Published in final edited form as:
*ACS Med Chem Lett*. 2013 August 8; 4(8): 710–714. doi:10.1021/ml4000904.

# Rational Design of Novel Pyridinol-Fused Ring Acetaminophen Analogues

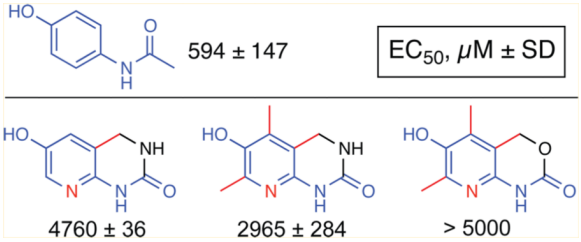
**Roman V. Shchepin**[†,*,‖], **Wei Liu**[†], **Huiyong Yin**[‡], **Irene Zagol-Ikapitte**[‡], **Taneem Amin**[‡], **Byeong-Seon Jeong**[†,⊥], **L. Jackson Roberts II**[‡,§], **John A. Oates**[‡], **Ned A. Porter**[†], and **Olivier Boutaud**[§]

[†]Department of Chemistry, Vanderbilt University, 7330 Stevenson Center, Station B 351822, Nashville, Tennessee 37235, United States

[‡]Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University Medical Center, 536 Robinson Research Building, Nashville, Tennessee 37232-6602, United States

[§]Department of Pharmacology, Division of Clinical Pharmacology, Vanderbilt University Medical Center, 536 Robinson Research Building, Nashville, Tennessee 37232-6602, United States

## Abstract




Acetaminophen (ApAP) is an electron donor capable of reducing radicals generated by redox cycling of hemeproteins. It acts on the prostaglandin H synthases (cyclooxygenases; COXs) to reduce the protoporphyrin radical cation in the peroxidase site of the enzyme, thus preventing the intra-molecular electron transfer that generates the Tyr385 radical required for abstraction of a hydrogen from arachidonic acid to initiate prostaglandin synthesis. Unrelated to this pharmacological action, metabolism of ApAP by CYPs yields an iminoquinone electrophile that is responsible for the hepatotoxicity, which results from high doses of the drug. We synthesized novel heterocyclic phenols predicted to be electron donors. Two of these inhibited the oxygenation of arachidonic acid by PGHS-1 and myoglobin and also were shown to be more metabolically stable and exhibited less direct cytotoxicity than acetaminophen. They are leading candidates for



**Corresponding Author**. * (R.V.S.) roman.shchepin@vanderbilt.edu.
‖(R.V.S.) Institute of Imaging Science, Vanderbilt University, 1161 21st Avenue South, MCN, AA-1105, Nashville, Tennessee 37232-2310, United States.
⊥(B.-S.J.) College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Republic of Korea.

**ASSOCIATED CONTENT**

**Supporting Information**

Experimental procedures and NMR spectra of key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

studies to determine whether they are free of the metabolism-based hepatotoxicity produced by acetaminophen.

**Keywords**

Acetaminophen; synthesis; heterocyclic phenols; toxicity; stability

Acetaminophen (ApAP; Paracetamol), with its analgesic and antipyretic properties, is one of the most widely used drugs. ApAP works by reducing the protoporphyrin radical cation in the peroxidase site of the prostaglandin $H_2$ synthases (also know as cyclooxygenases, COX-1 and COX-2),[1,2] thereby decreasing the production of the prostaglandins mediating fever and pain. We also have shown that acetaminophen can inhibit lipid peroxidation catalyzed by hemoglobin, myoglobin, and cytochrome c[3,4] and that acetaminophen can protect the kidney from oxidative damage associated with rhabdomyolysis.[3] This work provided the proof of concept that acetaminophen can be used in vivo to protect tissues from oxidative stress associated with hemeprotein redox cycling.

Unfortunately, acetaminophen is associated with hepatotoxicity, which may be severe,[5–7] occurring both in association with intentional and accidental overdose and with administration to susceptible individuals. It has been implicated in nearly 50% of all acute liver failure in the United States alone.[8] Acetaminophen's hepatotoxicity is due to its 2-electron oxidation by the phase I cytochrome P450 (CYP) 2E1 and CYP3A4 in the liver.[9–11] This leads to formation of the extremely reactive intermediate metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI), which conjugates to glutathione, resulting in glutathione depletion in the liver.[12] After GSH is depleted, NAPQI reacts with proteins[13,14] and impairs mitochondrial function,[15] leading to liver necrosis.

Taken together, the data indicate the need for a safer alternative to acetaminophen. In this letter, we describe the synthesis of acetaminophen analogues lacking the *p*-aminophenol structure that leads to formation of the toxic quinone imine electrophile metabolite. We also present the characterization of 2 lead compounds that are potent inhibitors of COX-1 and of myoglobin-induced lipid oxidation, but are metabolized by liver microsomes less rapidly than acetaminophen and do not exert direct cytotoxicity on cells in culture. Several nitrogen-containing highly potent phenolic antioxidants (**1**–**11**) have been prepared recently.[16,17]

For example, pyrimidinols (Figure 1, **1** and **2**) as well as pyridinols (**3** and **4**) were proven to be highly effective inhibitors of COX-1.[16]

A number of trends can be inferred from previously published results for pyrimidinols (**1** and **2**) and pyridinols (**3** and **4**)[16] as well as recently published vitamin $B_6$-derivatives (**5**–**11**).[17] In this letter, we evaluated efficiency to inhibit hemeproteins, cytotoxicity, and metabolic stability of those nitrogen-containing phenolic heterocycles seeking to identify the molecular structure motifs that correlate with higher activity and stability and lower levels of cytotoxicity. Our results provide information necessary for a rational design and synthesis of novel acetaminophen analogues with improved toxicity and stability profiles.

Cytotoxicity was assessed by measuring total intracellular ATP levels in HepG2 cells. This represents a good marker of the overall cellular health[18,19] as ATP levels are affected by mitochondrial integrity as well as by changes in cellular metabolism. Metabolic stability was assessed in vitro using rat liver microsomes.[20]

In addition to the hepatotoxicity caused by the toxic metabolite of acetaminophen, pyridine phenols (**3**–**4**) may produce direct cytotoxicity, especially in comparison with virtually

nontoxic pyrimidine phenols (**1**–**2**) as we have previously shown.[16] This can be explained by the formation of an unhindered cationic iminium ion (or quinone) and its subsequent reactivity toward nucleophiles.[21,22] Extensive metabolism by liver microsomes may be further evidence of cationic iminium ion formation. In this regard, significantly lower toxicity of pyridinols **5** and **7** may be attributed to the vital role of C(5) methyl substituent,[17] suggesting that a substituent in C(5) position can hinder this position for nucleophilic attack.

Excessively low ionization potential (IP), which leads to the formation of superoxide is the most likely cause of the instability and high direct cytotoxicity of compounds **10** and **11** as well as comparably elevated direct cytotoxicity of compound **6**.[17] Indeed, it has been shown that analogue **6** is a more potent antioxidant than **7** due to the higher degree of spin alignment between the aromatic ring and the adjacent nitrogen.[17] High degree of toxicity shown by compounds **8** and **9** especially in comparison with analogues **6** and **7** can be partially attributed to their low IP value. This argument is supported by the observation of large amounts of oxygenation products in the microsome stability assay (Supporting Information) as well as by the higher electron donating effect of an alkyl chain vs a methyl substituent.[23] Also, long alkyl chains of compounds **8** and **9** might result in a detergent-like cell membrane disruption, further elevating their toxicity.

The microsome stability assay showed that demethylation was a major metabolic path for almost all the NHMe or $N(Me)_2$ containing analogues. It also showed rapid metabolic consumption of the highly potent COX-1 inhibitor **2** by both demethylation and oxidation pathways. Demethylation may lead to increased toxicity by conversion, for example, from analogue **7** to **6** and **5**, both of which are highly cytotoxic.[17]

Three pyridinol analogues of acetaminophen (Figure 2) were designed based on the results described above. All compounds are related to acetaminophen. Their structures are designed to modulate the donor characteristics of the 6-amino group, similar to acetaminophen, and fix the geometry of ring substituents by the constraints of a fused ring. A similar structural motif can be found in uric acid, which is responsible for more than half of human blood plasma antioxidant capacity.[24] Thus, a urea bridge has been chosen to protect the free amine. This structural feature was intended to modulate the IP value of these novel analogues to alleviate the metabolic instability and cytotoxicity shown by analogues **10** and **11**. Since the presence or the absence of C(2) and C(4) substituents in the heterocyclic phenol ring did not drastically alter pyridines and pyrimidines toxicity profile, both analogues **12** (Scheme 1) and **13** have been prepared. The urethane analogue **14** of the urea analogue **13** was also prepared.

The previously known 2-amino-5-bromo-nicotinonitrile (**15**)[25] has been prepared from commercially available 2-amino-nicotinonitrile via an efficient nuclear monobromination, by treatment with *N*-bromosuccinimide (NBS) in the presence of a catalytic amount of $NH_4OAc$.[26] Reduction by the borane–tetrahydrofuran complex ($BH_3$-THF) followed by acidic deprotection of the resulting borane furnished the diamino compound (**16**), which was converted to the cyclic urea (**17**) by action of 1,1′-carbonyldiimidazole (CDI). *tert*-Butyloxycarbonyl (Boc) protection of exchangeable amide protons is important for success of the Pd catalyzed borylation reaction–oxidation two-step sequence.[27] Therefore, the Boc protected cyclic urea (**18**) was reacted with bis-(pinacolato)diboron, and the crude product was oxidized giving rise to a separable mixture of mono- and di-protected phenols (**19** and **20**). Either mono- or di-protected phenols (or their mixture) can be deprotected by the reaction with methanolic solution of hydrochloric acid followed by recrystallization.

The synthesis of analogues **13** (Scheme 2) and **14** originated from commercially available vitamin B6, which was converted to compound **21** by a highly efficient two-step sequence.[17]

Bromination with concentrated hydrobromic acid[28] afforded crude **22**, which was converted to compound **23** by reaction with sodium azide. Diazo-substitution furnished compound **24**. Both diazo and azido groups were simultaneously reduced by palladium-on-carbon catalyzed hydrogenation. Diamine **25** was converted to pyridinol-fused ring analogue **13** via CDI induced cyclic urea formation. Analogue **14** was produced from compound **21**, which was converted to 6-amino-5-(hydroxymethyl)-2,4-dimethylpyridin-3-ol by a known procedure[29] followed by protection with benzyl chloroformate (CbzCl) and subsequent cyclization under the basic conditions.

These three analogues have been evaluated for their microsomal stability and cellular toxicity as described in the experimental section. Analogues **12, 13**, and **14** have displayed a toxicity and stability profile, which is consistent with our structure-to-biological property relationship analysis described above. All three compounds have no direct cytotoxicity at concentrations below the millimolar range. Their $EC_{50}$ values for cytotoxicity are 5 to more than 10 times higher than that of acetaminophen (Table 1). All three compounds were metabolized by human liver microsomes to a lesser extent than acetaminophen, suggesting that in vivo metabolism by microsomes would be less as well.

Next, the efficiency of these ApAP analogues to inhibit hemeprotein-catalyzed lipid oxidation was tested using COX-1 and myoglobin as reported previously.[2,3] As shown in Table 2, all analogues are able to inhibit myoglobin-induced arachidonic oxidation within the same order of magnitude, with analogue **14** being the least potent. In contrary, analogue **14** is most potent in inhibiting COX-1 activity followed by ApAP and analogue **12**. Interestingly, analogue **13** was not able to significantly inhibit COX-1 at concentrations up to 1 mM.

The difference in the myoglobin-induced arachidonic oxidation assay values for analogues **13** and **14** can be explained by their electronic properties. Indeed, more electron-withdrawing oxygen would somewhat diminish analogue **14**'s ability to reduce myoglobin. While the reverse trend in COX-1 inhibition is a subject of further studies, we can speculate that it is related to the constrains of the COX-1 binding pocket; interactions with the heme pocket has been shown to affect bioflavonoid activities.[30] This possible mechanism is supported by the evidence showing that analogue **12** is more active that the dimethyl analogue **13**; the methyl group positive inductive effect would have predicted the opposite effect. Further computational studies, which are outside of this letter's scope, may shed some light on this phenomenon.

In conclusion, we investigated a number of potent phenolic heterocycles with respect to their efficiency in inhibiting hemeprotein-catalyzed lipid oxidation and also their metabolic stability and toxicity. Several important structure–stability and structure–toxicity relationship studies were used for the design of novel heterocyclic acetaminophen analogue series. Our results indicate that two analogues (**12** and **14**) may represent a good alternative to acetaminophen with a similar efficiency and better cytotoxicity profile. These analogues are promising candidates for studies in animal models of hepatotoxicity to determine whether they represent lead compounds for development of drugs that could replace acetaminophen.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We are thankful to Dr. Donald F. Stec for his help with 2D NMR characterization of the compounds.

**Funding**

This work was supported by NIGMS Center for Clinical Pharmacology and Drug Toxicology (GM-15431). J.A.O. is the Thomas F. Frist, Sr. Professor of Medicine.

## REFERENCES

1. Ouellet M, Percival MD. Mechanism of acetaminophen inhibition of cyclooxygenase isoforms. Arch. Biochem. Biophys. 2001; 387:273–280. [PubMed: 11370851]
2. Boutaud O, Aronoff DM, Richardson JH, Marnett LJ, Oates JA. Determinants of the cellular specificity of acetaminophen as an inhibitor of prostaglandin H(2) synthases. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:7130–7135. [PubMed: 12011469]
3. Boutaud O, Moore KP, Reeder BJ, Harry D, Howie AJ, Wang S, Carney CK, Masterson TS, Amin T, Wright DW, Wilson MT, Oates JA, Roberts LJ II. Acetaminophen inhibits hemoprotein-catalyzed lipid peroxidation and attenuates rhabdomyolysis- induced renal failure. Proc. Natl. Acad. Sci. U.S.A. 2010; 107:2699–2704. [PubMed: 20133658]
4. Yin H, Vergeade A, Shi Q, Zackert WE, Gruenberg KC, Bokiej M, Amin T, Ying W, Masterson TS, Zinkel SS, Oates JA, Boutaud O, Roberts LJ. Acetaminophen inhibits cytochrome c redox cycling induced lipid peroxidation. Biochem. Biophys. Res. Commun. 2012; 423:224–228. [PubMed: 22634010]
5. Larsson R, Ross D, Berlin T, Olsson LI, Moldeus P. Prostaglandin synthase catalyzed metabolic activation of *p*-phenetidine and acetaminophen by microsomes isolated from rabbit and human kidney. J. Pharmacol. Exp. Ther. 1985; 235:475–480. [PubMed: 3932643]
6. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO, Lee WM. Acetaminophen-induced acute liver failure: Results of a United States multicenter, prospective study. Hepatology. 2005; 42:1364–1372. [PubMed: 16317692]
7. Makin AJ, Wendon J, Williams R. A 7-year experience of severe acetaminophen-induced hepatotoxicity (1987–1993). Gastroenterology. 1995; 109:1907–1916. [PubMed: 7498656]
8. Lee WM. Acetaminophen and the U.S. Acute Liver Failure Study Group: lowering the risks of hepatic failure. Hepatology. 2004; 40:6–9. [PubMed: 15239078]
9. Nelson SD, Dahlin DC, Rauckman EJ, Rosen GM. Peroxidase-mediated formation of reactive metabolites of acetaminophen. Mol. Pharmacol. 1981; 20:195–199. [PubMed: 7290084]
10. Nelson SD. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Semin. Liver. Dis. 1990; 10:267–278. [PubMed: 2281334]
11. Mitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J. Pharmacol. Exp. Ther. 1973; 187:185–194. [PubMed: 4746326]
12. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J. Pharmacol. Exp. Ther. 1973; 187:211–217. [PubMed: 4746329]
13. Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. J. Pharmacol. Exp. Ther. 1973; 187:195–202. [PubMed: 4746327]
14. James LP, Letzig L, Simpson PM, Capparelli E, Roberts DW, Hinson JA, Davern TJ, Lee WM. Pharmacokinetics of acetaminophen–protein adducts in adults with acetaminophen overdose and acute liver failure. Drug Metab. Dispos. 2009; 37:1779–1784. [PubMed: 19439490]
15. Fannin RD, Russo M, O'Connell TM, Gerrish K, Winnike JH, Macdonald J, Newton J, Malik S, Sieber SO, Parker J, Shah R, Zhou T, Watkins PB, Paules RS. Acetaminophen dosing of humans results in blood transcriptome and metabolome changes consistent with impaired oxidative phosphorylation. Hepatology. 2010; 51:227–236. [PubMed: 19918972]

16. Nam TG, Nara SJ, Zagol-Ikapitte I, Cooper T, Valgimigli L, Oates JA, Porter NA, Boutaud O, Pratt DA. Pyridine and pyrimidine analogs of acetaminophen as inhibitors of lipid peroxidation and cyclooxygenase and lipoxygenase catalysis. Org. Biomol. Chem. 2009; 7:5103–5112. [PubMed: 20024105]
17. Serwa R, Nam TG, Valgimigli L, Culbertson S, Rector CL, Jeong BS, Pratt DA, Porter NA. Preparation and investigation of vitamin B6-derived aminopyridinol antioxidants. Chemistry. 2010; 16:14106–14114. [PubMed: 20967898]
18. Storer RD, McKelvey TW, Kraynak AR, Elia MC, Barnum JE, Harmon LS, Nichols WW, DeLuca JG. Revalidation of the in vitro alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutat. Res., Genet. Toxicol. 1996; 368:59–101.
19. Cree IA, Andreotti PE. Measurement of cytotoxicity by ATP-based luminescence assay in primary cell cultures and cell lines. Toxicol. In Vitro. 1997; 11:553–556. [PubMed: 20654351]
20. Mondal SK, Mazumder UK, Mondal NB, Banerjee S. Optimization of rat liver microsomal stability assay using HPLC. Int. J. Biol. Sci. 2008; 8:1110–1114.
21. Dahlin DC, Miwa GT, Lu AY, Nelson SD. *N*-Acetyl-*p*-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. Proc. Natl. Acad. Sci. U.S.A. 1984; 81:1327–1331. [PubMed: 6424115]
22. Shchepin R, Moller MN, Kim H-YH, Hatch DM, Bartesaghi S, Kalyanaraman B, Radi R, Porter NA. Tyrosine-lipid peroxide adducts from radical termination: Para coupling and intramolecular Diels–Alder cyclization. J. Am. Chem. Soc. 2010; 132:17490–17500. [PubMed: 21090613]
23. Carey, FA.; Sundberg, RJ. Advanced Organic Chemistry, Part A: Structure and Mechanisms. 5th ed.. New York: Springer-Verlag; 2007.
24. Maxwell SRJ, Thomason H, Sandler D, Leguen C, Baxter MA, Thorpe GHG, Jones AF, Barnett AH. Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. Eur. J. Clin. Invest. 1997; 27:484–490. [PubMed: 9229228]
25. Cai LS, Cuevas J, Temme S, Herman MM, Dagostin C, Widdowson DA, Innis RB, Pike VW. Synthesis and structure–affinity relationships of new 4-(6-iodo-*H*-imidazo[1,2-a]pyridin-2-yl)-*N*-dimethylbenzeneamine derivatives as ligands for human beta-amyloid plaques. J. Med. Chem. 2007; 50:4746–4758. [PubMed: 17722900]
26. Das B, Venkateswarlu K, Majhi A, Siddaiah V, Reddy KR. A facile nuclear bromination of phenols and anilines using NBS in the presence of ammonium acetate as a catalyst. J. Mol. Catal. A: Chem. 2007; 267:30–33.
27. Medina JR, Henry TA, Axten JM. A mild and general method for the synthesis of 2-substituted-5-hydroxypyrimidines. Tetrahedron Lett. 2006; 47:7363–7365.
28. Sakuragi T, Kummerow FA. The vitamin B6 derivatives structurally analogous to thiamine and their biological activity. Arch. Biochem. Biophys. 1957; 71:303–310. [PubMed: 13471031]
29. Nam T-G, Ku J-M, Rector CL, Choi H, Porter NA, Jeong B-S. Pyridoxine-derived bicyclic aminopyridinol antioxidants: synthesis and their antioxidant activities. Org. Biomol. Chem. 2011; 9:8475–8482. [PubMed: 22042081]
30. Wang P, Bai HW, Zhu BT. Structural basis for certain naturally occurring bioflavonoids to function as reducing co-substrates of cyclooxygenase I and II. PLoS One. 2010; 5

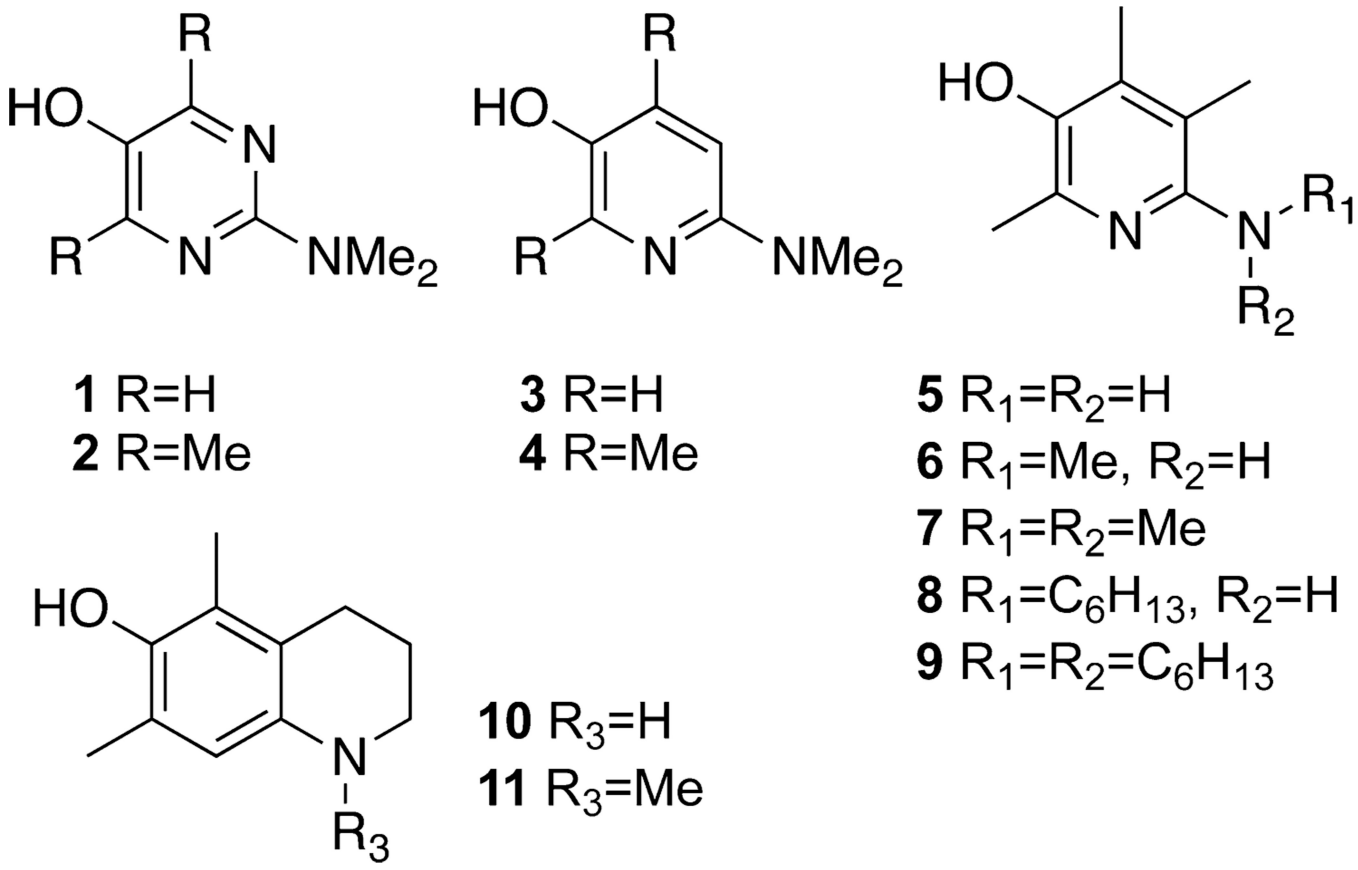



**Figure 1.**
Previously known pyrimidinols and pyridinols evaluated for their cytotoxicity and metabolic stability.

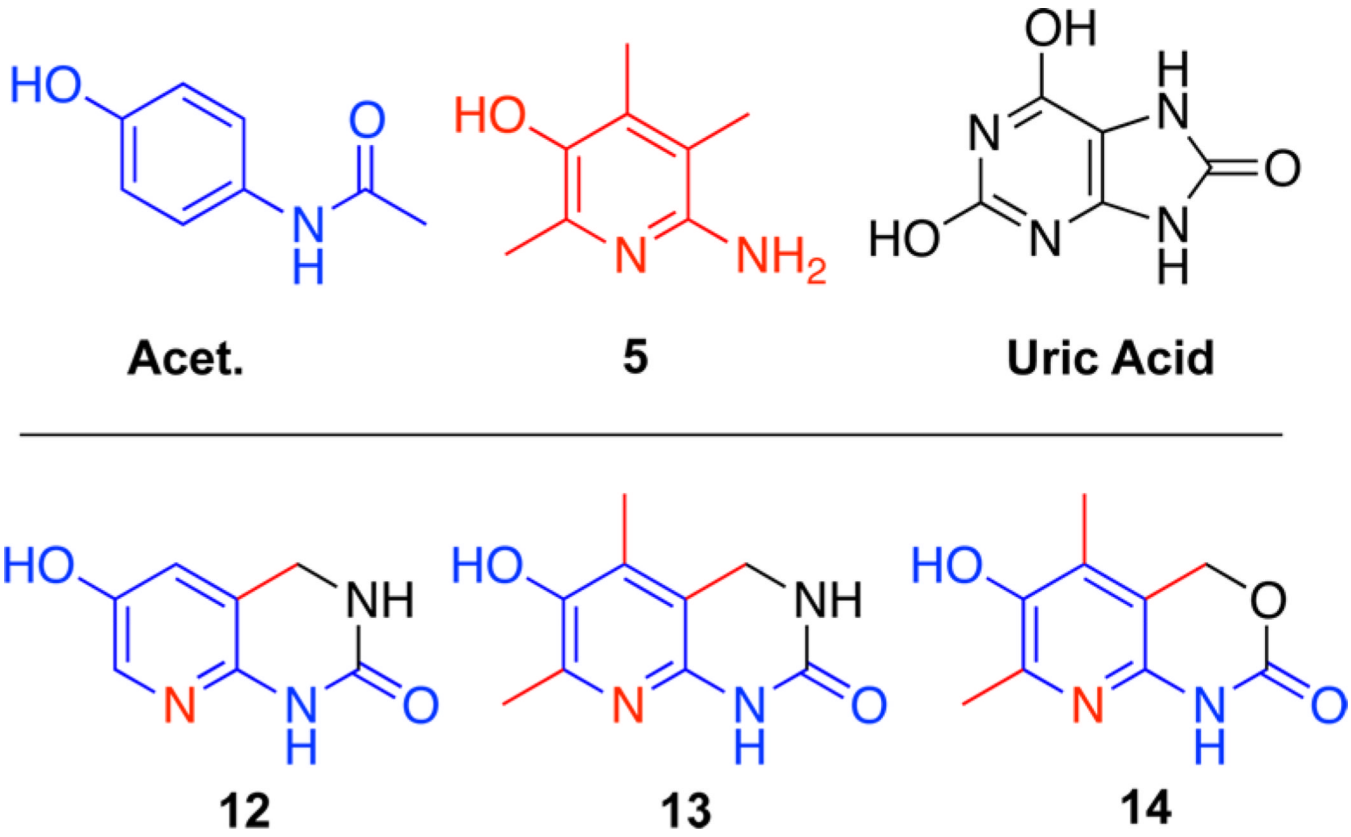
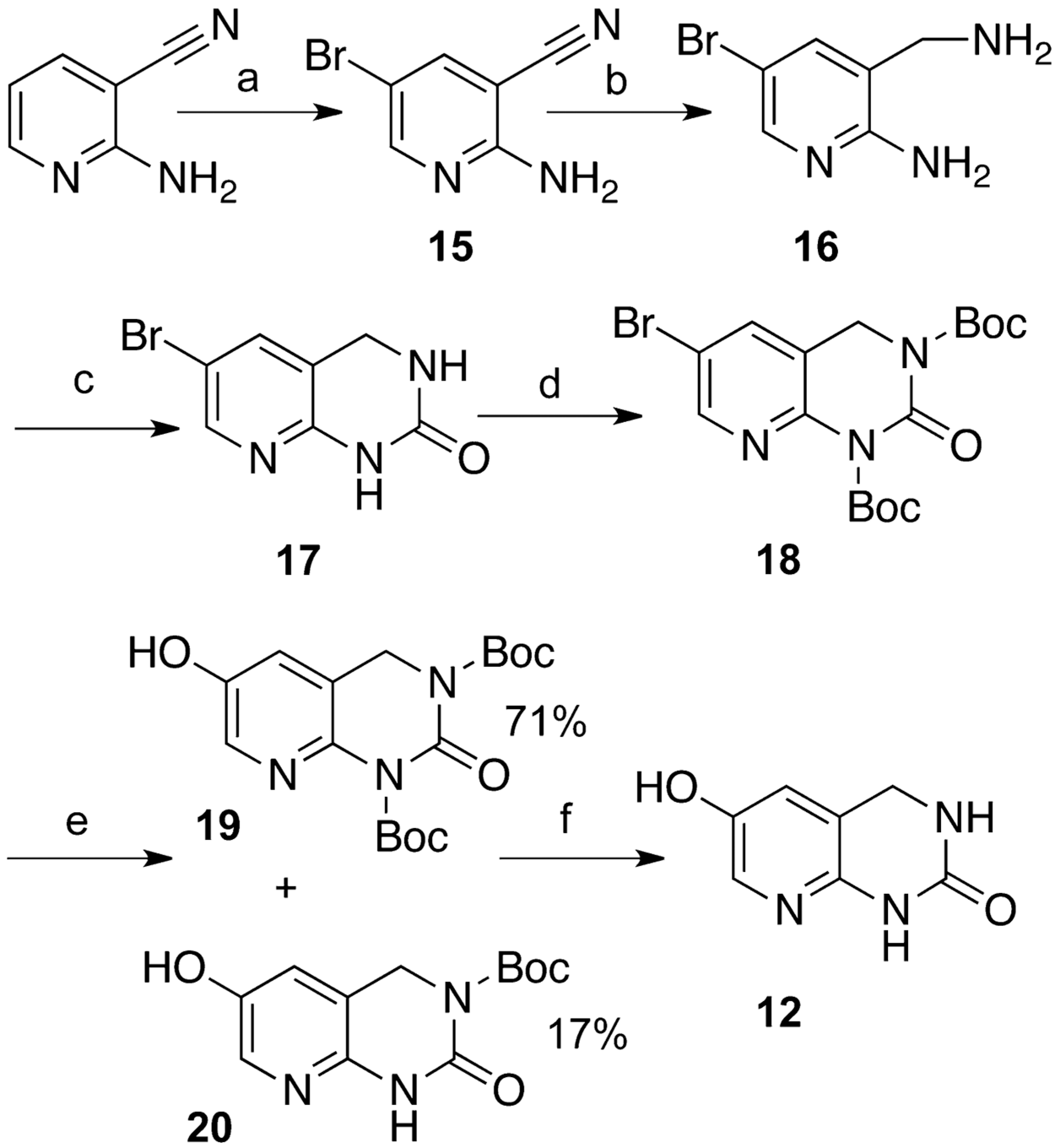


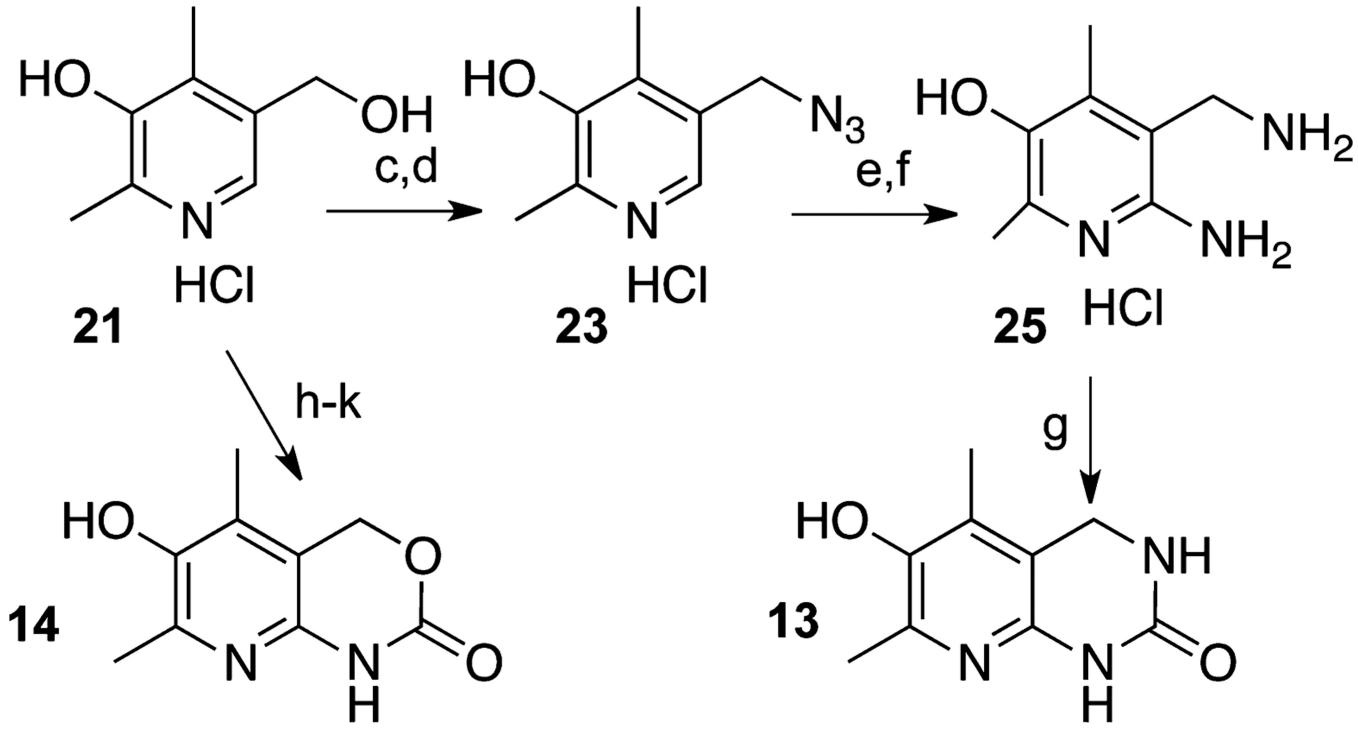

**Figure 2.**
Analogue design.




**Scheme 1.**

Synthesis of Pyridinol-Fused Ring Analogue 12a

[a]Reagents and conditions: (a) (i) NBS, $NH_4Ac$, MeCN, 0°C to RT, 12 h; (ii) 5% HCl, NaOH, pH > 10, $H_2O$, 0 °C, 20 min *91*%; (b) (i) $BH_3$-THF, THF, 0 °C to reflux, 12 h; (ii) 15% HCl in $H_2O$, 0 °C to reflux, 3 h; (iii) NaOH, to pH > 12, *71*%; (c) (i) CDI, DMF, RT; (ii) $H_2O$, RT to reflux to 0 °C, 20 min, *86*%. (d) $Boc_2O$, DMAP, THF, RT, 30 min, *97*%; (e) (i) KOAc, $Pd(OAc)_2$, bis-(pinacolato)diboron, DMF; (ii) $NaBO_3 \cdot 2H_2O$, THF/$H_2O$; (f) (i) HCl/MeOH, RT to 60 °C, 1.5 h; (ii) $NaHCO_3$, $H_2O$, 0 °C, 5 h, *75*%.




**Scheme 2.**

Synthesis of Pyridinol-Fused Ring Analogue 13a

[a]Reagents and conditions: (a) Zn, AcOH (Ac = acetyl), reflux, 3 h, *93*% over two steps; (b) 2 M HCl in ether, MeOH, reflux, 1 h; (c) HBr reflux, 20 min; (d) $NaN_3$, DMF, RT, 12 h, *66*% over two steps; (e) $PhNH_2$, $NaNO_2$, HCl then NaOH; (f) $H_2$, Pd/C, HCl, MeOH, 12 h, *57*% over two steps; (g) (i) CDI, DMF, RT, 12 h; (ii) AcOH, $H_2O$, RT, 8 h; (iii) $NaHCO_3$, 20% MeOH/$H_2O$, 3 h, *66*%; (h) $PhNH_2$, HCl, $NaNO_2$, NaOH, $H_2O$, 0 °C, 1 h, *95*%; (i) $H_2$,Pd/C, MeOH, RT, 6 h, *90*%; (j) CBzCl, $NaHCO_3$; (k) $K_2CO_3$, MeOH/$H_2O$.

**Table 1**

Stability and Direct Cytotoxicity of Novel Generation of Pyridinols[a]

| *N* | microsomal stability % left | cellular toxicity ($EC_{50}$, μM ± SEM) |
|---|---|---|
| ApAP | 65 ± 12 | 594 ± 147 |
| 12 | 108 ± 12 | 4760 ± 36 |
| 13 | 87 ± 7 | 2965 ± 284 |
| 14 | 97 ± 3 | >5000 |

[a]Microsomal stability is expressed as % of unmodified compound remaining after reaction. Microsomal stability values represent means ± SD. Cellular toxicity is expressed as the concentration causing a 50% decrease in total cellular ATP levels in HepG2 cells. Cellular toxicity values represent means ± SEM.

## Table 2

Inhibition by ApAP of Hemeprotein-Catalyzed Oxidation of Arachidonic Acid[a]

| analogue | ApAP | 12 | 13 | 14 |
|---|---|---|---|---|
| Mb (μM ± SEM) | 2.3 ± 0.2[b] | 1.2 ± 0.1 | 2.3 ± 0.4 | 5.3 ± 0.1 |
| COX-1 (μM ± SEM) | 372[c] | 451 ± 35 | >1000 | 198 ± 25 |

[a] The inhibition of the lipid peroxidation produced by myoglobin (Mb) and COX-1 by the different analogues was tested as described previously.[2,3] Values represent the $IC_{50}$ for each hemeprotein and are expressed as means ± SEM.

[b] Value reported in ref 3.

[c] Value reported in ref 2.