Supplementary Synthetic Procedures

An effective anti-viral approach targeting hepatitis B virus using NJK14047, a novel and selective biphenyl amide p38 MAPK inhibitor.

Running title: Anti-HBV activity of NJK14047, a p38 MAPK inhibitor

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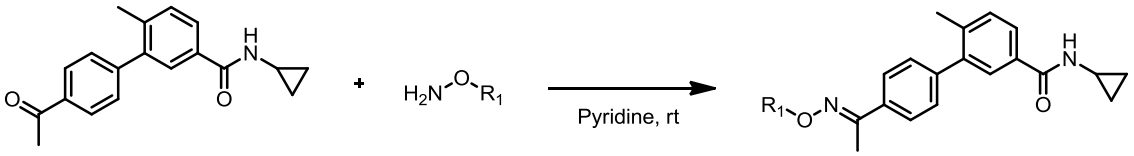
General Considerations

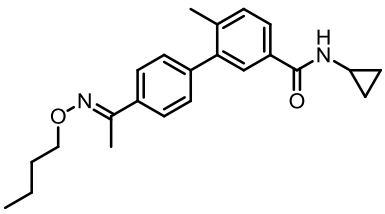
Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers (Aldrich, Alfa Aesar, and TCI) and were used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane, triethylamine and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 ℃. Air and moisture sensitive reactions were performed under argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates. Optical rotations were measured using 100 nm cell of 1~2 mL capacity. ^{1}H-NMR spectra were recorded on a Bruker Avance 400 (400 MHz for ^{1}H) spectrometer. ^{1}H-NMR chemical shifts are reported in parts per million (ppm) relative to TMS (tetramethylsilane), with the residual solvent peak used as an internal reference. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), bd (broad doublet), dd (doublet of doublets), dt (doublet of triplets), or dq (doublet of quartets); the coupling constants are reported in hertz (Hz). Low and High resolution mass spectra were obtained with JEOL JMS-700 instrument.

Experimental Procedure



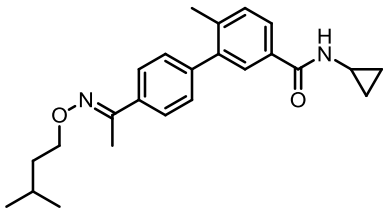
General procedure for the synthesis of NJK13018, NJK13020 and NJK13023

To a solution of 4'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (1 eq., ref: *Bioorg Med Chem.* 25 (2015), 3694-8.) in pyridine was added alkoxy amine (1.5 eq.). The reaction mixture was stirred at room temperature until complete consumption of the starting material on TLC. Then, the reaction mixture was acidified with 2N HCl, and diluted with EtOAc. The combined organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel (EtOAc/Hexanes) afforded *para*-oxime ether compounds.



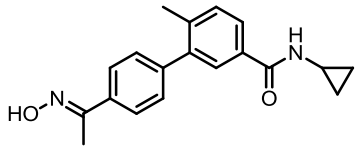
4'-(1-(butoxyimino)ethyl)-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (NJK13018)

Prepared from 4'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (78 mg, 0.267 mmol) and *O*-butylhydroxylamine (36 mg, 0.401 mmol) using the general procedure described above. Purification of the residue via flash column chromatography on silica afforded 44 mg (45%) of compound **NJK13018**; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, 2H, J = 8.2 Hz), 7.65 (dd, 1H, J = 8.2, 1.8 Hz), 7.51 (s, 1H), 7.33-7.29 (m, 3H), 6.38 (s, 1H), 4.22 (t, 2H, J = 6.6 Hz), 2.89-2.88 (m, 1H), 2.28 (s, 3H), 2.27 (s, 3H), 1.77-1.64 (m, 2H), 1.50-1.36 (m, 2H), 0.97 (t, 3H, J = 7.4 Hz), 0.85-0.84 (m, 2H), 0.61-0.60 (m, 2H); LR-MS (EI^+) *m/z* 364.3 (M^+); HR-MS (EI^+) calcd for $C_{15}H_{10}O_2$ (M^+) 364.2151; found 364.2154.



***N*-cyclopropyl-4'-(1-((isopentyloxy)imino)ethyl)-6-methyl-[1,1'-biphenyl]-3-carboxamide (NJK13020)**

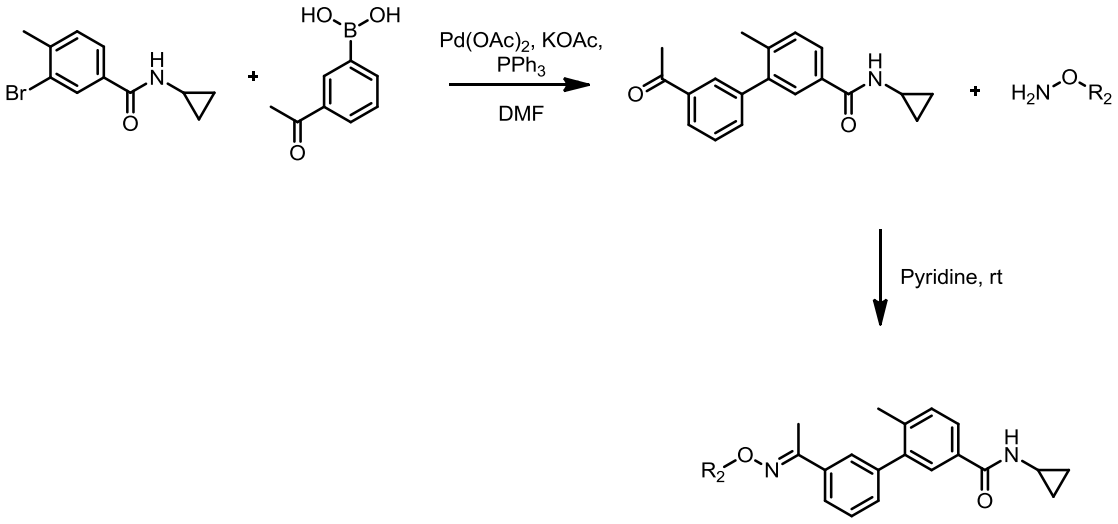
Prepared from 4'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (72 mg, 0.247 mmol) and *O*-isopentylhydroxylamine (38 mg, 0.370 mmol) using the general procedure described above. Purification of the residue via flash column chromatography on silica afforded 51 mg (54%) of compound **NJK13020**; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, 2H, J = 8.4 Hz), 7.65 (dd, 1H, J = 8.4, 1.8 Hz), 7.57 (d, 1H, J = 1.8 Hz), 7.31-7.29 (m, 3H), 6.36 (s, 1H), 4.25 (t, 2H, J = 6.8 Hz), 2.91-2.86 (m, 1H), 2.28 (s, 3H), 2.26 (s, 3H), 1.81-1.72 (m, 1H), 1.64 (dt, 2H, J = 6.9, 6.6 Hz), 0.97 (d, 6H, J = 6.4 Hz), 0.88-0.82 (m, 2H), 0.61-0.60 (m, 2H); LR-MS (EI^+) *m/z* 378.3 (M^+); HR-MS (EI^+) calcd for $C_{15}H_{10}O_2$ (M^+) 378.2307; found 378.2305.

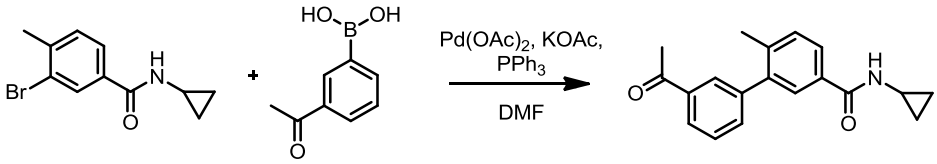


***N*-cyclopropyl-4'-(1-(hydroxyimino)ethyl)-6-methyl-[1,1'-biphenyl]-3-carboxamide (NJK13023)**

Prepared from 4'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (28 mg, 0.097 mmol) and Hydroxylamine (10 mg, 0.145 mmol) using the general procedure described above. Purification of the residue via flash column chromatography on silica afforded 28 mg (93%) of compound **NJK13023**; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 11.19 (s, 1H), 8.34 (d, 1H, J = 4.1 Hz), 7.67 (d, 3H, J = 8.2 Hz), 7.63 (d, 1H, J = 1.3 Hz), 7.32 (t, 3H, J = 7.8 Hz), 2.78-2.77 (m, 1H), 2.21 (s, 3H), 2.13 (s, 3H), 0.62-0.59 (m, 2H), 0.51-0.48 (m, 2H); LR-MS (EI^+) *m/z* 308.2 (M^+); HR-MS (EI^+) calcd for $C_{15}H_{10}O_2$ (M^+) 308.1525; found 308.1527.

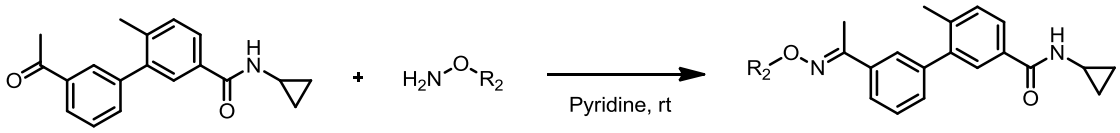
Synthesis scheme of NJK13031, NJK13032, NJK13034, NJK13035 and NJK13040





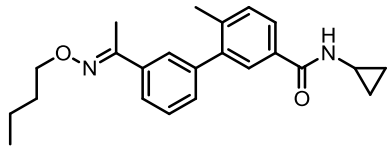
3'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide

To a solution of 3-bromo-*N*-cyclopropyl-4-methylbenzamide (857 mg, 3.370 mmol) in DMF/H_2O (10 mL/2 mL) was added 3-acetylphenyl boronic acid (663 mg, 4.045 mmol), $Pd(OAc)_2$ (8 mg, 0.034 mmol), KOAc (827 mg, 8.425 mmol), PPh_3 (44 mg, 0.169 mmol). The reaction mixture was stirred for overnight at 70 °C, cooled to rt, quenched with 2*N* HCl, and diluted with EtOAc. The combined organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica afforded 370 mg (37%) of the desired compound; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.97-7.95 (m, 1H), 7.89 (d, 1H, J = 1.3 Hz), 7.68 (dd, 1H, J = 8.0, 1.8 Hz), 7.59 (d, 1H, J = 1.8 Hz), 7.56-7.52 (m, 2H), 7.33 (d, 1H, J = 8.0 Hz), 6.36 (s, 1H), 2.93-2.88 (m, 1H), 2.64 (s, 3H), 2.28 (s, 3H), 0.89-0.84 (m, 2H), 0.64-0.60 (m, 2H);



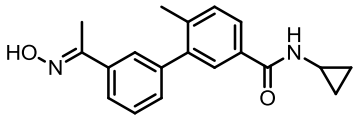
General procedure for the synthesis of NJK13031, NJK13032, NJK13034, NJK13035 and NJK13040

To a solution of 3'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (1 eq.) in pyridine were added alkoxy amine (1.5 eq.). The reaction mixture was stirred at room temperature until complete consumption of the starting material on TLC. Then, the reaction mixture was acidified with 2*N* HCl, and diluted with EtOAc. The combined organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel (EtOAc/Hexanes) afforded *meta*-oxime ether compounds.



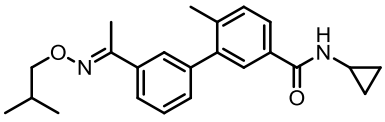
3'-(1-(butoxyimino)ethyl)-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (NJK13031)

Prepared from 3'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (40 mg, 0.135 mmol) and *O*-butylhydroxylamine (18 mg, 0.202 mmol) using the general procedure described above. Purification of the residue via flash column chromatography on silica afforded 35 mg (71%) of compound **NJK13031**; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.68-7.62 (m, 2H), 7.57 (s, 2H), 7.40 (t, 1H, J = 7.6 Hz), 7.31-7.26 (m, 2H), 6.44 (s, 1H), 4.19 (t, 2H, J = 6.7 Hz), 2.90-2.86 (m, 1H), 2.28 (s, 3H), 2.25 (s, 3H), 1.74-1.67 (m, 2H), 1.48-1.39 (m, 2H), 0.96 (t, 3H, J = 7.4 Hz), 0.86-0.81 (m, 2H), 0.62-0.58 (m, 2H); LR-MS (EI^+) *m/z* 364.2 (M^+); HR-MS (EI^+) calcd for $C_{15}H_{10}O_2$ (M^+) 364.2151; found 364.2152.



***N*-cyclopropyl-3'-(1-(hydroxyimino)ethyl)-6-methyl-[1,1'-biphenyl]-3-carboxamide (NJK13032)**

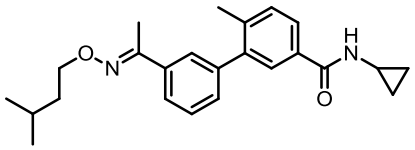
Prepared from 3'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (35 mg, 0.119 mmol) and Hydroxylamine (13 mg, 0.179 mmol) using the general procedure described above. Purification of the residue via flash column chromatography on silica afforded 32 mg (88%) of compound **NJK13032**; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.66 (d, 1H, J = 7.9 Hz), 7.57 (d, 3H, J = 7.1 Hz), 7.40 (t, 1H, J = 7.6 Hz), 7.28 (d, 2H, J = 8.2 Hz), 6.57 (s, 1H), 2.90-2.85 (m, 1H), 2.29 (s, 3H), 2.28 (s, 3H), 0.84-0.80 (m, 2H), 0.62-0.58 (m, 2H); LR-MS (EI^+) *m/z* 308.2 (M^+); HR-MS (EI^+) calcd for $C_{15}H_{10}O_2$ (M^+) 308.1525; found 308.1527.



***N*-cyclopropyl-3'-(1-(isobutoxyimino)ethyl)-6-methyl-[1,1'-biphenyl]-3-carboxamide (NJK13034)**

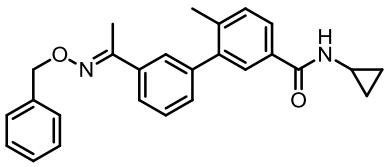
Prepared from 3'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (31 mg, 0.106 mmol) and *O*-isobutylhydroxylamine (14 mg, 0.159 mmol) using the general procedure described above. Purification of the residue via flash column chromatography on silica afforded 26 mg (69%) of compound **NJK13034**; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.68-7.62 (m, 2H), 7.58-7.56 (m, 2H), 7.41 (t, 1H, J = 7.7 Hz), 7.32-7.26 (m, 2H), 6.33 (s, 1H), 3.97 (d, 2H, J = 6.8 Hz), 2.92-2.86 (m, 1H), 2.28 (s, 3H), 2.26 (s, 3H), 2.09-2.02

(m, 1H), 0.96 (d, 6H, J = 6.7 Hz), 0.88-0.83 (m, 2H), 0.63-0.59 (m, 2H); LR-MS (EI^+) *m/z* 364.2 (M^+); HR-MS (EI^+) calcd for $C_{15}H_{10}O_2$ (M^+) 364.2151; found 364.2154.



_N_-cyclopropyl-3'-(1-((isopentyloxy)imino)ethyl)-6-methyl-[1,1'-biphenyl]-3-carboxamide (NJK13035)

Prepared from 3'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (33 mg, 0.113 mmol) and *O*-isopentylhydroxylamine (17 mg, 0.169 mmol) using the general procedure described above. Purification of the residue via flash column chromatography on silica afforded 24 mg (56%) of compound **NJK13035**; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.68-7.62 (m, 2H), 7.57 (d, 2H, J = 2.0 Hz), 7.41 (t, 1H, J = 7.6 Hz), 7.32-7.26 (m, 2H), 6.38 (s, 1H), 4.23 (t, 2H, J = 6.8 Hz), 2.91-2.86 (m, 1H), 2.28 (s, 3H), 2.24 (s, 3H), 1.79-1.72 (m, 1H), 1.64-1.59 (m, 2H), 0.95 (d, 6H, J = 6.6 Hz), 0.87-0.84 (m, 2H), 0.63-0.61 (m, 2H); LR-MS (EI^+) *m/z* 378.2 (M^+); HR-MS (EI^+) calcd for $C_{15}H_{10}O_2$ (M^+) 378.2307; found 378.2309.



3'-(1-((benzyloxy)imino)ethyl)-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (NJK13040)

Prepared from 3'-acetyl-*N*-cyclopropyl-6-methyl-[1,1'-biphenyl]-3-carboxamide (20 mg, 0.067 mmol) and *O*-benzylhydroxylamine (17 mg, 0.101 mmol) using the general procedure described above. Purification of the residue via flash column chromatography on silica afforded 23 mg (87%) of compound **NJK13040**; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.68-7.65 (m, 2H), 7.58-7.56 (m, 2H), 7.43-7.35 (m, 5H,), 7.33-7.27 (m, 3H), 6.31 (s, 1H), 5.24 (s, 2H), 2.90-2.88 (m, 1H), 2.29 (s, 3H), 2.28 (s, 3H), 0.88-0.83 (m, 2H), 0.62-0.58 (m, 2H); LR-MS (EI^+) *m/z* 398.3 (M^+); HR-MS (EI^+) calcd for $C_{15}H_{10}O_2$ (M^+) 398.1994; found 398.1993.