Zhang[‡], XiaocuiGuo[‡], Yuanju Zhu[‡], Sanming Li[†] and Hongzhuo Liu[†*]

[†]School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, P. R. China;

[‡] KBP Biosciences, 401, Buliding 2, Jinan Pharm Valley, North Section of Gangxing Three Road, Jinan,, Shandong,250101, P. R. China

Experimental details and characterization for compounds

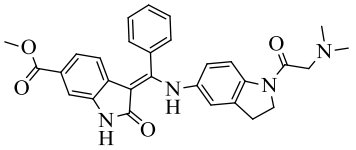
Experiments were generally conducted under inert atmosphere unless otherwise noted. Commercial solvents and reagents were generally used without further purification. All final products were characterized by ^{1}H NMR, LCMS and HPLC methods. ^{1}H NMR was recorded on a Bruker 400 spectrometer in the indicated solvent. Chemical shift (δ) were reported in parts per million relative to the internal standard tetramethylsilane. Tandem liquid chromatography/mass spectrometry (LCMS) was performed on Agilent 1260 separations module and Agilent 6120 mass detector. Compound purity and identity were determined by HPLC at 214 nm. Products were purified by column chromatography on silica gel by using the solvent systems indicated.

Synthesis of the key intermediates ***5***

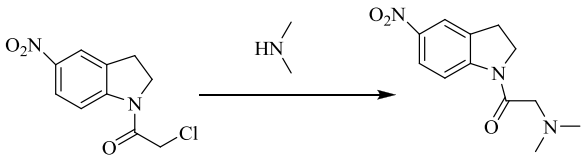
5-nitroindoline (164 g, 1 mol) was dissolved in 1 L DCM, and triethylamine (151.5 g, 1.5 mol) was added at -20℃. The mixture was stirred for 30 min and then 2-chloroacetyl chloride (134 g, 1.2 mol) was added dropwise. After 2 h HPLC analysis showed complete conversion, water were allowed to added the reaction system and the organic layer was separated, dried under vacuum to yield **5** (220 g, 91%).

General methods for the preparation and analytical data for compounds illustrated in Table 1.

(*Z*)-3-((1-(2-(dimethylamino)ethanoyl)indole-5-amide)(phenyl)methylene)-2-oxoindoline-6- carboxylate (compound *9*a) and its hydrochloride

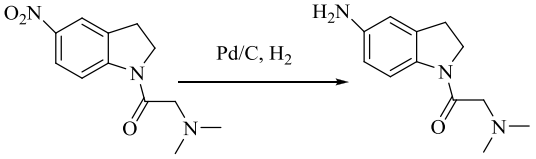


Step 1: 1-(2-(dimethylamino)ethanoyl)-5-nitroindoline (*6*a)



1-(2-chloracetyl)-5-nitroindoline (Compound ***5***, 22 g, 91 mmol), dimethylamine hydrochloride (22 g, 270 mmol) and K_2CO_3 (32.5 g, 235 mmol) were combined in toluene (200 mL). The reaction mixture was allowed to stir at 70℃ overnight and then concentrated in vacuum to provide the crude product. The solid product was purified by silica gel chromatography (Eluant: 10% methanol in DCM) to give the product (50% yield).

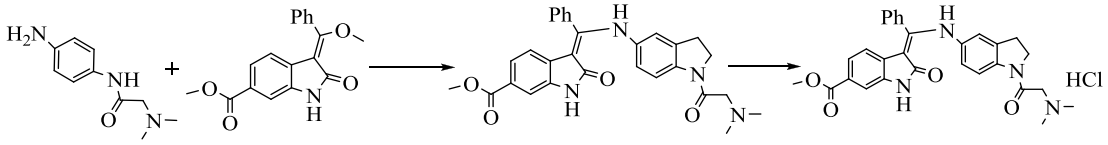
Step 2: 1-(2-(dimethylamino)ethanoyl)-5-aminoindolin (*7*a)



A heterogeneous mixture of 1-(2-(dimethylamino) ethanoyl)-5-nitroindoline (5 g, 20.1 mmol) and Pd/C (1 g) in methanol (200 mL) was allowed to react under H_2 atmosphere at RT overnight. The organic solvent was then moved by rotary evaporator to give the product as white solid (5 g, 99% yield).

Step 3:

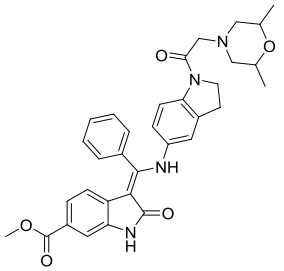
(*Z*)-3-((1-(2-(dimethylamino)ethanoyl)indole-5-amide)(phenyl)methylene)-2-oxoindoline-6-carbo xylate（compound *9*a）and its hydrochloride



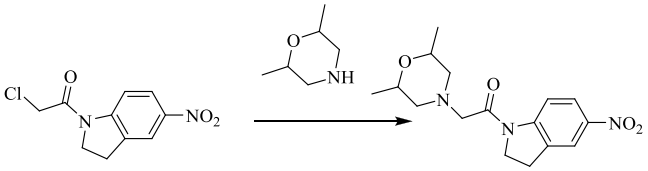
(*E*)-3-(methoxyl(phenyl)methylene)-2-oxoindoline-6-carboxylate (86 mg, 0.278 mmol) and 1-(2-(dimethylamino)ethanoyl)-5-aminoindoline (82 mg, 0.374 mmol) were combined in methanol (2 mL). The resulting solution was stirred at 70℃ for 7 h. Afterwards, the mixture was filtered and washed with cooled methanol to give ***9*a** (61 mg, 35.4% yield, 98.39% purity) as a yellow solid at RT. The obtained product was dissolved in 10 mL methanol, 1 mL of concentrated hydrochloric acid was

added at RT. The reaction was stirred for 1 h and then the resulting solution was filtered and washed with cooled methanol to its hydrochloride. LCMS(m/z) for $C_{29}H_{28}N_4O_4$ 497.3 (M+1). ^{1}H-NMR ($C_{29}H_{28}N_4O_4$·HCl, DMSO-d_6, 400MHz, δ; ppm): 12.16 (s, 1H), 10.96 (s, 1H), 9.77 (br. s., 1H), 7.79 (d, J = 8.8 1H), 7.56 (m, 3H), 7.45 (m, 2H), 7.26 (s, 1H), 7.17(m, 1H), 6.88 (s, 1H), 6.74 (s, 1H),5.81 (d, J = 8.4, 1H), 4.25 (m, 2H), 3.96 (m, 2H), 3.76 (s, 3H), 3.04 (m, 2H), 2.83 (m, 6H).

(*Z*)-3-((1-(2-(2,6-dimethylmorpholine)ethanoyl)dihydroindole-5-amide)(phenyl)methylene)-2-oxo indoline-6-carboxylate (compound *9*b) and its hydrochloride

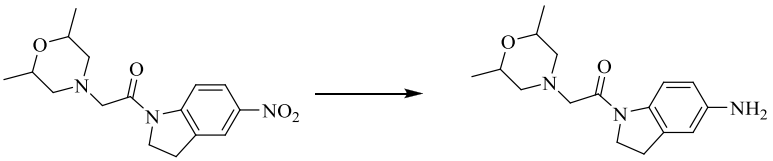


Step 1: 2-(2,6-dimethylmorpholine)-1-(5-nitrodihydroindole -1-)ethanone (*6*b)



To a solution of compound ***5*** (1.0 g, 4.2 mmol) and 2,6-dimethylmorpholine (1.15 g, 10 mmol) in DCM (50 mL) was added trimethylamine (1 mL, 7 mmol) dropwise. The reaction was allowed to stir at RT for 1 h. The reaction mixture was partitioned between DCM and water. The organic layer was washed with water, dried, concentrated and the product was used in the next step without purification.

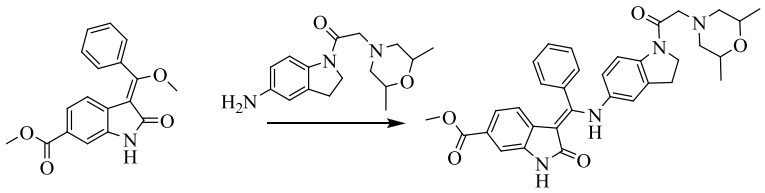
Step 2: 1-(5-amidedihydroindole -1-)-2-(2,6-dimethylmorpholine)ethanone (*7*b)



To a solution of the crude product (2-(2,6-dimethylmorpholine)-1-(5-nitrodihydroindole -1-yl)ethanone)in methanol (50 mL) was added Pd/C (0.2 g). The reaction mixture was allowed to stir

under H_2 atmosphere for 2 h, filtered and concentrated. The resulted filtrate was used in the next step without purification.

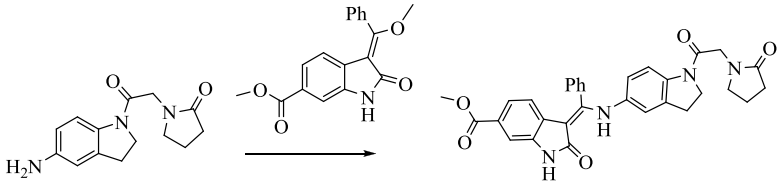
Step 3: (Z)-3-((1-(2-(2,6-dimethylmorpholine)ethanoyl)dihydroindole-5-amide)(phenyl) methylene)-2-oxoindoline-6-carboxylate(compound *9*b) and its hydrochloride



To a solution of the crude product from above step 1-(5-amidedihydroindole -1-yl)-2-(2,6-dimethylmorpholine)ethanone(82 mg, 0.28 mmol) and (*E*)-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (1.0 g, 3.2 mmol) in methanol (50 mL) was added KOH (57 mg, 1 mmol). The mixture was allowed to react at 60℃ for 2 h. Then the reaction mixture was washed with water and the product was extracted by DCM, which was further dried and concentrated. The resulted product was purified by silica gel chromatography (Eluant: 1% methanol in DCM) to give ***9***b.The obtained ***9***b was dissolved in 20 mL DCM, 1 mL hydrochloric acid (2N) was added at RT to give its hydrochloride as yellow solid (132 mg, 7.4% yield, 91.79% purity). LCMS(m/z) for $C_{33}H_{34}N_4O_5$ 567.1 (M+1).^{1}H-NMR ($C_{33}H_{34}N_4O_5$·HCl, DMSO-d_6, 400MHz, δ; ppm): 12.13 (s, 1H), 10.95 (s 1H), 10.50 (s, 1H), 7.79 (s, 1H), 7.51 (m, 6H), 7.18 (s, 1H), 6.89 (s, 1H), 6.74 (s, 1H), 5.81 (s, 1H), 4.29 (s, 2H), 3.98 (s, 4H), 3.76 (m, 3H), 3.16 (s, 2H), 3.06 (t, 2H), 2.74 (d, 2H), 1.09 (d, 6H).

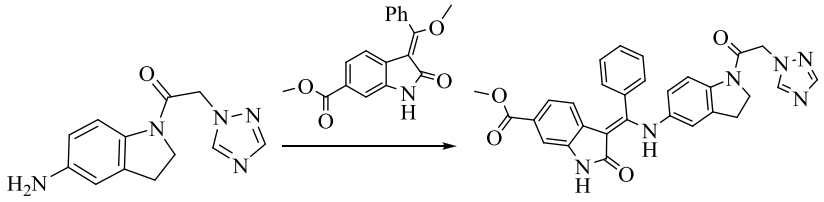
(*Z*)-3-((1-(2-(2-pyrrolidone-1-yl)acetyl)dihydroindole-5-amide)(phenyl)methylene)-2-oxoindoline -6-carboxylate (compound *9*c)

The compound ***6***c and ***7***c were prepared according Scheme 1 (analogous to ***6***b and ***7***b).



To a solution of (Z)-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (183 mg, 0.59 mmol) and (1-(5-amidedihydroindole-1-yl)-2-(2-pyrrolidone)ethanone(170 mg, 0.66 mmol) in methanol (20 mL) was added KOH(30 mg, 0.54 mmol). The mixture was allowed to react at RT for 12 h. Then the reaction mixture was washed with water and the product was extracted by DCM, which was further dried and concentrated. The resulted product was purified by silica gel chromatography (Eluant: 2% methanol in DCM) to give the compound ***9***c as yellow solid (90 mg, 43% yield, 92.23% purity). LCMS(m/z) for $C_{31}H_{28}N_4O_5$ 537 (M+1). ^{1}H-NMR ($C_{31}H_{28}N_4O_5$, DMSO-d_6, 400MHz, δ; ppm): 12.15 (s, 1H), 10.93（s, 1H）,7.73 (d, 1H), 7.55 (m, 3H), 7.43-7.40 (m, 2H), 7.41 (d, 1H), 7.17 (d, 1H), 6.83 (d, 1H), 6.67 (d, 1H), 5.81 (d, 1H), 4.10 (s, 2H), 4.04(t, 2H), 3.75(s, 3H), 3.00 (t, 2H), 2.24 (m, 2H), 1.95(m, 2H), 1.22(m, 2H).

(*Z*)-3-((1-(2-(1*H*-1,2,4-triazole-1-yl)acetyl)dihydroindole-5-amide)(phenyl)methylene)-2-oxoindoline-6-carboxylate (compound *9*d) and its hydrochloride



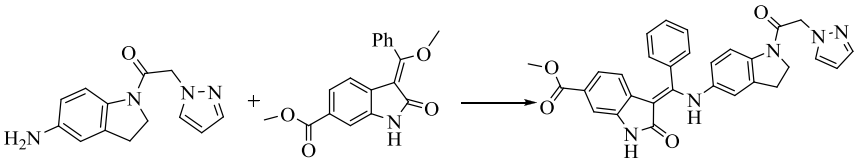
The compound 6d and 7d were prepared according Scheme 1(analogous to ***6***b and ***7***b).

To a solution of (Z)-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (3.2g, 10.3 mmol) and (1-(5-amidedihydroindole-1-yl)-2-(1H-1,2,4-triazole)ethanone (2.6 g, 10.7 mmol) in methanol (50 mL) was added KOH (203 mg, 3.6 mmol). The mixture was allowed to react at 60℃ for 2 h. Then the reaction mixture was cooled and washed with water and the product was extracted by methanol, which was further dried and concentrated to give the compound ***9***d. The obtained ***9***d was dissolved in 200 mL DCM and 50 mL methanol, 15mL of hydrochloric acid (1N) was added at RT to give its hydrochloride as yellow solid (5.2 g, 89% yield, 96.02% purity). LCMS(m/z) for $C_{29}H_{24}N_6O_4$ 521 (M+1). ^{1}H-NMR ($C_{29}H_{24}N_6O_4$·HCl, DMSO-d6, 400MHz, δ; ppm): 12.17(s, 1H), 10.93(s, 1H), 7.80(d,

1H), 7.50(m, 6H), 7.19(m, 1H), 6.83(s, 1H), 6.69(m, 1H), 5.83(d, 1H), 4.09(s, 2H), 3.92(t, 3H), 3.76(s, 3H), 2.96(t, 2H).

(*Z*)-3-((1-(2-(1*H*-pyrazol-1-yl)acetyl)dihydroindole-5-yl)(phenyl)methylene)-2-oxoindoline-6-carboxylate(compound *9*e)

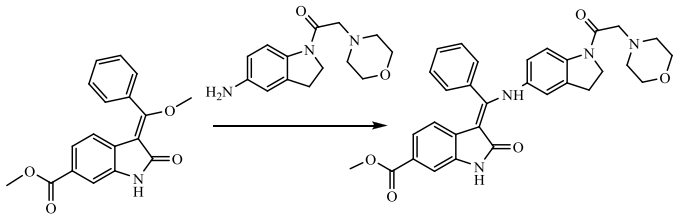
The compound 6e and 7e were prepared according Scheme 1.



To a solution of (Z)-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (72.5 mg, 0.234 mmol) and (1-(5-amidedihydroindole-1-yl)-2-(1H-pyrazol)ethanone (50 mg, 0.206 mmol) in methanol (20 mL) was added KOH (6 mg, 0.107 mmol). The mixture was allowed to react at RT for 4 h. Then the reaction mixture was washed with water and the product was extracted by DCM, which was further dried and concentrated. The resulted product was purified by silica gel chromatography (Eluant: 1% methanol in DCM) to give the compound ***9***e as yellow solid (70 mg, 65.5% yield, 98.78% purity). LCMS(m/z) for $C_{30}H_{25}N_5O_4$ 520 (M+1).^{1}H-NMR ($C_{30}H_{25}N_5O_4$, DMSO-d_6, 400MHz, δ; ppm): 12.09 (s, 1H), 10.93 (s, 1H), 7.69 (d, 1H), 7.64 (d, 1H), 7.52-7.54 (m, 3H), 7.42 (d, 4H), 7.14-7.18 (m, 1H), 6.83 (s, 1H), 6.65 (d, 1H), 6.26 (t, 1H), 5.80 (d, 1H), 5.14 (s, 2H), 4.09 (t, 2H), 3.74 (s, 3H), 3.01 (t, 2H).

(*Z*)-3-((1-(2-morpholinoacetyl)indole-5-amide)(phenyl)methylene)-2-oxoindoline-6-carboxylate (compound *3*, also named as KBP-7018) and its hydrochloride

The compound 6 and 7 were prepared according Scheme 1.

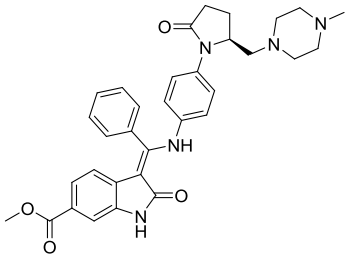


(Z)-methyl-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (130 g, 0.5 mol) and

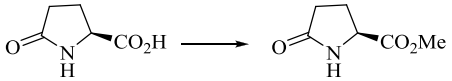
1-(5-aminoindolin-1-yl)-2-morpholinoethanone (154 g, 0.5 mol) were combined in methanol (1000 mL). The reaction was refluxed at RT for 12 h, then filtered and washed with cool methanol to provide the crude products. The residue was purified by silica gel chromatography (Eluant: 30% EtOAc in hexane) to afford the free base of the product (67% yield, 99.35% purity) as a yellow solid. A 1N solution of HCl in Et_2O was used to make the hydrochloride salt (15 mg, 6% over three steps). LCMS(m/z) for $C_{31}H_{30}N_4O_5$ 538.8 (M+1). ^{1}H-NMR ($C_{31}H_{30}N_4O_5 \cdot HCl$, DMSO-$d^6$, 400MHz, δ; ppm) 12.16 (s, 1H), 10.98 (s, 1H), 10.52 (brs, 1H), 7.78 (d, J=8.4Hz, 1H), 7.56-7.59 (m, 3H), 7.44-7.46 (m, 2H), 7.42 (d, J=1.2Hz, 1H), 7.17 (dd, J=1.2Hz, 8.0Hz, 1H), 6.88 (s, 1H), 6.74 (dd, J=1.6Hz, 8.4Hz, 1H), 4.36 (brs, 2H), 3.81-4.02 (m, 6H), 3.75 (s, 3H), 3.41 (m, 2H), 3.21 (m, 2H), 3.04 (t, J=8Hz, 1H).

General methods for the preparation and analytical data for compounds illustrated in Table 2.

(*S*,*Z*)-3-((4-(2-((4-methylpiperazine-1-yl)methyl)-5-carbonylpyrrolidine-1-yl)(phenyl)amide)methylene)-2-carbonylindole-6-carboxylate (compound *15*a)

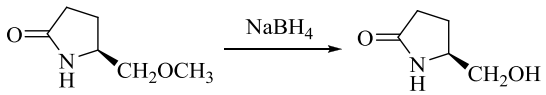


Step 1: (*S*)-5-oxopyrrolidin-2-carboxylate



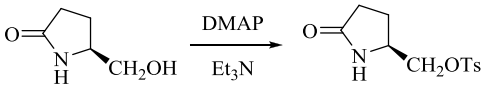
To 100 mL methanol was added 25 mL $SOCl_2$ slowly in an ice-cooled flask. (*S*)-5-oxopyrrolidin-2-methanoic acid (20 g, 0.155 mol) was dissolved in 20 mL methanol and then added to the reaction mixture dropwise. The mixture was gradually warmed to RT and allowed to stir overnight. The organic solvent was removed by rotary evaporator and the residue was taken up in ethyl acetate and saturated aqueous Na_2CO_3. After 1 h, the organic layer was concentrated to give the product (16 g, 72% yield). The product was directly to be used for the next step without any purification.

Step 2: (*S*)-5-hydroxymethylpyrrolidine-2-ketone



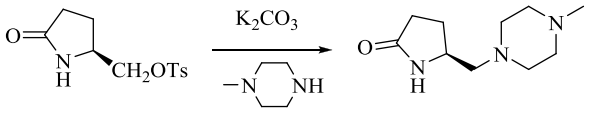
To a solution of above crude product (16 g, 0.112 mol) in ethanol (130 mL) was added $NaBH_4$ (4.25 g, 0.112 mol) in an ice-cooled flask. The reaction mixture was allowed to stir at RT overnight. Added 10% HCl solution (22 mL) and filtered. The filtrate was then concentrated to give the product (7.6 g, 59% yield). The product was directly to be used for the next step without any purification.

Step 3: (*S*)-4-methylbenzenesulfonic acid-(5-pyrrolidone-2-yl)methyl ester



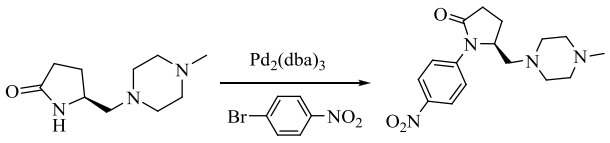
To a solution of above crude intermediates (7.6 g, 66 mmol) and TsCl (16.04 g, 84 mmol) in DCM (100 mL) under iced bath was added DMAP (1.6 g, 13 mmol) and Et_3N (7.6 g, 75 mmol). The reaction mixture was allowed to stir at RT overnight. The crude mixture was purified by column chromatography to give (*S*)-4-methylbenzenesulfonic acid-(5-pyrrolidone-2-yl)methyl ester (12.6 g, 71% yield).

Step 4: (*S*)-5-((4-methylpiperazine-1-)methyl)pyrrolidone-2-ketone (*11*a)



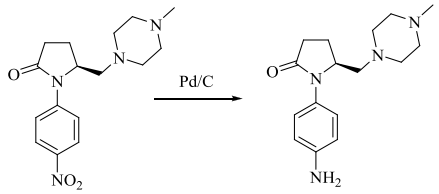
To a solution of (*S*)-4-methylbenzenesulfonic acid-(5-pyrrolidone-2-yl)methyl ester (5.38g , 20 mmol) and *N*-methylpiperazine (3.00 g, 30 mmol) in ACN (100 mL) was added K_2CO_3 (5.6 g, 41 mmol). The reaction mixture was refluxed overnight, then filtered to remove K_2CO_3. The filtrate was concentrated and the residue was dissolved in DCM. The white solid after sonication process was removed and then the filtrate was concentrated to give (*S*)-5-((4-methylpiperazine-1-yl)methyl)pyrrolidone-2-ketone (3.2 g, 81% yield).

Step 5: (*S*)-5-((4-methylpiperazine-1-yl)methyl)-1-(4-nitrophenyl)pyrrolidone-2-ketone (*13* a)



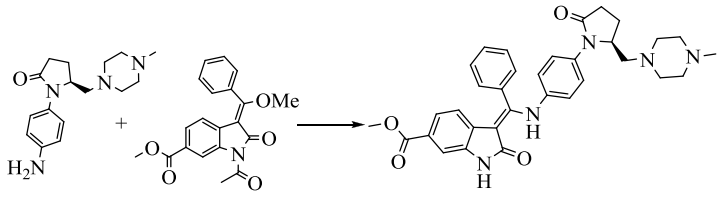
To a solution of (S)-5-((4- methylpiperazine-1-yl)methyl) pyrrolidone-2-ketone (1.6 g, 8.1 mmol), p-nitrobromobenzene (1.8 g, 8.9 mmol) and Dimethylbisdiphenylphosphinoxanthene (0.92 g, 1.6 mmol) in dioxane (20 mL) were added Cs_2CO_3 (3.9 g, 12 mmol) and $Pd_2(dba)_3$ (0.75 g, 8 mmol). The reaction mixture was refluxed overnight and the crude product was purified by column chromatography, eluting with DCM to give (S)-5-((4-methylpiperazine-1-yl)methyl)-1-(4-nitrophenyl) pyrrolidone-2-ketone (1.83 g, 72% yield).

Step 6: (*S*)-5-((4-methylpiperazine-1-yl)methyl)-1-(4-aminophenyl)pyrrolidone-2-ketone (*14*a)



To a solution of (S)-5-((4-methylpiperazine-1-yl)methyl)-1-(4-nitrophenyl) pyrrolidone-2-ketone (1.83 g, 5.75 mmol) in methanol (20 mL) was added 10% Pd/C (183 mg). The mixture was allowed to react under H_2 (1 atm) atmosphere overnight, then filtrate to remove Pd/C. The filtrate was concentrated and the crude product was purified by column chromatography to give (*S*)-5-((4-methylpiperazine-1-yl)methyl)-1-(4-aminophenyl)pyrrolidone-2-ketone (1.5 g, 90% yield).

Step 7: (*S*,*Z*)-3-((4-(2-((4-methylpiperazine-1-yl)methyl)-5-carbonylpyrrolidine-1-yl)(phenyl) amide) methylene)-2-carbonylindole -6-carboxylate (compound *15*a)

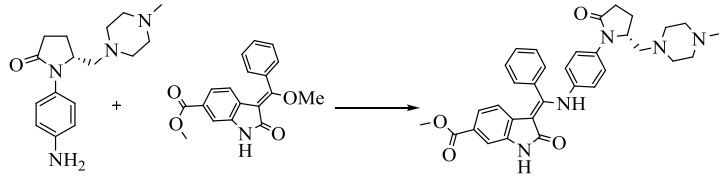


(*S*)-5-((4-methylpiperazine-1-yl)methyl)-1-(4-aminophenyl)pyrrolidone-2-ketone (144 mg, 0.45 mmol) and (*Z*)-1-acetyl-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylic methyl ether (0.175 g, 0.57 mmol) were dissolved in methanol (10 mL). The reaction mixture was refluxed overnight, and washed with water and extracted by DCM. The organic layer was concentrated and the crude product was purified by preparative liquid chromatography to give compound ***15***a as yellow solid (126 mg, 44% yield, 99.60% purity). LCMS(m/z) for $C_{33}H_{35}N_5O_4$ 566.3 (M+1). ^{1}H-NMR ($C_{33}H_{35}N_5O_4$, DMSO-d_6,

400MHz, δ; ppm): 2.02 (m, 2H), 2.32 (m, 14H), 2.63 (m, 2H), 3.86 (s, 3H), 4.22 (m, 1H), 5.96 (d, 1H), 6.78 (d,2H), 7.24 (d, 1H), 7.37 (m,3H), 7.44 (m, 4H), 7.82 (s, 1H), 12.15 (s, 1H).

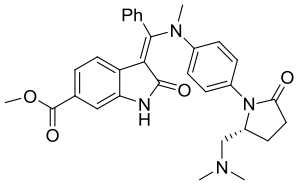
(*Z*)-3-((1-(2-(4-methylpiperazine-1-)-L-pyroglutamate-4-aminoethyl)(phenyl)methylene)-2-oxoindolin-6-carboxylate (compound *15*b)

The compound ***11***b, ***13***b and ***14***b were prepared according Scheme 2 (analogous to ***11***a, ***13***a and ***14***a except for R-intermediates used).

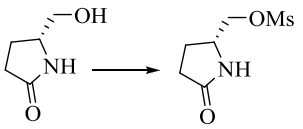


(*R*)-1-(4-aminophenyl)-5-(4-methylpiperazine-1-yl)methylpyrrolidine-2-ketone (144 mg, 0.5 mmol) was dissolved in methanol (10 mL) and (Z)-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (155 mg, 0.5 mmol) was added. The mixture was refluxed overnight and washed with water, followed by extraction by DCM and concentration. The resulting product was purified by preparative liquid chromatography to give compound ***15***b as yellow solid (126 mg, 44% yield, 97.16% purity). LCMS(m/z) for $C_{33}H_{35}N_5O_4$ 566 (M+1). ^{1}H-NMR ($C_{33}H_{35}N_5O_4$, DMSO-d_6, 400MHz, δ; ppm): 12.15 (s, 1H), 7.97 (s, 1H), 7.57-7.51(m, 8H), 6.80 (d, 2H), 5.97 (d, 1H), 4.25 (s, 1H), 3.86 (s, 3H), 2.67-2.22 (m, 16H), 2.09-2.02 (m, 2H).

(*R*,*Z*)-3-(((4-(2-((dimethylamino)methyl)-5-carbonylpyrrolidine-1-yl)phenyl)(methyl)amide)(phenyl)methylene)-2-oxoindoline-6-carboxylate (compound *15*c) and its chloride

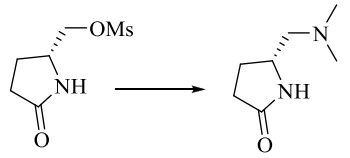


Step 1: (*R*)-(5-oxopyrrolin-2-yl)methyl methanesulfonate



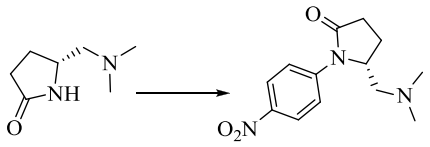
To a solution of (*R*)-5-(hydroxymethyl)pyrroline-2-ketone (23 g, 0.2mol) and TEA (56 mL) in DCM (200 mL) was added mesilatechloride dropwise (34 g, 0.3 mol). The reaction mixture was allowed to stir overnight, and filtrated to remove the solid. The resulting mixture was washed with HCl (1N), dried and then concentrated to give (*R*)-(5-oxopyrrolin-2-yl)methylmethanesulfonat (32.8 g, 85% yield).

Step 2: (*R*)-5-(dimethylaminemethyl) pyrroline-2-ketone (*11*c)



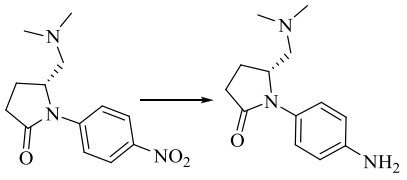
To a solution of (*R*)-(5-oxopyrrolin-2-yl)methylmethanesulfonat (5 g, 25.8 mmol) in THF (20 mL) was added 40% dimethylamine (1 mL). The reaction mixture was refluxed for 18 h and then diluted by ethyl acetate (200 ml). The resulting mixture was washed with saturated aqueous sodium chloride solution, and the solvent was removed to give (*R*)-5-(dimethylaminemethyl) pyrroline-2-ketone (3.38 g, 92% yield).

Step 3: (*R*)-5-((dimethylamino)methyl)-1-(4-nitrophenyl)pyrroline-2-ketone (*13*c)



To a solution of (*R*)-5-(dimethylaminemethyl)pyrroline-2-ketone (2.7 g, 19 mmol), 4-bromonitrobenzene (4.2 g, 20 mmol) and xanphos (0.66 g, 1.14 mmol) in 1,4-dioxane (20 mL) were added $Pd(dba)_3$ (0.27 g, 0.38 mmol) and Cs_2CO_3 (7.43 g, 22.8 mmol). The reaction mixture was refluxed at 120℃ for 18 h, and the solvent was removed. The crude product was then purified by silica gel with methanol/DCM (0~1:10) to give (*R*)-5-((dimethylamino)methyl)-1-(4-nitrophenyl)pyrroline-2-ketone (2.5 g, 50% yield).

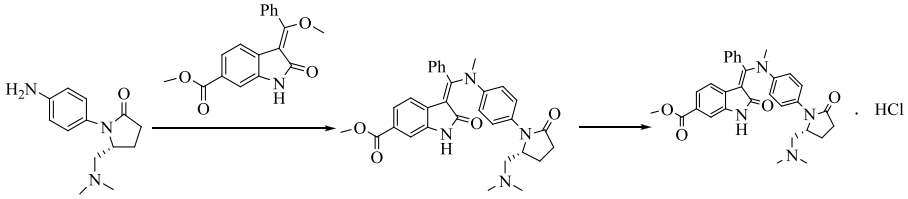
Step 4: (*R*)-5-((dimethylamino)methyl)-1-(4-aminophenyl)pyrroline-2-ketone (*14*c)



To a solution of (*R*)-5-((dimethylamino)methyl)-1-(4-nitrophenyl)pyrroline-2-ketone (1.2 g, 4.56 mmol) in methanol (10 mL) was added Pd/C (100 mg). The mixture was allowed to react under H_2 atmosphere for 72 h, and filtrated to remove Pd/C. The filtrate was concentrated to give (*R*)-5-((dimethylamino)methyl)-1-(4-aminophenyl)pyrroline-2-ketone (1.0 g, 99% yield).

Step5:

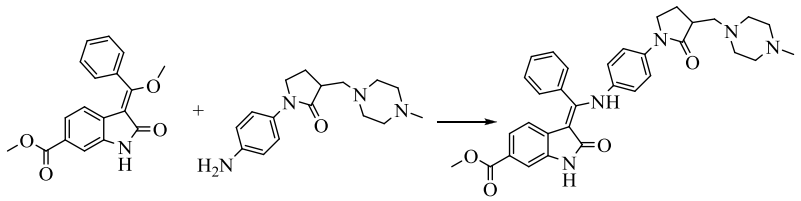
(*R*,*Z*)-3-(((4-(2-((dimethylamino)methyl)-5-carbonylpyrrolidine-1-yl)phenyl)(methyl)amide)(phenyl)methylene)-2-oxoindoline-6-carboxylate(compound *15*c) and its chloride



(*R*)-5-((dimethylamino)methyl)-1-(4-aminophenyl)pyrroline-2-ketone (82 mg, 0.35 mmol) and (*Z*)-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylic methyl ether (66 mg, 0.21 mmol) were dissolved in methanol (2 mL). The reaction mixture was refluxed at 70℃ for 7 h, and cooled to RT. The mixture was allowed to crystallize, filtrate, wash with methanol and dry to give (*R*,*Z*)-3-(((4-(2-((dimethylamino)methyl)-5-carbonylpyrrolidine-1-yl)phenyl)(methyl)amide)(phenyl) methylene)-2-oxoindoline-6-carboxylate as yellow solid (53 mg, 50% yield, 97.80% purity). To the solution of above product in methanol (10 mL) was added HCl (1 mL). The mixture was allowed to stir at RT for 2 h, filtrated and dried in vacuum to give its chloride (68 mg). LCMS(m/z) for $C_{31}H_{32}N_4O_4$ 524.2 (M+1). ^{1}H-NMR ($C_{31}H_{32}N_4O_4$, DMSO-d_6, 400MHz, δ; ppm): 12.25 (s, 1H), 10.98 (s, 1H), 10.24 (brs, 1H), 7.52-7.66 (m, 4H), 7.48 (d, J = 6.5 Hz, 1H), 7.41 (s, 1H), 7.35 (d, J = 8.5 Hz, 2H), 7.19 (d, J = 8.3 Hz, 1H), 6.88 (d, J = 8.5 Hz, 2H), 5.82 (d, J = 8.3 Hz, 1H), 4.68 (brs, 1H), 3.76 (s, 3H), 3.25 (t, J = 10.3 Hz, 1H), 2.83 - 2.96 (m, 1H), 2.72 (brs, 6H), 2.62 (dt, J = 17.1, 8.8 Hz, 1H), 2.12 - 2.42 (m, 3H), 1.93-2.08 (m, 1H).

(*Z*)-3-((4-(3-((4-methylpiperazine-1-yl)methyl)-2-oxopyrrolidin-1-)phenylamino)(phenyl)methylene)-2-oxoindoline-6-carboxylate (compound *17*a)

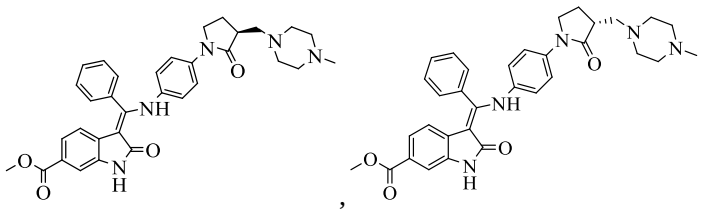
The intermediates (***11***, ***13*** and ***14***) were prepared according Scheme 2.



Toa solution of (*Z*)-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylic methyl ether (177 mg, 0.57 mmol) in methanol (20 mL) was added 1-(4-aminophenyl)-3-((4-methylpiperazin-1-yl)methyl)pyrrolidin-2-one (144 mg, 0.50 mmol). The reaction mixture was refluxed for 12 h. The mixture was cooled to RT, dried under reduced pressure. The residue was then purified by column chromatography to give Compound ***17***a as yellow solid (265 mg, 95% yield, 98.85% purity). LCMS(m/z) for $C_{33}H_{35}N_5O_4$ 566.3(M+1).^{1}H-NMR (DMSO-d6, 400MHz, δ; ppm): 12.19 (s, 1H), 10.95 (s, 1H), 7.56 (d, 2H), 7.47 (d, 4H), 7.40 (s, 1H), 7.17 (d, 1H), 6.88 (d, 2H), 5.79 (d,2H), 4.10 (d, 2H), 3.75 (s, 3H), 3.65 (m, 2H), 2.75 (m, 1H), 2.60 (dd, 1H), 2.39 (m, 8H), 2.17 (s, 3H), 1.82(m, 1H).

(*S*)-3-((4-(3-((4-methylpiperazine-1-)methyl)-2-oxopyrrolidin-1-yl)phenylamino)(phenyl)methylene)-2-oxoindoline-6-carboxylate and

(*R*)-3-((4-(3-((4-methylpiperazine-1-)methyl)-2-oxopyrrolidin-1-yl)phenylamino)(phenyl)methylene)-2-oxoindoline-6-carboxylate（compound *17*b or *17*c）



The compound ***17***a was separated by chiral column (chiralpakia, 15 cm×0.46 mm, 5 μM; eluent: LDCM/MeOH/DEA=98/2/0.1; flow rate: 1 ml/min; λ=214 nm; T=35℃): t_R=3.612 (***17***b, 99.81% purity**)**; t_R=4.236 (***17***c, 98.98% purity**)**. The resolution of ***17***b and***17***c was 1.97.

(*Z*)-N-methyl-2-(4-methylpiperazine-1-yl)-N-(4-((6-trifluoromethoxy)-2-oxoindoline -3-methylene)(phenyl)methylamino)phenyl)acetamide (Compound *21*b)

Step 1: N-(3-(trifluoromethoxy)phenyl)acetamide

A solution of sodium sulfate (10.0 g) in 120 mL of water was stirred until all the solids were dissolved. A solution of 4-(trifluoromethoxy)aniline (7.0 g, 40 mmol) in 60 mL of 1N aqueous HCl and 12 mL of EtOH was added to the resulting aqueous solution. The mixture was stirred and chloral hydrate (7.2 g, 44 mmol) was added. The resulting mixture was used for the next step without further treatment.

Step 2: (E)-2-(hydroxyimino)-N-(3-(trifluoromethoxy)phenyl)acetamide

A solution of hydroxylamine hydrochloride (9.2 g, 132 mmol) in 40 mL of water was added to the above solution of N-(3-(trifluoromethoxy)phenyl)acetamide. The mixture was heated with stirring to a gentle reflux until the solids were dissolved, and heating was continued for an additional 15 min. The resultant was poured on to 600 g of ice, stirred, and the product was precipitated from solution, collected by suction filtration, washed with water and dried to give (E)-2-(hydroxyimino)-N-(3-(trifluoromethoxy)phenyl)acetamide as yellow solid (8.5 g, 86%).

Step 3: 6-(trifluoromethoxy)indoline-2,3-dione

(E)-2-(hydroxyimino)-N-(3-(trifluoromethoxy)phenyl)acetamide (8.5 g, 34 mmol) was slowly added to 80 mL of concentrated sulphuric acid at 100℃. The reaction mixture was heated for 1 h and the resultant solution was poured into 560 g of ice and water, stirred for 1 h and filtered. The solid was washed with water and dried to give 6-(trifluoromethoxy)indoline-2,3-dione as brown solid (3.0 g, 38%).

Step 4: 6-(trifluoromethoxy)indolin-2-one (***18***b)

A stirred solution of 6-(trifluoromethoxy)indoline-2,3-dione (3.0 g, 13.0 mmol) in of hydrazine hydrate(60 mL) was heated to 140℃ for 4 h. The reaction mixture was cooled to room temperature, poured into 300 mL of ice water, and acidified to pH=2 with 6N hydrochloric acid. After standing at RT for 1 day the precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give 6-(trifluoromethoxy)indolin-2-one (1.1 g , 40%)

Step 5: 1-acetyl-6-(trifluoromethoxy)indolin-2-one

A solution of 6-(trifluoromethoxy)indolin-2-one (1.1 g, 5mol) in Acetic anhydride (20 mL) was stirred at 115℃ for 8 h. After cooled to RT, the resulting mixture was concentrated. The residue was purified by column(EtOAc: Petroleum ether=0~1:3) to give the product 1-acetyl-6-(trifluoromethoxy)indolin-2-one as brown solid (1.0 g, 75%).

Step 6: (E)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(trifluoromethoxy)indolin-2-one

A solution of 1-acetyl-6-(trifluoromethoxy)indolin-2-one (1.0 g, 3.9 mmol) in acetic anhydride (20 mL) was heated at 110℃. Trimethylorthobenzoate (2.1 g, 11.7 mmol) was then added dropwise over a period of 30 min. The mixture was stirred at 120℃ for 13 h. After cooled to RT, the mixture was concentrated. The residue was purified by column (EtOAc: Petroleum ether=0~1:3) to give the product (E)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(trifluoromethoxy)indolin-2-one as brown solid (800 mg, 54%).

Step 7: (E)-3-(methoxy(phenyl)methylene)-6-(trifluoromethoxy)indolin-2-one (***19*b**)

To a suspension of (E)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(trifluoromethoxy)indolin-2-one (800 mg, 2.1 mmol) in methanol (5 mL) was added KOH (40 mg, 0.7 mmol) in methanol (0.4 mL) dropwise at RT. The mixture was stirred at room temperature for 3 h and concentrated. The residue was then purified by column (EtOAc: Petroleum ether=0~1:2) to give (E)-3-(methoxy(phenyl)methylene)-6-(trifluoromethoxy)indolin-2-one as brown solid (650 mg, 91%).

Step 8: (Z)-N-methyl-2-(4-methylpiperazine-1-)-N-(4-((6-trifluoromethoxy)-2-oxoindoline -3-methylene)(phenyl)methylamino)phenyl)acetamide (Compound *21*b)

To a solution of (Z)-3-(methoxyl(phenyl)methylene)-6-trifluoromethoxyindole-2-ketone (350 mg, 1.0 mmol) in methanol (20 mL) was added N-(4-aminophenyl)-N-methyl-2-(1-methylpiperazin-4-yl)acetamide (262 mg, 1.0 mmol). The reaction mixture was allowed to stir at 70℃ for 13 h. The resulting solution was concentrated to give the crude product, which was then re-crystallized in ethyl acetate to give compound ***21***b as pale yellow solid (400 mg, 71% yield, 98.74% purity). LCMS(m/z) for $C_{30}H_{30}F_3N_5O_3$ 565.2 (M+1). ^{1}H-NMR (DMSO-d6, 400MHz, δ; ppm): 11.98 (s, 1H), 10.94 (s, 1H), 7.57(m, 3H), 7.53 (m, 2H), 7.10 (d,

J=8.4Hz, 2H), 6.84 (d, J=8.4Hz, 2H), 6.78 (d, J=0.8Hz, 1H), 6.54(m,1H), 5.75(d, J=8.4Hz, 1H), 3.03 (brs, 3H), 2.65 (brs, 2H), 2.12-2.22 (m, 6H), 2.09(m, 5H).

(*Z*)-N-methyl-2-(4-methylpiperazine-1-)-N-(4-((6-(1,2,4-oxadiazole-5-)-2-oxoindoline-3-methylene)(phenyl)methylamino)phenyl)acetamide (Compound *21*c)

Step 1: dimethyl 2-(4-cyano-2-nitrophenyl)malonate

To a stirred solution of potassium tert-butoxide (85.92 g, 767.12 mmol) in NMP (500 mL), was added dimethyl malonate (101.26 g, 767.12 mmol) dropwise over a period of 30 min. The mixture was stirred for another 1 h and 4-chloro-3-nitrobenzonitrile (70.00 g, 383.56 mmol) was then added in potions over a period of 30 min. The mixture was stirred at 90℃ for 1 h. After cooled to RT, the mixture was adjusted to pH=2 using 2N HCl, and filtered to give dimethyl 2-(4-cyano-2-nitrophenyl)malonate (90.2 g yield: 84.6%).

Step 2: dimethyl 2-(4-carbamoyl-2-nitrophenyl)malonate

A mixture of compound dimethyl 2-(4-cyano-2-nitrophenyl)malonate (20 g, 71.94 mmol) in concentrated sulfuric acid (50 mL) was stirred for 16 h. After the reaction completed, the mixture was poured into 500 mL cooled water and extracted with DCM, washed with water for twice with dried anhydrous Na_2SO_4, evaporated in vacuum to give dimethyl 2-(4-carbamoyl-2-nitrophenyl)malonate (21 g, 98.6%).

Step 3: (Z)-dimethyl 2-(4-((dimethylamino)methylenecarbamoyl)-2-nitrophenyl)malonate

A mixture of compound dimethyl 2-(4-carbamoyl-2-nitrophenyl)malonate (20 g, 67.56 mmol) in DMF-DMA (20 mL) was refluxed for 4 h, and cooled down to 0℃. Afterwards, the mixture was filtered to give (Z)-dimethyl 2-(4-((dimethylamino)methylenecarbamoyl)-2-nitrophenyl)malonate (19.1 g , 80.54%).

Step 4: dimethyl 2-(2-nitro-4-(1,2,4-oxadiazol-5-yl)phenyl)malonate

To a solution of hydroxylamine hydrochloride (2.45 g, 35.41 mmol) in a mixture of aqueous 5N sodium hydroxide solution (6.9 mL, 34.5 mmol), acetic acid (69 mL) and dioxane (59 mL) was added the compound (Z)-dimethyl 2-(4-((dimethylamino)methylenecarbamoyl)-2-nitrophenyl)malonate

(10 g, 29.51 mmol). The reaction was stirred at 90℃ for 2 h. Upon cooling to RT, the solvent was removed under vacuum and the residue was purified by column chromatography eluting with 20% ethyl actate/hexanes to give dimethyl 2-(2-nitro-4-(1,2,4-oxadiazol-5-yl)phenyl)malonate (2 g, 21.2%).

Step 5: Dimethyl 2-(2-amino-4-(1,2,4-oxadiazol-5-yl)phenyl)malonate

A mixture of dimethyl 2-(2-nitro-4-(1,2,4-oxadiazol-5-yl)phenyl)malonate (2 g, 6.23 mmol) and $SnCl_2 \cdot 2H_2O$ (4 g, 18.65 mmol) in ethyl acetate was stirred for 17 h, then washed with water (250 mL), dried with anhydrous Na_2SO_4, evaporated to give dimethyl 2-(2-amino-4-(1,2,4-oxadiazol-5-yl)phenyl)malonate for the next step without purification.

Step 6: 6-(1, 2, 4-oxadiazol-5-yl)indolin-2-one (*18*c)

Dimethyl 2-(2-amino-4-(1,2,4-oxadiazol-5-yl)phenyl)malonate from the last step in acetic acid was allow to react at 95℃ for 2 h. Upon cooling to RT, the mixture was filtered to give 6-(1, 2, 4-oxadiazol-5-yl)indolin-2-one (0.45 g, 35.6%).

Step 7: 1-acetyl-6-(1, 2, 4-oxadiazol-5-yl)indolin-2-one

A mixture of compound 6-(1,2,4-oxadiazol-5-yl)indolin-2-one (0.45 g, 2.23 mmol) in acetic anhydride (10 mL) was allow to react for 4 h, then cooled down to RT for the next step.

Step 8: (Z)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-5-yl)indolin-2-one (*19*c)

To a solution of the last step was added methyl orthobenzoate (0.608 mg, 3.345 mmol) dropwise. Afterwards, the mixture was allowed to react at 90℃ for 2 h. The solution was cooled down to RT and filtered to give (Z)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-5-yl)indolin-2-one (0.3 g, 37.3 %)

Step 9:

(Z)-N-(4-((1-acetyl-6-(1,2,4-oxadiazol-5-yl)-2-oxoindolin-3-ylidene)(phenyl)methylamino)phenyl)-N-methyl-2-(4-methylpiperazin-1-yl)acetamide

A mixture of compound of (Z)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-5-yl)indolin-2-one (0.30 g, 0.83 mmol) and compound ***20*** (0.26 g, 0.996 mmol) in MeOH (10 mL)was refluxed at 70℃ for 2hrs. The mixture

was cooled down to RT for the next step.

Step 10:

(*Z*)-N-methyl-2-(4-methylpiperazine-1-)-N-(4-((6-(1,2,4-oxadiazole-5-)-2-oxoindoline-3-methylene)(phenyl)methylamino)phenyl)acetamide (Compound *21*c)

To the reaction mixture wad added KOH(15.5 mg, 0.28 mmol) slowly. The mixture was allowed to stir for 3 h. The resulting solution was concentrated to give the crude product, which was then purified by silica gel column(eluent: DCM/MeOH=10:1) to give ***21*c** as yellow solid (80 mg, 17.6% yield, 83.45% purity). LCMS(m/z) for $C_{31}H_{31}N_7O_3$ 549.2 (M+1). ^{1}H-NMR (DMSO-d6, 400MHz, δ; ppm): 12.23 (s, 1H), 8.91 (brs, 1H), 8.41 (s, 1H), 7.71 (s, 1H), 7.55-7.59 (m, 3H), 7.41-7.49 (m, 3H), 6.97 (d, 2H), 6.82 (d,2H), 6.08(d, 1H), 3.12 (s, 3H), 2.84 (s, 2H), 2.57-2.64 (m, 8H), 2.42 (s, 3H).

(*Z*)-N-methyl-2-(4-methylpiperazine-1-yl)-N-(4-((6-(1,2,4-oxadiazole-3-yl)-2-oxoindoline-3-methylene)(phenyl)methylamino)phenyl)acetamide (Compound *21*d)

Step 1: (Z)-dimethyl 2-(4-(N'-hydroxycarbamimidoyl)-2-nitrophenyl)malonate

A mixture of dimethyl 2-(4-cyano-2-nitrophenyl)malonate (5.0g, 18 mmol), hydroxylamine hydrochloride (2.48 g, 36 mmol) and Na_2CO_3 (9.5 g, 90 mmol) in EtOH (150 mL) was stirred at 70℃ for 5 h in a sealed tube. After cooled to RT, the mixture was filtered. The filtrate was concentrated to give (Z)-dimethyl 2-(4-(N'-hydroxycarbamimidoyl)-2-nitrophenyl)malonateas orange solid (5.6 g, 100%).

Step 2: dimethyl 2-(2-nitro-4-(1,2,4-oxadiazol-3-yl)phenyl)malonate

To a stirred mixture of (Z)-dimethyl 2-(4-(N'-hydroxycarbamimidoyl)-2-nitrophenyl)malonate (5.6 g, 18 mmol) and trimethylorthoformate (7.6 g, 72 mmol) in THF (150 mL) was added $BF_3 \cdot Et_2O$ (3.1 mL, 21.6 mmol) dropwise at 0℃. Then, the mixture was stirred at 30℃ overnight. The resulting mixture was concentrated. The residue was purified by column (EtOAc/PE=0~1/5) to give dimethyl 2-(2-nitro-4-(1,2,4-oxadiazol-3-yl)phenyl)malonate as yellow oil (1.5 g, 26%).

Step 3: methyl 2-(2-amino-4-(1, 2, 4-oxadiazol-3-yl)phenyl)acetate

To a solution of dimethyl 2-(2-nitro-4-(1, 2, 4-oxadiazol-3-yl)phenyl)malonate (1.5 g, 4.7 mmol) in

EtOAc (200 mL) was added $SnCl_2 \cdot 2H_2O$ (5.3 g, 23.4 mmol). The reaction was stirred at RT for 48 h. The resulting solution was neutralized with saturated $NaHCO_3$. The resulting mixture was diluted with water (200 mL), filtered and extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine (200 mL), dried (Na_2SO_4) and concentrated to give methyl 2-(2-amino-4-(1, 2, 4-oxadiazol-3-yl)phenyl)acetate as yellow oil (1.0 g, 91%).

Step 4: 6-(1, 2, 4-oxadiazol-3-yl)indolin-2-one (*18*d)

A solution of methyl 2-(2-amino-4-(1, 2, 4-oxadiazol-3-yl)phenyl)acetate (1.0 g, 4.3 mmol) in acetic acid (20 mL) was stirred at 90℃for 1 h. After cooled to RT, the solution was concentrated. The residue was purified by column (EtOAc/PE=0~1/2) to give compound 6-(1, 2, 4-oxadiazol-3-yl)indolin-2-one as pale-white (700 mg, 61%).

Step 5: 1-acetyl-6-(1, 2, 4-oxadiazol-3-yl)indolin-2-one

A solution of 6-(1,2,4-oxadiazol-3-yl)indolin-2-one (700 mg, 3.5 mmol) in acetic anhydride (20 mL) was stirred at 110℃ for 2 h. After cooled to RT, the solution was concentrated to give 1-acetyl-6-(1,2,4-oxadiazol-3-yl)indolin-2-one as brown solid (850 mg, 100%).

Step 6: (E)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-3-yl)indolin-2-one

A suspension of 1-acetyl-6-(1,2,4-oxadiazol-3-yl)indolin-2-one (850 mg, 3.5 mmol) in acetic anhydride (50 mL) was heated to 110℃. Then, trimethylorthobenzoate (1.9 g, 10.5 mmol) was added dropwise over a period of 30 min. The mixture was stirred at 120℃ for 13 h. After cooled to RT, the mixture was concentrated. The residue was purified by column (EtOAc/PE=0~1/4) to give (E)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-3-yl)indolin-2-one as brown solid (600 mg, 47%).

Step 7: (E)-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-3-yl)indolin-2-one (*19*d)

To a solution of (E)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-3-yl)indolin-2-one (600 mg, 1.66 mmol) in methanol (10 mL) was added KOH (31 mg, 0.55 mmol) in methanol (0.31 mL) dropwise at RT. The mixture was stirred at RT for 2 h and then concentrated. The residue was purified by column (EtOAc/PE=0~1/2) to give (E)-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-3-yl)indolin-2-one as yellow solid (200 mg,

38%).

Step 8:

(*Z*)-N-methyl-2-(4-methylpiperazine-1-yl)-N-(4-((6-(1,2,4-oxadiazole-3-yl)-2-oxoindoline-3-methylene)(phenyl)methylamino)phenyl)acetamide (Compound *21*d)

To a solution of (E)-3-(methoxy(phenyl)methylene)-6-(1,2,4-oxadiazol-3-yl)indolin-2-one (200 mg, 0.62 mmol) in methanol (20 mL) was added N-(4-aminophenyl)-N-methyl-2-(4-methylpiperazin-1-yl)acetamide (160 mg, 0.61mmol). The reaction mixture was allowed to stir for 13 h at 70℃. The resulting solution was cooled to RT and concentrated under reduce pressure to give the crude product. The residue was purified by silica gel column (eluent: EtOAc:PE=0~80:100) to give ***21*d** as yellow solid (90 mg, 26% yield, 99.27% purity). LCMS(m/z) for $C_{31}H_{31}N_7O_3$ 549.2 (M+1). ^{1}H-NMR (DMSO-d6, 400MHz, δ; ppm): 12.20(s, 1H), 11.10 (s, 1H), 7.40-7.65(m, 6H), 7.10-7.18 (m, 3H), 6.98 (d, 1H), 6.89 (d, 2H), 5.78 (d,1H), 3.04(s, 3H), 2.66(s, 2H), 2.08-2.16(m, 11H).

(Z)-N-methyl-N-(4-((6-(2-methyl-2H-tetrazol-5-yl)-2-oxoindolin-3-ylidene)(phenyl)methylamino)phenyl)-2-(4-methylpiperazin-1-yl)acetamide (Compound *21*e) and its chloride

Step 1: Dimethyl 2-(4-cyano-2-nitrophenyl)malonate

To a stirred solution of potassium tert-butoxide (85.92 g, 767.12 mmol) in NMP (500 mL) was added dimethyl malonate (101.26 g, 767.12 mmol) dropwise over a period of 30 min. The mixture was stirred for another 1 h and 4-chloro-3-nitrobenzonitrile (70.00 g, 383.56 mmol) was added in potions over a period of 30 min. The mixture was stirred at 90℃ for 1 h. After cooled to RT, the mixture was adjusted to pH=2 using 2N HCl, and filtered to give dimethyl 2-(4-cyano-2-nitrophenyl)malonate (90.2 g, 84.6%).

Step 2: methyl 2-(4-cyano-2-nitrophenyl)acetate

A mixture of compound dimethyl 2-(4-cyano-2-nitrophenyl)malonate (70 g, 251.7 mmol) and LiCl (21.15 g, 503.4 mmol) was stirred at 90℃ for 4 h. Afterwards, the mixture was cooled down to RT, extracted with DCM and then washed with water for 2 times. The organic layer was dried with

anhydrous Na_2SO_4, evaporated in vacuum to give methyl 2-(4-cyano-2-nitrophenyl)acetate (50.0 g, 90%)

Step 3: methyl 2-(2-amino-4-cyanophenyl)acetate

A mixture of compound methyl 2-(4-cyano-2-nitrophenyl)acetate (50 g, 227 mmol) and Pd/C (2 g) in THF was stirred under H_2 atmosphere for 38 h. Afterwards, the mixture was filtered and then the solvent was evaporated in vacuum to give methyl 2-(2-amino-4-cyanophenyl)acetate (30.2 g 70%).

Step 4: 2-oxoindoline-6-carbonitrile

A mixture of methyl 2-(2-amino-4-cyanophenyl)acetate (30g, 157.89 mmol) in acetic acid was stirred at 95℃ for 2 h. Upon cooling to RT, the mixture was filtered to give 2-oxoindoline-6-carbonitrile (22.2 g, 89%)

Step 5: 1-acetyl-2-oxoindoline-6-carbonitrile

A mixture of 2-oxoindoline-6-carbonitrile (4 g, 25.31 mmol) in acetic anhydride (10 mL) was stirred for 4 h. The mixture was then cooled down to RT and filtered to give 1-acetyl-2-oxoindoline-6-carbonitrile (2 g, 39.5%).

Step 6: (Z)-1-acetyl-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carbonitrile

To a solution of 1-acetyl-2-oxoindoline-6-carbonitrile (2 g, 10 mmol) was added methyl orthobenzoate (2.73g, 15 mmol) dropwise. The mixture was allowed to react at 90℃ for 2 h. The solution was cooled down to RT and then filtered to give (Z)-1-acetyl-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carbonitrile (2g , 62.9%).

Step 7:
(Z)-N-(4-((1-acetyl-6-cyano-2-oxoindolin-3-ylidene)(phenyl)methylamino)phenyl)-N-methyl-2-(4-methylpiperazin-1-yl)acetamide

A mixture (Z)-1-acetyl-3-(methoxy(phenyl)methylene)-2-oxoindoline-6-carbonitrile (1 g, 3.14 mmol) and compound ***20*** (0.998 g, 3.77 mmol) in MeOH (10 mL) was refluxed at 70℃ for 2 h. The mixture was cooled down to RT for the next step.

Step 8:
(Z)-N-(4-((6-cyano-2-oxoindolin-3-ylidene)(phenyl)methylamino)phenyl)-N-methyl-2-(4-methylpiperazin-1-yl)acetamide

To the mixture of the last solution was added KOH (0.057 g, 1.03 mmol). Afterwards, the mixture was allowed to react for 3 h, filtered to give (Z)-N-(4-((6-cyano-2-oxoindolin-3-ylidene)(phenyl)methylamino)phenyl)-N-methyl-2-(4-methylpiperazin-1-yl)acetamide (1.2 g, 75.5%)**.**

Step 9:

(Z)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((2-oxo-6-(2H-tetrazol-5-yl)indolin-3-ylidene)(phenyl)methylamino)phenyl)acetamide

A mixture of (Z)-N-(4-((6-cyano-2-oxoindolin-3-ylidene)(phenyl)methylamino)phenyl)-N-methyl-2-(4-methylpiperazin-1-yl)acetamide (1 g ,1.96 mmol) and azidotributyltin (3.25 g 9.8 mmol) in methylbenzene (40 mL) was allowed to stir at 150℃ for 2 h. The resulting mixture was cooled down to RT and filtered to give(Z)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((2-oxo-6-(2H-tetrazol-5-yl)indolin-3-ylidene)(phenyl)methylamino)phenyl)acetamide (0.8 g, 74.3 %)

Step 10:

(Z)-N-methyl-N-(4-((6-(2-methyl-2H-tetrazol-5-yl)-2-oxoindolin-3-ylidene)(phenyl)methylamino)phenyl)-2-(4-methylpiperazin-1-yl)acetamide (Compound *21*e) and its chloride

To a solution of (Z)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((2-oxo-6-(2H-tetrazol-5-yl)indolin-3-ylidene)(phenyl)methylamino)phenyl)acetamide (276 mg, 0.77 mmol) in methanol (20 mL) at 40℃ was added KOH (21.5 mg, 0.38 mmol in 5 mL methanol) slowly with stirring for 0.5 h, followed by cooled to RT. N-(4-aminophenyl)-N-methyl-2-(1-methylpiperidin-4-yl)acetamide (200 mg, 0.76 mmol) was then added to the reaction mixture, which was allowed to stir at 70℃ for 10 h. The mixture was cooled to RT, concentrated under reduce pressure. The residue was purified by silica gel column (eluent: DCM/methanol =60:1) to give ***21*e** as yellow solid. The obtained product was dissolved in 20 mL methanol, then 2 drops of concentrated hydrochloric acid and DCM (2 mL) were added into the reaction mixture respectively. The mixture was allowed to stir at RT for 1 h. Then the solvent was removed under reduced pressure to give its chloride as yellow solid (100 mg, 24% yield, 98.11%

purity). LCMS (m/z) for $C_{32}H_{32}N_6O_3$ 548 (M+1).^{1}H-NMR ($C_{32}H_{32}N_6O_3$·HCl, DMSO-d6, 400MHz, δ; ppm): 12.20 (s, 1H), 11.10 (s, 1H), 7.40-7.65(m, 6H), 7.10-7.18 (m, 3H), 6.98 (d, 1H), 6.89 (d, 2H), 5.78 (d,1H), 3.04 (s, 3H), 2.66 (s, 2H), 2.08-2.16 (m, 11H).

((Z)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((6-(oxazol-5-yl)-2-oxoindolin-3-ylidene)(phenyl) methylamino)phenyl)acetamide (Compound *21*f)

Step 1: p-aminobenzamide

4-nitrophenylimidate (4 g, 26.29 mmol) was allowed to be reduced under H_2 atmosphere, resulting in p-aminobenzamide (2.5g , 78%).

Step 2: Tert-butyl (4-methylaminophenyl)amidemethylformate

To a solution of p-aminobenzamide (2.5 g, 20.46 mmol) in THF (50 mL) was added K_2CO_3 (5.66 g，40.93 mmol) and Boc_2O (4.47 g，20.46 mmol). The mixture was allowed to react at RT for 2 h. The resulting mixture was concentrated and then purified by column to give tert-butyl (4-methylaminophenyl)amidemethylformate (2 g, 44%).

Step 3: Tert-butyl 4-(N-methyl-2-(4-methylpiperazin-1-yl)acetamido)phenylcarbamate

To a solution of tert-butyl (4-Methylaminophenyl)amidemethylformate (1 g, 4.5 mmol) in THF (20 mL) was added 2-(4-methylpiperazin-1-yl)acetic acid (707 mg, 4.5 mmol), HATU (2.56 g，6.75 mmol) and K_2CO_3 (1.24 g，9 mmol). The reaction mixture was allowed to stir at 70 ℃ for 4 hr. The organic solvent was then evaporated and the product was purifed by column to give compound tert-butyl 4-(N-methyl-2-(4-methylpiperazin-1-yl)acetamido)phenylcarbamate (700 mg, 43%).

Step 4: N-(4-aminophenyl)-N-methyl-2-(1-methylpiperazin-4-yl)acetamide

Tert-butyl(4-(N-methy-2(1-Methylpiperazin-4-yl)acetamido)phenyl)amidemethylformat-e (700 mg，1.9 mmol) was allowed to react in TFA (10 mL) at 60℃ for 2 h. The solvent was removed under

vacuum to give N-(4-aminophenyl)-N-methyl-2-(1-methylpiperazin-4-yl)acetamide (400 mg, 79%).

Step 5:

(Z)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((6-(oxazol-5-yl)-2-oxoindolin-3-ylidene)(phenyl) methylamino)phenyl)acetamide (Compound *21*f)

To a solution of (*Z*)-1-acetyl-3-(methoxy(phenyl)methylene)-6-(oxazol-5-yl)indolin-2-one (280 mg, 0.78 mmol) in methanol (5 mL) at 40℃ was added KOH (22 mg, 0.4 mmol in 5 mL methanol) slowly with stir for 0.5 h, followed by cooled to RT. 1-(2-(4-methylpiperazin-1-yl)acetyl)-4-aminopiperazin (210 mg, 0.8 mmol) was then added to the reaction mixture, which was allowed to stir at 50℃ for 3 h. The resulting mixture was concentrated under reduce pressure. The residue was purified by silica gel column (eluent: DCM/methanol =60:1) to give ***21*f** as yellow solid (57 mg, 13% yield, 92.84% purity). LCMS (m/z) for $C_{32}H_{32}N_6O_3$ 548.2 (M+1).^{1}H-NMR (DMSO-d6, 400MHz, δ; ppm): 2.25(s, 3H), 2.42~2.47(m, 8H), 2.79 (s, 2H), 3.18 (s, 3H), 6.02 (d, 1H), 6.78(d, 2H), 6.98 (t, 3H), 7.20(d, 2H), 7.46(d, 2H), 7.52~7.59(m, 3H), 7.76(s, 1H), 7.86(s, 1H),12.01(s, 1H).

(*Z*)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((6-(furan-2-yl)-2-oxoindoline-3-methylene)(phen yl)methylamino)phenyl)acetamidinehydrochloride (Compound *21*g)

Step 1: Intermediate *21*a

***21*a** was prepared according to the previously published approach [1](as described in Scheme 3).

Step 2: Intermediate *22*

To a 50 mL round-bottomed flask were added (*Z*)-N-(4-((6-bromo-2-oxoindolin-3-ylidene)(phenyl)methylamino)phenyl)-N-methyl-2-(4-methylpipe razin-1-yl)acetamide (3.0 g, 5.3 mmol), bis(pinacolato)diboron (1.43 g, 5.6 mmol), KOAc (553 mg, 5.6 mmol), $PdCl_2$(dppf) (300 mg, 10%) and dioxane (16 mL). The mixture was stirred for 6 h at 100℃ under N_2 protection. The mixture was cooled to RT and then filtered. The filtrate was concentrated to dryness under reduce pressure. The solid was washed with methanol to give ***22*** as yellow solid (3.0 g, 92%).

Step 3:

(*Z*)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((6-(furan-2-yl)-2-oxoindoline-3-methylene)(phenyl)methylamino)phenyl)acetamidinehydrochloride (Compound *21*g)

To a solution of (*Z*)-N-methyl-N-(4-((6-borate-2-oxoindoline-3-ylmethylene)(phenyl)methylamino)(phenyl)-2-(4-methylpiperazin-1-yl)acetamide (121 6mg, 2 mmol) in dioxane (16 mL) was added water (1 mL), 2-bromofuran (900 mg, 6 mmol), Cs_2CO_3 (1960 mg, 6 mmol) and PdCl2(dppf) (120 mg, 10%). The reaction mixture was allowed to stir at 100℃ for 6h, and filtrated, concentrated to give the crude product. The residue was purified by silica gel column (eluent: DCM/MeOH=50:1) to give ***21***h as yellow solid (150 mg, 13.5% yield, purity 93.78%). The resulting product was dissolved in the mixture of 12 mL DCM and 2 mLmethanol and then 0.2 mL concentrated hydrochloric acid were added slowly. The above mixture was allowed to stir for 0.5 h. The resulting solution was then concentrated to give its chloride as yellow solid. LCMS (m/z) for $C_{33}H_{33}N_5O_3$·HCl 584.1 (M+1). ^{1}H-NMR ($C_{33}H_{33}N_5O_3$·HCl, DMSO-d6, 400MHz, δ; ppm): 2.74 (s, 3H), 3.09(m, 4H),3.15(m, 4H), 3.10(s, 2H), 3.48(s, 3H), 5.77(d, 1H), 6.51(d, 1H), 6.73(d, 1H), 6.83(m, 2H),6.93(d, 1H), 7.15(m, 3H), 7.54(d, 2H),7.62(m, 4H),10.88(s, 1H),12.06(s, 1H).

(*Z*)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((6-(4*H*-1,2,4-triazole-3-yl)-2-oxoindoline-3-ylmethylene)(phenyl)methylamino)phenyl)acetamidinehydrochloride (Compound *21*h)

Step 1: 3-chlorine-4-(4-methoxybenzyl)-4*H*-1,2,4-triazole

To a solution of 3-chlorine-4*H*-1,2,4-triazole (3 g, 29 mmol) and K_2CO_3 (5.25 g, 38 mmol) in DMF (30 mL) was added 4-bromomethylanisole (8.74 g, 44 mmol) slowly. The reaction mixture was allowed to stir at RT for 6 h, then filtrated and concentrated to give the crude product. The residue was then purified by silica gel column (eluent: DCM/MeOH=50:1) to give 3-chlorine-4-(4-methoxybenzyl)-4*H*-1,2,4-triazoleas white solid (5 g, 76.9%).

Step 2:

(*Z*)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((6-(4-(4-methoxybenzyl)-4*H*-1,2,4-triazole-3-yl)-2-oxoindoline-3-ylmethylene)(phenyl)methylamino)phenyl) acetamidine

To a solution of (*Z*)-N-methyl-N-(4-((6-borate-2-oxoindoline-3-ylmethylene)(phenyl)methylamino)(phenyl)-2-(4-methylpiperazin-1-yl)acetamide (***22***, 608 mg, 1 mmol) in dioxane (16 mL) was added water (1 mL), 3-chlorine-4-(4-methoxybenzyl)-4*H*-1,2,4-triazole(669mg,3mmol), Cs_2CO_3 (980 mg, 3 mmol) and $PdCl_2$(dppf) (60 mg, 10%). The reaction mixture was allowed to stir at 100 ℃ for 6 h. Then the mixture was filtrated and concentrated to give the crude product. The residue was purified by silica gel column (eluent: DCM/MeOH=50:1) to give (*Z*)-N-methyl -2-(4-methylpiperazin -1-yl)-N-(4-((6-(4-(4-methoxybenzyl)-4*H*-1,2,4-triazole-3-yl)-2-oxoindoline-3-ylmethylene)(phenyl) methylamino)phenyl) acetamidineas yellow solid (200 mg, 30%).

Step 3:

(*Z*)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((6-(4*H*-1,2,4-triazole-3-yl)-2-oxoindoline-3-ylmethylene)(phenyl)methylamino)phenyl)acetamidinehydrochloride (Compound 21h)

(*Z*)-N-methyl-2-(4-methylpiperazin-1-yl)-N-(4-((6-(4-(4-methoxybenzyl)-4*H*-1,2,4-triazole-3-yl)-2-oxoindoline-3-ylmethylene)(phenyl)methylamino)phenyl) acetamidine was dissovled in TFA (10 mL). The resulting solution was heated in a microwave at 110℃ for 0.5 h, then cooled to RT and concentrated to give the crude product. The residue was purified by silica gel column(eluent:DCM:MeOH:Et_3N=10:1:0.05) to give (*Z*)-N-methyl -2-(4-methylpiperazin-1-yl)-N-(4-((6-(4-(4-methoxybenzyl)-4*H*-1,2,4-triazole-3-yl)-2-oxoindoline-3-ylmethylene)(phenyl)methylamino)phenyl) acetamidine as yellow solid (120 mg, 35.8% yield, purity 95.45%). Its chloride was prepared in the same manner as described for ***21*** h chloride. LCMS (m/z) for $C_{31}H_{32}N_8O_2$ 549.3 (M+1). ^{1}H-NMR (DMSO-d6, 400MHz, δ; ppm): 2.74 (s, 3H), 3.09(m, 4H), 3.15(m, 4H), 3.35(s, 2H), 3.60(s, 3H), 5.83(d, 1H), 6.85(d, 2H), 7.20(d, 2H), 7.31(d, 1H), 7.65(m, 7H), 8.64(s, 1H),11.08(s, 1H),12.22(s, 2H).

Assay protocols for in vitro activity and PK profiles

In vitro kinase activity assays

The kinase inhibitory activities of KBP-7018 against 64 purified recombinant protein tyrosine kinases were examined by using an Off-Chip Mobility Shift Assay (MSA, Shanghai ChemPartner Co., Ltd, China) and anKinase-Glo Luminescent Assay (for PI3Ka and PI3Kδ assay only, Shanghai ChemPartner Co., Ltd, China) followed manufactory's protocols. Briefly, each test compound was mixed with enzyme, substrate, ATP, and Mg under appropriate buffer conditions for the MSA or ELISA. The readout value of the reaction control (complete reaction mixture) was set as 0% inhibition, and the readout value of the background (Enzyme (−)) was set as 100% inhibition; the percent inhibition of each test solution was then calculated. IC_{50} values (the half maximal inhibitory concentration) were calculated from concentrations versus % inhibition curves.

In vitro cellular activity assays

NIH3T3 cells (from ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) with L-glutamine supplemented with 10% calf serum (Gibco Lot# 8155322) and 100 units/ml penicillin, 100 μg/ml streptomycin. For activity assay, 3T3 cells were seeded plated into a 96-well plate (5000 cells/well) and allowed to attach overnight before being serum starved (0.5% calf serum, 24 h). Cells were then exposed to stimuli of recombinant human PDGF-BB (SBH Sciences Internal Standard) in the presence and absence of investigated compounds (0.002, 0.005, 0.014, 0.041, 0.123, 0.370, 1.111, 3.333 and 10 μM) for 42 hours before cell proliferation assay by Promega's Substrate Cell Titer 96 Aqueous One Solution (SBH Sciences).

In vivo animal model and experimental treatments

Adult C57 male mouse were purchased from Beijing Vital River Laboratories, Inc. This study was approved by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Science at Institute of Materia Medica Chinese Academy of Medical Sciences & Peking Union Meidcal College. All procedures on animals followed the guidelines for human treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Science at Institute of Materia Medica Chinese Academy of Medical Sciences & Peking Union Meidcal College.

For the induction of pulmonary fibrosis, mice were intratracheally injected with bleomycin (BLM, Nippon Kayaku, 5.0 mg/kg in 50 μL saline). To investigate the effects of compound *3* on BLM-induced pulmonary fibrosis, mice were grouped and lavage with KBP 7018 (10, 30 or 100 mg/kg) daily daily from day 0 to day 28. Thus, mice received a lavage saline and nintedanib (30 or 100 mg/kg) daily as a negative control and positive control. During the period of 28 days, the survival rate of each group was recorded.

Pharmacokinetics

The studies involving the CD-1 mice, SD male rats and beagle dogs were purchased from Beijing Vital River (mice and rats, China) and Marshall BioResource Inc. (dogs, Beijing, China) respectively. All the protocols were in accordance with the guidelines for laboratory animal care and use of KBP Biosciences Co. Ltd. The animals were single administered intravenously dosing at 10.0, 2.0 and 2.0 mg/kg of KBP 7018 for mice, rats and dogs respectively. To evaluate the oral bioavailability of molecules in above animal models, KBP 7018 was single administered orally by gavage at the dose of 50.0, 10.0 and 20.0 mg/kg for mice, rats and dogs respectively accordingly. The blood samples were taken at pre-determination time and the plasma were separated. The concentration of KBP 7018 in plasma was determined by UFLC-MS/MS (HPLC system: ShimadzuUFLC) after the samples preparations by proteins precipitation. Pharmacokinetics parameters were calculated by WinNolin 6.3.

References

1. Treu, M. K., Thomas; Reiser Ulrich, WO2010/012747A1 2010.

Table S1. The abbreviations of 64 kinases tested in a kinase panel.

Abbreviation			
ABL	Abl Protein Tyrosine Kinase	HER2	human epidermal growth factor receptor 2
AKT1	AKT serine/threonine kinase 1	HER4	human epidermal growth factor receptor 4
ALK	anaplastic lymphoma receptor tyrosine kinase	IGF1R	insulin-like growth factor 1 receptor
AMPKa1	AMP-activated protein kinase subunit a1	IKKβ	inhibitor of kappa B kinase beta
AURA	Aurora kinase A	INSR	Insulin receptor tyrosine kinase
AURB	Aurora kinase B	IRAK1	interleukin receptor associated kinase 1
BLK	B lymphocyte kinase	IRAK4	interleukin receptor associated kinase 2
BRAF	VRAF murine sarcoma viral oncogene homologue B1 kinase	ITK	IL2-inducible T-cell kinase
BRK	breast tumor kinase	JAK1	Janus kinase 1
BTK	Bruton' s tyrosine kinase	JAK2	Janus kinase 2
CDK2	cyclin-dependent kinase 2	JAK3	Janus kinase 3
CHK1	checkpoint kinase 1	JNK2	c-Jun N-terminal kinase 2
CK1d	casein kinase 1 delta	JNK3	c-Jun N-terminal kinase 3
CSK	C-terminal Src kinase	c-KIT	c-Kit proto-oncogene encoded protein tyrosine kinase
DYRK1a	dual-specificity tyrosine phosphorylation-regulated kinase 1a	LCK	lymphocyte-specific protein tyrosine kinase
EGFR	epidermal growth factor receptor	LYNa	LYN encoded protein tyrosine kinase a
EGFR (T790M)	epidermal growth factor receptor (T790M)	MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2
ERK2	extracellular signal-regulated kinase 2	MAPKAPK5	mitogen-activated protein kinase-activated protein kinase 5
FER	Fer protein tyrosine kinase	c-MET	MET proto-oncogene encoded protein kinase
FGR	Fgr protein tyrosine kinase	MSK1	serine/threonine protein kinase 2
FLT3	FMS-like tyrosine kinase 3	MST2	mammalian sterile 20-like kinases 1
FYN	FYN proto-oncogene encoded tyrosine kinase	NEK2	NIMA-related kinase 2
GSK3	glycogen synthase kinase	P38α	p38 mitogen-activated protein kinase α
HCK	Hemopoietic cell kinase	P38β	p38 mitogen-activated protein kinase β
p70S6K	ribosomal protein S6 kinase beta 1	PDGFRα	platelet-derived growth factor receptor α
PDK1	pyruvate dehydrogenase kinase 1	PI3Kα	phosphoinositide 3-kinase (PI3K)α
PI3Kδ	phosphoinositide 3-kinase (PI3K)δ	PKACa	PRKACA gene encoded kinase
PKCa	protein kinase C a	c-Raf	RAF proto-oncogene serine/threonine-protein kinase
RET	REarranged during Transfection (RET) proto-oncogene encoded protein tyrosine kinase	ROCK2	Rho-associated coiled-coil-containing protein kinase 2
RSK1	ribosomal protein S6 kinase alpha 1	SGK	serum/glucocorticoid regulated kinase 1
c-SRC	c-Src protein tyrosine kinase	SYK	spleen-associated tyrosine kinase
YES	YES proto-oncogene encoded tyrosine kinase	ZAP70	zeta-chain-associated protein kinase 70