6-Amino-3-Pyridinols: Towards Diffusion-Controlled Chain-Breaking Antioxidants

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6-Amino-3-Pyridinols: Towards Diffusion-Controlled Chain-Breaking Antioxidants**

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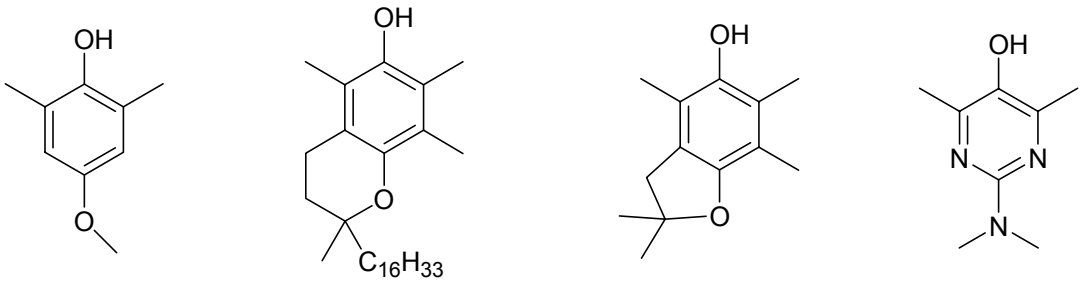
Peroxyl radicals ($LOO^{\bullet}$) are the chain-propagating species in the rate-determining step of lipid peroxidation (Eq. 1). Antioxidants, most commonly substituted phenols (ArOH) such as **1**-**3**, effectively intercept peroxyls by transferring the phenolic H-atom to the propagating radical (Eq. 2), at a rate (k_{inh}[ArOH]) faster than that of chain propagation (k_p[L-H]). The best-known example of a phenolic antioxidant is α-tocopherol (α-TOH, **2**), the most potent form of Vitamin E, Nature's primary defense against radical chain oxidation.[1] Recently, considerable effort has been devoted to the development of antioxidants more effective than α-TOH,[2],[3],[4],[5] since the *in-vivo* peroxidation of lipids has been suggested to be a major factor in the development of several degenerative diseases.[6]

$$LOO^{\bullet} + L\text{-}H \xrightarrow{k_p} LOOH + L^{\bullet} \qquad \text{Eq. 1}$$

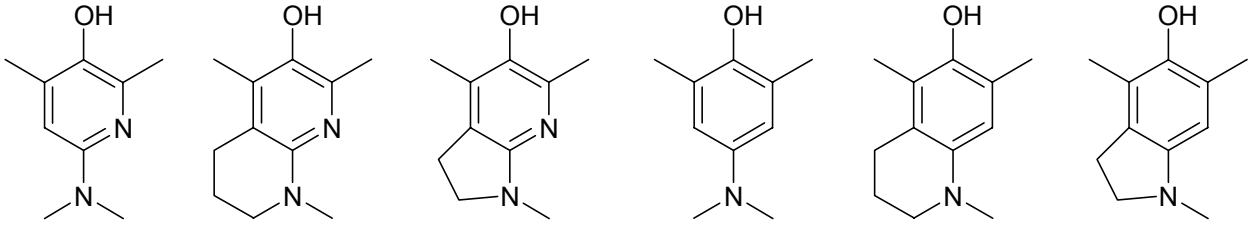
$$LOO^{\bullet} + ArOH \xrightarrow{k_{inh}} LOOH + ArO^{\bullet} \qquad \text{Eq. 2}$$

The bond dissociation enthalpy (BDE) of the phenolic O-H plays a central role in determining antioxidant efficacy with compounds having lower O-H BDEs generally being better antioxidants.[7],[8] Substitution with electron donating (ED) groups at the *o*- and *p*- positions to the phenolic O-H leads to compounds having lower BDEs. However good ED substituents also lead to a decrease in the ionization potential (IP) of the phenol, thereby rendering the resulting antioxidant directly reactive with oxygen. This results in limited air-stability of the antioxidant and in the potential generation of toxic products in biological environments. Calculations predicted that incorporation of two nitrogen atoms at the 3 and 5 positions of the phenolic ring significantly raises the IP and greatly improves the air stability of the antioxidant while only minimally lowering the O-H BDE.[4] Experiments confirmed this prediction: thus, for example, the 5-pyrimidinol **4** is perfectly stable in air and has a k_{inh} about twice that of α-TOH.[4]



1 **2** **3** **4**

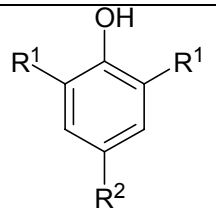
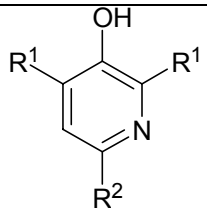
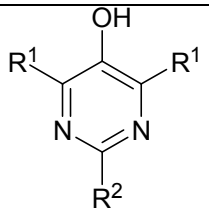
Calculations also predicted that 3-pyridinol would have a lower O-H BDE than the analogous 5-pyrimidinol and an IP intermediate to those of phenol and 5-pyrimidinol.[4] We therefore expected that 6-amino-3-pyridinols such as **5** would have lower O-H BDEs than the analogous 5-pyrimidinols (e.g. **4**) while maintaining improved air-stability compared to the corresponding phenols. Furthermore, the free 5-position in the pyridine-skeleton allows for fusion of aliphatic rings (i.e. to give **6** and **7**); structural changes that have been shown to increase k_{inh} significantly in the series **1**, **2** and **3** because of improved stereoelectronics.[2] In this communication we show by theory the effects of substitution on the O-H BDE (antioxidant activity) and IP (air stability) of a series of 3-pyridinols. We also report here the synthesis and experimental investigation of three new 3-pyridinol antioxidants that were selected on the basis of our calculations.



5 **6** **7** **8** **9** **10**

The calculated O-H BDEs and IPs of several substituted phenols, 3-pyridinols and 5-pyrimidinols were obtained using density functional theory models ((RO)B3LYP/6-311+G(2d,2p)//AM1/AM1 and B3LYP/6-31G(d)//AM1/AM1, respectively) and the values are given in Table 1.[9],[10]

Table 1. Calculated Substituent Effects on Gas Phase O-H BDEs at 298K and Adiabatic IPs at 0K of Substituted Phenols, 3-Pyridinols and 5-Pyrimidinols.[a]

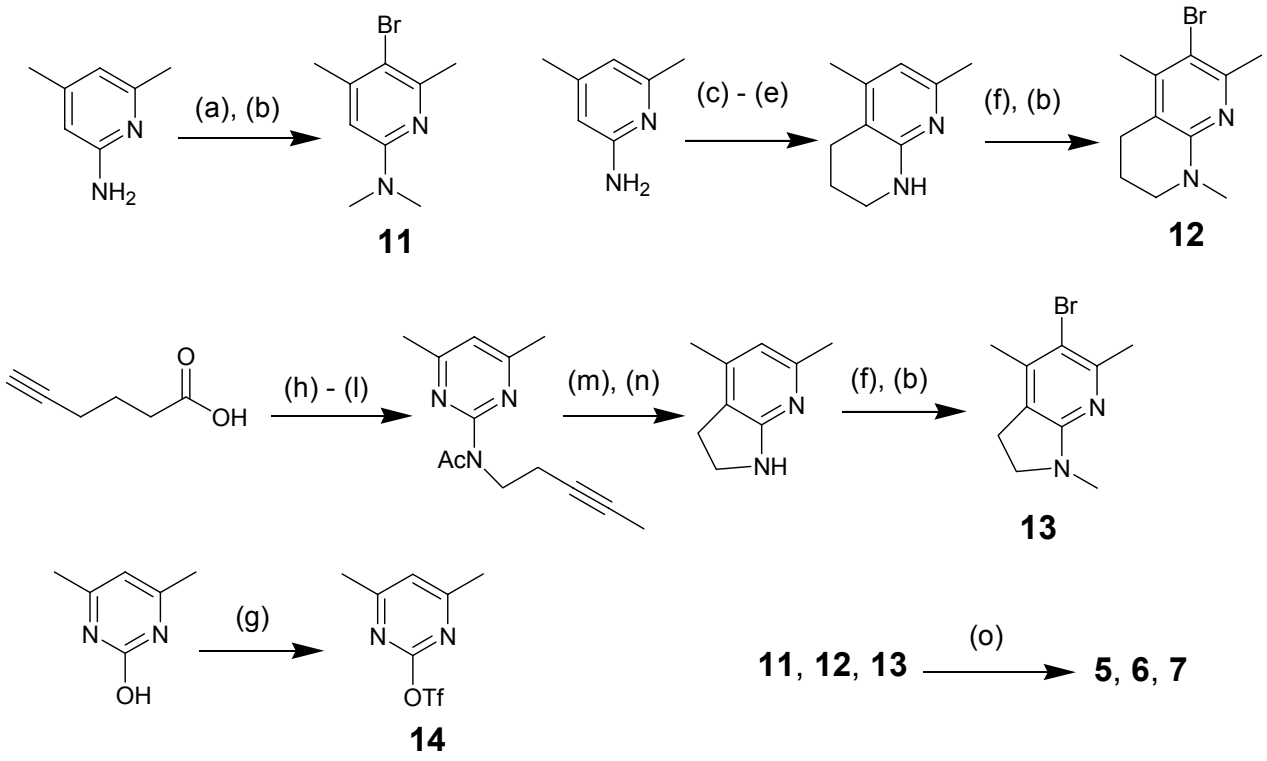
						
	BDE	IP	BDE	IP	BDE	IP
Substitution						
R^1=H, R^2=H	87.1 (0.0)	195.4 (0.0)	88.2 (0.0)	206.4 (0.0)	89.6 (0.0)	219.7 (0.0)
R^1=H, R^2=CH_3	84.6 (-2.5)	186.9 (-8.5)	85.4 (-2.8)	196.6 (-9.8)	86.8 (-2.8)	209.3 (-10.4)
R^1=CH_3, R^2=CH_3	80.4 (-6.7)	178.3 (-17.1)	81.1 (-7.1)	186.3 (-20.1)	83.2 (-6.4)	198.0 (-22.7)
R^1=H, R^2=OCH_3	81.0 (-6.1)	176.5 (-18.9)	82.0 (-6.2)	186.1 (-20.3)	83.6 (-6.0)	198.1 (-21.6)
R^1=CH_3, R^2=OCH_3	77.0 (-10.1)	169.2 (-26.2)	78.1 (-10.1)	177.4 (-29.0)	79.8 (-9.8)	188.3 (-31.4)
R^1=H, R^2=$N(CH_3)_2$	77.0 (-10.1)	157.7 (-37.7)	77.0 (-11.2)	164.6 (-41.8)	78.3 (-11.3)	174.6 (-45.1)
Compounds						
8, **5**, **4** (monocyclic)	72.3 (-14.8)	152.3 (-43.1)	73.5 (-14.7)	157.7 (-48.7)	74.1 (-15.5)	167.0 (-52.7)
9, **6** (fused 6-ring)	71.2 (-15.9)	148.3 (-47.1)	73.3 (-14.9)	154.6 (-51.8)		
10, **7** (fused 5-ring)	70.5 (-16.6)	145.2 (-50.3)	72.4 (-15.8)	152.3 (-54.1)		
2 (α-TOH)	74.8 (-12.3)	159.3 (-36.1)				

[a] Data for phenols and 5-pyrimidinols are from Ref. [4] except for **8**, **9** and **10**. All values are in kcal mol^{-1}. Substituent effects (relative to the unsubstituted parent) are in parentheses.

Clear trends can be seen from Table 1. For all three classes, the O-H BDE and IP values decrease by increasing the electron density into the aromatic ring. The order for the calculated values of these properties is always phenol < 3-pyridinol < 5-pyrimidinol. The calculations suggest that the highest antioxidant activities in conjunction with reasonable air-stability (IP values comparable to **2**)

are expected from pyridinols **5**-**7**. Therefore, we selected these three molecules as targets for synthesis and experimental investigations.

Scheme 1. Synthetic Approach to 3-Pyridinols **5**-**7**.



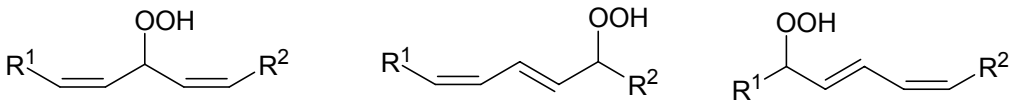
Key: (a) dibromodimethylhydantoin (DBDMH), CH_2Cl_2, -40 °C, 40 min, 67 %; (b) HCOOH, aq. H_2CO, reflux, 18 h, 100 % for **11**, 77 % for **12**, 84 % for **13**; (c) acrylic acid, pyridine, reflux, 24 h, 30 % after cryst.; (d) polyphosphoric acid, 125 °C, 40 min, 75 %; (e) BH_3·THF, THF, reflux, 18 h, 83 %; (f) DBDMH, CH_2Cl_2, -78 °C, 10-30 min, 93 % for **12**, 83 % for **13**; (g) Tf_2O, Et_3N, CH_2Cl_2, 0 °C, 1 h, 98 %; (h) 15 M aq. KOH, reflux, 4 h, 95 %; (i) $(PhO)_2PON_3$, *t*-BuOH, reflux, 30 h, 79 %; (j) ethereal HCl, 24 h, 70 % (k) 14, Et_3N, DMF, 1 d, 93 %; (l) Ac_2O, DMAP, 100 °C, 20 h, 91 %; (m) Ph_2O, reflux, 10 h, 82 %; (n) NaOH, MeOH, reflux, 20 h, 100 %; (o) 1. *n*-BuLi, THF, -78 °C, 30 min; 2. dry 2-nitro-*m*-xylene, THF, -78 °C, 1 to 3 h, 63 % for **5**, 25 % for **6**, 27 % for **7**.

There are few synthetic approaches to 6-amino-3-pyridinol structures described in the literature and **5**-**7** have never been reported.[11] We devised a synthetic sequence in which the reactive -OH moiety is introduced at low temperatures in the last step, thus minimizing potential decomposition of product

and/or intermediates. Only a brief description of the syntheses will be presented here; a forthcoming paper will report our extensive synthetic efforts in detail. Construction of the appropriate pyridine substructure involved a three-step Friedel-Crafts approach for **6** and a seven-step intramolecular Diels-Alder sequence for **7**. Construction of the pyridine was followed by bromination, methylation and hydroxylation (Scheme 1). The pyridinols **5**-**7** were obtained as yellow or orange solids.

Table 2 collects the O-H BDEs that were measured experimentally by radical equilibration EPR studies.[7b] The absolute experimental values (in benzene) were slightly higher (2 – 3 kcal mol^{-1}) than the calculated (gas-phase) values (Table 1), an issue already addressed in previous works.[4] However, the corresponding ΔBDE (BDE_{PyrOH} – $BDE_{\alpha-TOH}$) shows good agreement between experimental and calculated substituent effects for the 3-pyridinol series. The stability of the 3-pyridinols towards air was examined by monitoring their typical fluorescence and UV absorbance (see Supporting Information, SI) in aerated *tert*-butylbenzene solution (0.3 mM) at 37 °C. The simple 3-pyridinol **5** was stable over a 24 h period while **7** showed significant decomposition over the same time course, the compound **6** showing stability intermediate of **5** and **7**, in parallel with the calculated IPs for these compounds (Table 1).

Two different experimental approaches were used to determine absolute k_{inh} for the 3-pyridinols. First, we utilized a peroxyl radical clock based on the antioxidant-dependent trapping of bisallylic (11-OOH) and conjugated (9- and 13-OOH) hydroperoxide products in initiator-induced methyl linoleate autoxidations.[12] At low concentrations of antioxidant (10 – 100 mM), there is a linear correlation between concentration of antioxidant and ratio of trapping of 11-$OO^{\bullet}$ vs 9- and 13-$OO^{\bullet}$ with the slope of the line directly related to k_{inh} of the antioxidant.[12] Thus, by measuring the ratio of linoleate hydroperoxide products at various concentrations of an antioxidant it is possible to determine its k_{inh}.



11-OOH **9-OOH** **13-OOH**

$R^1=C_5H_{11}$, $R^2=(CH_2)_7COOMe$

3-Pyridinol **5** proved more effective than **4** or **2** in trapping 11-OO$^\bullet$ and a value of k_{inh} = 1.16 (± 0.04) x 10^7 M^{-1} s^{-1} was determined by this method (see SI). The total yield of hydroperoxide products formed from linoleate in the presence of **5** was ca. eight-fold lower than that observed for identical oxidations inhibited by **2** (data not shown). Interestingly, **6** and **7** proved to be such good inhibitors that we were unable to obtain reliable k_{inh} values for these compounds using the radical clock method.[13] Controlled styrene autoxidations inhibited by **5** – **7** were well behaved, however,[2] and allowed us to investigate the antioxidant activity of these compounds (see SI). The stoichiometric factor *n* (number of chains broken by one molecule of antioxidant) was determined to be 2 for all three compounds, similar to phenols and pyrimidinols.[4] The inhibition rate constants k_{inh} were determined from the slope of the inhibited oxygen consumption plots,[2],[4],[14] and the values obtained are presented in Table 2 together with previous data for **2** and **4**, reported for comparison. [4],[12]

Table 2. Solution Phase O-H BDEs and Inhibition Rate Constants for 3-Pyridinols **5**-**7**.

Antioxidant	O-H BDE (kcal mol^{-1})[a]	Δ(O-H BDE) (kcal mol^{-1})[b]	k_{inh} by clock (x 10^7 $M^{-1}s^{-1}$)[c]	k_{inh} by O_2 uptake (x 10^7 $M^{-1}s^{-1}$)[d]	$k_{inh}/k_{inh}(2)$[e]
5	77.0 ± 0.5	-1.3 / -1.3	1.16 ± 0.04	1.6 ± 0.6	5.0
6	76.3 ± 0.6	-2.0 / -1.5	N.D.[f]	8.8 ± 3.2	28
7	75.4 ± 0.7	-2.9 / -2.4	N.D.[f]	28.0 ± 18[g]	88
4	78.2 ± 0.3	-0.1 / -0.7	0.65 ± 0.08	0.86 ± 0.05	2.1
2 (α-TOH)	78.3 ± 0.3	0.0 / 0.0	0.38[h]	0.32[h]	1.0

[a] From EPR equilibration studies. [b] Experimental BDE (78.3 kcal mol^{-1}) or calculated BDE (74.8 kcal mol^{-1}) for α-TOH, **2**, as reference. Values are experimental/calculated ΔBDE. [c] In benzene at 37

°C by methyl linoleate radical clock. [d] In chlorobenzene by inhibited styrene autoxidation at 30 °C for **2**, **5**, **6**, **7**; in benzene at 50 °C for **4**. [e] k_{inh} from O_2 uptake were used. [f] See text. [g] The rather large error (=2SD) is the result of the large scatter in the measurements due to the dramatic inhibition by **7**; initiation rates as low as $1x10^{-10}$ s^{-1} had to be used in order to obtain measurable rates of oxygen consumption (as low as $1x10^{-9}$ $M^{-1}s^{-1}$) with concentrations of **7** as low as 2×10^{-7} M. [h] Value used to calibrate the radical clock. [h] Value from ref. [5a], re-confirmed in ref. [6]. The value of 0.41 ± 0.04 at 50 °C is used for comparison with **4**.

The 6-amino-3-pyridinols described here are a novel class of phenolic antioxidants that are more effective than any other phenolic class reported to date. The vacant 5-position on the pyridine ring of these compounds allows for fusion of an additional aliphatic ring, a substitution that further lowers the O-H BDE. Indeed, the pyridinols **6** and **7** are, to the best of our knowledge, the fastest chain-breaking antioxidants ever reported (previously 3,7-dimethoxy-phenothiazine with $k_{inh} = 5.5 \times 10^7$ $M^{-1}s^{-1}$).[15],[16] The air-stability of **7** is moderate but its k_{inh} exceeds 10^8 $M^{-1}s^{-1}$, thus approaching the diffusion-controlled limit for a bimolecular reaction.

Keywords: lipids · antioxidants · autoxidation · pyridines · ab initio calculations

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[16] This compound is quite reactive with air, much more so than **6** or **7**; quantitatively this is indicated by our calculated IP that is 7.8 and 5.5 kcal mol^{-1} lower than **6** and **7**, respectively.

SUPPORTING INFORMATION

for

6-Amino-3-Pyridinols: Towards Diffusion-Controlled Chain-Breaking Antioxidants**

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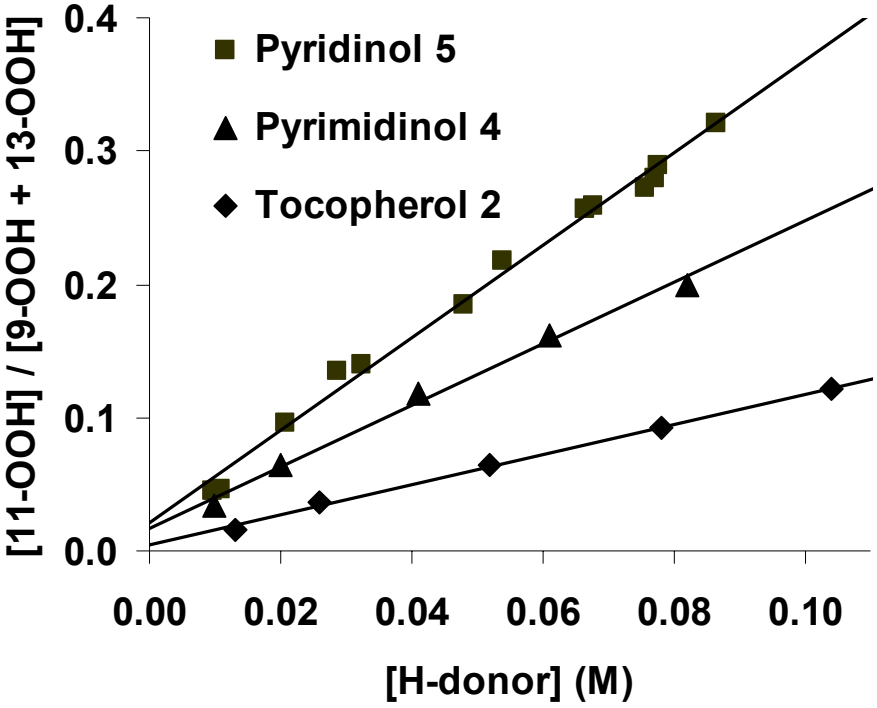

Figure S1. Determination of k_{inh} by the peroxyl radical clock method. Plotted are the values for [11-OOH] / ([9-OOH] + [13-OOH]) versus concentration of antioxidant in methyl linoleate autoxidations in benzene (200 mM) at 37 °C initiated by MeOAMVN (10 mM). Data for **2**, **4** and **5** are shown with the corresponding linear correlations from which the rate constants in Table 2 were derived.

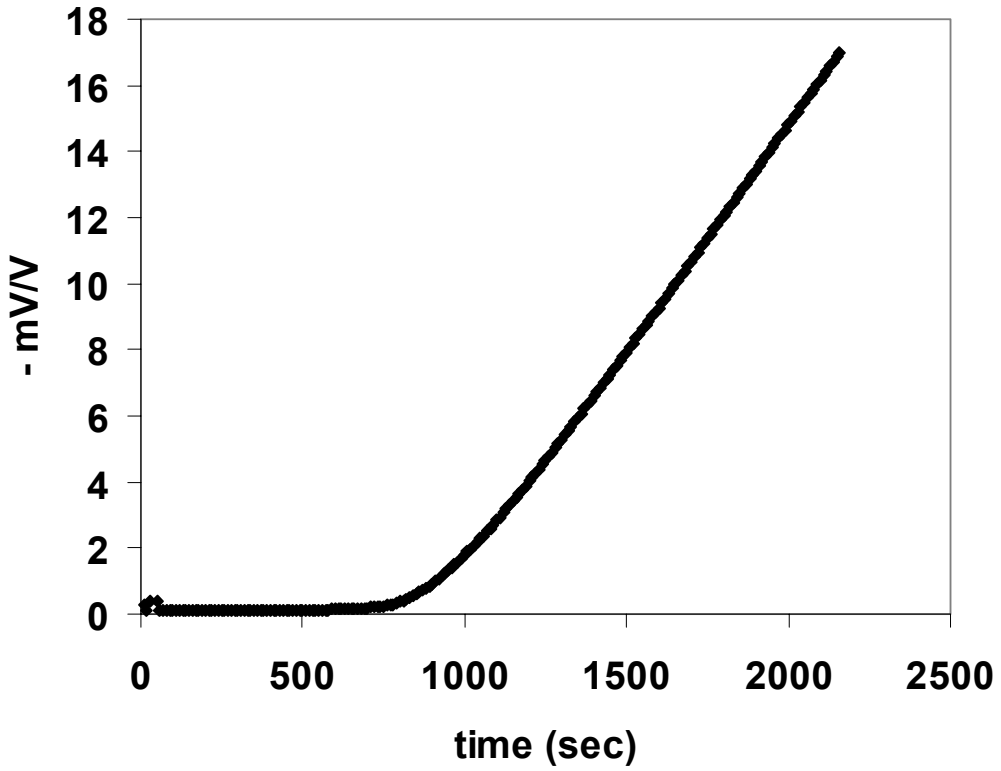

Figure S2. A typical oxygen uptake plot for an inhibited styrene autoxidation. This plot shows the oxygen consumption during autoxidation of styrene (8.6M) in chlorobenzene at 30°C initiated by AMVN (1.25 mM) in the presence of **6** (1.1 μM).

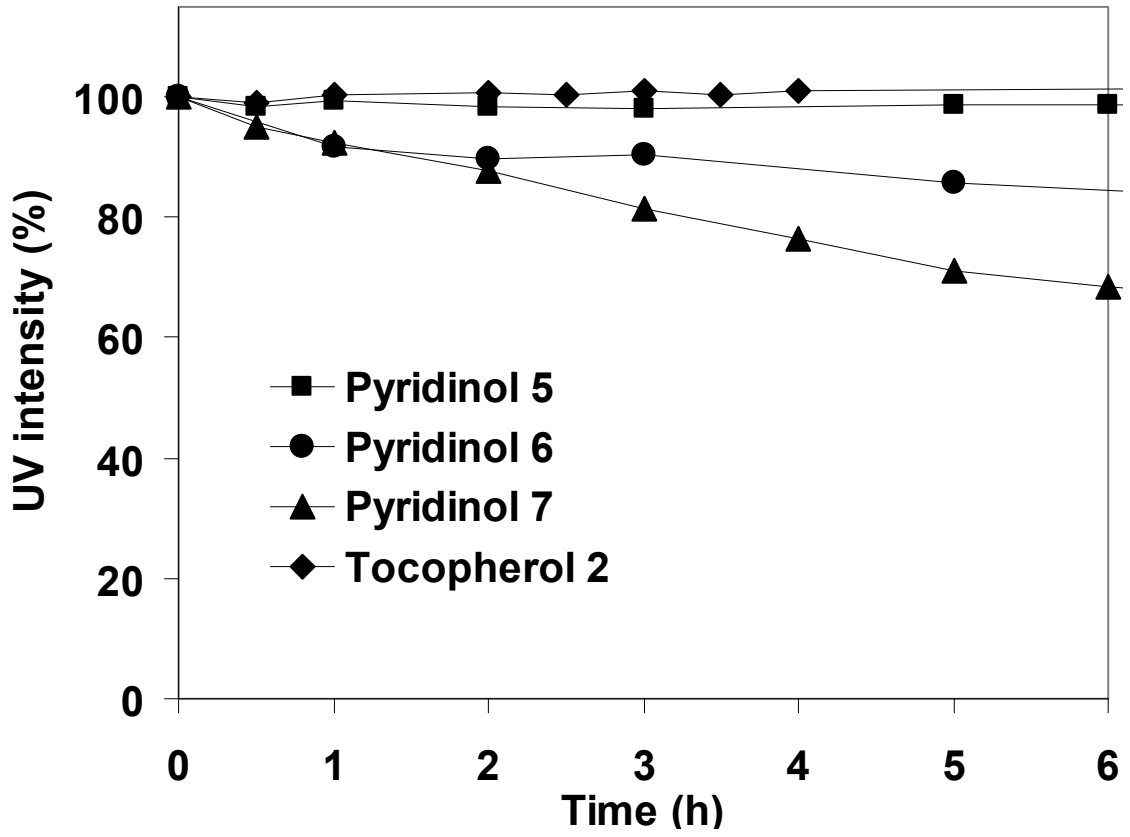

Figure S3. Decay of UV signal for 3-pyridinols **5**, **6** and **7** (@ 330 nm) and α-tocopherol **2** (@ 304 nm) in aerated *t*-butylbenzene (300 μM) at 37 °C.