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Maximizing the Reactivity of Phenolic and Aminic Radical-Trapping Antioxidants: Just Add Nitrogen!

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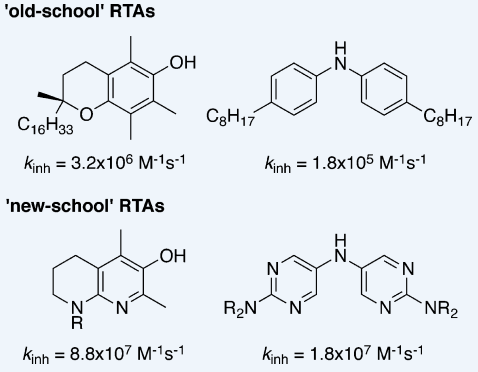
CONSPECTUS: Hydrocarbon autoxidation, the archetype free radical chain reaction, challenges the longevity of both living organisms and petroleum-derived products. The most important strategy in slowing this process is via the intervention of radical-trapping antioxidants (RTAs), which are abundant in nature and included as additives to almost every petroleum-derived product as well as several other commercial products. Accordingly, a longstanding objective of many academic and industrial scientists has been the design and development of novel RTAs that can outperform natural and industrial standards, such as α-tocopherol, the most biologically active form of vitamin E, and dialkylated diphenylamines, respectively.



Some time ago we recognized that attempts to maximize the reactivity of phenolic RTAs had largely failed because substitution of the phenolic ring with electron-donating groups to weaken the O−H bond and accelerate the rate of H atom transfer to radicals leads to compounds that are unstable in air. We surmised that incorporating nitrogen into the phenolic ring would render them more stable to one-electron oxidation, enabling their substitution with strong electron-donating groups. Guided by computational chemistry, we demonstrated that replacing the phenyl ring in very electron-rich phenols with either 3-pyridyl or 5-pyrimidyl rings leads to phenolic-like RTAs with good air stability and great reactivity. In fact, rate constants determined for the reactions of some compounds with peroxyl radicals were almost 2 orders of magnitude greater than those for α-tocopherol and implied that the reactions proceeded without an enthalpic barrier. Following extensive thermochemical and kinetic characterization, we took our studies of these compounds to more physiologically relevant media, such as lipid bilayers and human low density lipoproteins, where the heterocyclic analogues of vitamin E shone, displaying unparalleled abilities to inhibit lipid peroxidation and prompting their current investigation in animal models of degenerative disease. Moreover, we carried out studies of these compounds in several industrially relevant contexts and in particular demonstrated that they could be used synergistically with less reactive, less expensive, phenolic RTAs.

More recently, our attention has turned to the application of these ideas to maximizing the reactivity of diarylamine RTAs that are common in additives to petroleum-derived products, such as lubricating oils, transmission and hydraulic fluids, and rubber. In doing so, we have developed the most reactive diarylamines ever reported. The 3-pyridyl- and 5-pyrimidyl-containing diarylamines are easily accessed using Pd- and/or Cu-catalyzed cross-coupling reactions, and display an ideal compromise between reactivity and stability. The most reactive compounds are characterized by rate constants for reactions with peroxyl radicals that are independent of temperature, implying that—as for the most reactive heterocyclic phenols—these reactions proceed without an enthalpic barrier. Unprecedented reactivity was also observed when hydrocarbon autoxidations were carried out at elevated temperatures, real-world conditions where diarylamines are uniquely effective because of a catalytic RTA activity that makes use of the hydrocarbon substrate as a sacrificial reductant. Our studies to date suggest that heterocyclic diarylamines have real potential to increase the longevity of petroleum-derived products in a variety of applications where diphenylamines are currently used.

INTRODUCTION

Hydrocarbons and other molecules containing C−H bonds are prone to autoxidation, a process that results in the formal insertion of a molecule of O_2 into a C−H bond to yield a hydroperoxide. Autoxidation is the archetype free radical chain reaction, and follows the mechanism shown in Scheme 1 with alkyl and alkylperoxyl radicals as the chain-carrying species. The rate of autoxidation is controlled by the second propagation step, the abstraction of a H atom by a peroxyl radical, as well as the rates of radical generation (R_i) and termination. Since the reaction of alkyl radicals with O_2 takes place at (or near) the rate of diffusion, it does not usually figure into the kinetics of the process.

Organic materials possessing weak C−H bonds include petroleum-sourced materials such as oils and fuels, commercial products such as polymers and commodity chemicals, agricultural products, and manufactured foodstuffs. To inhibit

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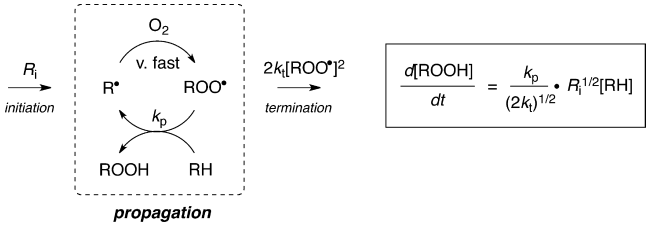
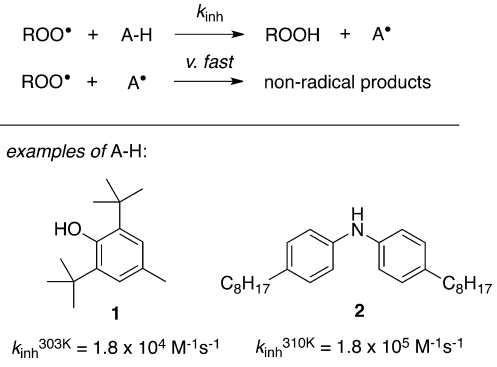
Scheme 1. Mechanism and Kinetics of Hydrocarbon Autoxidation

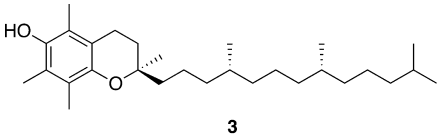


autoxidation, these materials are supplemented with significant amounts (up to 1−2 wt %) of chain-breaking antioxidants, also called radical-trapping antioxidants (RTAs). These compounds break the radical chain reaction by reducing peroxyl radicals to hydroperoxides and, in doing so, form an antioxidant-derived radical that is unreactive toward either the substrate or O_2. Instead, the antioxidant-derived radical generally reacts with a second peroxyl radical to yield non-radical products (Scheme 2).[1,2] The most widely used RTA additives are phenols and aromatic amines, examples of which are butylated hydroxytoluene (BHT, **1**) and 4,4′-dioctyldiphenylamine (**2**).

Scheme 2. Inhibition of Autoxidation by Radical-Trapping Antioxidants (A−H)



Since RTAs must outcompete the substrate for chain-carrying peroxyl radicals, both the relative reactivities and the concentrations of the RTA and substrate are key, i.e., $k_{inh}[A-H] \gg k_p[R-H]$. Thus, to minimize the amount of RTA needed to protect an oxidation-prone material, it is ideal to have a maximal k_{inh}. While efforts to develop optimal RTAs date back to the 1950s, the emerging biological significance of lipid autoxidation and its potential role in the pathophysiology of degenerative disease and aging have expanded interest in this area beyond a niche of industrial chemistry. Indeed, it was the efforts of Burton and Ingold[3] to understand the structure−activity relationships responsible for the unparalleled reactivity of α-tocopherol (α-TOH, **3**), the most biologically active form of vitamin E, that really set the mark.

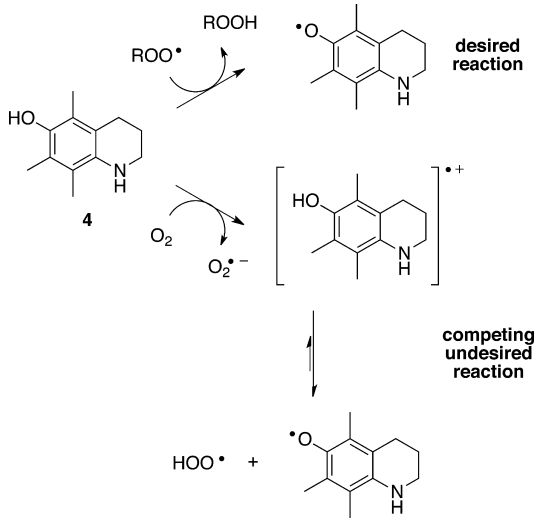


3

k_{inh}^{303K} = 3.2 x 10⁶ M⁻¹s⁻¹

On the basis of earlier observations that the k_{inh} values for substituted phenols correlate with the electron-donating abilities of the ring substituents (quantified by the Hammett-like electrophilicity parameter σ^+),[4] it was expected that replacement of the ring oxygen in the benzochroman ring of **3** with a nitrogen would yield the most reactive RTA ever. A N atom, being more electron-donating than an O atom, would better stabilize the electron-poor phenoxyl radical, weakening the O−H bond and accelerating the rate of the reaction.[5,6] Sadly, when tetrahydroquinoline **4** was synthesized and its reactivity evaluated, it became clear that the phenol had become too electron-rich; the compound was unstable in air and readily underwent autoxidation (Scheme 3).[7]

Scheme 3. Competing Antioxidant (Desired) and Pro-oxidant (Undesired) Reactions of Electron-Rich Phenolic Compounds



THE EARLY GOING

While an undergraduate researcher in the Ingold lab, one of us noticed that the vast majority of naturally occurring aromatic amines are heterocyclic and immediately wondered how much the incorporation of an electronegative heteroatom in the aromatic ring would destabilize the radical cation formed upon one-electron oxidation relative to the radical formed upon H atom abstraction by a radical. Curiosity was piqued when density functional theory calculations predicted O−H bond dissociation enthalpies (BDEs) of 87.1, 88.2, and 89.6 kcal/mol and ionization potentials of 195.4, 206.4, and 219.7 kcal/mol for the series phenol, 3-pyridinol, and 5-pyrimidinol, respectively (Table 1).[8] Subsequent calculations of the O−H BDEs in substituted phenols, 3-pyridinols, and 5-pyrimidinols revealed similar effects of ring substituents, with electron-

Table 1. Calculated O−H Bond Dissociation Enthalpies (BDEs) and Ionization Potentials (IPs) of Phenol, 3-Pyridinol, and 5-Pyrimidinol (in kcal/mol)[8]

			
O-H BDE	87.1	88.2	89.6
IP	195.4	206.4	219.7

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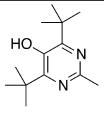
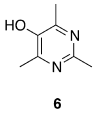
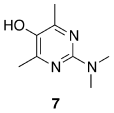
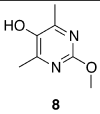
donating groups weakening the O−H bond to similar extents in each series ($\rho^+ \sim 7$).[8] These calculations suggested that 3-pyridinols and 5-pyrimidinols substituted with amine groups would be stable toward air oxidation, unlike their phenolic counterparts (i.e., **4**), but that their weak O−H bonds should lead to very fast reaction kinetics with peroxyl radicals.

In the summer of 1999, the authors were introduced to one another (by Ingold) at the Gordon Research Conference on Free Radical Chemistry, and hatched a plan to investigate the computational predictions by experiment. Two 5-pyrimidinols were initially synthesized by Giovanni Brigati, one with a substitution pattern equivalent to that of BHT and the other analogous to 2,4,6-trimethylphenol (**5** and **6**, respectively; cf. Table 2). The O−H BDEs of these compounds were

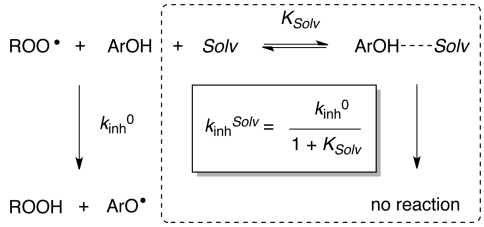
Table 2. Inhibition Rate Constants Determined at 323 K (k_{inh}^{323K}) and O−H Bond Dissociation Enthalpies (BDEs) Determined at 298 K for Some Substituted 5-Pyrimidinols in Benzene[8,11]

	 5	 6	 7	 8
k_{inh}^{323K} ($M^{-1}s^{-1}$)	2.2×10^4	3.3×10^4	8.6×10^6	2.1×10^5
O-H BDE[9] (kcal/mol)	84.1	85.2	77.1	82.5

determined experimentally by the radical equilibration electron paramagnetic resonance (REqEPR) technique and revealed that, as theory had predicted, the pyrimidinols each had O−H BDEs that were 2−3 kcal/mol larger than the equivalently substituted phenols.[8] Kinetics derived from inhibited autoxidations of styrene subsequently revealed that these compounds reacted roughly as quickly with peroxyl radicals as the phenols (within a factor of 3), despite the fact that their O−H bonds were slightly stronger.[8] This prompted the preparation of pyrimidinol **7**, a compound whose phenolic counterpart was too electron-rich to be isolated. Gratifyingly, **7** was indefinitely stable in an air atmosphere. Moreover, it was found to have an O−H BDE that was 0.1 kcal/mol lower than that of α-tocopherol (77.2 kcal/mol)[9] and reacted twice as quickly with peroxyl radicals.[8] It was remarkable that this simple compound, prepared by the trivial condensation of an easily accessed α-acyloxy-β-diketone with an alkylated guanidine, could eclipse α-tocopherol! Several other substituted pyrimidinols were subsequently investigated, including the methoxy-substituted derivative **8**, providing further validation of the computational predictions and enabling detailed studies of the kinetic and mechanistic details of their reactivity.[11]

The reactions of the pyrimidinols with peroxyl radicals were subject to primary H/D kinetic isotope effects (e.g., 3.1 for both **6** and **7**), consistent with the proton-coupled electron transfer (PCET) mechanism that is now widely believed to take place in the reaction of phenols with peroxyl radicals.[2] Likewise, these reactions were subject to the same type of kinetic solvent effect, one wherein interactions between the aryl alcohol and H-bond-accepting solvents slow the reaction through sequestration of the reactive H atom (Scheme 4).[12] The solvent effects

Scheme 4. Kinetic Solvent Effects in the Reactions of Aryl Alcohols (e.g., Phenols, 3-Pyridinols, and 5-Pyrimidinols) with Peroxyl Radicals



were more prominent for pyrimidinols because of the increased H-bond acidity of the key O−H moiety. For example, where the reaction of α-tocopherol with peroxyl radicals is slowed 4.9-fold on going from chlorobenzene to acetonitrile, the difference is 28-fold for the reaction of **8**. The increased acidity of the O−H bond in 5-pyrimidinol ($pK_a = 6.8$) relative to phenol ($pK_a = 9.9$) is the result of the inductive withdrawal of electron density by the electronegative ring nitrogens.[8,11]

The increased acidity of the pyrimidinols is believed to be key to the rather dramatic enhancement in their reactivity with alkyl radicals relative to their phenolic counterparts. For example, despite its greater H-bond acidity, pyrimidinol **7** was 5-fold more reactive toward primary alkyl radicals than α-tocopherol (in benzene). More dramatically, 5-pyrimidinol itself (i.e., no substitution) was estimated to be 100-fold more reactive than phenol in benzene despite the fact that the O−H bond in 5-pyrimidinol is 2 kcal/mol stronger than that in phenol.[8,11] The "enhanced" kinetics of the alkyl radical trapping by 5-pyrimidinols compared with phenols can be understood on the basis of a lowering of the enthalpic barrier to these reactions through increased polarization of the transition state, a so-called polar effect (cf. Figure 1).

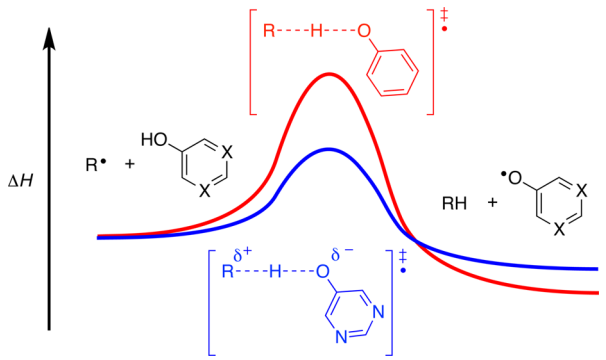


Figure 1. Schematic representation of a polar effect in the transition state of the reaction between either a 5-pyrimidinol or phenol with alkyl radicals.

■ PUSHING THE LIMIT: PYRIDINOLS

During this time, one of us was carrying out graduate research at Vanderbilt University under the supervision of Professor Ned Porter, who played a key role in the next phase of the project. It was anticipated that a pyridine ring in lieu of a pyrimidine ring would still offer favorable electronic characteristics (vide supra), but that annulation of the heterocycle to make bicyclic structures similar to α-tocopherol would now be possible. We first set out to prepare **9** in order to confirm that the substituted aminopyridines would be air-stable. Indeed they were, but less

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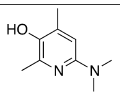
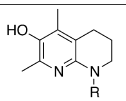
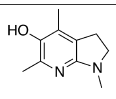
so than the corresponding pyrimidinols (several days vs indefinitely when in aerated organic solution), as expected on the basis of our computations.[13] The reactivity toward peroxyl radicals was also higher, as expected, with a rate constant of 1.6×10^7 M^{-1} s^{-1} (5-fold greater than that measured for α-tocopherol under the same conditions), due in large part to its weaker O−H bond (BDE of 75.9 kcal/mol, 1.3 kcal/mol lower than in α-tocopherol; cf. Table 3).[9]

Table 3. Inhibition Rate Constants Determined at 310 K (k_{inh}^{310K}) and O−H Bond Dissociation Enthalpies (BDEs) Determined at 298 K for Some 6-Amino-3-Pyridinols in Benzene[13,14]

			
	9	**10** R = CH_3 **12** R = $C_{16}H_{33}$	**11**
k_{inh}^{310K} ($M^{-1}s^{-1}$)	1.6×10^7	8.8×10^{7a}	2.8×10^8
O-H BDE[9] (kcal/mol)	75.9	75.2 [a]	74.3

[a]Determined for **10**.

With this precedent, we sought to prepare bicyclic compounds that more closely resembled α-tocopherol. Maikel Wijtmans was up to the task and completed the syntheses of **10** and **11**[13] along with a series of other substituted pyridinols[14] in order to confirm the predicted substituent effects. Enclosure of the amine in a six-membered ring as in **10** decreased the O−H BDE (to 75.2 kcal/mol)[9] and improved the reactivity 28-fold over that of α-tocopherol ($k_{inh} = 8.8 \times 10^7$ M^{-1} s^{-1}). Moreover, as expected on the basis of the earlier studies on phenols by Burton and Ingold,[3] contraction of the saturated ring as in **11** bolstered this even further (O−H BDE = 74.3 kcal/mol[9] and $k_{inh} = 2.8 \times 10^8$ M^{-1} s^{-1}) as a result of improved overlap between the amine lone pair and the aromatic π system.[13] As far as we are aware, compound **11** has the largest rate constant for a formal H atom transfer to a peroxyl radical, and given that log $A \sim 7-8$ for this type of reaction, it is likely that this reaction proceeds without an enthalpic barrier.

The study of a lipid-soluble variant of **10**, hexadecyl-substituted **12**, was subsequently carried out in suspensions of human low density lipoprotein (LDL) in phosphate-buffered saline.[15] LDL was chosen as a model system since the oxidation of cholesterol-esterified polyunsaturated lipids that make up the core of the LDL particle has been implicated in the onset and progression of atherosclerosis (cardiovascular disease). Incorporation of **12** into the LDL particles rendered the cholesterol-esterified lipids significantly more resistant to oxidation than in the presence of their native complement of α-TOH alone (cf. Figure 2). In fact, α-TOH is an ineffective inhibitor of lipid peroxidation in LDL (Figure 2A), instead mediating the process (so-called tocopherol-mediated peroxidation) as a result of the slow but kinetically significant reaction of the α-TOH-derived radical with lipids:[16]

$$\alpha\text{-TO}\cdot + \text{L}-\text{H} \rightarrow \alpha\text{-TOH} + \text{L}\cdot \quad (1)$$

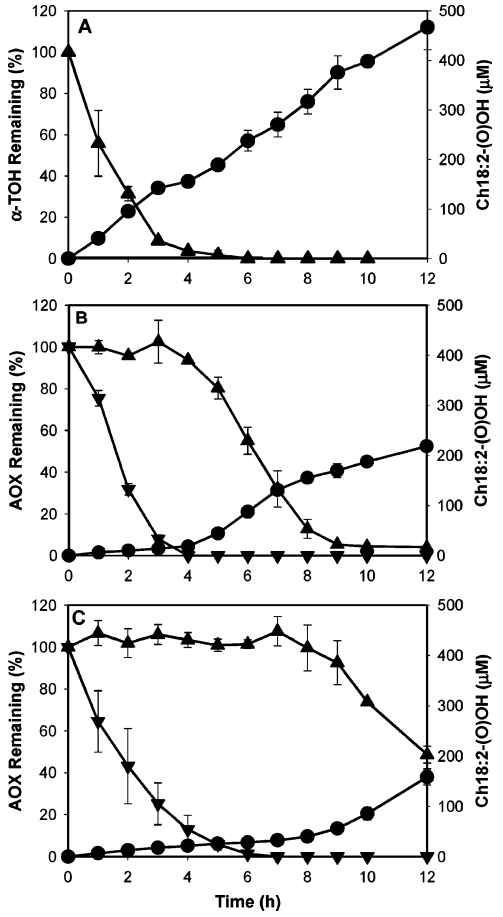
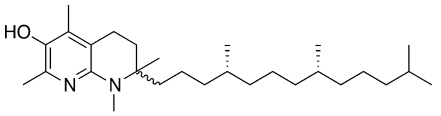


Figure 2. Oxidation of human LDL initiated by decomposition of the hydrophilic azo initiator C-0 (0.5 mM final concentration) at 37 °C. Shown are cholesterol linoleate hydroperoxides (●), α-TOH (▲), and **12** (▼) in (A) native LDL, (B) native LDL with 1.4:1 **12**/α-TOH, and (C) native LDL with 4:1 **12**/α-TOH.[15]

The analogous chain transfer reaction of the radical derived from **12** does not contribute significantly to the observed kinetics, presumably because the radical derived from **12** is ca. 2 kcal/mol more stable than α-TO·.

Given the exciting results with compound **12**, Tae-Gyu Nam was charged with the preparation of **13**, a proper aza analogue



13

of tocopherol (dubbed "N-tocopherol" or N-TOH)[17] featuring the same lipophilic side chain appended to the same quaternary position on the carbon skeleton as in α-tocopherol. While successful, this required a very lengthy synthesis (17 steps), making it impractical for most purposes, but at the very least it allowed the side-by-side comparison of an isosteric (di)aza analogue of α-tocopherol with the authentic natural product.[17] Moreover, it prompted us to think about whether these compounds could actually be used in vivo. An obstacle would be the bioavailability of the compound—an obvious issue considering that N-TOH is an electron-rich aromatic and various isoforms of hepatic cytochrome P450 have a well-established proclivity to metabolize such compounds. This question could be addressed (at least in part) if the compound

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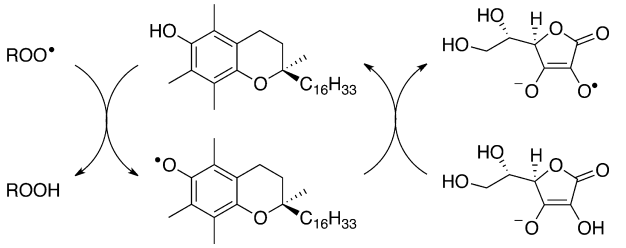
were a ligand for the tocopherol transport protein (TTP), the hepatic protein that binds α-tocopherol following its absorption in the liver, protecting it from oxidative metabolism and ensuring its delivery to peripheral tissues. Using a competitive binding assay developed by Jeffrey Atkinson at Brock University and recombinant human TTP, we were able to show that N-TOH was an equally good ligand for the protein as α-tocopherol—and several-fold better than the other naturally occurring congeners of vitamin E![17] This suggested that it may be possible to develop an RTA superior in reactivity to α-tocopherol and to use the machinery developed by the organism in order to promote its bioavailability.

A BIOLOGICAL PROBE?

Much has been made of the potential of RTAs as chemopreventive agents against degenerative disease, in particular cancer, heart, and neurodegenerative diseases. However, this potential has not obviously been realized. While many phytochemicals are believed to be RTAs, given the relatively poor reactivity of many of them and/or their poor solubility in the lipid bilayer and/or lipoproteins, it is doubtful that their biological activities can be ascribed to this reactivity. Indeed, the premier compound remains α-tocopherol, but even it suffers drawbacks, in particular its ability to mediate peroxidation (cf. eq 1) and its consequent need to be used in conjunction with ascorbate in order to subvert this process (Scheme 5).[16]

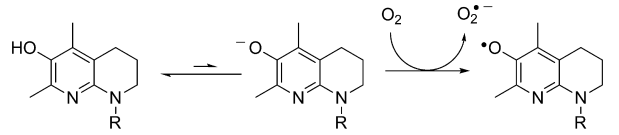
Scheme 5. Recycling of α-Tocopherol with Ascorbate



Building off our work with N-TOH, we sought to determine the optimal side chain for the naphthyridinol system, not only for efficacy of trapping radicals in lipid bilayers but also for regenerability by ascorbate and other water-soluble reductants. Moreover, we wondered whether simpler analogues that lacked the quaternary carbon at the 2-position would still bind to the TTP as do α-TOH and the isosteric analogue N-TOH. Hence, we synthesized a small library of N-alkylated analogues with varying side-chain length and branching and assayed their radical-trapping capabilities in unilamellar liposomes using a newly developed fluorogenic tocopherol derivative.[18] Interestingly, we found that while there was no effect of side-chain substitution on the radical-trapping activity of the compounds (all were at least 30-fold more reactive than α-tocopherol), there was a significant difference in the stoichiometries of the reactions due to the undesired pro-oxidant reaction shown in Scheme 6. While this reaction is not particularly relevant in nonpolar organic media, it becomes relevant in aqueous solution and/or biphasic media because of the ionization of the aryl alcohol ($pK_a \approx 10$[19]), which makes the naphthyridinol even more electron-rich, and accelerate the rate.

Thus, the more lipophilic compounds can trap two peroxyl radicals, as does α-TOH, whereas the less lipophilic compounds trap significantly fewer since they are readily consumed by

Scheme 6. Side-Chain Dependence of the Ease of Autoxidation of Naphthyridinols



R = CH_3, C_4H_9, C_5H_{11} partition extensively to aqueous phase - autoxidize readily
R = C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{15}H_{31}$, $C_{16}H_{33}$ (**12**) partition to lipid phase - stable to O_2

competing autoxidation. Moreover, the lipophilic compounds are readily regenerated in the presence of ascorbate, urate, or *N*-acetylcysteine as water-soluble reductants, and to our delight, they are good-to-excellent ligands for the TTP. In fact, compound **12** had an affinity for the TTP that was indistinguishable from that of α-tocopherol, and a branched *N*-$C_{15}H_{31}$ derivative was 10-fold better. We surmise that a hydrogen bond between an active-site serine and the sp^3-like ring nitrogen of the naphthyridinols makes up for the lack of the C2 quaternary carbon (with appropriate stereochemistry), the displaced side chain, and the differing side-chain substitution compared with the native ligand.

The foregoing collection of in vitro results have prompted us to examine the activity of these compounds in cell culture and transgenic (pro-atherogenic) mice. It is our hope that these studies will provide solid evidence in support of *or against* the hypothesis that oxidative damage is a key factor in the onset and progression of cardiovascular disease.

INDUSTRIAL RELEVANCE

Despite our focus on the potential applications of the novel compounds described above in the context of human health, we have long acknowledged that they are likely to be even more useful in industrial contexts.[20,21] The high reactivity of even simple substituted 5-pyrimidinols and 3-pyridinols with chain-carrying peroxyl radicals combined with their stability toward O_2 suggests a variety of applications in the protection of industrial materials and consumer goods from autoxidation. Moreover, the very high reactivity of the 5-pyrimidinols toward alkyl radicals suggests that they may be even more useful as inhibitors of radical polymerization, which accounts for roughly 40−45% of world polymer production.[22]

Of course, any discussion concerning the applications of pyridinols and/or pyrimidinols cannot avoid the issues of cost and availability. In fact, simple compounds such as **7** and **8** are easily accessible via relatively simple synthetic routes that have been progressively improved over the years.[11,14,23,24] While they remain more expensive than the standard phenolic compounds, it must be pointed out that these compounds are ideally suited for use in synergistic co-antioxidant mixtures with less expensive phenols, affording uncompromised performance at competitive cost.[24]

Since 3-pyridinols and 5-pyrimidinols have higher O−H BDEs than the corresponding phenols (by about 1.1 and 2.5 kcal/mol, respectively), but display similar or higher reactivities toward peroxyl radicals, they can be used synergistically with phenols. For example, binary systems comprising compound **14** and multiple equivalents of less effective 3,5-di-*tert*-butyl-4-hydroxyanisole (**15**) are equally effective in protecting styrene from autoxidation as multiple equivalents of **14** alone (cf. Figure 3). The mechanistic rationale is shown in Scheme 7, with the key features being the greater k_{inh} of **14** (3.6×10^6 M^{-1}

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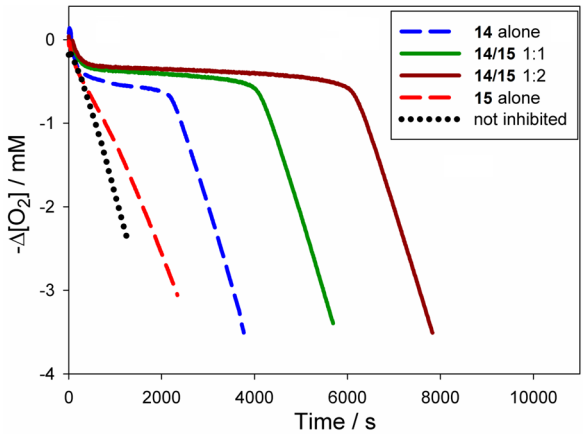
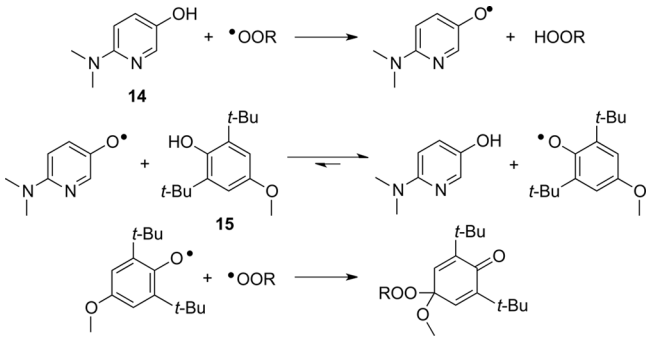


Figure 3. Example of synergism between pyridinol **14** and phenol **15** in the inhibited autoxidation of styrene.[24]

s^{-1}) relative to **15** (1.1×10^5 M^{-1} s^{-1}) and the favorable equilibrium to regenerate **14** from its corresponding aryloxyl radical using **15** ($\Delta G \approx \Delta H = -2.4$ kcal/mol).[24] The same approach can be exploited to create synergistic coinhibiting systems for radical polymerization and has in fact been exploited for use in high-performance polymer products following a joint project with the research groups of Professor Didier Gigmes at the University of Aix-Marseille and Dr. Armin Pfeil at HILTI Corporation.[25]

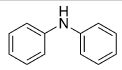
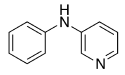
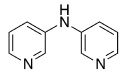
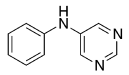
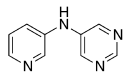
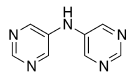
Scheme 7. Reactions Responsible for the Inhibition of Autoxidation by the Binary Co-antioxidant System of Pyridinol 14 and Phenol 15



HETEROCYCLIC DIARYLAMINES

After phenols, diphenylamines are easily the most common RTAs and in fact are the compounds of choice for applications where autoxidation at elevated temperatures is a particular problem. Diphenylamine reacts with peroxyl radicals by formal H atom transfer from the N−H group, which has a BDE of 84.7 kcal/mol;[26,9] this is slightly lower than the O−H BDE for phenol (87.2 kcal/mol),[10] accounting for its slightly higher reactivity. Similar to phenols, the BDE of diphenylamines can be modulated by substitution of the aromatic rings in conjugated positions.[27] However, unlike phenols, there has been very little success in the optimization of the chemical reactivity of diphenylamine antioxidants since their introduction in the 1950s, and 4,4′-dialkyldiphenylamines remain the industry standard. Among other reasons, we found that diphenylamines are less sensitive to substituent effects than phenols, a fact largely attributed to the decreased electron deficiency of the diphenylaminyl radical compared with a phenoxyl radical. For example, where a *p*-methoxy group weakens the O−H bond in phenol by 5.5 kcal/mol, the introduction of methoxy groups at each of the two para positions of diphenylamine weakens the N−H bond by only 4.0 kcal/mol.[5,27] Nonetheless, substitutions with strong electron-donating groups decrease their oxidation potential similarly to phenols, compromising their stability in an oxygen-rich environment and their usefulness as antioxidants. Consequently, diphenylamines were an obvious target of the approach presented above for phenols. Computation once again led the way, indicating that 3-pyridyl- and 5-pyrimidyl-containing diarylamines would have much higher ionization potentials than, but N−H BDEs that would be little different from, those of diphenylamines (Table 4).[28,29]

Table 4. Calculated N−H Bond Dissociation Enthalpies (BDEs) and Ionization Potentials (IPs) of a Variety of Heterocyclic Diarylamines (in kcal/mol)[29]

	N-H BDE	IP
	86.4	168.3
	86.8	174.7
	87.0	180.2
	86.4	179.3
	86.6	186.0
	86.5	193.0

Following up on these enticing computational results, Jason Hanthorn developed convenient means to access a wide variety of heterocyclic diarylamines[28,30] from their monoarylamine[23] precursors via Pd- and Cu-catalyzed reactions. The Pd-catalyzed aminations were enabled greatly by the use of an unconventional precatalyst, Pd(η^3-cinnamyl)(η^5-Cp), which cleanly gives the catalytically active PdL2 complex immediately upon exposure to a phosphine ligand (L). With a large library of symmetric and unsymmetric substituted diarylamines in hand, Hanthorn was able to determine the kinetics of their reactions with peroxyl radicals using a radical clock methodology he developed in parallel,[31] as well as the redox potentials of the compounds, some of which are shown in Table 5 along with N−H BDEs determined by the eminently reliable REqEPR method.[28,29]

The experimental data were again fully consistent with our theoretical predictions. For example, upon replacement of the two phenyl rings in the industrial standard dialkyldiphenyl-

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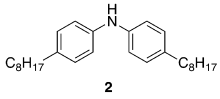
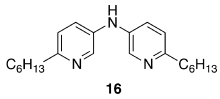
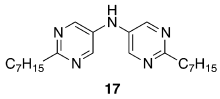
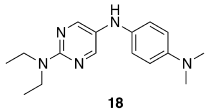
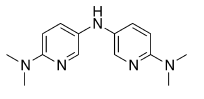
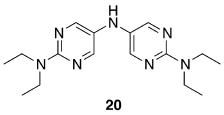
Table 5. Representative Heterocyclic Diarylamines and Some of Their Relevant Properties[28,29]

	k_{inh}^{310K} ($M^{-1}s^{-1}$)	E^0 (V)	BDE (kcal/mol)
 **2**	1.8×10^5	1.02[a]	82.2
 **16**	9.0×10^4	1.12[a]	83.1
 **17**	3.0×10^4	1.55[a]	84.1
 **18**	3.7×10^7	0.44	79.0
 **19**	3.4×10^7	0.44	78.8
 **20**	1.7×10^7	0.65	79.2

[a] E_{pa} values are given since the voltammagrams were not reversible.

amine **2** with two pyridyl rings (**16**), the oxidation potential increased by 100 mV, but the reactivity dropped only 2-fold.[28] More dramatically, two pyrimidyl rings (**17**) increased the oxidation potential by over 0.5 V relative to **2**, but the reactivity dropped only 6-fold.[28] These initial results indicated that heterocyclic diarylamines bearing strong electron-donating substituents (such as R_2N-) were likely to be stable to air oxidation, unlike the corresponding diphenylamines.

In fact, a series of heterocyclic diarylamines bearing *N*,*N*-dialkylamino substitution at the para positions with respect to the reactive N−H could be synthesized and the rate constants for their reactions with peroxyl radicals determined, revealing that they were up to 200-fold more reactive than the industrial standard 4,4′-dialkyldiphenylamines (e.g., compare compounds **18**−**20** with **2**). The high reactivities of these compounds, and the similarity in their reactivities, could clearly be attributed to their weak N−H BDEs, which were almost indistinguishable regardless of the extent of nitrogen incorporation in the ring (for comparison, the N−H BDE in the bis(*N*,*N*-dialkylamino)-substituted diphenylamine was 78.4 kcal/mol).[28,29] The temperature dependence of k_{inh} was determined for three representative diarylamines, including 4,4′-dioctyldiphenylamine **2**, and pre-exponential factors of log $A \approx 7$ were found, implying that the reactions of **18**−**20** with peroxyl radicals proceed with $E_a \approx 0$ and rates that are independent of temperature.

Consideration of all of the data we collected on the heterocyclic diarylamines revealed that, compared with phenols, insertion of a ring nitrogen into the diarylamine scaffold results in a much smaller increase in the BDE of the key bond (viz. 0.1−0.3 kcal/mol per N atom in diarylamines vs 1.1−1.5 kcal/mol per N atom in phenols).[29] We ascribed this difference to the fact that a diarylaminyl radical is inherently less electron-poor than a phenoxyl radical and is therefore destabilized to a lesser extent upon introduction of the more electronegative N atom(s) in the aromatic rings in place of carbon(s). Another intriguing finding was that diarylamines are inherently more reactive than phenols—that is, diarylamines with a given N−H BDE react more quickly with peroxyl radicals than phenols with the same (O−H) BDE. We explained this observation on the basis of the PCET mechanism of the formal H atom transfer between phenols and diphenylamines and peroxyl radicals. In the PCET transition state (Figure 4), the proton moves

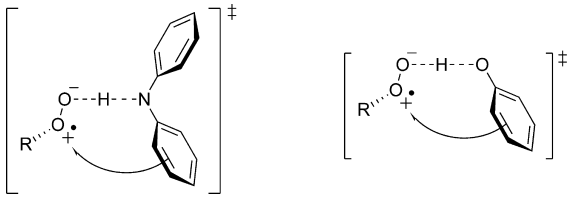
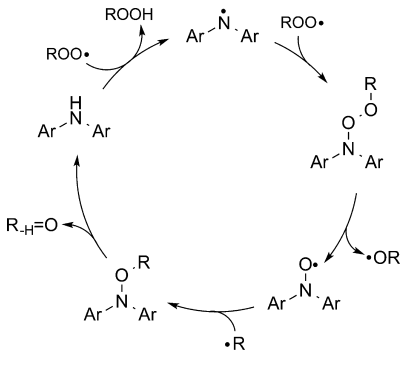


Figure 4. Schematic depictions of the transition states for the proton-coupled electron transfer from diphenylamine (left) and phenol (right) to a peroxyl radical.

between the diphenylamine (or phenol) and peroxyl radical via lone pairs on the N (or O) atom and the terminal oxygen atom of the peroxyl radical, respectively, while an electron moves from the π-HOMO centered on the aryl ring of the amine (or phenol) to the π-SOMO of the peroxyl radical. Since diarylamines have higher HOMO energies than phenols, they provide better orbital overlap with the π-SOMO of the peroxyl radical.[28,29]

More recently, we have expanded the study of these heterocyclic diarylamines to elevated temperatures, where diphenylamines are uniquely reactive because of the possibility for their regeneration via the catalytic cycle shown in Scheme 8. Elevated temperatures are required for this chemistry to occur since the N−O bond in the *N*,*N*-diarylalkoxyamine derived from combination of a diarylnitroxide and substrate-derived alkyl radical must be cleaved in order to reform the diarylamine.[32,33]

Scheme 8. Mechanism of Catalytic Activity of Diarylamine RTAs



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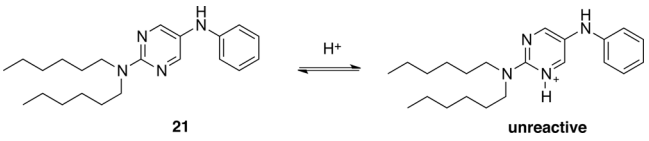
Following Ron Shah's synthesis of a series of heterocyclic diarylamines with higher alkyl substitution to ensure low volatility and good solubility of the compounds, he and Evan Haidasz carried out inhibited autoxidations of neat *n*-hexadecane in a stirred flow reactor at 160 °C and hydroperoxide production was assayed using a pro-fluorescent phosphine developed in one of our laboratories.[34] While most of the investigated compounds outperformed the industrial standard diphenylamine **2**,[35] the results were disappointing because the differences were not as dramatic as those we had observed at ambient temperatures (vide supra).

However, we soon realized that the heterocyclic diarylamines, such as **21**, which are much more basic than diphenylamine **2**, are deactivated by carboxylic acids formed upon autoxidation of hexadecane.[35] Protonation of the diarylamine strengthens the key N−H bond through destabilization of the electron-poor diarylaminyl radical, slowing the reaction with peroxyl radicals (Scheme 9).

Scheme 9. Protonation Deactivates Heterocyclic Diarylamines toward H Atom Transfer



Accordingly, the true reactivity of the diarylamines in these autoxidations could be revealed upon the addition of a base to neutralize the acids that are produced. For example, when 2,4,6-tri-*tert*-butylpyridine was added to an autoxidation inhibited by **21**, an unprecedented inhibition period was observed (cf. Figure 5).[35] No significant differences were observed in corresponding autoxidations in the absence of antioxidant or in the presence of the industry standard **2**, which is far less basic than **21** (the pK_a difference of their conjugate acids is ca. 4 units). This phenomenon was shown to be general, as other bases, such as cesium carbonate and a *tert*-alkyl primary amine, were also able to "rescue" the reactivity of **21** and the other heterocyclic amines. Whether the enhanced reactivity of these compounds can be exploited for real-world applications is currently under investigation.

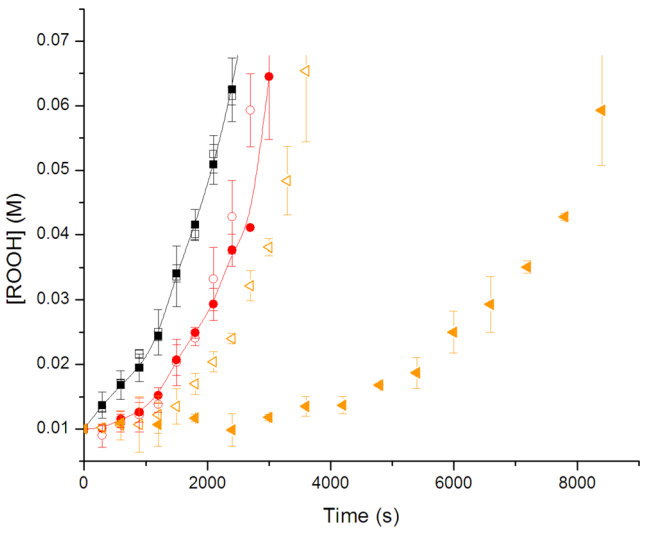


Figure 5. Hydroperoxide formation in the autoxidation of *n*-hexadecane at 160 °C initiated by 10 mM tetralin hydroperoxide (black squares) and inhibited by either **2** (red circles) or **21** (yellow triangles) (40 μM) in the presence (solid symbols) or absence (open symbols) of 2,4,6-tri-*tert*-butylpyridine (1 mM).[35]

PERSPECTIVE

The development of RTAs with optimal (i.e., barrierless) reactivity with peroxyl radicals has long been an objective of academic and industrial researchers. While the foregoing recounts our approach to achieve this objective, it remains to fully realize the potential of these structures in both commercial and industrial applications. While the 3-pyridinols and 5-pyrimidinols have been exploited in at least one industrial application of which we are aware, we anticipate that our recent demonstration of their synergistic potential and a presentation of the simple recipe for how this can be achieved is likely to prompt other applications. Likewise, our efforts to elucidate the structure−reactivity relationships that will govern the extension of their use from simple organic solutions to lipid bilayers and lipoproteins is likely to prompt the use of these compounds to probe the role of lipid peroxidation in pathophysiological processes.

Moderation of the electron-richness of phenols via inclusion of one or more heteroatoms in the aromatic ring can be utilized in a variety of contexts, not simply for the design of potent RTAs. For example, we employed this strategy to develop tyrosine analogues with varying redox and acid/base properties for mechanistic studies of proton-coupled electron transfer reactions in peptides and proteins[36] and also in the design of acetaminophen analogues with varying redox and acid/base properties for mechanistic studies on acetaminophen's ability to inhibit enzyme-catalyzed lipid peroxidation by cyclooxygenases and lipoxygenases.[19] Interestingly, it was the study of pyridinol analogues of organotellurophenols[37] that led to the recent discovery of an unusual mechanism underlying the impressive activity of recyclable organochalcogen-based glutathione peroxidase mimics.[38] This strategy will surely continue to be useful for us—and hopefully others—in mechanistic studies and/or the development of therapeutically and/or commercially relevant compounds.

Perhaps the most directly useful compounds that have come from our work in this area are the heterocyclic diarylamines introduced in the last section. However, it must be acknowledged that the mechanistic underpinnings of the reactivity of diarylamine antioxidants are still far from clear, despite our recent discovery that some of these compounds are regenerated in situ via an unprecendented retro-carbonyl-ene reaction.[33] Does the same chemistry operate with the heterocyclic compounds? Moreover, our preliminary work demonstrates that these compounds trap more than the classical "two" radicals even at ambient temperatures. Why? Is there something else to the reactivity of these compounds? We aim to find out!

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Notes

The authors declare no competing financial interest.

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Biographies

Luca Valgimigli (born 1969) received his M.Sci. (Med. Chem., Magna cum Laude) degree in 1994 and his Ph.D. degree (Org. Chem.) in 1998, both from the University of Bologna, after a visiting period (1995) at the National Research Council of Canada in Ottawa. In 1999 he joined the University of Bologna as a researcher and is currently Associate Professor of Organic Chemistry.

Derek Pratt (born 1976) received his B.Sc. (Hon. Chem.) degree from Carleton University in 1999 and his Ph.D. degree from Vanderbilt University in 2003. After completing postdoctoral work at the University of Illinois at Urbana−Champaign in 2005, he returned to Canada to take up a faculty position at Queen's University. In 2010 he moved to the University of Ottawa, where he is Associate Professor and Canada Research Chair in Free Radical Chemistry.

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First and foremost, we thank all of the co-workers that contributed to the scientific pursuits recounted above. We encourage the reader to consult the cited literature in order to identify them explicitly. We owe a particular debt of gratitude to Keith Ingold for introducing the co-authors to one another some 15 years ago (!) and also to Ned Porter, Gian Franco Pedulli, and Gino DiLabio for their support, encouragement, and contributions to this work. Lastly, we must acknowledge the funding agencies that have supported this project over the years, especially the Natural Sciences and Engineering Research Council (Canada), il Ministero dell' Istruzione, dell' Università e della Ricerca PRIN 2010−2011 2010PFLRJR (Italy), and the National Science Foundation (USA).

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