Design and synthesis of 2-alkylpyrimidine-4,6-diol and 6-alkylpyridine-2,4-diol as potent GPR84 agonists

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KEYWORDS: GPR84; agonists; 2-alkylpyrimidine-4,6-diol; 6-alkylpyridine-2,4-diol; structural modification

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1. Table S1. PK Parameters of compound 51 in ICR Mice

PK Parameters of Cpd 51 in ICR Mice (IV: 5 mg/kg)

PK Parameters		IV-1	IV-2	IV-3	Mean	SD	RSD (%)
Dose	$mg \cdot kg^{-1}$			5			
K_{el}	L/kg	0.183	0.212	0.374	0.256	0.103	40
$t_{1/2}$	hr	3.79	3.27	1.85	2.97	1.00	34
C_0	ng/mL	2262	2156	3748	2722	890	33
Cmax	ng/mL	1921	1752	2486	2053	384	19
AUC_{0-t}	hr*ng/mL	966	816	983	922	92.2	10
AUC_{0-inf}	hr*ng/mL	1035	839	1001	958	105	11
$AUMC_{0-t}$	hr*hr*ng/mL	876	477	462	605	235	39
$AUMC_{0-inf}$	hr*hr*ng/mL	1803	771	616	1063	645	61
CL	mL/min/kg	80.5	99.4	83.3	87.7	10.2	12
MRT_{IV}	hr	1.74	0.919	0.615	1.09	0.582	53
Vd_{SS}	L/kg	8.41	5.48	3.07	5.66	2.67	47

PK Parameters of Cpd 51 in ICR Mice (PO: 10mg/kg)

PK Parameters		PO-4	PO-5	PO-6	Mean	SD	RSD(%)
Dose	$mg \cdot kg^{-1}$			10			
K_{el}	h^{-1}	0.342	0.520	0.288	0.383	0.122	32
$t_{1/2}$	h	2.03	1.33	2.41	1.92	0.546	28
t_{max}	h	0.250	0.250	0.500	0.333	0.144	43
C_{max}	$ng \cdot mL^{-1}$	244	201	242	229	24.3	11
AUC_{0-t}	$h \cdot ng \cdot mL^{-1}$	434	295	467	399	91.4	23
AUC_{0-inf}	$h \cdot ng \cdot mL^{-1}$	504	303	591	466	148	32
$AUMC_{0-t}$	$h \cdot h \cdot ng \cdot mL^{-1}$	893	641	980	838	176	21
$AUMC_{0-inf}$	$h \cdot h \cdot ng \cdot mL^{-1}$	1516	718	2156	1463	721	49
MRT_{PO}	h	3.01	2.37	3.65	3.01	0.638	21
F	%	26.3	15.8	30.8	24.3	7.72	32

Route of Administration: p.o. (IG) and i.v.

Collection Means. 25 μL blood was collected from the orbital plexus into tubes after the animal was temporarily anesthetized by isoflurane inhalation. The tubes were filled full for about 12 h with a saline solution containing heparin (1000 units/mL), the saline was removed, and the tubes were dried at 105 ^{o}C before use.

Blood Samples. Immediately after collection, blood collection tubes were gently inverted at least five times, ensuring complete mixing, and were then immediately placed on ice. The blood was centrifuged for 10 min at 5000 rpm and 4 ^{o}C to separate the plasma from the red blood cells. Plasma was stored at −20 ^{o}C before LC−MS/MS

analysis for testing compound concentration.

Test compound (i.v.) was dissolved in 5% DMSO + 1% Cremophor EL + 94% Saline, and was administered to ICR mice (n = 3 per group) at the dose level of 5 mg/kg (Concentration: 1 mg/mL). Test compound (p.o.) was dissolved in 5% DMSO + 1% Cremophor EL + 94% (0.5% w/w) CMC-Na, and was administered orally to ICR mice (n = 3 per group) by gavage at the dose level of 10 mg/kg (Concentration: 1 mg/mL). The plasma samples (i.v.) were collected from the orbital plexus at 0.083, 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, 8.0, and 24 h postdose, and the plasma samples (p.o.) were collected from the orbital plexus at 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, 8.0, and 24 h postdose. The drug concentrations in the samples were determined using an established LC−MS/MS system after deproteinization. LC−MS/MS analysis was performed using a Agilent 1200 HPLC system and an API4000 Q-Trap mass spectrometer with an ESI source. Chromatographic separation was achieved using a Phenomonex Luna C8(2) column column (2.1 mm × 50 mm, 3 μm). The mobile phase consisted of 0.1% Formic acid (FA) H_2O / 0.1% Formic acid (FA) acetonitrile (85:15 ~5:95, v/v), and the flow rate was set at 0.3 mL/min. The injection cycle of each sample was set at 3 min. Noncompartmental pharmacokinetic parameters were calculated on the basis of the plasma concentration − time data using WinNonlin Professional 6.3 (Pharsight, CA).

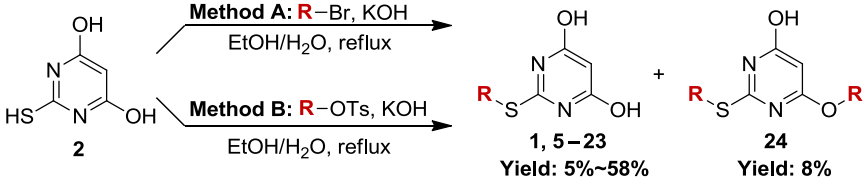
2. Synthesis and characterization of compounds

General Experimental

Starting materials, reagents and solvents were purchased from commercial suppliers and used without further purification. Anhydrous toluene and DCE were obtained from a distillation over sodium wire or CaH_2. All non-aqueous reactions were run under an inert atmosphere (nitrogen or argon) with rigid exclusion of moisture from reagents and all reaction vessels were oven-dried. Thin-layer chromatography (TLC) was carried out on pre-coated TLC plates with silica gel HSGF 254. Spots were visualized under UV at 254 nm. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Varian Mercury-VX 300, Varian MR 400, AVANCE III 500 or AVANCE III 600 spectrometer using deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), deuterated acetone (acetone-d_6) and deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent. Chemical shifts are expressed in δ (ppm.). Abbreviations for signal coupling are as follows: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; m, multiplet. Coupling constants (*J*) are given in Hz. HR-MS were measured on a Micromass Ultra Q-Tof. Purity was evaluated by analytical HPLC chromatograms using Agilent 1200 series LC system equipped with a degasser, a quaternary pump, an auto sampler, a column oven and a diode array detector. Analytes were separated on a Zorbax SB C18 column (4.6 × 150 mm, 5 μm). Solvent A was 0.1% trifluoroacetic acid in H_2O, and solvent B was 100% methanol. Gradient elution: 20% B for 2 min, then 20%-80% B from 2 to 20 min, 80% B was maintained for 5 min, then 80% -20% B from 25 min to 30 min. All compounds were monitored at 254 nm at room temperature. Flow rate: 1.0 mL/min. The following abbreviations for solvents and reagents are used; N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), sodium hydroxide (NaOH), 1,2-dichloroethane (DCE), tetrahydrofuran (THF).

Experimental procedure

General procedure for the preparation of compounds **1**, **5-11**, **17-24**.



Method A: A mixture of 2-mercaptopyrimidine-4,6-diol (1.0 mmol) and alkyl bromides (3 mmol) in the presence of KI (0.1 mmol) and KOH (3.0 mmol) in EtOH (10 mL)/H_2O (5 mL) was refluxed under a nitrogen atmosphere for 6 hrs. The reaction mixture was allowed to cool to room temperature, and ethyl acetate and water were added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to afford a crude residue, which was purified by silica gel column chromatography (DCM:MeOH = 20:1) to afford the desired products **1**, **5-11** and **17-23**.

Method B: Alkyl alcohol or benzyl alcohol (1.0 mmol) dissolved in DCM (5 mL) and pyridine (1.3 mmol) was added, then cooled to 0^{o}C and 4-toluene sulfonyl chloride in DCM (2 mL) was added slowly, after the addition, the reaction was heated to room temperature to react 12h, then sat $NaHCO_3$ solution was added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated, then the residue was dissolved in EtOH (10 mL)/H_2O (5 mL), and 2-mercaptopyrimidine-4,6-diol (0.5 mmol), KOH (1.5 mmol) was added, the mixture was refluxed overnight then cooled to room temperature, and ethyl acetate and water were added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to afford a crude residue, which was purified by silica gel column chromatography (DCM:MeOH = 20:1) to afford the desired products **12-16**.

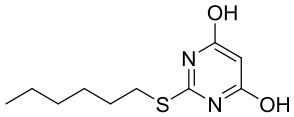
Notes: The two methods share almost the same reaction conditions, and display similar products profiles. The choice of method depends on the availability of the starting material.

The S,O-dialkylation products can be found in all the reactions, which have lower yields (e.g., **24**, 8%) compared to the S-alkylation products (e.g., **1**, 17%). No

O-alkylation products were isolated. The alkylation sites of products were determined by the ^{1}H NMR test, as the chemical shift of O-alkylation (3.8~4.3) is larger than that of S-alkylation (~3.0). And these products can also be distinguished in the TLC analysis: S,O-dialkylation products usually have larger retention factor (Rf) than S-alkylation products.

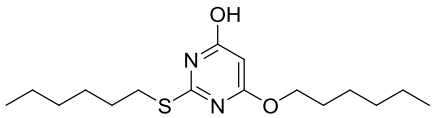
Because **24** lost activity in the biological test, no S,O-dialkylation products were collected in the further reactions.

2-(hexylthio)pyrimidine-4,6-diol (**1**)



Compound **1** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 1-bromohexane (0.3 ml, 2.07 mmol) as the starting material, product **1** was obtained as a white solid (26 mg, **yield:** 17%), HPLC purity: 99.4%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.28 (s, 1H), 5.11 (s, 1H), 3.08 (t, *J* = 6.0 Hz, 2H), 1.61 (m, 2H), 1.36 (m, 2H), 1.28 (m, 4H), 0.87 (t, *J* = 6.3 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 167.69, 163.56, 86.27, 31.43, 30.20, 29.37, 28.49, 22.70, 14.57. **HR-MS (ESI)** *m/z*: calcd for $C_{10}H_{17}N_2O_2S$ $[M+H]^+$:, 229.1011, found: 229.1003, error: −3.5 ppm.

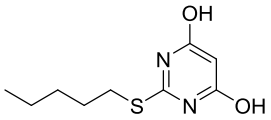
6-(hexyloxy)-2-(hexylthio)pyrimidin-4-ol (**24**)



In the same reaction preparing compound **1**, compound **24** can also be obtained as a white solid (17 mg, **yield:** 8%), HPLC purity: 99.1%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.32 (s, 1H), 5.36 (s, 1H), 4.18 (t, *J* = 6.6 Hz, 2H), 3.07 (t, *J* = 7.2 Hz, 2H), 1.63 (dd, *J* = 12.9, 6.3 Hz, 4H), 1.43 – 1.32 (m, 4H), 1.29 (d, *J* = 3.3 Hz, 8H), 0.87 (t, *J* = 4.8 Hz, 6H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 169.07, 166.05, 85.93, 66.55, 30.93, 30.79, 29.69, 29.05, 28.41, 27.96, 25.02, 22.05, 22.02, 13.84. **HR-MS**

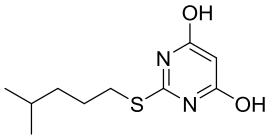
(ESI) *m/z*: calcd for $C_{16}H_{29}N_2O_2S$ $[M+H]^+$: 313.1950, found: 313.1945, error: −1.6 ppm.

2-(pentylthio)pyrimidine-4,6-diol (**5**):



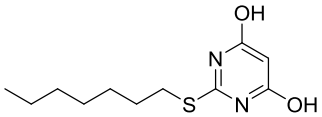
Compound **5** was prepared by the previously described General procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 1-bromopentane (0.26 ml, 2.07 mmol) as the starting material, product **5** was obtained as a white solid (16 mg, **yield:** 11%), HPLC purity: 97.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.28 (s, 1H), 5.11 (s,1H), 3.08 (t, *J* = 6.0 Hz, 2H), 1.61 (m, 2H), 1.36 (m, 2H), 1.28 (m, 2H), 0.87 (t, *J* = 6.3 Hz, 3H). **^{13}C NMR** (75 MHz, CD_3OD-d_4) δ 169.85, 164.03, 86.60, 32.08, 31.40, 30.16, 23.46, 14.44. **HR-MS (ESI)** *m/z*: calcd for $C_9H_{15}N_2O_2S$ $[M+H]^+$: 215.0854, found: 215.0847, error: −3.3 ppm.

2-((4-methylpentyl)thio)pyrimidine-4,6-diol (**6**)



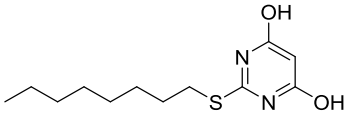
Compound **6** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 1-bromo-4-methylpentane (0.3 ml, 2.07 mmol) as the starting material, product **6** was obtained as a white solid (19 mg, **yield:** 12%), HPLC purity: 99.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.28 (s, 1H), 5.11 (s,1H), 3.00 (t, *J* = 6.0 Hz, 2H), 1.62 (m, 2H), 1.32 (m, 3H), 0.87 (d, *J* = 6.6 Hz, 6H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.2, 162.9, 85.3, 37.7, 29.7, 27.1, 26.6, 22.4. **HR-MS (ESI)** *m/z*: calcd for $C_{10}H_{17}N_2O_2S$ $[M+H]^+$: 229.1011, found: 229.1002, error: −3.9 ppm.

2-(heptylthio)pyrimidine-4,6-diol (**7**)



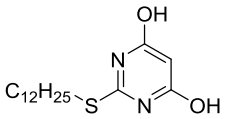
Compound **7** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 1-bromoheptane (0.33 ml, 2.07 mmol) as the starting material, product **7** was obtained as a white solid (27 mg, **yield:** 16%). HPLC purity: 93.4%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.28 (s, 1H), 5.11 (s, 1H), 3.08 (t, *J* = 6.0 Hz, 2H), 1.61 (m, 2H), 1.36 (m, 2H), 1.28 (m, 6H), 0.87 (t, *J* = 6.3 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 166.93, 162.91, 85.48, 31.41, 29.44, 28.68, 28.18, 28.06, 22.00, 13.91. **HR-MS (ESI)** *m/z*: calcd for $C_{11}H_{19}N_2O_2S$ $[M+H]^+$: 243.1167, found: 243.1162, error: −2.1 ppm.

2-(octylthio)pyrimidine-4,6-diol (**8**)



Compound **8** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 1-bromooctane (0.37 ml, 2.07 mmol) as the starting material, product **8** was obtained as a white solid (18 mg, **yield:** 10%). HPLC purity: 96.7%. **^{1}H NMR** (300 MHz, CD_3OD-d_4）δ 12.24 (s, 1H), 11.28 (s, 1H), 5.12 (s,1H), 3.11 (t, *J* = 6.0 Hz, 2H), 1.62 (m, 2H), 1.41 (m, 2H), 1.30 (m, 8H), 0.87 (t, *J* = 6.3 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO- d_6) δ 167.19, 162.73, 85.59, 31.19, 29.48, 28.67, 28.6, 28.51, 28.09, 22.07, 13.93. **HR-MS (ESI)** *m/z*: calcd for $C_{12}H_{21}N_2O_2S$ $[M+H]^+$: 257.1324, found: 257.1319, error: −1.9 ppm.

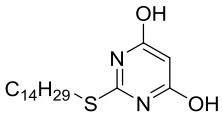
2-(dodecylthio)pyrimidine-4,6-diol (**9**)



Compound **9** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and

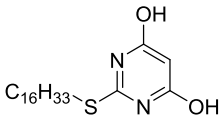
1-bromododecane (0.5 ml, 2.07 mmol) as the starting material, product **9** was obtained as a white solid (17 mg, **yield:** 8%). HPLC purity: 98.3%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.28 (s, 1H), 5.11 (s,1H), 3.07 (t, *J* = 6.9 Hz, 2H), 1.59 (m, 2H), 1.26 (m, 2H), 1.24 (m, 16H), 0.87 (t, *J* = 6.3 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.19, 162.91, 85.69, 31.22, 29.5, 29.00, 28.97, 28.94, 28.90, 28.67, 28.51, 28.08, 22.07, 13.93. **HR-MS (ESI)** *m/z*: calcd for $C_{16}H_{29}N_2O_2S$ $[M+H]^+$: 313.1950, found: 313.1940, error: −3.2 ppm.

2-(tetradecylthio)pyrimidine-4,6-diol (**10**)



Compound **10** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 1-bromotetradecaneb (0.62 ml 2.07 mmol) as the starting material, product **10** was obtained as a white solid (21 mg, **yield:** 9%), HPLC purity: 98.3%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.28 (s, 1H), 5.11 (s, 1H), 3.07 (t, *J* = 6.9 Hz, 2H), 1.59 (m, 2H), 1.26 (m, 2H), 1.24 (m, 20H), 0.87 (t, *J* = 6.3 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 166.98, 163.04, 85.59, 31.33, 29.52, 29.06, 29.02, 28.97, 28.75, 28.59, 28.16, 22.12, 13.95. **HR-MS (ESI)** *m/z*: calcd for $C_{18}H_{33}N_2O_2S$ $[M+H]^+$: 341.2263, found: 341.2257, error: −1.8 ppm.

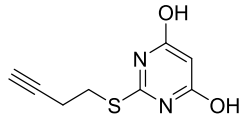
2-(hexadecylthio)pyrimidine-4,6-diol (**11**)



Compound **11** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 1-bromohexadecane (0.6 ml, 2.07 mmol) as the starting material, product **11** was obtained as a white solid (20 mg, **yield:** 8%), HPLC purity: 96.2%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.28 (s, 1H), 5.11 (s,1H), 3.07 (t, *J* = 6.9 Hz, 2H), 1.59 (m, 2H), 1.26 (m, 2H), 1.24 (m, 24H), 0.87 (t, *J* = 6.3 Hz, 3H). **^{13}C NMR** (75

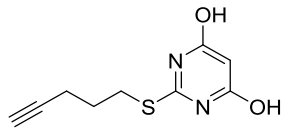
MHz, DMSO-d_6) δ 167.12, 162.80, 93.21, 85.45, 31.32, 29.51, 29.06, 28.98, 28.72, 28.59, 28.17, 22.12, 13.94. **HR-MS (ESI)** *m/z*: calcd for $C_{20}H_{37}N_2O_2S$ $[M+H]^+$: 369.2576, found: 369.2566, error: −2.7 ppm.

2-(but-3-yn-1-ylthio)pyrimidine-4,6-diol (**12**)



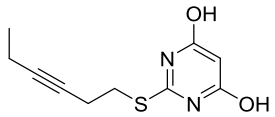
Compound **12** was prepared by the previously described General Procedure (Method B) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and but-3-yn-1-ol (96.6 mg, 1.38 mmol) as the starting material, product **12** was obtained as a white solid (8 mg, **yield:** 5%), HPLC purity: 97.8%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.30 (s, 1H), 5.15 (s, 1H), 3.21 (t, *J* = 6.3 Hz, 2H), 2.92 (s, 1H), 2.55 (m, 2H) **^{13}C NMR** (125MHz, DMSO-d_6) δ 167.2, 162.9, 85.6, 82.6, 72.5, 28.6, 18.7. **HR-MS (ESI)** *m/z*: calcd for $C_8H_9N_2O_2S$ $[M+H]^+$: 197.0385, found: 197.0377, error: −4.0 ppm.

2-(pent-4-yn-1-ylthio)pyrimidine-4,6-diol (**13**)



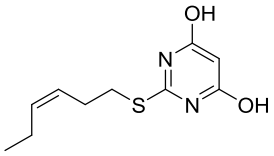
Compound **13** was prepared by the previously described General Procedure (Method B) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and pent-4-yn-1-ol (100 mg, 1.38 mmol) as the starting material, product **13** was obtained as a white solid (8 mg, **yield:** 7%), HPLC purity: 98.2%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.30 (s, 1H), 5.06 (s, 1H), 3.16 – 3.06 (t, *J* = 6.9 Hz, 2H), 2.82 (s, 1H), 1.87 – 1.74 (m, 2H), 1.24 (m, 2H). **^{13}C NMR** (125 MHz, CD_3OD-d_4) δ 161.56, 153.97, 82.29, 69.05, 29.28, 28.78, 27.87, 16.60. **HR-MS (ESI)** *m/z*: calcd for $C_9H_{10}N_2O_2SNa$ $[M+Na]^+$: 233.0361, found: 233.0353, error: −3.4 ppm.

2-(hex-3-yn-1-ylthio)pyrimidine-4,6-diol (**14**)



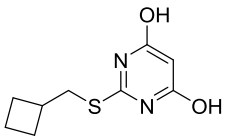
Compound **14** was prepared by the previously described General Procedure (Method B) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and hex-3-yn-1-ol (135 mg, 1.38 mmol) as the starting material, product **14** was obtained as a white solid (65 mg, **yield:** 42%), HPLC purity: 93.4%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.27 (s, 1H) , 5.12 (s, 1H), 3.18 (t, *J* = 6.3 Hz, 2H), 2.52-2.55 (m, 2H), 2.21 – 2.03 (m, 2H), 1.03 (t, *J* = 7.5 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 167.33, 162.79, 85.64, 83.08, 77.81, 29.07, 19.10, 14.00, 11.73. **HR-MS (ESI)** *m/z*: calcd for $C_{10}H_{13}N_2O_2S$ $[M+H]^+$: 225.0698, found: 225.0694, error: −1.7 ppm.

(Z)-2-(hex-3-en-1-ylthio)pyrimidine-4,6-diol (**15**)



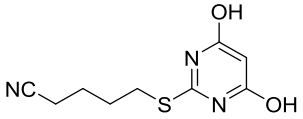
Compound **15** was prepared by the previously described General Procedure (Method B) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and (Z)-hex-3-en-1-ol (38 mg, 1.38 mmol) as the starting material, product **15** was obtained as a white solid (56 mg, **yield:** 35%), HPLC purity: 95.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.16 (s, 1H), 11.36 (s, 1H), 5.42 (d, *J* = 8.7 Hz, 1H), 5.39 (d, *J* = 7.2 Hz, 1H), 5.12 (s, 1H), 3.11 (t, *J* = 7.2 Hz, 2H), 2.38 (q, *J* = 6.9 Hz, 2H), 1.96-2.05 (m, 2H), 0.91 (t, *J* = 7.5 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 167.11, 162.85, 133.37, 126.50, 85.63, 29.56, 26.39, 20.17, 14.08. **HR-MS (ESI)** *m/z*: calcd for $C_{10}H_{15}N_2O_2S$ $[M+H]^+$: 227.0854, found:227.0849, error: −2.2 ppm.

2-((cyclobutylmethyl)thio)pyrimidine-4,6-diol (**16**)



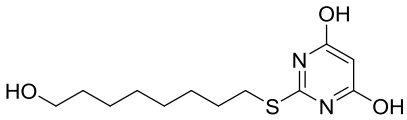
Compound **16** was prepared by the previously described General Procedure (Method B) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and cyclobutylmethanol (204 mg, 1.38 mmol) as the starting material, product **16** was obtained as a white solid (7 mg, **yield:** 5%), HPLC purity: 95.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.22 (s, 1H), 11.28 (s, 1H), 5.10 (s, 1H), 3.19 (d, *J* = 7.8 Hz, 2H), 1.98-2.05 (m, 1H), 1.90 – 1.61 (m, 6H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.0, 162.6, 85.5, 35.4, 34.1, 26.9, 17.4. **HR-MS (ESI)** *m/z*: calcd for $C_9H_{13}N_2O_2S$ $[M+H]^+$: 213.0698, found: 213.0690, error: −3.8 ppm.

2-(4-cyanobutyl)pyrimidine-4,6-diol (**17**)



Compound **17** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 5-bromopentanenitrile (0.23 ml, 2.07 mmol) as the starting material, product **17** was obtained as a white solid (8 mg, **yield:** 5%), HPLC purity: 99.3%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.22 (s, 1H), 11.28 (s, 1H), 5.11 (s,1H), 3.12 (t, *J* = 6.0 Hz, 2H), 2.56 (m, 2H), 1.60 (m, 4H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 162.36, 154.76, 119.59, 85.61, 28.81, 28.19, 23.99, 15.53. **HR-MS (ESI)** *m/z*: calcd for $C_9H_{12}N_3O_2S$ $[M+H]^+$: 226.0650, found: 226.0644, error: −2.7 ppm.

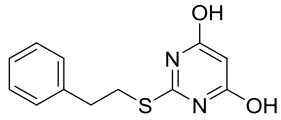
2-((8-hydroxyoctyl)thio)pyrimidine-4,6-diol (**18**)



Compound **18** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 8-bromooctan-1-ol (0.36 ml, 2.07 mmol) as the starting material, product **18** was obtained as a white solid (28 mg, **yield:** 15%), HPLC purity: 90.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.22 (s, 1H), 11.38 (s, 1H) 5.11 (s, 1H), 4.32 (s, 1H), 3.37 (t, *J* = 6.3 Hz, 2H), 3.08 (t, *J* = 7.2 Hz, 2H), 1.70 – 1.50 (m, 2H), 1.37 (m, 4H), 1.26 (m, 6H).

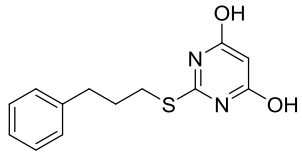
^{13}C NMR (75 MHz, DMSO-d_6) δ 1167.12, 162.80, 93.14, 85.61, 60.71, 32.52, 29.54, 28.86, 28.72, 28.62, 28.14, 25.46. **HR-MS (ESI)** *m/z*: calcd for $C_{12}H_{20}N_2O_3SNa$ $[M+Na]^+$: 295.1092, found: 295.1083, error: −3.5 ppm.

2-(phenethylthio)pyrimidine-4,6-diol (**19**)



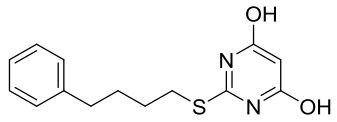
Compound **19** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and (2-bromoethyl)benzene (380 mg, 2.07 mmol) as the starting material, product **19** was obtained as a white solid (99 mg, **yield:** 58%), HPLC purity: 94.1%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.26 (s, 1H), 11.32 (s, 1H), 7.30 (m, 5H), 5.13 (s, 1H), 3.40-3.34 (m, 2H), 3.00 – 2.87 (m, 2H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.84, 163.25, 140.33, 129.11, 128.82, 126.84, 86.13, 35.20, 31.32. **HR-MS (ESI)** *m/z*: calcd for $C_{12}H_{13}N_2O_2S$ $[M+H]^+$: 249.0698, found: 249.0693, error: −2.0 ppm.

2-((3-phenylpropyl)thio)pyrimidine-4,6-diol (**20**)



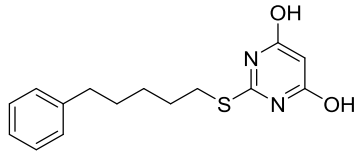
Compound **20** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and (3-bromopropyl)benzene (409 mg, 2.07 mmol) as the starting material, product **20** was obtained as a white solid (76 mg, **yield:** 42%), HPLC purity: 98.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.30 (s, 1H), 7.27 (m, 2H), 7.21 (m, 3H), 5.13 (s, 1H), 3.10 (t, *J* = 6.9 Hz, 2H), 2.75 – 2.60 (m, 2H), 2.01 – 1.83 (m, 2H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.56, 163.25, 141.57, 128.83, 128.76, 126.37, 85.99, 34.53, 30.84, 29.67. **HR-MS (ESI)** *m/z*: calcd for $C_{13}H_{15}N_2O_2S$ $[M+H]^+$: 263.0854, found: 263.0849, error: −1.9 ppm.

2-((4-phenylbutyl)thio)pyrimidine-4,6-diol (**21**)



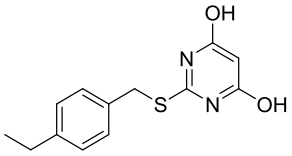
Compound **21** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and (4-bromobutyl)benzene (439 mg, 2.07 mmol) as the starting material, product **21** was obtained as a white solid (101 mg, **yield:** 53%), HPLC purity: 99.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.26 (s, 1H), 7.25 (m, 2H), 7.20 (m, 3H), 5.10 (s, 1H), 3.08 (t, *J* = 7.2 Hz, 2H), 2.61 – 2.53 (q, *J* = 6.6 Hz, 2H), 1.71 – 1.53 (m, 2H), 1.35-1.43 (m, 2H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.56, 163.30, 142.29, 128.94, 126.18, 86.08, 35.03, 30.44, 29.76, 28.89. **HR-MS (ESI)** *m/z*: calcd for $C_{14}H_{17}N_2O_2S$ $[M+H]^+$: 277.1011, found: 277.1006, error: −1.8 ppm.

2-((5-phenylpentyl)thio)pyrimidine-4,6-diol (**22**)



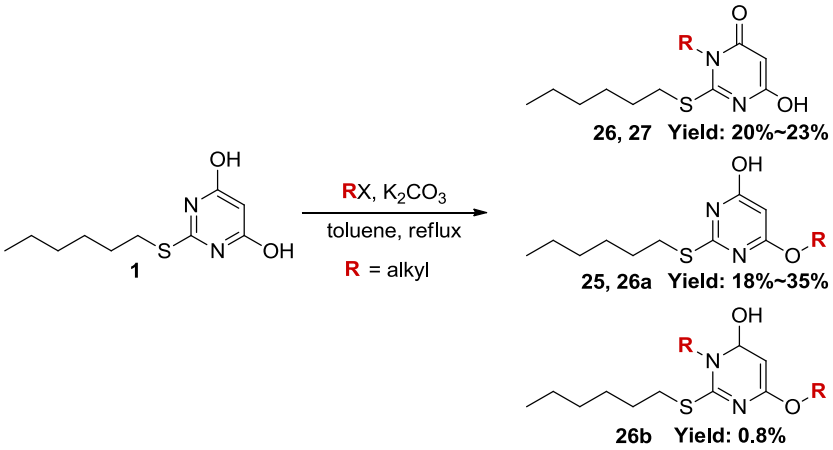
Compound **22** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and (5-bromopentyl)benzene (468 mg, 2.97 mmol) as the starting material, product **22** was obtained as a white solid (100 mg, **yield:** 50%), HPLC purity: 99.4%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.22 (s, 1H), 7.31 – 7.22 (m, 2H), 7.19 (m, 3H), 5.93 (s, 1H), 3.13 (t, *J* = 5.7, 2H), 2.61 (q, *J* = 6.3 Hz, 2H), 2.14 (m, 4H), 1.66 (s, 2H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.5, 163.3, 142.59, 128.76, 128.68, 126.10, 86.07, 35.49, 30.96, 29.92, 29.04, 28.22. **HR-MS (ESI)** *m/z*: calcd for $C_{15}H_{19}N_2O_2S$ $[M+H]^+$: 291.1167, found: 291.1161, error: −2.1 ppm.

2-((4-ethylbenzyl)thio)pyrimidine-4,6-diol (**23**)



Compound **23** was prepared by the previously described General Procedure (Method A) using 2-mercaptopyrimidine-4,6-diol (100 mg, 0.69 mmol) and 1-(bromomethyl)-4-ethylbenzene (410 mg, 2.07 mmol) as the starting material, product **23** was obtained as a white solid (87 mg, **yield:** 48%), HPLC purity: 96.3%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.34 (s, 1H), 11.40 (s, 1H), 7.34 (d, *J* = 7.8 Hz, 2H), 7.15 (d, *J* = 8.1 Hz, 2H), 5.19 (s, 1H), 4.33 (s, 2H), 2.62 – 2.52 (q, *J* = 7.5 Hz, 2H), 1.15 (t, *J* = 7.8 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ: 163.50, 152.32, 133.78, 129.05, 127.95, 85.93, 34.09, 28.26, 14.99. **HR-MS (ESI)** *m/z*: calcd for $C_{13}H_{15}N_2O_2S$ $[M+H]^+$: 263.0854, found: 263.0849, error: −1.9 ppm.

General procedure for the preparation of compounds **25-27**



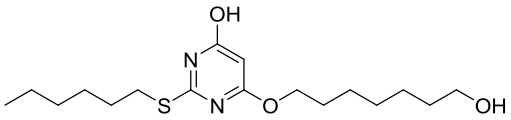
2-(hexylthio)pyrimidine-4,6-diol (**1**) (1 mmol) was dissolved in toluene (5 mL) and K_2CO_3 (2 mmol) was added under 0 °C, and halides (3 mmol) were added slowly. After the addition, the reaction was heated to reflux 3h, then cooled to room temperature. Ethyl acetate and water were added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to afford a crude residue, which was purified by silica gel column chromatography (DCM:MeOH = 30:1) to afford the desired products **25-27**.

Notes: In the reaction of synthesizing of compound 25 or 27, N-alkylation is the major product in the TLC analysis of the crude product. And for 26, N-alkylation

product and O-alkylation product have similar yields (26 *vs* 26a = 20% *vs* 18%), and the yield of N,O-dialkylation product is much lower (e.g., **26b**, 0.8%). N-alkylation product of 25 was not present in this manuscript, because of the impurity with the same Rf as N-alkylation product, which couldn't meet the purity requirement for the biological evalution.

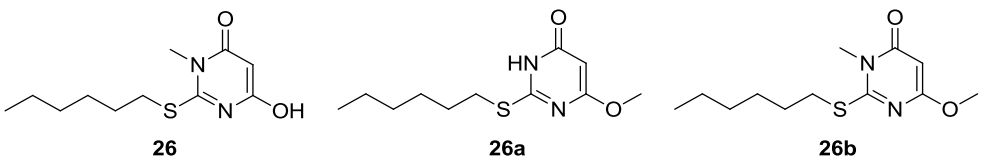
The alkylation sites of these products were determined by the ^{1}H NMR test, as the chemical shift of O-alkylation (~3.9) is larger than that of N-alkylation (~3.5). And these products can also be distinguished in the TLC analysis: O-alkylation products have the largest Rf; N-alkylation products and N,O-dialkylation products have almost the same Rfs, which were seperated by the preparative TLC.

2-(hexylthio)-6-hydroxy-3-(7-hydroxyheptyl)pyrimidin-4 (3H)-one (**25**)



Compound **25** was prepared by the previously described General Procedure using 2-(hexylthio)pyrimidine-4,6-diol (**1**) (100 mg, 0.44 mmol) and 8-bromooctan-1-ol (256 mg, 1.32 mmol) as the starting material, product **25** was obtained as a white solid (53 mg, **yield:** 35%), HPLC purity: 90.2%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.2 (s, 1H), 5.36 (s, 1H), 4.31 (t, *J* = 5.1 Hz, 2H), 4.16 (t, *J* = 6.6 Hz, 2H), 3.09 (t, *J* = 5.4 Hz, 2H), 1.66 (m, 2H), 1.36-1.42 (m,4H), 1.35 -1.29 (m, 12H), 0.87 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 166.57, 162.12, 85.60, 60.67, 32.47, 32.42, 31.37, 30.76, 28.63, 28.45, 27.95, 26.28, 25.46, 25.42, 22.00, 13.86. **HR-MS (ESI)** *m/z*: calcd for $C_{17}H_{31}N_2O_3S$ $[M+H]^+$: 343.2055, found: 343.2050, error: −1.5 ppm.

2-(hexylthio)-6-hydroxy-3-methylpyrimidin-4 (3H)-one (**26**)



26 **26a** **26b**

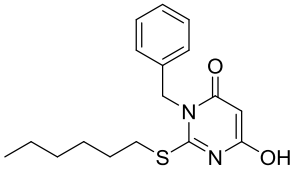
Compound **26** was prepared by the previously described General Procedure

using 2-(hexylthio)pyrimidine-4,6-diol (**1**) (100 mg, 0.44 mmol) and Iodomethane (186 mg, 1.32 mmol) as the starting material. Product **26** was obtained as a white solid (21 mg, **yield:** 20%), HPLC purity: 99.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.59 (s,1H), 3.47 (s, 3H), 3.16 (t, *J* = 6.0 Hz, 2H), 1.74 – 1.64 (m, 2H), 1.44 – 1.32 (m, 2H), 1.31 – 1.28 (m, 4H), 0.89 – 0.86 (t, *J* = 6.0 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 169.55, 85.55, 54.17, 30.72, 29.69, 28.85, 27.85, 21.96, 13.83. **HR-MS (ESI)** *m/z*: calcd for $C_{11}H_{19}N_2O_2S$ $[M+H]^+$: 243.1167, found: 243.1162, error: −2.1 ppm.

O-alkylation product **26a** was obtained in the same reaction (19 mg, **yield:** 18%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.49 (s,1H), 3.88 (s, 3H), 3.18 (t, *J* = 6.0 Hz, 2H), 1.77 – 1.68 (m, 2H), 1.44 – 1.32 (m, 2H), 1.31 – 1.28 (m, 4H), 0.89 – 0.86 (t, *J* = 6.0 Hz, 3H)

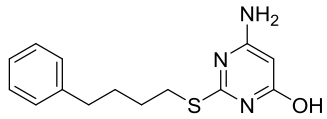
N,O-dialkylation product **26b** was obtained in the same reaction (1 mg, **yield:** 0.8%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.50 (s,1H), 3.85 (s, 3H), 3.46 (s, 3H), 3.18 (t, *J* = 6.0 Hz, 2H), 1.79 – 1.46 (m, 2H), 1.41 – 1.32 (m, 2H), 1.31 – 1.28 (m, 4H), 0.90 – 0.87 (t, *J* = 6.0 Hz, 3H).

3-benzyl-2-(hexylthio)-6-hydroxypyrimidin-4 (3H)-one (**27**)



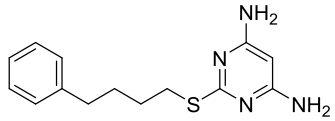
Compound **27** was prepared by the previously described General Procedure using 2-(hexylthio)pyrimidine-4,6-diol (**1**) (100 mg, 0.44 mmol) and benzyl bromide (224 mg, 1.32 mmol) as the starting material, product **27** was obtained as a white solid (32 mg, **yield:** 23%), HPLC purity: 97.2%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.26 (s, 1H), 7.17 (m, 5H), 5.29 (s, 1H), 3.55 (s, 2H), 3.11 (t, J = 7.2 Hz, 2H), 1.60 (m, 2H), 1.36 (m, 2H), 1.26 (m,4H), 0.86 (t, *J* = 6.3 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 164.88, 158.93, 141.11, 128.17, 127.98, 125.51, 97.49, 93.17, 30.79, 29.51, 28.73, 27.82, 27.76, 22.05, 13.90. **HR-MS (ESI)** *m/z*: calcd for $C_{17}H_{23}N_2O_2S$ $[M+H]^+$: 319.1480, found: 319.1476, error: −1.3 ppm.

6-amino-2-((4-phenylbutyl)thio)pyrimidin-4-ol (**28**)



A mixture of 6-amino-2-mercaptopyrimidin-4-ol (100 mg, 0.7 mmol) and (4-bromobutyl)benzene (445 mg, 2.1 mmol) in the presence of KI (12 mg, 0.07 mmol) and KOH (78 mg, 1.4 mmol) in EtOH (10 mL)/H_2O (5 mL) were refluxed under a nitrogen atmosphere for 6 hrs. The reaction mixture was allowed to cool to room temperature, and ethyl acetate and water were added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to afford a crude residue, which was purified by silica gel column chromatography (DCM:MeOH = 30:1) to afford a white solid (79 mg, **yield:** 41%), HPLC purity: 99.8%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 11.49 (s, 1H), 7.30 – 7.23 (m, 2H), 7.19 (m, 3H), 6.40 (s, 2H), 4.87 (s, 1H), 3.09 (t, *J* = 6.6 Hz, 2H), 2.59 (t, *J* = 6.6 Hz, 2H), 1.64 (m, 4H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 165.47, 164.51, 161.34, 142.00, 128.03, 127.93, 125.41, 81.29, 34.90, 30.17, 29.53, 28.55. **HR-MS (ESI)** *m/z*: calcd for $C_{14}H_{18}N_3OS$ $[M+H]^+$: 276.1171, found: 276.1164, error: −2.5 ppm.

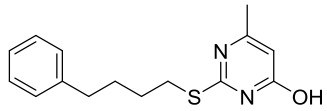
2-((4-phenylbutyl)thio)pyrimidine-4,6-diamine (**29**)



A mixture of 4,6-diaminopyrimidine-2-thiol (100 mg, 0.7 mmol) and (4-bromobutyl)benzene (445 mg, 2.1 mmol) in the presence of KI (12 mg, 0.07 mmol) and KOH (78 mg, 1.4 mmol) in EtOH (10 mL)/H_2O (5 mL) was refluxed under a nitrogen atmosphere for 6 hrs. The reaction mixture was allowed to cool to room temperature, and ethyl acetate and water were added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to afford a crude residue, which was purified by silica gel column chromatography (DCM:MeOH = 50:1) to afford a white solid (73 mg, **yield:** 38%), HPLC purity: 99.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 7.26 (m, 2H), 7.19 (m, 3H), 6.02 (s, 4H), 5.12 (s, 1H), 3.00 (t,

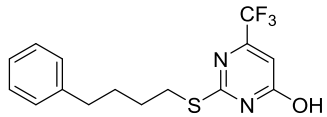
J = 6.6 Hz, 2H), 2.59 (t, *J* = 6.6 Hz, 2H), 1.62 (m, 4H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 168.90, 163.89, 142.57, 128.75, 128.70, 126.11, 79.49, 35.18, 30.65, 29.49. **HR-MS (ESI)** *m/z*: calcd for $C_{14}H_{19}N_4S$ $[M+H]^+$: 275.1330, found: 275.1323, error: −2.5 ppm.

6-methyl-2-((4-phenylbutyl)thio)pyrimidin-4-ol (**30**)



A mixture of 2-mercapto-6-methylpyrimidin-4-ol (100 mg, 0.7 mmol) and (4-bromobutyl)benzene (445 mg, 2.1 mmol) in the presence of KI (12 mg, 0.07 mmol) and KOH (78 mg, 1.4 mmol) in EtOH (10 mL)/H_2O (5 mL) was refluxed under a nitrogen atmosphere for 6 hrs. The reaction mixture was allowed to cool to room temperature, and ethyl acetate and water were added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to afford a crude residue, which was purified by silica gel column chromatography (DCM:MeOH = 40:1) to afford a white solid (59 mg, **yield:** 31%), HPLC purity: 99.0%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.49 (s, 1H), 7.26 (m, 2H), 7.19 (m, 3H), 5.92 (s, 1H), , 3.13 (t, *J* = 6.3 Hz, 2H), 2.61 (t, *J* = 6.9 Hz, 2H), 2.14 (s, 3H), 1.66 (m, 4H). **^{13}C NMR** (125 MHz, CD_3OD-d_4) δ 161.56, 141.95, 128.00, 127.92, 125.41, 106.68, 34.87, 30.15, 29.71, 28.46. **HR-MS (ESI)** *m/z*: calcd for $C_{15}H_{19}N_2OS$ $[M+H]^+$: 275.1218, found: 275.1226, error: −2.9 ppm.

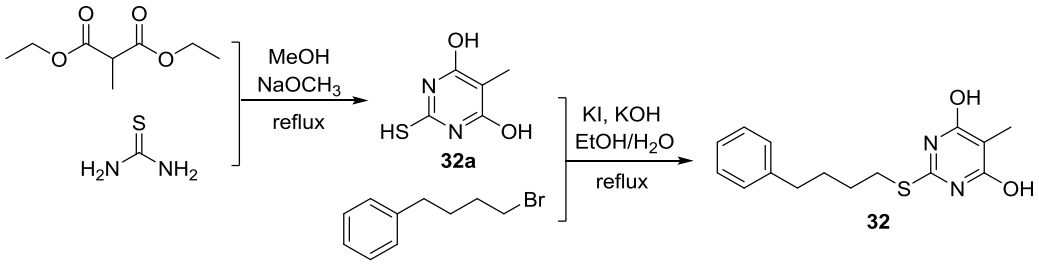
2-((4-phenylbutyl)thio)-6-(trifluoromethyl)pyrimidin-4-ol (**31**)



A mixture of 2-mercapto-6-(trifluoromethyl)pyrimidin-4-ol (100 mg, 0.5 mmol) and (4-bromobutyl)benzene (318 mg, 1.5 mmol) in the presence of KI (8 mg, 0.05 mmol) and KOH (84 mg, 1.5 mmol) in EtOH (10 mL)/H_2O (5 mL) was refluxed under a nitrogen atmosphere for 6 hrs. The reaction mixture was allowed to cool to room temperature, and ethyl acetate and water were added. The organic layer was

separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to afford a crude residue, which was purified by silica gel column chromatography (DCM:MeOH = 40:1) to afford desired product **31** (100 mg, **yield:** 61%) as a white solid, HPLC purity: 98.4%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 13.50 (s,1H), 7.32 – 7.22 (m, 2H), 7.17 (m, 3H), 6.58 (s, 1H), 3.16 (t, *J* = 9.6 Hz, 2H), 2.60 (t, *J* = 6.9 Hz, 2H), 1.67 (m, 4H). **HR-MS (ESI)** *m/z*: calcd for $C_{15}H_{16}F_3N_2OS$ $[M+H]^+$: 329.0935, found: 329.0931, error: −1.2 ppm.

5-methyl-2-((4-phenylbutyl)thio)pyrimidine-4,6-diol (**32**)

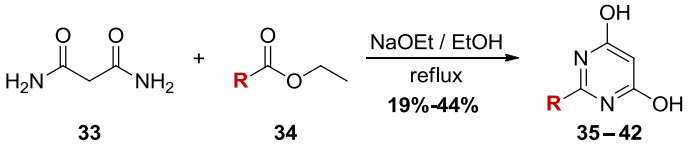


Diethyl 2-methylmalonate (1.0 g, 5.7 mmol) and thiourea (0.43 g, 5.7 mmol) dissolved in $NaOCH_3$/MeOH solution (sodium, 500 mg/MeOH, 15 mL) refluxed overnight and then cool to room temperature. MeOH was evaporated, then the residue was dissolved in H_2O (5 mL), and 3M HCl was added dropwise to acidified to pH = 1~2. The solid was filtered and dried under vacuum to afford product **32a** as a white solid (560 mg, **yield:** 59%).

A mixture of **32a** (100 mg, 0.6 mmol) and (4-bromobutyl)benzene (381 mg, 1.8 mmol) in the presence of KI (10 mg, 0.06 mmol) and KOH (101 mg, 1.8 mmol) in EtOH (10 mL)/H_2O (5 mL) was refluxed under a nitrogen atmosphere for 6 hrs. The reaction mixture was allowed to cool to room temperature, and ethyl acetate and water were added. The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered and concentrated to afford a crude residue, which was purified by silica gel column chromatography (DCM:MeOH = 30:1) to afford desired product **32** (47 mg, **yield:** 27%) as a white solid, HPLC purity: 99.6%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 12.22 (s, 1H), 11.01 (s, 1H), 7.26 (m, *J* = 7.2, 2H), 7.21 – 7.11 (m, 3H), 3.13 (t, *J* = 6.6, 2H), 2.59 (m, 2H), 1.71 (s, 3H), 1.69 – 1.60 (m, 4H). **^{13}C NMR** (125 MHz,

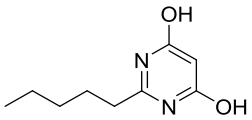
CD_3OD-d_4) δ 166.27, 157.82, 141.98, 128.00, 127.93, 125.41, 94.15, 87.12, 34.93, 30.17, 29.59, 28.52, 6.15. **HR-MS (ESI)** *m/z*: calcd for $C_{15}H_{19}N_2O_2S$ $[M+H]^+$: 291.1167, found: 291.1162, error: −1.7 ppm.

General procedure for the preparation of compounds **35-42**



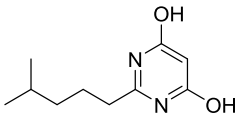
Malondiamide (**33**) (1.0 mmol), sodium ethoxide solution (sodium, 500 mg/ethanol, 15 mL), and ethyl alkylate (**34**) (1.2 mmol) were refluxed with stirring for 2 hrs. The filtrate was evaporated in vucuo and the residue in water was acidified to pH 2-5. The solid was filtered and dried under vacuum to afford **35-42** as a white solid.

2-pentylpyrimidine-4,6-diol (**35**)



Compound **35** was prepared by the previously described General Procedure using Malondiamide (**33**) (1.0 g, 9.8 mmol) and ethyl hexanoate (1.7g, 11.76 mmol) as the starting material, product **35** was obtained as a white solid (482 mg, **yield:** 27%), HPLC purity: 99.7%. **^{1}H NMR** (300 MHz, CD_3OD-d_4) δ 5.25 (s, 1H), 2.59 ((t, *J* = 8.1 Hz, 2H), 174 (q, *J* = 7.5 Hz, 2H), 1.42 – 1.31 (m, 4H), 0.93 (t, *J* = 6.6 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 166.32, 162.74, 87.06, 33.47, 30.58, 26.49, 21.72, 13.80. **HR-MS (ESI)** *m/z*: calcd for $C_9H_{14}N_2O_2Na$ $[M+Na]^+$: 205.0953, found: 205.0947, error: −2.9 ppm.

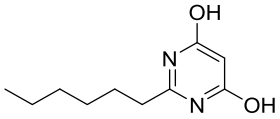
2-(4-methylpentyl)pyrimidine-4,6-diol (**36**)



Compound **36** was prepared by the previously described General Procedure

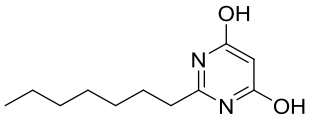
using Malondiamide (**33**) (1.0 g, 9.8 mmol) and ethyl 5-methylhexanoate (1.9 g, 11.76 mol) as the starting material, product **36** was obtained as a white solid (768 mg, **yield:** 40%), HPLC purity 98.5%. **^{1}H NMR** (300 MHz, CD_3OD-d_4) δ 5.25 (s, 1H), 2.56 (t, *J* = 7.8 Hz, 2H), 1.74 (q, *J* = 7.5 Hz, 2H), 1.57 (m, 1H), 1.25 (m, 2H), 0.90 (d, *J* = 6.6 Hz, 6H), **^{13}C NMR** (125 MHz, DMSO-d_6) δ 166.62, 163.20, 87.41, 38.15, 34.21, 27.59, 25.11, 22.83. **HR-MS (ESI)** *m/z*: calcd for $C_{10}H_{17}N_2O_2Na$ $[M+Na]^+$: 219.1109, found: 219.1100, error: −4.1 ppm.

2-hexylpyrimidine-4,6-diol (**37**)



Compound **37** was prepared by the previously described General Procedure using Malondiamide (**33**) (1.0 g, 9.8 mmol) and ethyl heptanoate (1.9 g, 11.76 mol) as the starting material, product **37** was obtained as a white solid (730 mg, **yield:** 38%), HPLC purity: 93.9%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 11.64 (s, 2H), 5.25 (s, 1H), 2.59 (t, *J* = 7.5 Hz, 2H), 1.73 (q, *J* = 7.5 Hz, 2H), 1.34 (m, 6H), 0.91 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 166.81, 163.11, 87.40, 35.59, 31.52, 28.86, 25.54, 22.48, 14.38. **HR-MS (ESI)** *m/z*: calcd for $C_{10}H_{17}N_2O_2$ $[M+H]^+$: 197.1290, found: 197.1282, error: −4.1 ppm.

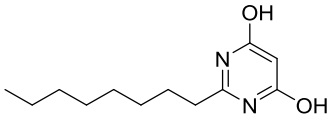
2-heptylpyrimidine-4,6-diol (**38**)



Compound **38** was prepared by the previously described General Procedure using Malondiamide (**33**) (1.0 g, 9.8 mmol) and ethyl octanoate (2.0 g, 11.76 mmol) as the starting material, product **38** was obtained as a white solid (885 mg, **yield:** 43%), HPLC purity: 95.8%. **^{1}H NMR** (300 MHz, CD_3OD-d_4) δ 5.25 (s, 1H), 2.59 (t, *J* = 7.5 Hz, 2H), 1.73 (q, *J* = 7.5 Hz, 2H), 1.34 (m, 8H), 0.91 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 166.79, 163.20, 87.40, 33.97, 31.54, 28.80, 28.72, 27.25, 22.50, 14.39. **HR-MS (ESI)** *m/z*: calcd for $C_{11}H_{18}N_2O_2Na$ $[M+Na]^+$: 233.1266,

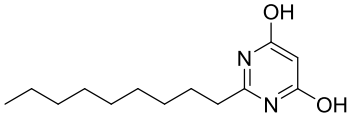
found: 233.1259, error: −3.3 ppm.

2-octylpyrimidine-4,6-diol (**39**)



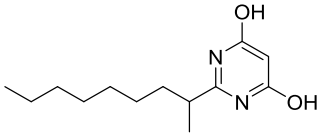
Compound **39** was prepared by the previously described General Procedure using Malondiamide (**33**) (1.0 g, 9.8 mmol) and ethyl nonanoate (2.2 g, 11.76 mmol) as the starting material, product **39** was obtained as a white solid (571 mg, **yield:** 26%), HPLC purity: 98.5%. **^{1}H NMR** (300 MHz, DMSO-d_4) δ 11.64 (s, 2H), 5.02 (s, 1H), 2.52 (t, *J* = 7.5 Hz, 2H), 1.62 (q, *J* = 6.6 Hz, 2H), 1.25 (m, 10H), 0.84 (t, *J* = 6.6 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 166.95, 163.41, 87.61, 34.19, 31.91, 29.24, 29.19, 29.06, 27.46, 22.76, 14.64. **HR-MS (ESI)** *m/z*: calcd for $C_{12}H_{21}N_2O_2$ $[M+H]^+$: 225.1603, found: 225.1598, error: −2.2 ppm.

2-nonylpyrimidine-4,6-diol (**40**)



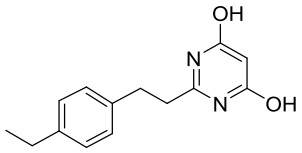
Compound **40** was prepared by the previously described General Procedure using Malondiamide (**33**) (1.0 g, 9.8 mmol) and ethyl decanoate (2.2 g, 11.76 mmol) as the starting material, product **40** was obtained as a white solid (560 mg, **yield:** 24%), HPLC purity: 98.3%. **^{1}H NMR** (300 MHz, CD_3OD-d_4) δ 5.25 (s, 1H), 2.54 (t, *J* = 7.5 Hz, 2H), 1.73 (q, *J* = 6.6 Hz, 2H), 1.30 (m, 12H), 0.89 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 167.08, 163.41, 87.60, 34.18, 31.95, 29.49, 29.33, 29.27, 29.04, 27.46, 22.83, 14.65. **HR-MS (ESI)** *m/z*: calcd for $C_{13}H_{23}N_2O_2$ $[M+H]^+$: 239.1760, found: 239.1751, error: −3.8 ppm.

2-(nonan-2-yl)pyrimidine-4,6-diol (**41**)



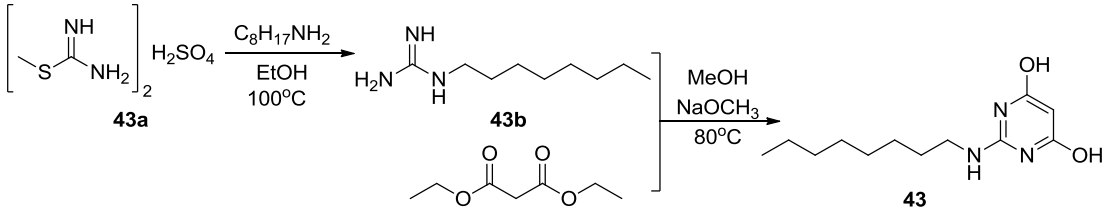
Compound **41** was prepared by the previously described General Procedure using Malondiamide (**33**) (1.0 g, 9.8 mmol) and ethyl 2-methylnonanoate (2.2 g, 11.76 mmol) as the starting material, product **41** was obtained as a white solid (443 mg, **yield:** 19%), HPLC purity: 99.9%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 11.58 (s, 2H), 5.06 (s, 1H), 2.62 (m, 1H), 1.63 (m, 2H), 1.32 (m, 2H), 1.19 (m, 8H), 1.15 (m, 3H), 0.84 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (125 MHz, CD_3OD-d_4) δ 168.18, 167.14, 87.27, 39.05, 34.66, 31.52, 29.08, 28.82, 26.99, 22.27, 17.66, 13.00. **HR-MS (ESI)** *m/z*: calcd for $C_{13}H_{23}N_2O_2$ $[M+H]^+$: 239.1760, found: 239.1755, error: −2.1 ppm.

2-(4-ethylphenethyl)pyrimidine-4,6-diol (**42**)



Compound **42** was prepared by the previously described General Procedure using Malondiamide (**33**) (1.0 g, 9.8 mmol) and ethyl 3-(4-ethylphenyl)propanoate (2.3 g, 11.76 mmol) as the starting material, product **42** was obtained as a white solid (1.1 g, **yield:** 44%), HPLC purity: 96.5%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 11.50 (s, 2H), 7.12 (m, 4H), 5.06 (s, 1H), 2.95 – 2.88 (t, *J* = 7.5 Hz, 2H), 2.78 – 2.69 (t, *J* = 8.1 Hz, 2H), 2.60 – 2.53 (q, *J* = 7.5 Hz, 2H), 1.15 (t, *J* = 7.5 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.24, 162.32, 142.02, 137.94, 128.66, 128.24, 87.73, 35.95, 32.58, 28.23, 16.14. **HR-MS (ESI)** *m/z*: calcd for $C_{14}H_{17}N_2O_2$ $[M+H]^+$: 245.1290, found: 245.1284, error: −2.4 ppm.

2-(octylamino)pyrimidine-4,6-diol (**43**)

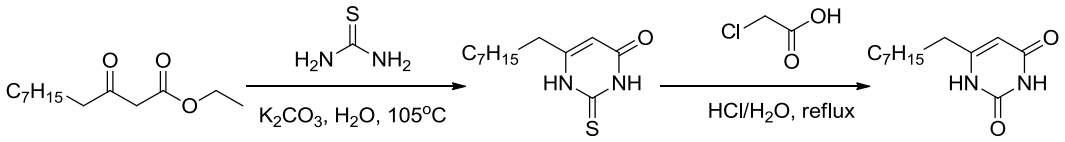


A solution of octylamine (387 mg, 3 mmol) and methylisothiouronium sulphate (**43a**) (500 mg, 1.8mmol) in 15 mL absolute ethanol was heated at 100 °C for 18 hrs.

The solvent was removed under vacuum and insoluble matter was filtered off. The crude oil was dissolved in EtOH/H_2O and left in a refridgerator. The solid that crystallised was rinsed with EtOH, Et_2O and dried under vacuum to afford 1-octylguanidine (**43b)** as a white solid (700 mg, crude product).

Then diethyl malonate (656 mg, 4.1 mmol), sodium ethoxide solution (sodium, 500 mg/ethanol, 15 mL), and **43b** (700 mg, 4.1 mmol) were refluxed with stirring for 16 hrs. The filtrate was evaporated in vacuo and the residue in water (5 mL) brought to pH 2-5, The solid was filtered and dried under vacuum to afford product **43** as a white solid (64 mg, **yield:** 15% in two steps), HPLC purity: 95.9%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 10.29 (s, 2H), 6.48 (s, 1H), 4.58 (s, 1H), 3.20 (dd, *J* = 12.9, 6.9 Hz, 2H), 1.46 (m, 2H), 1.26 (m, 10H), 0.85 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 154.15, 78.96, 31.22, 28.84, 28.68, 26.25, 22.07, 13.92. **HR-MS (ESI)** *m/z*: calcd for $C_{12}H_{22}N_3O_2$ $[M+H]^+$: 240.1712, found: 240.1708, error: −1.7 ppm.

6-octylpyrimidine-2,4-diol (**44**)

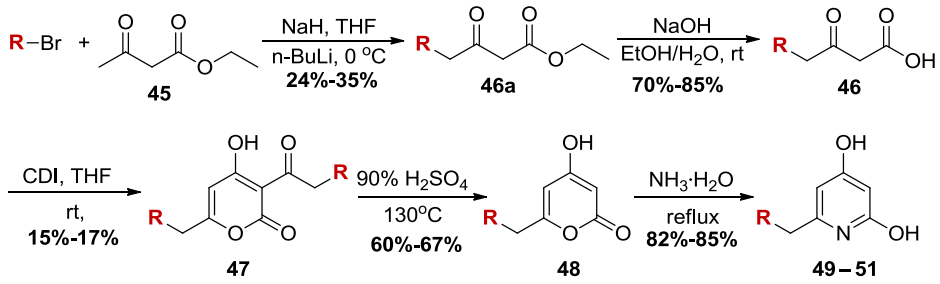


44a **44**

Thiourea (350 mg, 1.54 mmol) was dissolved in 1.0 mL of water at 70 °C ethyl 3-oxoundecanoate (76 mg, 1.0 mmol) was added followed by K_2CO_3 (213 mg, 1.54 mmol), upon which the solution became cloudy. It was heated to 105 ^{o}C, where the precipitate dissolved. The reaction was heated at this temperature open to air for 1 hr, boiling off any remaining ethanol and leaving a light yellow solid. Heat was removed and the reaction allowed to cool to room temperature over a period of 2 hrs. Water (6.6 mL) was added followed by 1M HCl to pH=1-2, then extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and solvent removed in vacuo. The crude solid was purified by column chromatography (PE: EA = 10:1) to afford product **44a** as a white solid (56 mg, **yield:** 23.3%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.30 (s, 1H), 12.19 (s, 1H), 5.67 (s, 1H), 2.33 (t, J = 8.1 Hz, 2H),

1.51 (m, 2H), 1.26 (m, 10H), 0.86 (t, J = 6.3 Hz, 3H).Chloroacetic acid (31 mg, 0.42mmol) was dissolved in H_2O (1 mL), compound **44a** (50 mg, 0.21 mmol) was dissolved in H_2O/THF (5 mL/2 mL) and added to the stirring solution. The reaction mixture was refluxed for 6 hrs. Concentrated HCl (0.1 mL) was added carefully, and the reaction returned to reflux for 12 hrs. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and solvent removed in vacuo. The crude solid was purified by column chromatography (DCM:MeOH = 20:1) to afford desired product **44** (5 mg, **yield:** 10.6%) as a white solid, HPLC purity: 99.4%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 10.87 (s, 1H), 10.77 (s, 1H), 5.31 (s, 1H), 2.26 (t, *J* = 7.5 Hz, 2H), 1.51 (m, 2H), 1.25 (m, 10H), 0.86 (t, *J* = 6.6 Hz, 3H). **^{13}C NMR** (125 MHz, CD_3OD-d_4) δ 166.04, 158.07, 152.23, 97.90, 32.13, 31.57, 28.92, 28.88, 28.65, 27.27, 22.29, 13.01. **HR-MS (ESI)** *m/z*: calcd for $C_{12}H_{21}N_2O_2$ $[M+H]^+$: 225.1603, found:225.1595, error: −3.6 ppm.

General procedure for the preparation of compounds **49-51**



NaH (1.1 mmol) was suspended in THF (10 mL) and cooled to 0°C, ethyl 3-oxobutanoate (**45**) (1 mmol) was added slowly. After the addition, the reaction was stirred under 0 °C for 10 min, then n-BuLi (1.1 mmol) was added slowly and the reaction was allowed to stir for another 10 min before alkyl bromides (1 mmol) was added. The reaction was stirred at 0°C for 24 hrs, then quenched by H_2O (5 mL), extracted with EtOAc. The organic layers were combined, washed with water and brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure to give an orange oil, which was subjected to column chromatography (PE:EA = 50:1) to give ester **46a** (**yield:** 24%-35%).

Ester **46a** (1 mmol) and NaOH (3 mmol) were dissolved in methanol (5

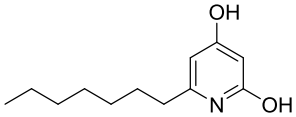
mL)/Water (5 mL), and the mixture was stirred for 18 hrs at room temperature, Methanol and water were removed under reduced pressure to give an orange oil, which was diluted by water (5 mL), followed by the addition of 4M HCl (10% v/v, 15 mL) to acidify the mixture to pH 1-2, and product **46** was precipitated as a yellow solid (**yield:** 70%-85%).

To a stirred solution of **46** (1 mmol) in dry tetrahydrofuran (6 mL) 1,1'-carbonyldiimidazole (1.4 mmol) in dry tetrahydrofuran (3 mL) was added at room temperature in the atomosphere of nitrogen. The resulting mixture was left stirring at room temperature for 24 hrs. The mixture was then acidified to pH = 1 with 0.5 M HCl and extracted with ethyl acetate. The organic layer was washed with brine , dried over anhydrous $MgSO_4$ and solvent was removed in vacuo. The crude solid was recrystallised from methanol to yield **47** as pale yellow crystals (**yield:** 15%-17%).

Sulfuric acid (90% v/v) was added to pyrone **47** (1 mmol) and the solution was heated at 130 ^{o}C for 1 hr. The reaction mixture was then cooled to room temperature and poured into ice water in 0 ^{o}C. The oily residue was extracted with ethyl acetate and the combined organic layers washed with water, dried over anhydrous $MgSO_4$ and concentrated in vacuo to give a brown oil, which was purified by column chromatography (DCM:MeOH = 20:1) to afford **48** as a white solid (**yield:** 60%-67%).

Ammonia (28% w/w, 5 mL), H_2O (2 mL) was added to 48 (1 mmol) and the solution heated at 130^{o}C for 6 hrs. The reaction was diluted with water (4 mL) and cooled to room temperature. The mixture was then acidified to pH = 1 with 0.5 M HCl and the resulting solid was filtered and dried in vacuo, which was purified by column chromatography (DCM:MeOH = 20:1) to afford **49-51** as a white solid. (**yield:** 82%-85%).

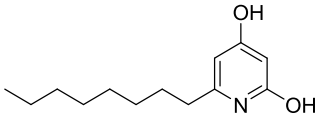
6-heptylpyridine-2,4-diol (**49**)



Compound **49** was prepared by the previously described General Procedure

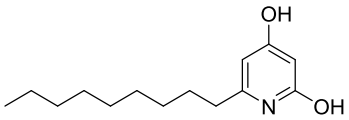
using ethyl 3-oxobutanoate (**45**) (1.0 g, 7.7 mmol) and 1-bromohexane (1.3 g, 7.7 mmol) as the starting material, product **49** was obtained as a white solid (42 mg, **yield:** 2.6% for 5 steps), HPLC purity: 99.9%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 10.85 (s, 1H), 10.25 (s, 1H), 5.58 (s, 1H), 5.32 (s, 1H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.51 (m, 2H), 1.24 (m, 8H), 0.86 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ 167.45, 164.74, 150.09, 97.31, 95.94, 32.08, 31.14, 28.31, 28.05, 22.04, 13.94. **HR-MS (ESI)** *m/z*: calcd for $C_{12}H_{20}NO_2$ $[M+H]^+$: 210.1494, found: 210.1488, error: −2.9 ppm.

6-octylpyridine-2,4-diol (**50**)



Compound **50** was prepared by the previously described General Procedure using ethyl 3-oxobutanoate (**45**) (1.0 g, 7.7 mmol) and 1-bromoheptane (1.4 g, 7.7 mmol) as the starting material, product **50** was obtained as a white solid (46 mg, **yield:** 2.7% for 5 steps), HPLC purity: 99.4%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 10.85 (s, 1H) 10.27 (s,1H), 5.58 (s, 1H), 5.33 (s, 1H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.51 (q, *J* = 6.6 Hz,2H), 1.24 (m, 10H), 0.85 (t, *J* = 6.0 Hz, 3H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ 167.88, 165.22, 150.55, 97.77, 96.42, 32.57, 31.71, 29.10, 29.04, 28.82, 28.52, 22.55, 14.42. **HR-MS (ESI)** *m/z*: calcd for $C_{13}H_{22}NO_2$ $[M+H]^+$: 224.1651, found: 224.1644, error: −3.1 ppm.

6-nonylpyridine-2,4-diol (**51**)



Compound **51** was prepared by the previously described General Procedure using ethyl 3-oxobutanoate (**45**) (1.0 g, 7.7 mmol) and 1-bromooctane (1.5 g, 7.7 mmol) as the starting material, product **51** was obtained as a white solid (38 mg, **yield:** 2.1% for 5 steps), HPLC purity: 99.8%. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 10.85 (s,1H),10.27 (s,1H), 5.58 (s, 1H), 5.33 (s, 1H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.51 (q, *J* = 6.6 Hz,2H), 1.24 (m, 12H), 0.85 (t, *J* = 6.0 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ

168.16, 165.46, 150.76, 98.02, 96.62, 32.78, 31.95, 29.56, 29.35, 29.03, 28.74, 22.78, 14.64. **HR-MS (ESI)** *m/z*: calcd for $C_{14}H_{24}NO_2$ $[M+H]^+$: 238.1807, found: 238.1801, error: −1.8 ppm.

3. Calcium mobilization assay

HEK293 cells stably expressing GPR84 and Gα16 were seeded onto 96-well plates at a density of 4×10^4 cells/well and cultured overnight. Then the cells were incubated with Hanks' balanced salt solution (HBSS) buffer containing 5.6 mM D-glucose, 250 μM sulfinpyrazone, 0.03% Cremophor EL and 2 mM Fluo-4 AM at 37 °C for 45 min. After a thorough washing, 50 μL of new HBSS buffer was added. 25 μL of Chemicals prepared in HBSS buffer were then dispensed into the well using a FlexStation III microplate reader (Molecular Devices), and intracellular calcium change was recorded at an excitation wavelength of 485 nm and an emission wavelength of 525 nm.

4. HTRF cAMP assay

Intracellular cAMP levels were detected with a HTRF cAMP kit (Cisbio) according to the manufacture's instruction. In brief, cells were harvested and resuspended in DMEM containing 500μM IBMX to obtain a final cell count of 4×10^5 cells/mL. The cells were dispensed into 384-well plates at 2,000 cells/5 μL/well. Then 2.5 μL of DMEM containing compounds of various concentrations was added to the plates. After incubation at room temperature for 30 minutes, 2.5 μL DMEM containing 10 μM forskolin was added and the incubation was continued for another 30 minutes. Lysis buffer containing anti-cAMP antibody and d2-cAMP (a fluorescent conjugated cAMP) was added to stop the reaction. After 60-minute incubation in the dark, Signals were detected with an Envision 2101 plate reader (PerkinElmer).

5. Statistical Analysis

Data were analyzed with GraphPad Prism software (GraphPad Software, Inc.). Nonlinear regression analysis was performed to generate dose-response curves and calculate concentration for 50% of the maximal effect (EC_{50}). The data are presented as the mean ± SEM.