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BRIEF REPORT



Discovery of novel FGFR4 inhibitors through a build-up fragment strategy

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ABSTRACT

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death. FGFR4 has been implicated in HCC progression, making it a promising therapeutic target. We introduce an approach for identifying novel FGFR4 inhibitors by sequentially adding fragments to a common warhead unit. This strategy resulted in the discovery of a potent inhibitor, **4c**, with an IC_{50} of 33 nM and high selectivity among members of the FGFR family. Although further optimisation is required, our approach demonstrated the potential for discovering potent FGFR4 inhibitors for HCC treatment, and provides a useful method for obtaining hit compounds from small fragments.

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FGFR4; small molecule; hit identification; kinase inhibitor; fragment

Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, which is the sixth most common cause of cancer-related death in 2022¹. HCC is reported to be typically developed due to chronic liver inflammation caused by hepatitis B or C infection or non-alcoholic steatohepatitis (NASH) or cirrhosis^{2–3}. Regarding therapies for advanced HCC, multi-kinase inhibitors (TKIs), sorafenib⁴ and regorafenib⁵ have been approved for clinical use. Although they significantly improve the overall survival and quality of life of patients over conventional chemotherapies, the median survival time is less than one year⁶.

The Fibroblast Growth Factor Receptor 4 (FGFR4), a member of the FGFR family with tyrosine kinase domains, is activated by its specific ligand, FGF19⁷. FGFR4 plays an important role in the regulation of cell growth, differentiation, and survival. Aberrant expression or dysregulation of FGFR4 has been observed in several solid tumours and has been implicated to the development and progression of various types of cancer, including liver, lung, and breast cancer⁸. Abnormal activation of FGF19-FGFR4 signalling is closely associated with HCC progression⁹. FGFR4 inhibitors have demonstrated promise as a potential treatment for HCC¹⁰. In a phase I trial, fsgatinib (BLU-554), a highly potent and selective FGFR4 inhibitor, showed anti-tumor activity in patients with over-expression of FGF19 in advanced HCC¹¹. FGF401, a reversible-covalent inhibitor for FGFR4, was investigated in clinical trial, and its treatment was safe and showed preliminary clinical efficacy¹². H3B-6527 is a potent and selective FGFR4 inhibitor that has shown promising results in preclinical studies¹³ and is being evaluated in clinical trials for the treatment of advanced HCC¹⁴. Therefore, inhibition of FGFR4 can be an effective strategy for treating HCC. In addition, Studies on the binding mode of the known inhibitors have suggested that the 1,3-diamino group for BLU554¹⁵ and H3B-6527¹³ or the pyridine group for FGF401¹⁶ would be placed towards the hinge region. We tried to discover

novel FGFR4 inhibitors through an extension strategy as a hit identification strategy, starting from a small and commonly used warhead moiety that was expected to be favourable for target binding. Herein, we described the discovery of novel FGFR4 inhibitors through building up from a small fragment. This strategy commenced with the addition of heterocycle, which may be involved in hydrogen bonding interactions, as shown in Figure 1.

Materials and methods

Chemistry

General information

Unless otherwise noted, all reagents and solvents were purchased from Alfa Aesar, Combi Blocks, Fisher Scientific, Samchun Pure Chemical, Sigma Aldrich, or TCI and used without additional processing. Reactions were monitored by thin-layer chromatography (TLC) using Merck TLC silica gel 60F₂₅₄ 250 μ m plates. Flash column chromatography was performed using ZEOprep 60 silica gel (Zeochem, 40–63 μ m) and a CombiFlash system (Teledyne ISCO) loaded with pre-packed silica gel flash column cartridges (Welux™). ¹H and ¹³C NMR spectra were obtained using a 600 MHz NMR spectrometer (JEOL) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm, δ) downfield of TMS, and the coupling constant (*J*) is reported in hertz (Hz). Splitting patterns are reported with the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; td, triplet of doublets; m, multiplet; b, broad signal.

Methyl 2-acrylamidobenzoate (1a)

To a solution of methyl anthranilate (130 μ L, 1 mmol) in anhydrous dichloromethane (2 mL) at 0 °C, was added acryloyl chloride (100 μ L, 1.2 mmol) and triethylamine (280 μ L, 2 mmol). Then the reaction

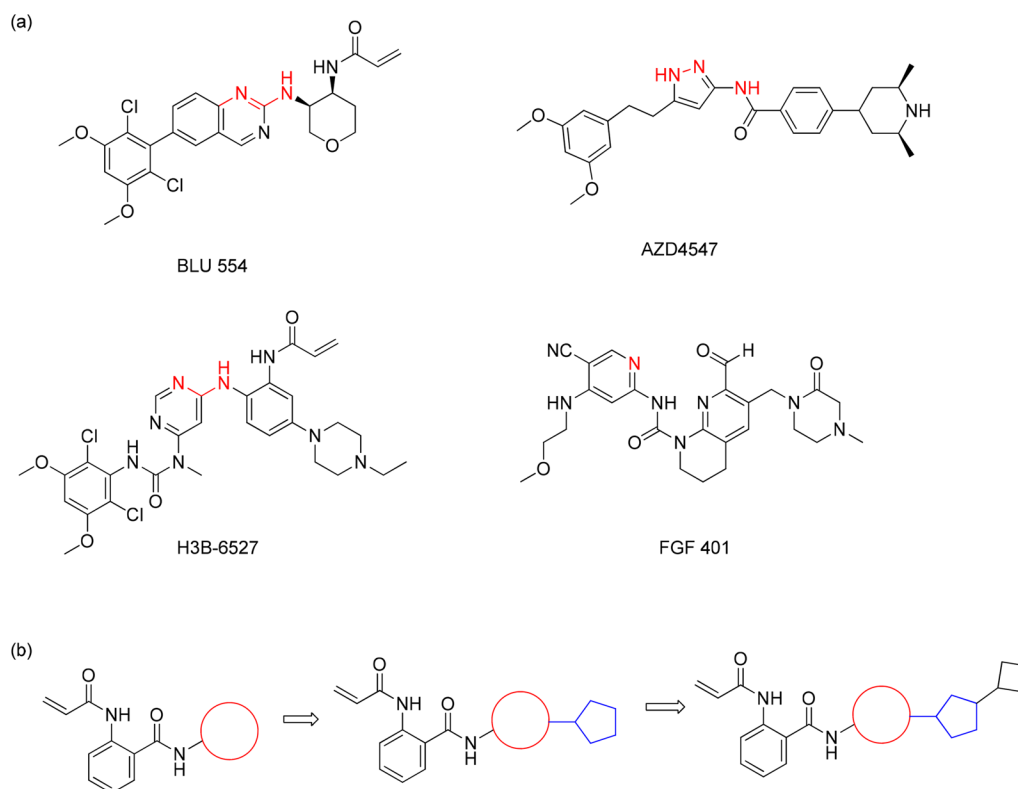


Figure 1. (a) Structures of selective FGFR4 kinase inhibitors. Atoms in red were putatively placed towards hinge region (b) Schematic structure of designed molecules using fragment-assembly strategy.

mixture was purged with N_2 gas and stirred at 25°C for 3 h. After confirming the reaction was complete by TLC, the mixture was concentrated *in vacuo* and solid loaded without work up. It purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=10: 90 to 20: 80) to obtain **1a** as a white solid (90 mg, 44%).

1H -NMR (600 MHz, $CDCl_3$) δ 11.34 (s, 1H), 8.81 (d, $J=7.2$ Hz, 1H), 8.03 (dd, $J=8.4, 1.2$ Hz, 1H), 7.56 (ddd, $J=8.4, 7.2, 1.2$ Hz, 1H), 7.09 (t, $J=7.2$ Hz, 1H), 6.43 (dd, $J=16.8, 1.2$ Hz, 1H), 6.32 (dd, $J=16.8, 10.2$ Hz, 1H), 5.79 (dd, $J=10.2, 1.8$ Hz, 1H), 3.92 (s, 3H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 168.8, 164.1, 141.5, 134.7, 132.5, 130.9, 127.2, 122.6, 120.4, 115.0, 52.3.

2-(Propanoyl amino)-N-pyridazin-3-benzamide (1b)

To a solution of the compound **2a** (106.2 mg, 0.5 mmol) in methanol (5 ml) at 25°C, was added palladium on carbon (30.8 mg, 0.3 wt.%). Then the reaction mixture was purged with H_2 gas and stirred at 25°C for 3 h. After confirming the reaction was complete by TLC, the mixture was filtered through Celite™ and concentrated *in vacuo* to obtain **1b** as a white solid (100.3 mg, 97%).

1H -NMR (600 MHz, $CDCl_3$) δ 8.74 (d, $J=8.3$ Hz, 1H), 8.03 (dd, $J=8.3, 1.4$ Hz, 1H), 7.53–7.56 (m, 1H), 7.06–7.09 (m, 1H), 3.93 (s, 3H), 2.49 (q, $J=7.6$ Hz, 2H), 1.28 (t, $J=7.6$ Hz, 3H)

2-Acrylamido-N-(pyridazin-3-yl)benzamide (2a)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 3-aminopyridazine (19 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the

sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2a** as a pale yellow solid (6.3 mg, 12%).

1H -NMR (600 MHz, $CDCl_3$) δ 11.05 (s, 1H), 9.25 (s, 1H), 9.01 (d, $J=4.2$ Hz, 1H), 8.80 (d, $J=7.8$ Hz, 1H), 8.54 (d, $J=9$ Hz, 1H), 7.81 (d, $J=7.2$ Hz, 1H), 7.61 (td, $J=7.8, 1.2$ Hz, 1H), 7.58 (dd, $J=9.6, 4.8$ Hz, 1H), 7.21 (td, $J=7.8, 1.2$ Hz, 1H), 6.44 (d, $J=16.8$ Hz, 1H), 6.31 (dd, $J=16.8, 10.2$ Hz, 1H), 5.80 (d, $J=10.2$ Hz, 1H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 164.1, 155.1, 140.6, 134.4, 132.3, 128.5, 127.7, 127.3, 127.0, 123.5, 122.2, 119.3, 119.1; HRMS (ESI): m/z calcd for $C_{14}H_{12}N_4O_2$ $[M-H]^-$ 267.0887, found 267.0899.

2-Acrylamido-N-(pyridin-2-yl)benzamide (2b)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 2-aminopyridine (19 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2b** as a white solid (5.8 mg, 11%).

1H -NMR (600 MHz, $CDCl_3$) δ 11.13 (s, 1H), 8.76–8.78 (m, 2H), 8.29–8.30 (m, 2H), 7.79 (td, $J=7.8, 1.8$ Hz, 1H), 7.71 (dd, $J=7.2, 1.2$ Hz, 1H), 7.57 (td, $J=7.8, 1.2$ Hz, 1H), 7.17 (td, $J=7.8, 1.8$ Hz, 1H), 7.11–7.13 (m, 1H), 6.44 (dd, $J=16.8, 1.2$ Hz, 1H), 6.32 (dd, $J=16.8, 10.2$ Hz, 1H), 5.79 (dd, $J=10.2, 1.2$ Hz, 1H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 167.7, 164.2, 151.1, 148.3, 140.2, 138.7, 133.7, 132.4, 127.6,

127.0, 123.3, 122.1, 120.6, 120.2, 114.5; HRMS (ESI): m/z calcd for $C_{15}H_{13}N_3O_2$ $[M-H]^-$ 266.0935, found 266.0939.

2-Acrylamido-N-(pyridin-3-yl)benzamide (2c)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 3-aminopyridine (19 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2c** as a white solid (5.7 mg, 11%).

1H -NMR (600 MHz, $CDCl_3$) δ 10.7 (s, 1H), 9.06 (s, 1H), 8.93 (d, $J=3$ Hz, 1H), 8.46 (dd, $J=4.8, 1.8$ Hz, 1H), 8.39 (d, $J=7.8$ Hz, 1H), 8.24 (dt, $J=14.4, 2.4$ Hz, 1H), 7.52 (dd, $J=8.4, 1.2$ Hz, 1H), 7.38 (dd, $J=8.4, 4.2$ Hz, 1H), 7.32 (td, $J=7.8, 1.2$ Hz, 1H), 7.02 (t, $J=7.8$ Hz, 1H), 6.47 (d, $J=16.8$ Hz, 1H), 6.26 (dd, $J=16.8, 10.2$ Hz, 1H), 5.84 (d, $J=10.2$ Hz, 1H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 167.8, 164.6, 146.0, 142.2, 138.7, 134.8, 132.7, 132.0, 128.2, 127.7, 127.6, 123.9, 123.5, 122.3, 121.4;

HRMS (ESI): m/z calcd for $C_{15}H_{13}N_3O_2$ $[M-H]^-$ 266.0935, found 266.0936.

2-Acrylamido-N-(pyrimidin-2-yl)benzamide (2d)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 2-aminopyrimidine (19 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2d** as a white solid (7.4 mg, 14%).

1H -NMR (600 MHz, $CDCl_3$) δ 11.12 (s, 1H), 8.92 (s, 1H), 8.77 (d, $J=8.4$ Hz, 1H), 8.71 (d, $J=4.8$ Hz, 2H), 7.73 (dd, $J=7.2, 1.2$ Hz, 1H), 7.57 (td, $J=8.4, 1.2$ Hz, 1H), 7.16 (t, $J=7.2$ Hz, 1H), 7.13 (t, $J=4.8$ Hz, 1H), 6.45 (d, $J=16.8$ Hz, 1H), 6.31 (dd, $J=16.8, 10.2$ Hz, 1H), 5.78 (d, $J=10.2$ Hz, 1H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 167.1, 164.4, 158.7, 157.6, 140.7, 134.1, 132.2, 128.0, 127.4, 123.3, 122.3, 119.7, 117.5; HRMS (ESI): m/z calcd for $C_{14}H_{12}N_4O_2$ $[M-H]^-$ 267.0887, found 267.0899.

2-Acrylamido-N-(pyrimidin-4-yl)benzamide (2e)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 4-aminopyrimidine (19 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2e** as a pale yellow solid (18.8 mg, 35%).

1H -NMR (600 MHz, $CDCl_3$) δ 10.88 (s, 1H), 9.06 (s, 1H), 8.92 (d, $J=1.8$ Hz, 1H), 8.73 (s, 1H), 8.72 (s, 1H), 8.25 (dd, $J=3.6, 1.2$ Hz, 1H), 7.68 (dd, $J=7.8, 1.2$ Hz, 1H), 7.55 (td, $J=7.8, 1.8$ Hz, 1H), 7.16 (td, $J=7.8, 1.2$ Hz, 1H), 6.45 (d, $J=16.8$ Hz, 1H), 6.25 (dd, $J=16.8, 10.2$ Hz,

1H), 5.82 (d, $J=10.2$ Hz, 1H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 168.5, 164.2, 158.8, 158.7, 157.1, 140.2, 134.3, 132.2, 127.9, 127.3, 123.4, 122.3, 119.4, 110.7; HRMS (ESI): m/z calcd for $C_{14}H_{12}N_4O_2$ $[M-H]^-$ 267.0887, found 267.0899.

2-Acrylamido-N-(pyrazin-2-yl)benzamide (2f)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 2-aminopyrazine (19 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2f** as a white solid (8.1 mg, 15%).

1H -NMR (600 MHz, $CDCl_3$) δ 10.98 (s, 1H), 9.62 (d, $J=1.2$ Hz, 1H), 8.86 (s, 1H), 8.72 (d, $J=9$ Hz, 1H), 8.43 (d, $J=3$ Hz, 1H), 8.32 (dd, $J=3, 1.2$ Hz, 1H), 7.69 (dd, $J=7.2, 1.2$ Hz, 1H), 7.53 (td, $J=7.8, 1.2$ Hz, 1H), 7.15 (t, $J=7.8$ Hz, 1H), 6.45 (d, $J=16.8$ Hz, 1H), 6.32 (dd, $J=16.8, 10.2$ Hz, 1H), 5.82 (d, $J=10.2$ Hz, 1H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 167.6, 164.3, 148.1, 142.5, 141.0, 140.2, 137.5, 134.0, 132.3, 127.8, 127.3, 123.4, 122.3, 119.5; HRMS (ESI): m/z calcd for $C_{14}H_{12}N_4O_2$ $[M-H]^-$ 267.0887, found 267.09.

2-Acrylamido-N-(6-chloropyridazin-3-yl)benzamide (2g)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 3-amino-6-chloropyridazine (26 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2g** as a white solid (7.1 mg, 11%).

1H -NMR (600 MHz, $CDCl_3$) δ 10.90 (s, 1H), 9.40 (s, 1H), 8.77 (d, $J=7.8$ Hz, 1H), 8.55 (d, $J=10.8$ Hz, 1H), 7.81 (dd, $J=8.4, 1.2$ Hz, 1H), 7.59–7.62 (m, 2H), 7.21 (td, $J=7.2, 1.8$ Hz, 1H), 6.43 (d, $J=16.8$ Hz, 1H), 6.29 (dd, $J=16.8, 10.2$ Hz, 1H), 5.80 (d, $J=10.2$ Hz, 1H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 168.3, 164.1, 154.3, 153.0, 140.5, 134.5, 132.3, 130.1, 127.8, 127.4, 123.5, 122.2, 121.7, 119.0; HRMS (ESI): m/z calcd for $C_{14}H_{11}ClN_4O_2$ $[M-H]^-$ 301.0497, found 301.0504.

2-Acrylamido-N-(6-ethynylpyridin-3-yl)benzamide (2h)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 6-ethynylpyridin-3-amine (24 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2h** as an ivory solid (19.7 mg, 34%).

1H -NMR (600 MHz, $CDCl_3$) δ 10.64 (s, 1H), 9.12 (s, 1H), 8.88 (d, $J=3$ Hz, 1H), 8.34 (d, $J=8.4$ Hz, 1H), 8.27 (dd, $J=9, 2.4$ Hz, 1H), 7.55 (d, $J=9$ Hz, 1H), 7.50 (dd, $J=7.8, 1.2$ Hz, 1H), 7.32 (td, $J=7.8, 1.2$ Hz, 1H), 7.02 (t, $J=7.2$ Hz, 1H), 6.47 (d, $J=16.8$ Hz, 1H), 6.26 (dd, $J=16.8, 10.2$ Hz, 1H), 5.85 (d, $J=10.2$ Hz, 1H), 3.18 (s, 1H); ^{13}C -NMR (150 MHz, $CDCl_3$) δ 167.7, 164.7, 142.1, 138.7, 138.1, 134.7, 132.8, 132.0, 128.3,

127.9, 127.6, 127.3, 123.6, 122.5, 121.4, 82.7, 76.8; HRMS (ESI): m/z calcd for $C_{17}H_{13}N_3O_2$ [M-H]⁻ 290.0934, found 290.0934.

2-Acrylamido-N-(6-ethynylpyridazin-3-yl)benzamide (2i)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 6-ethynylpyridazin-3-amine (24 mg, 0.2 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2i** as a pale yellow solid (8.1 mg, 14%).

¹H-NMR (600 MHz, CDCl₃) δ 11.00 (s, 1H), 9.19 (s, 1H), 8.80 (d, J =9 Hz, 1H), 8.54 (d, J =9 Hz, 1H), 7.78 (d, J =6.6 Hz, 1H), 7.68 (d, J =8.4 Hz, 1H), 7.62 (td, J =7.8, 1.2 Hz, 1H), 7.22 (td, J =7.8, 1.2 Hz, 1H), 6.43 (d, J =16.8 Hz, 1H), 6.30 (dd, J =16.8, 10.2 Hz, 1H), 5.80 (d, J =10.2 Hz, 1H), 3.42 (s, 1H); ¹³C-NMR (150 MHz, CDCl₃) δ 168.1, 164.1, 153.5, 144.4, 140.7, 134.6, 132.3, 132.0, 127.8, 127.2, 123.5, 122.3, 118.9, 118.1, 81.6, 79.7; HRMS (ESI): m/z calcd for $C_{16}H_{12}N_4O_2$ [M-H]⁻ 291.0887, found 291.0894.

2-Acrylamido-N-(5-methylisoxazol-3-yl)benzamide (2j)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 3-amino-5-methylisoxazole (25 mg, 0.24 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2j** as a white solid (12.1 mg, 22%).

¹H-NMR (600 MHz, CDCl₃) δ 11.11 (s, 1H), 9.81 (s, 1H), 8.75 (d, J =9.6 Hz, 1H), 7.80 (dd, J =7.8, 1.2 Hz, 1H), 7.57 (td, J =7.8, 1.2 Hz, 1H), 7.17 (td, J =7.8, 1.8 Hz, 1H), 6.82 (s, 1H), 6.43 (dd, J =16.8, 1.2 Hz, 1H), 6.30 (dd, J =16.8, 10.2 Hz, 1H), 5.79 (d, J =10.2 Hz, 1H), 2.46 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 170.4, 167.4, 164.2, 158.2, 140.4, 134.0, 132.4, 127.8, 127.6, 123.3, 122.0, 119.1, 97.0, 12.9; HRMS (ESI): m/z calcd for $C_{14}H_{13}N_3O_3$ [M-H]⁻ 270.0883, found 270.0889.

2-Acrylamido-N-(1H-indazol-3-yl)benzamide (2k)

To a solution of the compound **1a** (40 mg, 0.2 mmol), 1H-indazol-3-amine (32 mg, 0.24 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2k** as a white solid (20.0 mg, 33%).

¹H-NMR (600 MHz, CDCl₃) δ 11.10 (s, 1H), 9.60 (s, 1H), 8.67 (d, J =1.8 Hz, 1H), 7.90 (d, J =8.4 Hz, 1H), 7.73 (d, J =7.8 Hz, 1H), 7.45 (t, J =7.8 Hz, 1H), 7.37 (s, 1H), 7.37 (s, 1H), 7.15–7.17 (m, 1H), 7.04 (t, J =7.8 Hz, 1H), 6.39 (d, J =16.8 Hz, 1H), 6.21 (dd, J =16.8, 10.2 Hz, 1H), 5.70 (d, J =10.2 Hz, 1H); ¹³C-NMR (150 MHz, CDCl₃) δ 167.8, 164.5, 141.7, 140.6, 139.9, 133.4, 132.2, 128.0, 127.8, 127.8, 123.2,

122.4, 122.1, 121.2, 120.1, 116.4, 110.3; HRMS (ESI): m/z calcd for $C_{17}H_{14}N_4O_2$ [M-H]⁻ 305.1043, found 305.1056.

2-Acrylamido-N-(quinazolin-4-yl)benzamide (2l)

To a solution of the compound **1a** (40 mg, 0.2 mmol), quinazolin-4-ylamine (35 mg, 0.24 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **2l** as a white solid (19.1 mg, 30%).

¹H-NMR (600 MHz, CDCl₃) δ 12.26 (s, 1H), 8.83 (d, J =8.4 Hz, 1H), 8.72 (d, J =6 Hz, 1H), 8.67 (d, J =4.2 Hz, 1H), 8.32 (s, 1H), 7.94 (td, J =7.8, 1.8 Hz, 1H), 7.89 (d, J =8.4 Hz, 1H), 7.69 (t, J =7.8 Hz, 1H), 7.56 (td, J =7.8, 1.8 Hz, 1H), 7.17 (td, J =7.8, 1.2 Hz, 1H), 6.45 (dd, J =16.8, 1.2 Hz, 1H), 6.36 (dd, J =16.8, 10.2 Hz, 1H), 5.78 (dd, J =10.2, 1.2 Hz, 1H); ¹³C-NMR (150 MHz, CDCl₃) δ 164.2, 157.4, 148.8, 141.6, 141.2, 135.9, 134.3, 132.9, 132.6, 132.6, 128.6, 128.4, 127.2, 126.4, 122.9, 122.8, 121.5, 120.7; HRMS (ESI): m/z calcd for $C_{18}H_{14}N_4O_2$ [M-H]⁻ 317.1043, found 317.1055.

6-(3-(Dimethylamino)prop-1-yn-1-yl)pyridazin-3-amine (3a)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), N,N-dimethylpropargylamine (107 μ L, 1 mmol), and TEA (420 μ L, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25 °C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=3: 97 to 6: 94) to obtain **3a** as a brown solid (93 mg, 53%).

¹H-NMR (600 MHz, CDCl₃) δ 7.25 (d, J =9 Hz, 1H), 6.82 (d, J =9 Hz, 1H), 5.80 (b, 2H), 3.53 (s, 2H), 2.40 (s, 6H); ¹³C-NMR (150 MHz, CDCl₃) δ 158.6, 139.3, 131.4, 114.0, 86.4, 82.6, 48.5, 44.3.

6-(3-Methoxyprop-1-yn-1-yl)pyridazin-3-amine (3b)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), methyl propargyl ether (84 μ L, 1 mmol), and TEA (420 μ L, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25 °C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=3: 97 to 6: 94) to obtain **3b** as a yellow solid (132 mg, 80%).

¹H-NMR (600 MHz, CDCl₃) δ 7.29 (d, J =9.6 Hz, 1H), 6.91 (d, J =8.4 Hz, 1H), 5.71 (b, 2H), 4.34 (s, 2H), 3.45 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 158.5, 139.1, 131.5, 114.3, 87.1, 83.2, 60.3, 58.0.

6-((4-Methoxyphenyl)ethynyl)pyridazin-3-amine (3c)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 4-ethynylanisole (132 mg, 1 mmol), and TEA (420 μ L, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25 °C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC

with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 80: 20) to obtain **3c** as a yellow solid (166 mg, 74%).

¹H-NMR (600 MHz, CDCl₃) δ 7.47 (d, *J*=9 Hz, 2H), 7.33 (d, *J*=9 Hz, 1H), 7.04 (d, *J*=9 Hz, 1H), 6.87 (d, *J*=9 Hz, 2H), 5.96 (b, 2H), 3.82 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 160.2, 158.3, 137.8, 133.3, 131.5, 114.8, 114.2, 114.0, 92.5, 84.9, 55.4.

6-((3-Methoxyphenyl)ethynyl)pyridazin-3-amine (3d)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 3-ethynylanisole (127 μL, 1 mmol), and TEA (420 μL, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25°C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=3: 97 to 6: 94) to obtain **3d** as a brown gel (180 mg, 80%).

¹H-NMR (600 MHz, CDCl₃) δ 7.35 (d, *J*=9 Hz, 1H), 7.26 (t, *J*=7.8 Hz, 1H), 7.14 (d, *J*=7.8 Hz, 1H), 7.12 (d, *J*=7.2 Hz, 1H), 7.05 (s, 1H), 6.92 (dd, *J*=8.4, 2.4 Hz, 1H), 6.25 (b, 2H), 3.79 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 159.1, 158.5, 138.8, 131.2, 129.4, 123.9, 122.7, 116.3, 115.3, 114.5, 90.7, 85.7, 55.1.

6-((2-Methoxyphenyl)ethynyl)pyridazin-3-amine (3e)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 1-ethynyl-2-methoxybenzene (130 μL, 1 mmol), and TEA (420 μL, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25°C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 80: 20) to obtain **3e** as a yellow solid (160 mg, 71%).

¹H-NMR (600 MHz, CDCl₃) δ 7.52 (dd, *J*=7.2, 1.2 Hz, 1H), 7.41 (d, *J*=9 Hz, 1H), 7.36 (td, *J*=8.4, 1.2 Hz, 1H), 7.09 (d, *J*=9.6 Hz, 1H), 6.95 (t, *J*=7.2 Hz, 1H), 6.93 (d, *J*=9 Hz, 1H), 5.80 (b, 2H), 3.91 (s, 2H); ¹³C-NMR (150 MHz, CDCl₃) δ 160.3, 158.0, 139.8, 133.8, 131.9, 130.8, 120.7, 115.0, 111.2, 110.9, 89.7, 88.3, 55.9.

6-((3,5-Dimethoxyphenyl)ethynyl)pyridazin-3-amine (3f)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 1-ethynyl-3,5-dimethoxybenzene (162 mg, 1 mmol), and TEA (420 μL, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25°C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 80: 20) to obtain **3f** as a yellow gel (183.7 mg, 82%).

¹H-NMR (600 MHz, CDCl₃) δ 7.35 (d, *J*=9 Hz, 1H), 7.10 (d, *J*=9 Hz, 1H), 6.67 (d, *J*=2.4 Hz, 2H), 6.47 (t, *J*=1.8 Hz, 1H), 6.29 (b, 2H), 3.77 (s, 6H); ¹³C-NMR (150 MHz, CDCl₃) δ 160.3, 158.6, 137.5, 131.3, 123.1, 114.5, 109.3, 102.1, 90.9, 85.5, 55.3.

6-((3-(Dimethylamino)phenyl)ethynyl)pyridazin-3-amine (3g)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 3-ethynyl-*N,N*-dimethylaniline (145 mg, 1 mmol), and TEA (420 μL, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI

(38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25°C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 80: 20) to obtain **3g** as a yellow gel (207 mg, 87%).

¹H-NMR (600 MHz, CDCl₃) δ 7.35 (d, *J*=8.4 Hz, 1H), 7.20 (t, *J*=8.4 Hz, 1H), 7.06 (d, *J*=9 Hz, 1H), 6.86–6.88 (m, 2H), 6.73 (dd, *J*=8.4, 3 Hz, 1H), 6.05 (b, 2H), 2.94 (s, 6H); ¹³C-NMR (150 MHz, CDCl₃) δ 159.4, 158.4, 150.2, 137.7, 131.6, 129.1, 122.2, 119.7, 115.2, 113.4, 95.5, 84.8, 40.4.

6-((4-(Dimethylamino)phenyl)ethynyl)pyridazin-3-amine (3h)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 4'-dimethylaminophenylacetylene (145 mg, 1 mmol), and TEA (420 μL, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25°C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=8: 92 to 10: 90) to obtain **3h** as a brown solid (166 mg, 70%).

¹H-NMR (600 MHz, CDCl₃) δ 7.40 (d, *J*=9 Hz, 2H), 7.37 (d, *J*=9.6 Hz, 1H), 7.13 (d, *J*=9 Hz, 1H), 6.65 (d, *J*=9 Hz, 2H), 3.01 (s, 6H); ¹³C-NMR (150 MHz, CDCl₃) δ 157.8, 150.6, 137.8, 133.0, 131.7, 129.5, 115.6, 111.5, 93.6, 83.9, 40.1.

6-(Phenylethynyl)pyridazin-3-amine (3i)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), ethynylbenzene (131 μL, 1.2 mmol), and TEA (420 μL, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25°C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 80: 20) to obtain **3i** as a yellow solid (185 mg, 95%).

¹H-NMR (600 MHz, CDCl₃) δ 7.56–7.57 (m, 2H), 7.35–7.37 (m, 4H), 6.79 (d, *J*=9 Hz, 1H), 5.52 (b, 2H); ¹³C-NMR (150 MHz, CDCl₃) δ 158.2, 140.1, 132.0, 131.5, 129.1, 128.5, 122.3, 113.9, 91.3, 86.3.

6-(Pyridin-3-ylethynyl)pyridazin-3-amine (3j)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 3-ethynylpyridine (103 mg, 1 mmol), and TEA (420 μL, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25°C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=3: 97 to 6: 94) to obtain **3j** as a yellow solid (122 mg, 62%).

¹H-NMR (600 MHz, DMSO-d₆) δ 8.80 (s, 1H), 8.63 (d, *J*=4.2 Hz, 1H), 8.02 (dt, *J*=8.4, 1.8 Hz, 1H), 7.49–7.52 (m, 2H), 6.94 (s, 2H), 6.84 (d, *J*=8.4 Hz, 1H); ¹³C-NMR (150 MHz, DMSO-d₆) δ 159.4, 151.6, 149.2, 138.6, 137.0, 130.9, 123.7, 119.0, 112.8, 90.4, 86.3.

6-(Cyclohexylethynyl)pyridazin-3-amine (3k)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), ethynylcyclohexane (131 μL, 1.2 mmol), and TEA (420 μL, 3 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (70 mg, 0.1 mmol) and CuI (38 mg, 0.2 mmol), purged by N₂ gas, stirred at 25°C for 24 h. The mixture

was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 80: 20) to obtain **3k** as a yellow gel (178 mg, 89%).

¹H-NMR (600 MHz, CDCl₃) δ 7.24 (d, *J*=9 Hz, 1H), 7.05 (d, *J*=9.6 Hz, 1H), 5.96 (b, 2H), 2.61 (tt, *J*=9.6, 3.6 Hz, 1H), 1.88–1.90 (m, 2H), 1.74–1.76 (m, 2H), 1.51–1.56 (m, 3H), 1.33–1.37 (m, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 158.2, 137.7, 131.6, 117.5, 115.0, 96.9, 32.2, 29.5, 25.6, 24.8.

6-(Naphthalen-1-ylethynyl)pyridazin-3-amine (3l)

6-ethynylpyridazin-3-amine (36 mg, 0.3 mmol), 1-iodonaphthalene (44 μL, 0.3 mmol), and TEA (120 μL, 0.9 mmol) were dissolved in dry THF (2 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (21 mg, 0.03 mmol) and CuI (12 mg, 0.014 mmol), purged by N₂ gas, stirred at 25 °C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=2: 98 to 5: 95) to obtain **3l** as a yellow solid (60 mg, 81%).

¹H-NMR (600 MHz, CDCl₃) δ 8.46 (d, *J*=8.4 Hz, 1H), 7.88 (d, *J*=3.6 Hz, 1H), 7.87 (d, *J*=3.6 Hz, 1H), 7.82 (d, *J*=7.2 Hz, 1H), 7.60 (t, *J*=7.8 Hz, 1H), 7.54 (t, *J*=7.8 Hz, 1H), 7.46–7.49 (m, 2H), 6.85 (d, *J*=9 Hz, 1H), 5.28 (b, 2H); ¹³C-NMR (150 MHz, CDCl₃) δ 158.1, 140.4, 133.3, 133.2, 131.5, 131.1, 129.6, 128.4, 127.1, 126.7, 126.3, 125.4, 120.0, 113.6, 91.2, 89.4.

6-(3-(3-Methoxyphenoxy)prop-1-yn-1-yl)pyridazin-3-amine (3m)

The solution of 3-methoxyphenol (120 μL, 1 mmol), propargyl bromide (80 μL, 1 mmol), potassium carbonate (276 mg, 2 equiv) and acetone was stirred at 25 °C for 6 h. Then the reaction mixture was filtered, and the filtrate was evaporated *in vacuo*. To reaction vial, 6-iodopyridazin-3-amine (154 mg, 0.7 mmol), and TEA (280 μL, 2.0 mmol) were dissolved in dry THF (3 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (49 mg, 0.07 mmol) and CuI (27 mg, 0.14 mmol), purged by N₂ gas, stirred at 25 °C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 80: 20) to obtain **3m** as a brown solid (111 mg, 44%).

¹H-NMR (600 MHz, CDCl₃) δ 7.17–7.21 (m, 2H), 6.74 (d, *J*=9 Hz, 1H), 6.60 (dd, *J*=8.4, 2.4 Hz, 1H), 6.56 (t, *J*=1.8 Hz, 1H), 6.54 (dd, *J*=7.8, 1.8 Hz, 1H), 5.78 (b, 2H), 4.88 (s, 2H), 3.77 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 160.9, 158.9, 158.7, 138.7, 131.5, 130.1, 114.0, 107.3, 107.0, 101.6, 85.9, 84.0, 56.5, 55.4.

6-(3-Methoxyphenoxy)pyridazin-3-amine (3n)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 3-methoxyphenol (118 μL, 1 mmol), picolinic acid (25 mg, 0.2 mmol), potassium phosphate (424 mg, 2 mmol) and CuI (20 mg, 0.1 mmol) were dissolved in DMSO (3 ml), purged by N₂ gas, stirred at 100 °C for overnight. Then a reaction mixture was added distilled water and the aqueous layer was washed with dichloromethane. The organic layer was evaporated and purified by MPLC with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 80: 20) to obtain **3n** as a brown solid (113 mg, 52%).

¹H-NMR (600 MHz, CDCl₃) δ 6.75 (t, *J*=8.4 Hz, 1H), 6.51 (d, *J*=9 Hz, 1H), 6.46 (d, *J*=9 Hz, 1H), 6.20 (dd, *J*=8.4, 1.2 Hz, 1H), 6.15–6.16 (m, 2H), 5.31 (b, 2H), 3.29 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 159.7, 158.6, 157.8, 155.5, 129.0, 119.6, 118.8, 110.7, 108.6, 104.7, 54.3.

6-(3-Methoxyphenyl)pyridazin-3-amine (3o)

6-Iodopyridazin-3-amine (221 mg, 1 mmol), 3-methoxyphenyl boronic acid (180 mg, 1.2 mmol) and potassium carbonate (276 mg, 2 mmol) were dissolved in the solution that mixed THF and distilled water by 2 ml and 1 ml, then degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₄ (110 mg, 0.1 mmol) and purged by N₂ gas, heated with stirring in microwave reactor at 130 °C for 1 h. The mixture was filtered over Celite and the filtrate was evaporated *in vacuo* and purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=3: 97 to 6: 94) to obtain **3o** as a white solid (137 mg, 68%).

¹H-NMR (600 MHz, CDCl₃) δ 7.59–7.62 (m, 2H), 7.44 (d, *J*=7.8 Hz, 1H), 7.37 (t, *J*=7.8 Hz, 1H), 6.96 (dd, *J*=8.4, 3 Hz, 1H), 6.85 (d, *J*=9 Hz, 1H), 5.09 (b, 2H), 3.88 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 160.2, 158.8, 152.3, 137.9, 129.9, 126.3, 118.5, 115.1, 115.0, 111.3, 55.5.

6-[2-(3-Fluoro-4-methoxyphenyl)ethynyl]pyridazin-3-amine (3r)

2-fluoro-4-iodo-1-methoxybenzene (63.5 mg, 0.25 mmol), 6-ethynylpyridazin-3-amine (30 mg, 0.25 mmol), and diisopropylamine (71 μL, 0.504 mmol) were dissolved in dry THF (2 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (17.7 mg, 0.025 mmol) and CuI (9.6 mg, 0.05 mmol), purged by N₂ gas, stirred at 25 °C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=3: 97 to 6: 94) to obtain **3r** as a yellow solid (25 mg, 41%).

¹H-NMR (600 MHz, MeOD-d₄) δ 7.43 (d, *J*=9.6 Hz, 1H), 7.27–7.34 (m, 2H), 7.11 (t, *J*=8.6 Hz, 1H), 6.88–6.92 (m, 1H), 3.91 (s, 3H)

6-[2-(1,3-Benzodioxol-5-yl)ethynyl]pyridazin-3-amine (3s)

5-Ethynyl-1,3-benzodioxole (128.1 mg, 0.88 mmol), 6-iodopyridazin-3-amine (194 mg, 0.88 mmol), and TEA (368 μL, 2.64 mmol) were dissolved in dry THF (2 ml) and degassed with N₂ balloon for 5 min. To a mixture were added Pd(PPh₃)₂Cl₂ (63.17 mg, 0.09 mmol) and CuI (34.3 mg, 0.18 mmol), purged by N₂ gas, stirred at 25 °C for 24 h. The mixture was filtered over Celite and the filtrate was purified using MPLC with gradient concentration of ethyl acetate/hexane (v: v=60: 40 to 80: 20) to obtain **3s** as a yellow solid (48 mg, 23%).

¹H-NMR (600 MHz, DMSO-d₆) δ 7.40 (d, *J*=9.0 Hz, 1H), 7.09–7.11 (m, 2H), 6.98 (d, *J*=8.3 Hz, 1H), 6.75 (d, *J*=9.0 Hz, 3H), 6.09 (s, 2H)

2-Acrylamido-N-(6-(3-(dimethylamino)prop-1-yn-1-yl)pyridazin-3-yl)benzamide (4a)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3a** (35 mg, 0.2 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μL, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=5: 95 to 8: 92) to obtain **4a** as a brown solid (7.0 mg, 10%).

¹H-NMR (600 MHz, CDCl₃) δ 11.02 (s, 1H), 9.32 (b, 1H), 8.79 (d, *J*=8.4 Hz, 1H), 8.50 (d, *J*=9.6 Hz, 1H), 7.80 (d, *J*=7.2 Hz, 1H), 7.62 (d, *J*=9 Hz, 1H), 7.61 (td, *J*=7.8, 1.2 Hz, 1H), 7.21 (t, *J*=8.4 Hz, 1H), 6.43 (d, *J*=16.8 Hz, 1H), 6.30 (dd, *J*=16.8, 10.2 Hz, 1H), 5.80 (d, *J*=10.2 Hz,

1H), 3.58 (s, 2H), 2.42 (s, 6H); ^{13}C -NMR (150 MHz, CDCl_3) δ 168.2, 164.1, 153.2, 145.2, 140.6, 134.5, 132.3, 131.7, 127.7, 127.3, 123.5, 122.2, 119.1, 118.3, 89.9, 81.8, 48.7, 44.5; HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{19}\text{N}_5\text{O}_2$ [M-H] $^-$ 348.1465, found 348.1482.

2-Acrylamido-N-(6-((3-methoxyprop-1-yn-1-yl)pyridazin-3-yl)benzamide (4b)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3b** (33 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μL , 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=5: 95 to 8: 92) to obtain **4b** as a yellow solid (7.4 mg, 11%).

^1H -NMR (600 MHz, CDCl_3) δ 11.00 (s, 1H), 9.31 (s, 1H), 8.79 (d, $J=8.4\text{ Hz}$, 1H), 8.52 (d, $J=9\text{ Hz}$, 1H), 7.80 (d, $J=7.2\text{ Hz}$, 1H), 7.65 (d, $J=9.6\text{ Hz}$, 1H), 7.61 (td, $J=7.8, 1.2\text{ Hz}$, 1H), 7.21 (td, $J=7.8, 1.2\text{ Hz}$, 1H), 6.43 (d, $J=16.8\text{ Hz}$, 1H), 6.30 (dd, $J=16.8, 10.2\text{ Hz}$, 1H), 5.80 (d, $J=10.2\text{ Hz}$, 1H), 4.41 (s, 2H), 3.50 (s, 3H); ^{13}C -NMR (150 MHz, CDCl_3) δ 168.2, 164.1, 153.4, 144.7, 140.6, 134.5, 132.3, 131.7, 127.8, 127.3, 123.5, 122.2, 119.0, 118.3, 89.7, 82.6, 60.3, 58.3; HRMS (ESI): m/z calcd for $\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}_3$ [M-H] $^-$ 335.1149, found 335.1163.

2-Acrylamido-N-(6-((4-methoxyphenyl)ethynyl)pyridazin-3-yl)benzamide (4c)

To a solution of compound **1a** (40 mg, 0.2 mmol), **3c** (45 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μL , 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4c** as a pale yellow solid (2.7 mg, 3.3%).

^1H -NMR (600 MHz, CDCl_3) δ 11.05 (s, 1H), 9.22 (s, 1H), 8.81 (d, $J=8.4\text{ Hz}$, 1H), 8.54 (d, $J=9\text{ Hz}$, 1H), 7.80 (d, $J=7.8\text{ Hz}$, 1H), 7.70 (d, $J=9\text{ Hz}$, 1H), 7.62 (td, $J=8.4, 1.2\text{ Hz}$, 1H), 7.59 (d, $J=9\text{ Hz}$, 2H), 7.23 (t, $J=7.2\text{ Hz}$, 1H), 6.93 (d, $J=8.4\text{ Hz}$, 2H), 6.44 (d, $J=17.4\text{ Hz}$, 1H), 6.32 (dd, $J=17.4, 10.2\text{ Hz}$, 1H), 5.81 (d, $J=10.2\text{ Hz}$, 1H), 3.86 (s, 3H); ^{13}C -NMR (150 MHz, CDCl_3) δ 168.0, 164.1, 160.7, 152.8, 145.7, 140.6, 134.4, 133.8, 132.3, 131.4, 127.7, 127.1, 123.4, 122.2, 119.0, 118.1, 114.3, 113.6, 94.1, 84.5, 55.5; HRMS (ESI): m/z calcd for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_3$ [M-H] $^-$ 397.1305, found 397.1320.

2-Acrylamido-N-(6-((3-methoxyphenyl)ethynyl)pyridazin-3-yl)benzamide (4d)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3d** (45 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μL , 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4d** as a yellow solid (5.1 mg, 6%).

^1H -NMR (600 MHz, CDCl_3) δ 11.04 (s, 1H), 9.25 (s, 1H), 8.81 (d, $J=8.4\text{ Hz}$, 1H), 8.55 (d, $J=9\text{ Hz}$, 1H), 7.81 (d, $J=8.4\text{ Hz}$, 1H), 7.73 (d,

$J=9\text{ Hz}$, 1H), 7.62 (td, $J=7.8, 1.2\text{ Hz}$, 1H), 7.31 (t, $J=7.8\text{ Hz}$, 1H), 7.21–7.25 (m, 2H), 7.17 (dd, $J=3, 1.2\text{ Hz}$, 1H), 6.98 (ddd, $J=8.4, 3, 1.8\text{ Hz}$, 1H), 6.44 (d, $J=16.8\text{ Hz}$, 1H), 6.31 (dd, $J=16.8, 10.2\text{ Hz}$, 1H), 5.81 (d, $J=10.2\text{ Hz}$, 1H), 3.85 (s, 3H); ^{13}C -NMR (150 MHz, CDCl_3) δ 168.1, 164.2, 159.6, 153.1, 145.5, 140.7, 134.5, 132.3, 131.7, 129.8, 127.8, 127.2, 124.8, 123.5, 122.6, 122.2, 119.1, 118.2, 116.9, 116.5, 93.6, 85.3, 55.5; HRMS (ESI): m/z calcd for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_3$ [M-H] $^-$ 397.1305, found 397.1324.

2-Acrylamido-N-(6-((2-methoxyphenyl)ethynyl)pyridazin-3-yl)benzamide (4e)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3e** (45 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μL , 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4e** as a white solid (16.4 mg, 21%).

^1H -NMR (600 MHz, CDCl_3) δ 11.05 (s, 1H), 9.49 (s, 1H), 8.79 (d, $J=7.2\text{ Hz}$, 1H), 8.53 (d, $J=8.4\text{ Hz}$, 1H), 7.87 (dd, $J=8.4, 1.2\text{ Hz}$, 1H), 7.75 (d, $J=9\text{ Hz}$, 1H), 7.59–7.62 (m, 2H), 7.40 (td, $J=8.4, 1.8\text{ Hz}$, 1H), 7.23 (t, $J=8.4\text{ Hz}$, 1H), 6.99 (t, $J=7.8\text{ Hz}$, 1H), 6.95 (d, $J=7.8\text{ Hz}$, 1H), 6.44 (d, $J=16.8\text{ Hz}$, 1H), 6.32 (dd, $J=16.8, 10.2\text{ Hz}$, 1H), 5.80 (d, $J=10.2\text{ Hz}$, 1H), 3.95 (s, 3H); ^{13}C -NMR (150 MHz, CDCl_3) δ 168.2, 164.2, 160.7, 153.1, 145.7, 140.5, 134.4, 134.2, 132.3, 131.8, 131.3, 127.7, 127.5, 123.5, 122.1, 120.7, 119.2, 118.4, 118.4, 110.9, 90.5, 89.4, 56.0; HRMS (ESI): m/z calcd for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_3$ [M-H] $^-$ 397.1305, found 397.1315.

2-Acrylamido-N-(6-((3,5-dimethoxyphenyl)ethynyl)pyridazin-3-yl)benzamide (4f)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3f** (51 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μL , 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4f** as a yellow solid (15.1 mg, 18%).

^1H -NMR (600 MHz, CDCl_3) δ 11.03 (s, 1H), 9.42 (s, 1H), 8.79 (d, $J=9\text{ Hz}$, 1H), 8.54 (d, $J=9\text{ Hz}$, 1H), 7.84 (d, $J=7.8\text{ Hz}$, 1H), 7.72 (d, $J=9\text{ Hz}$, 1H), 7.61 (td, $J=7.8, 1.2\text{ Hz}$, 1H), 7.22 (t, $J=7.2\text{ Hz}$, 1H), 6.79 (d, $J=1.8\text{ Hz}$, 2H), 6.54 (t, $J=2.4\text{ Hz}$, 1H), 6.44 (d, $J=16.8\text{ Hz}$, 1H), 6.31 (dd, $J=16.8, 10.2\text{ Hz}$, 1H), 5.81 (d, $J=10.2\text{ Hz}$, 1H), 3.82 (s, $J=6\text{ Hz}$); ^{13}C -NMR (150 MHz, CDCl_3) δ 168.2, 164.1, 160.8, 153.2, 145.3, 140.6, 134.5, 132.3, 131.7, 127.8, 127.4, 123.5, 122.9, 122.2, 119.1, 118.4, 110.0, 103.3, 93.6, 85.0, 55.7; HRMS (ESI): m/z calcd for $\text{C}_{24}\text{H}_{20}\text{N}_4\text{O}_4$ [M-H] $^-$ 427.1411, found 427.1429.

2-Acrylamido-N-(6-((3-(dimethylamino)phenyl)ethynyl)pyridazin-3-yl)benzamide (4g)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3g** (47 mg, 0.2 mmol) in dry toluene purged with N_2 gas, was added trimethylaluminum (150 μL , 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C,

concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4g** as a yellow solid (10.0mg, 12%).

¹H-NMR (600MHz, CDCl₃) δ 11.04 (s, 1H), 9.34 (s, 1H), 8.80 (d, *J*=7.8Hz, 1H), 8.53 (d, *J*=9Hz, 1H), 7.83 (d, *J*=7.8Hz, 1H), 7.72 (d, *J*=9.6Hz, 1H), 7.61 (t, *J*=7.8Hz, 1H), 7.21–7.25 (m, 2H), 6.98–7.00 (m, 2H), 6.79 (dd, *J*=7.8, 1.8Hz, 1H), 6.44 (d, *J*=16.8Hz, 1H), 6.31 (dd, *J*=16.8, 10.2Hz, 1H), 5.80 (d, *J*=10.2Hz, 1H), 2.98 (s, 3H); ¹³C-NMR (150MHz, CDCl₃) δ 168.2, 164.1, 153.0, 150.4, 145.7, 140.6, 134.4, 132.3, 131.7, 129.3, 127.7, 127.4, 123.5, 122.2, 122.0, 120.3, 119.1, 118.3, 115.8, 114.1, 94.9, 84.5, 40.6; HRMS (ESI): *m/z* calcd for C₂₄H₂₁N₅O₂ [M-H]⁻ 410.1622, found 410.1631.

2-Acrylamido-N-(6-((4-(dimethylamino)phenyl)ethynyl)pyridazin-3-yl)benzamide (4h)

To a solution of the compound **1a** (40mg, 0.2mmol), compound **4h** (47mg, 0.2mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150μL, 2.0M in toluene) dropwise. The reaction mixture was heated at 110°C for 3h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4h** as a yellow solid (13.8mg, 17%).

¹H-NMR (600MHz, CDCl₃) δ 11.07 (s, 1H), 9.40 (s, 1H), 8.79 (d, *J*=8.4Hz, 1H), 8.48 (d, *J*=9Hz, 1H), 7.84 (d, *J*=7.2Hz, 1H), 7.66 (d, *J*=9.6Hz, 1H), 7.59 (t, *J*=8.4Hz, 1H), 7.51 (d, *J*=9Hz, 1H), 7.21 (t, *J*=7.2Hz, 1H), 6.67 (d, *J*=9Hz, 2H), 6.43 (d, *J*=16.8Hz, 1H), 6.31 (dd, *J*=16.8, 10.2Hz, 1H), 5.80 (d, *J*=10.2Hz, 1H), 3.02 (s, 6H); ¹³C-NMR (150MHz, CDCl₃) δ 168.1, 164.1, 152.6, 151.0, 146.2, 140.5, 134.3, 133.6, 132.4, 131.3, 127.7, 127.5, 123.5, 122.1, 119.2, 118.5, 111.8, 107.9, 96.0, 84.3, 40.2; HRMS (ESI): *m/z* calcd for C₂₄H₂₁N₅O₂ [M-H]⁻ 410.1622, found 410.1637.

2-Acrylamido-N-(6-(phenylethynyl)pyridazin-3-yl)benzamide (4i)

To a solution of the compound **1a** (40mg, 0.2mmol), compound **4i** (39mg, 0.2mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150μL, 2.0M in toluene) dropwise. The reaction mixture was heated at 110°C for 3h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4i** as a yellow solid (22.5mg, 31%).

¹H-NMR (600MHz, CDCl₃) δ 11.03 (s, 1H), 9.60 (s, 1H), 8.78 (d, *J*=8.4Hz, 1H), 8.53 (d, *J*=9.6Hz, 1H), 7.87 (dd, *J*=8.4, 1.2Hz, 1H), 7.72 (d, *J*=9Hz, 1H), 7.64 (dd, *J*=7.8, 2.4Hz, 1H), 7.59 (t, 7.8Hz, 1H), 7.39–7.43 (m, 3H), 7.21 (t, *J*=7.8Hz, 1H), 6.44 (d, *J*=16.8Hz, 1H), 6.31 (dd, *J*=16.8, 10.2Hz, 1H), 5.80 (d, *J*=10.2Hz, 1H); ¹³C-NMR (150MHz, CDCl₃) δ 168.3, 164.1, 153.2, 145.4, 140.5, 134.4, 132.3, 132.3, 131.7, 129.7, 128.7, 127.7, 127.6, 123.5, 122.1, 121.7, 119.2, 118.6, 93.6, 85.5; HRMS (ESI): *m/z* calcd for C₂₂H₁₆N₄O₂ [M-H]⁻ 367.1200, found 367.1209.

2-Acrylamido-N-(6-(pyridin-3-ylethynyl)pyridazin-3-yl)benzamide (4j)

To a solution of the compound **1a** (40mg, 0.2mmol), compound **3j** (40mg, 0.2mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150μL, 2.0M in toluene) dropwise. The reaction mixture was heated at 110°C for 3h in the sand bath. After

confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=8: 92 to 10: 90) to obtain **4j** as a yellow solid (16.3mg, 22%).

¹H-NMR (600MHz, CDCl₃) δ 11.00 (s, 1H), 9.55 (s, 1H), 8.88 (s, 1H), 8.79 (d, *J*=9Hz, 1H), 8.65 (s, 1H), 8.58 (d, *J*=9Hz, 1H), 7.93 (dt, *J*=8.4, 1.8Hz, 1H), 7.85 (dd, *J*=7.2, 1.2Hz, 1H), 7.75 (d, *J*=8.4Hz, 1H), 7.61 (td, *J*=7.8, 1.2Hz, 1H), 7.36 (dd, *J*=7.8, 6.4Hz, 1H), 7.22 (t, *J*=7.2Hz, 1H), 6.44 (d, *J*=16.8Hz, 1H), 6.31 (dd, *J*=16.8, 10.2Hz, 1H), 5.81 (d, *J*=10.2Hz, 1H); ¹³C-NMR (150MHz, CDCl₃) δ 168.3, 164.1, 153.5, 152.7, 149.9, 144.8, 140.6, 139.1, 134.5, 132.3, 131.7, 127.8, 127.5, 123.5, 123.4, 122.2, 119.1, 119.0, 118.4, 89.9, 88.6; HRMS (ESI): *m/z* calcd for C₂₁H₁₅N₅O₂ [M-H]⁻ 368.1152, found 368.1166.

2-Acrylamido-N-(6-(cyclohexylethynyl)pyridazin-3-yl)benzamide (4k)

To a solution of the compound **1a** (40mg, 0.2mmol), compound **3k** (40mg, 0.2mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150μL, 2.0M in toluene) dropwise. The reaction mixture was heated at 110°C for 3h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=20: 80 to 50: 50) to obtain **4k** as a red gel (12.7mg, 17%).

¹H-NMR (600MHz, CDCl₃) δ 11.04 (s, 1H), 9.50 (s, 1H), 8.77 (d, *J*=7.8Hz, 1H), 8.45 (d, *J*=9.6Hz, 1H), 7.84 (dd, *J*=7.8, 1.2Hz, 1H), 7.56–7.60 (m, 2H), 7.20 (t, *J*=8.4Hz, 1H), 6.43 (dd, *J*=16.8, 1.2Hz, 1H), 6.30 (dd, *J*=16.8, 10.2Hz, 1H), 5.79 (d, *J*=10.2Hz, 1H), 2.68 (tt, *J*=9.6, 4.2Hz, 1H), 1.93–1.95 (m, 2H), 1.77–1.81 (m, 2H), 1.58–1.63 (m, 3H), 1.37–1.41 (m, 3H); ¹³C-NMR (150MHz, CDCl₃) δ 168.2, 164.1, 152.9, 145.8, 140.4, 134.2, 132.3, 131.6, 127.6, 127.5, 123.4, 122.0, 119.1, 118.5, 99.6, 60.5, 32.3, 29.9, 25.9, 25.0; HRMS (ESI): *m/z* calcd for C₂₂H₂₂N₄O₂ [M-H]⁻ 373.1669, found 373.1679.

2-Acrylamido-N-(6-(naphthalen-1-ylethynyl)pyridazin-3-yl)benzamide (4l)

To a solution of the compound **1a** (40mg, 0.2mmol), compound **3l** (49mg, 0.2mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150μL, 2.0M in toluene) dropwise. The reaction mixture was heated at 110°C for 3h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4l** as a white solid (19.2mg, 23%).

¹H-NMR (600MHz, CDCl₃) δ 11.04 (s, 1H), 9.38 (s, 1H), 8.80 (d, *J*=8.4Hz, 1H), 8.58 (d, *J*=9.6Hz, 1H), 8.48 (d, *J*=8.4Hz, 1H), 7.93 (d, *J*=8.4Hz, 1H), 7.90 (d, *J*=8.4Hz, 1H), 7.89 (d, *J*=6Hz, 1H), 7.85 (dd, *J*=8.4, 1.8Hz, 1H), 7.82 (d, *J*=9Hz, 1H), 7.56–7.66 (m, 3H), 7.50 (t, *J*=7.2Hz, 1H), 7.23 (td, *J*=7.8, 1.2Hz, 1H), 6.44 (d, *J*=16.8Hz, 1H), 6.32 (dd, *J*=16.8, 10.2Hz, 1H), 5.81 (d, *J*=10.2Hz, 1H); ¹³C-NMR (150MHz, CDCl₃) δ 168.2, 164.2, 153.2, 145.6, 140.6, 134.5, 133.4, 133.3, 132.3, 131.8, 131.7, 130.4, 128.6, 127.8, 127.5, 127.4, 126.9, 126.2, 125.4, 123.5, 122.2, 119.3, 119.1, 118.4, 92.0, 90.3; HRMS (ESI): *m/z* calcd for C₂₆H₁₈N₄O₂ [M-H]⁻ 417.1356, found 417.1370.

2-Acrylamido-N-(6-(3-(3-methoxyphenoxy)prop-1-yn-1-yl)pyridazin-3-yl)benzamide (4m)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3m** (51 mg, 0.2 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4m** as a yellow solid (9.4 mg, 11%).

¹H-NMR (600 MHz, CDCl₃) δ 11.00 (s, 1H), 9.16 (s, 1H), 8.80 (d, *J*=8.4 Hz, 1H), 8.51 (d, *J*=9 Hz, 1H), 7.77 (d, *J*=7.8 Hz, 1H), 7.63 (d, *J*=9 Hz, 1H), 7.61 (td, *J*=7.8, 1.2 Hz, 1H), 7.23 (t, *J*=8.4 Hz, 1H), 7.21 (t, *J*=7.8 Hz, 1H), 6.64 (dd, *J*=7.8, 1.8 Hz, 1H), 6.57–6.60 (m, 2H), 6.43 (dd, *J*=16.8, 1.2 Hz, 1H), 6.30 (dd, *J*=16.8, 10.2 Hz, 1H), 5.80 (d, *J*=10.2 Hz, 1H), 4.98 (s, 2H), 3.81 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 168.1, 164.1, 161.1, 158.9, 153.4, 144.5, 140.7, 134.6, 132.3, 131.9, 130.2, 127.8, 127.2, 123.5, 122.3, 119.0, 118.1, 107.5, 107.0, 101.7, 88.5, 83.2, 56.5, 55.5; HRMS (ESI): *m/z* calcd for C₂₄H₂₀N₄O₄ [M-H]⁻ 427.1411, found 427.1431.

2-Acrylamido-N-(6-(3-methoxyphenoxy)pyridazin-3-yl)benzamide (4n)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3n** (49 mg, 0.2 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=5: 95 to 8: 92) to obtain **4n** as a red gel (20.9 mg, 27%).

¹H-NMR (600 MHz, CDCl₃) δ 11.02 (s, 1H), 9.70 (s, 1H), 8.72 (d, *J*=9 Hz, 1H), 8.49 (d, *J*=9.6 Hz, 1H), 7.81 (d, *J*=7.8 Hz, 1H), 7.54 (t, *J*=7.8 Hz, 1H), 7.32 (t, *J*=8.4 Hz, 1H), 7.27 (d, *J*=9.6 Hz, 1H), 7.08 (t, *J*=7.2 Hz, 1H), 6.75–6.80 (m, 3H), 6.41 (d, *J*=16.8 Hz, 1H), 6.28 (dd, *J*=16.8, 10.2 Hz, 1H), 5.77 (d, *J*=10.2 Hz, 1H), 3.81 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 168.2, 164.1, 163.8, 161.1, 154.9, 152.3, 140.3, 134.0, 132.4, 130.4, 127.7, 127.6, 124.2, 123.4, 122.0, 119.7, 119.4, 113.0, 111.2, 107.1, 55.6; HRMS (ESI): *m/z* calcd for C₂₁H₁₈N₄O₄ [M-H]⁻ 389.1255, found 389.1269.

2-Acrylamido-N-(6-(3-methoxyphenyl)pyridazin-3-yl)benzamide (4o)

To a solution of the compound **1a** (40 mg, 0.2 mmol), compound **3o** (40 mg, 0.2 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4o** as a white solid (20.5 mg, 27%).

¹H-NMR (600 MHz, CDCl₃) δ 11.11 (s, 1H), 9.87 (s, 1H), 8.79 (d, *J*=8.4 Hz, 1H), 8.57 (d, *J*=8.4 Hz, 1H), 7.95–7.96 (m, 2H), 7.67 (s, 1H), 7.55–7.59 (m, 2H), 7.44 (t, *J*=7.8 Hz, 1H), 7.17 (t, *J*=7.8 Hz, 1H), 7.05 (dd, *J*=7.8, 2.4 Hz, 1H), 6.44 (d, *J*=16.8 Hz, 1H), 6.31 (dd, *J*=16.8, 10.2 Hz, 1H), 5.80 (d, *J*=10.2 Hz, 1H), 3.90 (s, 3H); ¹³C-NMR (150 MHz,

CDCl₃) δ 168.6, 164.1, 160.4, 156.9, 154.2, 140.5, 137.3, 134.2, 132.4, 130.3, 127.8, 127.7, 126.2, 123.4, 122.0, 120.0, 119.4, 119.2, 116.2, 112.0, 55.6; HRMS (ESI): *m/z* calcd for C₂₁H₁₈N₄O₃ [M-H]⁻ 373.1305, found 373.1317.

2-Acrylamido-N-(6-(3-methoxyphenethyl)pyridazin-3-yl)benzamide (4p)

Compound **3d** (45 mg, 0.2 mmol) was dissolved in MeOH as a solvent, add Pd/C to a mixture, purged by H₂ gas and stirred for 3 h at 25°C. The solution was filtered, and the filtrate was evaporated *in vacuo*. To the vial, a solution of the compound **1a** (40 mg, 0.2 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=40: 60 to 70: 30) to obtain **4p** as a white solid (4.4 mg, 5%).

¹H-NMR (600 MHz, CDCl₃) δ 11.07 (b, 1H), 9.17 (s, 1H), 8.79 (d, *J*=8.4 Hz, 1H), 8.38 (d, *J*=9 Hz, 1H), 7.81 (d, *J*=7.2 Hz, 1H), 7.60 (td, *J*=7.8, 1.2 Hz, 1H), 7.28 (d, *J*=9 Hz, 1H), 7.19–7.23 (m, 2H), 6.79 (d, *J*=7.8 Hz, 1H), 6.76–6.77 (m, 2H), 6.43 (d, *J*=16.8 Hz, 1H), 6.30 (dd, *J*=16.8, 10.2 Hz, 1H), 5.79 (d, *J*=10.2 Hz, 1H), 3.79 (s, 3H), 3.28 (dd, *J*=9.6, 7.2 Hz, 2H), 3.10 (dd, *J*=9, 6.6 Hz, 2H); ¹³C-NMR (150 MHz, CDCl₃) δ 164.1, 159.9, 153.4, 151.1, 142.3, 140.5, 134.2, 132.4, 129.7, 128.7, 127.6, 127.3, 123.5, 122.1, 121.0, 119.2, 114.5, 113.9, 111.8, 107.5, 55.3, 37.5, 35.8; HRMS (ESI): *m/z* calcd for C₂₃H₂₂N₄O₃ [M-H]⁻ 401.1618, found 401.1632.

2-Acrylamido-N-(6-(3-(dimethylamino)phenethyl)pyridazin-3-yl)benzamide (4q)

Compound **3g** (47 mg, 0.2 mmol) was dissolved in MeOH as a solvent, add Pd/C to a mixture, purged by H₂ gas and stirred for 3 h at 25°C. The solution was filtered, and the filtrate was evaporated *in vacuo*. To the vial, a solution of the compound **1a** (40 mg, 0.2 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (150 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110°C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25°C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of methanol/dichloromethane (v: v=8: 92 to 10: 90) to obtain **4q** as a white solid (7.0 mg, 8%).

¹H-NMR (600 MHz, CDCl₃) δ 11.09 (s, 1H), 9.24 (s, 1H), 8.79 (d, *J*=7.8 Hz, 1H), 8.37 (b, 1H), 7.83 (d, *J*=7.8 Hz, 1H), 7.59 (td, *J*=7.8, 1.2 Hz, 1H), 7.31 (d, *J*=9.6 Hz, 1H), 7.16–7.21 (m, 2H), 6.61 (dd, *J*=7.2, 2.4 Hz, 1H), 6.58–6.59 (m, 2H), 6.43 (d, *J*=16.8 Hz, 1H), 6.26 (dd, *J*=16.8, 10.2 Hz, 1H), 5.79 (d, *J*=10.2 Hz, 1H), 3.29 (t, *J*=7.8 Hz, 2H), 3.06 (t, *J*=7.8 Hz, 2H), 2.93 (s, 6H); ¹³C-NMR (150 MHz, CDCl₃) δ 164.1, 153.5, 151.0, 143.0, 141.6, 140.5, 134.2, 132.4, 129.4, 128.8, 127.6, 127.4, 127.4, 123.4, 122.1, 119.5, 119.3, 117.0, 112.9, 110.8, 40.8, 37.7, 36.4; HRMS (ESI): *m/z* calcd for C₂₄H₂₅N₅O₂ [M-H]⁻ 414.1935, found 414.1939.

2-Acrylamido-N-(6-(2-(3-fuloro-4-methoxyphenylethynyl)pyridazin-3-yl)benzamide (4r)

To a solution of the compound **1a** (21 mg, 0.1 mmol), compound **3r** (25 mg, 0.1 mmol) in dry toluene purged with N₂ gas, was

added trimethylaluminum (80 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 80 °C for 20 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with with gradient concentration of ethyl acetate/hexanes (v: v=50: 50 to 70: 30) to obtain **4r** as a white solid (6.3 mg, 15%).

¹H-NMR (600 MHz, CDCl₃) δ 11.03 (s, 1H), 9.27 (s, 1H), 8.80 (d, J=7.6 Hz, 1H), 8.54 (d, J=9.0 Hz, 1H), 7.80–7.82 (m, 1H), 7.69–7.70 (m, 1H), 7.60–7.63 (m, 1H), 7.39–7.40 (m, 1H), 7.35 (dd, J=11.7, 2.1 Hz, 1H), 7.21–7.23 (m, 1H), 6.97 (t, J=8.6 Hz, 1H), 6.44 (d, J=17.9 Hz, 1H), 6.31 (dd, J=17.2, 10.3 Hz, 1H), 5.80 (d, J=10.3 Hz, 1H), 3.94 (s, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 164.15, 153.07, 151.11, 145.39, 140.63, 134.49, 132.33, 131.56, 129.21, 129.19, 127.76, 127.28, 123.51, 122.24, 119.80, 119.66, 119.07, 118.26, 114.05, 113.39, 92.60, 85.03, 56.41; HRMS (ESI): *m/z* calcd for C₂₃H₁₇N₄O [M-H]⁺ 415.1212, found 415.1227.

2-Acrylamido-N-(6-(2-(1,3-benzodioxol-5-yl)ethynyl)pyridazin-3-yl)benzamide (4s)

To a solution of the compound **1a** (41 mg, 0.2 mmol), compound **3s** (48 mg, 0.2 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (203 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 80 °C for 10 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with with gradient concentration of ethyl acetate/hexanes (v: v=20: 80 to 50: 50) to obtain **4s** as a white solid (5.8 mg, 7%).

¹H-NMR (600 MHz, DMSO-d₆) δ 8.39 (d, J=9.6 Hz, 1H), 7.97 (d, J=9.0 Hz, 1H), 7.92 (d, J=9.0 Hz, 1H), 7.80 (d, J=8.3 Hz, 1H), 7.56 (t, J=7.2 Hz, 1H), 7.21–7.27 (m, 4H), 7.03 (d, J=8.3 Hz, 1H), 6.42 (dd, J=17.2, 10.3 Hz, 1H), 6.17–6.21 (m, 1H), 6.12 (s, 2H); ¹³C-NMR (150 MHz, DMSO-d₆) δ 167.84, 163.30, 154.24, 148.80, 147.54, 143.79, 136.93, 131.97, 131.32, 129.34, 127.15, 127.06, 125.48, 123.83, 122.62, 118.90, 118.05, 113.99, 111.27, 108.99, 101.78, 92.22, 84.67, 54.90 HRMS (ESI): *m/z* calcd for C₂₃H₁₆N₄O₄ [M-H]⁺ 411.1098, found 411.1117.

N-[6-[2-(4-methoxyphenyl)ethynyl]pyridazin-3-yl]-2-(propanoylamino)benzamide (4t)

To a solution of compound **1b** (100 mg, 0.5 mmol), **3c** (109 mg, 0.5 mmol) in dry toluene purged with N₂ gas, was added trimethylaluminum (360 μ L, 2.0 M in toluene) dropwise. The reaction mixture was heated at 110 °C for 3 h in the sand bath. After confirming the reaction was complete by TLC, the mixture was cooled at 25 °C, concentrated *in vacuo*, and solid loaded without work up. It was purified using MPLC with gradient concentration of ethyl acetate/hexanes (v: v=0: 100 to 60: 40) to obtain **4t** as a pale white solid (44 mg, 22.9%).

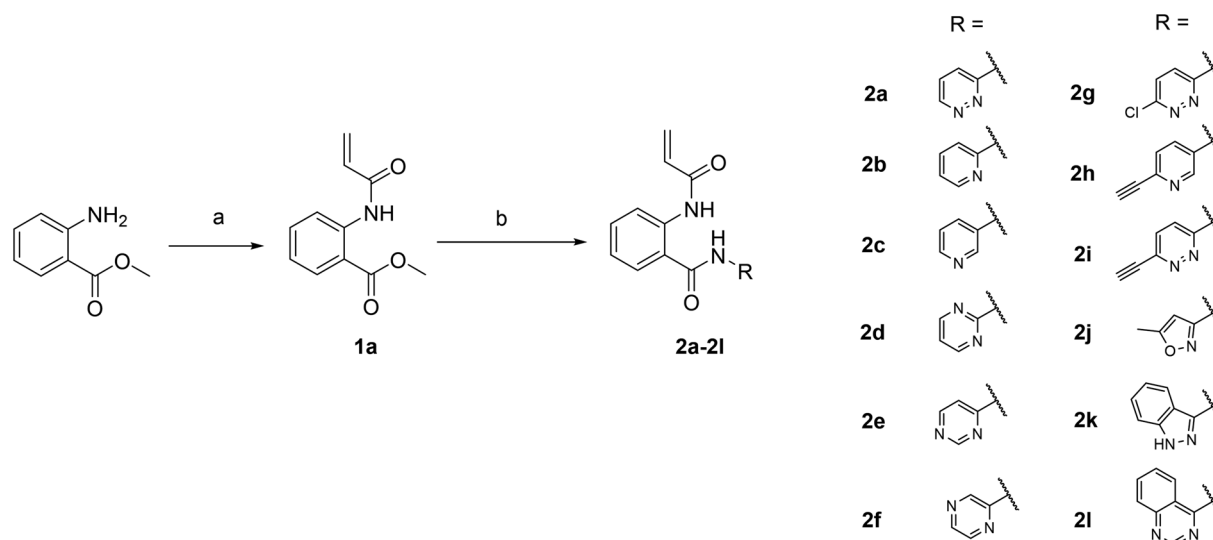
¹H-NMR (600 MHz, CDCl₃) δ 10.75 (s, 1H), 9.13 (s, 1H), 8.72 (d, J=8.3 Hz, 1H), 8.53 (d, J=9.6 Hz, 1H), 7.76 (d, J=6.9 Hz, 1H), 7.69 (d, J=9.6 Hz, 1H), 7.57–7.63 (m, 3H), 7.18–7.21 (m, 1H), 6.92 (d, J=9.3 Hz, 2H), 3.86 (d, J=6.9 Hz, 3H), 2.48 (q, J=7.6 Hz, 2H), 1.28 (t, J=7.6 Hz, 3H); ¹³C-NMR (150 MHz, CDCl₃) δ 172.87, 168.24, 160.77, 153.07, 145.62, 140.54, 134.21, 133.93, 131.49, 127.49, 122.01, 119.11, 118.51, 114.66, 114.14, 113.63, 94.02, 84.59, 77.16, 55.51, 31.65, 9.71; HRMS (ESI): *m/z* calcd for C₂₃H₂₀N₄O₃ [M-H]⁺ 399.14624, found 399.1461.

In vitro kinase assay

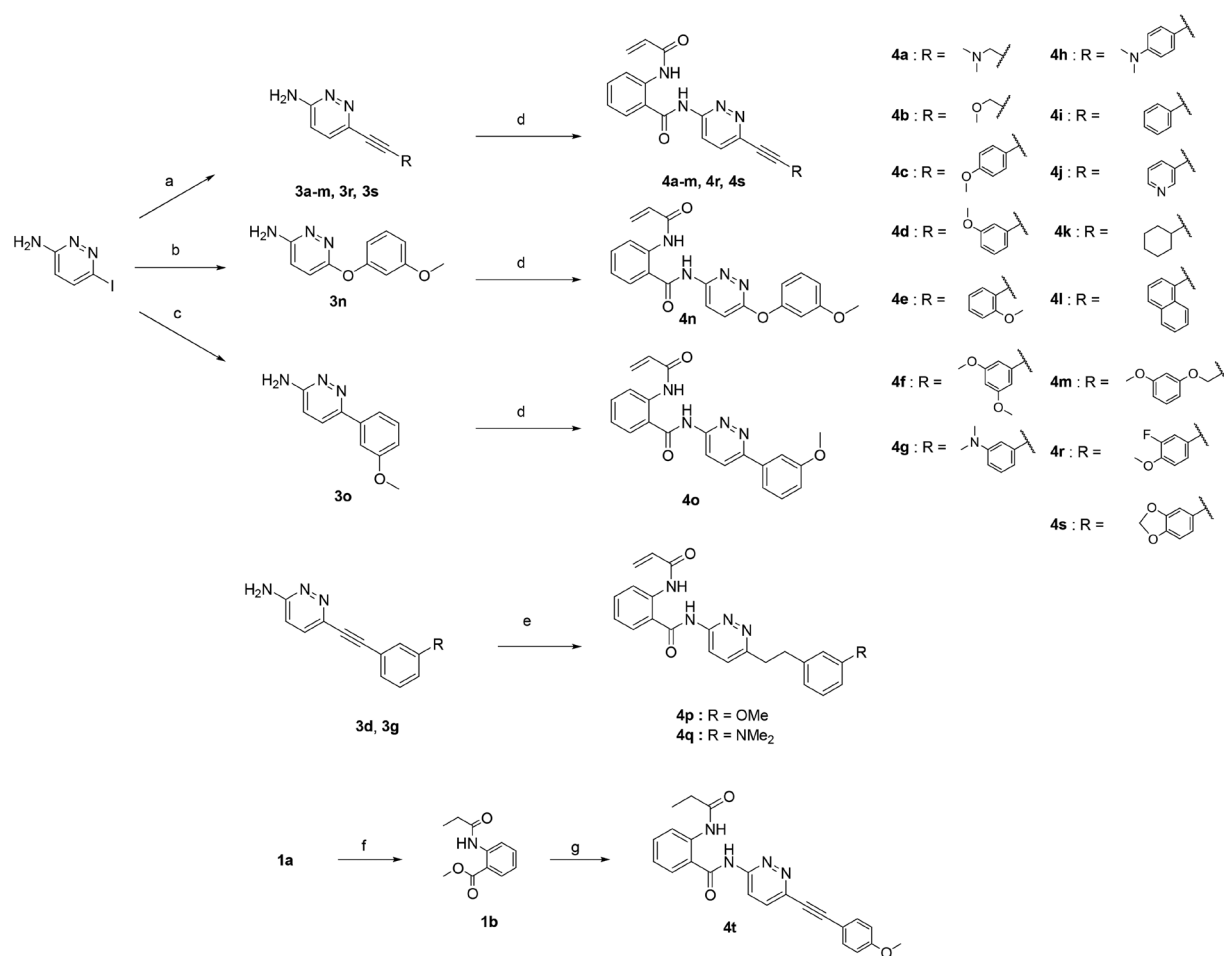
In vitro kinase assay of compound **1**, **24–56**, **58** were performed through Kinase HotSpot Profiling service of Reaction Biology Corp. (USA). % inhibition of compounds was measured in the presence of a Km concentration of ATP at 0.1 and 1 μ M.

Metabolic stability assay

Two types of liver microsomes (Human, Mouse, 0.5 mg/ml; Corning, cat No. #452117, #452701) were pre-incubated for 5 min at 37 °C after adding compounds at a concentration of 1 μ M in 0.1 M phosphate buffer (pH 7). Subsequently, the reaction was initiated by adding the NADPH Regeneration System solution (Promega, #V9510) and allowed to incubate for 30 min at 37 °C. To terminate the reaction, an acetonitrile solution containing an internal standard (chlorpropamide) was added, followed by centrifugation for 15 min at 15,000 rpm and 4 °C. The supernatant was then injected into the LC-MS/MS system (Nexera XR system, Shimadzu) for the



Scheme 1. Reagents and conditions: (a) acryloyl chloride, Et₃N, CH₂Cl₂, rt, 3 h, 44%; (b) arylamine, trimethylaluminum 2 M in toluene, dry toluene, 110 °C, 3 h, 11–35%.



Scheme 2. Reagents and conditions: (a) arylacetylene, $(\text{Ph}_3\text{P})_2\text{PdCl}_2$, CuI, dry 1,4-dioxane, rt, overnight, 44–95%; (b) 3-methoxyphenol, picolinic acid, K_3PO_4 , CuI, DMSO, 100°C, overnight, 52%; (c) 3-methoxyphenylboronic acid, $\text{Pd}(\text{PPh}_3)_4$, K_2CO_3 , THF, water, 130°C, 1 h, microwave irradiation, 68%; (d) **1a**, $\text{Al}(\text{CH}_3)_3$ 2M in toluene, dry toluene, 110°C, 3 h, 11–31%. (e) i) Pd/C , H_2 , CH_3OH , rt, 3 h; ii) **1a**, $\text{Al}(\text{CH}_3)_3$ 2M in toluene, dry toluene, 110°C, 3 h, 5–8%. (f) Pd/C , H_2 , CH_3OH , rt, 3 h, 97%; (g) **3c**, $\text{Al}(\text{CH}_3)_3$ 2M in toluene, dry toluene, 110°C, 3 h, 23%.

analysis of the parent compound. The experiments were conducted in duplicate, and the variation was within 15%. The results were quantified by comparing the values obtained after a 30-min reaction with those at 0 min, expressed as % Remaining (note that when results are unstable, the deviation may exceed 15%). To confirm the accuracy of the experiments, a positive control group, verapamil (1 μM), was conducted only in human microsomes, and the internal reference value was within 15% (± 1), confirming the suitability of the experimental results.

Results and discussion

As shown in Scheme 1, key intermediate **1a** was prepared by coupling with acryloyl chloride. Then, first blocks were introduced by adding a variety of aryl amines in the presence of trimethylaluminum to provide **2a–2l**. The second blocks were linked to pyridazine group via metal catalysed coupling reactions, as described in Scheme 2. A variety of acetylenes **3a–m**, **3r**, **3s**, arylether **3n** and aryl derivative **3o** were synthesised by Sonogashira reaction, Ullmann type reaction, and Suzuki-Miyaura coupling reaction, respectively. Amide formation in the presence of Lewis acid provided the target derivatives **4a–s**. Saturated alkyl derivatives **4p** and **4q** were prepared by hydrogenation of **3d** and **3g**. Finally, compound **4t**, a propionamide derivative with a reduced vinyl group in the acrylamide moiety, was synthesised through reduction of compound **1a**.

Table 1. In vitro inhibitory activity of compound **24–32**, **54–56** against FGFR4 kinase at 1 μM .

compound	R	% inhibition	compound	R	% inhibition
2a		21	2g		30
2b		9	2h		44
2c		7	2i		49
2d		6	2j		5
2e		12	2k		4
2f		9	2l		0

Table 2. In vitro inhibitory activity of compounds **4a–4s** against FGFR4 kinase at 1 μ M.

compound	R	% Inhibition	compound	R	% Inhibition
4a		10	4b		47
4c		97	4d		86
4e		42	4f		80
4g		59	4h		92
4i		91	4j		13
4k		25	4l		17
4m		37	4n		7
4o		39	4p		26
4q		13	4r		56
4s		92	4t	-	0
BLU554	-	97			

Table 3. IC₅₀ values of selected compounds (**4a**, **4d**, **4h** and **4s**).

	4c	4d	4i	4s	Staurosporine
IC ₅₀ (nM)	33	424	235	319	151

Table 4. Kinase selectivity of **4c** against FGFR subtypes.

Compound	% inhibition at 1 μ M			
	FGFR1	FGFR2	FGFR3	FGFR4
4c	3	1	0	97
BLU554	29	11	10	97

First, small aromatic fragments were added to the acrylamidophenyl moiety as a common irreversible warhead essential for activity. As shown in Table 1, the introduction of pyridazine displayed slightly better activity compared to pyridine (**2b**, **2c**), and pyrimidines (**2d**, **2e**) and

Table 5. Metabolic stability of **4c** in human and mouse liver microsomes.

Compounds	Remaining % after 30 min	
	Human	Mouse
4c	7.5	25.2
4s	62.1	53.9
Verapamil	17.4	–

pyrazine (**2f**). The addition of an acetylene group (**2h** and **2i**) or chlorination (**2g**) led to increased activity, but methylisoxazole (**2j**) and bicyclic compounds (**2k** and **2l**) did not exhibit any activity. Therefore, further addition of fragments was carried out at terminal end of the acetylene moiety of **2i**. As shown in Table 2, the introduction of non-aromatic groups (**4a** and **4b**) exhibited weak activity, but *p*-methoxyphenyl derivative **4c** displayed significant activity, surpassing all other alkoxy phenyl derivatives (**4d**, **4e** and **4f**). Replacement of

methoxy group with dimethylamino group did not improve activity, although *p*-amino derivative **4h** retained its activity. Unsubstituted phenyl derivative **4i** showed similar activity to **4h**, indicating that meta substitution at the terminal phenyl group would be unfavourable. cyclohexyl (**4k**) and pyridinyl (**4j**) and naphthyl (**4l**) derivatives showed poor activity. One-carbon-extension (**4m**) led to a decrease activity. Removal of acetylene moiety (**4n** and **4o**) did not improve activity either. Hydrogenation of acetylene (**4p** and **4q**) resulted in a significant decrease in activity. The introduction of fluoride (**4r**) decreased activity by half, while the alkoxy group (**4s**) caused a slight decrease in activity.

IC₅₀ values for some selected compounds were described in Table 3. IC₅₀ value of **4c** exhibited about a 10-fold improvement compared to that of other derivatives and was 5-fold more potent than staurosporin (Table 3). As shown in Table 4, at 1 μM **4c** demonstrated excellent selectivity of for the FGFR family. However, its metabolic stability was not great (Table 5). In contrast, **4s** showed improved stability, suggesting that methoxyphenyl group would be susceptible to metabolic processes. Further investigation would be necessary to optimise its pharmacokinetic properties.

Conclusion

We have successfully identified potent and selective FGFR4 inhibitors using a strategy that involves the sequential addition and selection of fragments to a covalent warhead unit. Among the approaches employed to discover active compounds, our method has demonstrated its effectiveness for obtaining hit compounds from small fragments. This approach would be useful to researchers who do not have access to a substantial chemical library.

Authors contributions

J.K. mainly conducted the experimental work with support from C.G.I., K.O., J.M.L., and F.A.R. in the preparation of some derivatives, as well as in the data collection and interpretation. K.H.M. conceptualised and designed the experiment, and prepared the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data supporting the findings of this study are available within the article.

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