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Synthesis and biological evaluation of fluorinated 3,4-dihydroquinolin-2(1H)-ones and 2-oxindoles for anti-hepatic fibrosis

Man Li, Fu-Sheng He, Longshan Ji, Ya-Ting Gao, Xin Zhang, Zhuo Yu, Miao Fang, Jie Wu* and Yue-Qiu Gao*

Supporting Information

Materials and methods

General experimental procedure for the synthesis of (*Z*)-4-(iodomethylene)-3-(2,2,2-trifluoroethyl)-3,4-dihydroquinolin-2(1*H*)-ones

Benzene-tethered 1,7-enynes **1** (0.2 mmol) was added to a mixture of Togni reagent **2** (0.3 mmol), NaI (0.2 mmol) and PhCOOH (0.2 mmol) in DCE (4.0 mL). The mixture which placed around the mercury lamp (purchased from Yuming, Shanghai) with a distance of 10 centimeters was stirred under UV irradiation (0.67 W cm^{-1}) for 12 hours at room temperature. After completion of reaction as indicated by TLC, the solvent was evaporated, and the residue was purified directly by flash column chromatograph (EtOAc/*n*-hexane, 1:8) to give the desired products **3**.

(*E*)-6-Fluoro-4-(iodo(phenyl)methylene)-1,3-dimethyl-3-(2,2,2-trifluoroethyl)-3,4-dihydroquinolin-2(1*H*)-one (3a)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 3H), 1.95-2.02 (m, 1H), 2.16-2.22 (m, 1H), 3.39 (s, 3H), 6.99 (dd, J_1 = 8.9 Hz, J_2 = 4.6 Hz, 1H), 7.10-7.15 (m, 1H), 7.26-7.34 (m, 5H), 7.61 (dd, J_1 = 8.9 Hz, J_2 = 2.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 31.2, 41.0 (q, J = 27.3 Hz), 48.1, 102.3, 115.6 (d, $^3J_{CF}$ = 8.0 Hz), 116.5 (d, $^2J_{CF}$ = 22.8 Hz), 119.3 (d, $^2J_{CF}$ = 24.4

Hz), 124.3, 127.1, 127.4, 127.7, 128.1, 128.3, 131.7, 134.3, 138.2, 146.1, 158.3 (d, $^{1}J_{CF}$ = 243.5 Hz), 169.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.7 (t, *J* = 10.4 Hz), -118.7 (td, J_1 = 8.3 Hz, J_2 = 4.9 Hz); HRMS (ESI) calcd for $C_{20}H_{16}F_4INO$: 490.0285 (M + H^+), found: 490.0281.

(*E*)-4-(Iodo(phenyl)methylene)-1,3-dimethyl-3-(2,2,2-trifluoroethyl)-6-(trifluoromethyl)-3,4-dihydroquinolin-2(1*H*)-one (3b)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 3H), 1.96-2.07 (m, 1H), 2.11-2.23 (m, 1H), 3.44 (s, 3H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.26-7.35 (m, 5H), 7.67 (d, *J* = 8.4 Hz, 1H), 8.16 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.4, 31.1, 41.1 (q, *J* = 27.6 Hz), 48.1, 103.3, 114.3, 124.1, 124.9, 125.2, 126.8, 127.4, 127.8, 128.0, 128.1, 128.4, 129.8, 130.2, 137.6, 140.8, 146.0, 169.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.9 (t, *J* = 10.4 Hz), -62.2; HRMS (ESI) calcd for $C_{21}H_{16}F_6INO$: 540.0254 (M + H^+), found: 540.0254.

(*E*)-4-(Iodo(*p*-tolyl)methylene)-1,3-dimethyl-3-(2,2,2-trifluoroethyl)-3,4-dihydroquinolin-2(1*H*)-one (3c)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (s, 3H), 1.92-2.03 (m, 1H), 2.13-2.25 (m, 1H), 2.36 (s, 3H), 3.40 (s, 3H), 7.03 (d, *J* = 8.1 Hz, 1H), 7.10-7.19 (m, 4H), 7.25 (d, *J* = 9.2 Hz, 1H), 7.41 (t, *J* = 7.3 Hz, 1H), 7.86 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3, 21.3, 30.9, 41.0 (q, *J* = 27.4 Hz), 48.1, 101.7, 114.2, 123.0, 124.4, 127.2, 127.5, 128.2, 128.3, 128.8, 129.9, 130.4, 132.4, 138.2, 138.9, 143.7, 169.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.7 (t, *J* = 10.4 Hz); HRMS (ESI) calcd for $C_{21}H_{19}F_3INO$: 486.0536 (M + H^+), found: 486.0519.

(*E*)-4-(Iodo(phenyl)methylene)-1,3,6-trimethyl-3-(2,2,2-trifluoroethyl)-3,4-dihydroquinolin-2(1*H*)-one (3d)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 3H), 1.91-2.03 (m, 1H), 2.16-2.27 (m, 1H), 2.41 (s, 3H), 3.38 (s, 3H), 6.92 (d, *J* = 8.2 Hz, 1H), 7.20 (d, *J* = 8.2 Hz, 1H), 7.24-7.34 (m, 5H), 7.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 21.3, 30.9, 41.0 (q, *J* = 27.1 Hz), 48.1, 100.9, 114.1, 124.4, 127.6, 127.7, 128.0, 128.1, 128.3, 130.0, 130.4, 132.6, 132.7, 135.6,

139.3, 146.5, 169.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.7 (t, *J* = 10.5 Hz); HRMS (ESI) calcd for $C_{21}H_{19}F_3INO$: 486.0536 (M + H^+), found: 486.0534.

(*E*)-4-((4-Chlorophenyl)iodomethylene)-1,3-dimethyl-3-(2,2,2-trifluoroethyl)-3,4-dihydroquinolin-2(1*H*)-one (3e)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 3H), 1.92-2.04 (m, 1H), 2.14-2.26 (m, 1H), 3.40 (s, 3H), 7.04 (d, *J* = 8.1 Hz, 1H), 7.19 (dd, J_1 = 16.3 Hz, J_2 = 8.1 Hz, 2H), 7.29-7.33 (m, 3H), 7.41 (dd, J_1 = 11.3 Hz, J_2 = 4.3 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 31.0, 41.0 (q, *J* = 27.5 Hz), 48.3, 99.2, 114.3, 123.1, 124.4, 127.1, 127.9, 128.4, 128.9, 129.7, 130.2, 132.2, 134.0, 138.0, 140.0, 144.9, 169.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.7 (t, *J* = 10.5 Hz); HRMS (ESI) calcd for $C_{20}H_{16}ClF_3INO$: 505.9990 (M + H^+), found: 505.9990.

Methyl (*E*)-4-((1,3-dimethyl-2-oxo-3-(2,2,2-trifluoroethyl)-2,3-dihydroquinolin-4(1*H*)-ylidene) iodomethyl) benzoate (3f)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 3H), 1.93-2.04 (m, 1H), 2.15-2.24 (m, 1H), 3.41 (s, 3H), 3.93 (s, 3H), 7.05 (d, *J* = 8.1 Hz, 1H), 7.20 (t, *J* = 7.5 Hz, 1H), 7.35-7.37 (m, 1H), 7.42-7.45 (m, 2H), 7.86 (d, *J* = 7.6 Hz, 1H), 8.01 (dd, J_1 = 5.6 Hz, J_2 = 2.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.5, 31.0, 41.0 (q, *J* = 27.6 Hz), 48.3, 52.2, 99.0, 123.1, 124.3, 127.1, 127.6, 128.4, 129.1, 129.3, 129.6, 129.8, 130.2, 132.2, 138.0, 140.0, 150.8, 166.3, 169.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.7 (t, *J* = 10.2 Hz); HRMS (ESI) calcd for $C_{22}H_{19}F_3INO_3$: 530.0434 (M + H^+), found: 530.0423.

General experimental procedure for the synthesis of fluorinated 3,3-disubstituted 2-oxindoles

Fluorinated alkyl iodide (0.36 mmol) was added to a solution of *N*-arylacrylamides **4** (0.3 mmol), Na_2CO_3 (0.3 mmol) in CH_3CN (4.0 mL) under N_2 atmosphere. The mixture was stirred under ultraviolet irradiation for 12 hours. After completion of reaction as indicated

by TLC, the solvent was evaporated, and the residue was purified directly by flash column chromatograph (EtOAc/*n*-hexane, 1:8) to give the desired products **5**.

1,3-Dimethyl-3-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)indolin-2-one (5a)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 3H), 2.55-2.68 (m, 1H), 2.83-2.95 (m, 1H), 3.25 (s, 3H), 6.89 (d, *J* = 7.8 Hz, 1H), 7.09 (d, *J* = 7.4 Hz, 1H), 7.28-7.33 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 26.3, 36.8 (t, *J* = 20.2 Hz), 44.1, 108.4, 114.4-118.8 (m), 122.5, 123.5, 128.4, 131.2, 142.7, 178.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -81.2 (t, *J* = 9.5 Hz, 3F), -108.9 (d, $J_{F\text{-}F}$ = 275.1 Hz, 1F), -114.7(d, $J_{F\text{-}F}$ = 269.7 Hz, 1F), -123.7 (br, 2F), -125.9--126.1 (m, 2F); HRMS (ESI) calcd for $C_{15}H_{12}F_9NO$: 394.0860 (M + H^+), found: 394.0860.

4-Methoxy-1,3-dimethyl-3-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)indolin-2-one (5b)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 3H), 2.77-2.89 (m, 1H), 2.90-3.02 (m, 1H), 3.22 (s, 3H), 3.86 (s, 3H), 6.54 (d, *J* = 7.8 Hz, 1H), 6.63 (d, *J* = 8.5 Hz, 1H), 7.28 (t, *J* = 8.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.5, 26.6, 29.6, 35.4 (t, *J* = 20.0 Hz), 43.7, 55.3, 101.7, 105.7, 109.9, 115.8-118.7 (m), 129.6, 143.9, 156.3, 178.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ -81.1 (t, *J* = 9.8 Hz, 3F), -113.1 (d, $J_{F\text{-}F}$ = 271.6 Hz, 1F), -116.0 (d, $J_{F\text{-}F}$ = 272.2 Hz, 1F), -124.8 (br, 2F), -125.9--126.1 (m, 2F); HRMS (ESI) calcd for $C_{16}H_{14}F_9NO_2$: 424.0954 (M + H^+), found: 424.0943.

1,3-Dimethyl-3-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)-1*H*-benzo[g]indol-2(3*H*)-one (5c)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74 (s, 3H), 2.69-2.82 (m, 1H), 3.51- 3.63 (m, 1H), 3.55 (s, 3H), 6.98 (d, *J* = 7.5 Hz, 1H), 7.42-7.46 (m, 2H), 7.52-7.55 (m, 2H), 7.75 (d, *J* = 8.1 Hz，1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.8, 34.0, 41.4 (t, *J* = 19.3 Hz), 43.5, 108.7, 117.7, 118.8, 122.8, 123.4, 124.3, 126.4, 126.5, 126.7, 127.9, 133.4, 135.1, 136.2, 171.4；^{19}F NMR (376 MHz, $CDCl_3$) δ -81.1 (t, *J* = 9.8 Hz, 3F), -107.6 (d, $J_{F\text{-}F}$ = 284.9 Hz, 1F), -113.0 (d, $J_{F\text{-}F}$ = 273.7 Hz, 1F), -124.8 (br, 2F), -125.8--126.1 (m, 2F); HRMS (ESI) calcd for $C_{19}H_{14}F_9NO_2$: 444.1004 (M + H^+), found: 444.1018.

1,3-Dimethyl-3-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)-5-(trifluoromethyl)indolin-2-one (5d)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3H), 2.58-2.71 (m, 1H), 2.88-3.00 (m, 1H), 3.29 (s, 3H), 6.98 (d, *J* = 8.2 Hz, 1H), 7.52 (s, 1H), 7.61 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 26.6, 37.0 (t, *J* = 20.4 Hz), 44.1, 108.3, 120.7, 122.9, 124.5, 124.8, 125.2,125.6, 126.3,126.4, 131.8, 145.8, 178.4; ^{19}F NMR (376 MHz, $CDCl_3$) δ -61.6 (s, 3F), -81.2 (t, *J* = 9.8 Hz, 3F), -108.6 (d, J_{F-F} = 272.4 Hz, 1F), -114.7(d, J_{F-F} = 271.9 Hz, 1F), -124.6 (br, 2F), -125.9--126.1 (m, 2F); HRMS (ESI) calcd for $C_{16}H_{12}F_{12}NO$: 462.0722 (M + H^+), found: 462.0726.

1,3,5-Trimethyl-3-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)indolin-2-one (5e)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 3H), 2.36 (s, 3H), 2.51-2.65 (m, 1H), 2.81-2.93 (m, 1H), 3.23 (s, 3H), 6.78 (d, *J* = 7.8 Hz, 1H), 7.11 (d, *J* = 8.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 25.9, 26.5, 36.9 (t, *J* = 20.2 Hz), 44.2, 108.2, 115.8-120.1 (m), 124.3, 128.7, 131.3, 132.2, 140.4, 178.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -81.1 (t, J = 9.8 Hz, 3F), -108.9 (d, J_{F-F} = 275.3 Hz, 1F), -114.8(d, J_{F-F} = 268.9 Hz, 1F), -124.6 (br, 2F), -125.9--126.0 (m, 2F); HRMS (ESI) calcd for $C_{16}H_{15}F_9NO$: 408.1005 (M + H^+), found: 408.1002.

5-(*tert*-Butyl)-1,3-dimethyl-3-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)indolin-2-one (5f)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 9H), 1.44 (s, 3H), 2.55-2.66 (m, 1H), 2.81-2.94 (m, 1H), 3.23 (s, 3H), 6.81 (d, *J* = 8.7 Hz, 1H), 7.33-7.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 26.4, 31.4, 34.5, 36.9 (t, *J* = 20.4 Hz), 44.4, 107.7, 114.9-120.9 (m), 124.9, 130.9, 140.3, 145.8, 178.7； ^{19}F NMR (376 MHz, $CDCl_3$) δ -81.2 (t, *J* = 9.8 Hz, 3F), -109.0 (d, J_{F-F} = 270.8 Hz, 1F), -114.6(d, J_{F-F} = 272.1 Hz, 1F), -124.7 (br, 2F), -125.8--126.0 (m, 2F); HRMS (ESI) calcd for $C_{19}H_{20}F_9NO$: 450.1474 (M + H^+), found: 450.1469.

5-Acetyl-1,3-dimethyl-3-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)indolin-2-one (5g)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3H), 2.61 (s, 3H), 2.65-2.74 (m, 1H), 2.88-3.01 (m, 1H), 3.30 (s, 3H), 6.95 (d, *J* = 8.2 Hz, 1H), 7.95 (s, 1H), 7.99 (dd, J_1 = 1.6 Hz, J_2 = 8.2

Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.9, 26.3, 26.7, 37.0 (t, *J* = 20.3 Hz), 43.9, 107.9, 114.8-118.6 (m), 123.4, 130.5, 131.6, 132.2, 147.1, 178.8, 196.9； ^{19}F NMR (376 MHz, $CDCl_3$) δ -81.1 (t, *J* = 9.8 Hz, 3F), -108.6 (d, J_{F-F} = 271.6 Hz, 1F), -114.7 (d, J_{F-F} = 271.9 Hz, 1F), -124.6 (br, 2F), -125.8--126.0 (m, 2F); HRMS (ESI) calcd for $C_{17}H_{14}F_9NO_2$: 436.0954 ($M + H^+$), found: 436.0940.

5-Chloro-1,3-dimethyl-3-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)indolin-2-one (5h)

Purity: >98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 3H), 2.52-2.65 (m, 1H), 2.83-2.96 (m, 1H), 3.24 (s, 3H), 6.82 (d, *J* = 8.2 Hz, 1H), 7.27 (s, 1H), 7.30 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 26.5, 36.9 (t, *J* = 20.4 Hz), 44.3, 109.4, 115.8-121.5 (m), 124.1, 128.0, 128.5, 132.9, 141.3, 178.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ -81.1 (t, *J* = 9.8 Hz, 3F), -108.6 (d, J_{F-F} = 272.2 Hz, 1F), -114.7(d, J_{F-F} = 271.7 Hz, 1F), -124.6 (br, 2F), -125.8--126.0 (m, 2F); HRMS (ESI) calcd for $C_{15}H_{12}ClF_9NO$: 428.0459 ($M + H^+$), found: 428.0462.

Cell Culture and experimental design

The human LX-2 cell line was a well characterized human HSC cell line, that could transdifferentiate in vitro from a quiescent-like phenotype to a more proliferative and activated behavior, and it provided a useful platform to assess anti-fibrotic drugs. LX-2 cells were donated by Institute of Liver Diseases Affiliated to Shanghai University of Traditional Chinese Medicine. LX-2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientific), containing penicillin (100 U/mL), and streptomycin (100 μg/mL) and fetal bovine serum(FBS) at 10% final concentration. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO2, and the medium was changed once per day. When the cell density reached 80%–90%, the cells were passaged. To evaluate the effects of chemicals on the activation of LX-2 cells, cells were cultivated in media containing 10% FBS in the presence of TGF-β and chemicals.

Drug Concentration

All chemicals were dissolved in DMSO at 0.05% (v/v). The concentrations of all chemicals were tested for the measurement of cell viability at 0.1μM, 1 μM ,10 μM and 100 μM.

Cytotoxicity Assay

The cytotoxicity analyses were performed by a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Gaithersburg, MD, USA). For the assay, 3×10^3 LX-2 cells were cultured in wells of 96-well plates for 12h, followed by the incubation of different concentrations of chemicals (0.1μM, 1 μM ,10 μM and 100 μM) for extra 72h. Next, the cultured cell medium was collected, and CCK-8 solution（10μl）was added to the culture medium, and the cultures were incubated for 2h at 37°C. The absorbance was detested by Microplate Reader (Bio-Rad; Hercules, CA, USA) under the optical density (OD) at 450 nm.

Activation of LX-2 cells

To activate LX-2 cells, TGF- β was added to the culture medium at the final concentration of 10 μg/ml.

ELISA

Collagen I and fibrosin levels in culture medium were tested by ELISA. Human collagen I ELISA kit was purchased from Abcam (ab210966). Human fibrosin ELISA kit was purchased from Shanghai Ji Ning Technology Co., Ltd. In brief, 100 μl of sample was added into every well. After incubating 2h at room temperature, the plate was washed and100μl antibody was added to each well. After incubating 1h at room temperature, the plate was washed and 200μl substrate solution was added to each well. After incubating for 20 minutes at room temperature，50 μl of stop solution was added to each well. The absorbance was detested by Microplate Reader (Bio-Rad; Hercules, CA, USA) under the optical density (OD) at 450 nm.

RNA extraction and Real-time-PCR analyses

Total RNA was isolated from LX-2 cells using Trizol Reagent(Invitrogen) and quantified. RNA was synthesized from 1 μg of total RNA using cDNA Reverse Transcription kit (Invitrogen). Real-time PCR was performed using the Mastercycler ep realplex 4 real-time PCR system (Eppendorf) with

an SYBR Green qPCR Master Mix (Fermentas) according to the manufacturer's protocol. The sequences of primers for human α-SMA (NC_000010.11) were 5'-CCACCGCAAATGCTTCTAAGT-3'(forword) and 5'-GGCAGGAATGATTTGGAAA
GG--3' (reverse). The primers for human GAPDH (NM_002046.3) were 5'–TGCACCACCAACTGCTT AGC-3' (forward) and 5'-GGCATGGACTGTGGTCATGAG-3' (reverse). The amplification conditions were as follows: 95 1C for 10min, and 40 cycles of 951C for 15s, 641C for 30s and 72 1C 20 s. The amount of α-SMA transcripts of individual samples was normalized to GAPDH.