SUPPORTING INFORMATION

EXPERIMENTAL

General

All solvents were purchased from common vendors and were used as received. All reactions were performed under N_2 in oven-dried or flame-dried glassware. All new compounds were characterized by ^{1}H, ^{13}C and HRMS. High-resolution mass spectra were recorded using a Synapt hybrid quadrupole/oa-TOF Mass Spetrometer (Waters Corp., Milford, MA) equipped with a dual chemical ionization/electrospray (ESCI) source. A post-acquisition gain correction was applied using a solution of 3-[(3- holamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, FW 614.88) as the lock mass spray.

Biological assays

Microsomal Metabolism of Antioxidants

The incubation of antioxidants with rat liver microsomes (BD Gentest, Woburn, MA) contained the following: 10 μM substrate, 1 mg/mL rat liver microsome protein, 50 mM potassium phosphate buffer (pH 7.4) and/or 1 mM GSH. This mixture was preincubated for 2 min at 37 °C. The reaction was initiated by the addition of NADPH to reach a final concentration of 1 mM. The final incubation volume was 0.25 mL. Samples without NADPH were used as controls. The substrates were dissolved in MeOH; the concentration of solvent was kept under 1% in all incubations. After 30 min of incubation, the reaction was terminated by adding 0.25 mL of ice-cold acetonitrile containing 1μM d_3-acetaminophen as internal standard. After vortexing and centrifugation, 10 μL of the resulting supernatant was analyzed by LC-MS analysis.

LC-MS Analysis

Samples were subjected to chromatographic separation on a 250 × 2.0 mm Luna 5 μm C18 column (Phenomenex, Torrance, CA), and eluates were analyzed with a Thermo Quantum Ultra triple quadrupole mass spectrometer. Elution solvents were 5% acetonitrile, 95% H_2O, and 0.1% acetic acid (solvent A), and 95% acetonitrile, 5% H_2O, and 0.1% acetic acid (solvent B). The gradient started with 100% A and increased to 100% B in 7 min and held for 3 min before it returned to 100% A. The MS was operated in the positive ion mode using electrospray ionization (ESI) in the selective reaction monitoring (SRM) mode and full scan mode. MS parameters were optimized for acetaminophen and were as follows: auxiliary gas pressure was set at 55 psi, sheath gas pressure was 60 psi, utilizing nitrogen for both. Discharge current was set at 12 eV and the vaporizer temperature was set at 300 °C. Collision induced dissociation (CID) for the antioxidants and their metabolites were optimized respectively under 1.0 mTorr of argon. Data acquisition and analysis were performed using Xcaliber software, version 2.0 (San Jose, CA).

Cell-based cytotoxicity assays

Human hepatoma HepG2 cells were obtained from American Type Tissue Collection (ATCC, Manassas, VA, USA). HepG2 cells were cultured in the complete medium containing 10% FBS, 0.1 M non-essential amino acids, 100 U/mL penicillin-streptomycin, with 5% CO_2 and 95% air at 37 °C.

In each 96-well microplate, HepG2 cells were suspended at a density of 10^5 cells/ml in a volume of 100 μL per well. An additional 100 μL of medium was added to the wells that contained vehicle controls (3 uL of DMSO) that comprised less than 0.5% of the total volume or compound (1uL). Serial dilutions of each compound were used to construct the dose-response curves for each test compound. Three replicates were performed per dose of compound.

Cytotoxicity was assessed by measuring intracellular ATP levels using the luciferin-luciferase reaction. This assay is based on the knowledge that ATP is present in all metabolically active cells and that its levels are affected by alteration in membrane integrity, mitochondrial function and metabolic activity. Therefore, a reduction in cellular ATP is indicative of a cytotoxic effect. Cellular ATP content was measured using Cytolux L001-100® assay kits with modifications (EG&G Wallac, Finland). The emitted light intensity is linearly related to the ATP concentration and is measured using a luminometer (Model Luminoskan RS, Labsystems, Finland). The number of cells plated per well and the amount of reagent used were optimized to produce a high signal/background ratio. Cells were exposed to compounds for 24 h. After incubation, 100 µL of medium was removed from each well. 50 µL of Somalyze® reagent was then added for 5 min to lyse cells. 50 µL/well of luciferase/luciferin reagent was added and shake for 5 min. Luminescence was measured after dark adapter plate for 10 min.

Luminescence was measured in terms of relative light units (RLUs), which are used to quantify the level of ATP in a sample. Cytotoxicity was calculated based on the percent cellular ATP in compound treated wells (in triplicate) relative to solvent controls (defined as 100% ATP level). EC_{50} values were determined by the log-logit function at 50% viability. Values are presented as means ± S.E. (standard error) from at least three separate experiments.

Inhibition of COX-1-induced oxidation of AA

Ovine COX-1 (specific activity 202 mol AA/min/mol enzyme) was preincubated on ice for 20 min with 2 molar equivalents of hematin in Tris HCl buffer pH 8.0, 500 µM phenol. This solution was then warmed for 5 min at 37°C in the presence or absence of various concentrations of analogs. [^{14}C] AA (4.8 nCi, 0.5 µM final concentration) in Tris HCl buffer pH 8.0 was preincubated at 37°C for 2 min. The reaction was initiated by adding COX-1 (5.4 nM final concentration) to a total volume of 200 µl and terminated after 8 sec by the addition of 200 µl of ice-cold diethyl ether: methanol: 4 M citric acid (30:4:1) containing 8 µg of butylated hydroxyanisole as antioxidant and 8 µg of unlabelled AA as a carrier. The organic layer was collected and oxidation products were analyzed by GC/NICI/MS as described below.

Inhibition of Mb-induced oxidation of AA.

Concentration of Mb was determined optically by reducing an aliquot to deoxymyoglobin with sodium dithionite (ε_{435nm}=121mM^{-1}cm^{-1}) [1]. The Mb was stored at 4°C and used within 3 days of preparation. Ferric Mb 10 µM in PBS pH 7.4, 10 µM EDTA was incubated at 37°C with various concentrations of analogs and with 10 µM [^{14}C] AA (51 Ci/mol). The reaction was initiated with the addition of H_2O_2 at the final concentration of 5 µM and allowed to proceed for 3 h in a total volume of 100 µl. At this time, the reaction was terminated by the addition of 400 µl of ice-cold diethyl ether: methanol: 4 M citric acid (30:4:1) containing 8 µg of butylated hydroxyanisole as antioxidant and 8 µg of unlabelled AA as a carrier. The organic layer was collected and oxidation products were analyzed by GC/NICI/MS as described below.

GC/NICI/MS analysis of oxidation products of AA.

The organic layer was loaded on a silica plate and eluted with the organic phase of ethyl acetate: isooctane: water: glacial acetic acid (45:25:50:1). TLC plates were analyzed for radioactivity by a Bioscan AR-2000 imaging scanner (Bioscan, Washington, DC). When indicated, ApAP, vitamin C, or 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox™) was added at the same time as arachidonic acid, before adding H_2O_2. The activity was determined as nmol AA oxidized by Mb during the time of the reaction. The results were expressed as percent of the control oxidation where no antioxidant was added. Each experiment was performed in duplicate on three or four separate occasions.

Microsome Stability and direct cytotoxicity of several previously published phenolic heterocycles

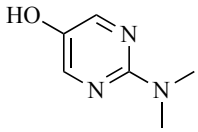
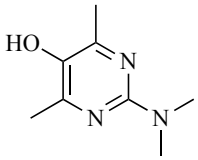
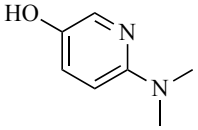
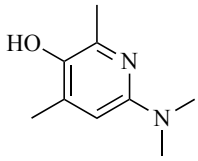
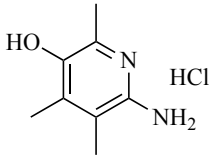
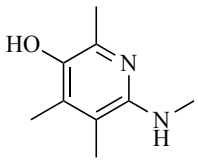
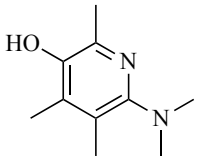
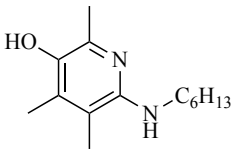
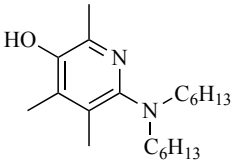
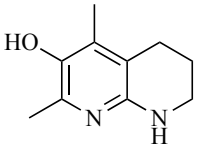
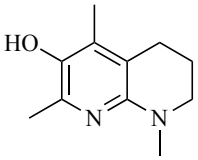
Table 1. (Stability and toxicity essay)			
N	Structure	Microsome Metabolism % of analog left *(Presumed decomposition pathway)*	Half maximal toxic concentration (EC_{50}, uM)
1		82 ± 11	1500 [a]
2		14 ± 4 *(-2Me, +O)*	>2,000
3		35 ± 3	250 [a]
4		1 ± 0.3 *(-2Me)*	76.4 ± 9.1
5		112 ± 17	>2,000
6		56 ± 4 *(-2Me)*	411.1 ± 60.3
7		51 ± 4 *(-2Me)*	>2,000
8		45 ± 5 *(+O)*	69.9 ± 0.9

Table 1. (continued)			
N	Structure	Microsome Metabolism % of analog left *(Presumed decomposition pathway)*	Half maximal toxic concentration (EC_{50}, uM)
9		7 ± 4 *(+O)*	25 ± 5
10		28 ± 11	411.1 ± 123.1
11		6 ± 1 *(-2Me)*	280.4 ± 17.4
[a] Toxicity values from previously published data [2]			

Chemistry

2-amino-5-bromonicotinonitrile (15) [3] To a solution of 2-aminonicotinonitrile (10g, 84mmol) in dry MeCN (200ml) NH_4Ac (0.64g, 8.2mmol) was added. The reaction was cooled to 0°C and NBS (16.44g, 92.4mmol). After 12h, solvent was removed under the reduced pressure and 5% hydrochloric acid was added until most of product was redissolved. Resulting solution was filtered, cooled to 0°C and basified to pH>10 with NaOH (6M). In 20 minutes crystals were collected, washed on the filter with large amount of water and dried in vacuo affording 2-amino-5-bromonicotinonitrile, **14** (15.1g, 76.3mmol, 91% yield). R_f = 0.5 (5% EtOH/CH_2Cl_2); ^{1}H NMR (400Mz, *d*-DMSO) δ 8.26 (d, 1H, *J* = 2.4), 8.13 (d, 1H, *J* = 2.4), 7.11 (s, 2H); ^{13}C δ 158.8, 153.7, 143.5, 115.7, 103.8, 90.9;

3-(aminomethyl)-5-bromopyridin-2-amine (16) Suspension of 2-amino-5-bromonicotinonitrile **15** (7.3g, 0.037mol) in dry THF (20ml) was cooled to 0°C and BH_3·THF (147ml, 0.147mol of 1M in THF) was added slowly. After 1h at RT, reaction was set to reflux over night. It was cooled to 0°C and water (50ml), followed by HCl (20ml, 15% in H_2O) were added slowly. Bulk of the solvent was removed under the reduced pressure and the residue was resuspended in HCl (100ml, 15% in H_2O). After 3h of reflux, solvent was removed *in vacuo*, resulting residue was redissolved in water (20ml) and cooled to 0°C. Dry pellets of NaOH were added until pH of the solution rose above 12. Crystals were collected, washed with small amount of ice-cold water and dried *in vacuo* furnishing 3-(aminomethyl)-5-bromopyridin-2-amine, **16** (5.20g, 25.7mmol, 71% yield). ^{1}H NMR (400Mz, *d*-DMSO) δ 7.85 (d, 1H, *J* = 2.8), 7.52 (d, 1H, *J* = 2.8), 6.06 (s, 2H), 3.51 (s, 2H); ^{13}C δ 156.6, 145.3, 136.5, 124.0, 105.6, 41.6; HRMS calcd for $C_6H_9N_3Br$ (MH^+): 201.9980; found 201.9981 (0.5 ppm);

6-bromo-3,4-dihydropyrido[2,3-d]pyrimidin-2(1H)-one (17) To a solution of 3-(aminomethyl)-5-bromopyridin-2-amine, **16** (9.66g, 0.048mol) in dry DMF (100ml) CDI (9.3g, 0.057mol) was added. After 12h, reaction was quenched with water (20ml), solvent was removed under reduced pressure and the residue was resuspended in boiling water (200ml). After 20 minutes, solvent was cooled to 0°C, crystals were collected, washed with water and dried under reduced pressure furnishing 6-bromo-3,4-dihydropyrido[2,3-d]pyrimidin-2(1H)-one, **16** (9.40g,

41.2mmol, 86% yield). R_f = 0.33(5% EtOH/CH_2Cl_2); ^{1}H NMR (400Mz, *d*-DMSO) δ 9.65 (s, 1H), 8.13 (s, 1H), 7.74 (s, 1H), 7.02 (s, 1H), 4.31 (s, 2H); ^{13}C δ 154.2, 150.1, 147.0, 136.4, 116.0, 111.1, 40.9; HRMS calcd for $C_7H_7N_3OBr$ (MH^+): 227.9772; found 227.9779 (3.1 ppm);

di-tert-butyl 6-bromo-2-oxopyrido[2,3-d]pyrimidine-1,3(2H,4H)-dicarboxylate (18) To a solution of **17** (9.4g, 41mmol) in dry THF (100ml) N,N-dimethylpyridin-4-amine (1g, 8.2mmol) was added followed by Boc_2O (19.8g, 90.2mmol). Upon completion (~30min, judged by TLC) reaction was concentrated *in vacuo,* suspended in water (300ml) and extracted with 70% EA/Hex (3 x 350ml). Combined organic fractions were extracted with NH_4Cl (100ml, sat. aq.), brine (2 X 100ml) and dried over sodium sulfate. Solvent was removed under reduced procure and crude product was purified by flash chromatography (1% EtOH/CH_2Cl_2) furnishing di-tert-butyl 6-bromo-2-oxopyrido[2,3-d]pyrimidine-1,3(2H,4H)-dicarboxylate, **17** (17.1g, 40.0mmol, 97% yield). R_f = 0.8(5% EtOH/CH_2Cl_2); ^{1}H NMR (400Mz, $CDCl_3$) δ 8.30 (d, 1H, *J* = 2.4), 7.63 (d, 1H, *J* = 2.4), 4.74 (s, 2H), 1.60 (s, 9H), 1.47 (s, 9H); ^{13}C δ 151.6, 148.8, 148.5, 148.0, 147.5, 136.4, 116.8, 114.6, 85.8, 84.3, 43.8, 28.0, 27.5; HRMS calcd for $C_{17}H_{22}N_3O_5BrNa$ ($M+Na^+$): 450.0641; found 450.0650 (2.0 ppm);

di-*tert*-butyl 6-hydroxy-2-oxopyrido[2,3-*d*]pyrimidine-1,3(*2H,4H*)-dicarboxylate (19) and *tert*-butyl 6-hydroxy-2-oxo-1,2-dihydropyrido[2,3-*d*]pyrimidine-3(4*H*)-carboxylate (20) In a dry single necked **18** (8.4g, 19.6mmol), bis-(pinacolato)diboron (20g, 40mmol), potassium acetate (5.77g, 60mmol) and $Pd(OAc)_2$ (0.132g, 0.6mmol) were combined, sealed with robber septa and flashed with nitrogen for 20min. After that, dry DMF (75ml) was added to the reaction and it was heated at 85°C on an oil bath over night. After cooling to R.T., dark suspension was diluted with water (150ml) and extracted (3 x 150) with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered, concentrated, and further dried *in vacuo*. The dark oil was dissolved in THF (150 mL) and water (150 mL). Sodium perborate dihydrate (6.0g, 50 mmol) was added and the reaction mixture was stirred overnight. Saturated aqueous ammonium chloride solution was added and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by silica gel column chromatography (5% EtOH/CH_2Cl_2) afforded di-*tert*-butyl 6-hydroxy-2-oxopyrido[2,3-*d*]pyrimidine-1,3(*2H,4H*)-dicarboxylate, **19** (5.08g, 13.9mmol, 71%) and *tert*-butyl 6-hydroxy-2-oxo-1,2-dihydropyrido[2,3-*d*]pyrimidine-3(4*H*)-carboxylate, **20** (0.87g, 3.30mmol, 17%).

di-*tert*-butyl 6-hydroxy-2-oxopyrido[2,3-*d*]pyrimidine-1,3(*2H,4H*)-dicarboxylate **19** R_f = 0.56 (5% EtOH/CH_2Cl_2); ^{1}H NMR (400Mz, $CDCl_3$) δ 7.91 (d, 1H, *J* = 2.8), 7.48 (s, 1H), 7.11 (d, 1H, *J* = 2.4), 4.70 (s, 2H), 1.57 (s, 9H), 1.52 (s, 9H); ^{13}C δ 151.7, 151.7, 150.5, 149.7, 149.2, 141.3, 135.3, 121.6, 116.6, 85.7, 84.3, 44.4, 27.9, 27.6; HRMS calcd for $C_{17}H_{23}N_3O_6Na$ ($M+Na^+$): 388.1485; found 388.1489 (1.0 ppm);

tert-butyl 6-hydroxy-2-oxo-1,2-dihydropyrido[2,3-*d*]pyrimidine-3(4*H*)-carboxylate **20** R_f = 0.16 (5% EtOH/CH_2Cl_2); ^{1}H NMR (400Mz, *d*-DMSO) δ 10.19 (s, 1H), 9.62 (s, 1H), 7.69 (d, 1H, *J* = 2.8), 7.13 (d, 1H, *J* = 2.7), 4.63 (s, 2H), 1.43 (s, 9H); ^{13}C δ 151.9, 150.4, 149.7, 142.2, 134.0, 121.5, 116.0, 81.8, 44.2, 27.7; HRMS calcd for $C_{12}H_{15}N_3O_4Na$ ($M+Na^+$): 288.0960; found 288.0959 (0.3 ppm);

6-hydroxy-3,4-dihydropyrido[2,3-d]pyrimidin-2(1H)-one (12) To a solution **19** (5.04g, 13.8mmol) in MeOH (20ml) was added HCl in MeOH(30ml, 1.2M). The solution was gently warmed to ~60°C and reaction was monitored by TLC for until completion (1.5h). Solvent was removed under reduced pressure and the residue was resuspended in water (20ml) naturalize with sat. $NaHCO_3$ to pH~7. Solution was cooled to 0°C, crystals collected and washed with small amount ice cold water (*Note: Crystals may not need further purification*). Crystals were recrystallized from boiling 10% MeOH/H_2O. After 5h, solvent was cooled to 0°C, crystals filtered, washed with small amount of ice cold water and dried under the reduced pressure furnishing 6-hydroxy-3,4-dihydropyrido[2,3-d]pyrimidin-2(1H)-one , **12** (1.70g, 10.3mmol, 75% yield). (*Note: Similar procedure can be applied to compound **20***). Product structure was analyzed by means of ^{1}H, ^{13}C and 2D NMR (NOESY, HSQC, HMBC).

R_f = 0.38 (20% EtOH/ CH_2Cl_2); ^{1}H NMR (400Mz, *d*-DMSO) δ 9.34 (s, 1H, -O<u>H</u>), 9.11 (s, 1H, C-N<u>H</u>-C=O), 7.60 (d, 1H, *J* = 2.8, -C(OH)-C<u>H</u>-N), 6.96 (d, 1H, *J* = 2.0, -C(OH)-C<u>H</u>-C), 6.77(s, 1H, CH_2-N<u>H</u>-C=O), 4.24 (s, 2H, C-C<u>H</u>$_2$-NH-); ^{13}C δ 154.8 (NH-<u>C</u>O-NH), 148.9(-<u>C</u>OH), 143.4 (=N-<u>C</u>-NH-), 133.2 (-COH-<u>C</u>H-N), 121.6(-COH-<u>C</u>H-C-), 114.0 (-CH-<u>C</u>-CH_2-), 41.3 (C-<u>C</u>H$_2$-NH-); Structure was further analyzed by means NOESY, HSQC, HMBC. HRMS calcd for $C_7H_8N_3O_2$ (MH^+): 166.0617; found 166.0619 (1.2 ppm);

5-(bromomethyl)-2,4-dimethylpyridin-3-ol hydrobromide (22) was made by a previously

described procedure from compound **21** [4]. No recrystallization was performed. ^{1}H NMR (300Mz, *d*-DMSO) δ 10.74(brs, 1H), 8.51(s, 1H), 4.87(s, 2H), 2.58(s, 3H), 2.41(s, 3H); ^{13}C δ 152.4, 143.5, 141.8, 134.6, 131.7, 27.8, 15.4, 12.9

5-(azidomethyl)-2,4-dimethylpyridin-3-ol hydrochloride (23) To a DMF (150ml) solution of crude 5-(bromomethyl)-2,4-dimethylpyridin-3-ol hydrobromide, **22** (32.9g, 0.112mol) sodium azide (7.28g, 0.112mol) was added. After 12h, reaction was concentrated under the reduced pressure. The residue was extracted with methanol (2 x 250ml). To the combined extracts HCl in Et_2O (200ml, 2M) was added. Solvent was removed *in vacuo* and residue was recrystallize from boiling *i*-PrOH. After 12 h at R.T. it was cooled to 0°C, crystals were filtered and washed with ice-cold ethyl ether. Drying under reduced pressure afforded 5-(azidomethyl)-2,4-dimethylpyridin-3-ol hydrochloride **23**, (15.8g, 73.6mmol) in 66% yield (*over two steps*). R_f = 0.41 (10% EtOH/CH_2Cl_2); ^{1}H NMR (400Mz, *d*-DMSO) δ 10.76 (br. s, 1H), 8.31 (s, 1H), 4.70 (s, 2H), 3.68 (v. br. s, 1H), 2.60 (s, 3H), 2.37 (s, 3H); ^{13}C δ 152.2, 143.0, 141.8, 132.4, 130.9, 48.5, 15.3, 12.9; HRMS calcd for $C_8H_{11}N_4O$ (MH^+): 179.0933; found 179.0930 (1.7 ppm).

(E)-5-(azidomethyl)-2,4-dimethyl-6-(phenyldiazenyl)pyridin-3-ol three hydrochloride (24) In a beaker equipped with a magnetic stirrer and pH electrode and immersed in an ice bath, substrate **23** (15g, 0.070mol) was dissolved in water/methanol (200/25 mL) and the pH of the solution was adjusted to 8 with 2.5M NaOH. Diazonium salt, freshly prepared by slow mixing of aniline in 6M HCl (6mL, 0.066mol; 68 mL) with aqueous $NaNO_2$ (4.5g, 0.066mol; 22mL) in ice bath, was added to the substrate solution portionwise along with 2.5M NaOH to maintain pH 8. After 3h, a red precipitate that formed in the reaction, it was filtered off and partially dissolved in dichloromethane (4 x 500ml). The organic solvent was dried over Na_2SO_4, and condensed under vacuum forming dark red powder. The residue was redissolved in MeOH and HCl in Et_2O (100ml, 2M) was added and the solvent was removed under reduced pressure. Product was used for the next step without further purification. Small, apparently more pure crystals were scooped for NMR and MS analysis. R_f=0.74 (10% EtOH/CH_2Cl_2); ^{1}H NMR (600Mz, *d*-DMSO) δ 7.96 (dd, 2H, $J_{1,2}$ = 1.8 and 7.2), 7.60 (m, 3H), *6.05(br. s, 4H)*, 4.98 (s, 2H), 2.53(s, 3H), 2.36(s, 3H); ^{13}C δ 154.1(v. br.), 151.9, 149.0, 146.6, 136.4, 131.7, 129.9(v. br), 122.7, 45.4, 18.9, 12.3; HRMS calcd for $C_{14}H_{15}N_6O$ (MH^+): 283.1307; found 283.1309 (0.7 ppm).

6-amino-5-(aminomethyl)-2,4-dimethylpyridin-3-ol hydrochloride (25) To a solution of crude **24** (from the previous step) in MeOH (1.5L) HCl in MeOH(20ml, 1.2M) was added. After flashing reaction with nitrogen Pd/C (1g, wet, 10 % load) was added and the reaction was left under hydrogen for 12h. After that, the solution was filtered and HCl in Ethyl ether (120ml, 2M) was added and the solution was concentrated *in vacuo*. The residue was redissolved in 250ml of MeOH and cooled to 0°C and 350ml of ethyl ether was added slowly. Two hours later, crystals were filtered and washed with 100ml of ice cold ether furnishing 6-amino-5-(aminomethyl)-2,4-dimethylpyridin-3-ol hydrochloride (8.13g, 39.9mmol, 57% *over two steps*). ^{1}H NMR (400Mz, *d*-DMSO) δ 9.22 (br. s, 1H), 8.43 (s, 3H), 7.54 (s, 2H), 4.05 (d, 2H, *J* = 4.4), 2.38 (s, 3H), 2.36 (s, 3H); ^{13}C δ 149.8, 148.8, 140.9, 133.8, 113.2, 33.7, 14.3, 14.0; HRMS calcd for $C_8H_{14}N_3O$ (MH^+): 168.1137; found 168.1138 (0.6 ppm).

6-hydroxy-5,7-dimethyl-3,4-dihydropyrido[2,3-d]pyrimidin-2(1H)-one (13) To solution **25** (7.62g, 37.4mmol) in dry DMF (170ml) CDI (10.37g, 64.0mmol) was added. After 12h, water (100ml) and glacial acetic acid (3ml) were added to the reaction. Eight hours later reaction was concentrated *in vacuo*. The residue was resuspended in 200ml of H_2O and basified to pH ~ 7-8 with sodium bicarbonate. After 3 hours, it was concentrated under reduced pressure and set to recrystallized from boiling 20 % MeOH/H_2O (500ml). After 10h at RT, solvent was cooled down to 0°C, crystal filtered and washed with small amount of ice cold water affording 6-hydroxy-5,7-dimethyl-3,4-dihydropyrido[2,3-d]pyrimidin-2(1H)-one (4.8g, 24.8mmol, 66% yield). Product structure was analyzed by means of ^{1}H, ^{13}C and 2D NMR (NOESY). R_f = 0.56 (20% EtOH/ CH_2Cl_2); ^{1}H NMR (400Mz, *d*-DMSO) δ 8.88 (s, 1H, -C-N<u>H</u>-C=O), 8.13 (s, 1H, -CH-CO<u>H</u>-CH), 6.77(s, 1H, -CH_2-N<u>H</u>-C=O), 4.22 (s, 2H, C-C<u>H_2</u>-NH-), 2.23 (s, 3H, -COH-C<u>Me</u>-N), 1.99 (s, 3H, -COH-C<u>Me</u>-C); ^{13}C δ 154.2, 144.2, 142.5, 142.4, 132.1, 109.7, 40.1, 19.0, 11.2; HRMS calcd for $C_9H_{12}N_3O_2$ (MH^+): 194.0930; found 194.0928 (1.0 ppm); UV ε(202) = 1.3E+05, ε (250) = 6.8E+04, ε (305) = 6.6+04E;

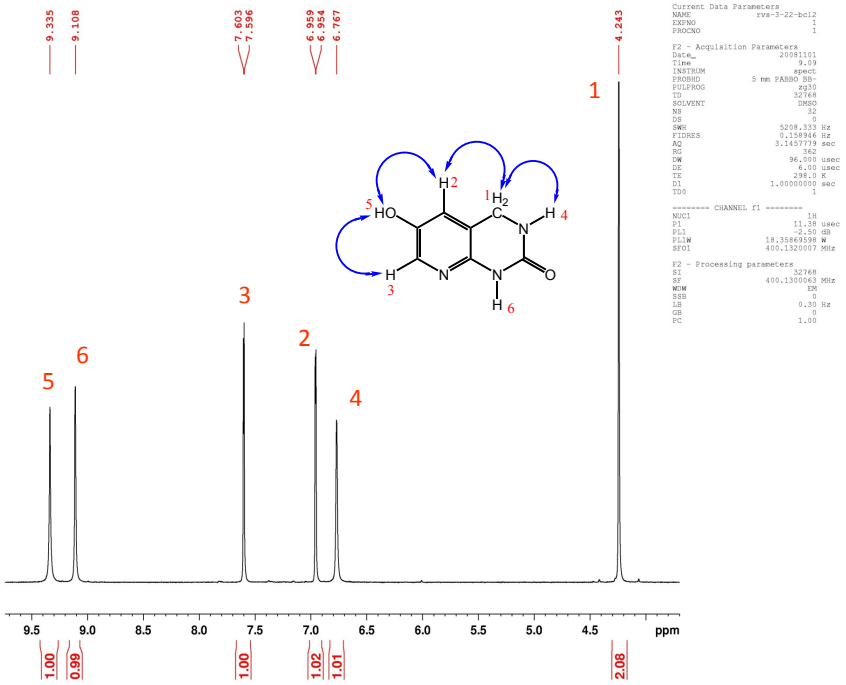
6-hydroxy-5,7-dimethyl-1H-pyrido[2,3-d][1,3]oxazin-2(4H)-one (14)

To a solution of 6-amino-5-(hydroxymethyl)-2,4-dimethylpyridin-3-ol [5], **21** (0.1 g, 0.59 mmol) in THF/$NaHCO_3$ (5ml/1.5ml) CBzCl(2.52mmol, 0.38 g) was added. The mixture was left over night at

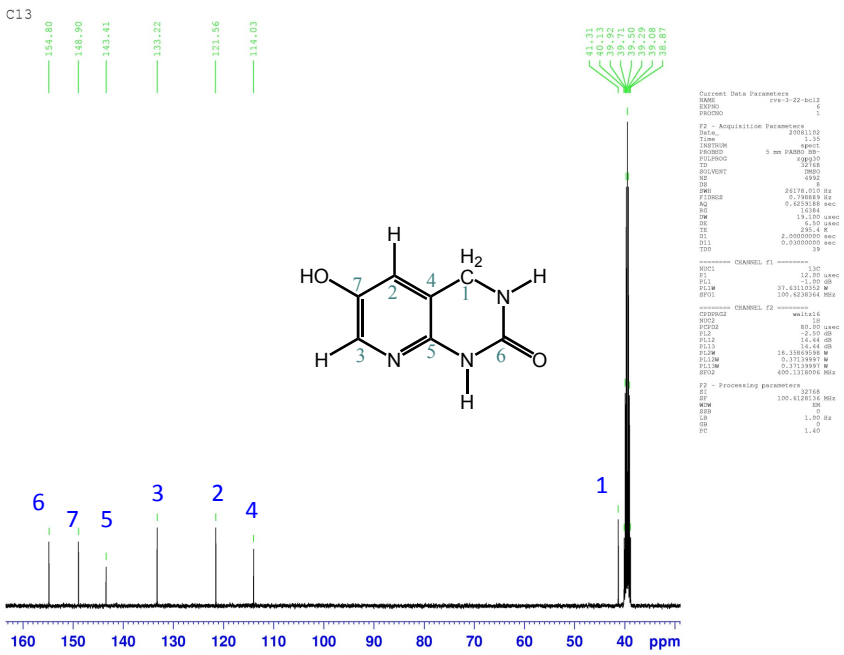
RT and solvent was evaporated under the reduced pressure. The residue was redissolved in MeOH/H_2O (5ml/5ml) and K_2CO_3 (0.2g) was added. After 2h reflux, the solvent was removed under the reduced pressure. Solid residue was extracted with two portions of EtOH (10ml), which were combined and concentrated *in vacuo*. Crude product was purified by flash chromatography (5% EtOH/CH_2Cl_2) furnishing 6-hydroxy-5,7-dimethyl-1H-pyrido[2,3-d][1,3]oxazin-2(4H)-one (62mg, 54%); R_f = 0.31 (5% EtOH/CH_2Cl_2); ^{1}H NMR (400Mz, *d*-DMSO) δ 10.19 (s, 1H), 8.42 (s, 1H), 5.25 (s, 2H), 2.28 (s, 3H), 2.06 (s, 3H), ^{13}C δ 152.0, 145.7, 143.9, 141.1, 131.7, 110.4, 65.3, 19.5, 11.5. $C_9H_{11}N_2O_3$ (MH^+): 195.0770; found 195.0764 (3.1 ppm);

NMR SPECTRA OF KEY ANALOOGS

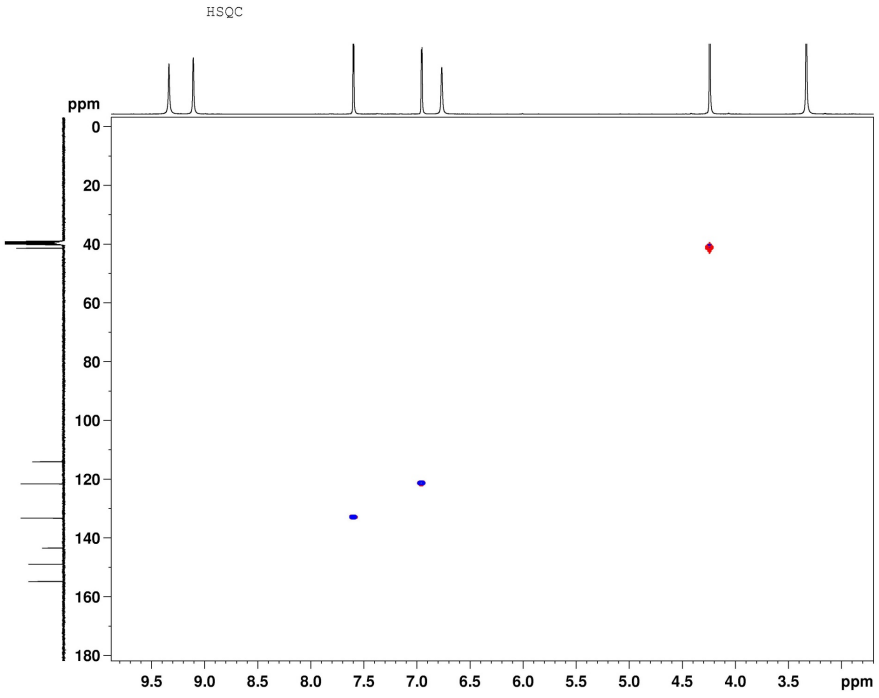
Analog **12** (^{1}H spectrum, DMSO-d_6)



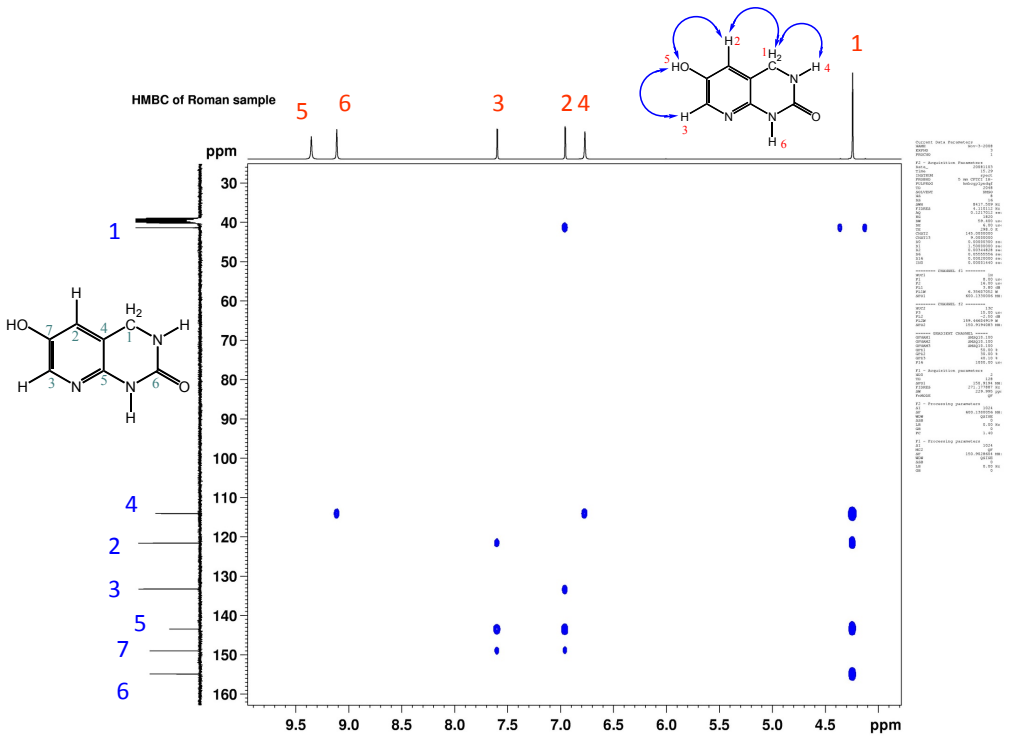
Analog **12** (^{13}C spectrum, DMSO-d_6)



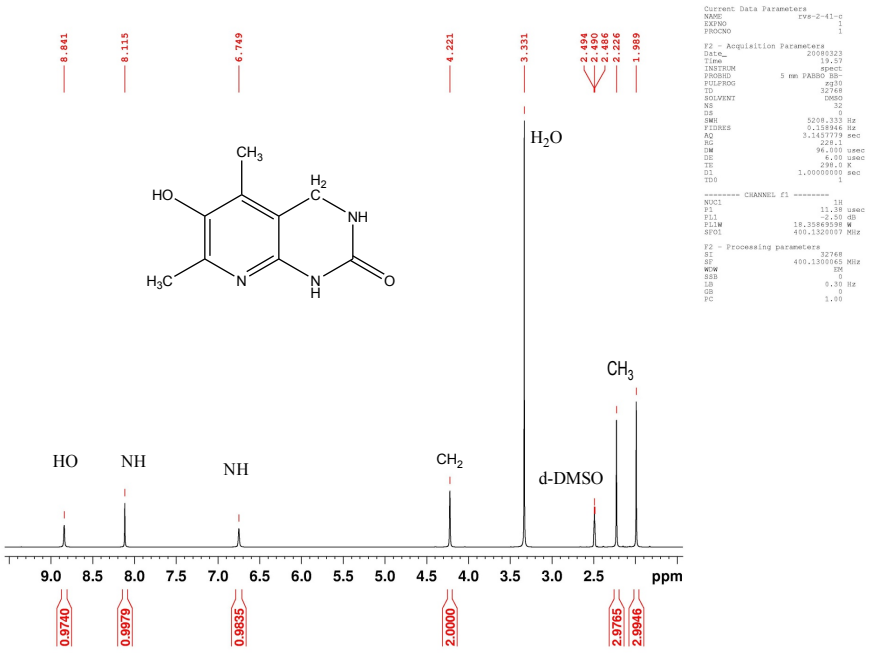
Analog **12** (HSQC, DMSO-d_6)



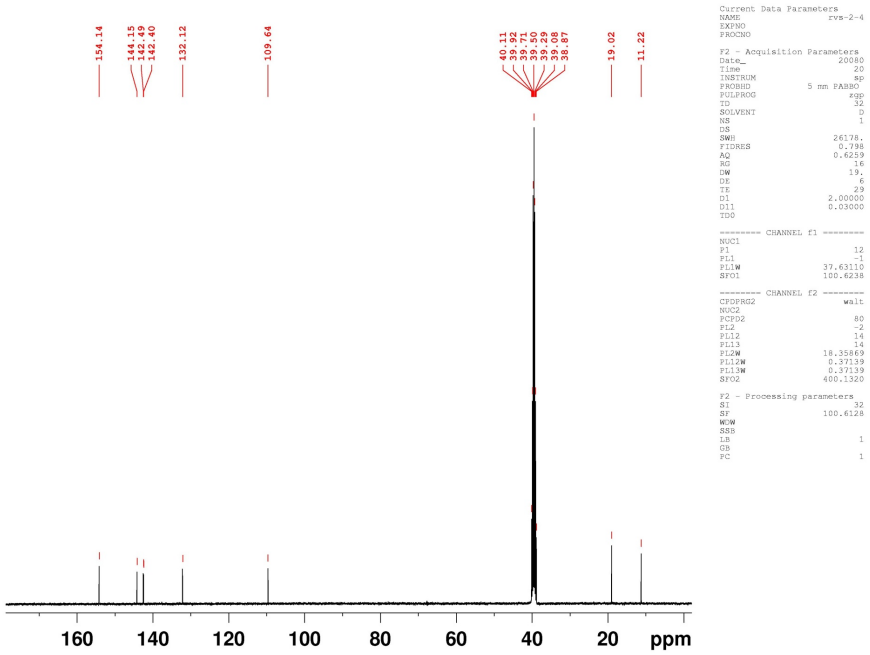
Analog **12** (HMBC, DMSO-d_6)



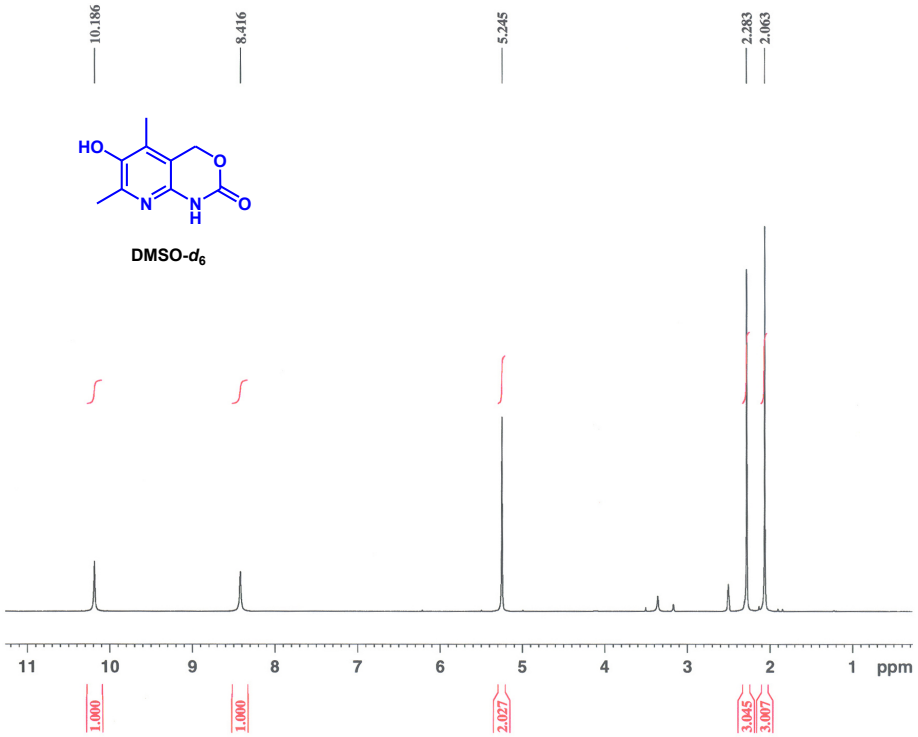
Analog **13** (^{1}H spectrum, DMSO-d$_6$)



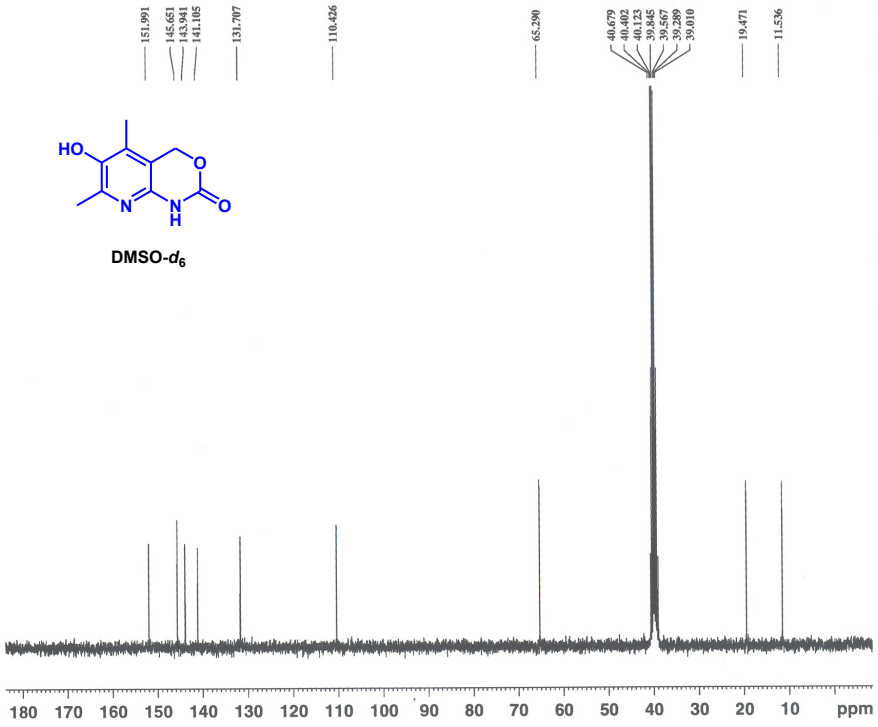
Analog **13** (^{13}C spectrum, DMSO-d$_6$)



Analog **14** (^{1}H spectrum)



Analog **14** (^{13}C spectrum)



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