Synthesis, *in silico*, & *in vitro* characterization of novel dihydropyrimidones that target peroxisome proliferator-activated receptor-γ

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Supplementary Data

Chemistry

Materials and methods: All the chemicals are purchased from Sigma-Aldrich unless otherwise mentioned specifically. Melting points were determined in capillaries on a Tottoli apparatus and are uncorrected. The NMR experiments ^{1}H, ^{13}C, were carried out at 400 MHz and the reported chemical shifts (δ) are given in ppm and the coupling constants (J) in Hertz (Hz). Multiplicities of NMR signals are designed as s (singlet), d (doublet), m (multiplet, for unresolved lines). Mass spectra were recorded on a Trio 1000 Thermo Quest spectrometer in the electron impact mode or a Platform Micromass spectrometer in the electro spray mode. TLC was performed on silica gel Alugram SilG/UV254 (Macherey-Nagel).

General Procedure for the Synthesis of dihydropyrimidones

To a 50 mL round bottom flask, aldehyde (1.0 equiv), urea (1.5 equiv), diketone (1.0 equiv), ZnO (3 molecular %) and water (10 mL) were added. The reaction mixture was refluxed for 3h. Then the solid was filtered, dried and purified by column chromatography (Figure 1).

1. Synthesis of ethyl 6-methyl-2-oxo-4-(4-oxo-4H-chromen-3-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a):

The product **4a** was obtained from 4-oxo-4H-chromene-3-carbaldehyde **1a** (174 mg, 1 eq), urea (90mg, 1.5eq) and ethyl 3-oxobutanoate (130mg 1eq). This compound was obtained as light brown color solid in 95% yield. Melting point: 288-290 ^{0}C

IR νmax (KBR,cm^{-1}): 3234(N-H), 1722(ester), 1643(Amide)

^{1}H NMR (400 MHz, DMSO δ in ppm) 1.09 (3H,s,methyl) , 2.26 (3H,s, methyl) 4.01 (2H,q,methylene), 5.24 (1H,s,methyne), 7.23(1H,s,Aromatic H) 7.51 (1H,t, Aromatic H), 7.66 (1H,d,Aromatic H), 7.79(1H,t, Aromatic H), 8.08(1H,d, Aromatic H), 8.16(1H, s, N-H), 9.23(1H,s,N-H) ;

C^{13}NMRδ:14.09,17.95,48.11,59.02,94.95,118.39,123.70,124.57,124.98,125.49,134.17,150.09,151.96,154.73,155.66,165.05,175.38

Anal. Calcd.for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 08.53 ; found C, 61.89; H, 4.58; N, 4.78%

MASS; m/z found for $C_{17}H_{16}N_2O_5$ **329.2** ($[M+1]^+$).

2. **Synthesis of 5-acetyl-6-methyl-4-(4-oxo-4H-chromen-3-yl)-3,4-dihydropyrimidin-2(1H)-one(4b):**

The product **4b** was obtained from 4-oxo-4H-chromene-3-carbaldehyde **1b** (174 mg, 1 eq), urea (90mg, 1.5eq) and acetyl acetone (100 mg 1eq). This compound was obtained as brown color solid in 92% yield. Melting point: 138-140^{0}C

IR νmax (KBR,cm^{-1}): 3330(N-H), 1710(ketone), 1697(Amide).

^{1}H NMR (400 MHz, DMSO δ in ppm): 2.26 (3H,s, methyl) 3.60 (3H,s, methyl), 5.37 (1H,s,methyne), 7.23(1H,s,Aromatic H) 7.53 (1H,t, Aromatic H), 7.64 (1H,d,Aromatic H), 7.81(1H,t, Aromatic H), 8.10(1H,d, Aromatic H), 8.16(1H, s, N-H), 9.23(1H,s,N-H).

C^{13}NMRδ:193.78,154.63,152.13,149.57,134.28,130.91,125.52,124.99,124.10,123.52,119.48,118.42,106.16,47.32,30.22,19.06**.**

Anal. Calcd.for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 09.39 ; found C, 64.23; H, 4.58; N, 09.15%

MASS; m/z found for **$C_{16}H_{14}N_2O_4$** : 297.4 ($[M-1]^+$).

3. **Synthesis of 5-acetyl-4-(2,6-dichlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one(4c):**

The product **4c** was obtained from 2,6-dichlorobenzaldehyde **1c** (175 mg, 1 eq), urea (90mg, 1.5eq) and acetyl acetone (100 mg 1eq). This compound was obtained as brown color solid in 93% yield. Melting point: 143-145 ^{0}C; **IR νmax (KBR,cm^{-1}):** 3320(N-H), 1715(ketone), 1685(Amide); **^{1}H NMR (400 MHz, DMSO δ in ppm):** 1.71 (3H,s, methyl) 2.40(3H,s, acetyl), 5.25 (1H,s,methyne), 7.50 (3H,m, Aromatic H), 8.15(2H,s,N- H); **Anal. Calcd.for $C_{13}H_{12}N_2O_2Cl_2$**: C, 52.19; H, 4.04; N, 09.36 ; found C, 52.02; H, 4.00; N, 09.15%; **MASS**; m/z found for **$C_{13}H_{12}N_2O_2Cl_2$**: 298,300.02 ($[M+1]^+$).

4. **Synthesis of 3-(5-acetyl-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-4-yl)-4H-chromen-4-one(4d):**

The product **4d** was obtained from 4-oxo-4H-chromene-3-carbaldehyde **1d** (174 mg, 1 eq), thio urea (114 mg, 1.5eq) and acetyl acetone (100 mg 1eq). This compound was obtained as dark brown color solid in 94% yield. Melting point: 190-192^{0}C

IR νmax (KBR,cm^{-1}): 3310(N-H), 1720(ester), 1690(Amide)

^{1}H NMR (400 MHz, DMSO δ in ppm): 8.01(1H,s,Aromatic H) 7.0-7.9 (4H,m, Aromatic H), 4.6 (1H,s,methyne), 2.88 (3H,s, -CH_3) 2.72 (2H,s, N-H), 1.72(3H,s,CH3) .

Anal. Calcd.for $C_{16}H_{14}N_2O_3S$: C, 61.13; H, 4.49; N, 08.91 ; found C, 59.92; H, 4.30; N, 09.05%

MASS; m/z found for **$C_{16}H_{14}N_2O_3S$**: 313.12 ($[M-1]^+$)

5. **Synthesis of ethyl 6-methyl-4-(4-oxo-4H-chromen-3-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate(4e)**

The product **4e** was obtained from 4-oxo-4H-chromene-3-carbaldehyde **1e** (174 mg, 1 eq), thio urea (114mg, 1.5eq) and ethyl 3-oxobutanoate (130mg 1eq). This compound was obtained as brown color solid in 96% yield. Melting point: 145-147^{0}C

IR νmax(KBR,cm^{-1}): 3325(N-H), 1743(ester), 690(C=S)

^{1}H NMR (400 MHz, DMSO δ in ppm): 8.1(1H, s, Aromatic H) 7.0-8.1 (4H,m, Aromatic H), 4.7 (1H,s,methyne), 2.20 (2H,q, -CH_2) 2.70 (2H,s, N-H), 1.80(3H,s,CH3),1.35(3H,t, CH_3)

Anal. Calcd.for $C_{17}H_{16}N_2O_4S$: C, 52.29; H, 4.68; N, 08.13 ; found C, 52.42; H, 4.30; N, 08.05%

MASS; m/z found for **$C_{17}H_{16}N_2O_4S$**: 343.0 ($[M-1]^+$)

6. Synthesis of 5-acetyl-4-(1H-indol-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one(4f):

The product **4f** was obtained from 2,3-dihydrobenzofuran-5-carbaldehyde **1f** (148 mg, 1 eq), urea (90mg, 1.5eq) and acetyl acetone (100 mg 1eq). This compound was obtained as brown color solid in 92% yield. Melting point: 255-257^{0}C

IR νmax (KBR,cm^{-1}): 3325(N-H), 1720(ketone), 1695(Amide)

^{1}H NMR (400 MHz, DMSO δ in ppm): 1.9 (3H,s,methyl) , 2.5 (3H,s,methyl) 3.25 (2H,t,methylene), 4.05 (2H,t,methylene), 5.20(1H,s,methyne), 6.65(1H,d,Aromatic H) 6.90(1H,d, Aromatic H), 7.01 (1H,s,Aromatic H), 7.8(2H,s, N-H).

Anal. Calcd.for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29 ; found C, 65.98; H, 5.85; N, 10.05%

MASS; m/z found for **$C_{15}H_{16}N_2O_3$**: 273.2 ($[M+1]^+$)

7. **Synthesis of 5-acetyl-4-(2,3-dihydrobenzofuran-5-yl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one (4g):**

The product **4g** was obtained from indole-3-carbaldehyde **1g** (145 mg, 1 eq), urea (90mg, 1.5eq) and acetyl acetone (100 mg 1eq). This compound was obtained as brown color solid in 92% yield. Melting point: 165-168 ^{0}C

IR νmax (KBR,cm^{-1}): 3320(N-H), 1725(ketone), 1690(Amide)

^{1}H NMR (400 MHz, DMSO δ in ppm): 8.93(1H,s,indole N-H),8.08(1H,s,N-CH),8.26(2H,s,N-H) 7.15-7.45 (4H,m, Aromatic H), 5.45 (1H,s,methyne), 2.01 (3H,s, adetyl-H),1.2(3H,s,methyl).

C^{13} NMR: δ 184,150,138,137,124,123,122,120,118,114,112,108,49,28,15.

Anal. Calcd.for $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60 ; found C, 66.78; H, 5.55; N, 15.45%

MASS; m/z found for **$C_{15}H_{15}N_3O_2$**: 269.2 ([M+1]$^+$)

8. **Synthesis of ethyl 4-(2,3-dihydrobenzofuran-5-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4h)**

The product **4h** was obtained from 2,3-dihydrobenzofuran-5-carbaldehyde **1h** (148 mg, 1 eq), urea (90mg, 1.5eq) and ethyl 3-oxobutanoate (130mg 1eq). This compound was obtained as brown color solid in 94% yield. Melting point: 139-141^{0}C

IR νmax(KBR): 3325(N-H), 1725(ester), 1695(Amide)

^{1}H NMR (400 MHz, DMSO δ in ppm):

1.11 (3H,t,methyl) , 3.18 (3H,s,methyl) 3.24 (2H,t,methylene), 4.02 (2H,q,methylene), 4.53(2H,t, Methylene), 5.06(1H,s,methane), 6.67(1H,d,Aromatic H) 6.96(1H,d, Aromatic H), 7.09 (1H,s,Aromatic H), 7.59(1H,s, N-H), 9.08(1H,s, N-H).

Anal. Calcd.for $C_{16}H_{18}N_2O_4$: C, 63.56; H, 6.00; N, 09.27 ; found C, 63.18; H, 5.85; N, 09.05%

MASS; m/z found for **$C_{16}H_{18}N_2O_4$**: 303.13 ([M+1]$^+$)

9. **Synthesis of 1-(4-(2,3-dihydrobenzofuran-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethanone (4i):**

The product **4i** was obtained from 2,3-dihydrobenzofuran-5-carbaldehyde **1i** (148 mg, 1 eq), thio urea (114mg, 1.5eq) and acetyl acetone (100 mg 1eq). This compound was obtained as brown color solid in 93% yield. Melting point: 220-222^{0}C;

IR νmax(KBR): 3340(N-H), 1722(ketone), 670(C=S)

^{1}H NMR (400 MHz, DMSO δ in ppm): 1.85 (3H,s,methyl) , 2.55 (2H,s,N-H), 2.45 (3H,s,methyl) 3.20 (2H,t,methylene), 4.10 (2H,t,methylene), 5.20(1H,s,methyne), 6.60(1H,d,Aromatic H) 6.95(1H,d, Aromatic H), 7.01 (1H,s,Aromatic H).

Anal. Calcd.for $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 09.71 ; found C, 61.98; H, 5.85; N, 10.05%

MASS; m/z found for **$C_{15}H_{16}N_2O_2S$**: 289.09 ($[M+1]^+$)

10. **Synthesis of ethyl 4-(2,3-dihydrobenzofuran-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4j):**

The product **4j** was obtained from 2,3-dihydrobenzofuran-5-carbaldehyde **1j** (148 mg, 1 eq), thio urea (114mg, 1.5eq) and ethyl 3-oxobutanoate (130mg 1eq). This compound was obtained as brown color solid in 91% yield. Melting point: 298-300^{0}C

IR νmax (KBR): 3350(N-H), 1745(ester), 675(C=S)

^{1}H NMR (400 MHz, DMSO δ in ppm): 1.15 (3H,t,methyl) , 2.50 (2H,s,N-H) 3.20 (3H,s,methyl) 3.30 (2H,t,methylene), 4.05 (2H,q,methylene), 4.53(2H,t, Methylene), 5.06(1H,s,methane), 6.70(1H,d,Aromatic H) 6.95(1H,d, Aromatic H), 7.05 (1H,s,Aromatic H), 7.60(1H,s, N-H).

Anal. Calcd.for $C_{16}H_{18}N_2O_3S$: C, 60.36; H, 5.70; N, 08.80 ; found C, 60.08; H, 5.85; N, 09.05%

MASS; m/z found for **$C_{16}H_{18}N_2O_3S$**: 319.10 ($[M+1]^+$)

11. **Synthesis of ethyl 6-(chloro methyl)-4-(2,3-dihydrobenzofuran-5-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate(4k):**

The product **4k** was obtained from 2,3-dihydrobenzofuran-5-carbaldehyde **1k** (148 mg, 1 eq), urea (90mg, 1.5eq) and ethyl 4-chloro-3-oxobutanoate (164mg 1eq). This compound was obtained as grey color solid in 95% yield. Melting point: 179-181^{0}C

IR νmax(KBR): 3320(N-H), 1730(ester), 1697(amide)

^{1}H NMR (400 MHz, DMSO δ in ppm): 8.03(1H,s,Aromatic H) 7.6-7.8 (2H,m, Aromatic H), 7.5 (2H,s,N-H), 5.50 (1H,s,methyne),4.5(2H,t,CH_2-CH_2), 4.2(2H,q,CH_3-CH_2) 4.1(2H, s, CH_2-Cl) 3.0 (3H,t, CH_2-CH_2),1.05(3H,t,CH_2-CH_3).

Anal. Calcd.for $C_{16}H_{17}N_2O_4$Cl: C, 57.06; H, 5.09; N, 08.32 ; found C, 56.98; H, 5.25; N, 08.05%

MASS; m/z found for **$C_{16}H_{17}N_2O_4$**Cl:336,338 ($[M+1]^+$)

12. **Synthesis of ethyl 6-(chloromethyl)-4-(2,3-dihydrobenzofuran-5-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate(4l):**

The product **4l** was obtained from 2,3-dihydrobenzofuran-5-carbaldehyde **1l** (148 mg, 1 eq), thio urea (114mg, 1.5eq) and ethyl 4-chloro-3-oxobutanoate (164mg, 1eq). This compound was obtained as light brown color solid in 92% yield. Melting point: 131-133^{0}C

IR νmax(KBR): 3320(N-H), 1745(ester), 680(C=S)

^{1}H NMR (400 MHz, DMSO δ in ppm): 8.1(1H,s,Aromatic H) 7.3-7.5 (2H,m, Aromatic H), 5.55 (1H,s,methyne),4.32(2H,t,CH_2-CH_2), 4.25(2H,q,CH_3-CH_2) 4.18(2H, s, CH_2-Cl) 3.12 (3H,t, CH_2-CH_2), 2.4 (2H,s,N-H),1.08(3H,t,CH_2-CH_3).

Anal. Calcd.for $C_{16}H_{17}N_2O_3$ClS: C, 54.46; H, 4.86; N, 07.94 ; found C, 54.08; H, 4.95; N, 08.05%; **MASS**; m/z found for **$C_{16}H_{17}N_2O_3$**ClS: 352,354 ($[M+1]^+$)

13. **Synthesis of 4-(2,3-dihydrobenzofuran-5-yl)-7,7-dimethyl-2-thioxo-1,2,3,4,7,8-hexahydroquinazolin-5(6H)-one(4m):**

The product **4m** was obtained from 2,3-dihydrobenzofuran-5-carbaldehyde **1m** (148 mg, 1 eq), thio urea (114mg, 1.5eq) and dimedone (140 mg, 1eq). This compound was obtained as light brown color solid in 95% yield. Melting point: 260-262^{0}C

IR νmax(KBR): 3326(N-H), 1720(ketone), 690(C=S)

^{1}H NMR (400 MHz, DMSO δ in ppm): 7.07(1H,s,Aromatic H) 6.92 (1H,d, Aromatic H), 6.65 (1H,d,Aromatic H), 5.37 (1H,s,methyne),4.5(2H,t,CH_2-CH_2), 3.1(2H, t, CH_2-CH_2) 2.4 (2H,s, C-CH_2) 2.3 (2H,s, N-H), 2.1(2H,s,C-CH_2), 1.05(6H,s,CH3[2]).

C^{13} NMR: δ 196,174,162,159,148,135,127,126,125,114,71,51,50,41,32,30,28,26**.**

Anal. Calcd.for $C_{18}H_{20}N_2O_2$S: C,65.83; H, 6.14; N, 08.53 ; found C, 65.42; H, 6.25; N, 08.35%

MASS; m/z found for **$C_{18}H_{20}N_2O_2$**S: 329.2 ($[M+1]^+$)

14. **Synthesis of 4-(2,3-dihydrobenzofuran-5-yl)-7,7-dimethyl-3,4,7,8-tetrahydroquinazoline-2,5(1H,6H)-dione(4n):**

The product **4n** was obtained from 2,3-dihydrobenzofuran-5-carbaldehyde **1n** (148 mg, 1 eq), urea (90mg, 1.5eq) and dimedone (140 mg, 1eq). This compound was obtained as colorless solid in 90% yield. Melting point: 163-165^{0}C

IR νmax(KBR): 3335(N-H), 1720(ketone), 1685(amide)

^{1}H NMR (400 MHz, DMSO δ in ppm): 9.35 (2H,s, N-H) 7.05(1H,s,Aromatic H) 6.79 (1H,d, J=8.0,Aromatic H), 6.64 (1H,d, J=8.0,Aromatic H), 5.05 (1H,s,methyne),4.4(2H,t,CH_2-CH_2), 3.1-3.4(4H, m, CH_2,CH_2-CH_2) 2.09 (2H,s, C-CH_2), 1.1(6H,s,CH3[2]).

Anal. Calcd.for $C_{18}H_{20}N_2O_3$: C,69.21; H, 6.45; N, 08.97 ; found C, 69.42; H, 6.15; N, 08.75%

MASS; m/z found for **$C_{18}H_{20}N_2O_3$**: 313.2 ([M-1]$^+$)

15. **Synthesis of 5-acetyl-4-(7-hydroxy-4-oxo-4H-chromen-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one(4o):**

The product **4o** was obtained from 7-hydroxy-4-oxo-4H-chromene-3-carbaldehyde **1o**(190 mg, 1 eq), urea (90mg, 1.5eq) and ethyl 3-oxobutanoate (130mg, 1eq). This compound was obtained as colorless solid in 90% yield. Melting point: 212-214^{0}C

IR νmax(KBR): 3330(N-H), 1725(ketone), 1690(amide)

^{1}H NMR (400 MHz, DMSO δ in ppm): 9.90(1H,s,O-H) 9.12(1H,s, Aromatic H), 8.10 (1H,s,Aromatic H), 7.59 (2H,s,N-H),7.10(2H,m,Aromatic-H),5.25 (1H,s,-CH),4.20(2H, q, CH_2) 1.95(3H,s,CH3),1.20(3H,t,CH_3)

Anal. Calcd.for $C_{16}H_{14}N_2O_5$: C,61.14; H, 4.49; N, 08.91 ; found C, 61.32; H, 4.25; N, 08.75%

MASS; m/z found for **$C_{16}H_{14}N_2O_5$**: 345.10 ([M+1]$^+$).

16. **Synthesis of ethyl 4-(7-hydroxy-4-oxo-4H-chromen-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4p):**

The product **4p** was obtained from 7-hydroxy-4-oxo-4H-chromene-3-carbaldehyde **1p** (190 mg, 1 eq), urea (90mg, 1.5eq) and acetyl acetone (100 mg 1eq). This compound was obtained as brown color solid in 91% yield. Melting point: 251-253^{0}C

IR νmax(KBR): 3310(N-H), 1735(ester), 1690(amide)

^{1}H NMR (400 MHz, DMSO δ in ppm): 9.99(1H,s,O-H) 9.17(1H,s, Aromatic H), 8.05 (1H,s,Aromatic H), 7.5 (2H,s,N-H),7.19(2H,m,Aromatic-H),5.21 (1H,s,-CH),2.23(3H, s, CH_3) 1.08(3H,s,CH3).

Anal. Calcd.for $C_{17}H_{16}N_2O_6$: C,59.30; H, 4.68; N, 08.14 ; found C, 59.10; H, 4.55; N, 08.05%

MASS; m/z found for **$C_{17}H_{16}N_2O_6$**: 343.0 ([M-1]$^+$)

17. **Synthesis of 5-acetyl-4-(1-(4-(2-cyanophenyl)1-benzyl-1H-indol-3-yl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one(4q)**

The product **4q** was obtained from 2-(1-(4-(2-cyanophenyl)1-benzyl-1H-indole-3-carbaldehyde **1q** (460 mg, 1 eq), urea (90mg, 1.5eq) and acetyl acetone (100 mg 1eq).This compound was obtained as colorless solid in 90% yield. Melting point: 195-197 ^{0}C

IR νmax(KBR): 3335(N-H), 1720(ketone), 1685(amide)

^{1}H NMR (400 MHz, DMSO δ in ppm): 8.13(1H,d, Aromatic-H) 7.90(1H,d, Aromatic H), 7.77 (2H,d,Aromatic H), 7.5-7.6 (4H,m, Aromatic H),7.2-7.4(4H,m,Aromatic-H),6.35(2H,s, N-H) 5.69 (2H,s, Ph -CH_2),5.49(1H,s,methyne), 2.75(3H, s, O=C-CH_3) 2.40(3H,s,CH3).

C^{13} NMR: δ 163.21, 149.61, 148.47, 143.41, 142.62, 139.10, 138.03, 137.07, 133.83, 130.04, 129.22,128.96,126.66,124.80,123.73,122.99,120.48,119.28,118.46,111.88,110.08,109.56,108.83, 100.14,99.2846.35,45.34,19.62,12.47.

Anal. Calcd.for $C_{29}H_{24}N_4O_2$: C,75.63; H, 5.25; N, 12.17 ; found C, 75.50; H, 5.15; N, 12.05%

MASS; m/z found for **$C_{29}H_{24}N_4O_2$**: 458.4 ($[M-1]^+$).

Biology

Cell Lines: Human hepatocellular carcinoma (HCC) cell line HepG2 was obtained from American Type Culture Collection (Manassass, VA). HCC cell lines, HCCLM3 and HUH-7, and normal human liver cell line, LO2, were kindly provided by Prof. Kam Man Hui, National Cancer Centre, Singapore. All the cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 1X antibiotic-antimycotic solution with 10% FBS.

Cytotoxicity Studies: The anti-proliferative effect of dihydropyrimidones against various HCC cell lines (HepG2, HCCLM3 and HUH-7) and normal human liver cells, LO2, was determined by the MTT dye uptake method. The cells (5×10^3/well) were incubated in triplicate experiments in a 96-well plate in the presence or absence of indicated concentrations of the pyrimidones in a final volume of 0.2 ml for indicated time points at 37 °C. Thereafter, 20 µl of MTT solution (5 mg/ml in PBS) was added to each well. After a 4-h incubation in the dark at 37 °C, 0.1 ml of lysis buffer (20% SDS, 50% dimethylformamide) was added and incubated for 2 h at 37 °C, followed by measurement of optical density at 570 nm by Tecan plate reader (Durham, NC).

In silico ligand similarity studies: Given the constantly increasing amount of bioactivity

data available, we attempted to rationalize the mode-of-action of the experimentally active compounds using *in silico* approaches, which are currently the topic of many chemogenomics studies.[1,2] In order to achieve this, we applied the Laplacian-modified Naïve Bayesian classifier to predict potential targets for the compounds which were experimentally tested, as deployed by Koutsoukas *et al*.[3] This classifier was trained on a large dataset extracted from ChEMBL[4] comprising approximately 190,000 bioactive compounds covering 477 human protein targets. Proteins with a minimum log-odds score of 10 were considered as possible targets.

Westernblot analysis: We performed Westernblot analysis for detection of various proteins. Compound **4g** treated whole-cell extracts were lysed in lysis buffer (20 mMTris (pH 7.4), 250 mM NaCl, 2 mM EDTA (pH 8.0), 0.1% TritonX-100, 0.01 mg/ml aprotinin, 0.005 mg/ml leupeptin, 0.4 mM PMSF, and 4 mM NaVO4). Lysates were then spun at 14,000 rpm for 10min and resolved on a 10% SDS gel. After electrophoresis, the proteins were electrotransferred to a nitrocellulose membrane, blocked with Blocking One (Nacalai Tesque Inc, Kyoto, Japan), and probed with various primary antibodies (1:1000) overnight at 4°C. The blot was washed, exposed to HRP-conjugated secondary antibodies for 1 h, and finally examined by chemiluminescence (ECL; GE Healthcare, Little Chalfont, Buckinghamshire, UK).

PPR-γ activation studies: To determine PPAR-γ activation, we performed DNA binding assays using TransAM PPAR-γ Transcrption Factor Kit from Active Motif according to the manufacturer's instructions. Nuclear extract of HepG2 cells treated with compound **4g** for different time periods was prepared using nuclear extraction kit from Active Motif, according to the manufacturer's protocol. Briefly, 10 mg of nuclear proteins were added into

96-well plate coated with an unlabeled oligonucleotide containing the consensus binding site for PPAR-γ (5′-AACTAGGNCAAAGGTCA-3′) and incubated for 1 h. The wells were washed and incubated with antibodies against PPAR-γ for 1 h. A horseradish peroxidase (HRP) conjugated secondary antibody was then applied to detect the bound primary antibody and provided the basis for colorimetric quantification. The enzymatic product was measured at 450 nm by microplate reader (Tecan Systems).

In silico molecular docking studies: The co-crystal structure of human PPAR-γ in complex with rosiglitazone (PDB: 4EMA) was used for the structure-based molecular docking studies.[4] The software InsightII from Accelrys was used to obtain a full set of tools for molecular modeling, which includes molecular graphics and forcefield-based simulations. Molecular modeling was performed with the commercially available InsightII packages from Discovery Studio (DS) Version 2.5. Before performing the CDOCKER protocol of DS, the 3-D structure of the rosiglitazone bound PPAR-γ copy was cleaned, and the size and spatial orientation of the LBD was identified. All calculations were performed using the CHARMM force field. Each energy-minimized final docking position of the dihydropyrimidones was evaluated using the interaction score function implemented in the CDOCKER module. The predicted binding pose of compound **4g** was compared against the known binding pose of rosiglitazone (PDB: 4EMA).

Migration Assay: The migration of HepG2 cells was investigated using a wound healing assay. An IBIDI culture insert (ibidi GmbH, Munich, Germany) consists of two reservoirs separated by a 500 µm thick wall created by a culture insert in a 35mm petri dish. For the migration assay, an equal number of HepG2 cell (70 µl; 5×10^5 cells/ml) was added into the two reservoirs of the same insert and incubated at 37°C/5% CO2. After 12 h, the insert was

gently removed creating a gap of ~500 μm. The cells were pretreated with the PPAR-γ inhibitor GW9662 (20 μM for 2h), followed by incubation with compound **4g** for 8 h. The cells were also treated either with 25 μM compound **4g** alone for 8 h or GW9662 alone (20 μM for 2h). After incubation, the wounds were observed using bright field microscopy and multiple images were taken at areas flanking the intersections of the wound. The gap distance of the wound was measured at three different sites using Photoshop software, and data were normalized to the average of the control. Graphs were plotted against the percentage of migration distance the cells moved before and after treatment, as well normalized to the control.

Invasion Assay: The *in vitro* tumor cell invasion assay was performed using the Bio-Coat Matrigel invasion assay system (BD Biosciences, San Jose, CA, USA), according to the manufacturer's instructions. 1×10^5 HepG2 cells were suspended in serum-free DMEM and seeded into the Matrigel transwell chambers consisting of polycarbonate membranes with 8-μm pores. The cells were pretreated with GW9662 (20 μM for 2h), followed by incubation with compound **4g** for 8 h. The cells were also treated either with 25 μM compound **4g** alone for 8 h or GW9662 alone (20 μM for 2 h). After incubation, the upper surfaces of the transwell chambers were wiped with cotton swabs, and the invading cells were fixed and stained with crystal violet solution. The invading cells were then counted in five randomly selected areas under microscopic observation.

Statistical analysis was done using a software Origin 8 (OriginLab). The Mann–Whitney U test was used to determine P-values

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Supplementary Table 1: The physical parameters for the newly synthesized pyrimidones

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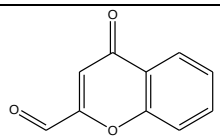
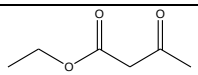
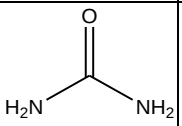
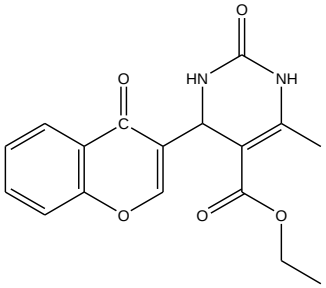
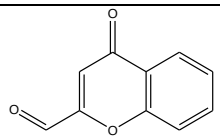
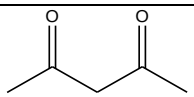
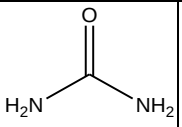
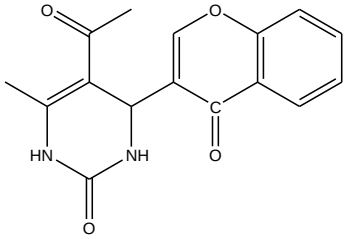
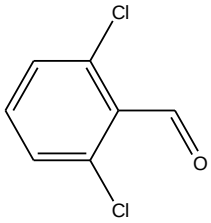
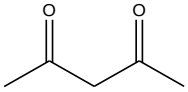
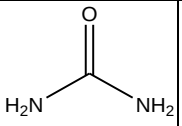
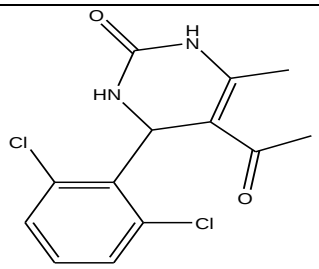
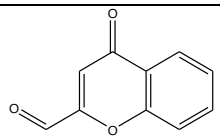
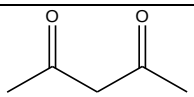
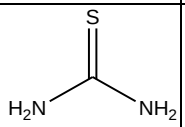
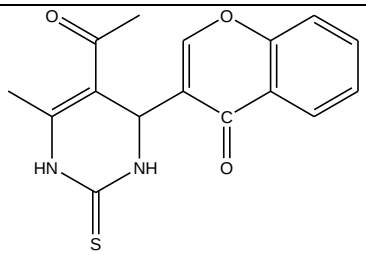
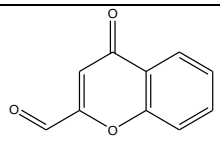
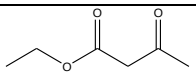
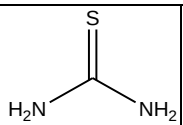
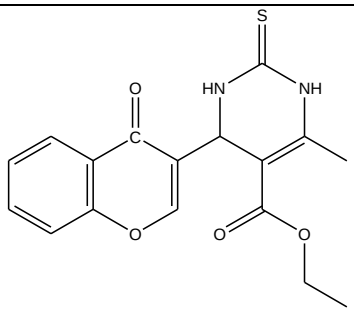
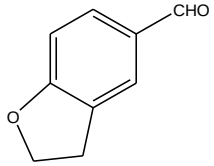
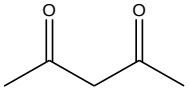
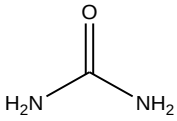
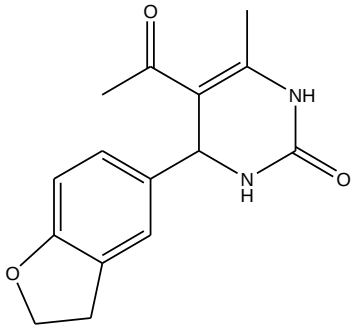
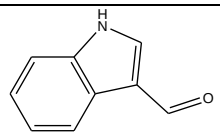
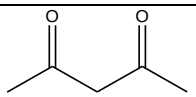
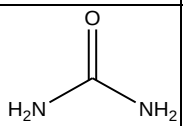
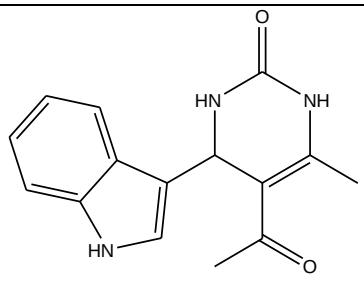
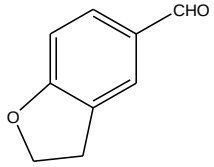
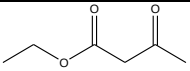
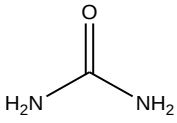
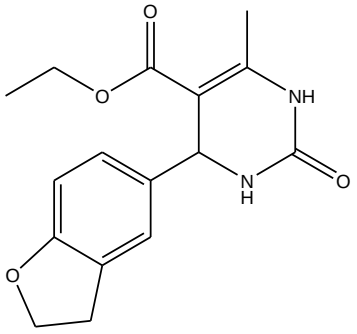
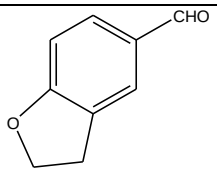
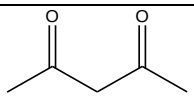
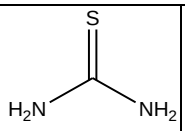
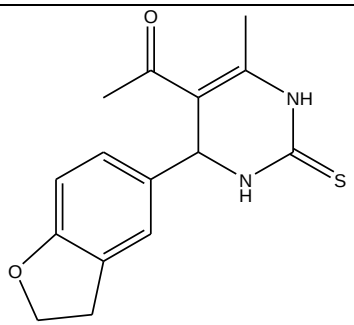
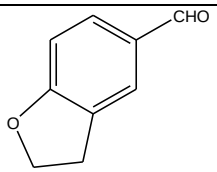
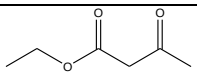
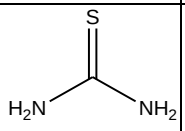
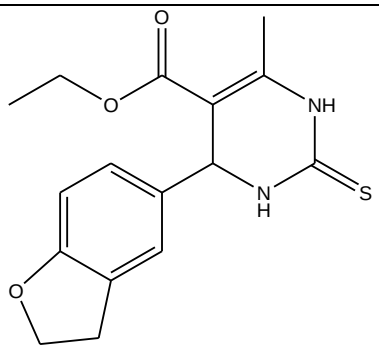
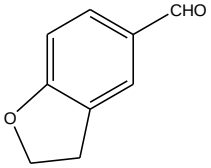
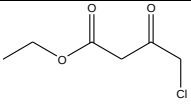
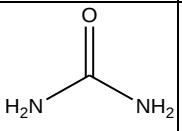
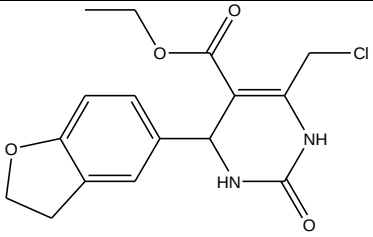
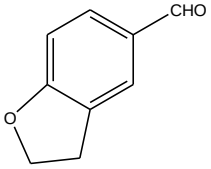
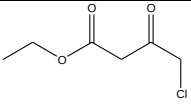
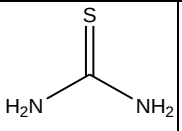
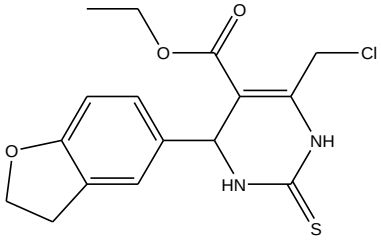
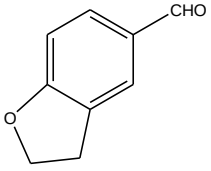
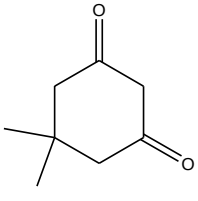
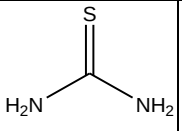
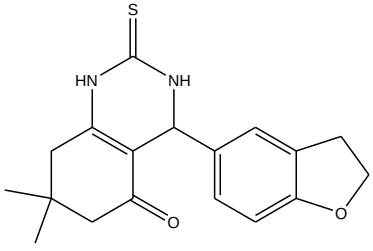
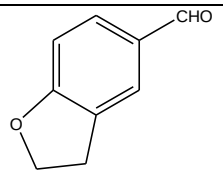
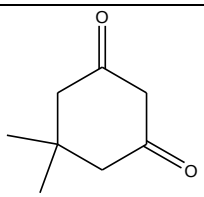
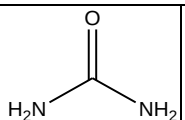
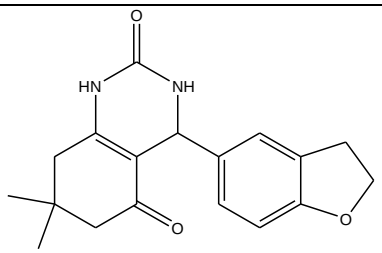
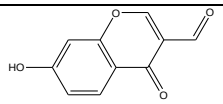
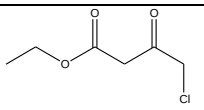
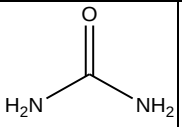
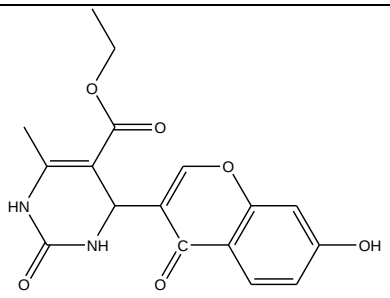
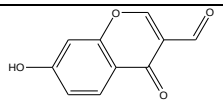
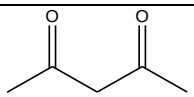
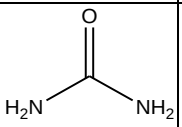
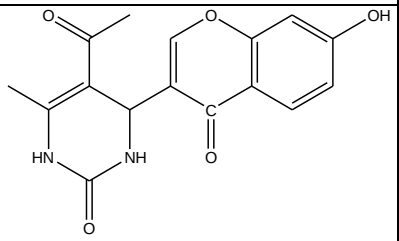
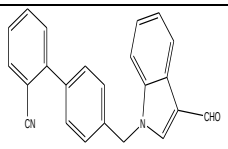
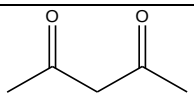
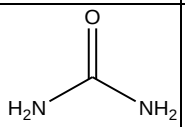
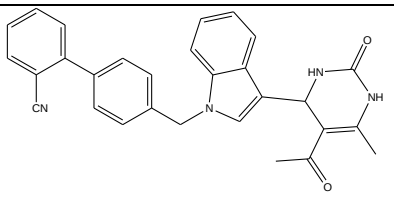
4a					83%
4b					82%
4c					87%
4d					86%

4e					89%
4f					87%
4g					70%
4h					85%

4i					89%
4j					83%
4k					**80 %**
4l					86%
4m					88%

4n					90%
4o					94%
4p					91%
4q					93%

Supplementary table 2: The comparison of the cytotoxicity data for the synthesized pyrimidones (4a-q) against HepG2 and normal LO2 cells compounds.

DHPs	HepG2 IC_{50} (μM)	LO2 IC_{50} (μM)	Fold difference
4a	226.67	343.97	1.52
4b	420.86	282.91	0.67
4c	767.92	481.37	0.63
4d	519.99	242.99	0.47
4e	114.11	212.36	1.86
4f	121.41	250.19	2.06
4g	17.9	136.93	7.6
4h	166.49	323.84	1.95
4i	304.98	452.00	1.48
4j	140.03	182.93	1.31
4k	56.27	71.99	1.28
4l	91.97	172.95	1.88
4m	94.69	142.45	1.50
4n	73.91	219.29	2.97
4o	38.85	71.51	1.84
4p	47.24	106.07	2.25
4q	92.49	120.67	1.30

Supplementary table 3: The predicted human targets for the compound 4g.

Predicted target	**Log odds score**
Peroxisome proliferator-activated receptor gamma	26.02
Alpha-1B adrenergic receptor	9.22
Alpha-1D adrenergic receptor	8.94
Motilin receptor	8.53
Alpha-1A adrenergic receptor	7.41
Sodium/glucose cotransporter 1	6.77
Cytosolic phospholipase A2	6.11
Peroxisome proliferator-activated receptor alpha	3.68
Prostaglandin E2 receptor EP4 subtype	3.68
Chymase	3.55
Tryptase alpha/beta-1	3.22
Prostacyclin receptor	2.96
Prostaglandin E2 receptor EP1 subtype	2.34
Tumor necrosis factor	2.01
Sodium/glucose cotransporter 2	0.84
Acetyl-CoA carboxylase 1	0.82
Oxytocin receptor	0.71
Gastric inhibitory polypeptide receptor	0.68
Amine oxidase [flavin-containing] A	0.68

Mineralocorticoid receptor	0.64
Prostaglandin E2 receptor EP2 subtype	0.38
Cytochrome P450 2D6	0.11
Apoptosis regulator Bcl-2	0.10
Sterol O-acyltransferase 1	0.08
Vasopressin V1b receptor	0.06

Supplementary Table 4: The in silico binding analysis (CDOCER Score) of the pyrimidones that bound to PPARgamma

Compounds	CDOCKER ENEGRY	CDOCKER INTERACTION ENERGY
4a	-20.7	-40.4
4b	-21.0	-36.0
4c	-12.2	-27.7
4d	-19.0	-35.5
4e	-15.0	-32.7
4f	-22.2	-38.0
4g	-24.0	-33.7
4h	-11.8	-36.5
4i	-10.4	-34.4
4j	-12.9	-36.2
4k	-18.9	-40.1
4l	-13.0	-38.7
4m	-5.7	-35.6
4n	-7.7	-37.1
4o	-23.4	-33.0
4q	1.8	-44.8