

Supporting Information

Inhibition of Cancer-Associated Mutant Isocitrate Dehydrogenases: Synthesis, SAR and Selective Antitumor Activity

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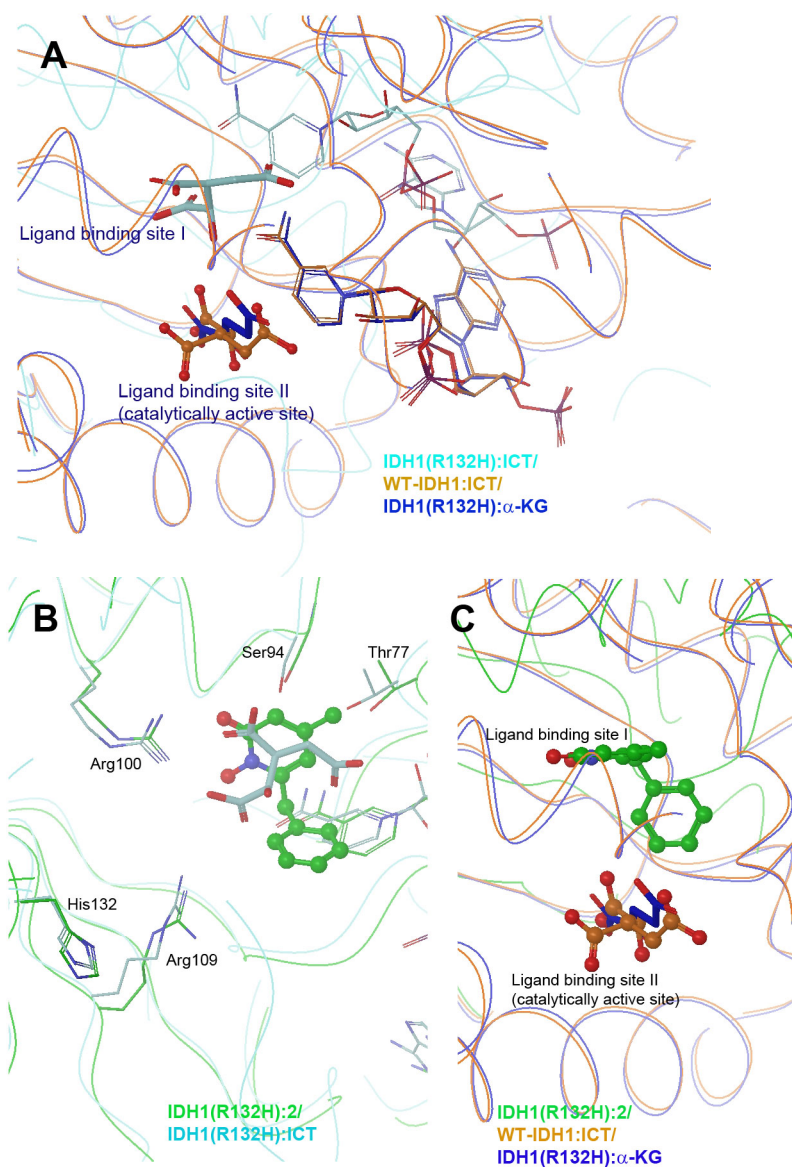


Figure S1. (A) Alignment of the crystal structures of IDH1(R132H):ICT (in cyan, PDB: 3MAP), WT-IDH1:ICT (in orange, PDB: 1T0L) and IDH1(R132H):α-KG (in blue, PDB: 3INM), showing that ICT binds to the ligand binding site I in IDH1(R132H), while it binds to the site II in WT IDH1. α-KG is located in the binding site II of IDH1(R132H); (B) The aligned structures of IDH1(R132H):2 (with carbon and protein chain shown in green, PDB: 4I3L) and IDH1(R132H):ICT (in cyan) and (C) The aligned structures of IDH1(R132H):2 (in green), WT-IDH1:ICT (in orange) and IDH1(R132H):α-KG (in blue), showing compound **2** is located in the ligand binding site I of IDH1(R132H).

Experimental Section

All reagents were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich (Milwaukee, WI). Compounds were characterized by ^1H NMR on a Varian (Palo Alto, CA) 400-MR spectrometer. The purities of all compounds were determined by a Shimadzu Prominence HPLC with a Zorbax C18 or C8 column (4.6 x 250 mm) monitored by UV absorbance at 254 nm. The purities of all compounds were found to be >95%.

General method A: Conversion of 2-methoxypyridine to 1-hydroxypyridin-2-one.

A substituted 2-methoxypyridine (1 mmol) were dissolved in CHCl_3 (5.0 mL), then 3-chloroperbenzoic acid (258.9 mg, 1.5 mmol) was added to the reaction system. The reaction solution was stirred at 50 °C. After the reaction completed (in ~12 h monitored by TLC), the reaction system was purified with flash column chromatography (silica gel, ethyl acetate/MeOH 1/0 – 2/1, v/v) to afford the N-oxide compound (50 – 70% yield). The N-oxide thus obtained (0.3 mmol) was dissolved in AcCl (5.0 mL) and the reaction solution was stirred at 50 °C overnight. The solvent was removed under reduced pressure and the residue was dissolved in MeOH (5.0 mL) and stirred overnight. MeOH was removed under reduced pressure and the resulting powder was washed with Et_2O to afford the 1-hydroxypyridin-2-one as a pale-yellow or off-white powder in 90 - 95% yield.

General method B: Pd(II) mediated coupling reactions.

General method B1. To a solution of a bromo-substituted 2-methoxypyridine (2.5 mmol), $\text{Pd}(\text{PPh}_3)_4$ (144 mg, 0.125 mmol) in anhydrous THF (5 mL) was added an organozinc reagent such as benzylzinc bromide (8 mmol, 8 mL of 1 M solution in THF). The reaction mixture was refluxed for 12 h and the solvent was removed under reduced pressure. The residue was purified with flash chromatography (silica gel, hexane/ethyl acetate = 10/1) to give a substituted 2-methoxypyridine.

General method B2. A mixture of a bromo-substituted 2-methoxypyridine (376.0 mg, 2 mmol), a (substituted) styrene (10 mmol), $\text{Pd}(\text{dppf})\text{Cl}_2$ (146.3 mg, 0.2 mmol), and Cs_2CO_3 (1.95 g, 6 mmol) was stirred in anhydrous 1,4-dioxane (8 mL) at 120 °C for 12 h. The reaction was quenched by addition of 14 mL of H_2O and the organic product was extracted with CH_2Cl_2 (3 x 50 mL) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and the residue was purified with flash chromatography (silica gel, hexane/ethyl acetate = 10/1) to give the coupling product, which was subjected to Pd/C (10%) catalyzed hydrogenation to afford a (2-phenyl)ethyl substituted 2-methoxypyridine as a colorless oil.

General method C: Synthesis of a 6-substituted 4-methyl-1-hydroxypyridin-2-one.

A mixture of AlCl_3 (5.98 g, 45 mmol), chloroacetyl chloride (2.26 g, 1.5 mL, 20 mmol) and ethyl 3-methylbut-2-enoate (2.56 g, 2.8 mL, 20 mmol) in CH_2Cl_2 (50 mL) was stirred at 40 °C for 3 h. Upon cooling in an ice bath, the mixture was quenched by concentrated HCl (37% in water, 5 mL) and 5 mL ice water. The organic product was extracted with CH_2Cl_2 (3 x 50 mL) and washed with 10% NaHCO_3 and water. The solvent was removed under reduced pressure to afford a mixture of isomeric esters, which were heated in a mixture of HOAc (13 mL) and H_2SO_4 (2 mL) at 95 °C for 2 h. The mixture was poured into ice water and neutralized cautiously by NaHCO_3 . The organic product was extracted with CH_2Cl_2 (3 x 50 mL) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and the residue was purified with flash chromatography (silica gel, hexane/ethyl acetate = 3/1) to give the 6-(chloromethyl)-4-methyl-2H-pyran-2-one (1.62 g, 51% yield).

A mixture of 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol), an organoboronic acid (2.52 mmol), $\text{Pd}(\text{dppf})\text{Cl}_2$ (46.1 mg, 0.063 mmol) and Cs_2CO_3 (821.1 mg, 2.52 mmol) was refluxed in anhydrous 1,4-dioxane (6 mL) at 110 °C for 12 h. The reaction was quenched

with 14 mL of H₂O and the organic product was extracted with CH₂Cl₂ (3 x 50 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the residue was purified with flash chromatography (silica gel, hexane/ethyl acetate = 10/1) to give a substituted 2H-pyran-2-one as a colorless oil.

A mixture of a substituted 2H-pyran-2-one (0.5 mmol), P₄S₁₀ (2.22g, 5 mmol) in C₆H₆ (10 mL) was stirred at 80 °C for 12 h. Upon removal of the solvent under reduced pressure, the residual oil was purified with flash chromatography on silica gel (eluent: hexane/ethyl acetate = 5/1) to give the corresponding 2H-pyran-2-thione in 53 – 78% yield. To a solution of the substituted 2H-pyran-2-thione (0.2 mmol) in pyridine (8 mL) was added NH₂OH·HCl (69.5 mg, 1.0 mmol). The reaction mixture was stirred at 115 °C for 12 h. Pyridine was removed under reduced pressure and the solid obtained was washed with Et₂O to afford the substituted 1-hydroxypyridin-2-one in 90 - 96% yield as a pale-yellow or off-white powder.

If there is a -OMe in the phenyl ring of the above product, the methyl group can also be removed by treatment with BBr₃ to give a -OH. To a solution of a OMe-containing 1-hydroxypyridin-2-one (0.1 mmol) in CH₂Cl₂ (5 mL) was added BBr₃ (125.2 mg, 48 μL, 0.5 mmol). The reaction mixture was stirred at room temperature for 12 h. The reaction was quenched with H₂O and the organic product was extracted with CH₂Cl₂ (3 x 50 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the solid was washed with Et₂O to afford the OH-containing 1-hydroxypyridin-2-one in 95 – 97% yield as a pale-yellow or off-white powder.

General Method D: A mixture of a carboxylic acid (1 mmol), an amine (1.2 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (290 mg, 1.5 mmol) and 1-hydroxybenzotriazole (135 mg, 1 mmol) in dry CH₂Cl₂ (5 mL) was stirred overnight. Ethyl acetate and water (1/1, 30 mL) were added. The organic layer was separated, washed with 1M HCl, saturated sodium bicarbonate, dried over sodium sulfate and concentrated *in vacuo*. The amide product was purified with appropriate flash chromatography (silica gel).

Synthesis of Compounds 2-5, 7, 21-26 and 33 were reported in our previous publications (Ref. 21 and 22). Compound 9, ciclopirox was purchased from AK Scientific (Union City, CA).

Compound 6 (C₁₁H₉NO₃): To a mixture of 4-hydroxy-2-methoxypyridine (375 mg, 3 mmol), bromobenzene (263 μL, 2.5 mmol) and 2,2,6,6-tetramethylheptane-3,5-dione (20 μL, 8 mmol%) in DMF (2.5 mL) were added CuI (10 mg, 2 mmol%) and K₃PO₄ (1.27 g, 6 mmol), respectively. The reaction mixture was warmed to 110 °C and stirred for 24 h, and then cooled to room temperature. Ethyl acetate and water were added. The organic layer was separated, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, hexane/ethyl acetate = 50/1) to give 2-methoxy-5-phenoxy-pyridine as a colorless oil (384 mg, 77%), which was converted into compound 6 as a white solid (332 mg, 91%) by following general method A. ¹H NMR (400 MHz, d₆-DMSO): δ 7.98 (d, *J* = 3.2 Hz, 1 H), 7.35-7.30 (m, 3 H), 7.07-7.05 (t, *J* = 6.0 Hz, 1 H), 7.03-6.92 (m, 2 H), 6.56-6.54 (m, 1 H); MS (ESI): *m/z* 204.1 (M + H)⁺.

Compound 8 (C₆H₆N₂O₄): ¹H NMR (400 MHz, D₂O): δ 7.51 (d, *J* = 4.0 Hz, 1H), 7.03 (d, *J* = 3.6 Hz, 1H), 6.71 (m, 1H); MS (ESI): *m/z* 170.3 M⁺.

Compound 10 (C₁₃H₁₃NO₂): It was prepared from 5-bromo-2-methoxypyridine (190 mg, 1.0 mmol) and styrene, following general methods B2 and A, as an off-white powder (110.5 mg, 51%). ¹H NMR (400 MHz, CDCl₃): δ 7.50 (s, 1H), 7.30-7.18 (m, 5H), 7.11 (d, *J* = 7.0, 2H), 6.70 (d, *J* = 9.2 Hz, 1H), 2.87-2.81 (m, 2H), 2.88-2.72 (m, 2H); MS (ESI): *m/z* 216.1 (M + H)⁺.

Compound 11 (C₁₄H₁₅NO₂): A mixture of 1-allylbenzene (0.198 mL, 1.5 mmol) and 9-BBN (4.5 mL, 0.5 M solution, 2.25 mmol) was refluxed in tetrahydrofuran for 3h, after which to the resulting solution, Pd(dppf)Cl₂ (41 mg, 5 mol%), tripotassium phosphate (0.636 g, 3 mmol) and 5-bromo-2-methoxypyridine (0.129 mL, 1 mmol) were added. The mixture was heated to 80 °C for 8h. Upon cooling, the solvent was evaporated and the residue was subjected to a flash column chromatography (silica gel, ethyl acetate:hexane (1:5) to give 5-(3-phenylpropyl)-2-methoxypyridine as yellow oil (0.190 g, 83.7%), which was converted to compound 11 (0.11 g, 60%) using the general method A. ¹H NMR (400 MHz, CDCl₃): 7.96 (d, *J* = 2.4 Hz, 1H), 7.39 (dd, *J* = 2.8, 8.4 Hz, 1H), 7.30-7.16 (m, 5H), 6.66 (d, *J* = 8.4 Hz, 1H), 3.91 (s, 3H), 2.64 (t, *J* = 7.6 Hz, 2H), 2.56 (t, *J* = 8.0 Hz, 2H), 1.91 (m, 2H); MS (ESI): *m/z* 230.1 (M + H)⁺.

Compound 12 (C₆H₆N₂O₄): It was prepared from 6-methoxynicotinic acid (150 mg, 1.0 mmol) and hydroxylamine hydrochloride, following general methods D and A, as an off-white powder (106.9 mg, 63%). ¹H NMR (400 MHz, d₆-DMSO): δ 7.95 (d, *J* = 7.2 Hz, 1H), 6.77 (d, *J* = 2.0 Hz, 1H), 6.42 (dd, *J* = 7.2, 2.0 Hz, 1H); MS (ESI): *m/z* 171.0 (M + H)⁺.

Compound 13 (C₁₃H₁₂N₂O₃): It was prepared from 6-methoxynicotinic acid (150 mg, 1.0 mmol) and benzylamine, following general methods D and A, as an off-white powder (178.3 mg, 73%). ¹H NMR (400 MHz, D₂O): δ 8.17 (d, *J* = 4.4 Hz, 1H), 7.62 (dd, *J* = 2.8, 7.2 Hz, 1H), 7.28-7.04 (m, 5 H), 6.21 (d, *J* = 9.2 Hz, 1 H), 4.38 (s, 2 H); MS (ESI): *m/z* 245.5 (M + H)⁺.

Compound 14 (C₁₃H₁₂N₂O₃): It was prepared from 5-amino-2-methoxypyridine (120 mg, 1.0 mmol) and phenylacetic acid, following general methods D and A, as an off-white powder (104.8 mg, 43%). ¹H NMR (400 MHz, CDCl₃): 8.04 (d, *J* = 2.4 Hz, 1H), 7.79 (dd, *J* = 2.8, 9.2 Hz, 1H), 7.41-7.32 (m, 5H), 6.99 (s, br, 1H), 6.68 (d, *J* = 8.8 Hz, 1H), 3.88 (s, 3H), 3.74 (s, 2H); MS (ESI): *m/z* 245.5 (M + H)⁺.

Compound 15 (C₁₁H₉NO₂): It was prepared from 6-bromo-2-methoxypyridine (190 mg, 1.0 mmol) and phenylboronic acid, following general methods B1 and A, as an off-white powder (165.2 mg, 88%). ¹H NMR (400 MHz, d₆-DMSO): δ 8.01 (d, *J* = 3.2 Hz, 1H), 7.59-7.41 (m, 7 H); MS (ESI): *m/z* 188.2 (M + H)⁺.

Compound 16 (C₁₃H₁₃NO₂): To a solution of 6-bromo-2-methoxypyridine (1.88 g, 1.2 mL, 10 mmol), TMEDA (1.73 g, 2.2 mL, 15 mmol) in anhydrous THF (10 mL) was added nBuLi (2.5 M, 6 mL, 15 mmol) at -78 °C. The reaction mixture was stirred for 1 h. Acetophenone (30 mmol) was added and allowed to warm to room temperature slowly and stirred overnight. The reaction was quenched by addition of H₂O (10 mL) and the organic product was extracted with CH₂Cl₂ (3 x 50 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the residue was purified with flash chromatography (silica gel, hexane/ethyl acetate = 3/1) to give the corresponding secondary alcohol. A mixture of the alcohol (5 mmol), MsCl (1.14g, 0.8 mL, 10 mmol), Et₃N (1.52 g, 2.1 mL, 15 mmol) in CH₂Cl₂ (10 mL) was stirred at 50 °C for 12 h and then the solvent was removed under reduced pressure. The residue was purified with flash chromatography (silica gel, hexane/ethyl acetate = 10/1) to give the eliminated compound, which was hydrogenated for 12 h, catalyzed with Pd(OH)₂ (35.1 mg, 20% on Charcoal). Upon filtering off the catalyst, the solvent was removed under reduced pressure to afford 6-(1-phenylethyl)-2-methoxypyridine, which was converted to compound 16 as a pale yellow powder (664.3 mg, 62%), using the general method A. ¹H NMR (CDCl₃, 400 MHz, TMS): δ 1.66 (3H, d, *J* = 6.8 Hz), 4.61 (1H, q, *J* = 6.8 Hz), 6.24-6.26 (1H, m), 6.69-6.71 (1H, m), 7.24-7.28 (3H, m), 7.32-7.40 (3H, m); MS (ESI): *m/z* 216.4 (M + H)⁺.

Compound 17 (C₁₃H₁₃NO₂): It was prepared from 6-bromo-2-methoxypyridine (190 mg, 1 mmol) and styrene, following general methods B2 and A, as a yellow powder (110 mg, 51% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 3.06-3.07 (2H, m), 3.15-3.17 (2H, m), 6.17-6.33 (1H, m), 6.81-6.82 (1H, m), 7.18-7.23 (3H, m), 7.27-7.31 (2H, m), 7.37 (1H, s), 7.71 (1H, s); MS (ESI): m/z 216.3 (M + H)⁺.

Compound 18 (C₁₅H₁₅NO₄): It was prepared from 6-bromo-2-methoxypyridine (190 mg, 1 mmol) and 4-methoxycarbonylstyrene, following general methods B2 and A, as a yellow powder (123.0 mg, 45% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 3.10 (4H, s), 3.89 (3H, s), 6.03 (1H, s), 6.63-6.65 (1H, m), 7.22-7.25 (3H, m), 7.93-7.95 (2H, m); MS (ESI): m/z 274.1 (M + H)⁺.

Compound 19 (C₁₃H₁₃NO₃): A mixture of 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) obtained in the general method C, Phenol (240 mg, 2.5 mmol) and KOH (2.5 mmol) was reflux in EtOH overnight, followed by chromatography (silica gel, hexane/ethyl acetate = 8/1) to give 6-phenoxy-methyl-4-methyl-2H-pyran-2-one, which was converted to compound 19 (272.0 mg, 93%) as a pale yellow powder, following the general method C. ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.26 (3 H, s), 5.21 (2 H, s), 6.41 (1 H, s), 6.50 (1 H, s), 6.93-7.05 (3 H, m), 7.31-7.35 (2H, m); MS (ESI): m/z 232.1 (M + H)⁺.

Compound 20 (C₇H₉NO₂): It was prepared from acetyl chloride, following general method C, as a yellow powder. ¹H NMR (400 MHz, d₆-DMSO): δ 6.16 (s, 1 H), 5.93 (s, 1 H), 2.24 (s, 3 H), 2.06 (s, 3 H); MS (ESI): m/z 140.5 (M + H)⁺.

Compound 28 (C₁₃H₁₂O₂): To a solution of phenylacetic acid (1.36 g, 10 mmol) in CH₂Cl₂ (10 mL) was added oxalyl chloride (30 mmol, 8.6 mL) followed by 0.05 mL of DMF. After 3 h, the solvents were removed under reduced pressure and the acid chloride thus obtained was dissolved in CH₂Cl₂ (10 mL), followed by addition of ethyl 3-methyl-but-2-enate (10 mmol, 1.3 g) and AlCl₃ (35 mmol, 4.7 g). The reaction mixture was refluxed for 5 h and the reaction quenched by HCl (aq.) and ice. The product was extracted with CH₂Cl₂ (50 mL x3) and the combined organic phases were washed with saturated NaHCO₃, dried over sodium sulfate, and evaporated to dryness to give a mixture of esters. It was dissolved in CH₃COOH (10 mL) followed by adding concentrated H₂SO₄ (3 mL). The reaction mixture was heated to 100 °C for 5 h before carefully quenched with NaHCO₃ (aq.) at 0 °C. The product was extracted with EtOAc (50 mL x3) and the organic layers were dried over sodium sulfate, evaporated, and purified with a flash column chromatography (silica gel, EtOAc/Hexane 3/1) to give compound 28 (1.5 g, 75%) as a white solid. ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.02 (s, 3 H), 3.74 (s, 2 H), 5.74 (s, 1 H), 5.87 (s, 1 H), 7.16-7.38 (m, 5 H); MS (ESI): m/z 201.3 (M + H)⁺.

Compound 29 (C₁₃H₁₂SO): To a solution of 28 (400 mg, 2 mmol) in C₆H₆ (10 mL) was added P₄S₁₀ (5 mmol, 2.2 g) and the reaction mixture was stirred at 80 °C for 5 h. Upon removal of the solvent, the residue was subjected to a flash column chromatography (silica gel, EtOAc/hexane 10/1, v/v) to give compound 29 (33.72 mg, 78%) as a white solid. ¹H NMR (CDCl₃, 400 MHz, TMS): δ 1.99 (s, 3 H), 3.72 (s, 2 H), 5.70 (s, 1 H), 5.82 (s, 1 H), 7.16-7.36 (m, 5 H); MS (ESI): m/z 217.5 (M + H)⁺.

Compound 27 (C₁₃H₁₄N₂O): To a solution of compound 29 (43.3 mg, 0.2 mmol) in pyridine (8 mL) was added N₂H₄·H₂O (50.1 mg, 1.0 mmol). The reaction mixture was stirred at 115 °C for 12 h. Pyridine was removed under reduced pressure and the powder was washed with Et₂O to afford compound 27 (38 mg, 89%) as a pale yellow powder. ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.17 (3H, s), 4.10 (2H, s), 4.96 (2H, s), 5.90 (1H, s), 6.36 (1H, s), 7.10-7.42 (5H, m); MS (ESI): m/z 215.1 (M + H)⁺.

Compound 30 (C₁₃H₁₃NO₂): It was prepared from 3-bromo-4-methyl-2-methoxypyridine (200 mg, 1 mmol) and phenylzinc bromide, following general methods B1 and A, as a yellow powder (114.2 mg, 53% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.31 (3H, s), 4.10 (2H, s), 6.31 (1H, s), 7.21-7.32 (5H, m), 7.72 (1H, s), 9.30 (1H, s); MS (ESI): m/z 216.3 (M + H)⁺.

Compound 31 (C₁₄H₁₅NO₃): It was prepared from 3-bromo-4-methyl-2-methoxypyridine (200 mg, 1 mmol) and 3-methoxyphenylzinc bromide, following general methods B1 and A, as a yellow powder (116.7 mg, 48% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.25 (3H, s), 3.75 (3H, s), 4.02 (2H, s), 6.29 (1H, s), 6.70-6.77 (3H, m), 7.13-7.17 (1H, m), 7.66 (1H, s); MS (ESI): m/z 246.1 (M + H)⁺.

Compound 32 (C₁₃H₁₃NO₃): It was prepared from compound **31** (123 mg, 0.5 mmol) and BBr₃, following the deprotection procedure described in the last paragraph of the general method C, as a yellow powder (110 mg, 95% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.22 (3H, s), 3.97 (2H, s), 6.20-6.21 (1H, m), 6.63-6.81 (3H, m), 7.07-7.11 (1H, m), 7.60-7.62 (1H, m); MS (ESI): m/z 232.1 (M + H)⁺.

Compound 35 (C₁₃H₁₃NO₃): To a solution of 2,6-dibromopyridine (4.74 g, 20 mmol) in TFA (50 mL) was added H₂O₂ (35%, 50 mL) at room temperature. Then the reaction mixture was stirred at 100 °C for 12 h. The reaction system was then poured into 500 mL water and the precipitated unreacted starting material was filtered off. The product was extracted with 500 mL of CH₂Cl₂ three times and the solvents were removed under reduced pressure to afford N-oxide 2,6-dibromopyridine-1-oxide (4.20 g, 83% yield). To a solution of N-oxide (4.05 g, 16 mmol) in H₂SO₄ (30 mL) was added HNO₃ (8 mL) at 0 °C. The reaction mixture was stirred at 60 °C for 12 h and neutralized carefully with NaHCO₃ (aq.) at 0 °C. The product was extracted with 200 mL of CH₂Cl₂ three times and the solvent was removed under reduced pressure to afford compound 2,6-dibromo-4-nitropyridine-1-oxide (4.15 g, 87% yield). To a solution of above product (3.87 g, 13 mmol) in CHCl₃ (50 mL) was added PBr₃ (15 mL) at room temperature and the reaction mixture was stirred at 60 °C for 12 h, before quenched with H₂O at 0 °C. The product was extracted with 100 mL of CH₂Cl₂ three times and the solvents were removed under reduced pressure. The residue was purified with flash column chromatography (silica gel, ethyl acetate/hexane: 1/5) to afford the compound 2,6-dibromo-4-nitropyridine (2.93 g, 80% yield). To a solution of compound 2,6-dibromo-4-nitropyridine (2.82 g, 10 mmol) in THF (30 mL) and MeOH (10 mL) was added NaOMe (1.13 g, 21 mmol) at room temperature. The reaction mixture was stirred at 40 °C for 12 h and the solvents were removed under reduced pressure. The residue was purified with flash column chromatography (silica gel, ethyl acetate/hexane: 1/3) to afford the compound 2,6-dibromo-4-methoxypyridine (1.7 g, 78% yield). Compound **35** was prepared from compound 2,6-dibromo-4-methoxypyridine (1.53 g, 7 mmol), following the general methods B1 and A, as an off-white powder (582.8 mg, 63%). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 3.85 (3H, s), 4.25 (2H, s), 6.00 (1H, s), 6.86 (1H, s), 7.25-7.26 (2H, m), 7.30-7.38 (3H, m), 7.79 (1H, s); MS (ESI): m/z 232.1 (M + H)⁺.

Compound 34 (C₁₂H₁₁NO₃): It was prepared from compound **35** (231 mg, 1 mmol) and BBr₃, following the deprotection procedure described in the last paragraph of the general method C, as a yellow powder (200.0 mg, 92% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 4.06 (2H, s), 5.61 (1H, s), 6.00 (1H, s), 7.26-7.34 (5H, m); MS (ESI): m/z 218.4 (M + H)⁺.

Compound 37 (C₁₅H₁₅NO₄): To a solution of methyl 2,6-dibromoisonicotinate (5.90 g, 20 mmol) in EtOH (50 mL) was slowly added NaBH₄ (1.89 g, 50 mmol) at room temperature. Then the reaction mixture was stirred at 78 °C for 12 h, before quenched by addition of H₂O (10 mL). The product was extracted with 50 mL of CH₂Cl₂ three times and the organic layers were evaporated to dryness to give

crude (2,6-dibromopyridin-4-yl)methanol (4.96 g, 93%). To a solution of the aforementioned compound, Et₃N (2.73 g, 3.8 mL, 27 mmol) in CH₂Cl₂ (30 mL) was added methoxymethyl chloride (2.17 g, 2.1 mL, 27 mmol) at room temperature and stirred for 12 h. The product was extracted with 50 mL of CH₂Cl₂ three times and the organic layers were evaporated to give 2,6-dibromo-4-((methoxymethoxy)methyl)pyridine (5.15 g, 92%). The compound thus obtained (2.5 g, 9 mmol) and NaOMe (13.5 mmol) were dissolved in MeOH (10 mL) and the reaction solution was stirred at 65 °C overnight. The solvent was removed under reduced pressure and the residue was purified with flash column chromatography (silica gel, ethyl acetate/hexane: 1/5) to afford the 2-bromo-6-methoxy-4-((methoxymethoxy)methyl)pyridine as a white solid. Following the general methods B1, 6-benzyl-4-((methoxymethoxy)methyl)-2-methoxypyridine can be obtained (1.36 g, 31% yield). To its solution (1.09 g, 4 mmol) in MeOH (10 mL) was added 3 N HCl (3 mL) at room temperature. Then the reaction mixture was stirred at the same temperature for 12 h. The product was extracted with 50 mL of CH₂Cl₂ three times and the organic layers were evaporated to dryness to give 6-benzyl-4-(hydroxymethyl)-2-methoxypyridine (843.7 mg, 92%), which was converted to compound **37** following the general method A as a pale yellow powder (424.5 mg, 52% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.09 (3 H, s), 4.09 (2 H, s), 4.34 (1 H, s), 4.64 (1 H, s), 5.84 (1 H, s), 6.57 (1 H, s), 7.30-7.37 (5 H, m); MS (ESI): m/z 274.1 (M + H)⁺.

Compound 36 (C₁₃H₁₃NO₃): Compound **37** (110 mg, 0.4 mmol) was hydrolyzed by 1 equivalent of NaOH in MeOH/H₂O to give compound **36** as a yellow powder in quantitative yield (87.1 mg, 92% yield). ¹H NMR (CDCl₃, 400 MHz): δ 4.13 (2 H, s), 4.39 (1 H, s), 4.52 (1 H, s), 5.95 (1 H, s), 6.61 (1 H, s), 7.29-7.36 (5 H, m); MS (ESI): m/z 232.2 (M + H)⁺.

Compound 38 (C₁₄H₁₅NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 4-methoxybenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (161.0 mg, 52% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.18 (3 H, s), 3.80 (3 H, s), 4.05 (2 H, m), 5.83 (1 H, s), 6.47 (1 H, s), 6.88-6.90 (2 H, m), 7.17-7.19 (2 H, m); MS (ESI): m/z 246.1 (M + H)⁺.

Compound 39 (C₁₄H₁₅NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 3-methoxybenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (198.1 mg, 63% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.37 (3 H, s), 4.02 (3 H, s), 4.30 (2 H, s), 6.04 (1 H, s), 6.62 (1 H, s), 7.04-7.08 (3 H, m), 7.46-7.50 (1 H, m); MS (ESI): m/z 246.1 (M + H)⁺.

Compound 40 (C₁₂H₁₁NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (100 mg, 0.63 mmol) and 3-methoxybenzeneboronic acid (1.26 mmol), following the general method C as a yellow powder (70.0 mg, 48% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.16 (3 H, s), 4.04 (2H, s), 5.95 (1 H, s), 6.46 (1 H, s), 6.68-6.88 (3 H, m), 7.18 (1 H, s); MS (ESI): m/z 232.3 (M + H)⁺.

Compound 41 (C₁₄H₁₅NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 2-methoxybenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (167.7 mg, 54% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.12 (3 H, s), 3.80 (3 H, s), 4.10 (2 H, s), 5.69 (1 H, s), 6.38 (1 H, s), 6.90-6.96 (2 H, m), 7.15-7.17 (1 H, m), 7.26-7.31 (1 H, m); MS (ESI): m/z 246.1 (M + H)⁺.

Compound 42 (C₁₂H₁₁NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (100 mg, 0.63 mmol) and 2-methoxybenzeneboronic acid (1.26 mmol), following the general method C as a

yellow powder (82.6 mg, 57% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.42 (3 H, s), 4.39 (2 H, s), 6.45 (1 H, s), 6.75 (1 H, s), 7.08-7.16 (2 H, m), 7.39-7.40 (2 H, m), 7.49 (1 H, s); MS (ESI): m/z 232.3 ($\text{M} + \text{H}$) $^+$.

Compound 43 ($\text{C}_{13}\text{H}_{12}\text{FNO}_2$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 3-fluorobenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (161.5 mg, 55% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.17 (3 H, s), 4.08 (2 H, s), 5.83 (1 H, s), 6.42 (1 H, s), 6.96-6.98 (2 H, m), 7.03-7.05 (1 H, m), 7.28-7.33 (1 H, m); ^{19}F NMR (CDCl_3 , 376 MHz, CFCl_3): δ -112.7; MS (ESI): m/z 234.1 ($\text{M} + \text{H}$) $^+$.

Compound 44 ($\text{C}_{13}\text{H}_8\text{F}_5\text{NO}_2$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and pentafluorobenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (222.0 mg, 57% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.20 (3 H, s), 4.18 (2 H, s), 5.77 (1 H, s), 6.48 (1 H, s). ^{19}F NMR (CDCl_3 , 376 MHz, CFCl_3): δ -161.46, -161.48, -154.4, -141.42, -141.38; MS (ESI): m/z 306.1 ($\text{M} + \text{H}$) $^+$.

Compound 45 ($\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_2$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 4-cyanofluorobenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (205.7 mg, 68% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.20 (3 H, s), 4.14 (2 H, s), 5.85 (1 H, s), 6.45 (1 H, s), 7.37-7.39 (1 H, m), 7.52-7.54 (1 H, m), 7.63-7.65 (1 H, m), 7.69-7.72 (1 H, m); MS (ESI): m/z 241.2 ($\text{M} + \text{H}$) $^+$.

Compound 46 ($\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_2$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 3-cyanofluorobenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (199.6 mg, 65% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.20 (3 H, s), 4.12 (2 H, s), 5.88 (1 H, s), 6.45 (1 H, s), 7.43-7.47 (1 H, m), 7.51-7.59 (3 H, m); MS (ESI): m/z 241.2 ($\text{M} + \text{H}$) $^+$.

Compound 47 ($\text{C}_{14}\text{H}_{13}\text{NO}_4$): To a solution of 3-((1-hydroxy-4-methyl-6-oxo-1,6-dihydropyridin-2-yl)methyl)benzonitrile (48.1 mg, 0.2 mmol) in EtOH (5 mL) was added 3 M NaOH (3 mL). The reaction mixture was stirred at 78 $^\circ\text{C}$ for 12 h. The reaction system was acidified by HCl (aq.), the organic compound was extracted with CH_2Cl_2 (3 x 50 mL) and dried over anhydrous Na_2SO_4 . CH_2Cl_2 was removed under reduced pressure to afford the acid compound as a light yellow solid (46.7 mg, 90% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.20 (3 H, s), 4.17 (2 H, s), 5.92 (1 H, s), 6.48 (1 H, s), 7.43-7.47 (1 H, m), 7.51-7.53 (1 H, m), 8.02-8.05 (2 H, m); MS (ESI): m/z 260.1 ($\text{M} + \text{H}$) $^+$.

Compound 48 ($\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_3$): To a solution of 3-((1-hydroxy-4-methyl-6-oxo-1,6-dihydropyridin-2-yl)methyl)benzonitrile (48.1 mg, 0.2 mmol) in EtOH (5 mL) was added H_2O_2 (35%, 0.3 mL), Na_2CO_3 (210 mg, 2 mmol). The reaction mixture was stirred at room temperature for 12 h. The reaction system was acidified by HCl (aq.), the organic compound was extracted with CH_2Cl_2 (3 x 50 mL) and dried over anhydrous Na_2SO_4 . CH_2Cl_2 was removed under reduced pressure to afford the amide as an off-white solid (44.9 mg, 87% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.27 (3 H, s), 4.12 (2 H, s), 5.88 (1 H, s), 6.44 (1 H, s), 7.36-7.46 (2 H, m), 7.52-7.56 (2 H, m); MS (ESI): m/z 259.1 ($\text{M} + \text{H}$) $^+$.

Compound 49 ($\text{C}_{14}\text{H}_{12}\text{F}_3\text{NO}_2$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 4-trifluoromethylbenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (224.7 mg, 63% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.19 (3 H, s),

4.14 (2 H, s), 5.83 (1 H, s), 6.43 (1 H, s), 7.37-7.39 (2 H, m), 7.59-7.61 (2 H, m); ^{19}F NMR (CDCl_3 , 376 MHz, CFCl_3): δ -62.6; MS (ESI): m/z 284.1 ($\text{M} + \text{H}$) $^+$.

Compound 50 ($\text{C}_{15}\text{H}_{17}\text{NO}_2$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 3,5-dimethylbenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (215.4 mg, 66% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.17 (3 H, s), 2.30 (6 H, s), 4.01 (2 H, s), 5.81 (1 H, s), 6.40 (1 H, s), 6.86 (2 H, s), 6.92 (1 H, s); MS (ESI): m/z 244.1 ($\text{M} + \text{H}$) $^+$.

Compound 51 ($\text{C}_{11}\text{H}_{11}\text{NO}_2\text{S}$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 3-thiophenboronic acid (2.52 mmol), following the general method C as an off-white powder (170.0 mg, 61% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.20 (3 H, s), 4.14 (2 H, s), 5.90 (1 H, s), 6.49 (1 H, s), 7.00-7.01 (1 H, m), 7.14 (1 H, s), 7.32-7.34 (1 H, m); MS (ESI): m/z 222.2 ($\text{M} + \text{H}$) $^+$.

Compound 52 ($\text{C}_{19}\text{H}_{17}\text{NO}_2$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 3-biphenylboronic acid (2.52 mmol), following the general method C as an off-white powder (231.1 mg, 63% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.16 (3 H, s), 4.17 (2 H, s), 5.85 (1 H, s), 6.40 (1 H, s), 7.24-7.26 (1 H, m), 7.34-7.37 (1 H, m), 7.40-7.46 (3 H, m), 7.50-7.54 (2 H, m), 7.56-7.60 (2 H, m); MS (ESI): m/z 292.1 ($\text{M} + \text{H}$) $^+$.

Compound 53 ($\text{C}_{19}\text{H}_{17}\text{NO}_3$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 4-phenoxybenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (194.2 mg, 54% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.17 (3 H, s), 4.06 (2 H, s), 5.82 (1 H, s), 6.40 (1 H, s), 6.97-7.03 (4 H, m), 7.09-7.13 (1 H, m), 7.21-7.23 (2 H, m), 7.31-7.36 (2 H, m); MS (ESI): m/z 308.1 ($\text{M} + \text{H}$) $^+$.

Compound 54 ($\text{C}_{17}\text{H}_{15}\text{NO}_2$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 1-naphthylboronic acid (2.52 mmol), following the general method C as an off-white powder (203.7 mg, 61% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.01 (3 H, s), 4.56 (2 H, s), 5.50 (1 H, s), 6.40 (1 H, s), 7.35-7.37 (1 H, m), 7.45-7.52 (4 H, m), 7.84-7.91 (2 H, m); MS (ESI): m/z 266.1 ($\text{M} + \text{H}$) $^+$.

Compound 55 ($\text{C}_{18}\text{H}_{17}\text{NO}_3$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 6-methoxy-1-naphthylboronic acid (2.52 mmol), following the general method C as an off-white powder (226.8 mg, 61% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.24 (3 H, s), 3.95 (3 H, s), 4.20 (2 H, s), 5.79 (1 H, s), 6.40 (1 H, s), 7.12-7.17 (2 H, m), 7.30-7.32 (1 H, m), 7.60-7.63 (1 H, m), 7.68-7.72 (2 H, m); MS (ESI): m/z 296.1 ($\text{M} + \text{H}$) $^+$.

Compound 56 ($\text{C}_{17}\text{H}_{15}\text{NO}_3$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (100 mg, 0.63 mmol) and 6-methoxy-1-naphthylboronic acid (1.26 mmol), following the general method C as an off-white powder (83.3 mg, 47% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.36 (3 H, s), 3.88 (2 H, s), 5.96 (1 H, s), 6.95 (1 H, s), 7.52-7.76 (6 H, m); MS (ESI): m/z 282.3 ($\text{M} + \text{H}$) $^+$.

Compound 57 ($\text{C}_{15}\text{H}_{13}\text{NO}_2\text{S}$): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 2-benzothiophenboronic acid (2.52 mmol), following the general method C as an off-white powder (222.3 mg, 65% yield). ^1H NMR (CDCl_3 , 400 MHz, TMS): δ 2.18 (3 H, s), 4.37 (2 H,

s), 6.01 (1 H, s), 6.45 (1 H, s), 7.33-7.37 (2 H, m), 7.52-7.54 (1 H, m), 7.70-7.73 (1 H, m), 7.78-7.80 (1 H, m); MS (ESI): m/z 272.3 ($M + H$)⁺.

Compound 58 (C₂₀H₁₉NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 4-(4-methoxyphenyl)boronic acid (2.52 mmol), following the general method C as an off-white powder (275.1 mg, 68% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.15 (3 H, s), 3.84 (3 H, s), 4.12 (2 H, s), 5.85 (1 H, s), 6.40 (1 H, s), 6.96-6.98 (2 H, m), 7.29-7.31 (2 H, m), 7.51-7.53 (4 H, m); MS (ESI): m/z 322.1 ($M + H$)⁺.

Compound 59 (C₁₉H₁₇NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (100 mg, 0.63 mmol) and 4-(4-methoxyphenyl)boronic acid (1.26 mmol), following the general method C as an off-white powder (95.0 mg, 49% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.18 (3 H, s), 4.12 (2 H, s), 5.89 (1 H, s), 6.45 (1 H, s), 6.91-6.93 (2 H, m), 7.28-7.30 (2 H, m), 7.45-7.47 (2 H, m), 7.50-7.52 (2 H, m); MS (ESI): m/z 308.1 ($M + H$)⁺.

Compound 60 (C₂₀H₁₉NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 4-(3-methoxyphenyl)boronic acid (2.52 mmol), following the general method C as an off-white powder (261.1 mg, 63% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.17 (3 H, s), 3.86 (3 H, s), 4.13 (2 H, s), 5.85 (1 H, s), 6.41 (1 H, s), 6.89-6.91 (1 H, m), 7.12-7.18 (2 H, m), 7.31-7.38 (3 H, m), 7.52-7.58 (2 H, m); MS (ESI): m/z 322.1 ($M + H$)⁺.

Compound 61 (C₁₉H₁₇NO₃): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (100 mg, 0.63 mmol) and 4-(3-methoxyphenyl)boronic acid (1.26 mmol), following the general method C as an off-white powder (95.5 mg, 49% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.17 (3 H, s), 4.12 (2 H, s), 5.90 (1 H, s), 6.45 (1 H, s), 6.83-6.84 (1 H, m), 7.08-7.14 (2 H, m), 7.29-7.30 (3 H, m), 7.53-7.54 (2 H, m); MS (ESI): m/z 308.1 ($M + H$)⁺.

Compound 62 (C₂₅H₂₁NO₂): It was prepared from 6-(chloromethyl)-4-methyl-2H-pyran-2-one (200 mg, 1.26 mmol) and 3,5-diphenylbenzeneboronic acid (2.52 mmol), following the general method C as an off-white powder (314.6 mg, 68% yield). ¹H NMR (CDCl₃, 400 MHz, TMS): δ 2.16 (3 H, s), 4.23 (2 H, s), 5.91 (1 H, s), 6.41 (1 H, s), 7.48-7.54 (8 H, m), 7.63-7.74 (5 H, m); MS (ESI): m/z 368.1 ($M + H$)⁺.