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The L-Type Calcium Channel Blockers, Hantzsch 1,4-Dihydropyridines, Are Not Peroxyl Radical-Trapping, Chain-Breaking Antioxidants

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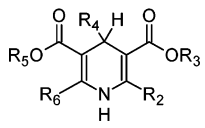
The antioxidant properties of Hantzsch 1,4-dihydropyridine esters and two dibenzo-1,4-dihydropyridines, 9,10-dihydroacridine (DHAC) and *N*-methyl-9,10-dihydroacridine (*N*-Me-DHAC), have been explored by determining whether they retard the autoxidation of styrene or cumene at 30 °C. Despite a claim to the contrary [(2003) *Chem. Res. Toxicol. 16*, 208−215], the Hantsch esters were found to be virtually inactive as chain-breaking antioxidants (CBAs), their reactivity toward peroxyl radicals being some 5 orders of magnitude lower than that of the excellent CBA, 2,2,5,7,8-pentamethyl-6-hydroxy-chroman (PMHC). DHAC was found to be about a factor of 10 less reactive than PMHC. From kinetic measurements using DHAC, *N*-deuterio-DHAC, and *N*-Me-DHAC, it is concluded that it is the N−H hydrogen in DHAC that is abstracted by peroxyl radicals, despite the fact that in DHAC the calculated C−H bond dissociation enthalpy (BDE) is about 11 kcal/mol lower than the N−H BDE. The rates of hydrogen atom abstraction by the 2,2-diphenyl-1-picrylhydrazyl radical (dpph$^\bullet$) have also been determined for the same series of compounds. The trends in the peroxyl and dpph$^\bullet$ rate constants are similar.

Introduction

1,4-Dihydropyridine (DHP) calcium channel modulators also known as Hantzsch esters, **1**, interact strongly with the α-1 subunit of voltage-dependent L-type Ca^{2+} channels and are used in the treatment of hypertension (*1*).



1, commonly

$R_2 = R_6 = Me$

$R_3 = R_5 = Me$ or Et

$R_4 =$ aryl

Because changes in calcium ion concentrations are often associated with cell injuries induced by oxidative stress (*2*, *3*), it is noteworthy that Hantzsch esters not only are "calcium blockers" but also are able to retard the peroxidation in vitro of biological lipids (*4*, *5*), low-density lipoprotein (*6*), mitochondria (*7*), and whole cells in culture (*8*). With one exception (*5*), in all of this work, it has been explicitly (or, at least, implicitly) presumed that Hantzsch esters function as "chain-breaking", peroxyl radical-trapping antioxidants. The different mechanisms of action of chain-breaking and "preventive" antioxidants have been described in several of our earlier publications (*9*). The possibility that **1** is actually a preventive antioxidant of the metal ion-chelating subclass has been largely ignored. This is surprising because Fe^{2+} or Cu^{2+} were used to initiate all of the cited peroxidations (*4*−*8*), and in such systems, it is difficult, if not impossible, to decide whether a retarder of peroxidation is a chain-breaking or a preventive antioxidant (or both).

Scheme 1. Lipid Peroxidation in the Presence of a CBA

Chain Propagation:

$$L^\bullet + O_2 \xrightarrow{v.\,fast} LOO^\bullet \quad (1)$$

$$LOO^\bullet + LH \xrightarrow{k_{prop}} LOOH + L^\bullet \quad (2)$$

Chain Termination:

$$LOO^\bullet + InH \xrightarrow{k_{inh}} LOOH + In^\bullet \quad (3)$$

$$LOO^\bullet + In^\bullet \xrightarrow{v.\,fast} \text{non-radical products} \quad (4)$$

A necessary, but by no means sufficient (*9*, *10*) condition for an inhibitor, InH, of lipid (LH) peroxidation to be a peroxyl radical-trapping, chain-breaking antioxidant (CBA henceforth) is that the peroxyl radicals, LOO$^\bullet$, react with InH much more rapidly than they react with LH; that is, it is necessary that the rate constant for inhibition, k_{inh}, is much larger than the rate constant for propagation, k_{prop}; see Scheme 1.

There have been only two attempts to determine whether Hantzsch esters actually are CBAs and, if so, how efficient they are in comparison with known phenolic CBAs (*11*, *12*). On first reading, these two reports are completely contradictory. The first, by Ursini and co-workers (*11*), employed the "crocin bleaching" assay of Bors et al. (*13*). In this assay, crocin (a water-soluble carotenoid) is employed together with a putative InH under competitive conditions in a system in which free

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Scheme 2. Crocin Bleaching Antioxidant Assay

$$[(H_2N)^+_2CC(CH_3)_2N=]_2 \xrightarrow{\Delta,\ k_d} 2\ e\ (H_2N)^+_2CC(CH_3)^\bullet_2$$
$$^+ANNA^+ \text{ (or ABAP)} \qquad 2\ e\ {}^+A^\bullet$$

$$\xrightarrow{O_2} 2\ e\ (H_2N)^+_2CC(CH_3)_2OO^\bullet \qquad (5)$$
$$2\ e\ {}^+AOO^\bullet$$

$$^+AOO^\bullet + InH \xrightarrow{k_6} {}^+AOOH + In^\bullet \qquad (6)$$

$$^+AOO^\bullet + \text{Crocin} \xrightarrow{k_7} \text{Bleached Crocin} \qquad (7)$$

Table 1. Crocin Bleaching Assay of Relative $^+AOO^\bullet$ Trapping Rate Constants, k_6/k_7, by Two Hantzsch Esters and Two Phenolic CBAs (from Ref *11*)

compound	k_6/k_7
lacidipine[a]	3.4×10^{-3}
nicardipine[b]	"inactive"
probucol[c]	0.05
Trolox[d]	1.8

[a] **1** ($R_2 = R_6 =$ Me; $R_3 = R_5 =$ Et; $R_4 =$ 2-*tert*-BuOCOCH=CHC_6H_4). [b] **1** ($R_2 = R_6 =$ Me; $R_3 =$ Me; $R_5 = CH_2CH_2N(CH_3)CH_2C_6H_5$; $R_4 =$ 3-$NO_2C_6H_4$). [c] (1-HO-2,6-*tert*-Bu_2−C_6H_2-4-S)$_2C(CH_3)_2$. [d] 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate.

radicals of some desired type are generated at a constant rate. In the present case, peroxyl radicals, $^+AOO^\bullet$, were generated by the thermal decomposition of azo-bis-2-amidinopropane, $^+ANNA^+$ [1] (or ABAP), at 40 °C in oxygen-containing 10% ethanol in water. Rates of crocin bleaching were measured at different concentrations of "antioxidant" with the same crocin concentration, which allowed the rate constant ratio, k_6/k_7, to be determined for $^+AOO^\bullet$; see Scheme 2.

In this scheme, k_d is the rate constant for decomposition of $^+ANNA^+$ and e is the efficiency of escape of the geminate pair of $^+A^\bullet$ radicals from the solvent cage. Relevant results are summarized in Table 1. According to the crocin bleaching assay, the two Hantzsch esters were only 0.2% (laridipine) or <0.2% (nicardipine) as active at trapping $^+AOO^\bullet$ radicals as Trolox (*11*), the water-soluble vitamin E analogue, a phenol that is acknowledged to be a very good water-soluble peroxyl radical-trapping CBA.

In the second and much more recent report, Núñez-Vergara and co-workers (*12*) also employed $^+AOO^\bullet$ radicals generated by thermal decomposition of $^+ANNA^+$. In oxygen-saturated buffer/DMF (70/30) at pH 7.4 and 37 °C, the oxidation of nine **1**'s (six having the NH group and three an NC_2H_5 group) was followed by UV/vis spectroscopy. The rates of oxidation of the six NH **1**'s varied from 20 to 68% and of the three NC_2H_5 **1**'s from 7 to 37% of the rate at which Trolox was oxidized under the same conditions. It was claimed (*12*) that this protocol yielded relative rate constants for $^+AOO^\bullet$ radicals and these were then converted to an absolute scale using an experimental determination of k_{inh} for Trolox ($4.9 \times 10^3\ M^{-1}\ s^{-1}$) made by one of us (*17*). Further evidence purporting to show that the inhibition kinetic rate constants reported reflected hydrogen atom abstraction from the NH groups of **1** by $^+AOO^\bullet$ (reaction 6) was an NH/ND deuterium kinetic isotope effect (DKIE) of 1.2, the high reactivity of the NC_2H_5 **1**'s being left unexplained. Thus, on first reading, Núñez-Vergara's results (*12*) suggest that compounds having the general structure **1** (both NH and NC_2H_5) have a reactivity toward $^+AOO^\bullet$ comparable to that of Trolox, whereas Ursini's results (*11*) indicate that two NH **1**'s have a reactivity toward $^+AOO^\bullet$ that is smaller by a factor of 500 or more than Trolox.

It should be recognized that Núñez-Vergara and co-workers (*12*) were (primarily) titrating for **1** with the $^+AOO^\bullet$ radicals, not (as they claimed) measuring the inhibition kinetic rate constants, k_{inh}. This is because there is no substrate, LH, to compete with **1** (or Trolox) for the $^+AOO^\bullet$ radicals. Indeed, the only competition with $^+AOO^\bullet$ consumption by the various **1** or by Trolox is $^+AOO^\bullet$ consumption in their bimolecular self-reaction, reaction 8:

$$^+AOO^\bullet + {}^+AOO^\bullet \rightarrow \text{nonradical products} \qquad (8)$$

Indeed, the Núñez-Vergara publication actually provides excellent evidence that in the **1** + $^+AOO^\bullet$ reactions it was the quantity of **1** present that was being measured, not the rate constant for the reaction of **1** with $^+AOO^\bullet$. Thus, the time course of the reaction of 200 μM **1** (R_2 to R_6 were five methyl groups) with 20 mM $^+ANNA^+$ in buffered (pH 7.4) aqueous/ethanol (70/30) at 37 °C in the presence of oxygen (i.e., for the reaction with $^+AOO^\bullet$) is shown in Figure 4B of ref *12*. Under these experimental conditions, the rate of initiation, i.e., the formation rate of $^+AOO^\bullet$ radicals, is given by $R_i = 2ek_d[^+ANNA^+]$ in which k_d is the rate constant for $^+ANNA^+$ decomposition ($1.3 \times 10^{-6}\ s^{-1}$) and e is the cage escape efficiency (≈0.5) (*18*); see eq 5 (*14*). The rate of formation of $^+AOO^\bullet$ radicals with 20 mM of $^+ANNA^+$ will be

$$2 \times 0.5 \times 1.3 \times 10^{-6} \times 20 \times 10^{-3} = 2.6 \times 10^{-8}\ M\,s^{-1}$$

The reaction was observed to continue for about 4 h and then cease (quite abruptly). The quantity of $^+AOO^\bullet$ radicals generated in this time will be $2.6 \times 10^{-8} \times 4 \times 3600 = 374\ \mu M$. The majority of phenolic CBAs trap about two peroxyl radicals; see reactions 3 and 4; that is, the stoichiometric factor, n, for most CBAs is ca. 2.0 (*19*). The reaction of **1** with $^+AOO^\bullet$ would also appear to have an n value of ~2.0 because the products from the reactions of $^+AOO^\bullet$ with the six NH Hantsch esters were shown to be the corresponding pyridines (*12*); that is, these **1** all lose two hydrogen atoms per molecule to the attacking radicals. Thus, all of the 200 μM **1** used in the experiment under consideration would be consumed by 400 μM $^+AOO^\bullet$. This quantity of radicals is not significantly different from the calculated 374 μM of radicals generated over 4 h, and the derived n value of 1.87 is well within the normal range (*19*, *20*). Unfortunately, there is also a similar confusion between competitive pairs of reactions from which relative kinetic data can be derived and titration for a single added reagent in some related publications on the oxidation of **1** by the Núñez-Vergara group (*21*).

The foregoing analysis of the literature (*11*, *12*) strongly implies that Hantzsch DHP calcium channel blockers, **1**, are unlikely to be CBAs in either biologically mimetic systems or in vivo. However, there is still some uncertainty about the CBA activities of **1** because, as we have pointed out elsewhere (*14*−*16*), $^+AOO^\bullet$ radicals are not biomimetic peroxyl radicals because they bear a positive charge whereas most biologically relevant, small peroxyl radicals will be neutral or negatively charged. Moreover, $^+AOO^\bullet$ radicals almost certainly have a different (higher) reactivity in H-atom abstraction reactions from the reactivities of biologically relevant $LOO^\bullet$ and $HOO^\bullet$ peroxyls (*16*). This uncertainty stimulated us to investigate the abilities

[1] The $^+ANNA^+$, $^+A^\bullet$, and $^+AOO^\bullet$ designations were introduced (*14*, *15*) because the presence and sign of the Coulombic charge, or lack thereof, on water-soluble radicals can become extremely important in determining how reactive the radicals are towards certain biologically relevant, charged substrates, e.g., DNA (*14*), in determining the cage escape efficiency, e, of the geminate carbon-centered radical pair, in determining the absolute rate constants for H-atom abstractions by the peroxyl radicals (*16*) and probably in determining the rate constants for peroxyl/peroxyl chain termination reactions (*14*).

of two typical Hantzsch esters, **1a** ($R_2 = R_6 =$ Me, $R_3 = i$-Pr, $R_5 = CH_2CH_2OMe$, $R_4 =$ 3-NO_2-phenyl, the pharmaceutical active Nimodipine) and **1b** ($R_2 = R_4 = R_6 =$ Me, $R_3 = R_5 =$ Et) to act as CBAs in an extremely well-understood and quantified chemical peroxidation system. Indeed, this system (see Materials and Methods) has been in use for over 40 years (*22*), and during this time, it has provided most of the absolute rate constants for the reactions of uncharged alkylperoxyl radicals with phenols, aromatic amines, and other types of CBAs (*23*). This system has also proved to be a powerful diagnostic tool for distinguishing between peroxyl radical-trapping CBAs and compounds claimed to belong to this class of antioxidants but which are not, in fact, CBAs (*9c*).

We have also investigated the reactivities of **1b** and **1c** ($R_2 = R_3 = R_5 = R_6 =$ Me, $R_4 =$ 2-NO_2-phenyl, the pharmaceutical active Nifedipine) toward the 2,2-diphenyl-1-picrylhydrzyl radical (dpph$^\bullet$). This radical was used for two reasons: (i) Kinetic studies on the reactions of phenols with dpph$^\bullet$ revealed anomalously high rate constants in alcohol solvents (*24*) that were later observed to be mirrored in the reactions of phenols with uncharged alkylperoxyl radicals (LOO$^\bullet$) (*25*). Further work with dpph$^\bullet$ eventually provided the explanation for these anomalies (*26*).[2](ii) Kinetic measurements with dpph$^\bullet$ are much easier to carry out than those with LOO$^\bullet$.

The reactivities toward peroxyl and dpph$^\bullet$ radicals of two dibenzo-1,4-dihydropyridines, 9,10-dihydroacridine (DHAC) and *N*-methyl-9,10-dihydroacridine (*N*-Me-DHAC), were also determined. This pair of compounds was selected with the expectation that they would demonstrate a very important, but all too frequently overlooked, aspect of radical kinetics. This is that the bond dissociation enthalpies (BDEs) are not the only factor determining relative H-atom donor abilities and hence CBA activities. In DHAC, the calculated C−H and N−H BDEs are 71.0 and 82.3 kcal/mol, respectively (*27*). Nevertheless, it was anticipated that the NH group in DHAC would make this compound a relatively good H-atom donor (like Ph_2NH) whereas the absence of this group in *N*-Me-DHAC would make this compound a relatively poor H-atom donor (like Ph_2CH_2). This is clearly a matter of some importance whenever one is considering the possible CBA activities of compounds such as DHP (*10*) and substituted dihydropyridines, including **1**. That is, do such compounds react with LOO$^\bullet$ and with dpph$^\bullet$ radicals by donating H-atoms from their weaker C−H bond or from their stronger N−H bond (*27*).

To validate our kinetic results, the reactivities of an excellent CBA, 2,2,5,7,8-pentamethyl-6-hydroxy-chroman (PMHC), and a moderately weak CBA, 2,6-di-*tert*-butyl-4-methylphenol (BHT), toward LOO$^\bullet$ and dpph$^\bullet$ radicals were also determined under the same experimental conditions as had been used with **1**, *N*-Me-DHAC, and DHAC.

Results

Rate Constants for Hydrogen Abstraction by LOO$^\bullet$. The rate constants, k_9^S (where the superscript, S, refers to the solvent since the magnitude of k_9^S will be solvent-dependent), for hydrogen atom abstraction from potential CBAs by peroxyl radicals are best determined using styrene or cumene (see below) as the oxidizable substrate (LH) in autoxidations initiated by the thermal decomposition of azo-bis-isobutyronitrile (AIBN) at 30 °C.

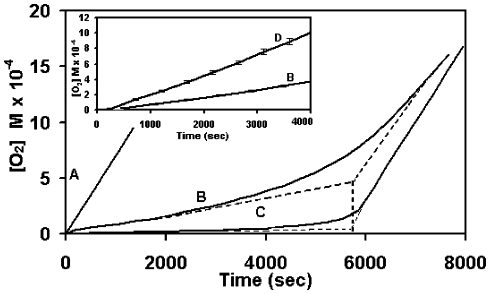


Figure 1. Oxygen uptake profiles for the inhibited autoxidation of styrene, 4.35 M, in chlorobenzene initiated by AIBN, 2.12×10^{-2} M, in a sample volume of 2.0 mL at 30 °C: curve A, uninhibited; curve B, inhibited by DHAC, 6.60 μM; and curve C, inhibited by PMHC, 6.30 μM. The inset shows oxygen uptake over the first 4000 s with DHAC, 6.60 μM (B) and with *N*-deuterio-DHAC, 6.60 μM, in the presence of 50 μL of D_2O (D). The error bars represent the average of three separate experiments.

$$\mathrm{LOO^\bullet + XH \xrightarrow{k_9^S} LOOH + X^\bullet} \qquad (9)$$

Styrene is the preferred substrate for good CBAs, i.e., for CBAs having large k_9^S (k_{inh}) values, because the rate constant for propagation, k_{10}, is larger (41 M^{-1} s^{-1} at 30 °C) (*28*, *29*) than for almost all other hydrocarbons. This allows a chain oxidation

$$\mathrm{LOO^\bullet + H_2C{=}CHPh \xrightarrow{k_{10}} LOOCH_2CH(^\bullet)Ph} \qquad (10)$$

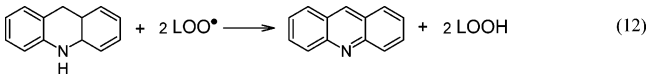
of styrene to occur in the presence of (low concentrations) of CBAs having rate constants for inhibition, k_9^S, as high as 5×10^6 to $\sim 3 \times 10^8$ M^{-1} s^{-1} (*30*, *31*). By measuring the rate of oxygen uptake for the inhibited reaction (at known inhibitor concentrations and at a known rate of chain initiation), it is simple to calculate the rate constant ratio, k_{10}/k_9^S, and hence k_9^S.

Very weak CBAs ($k_9^S <$ ca. 10^3 M^{-1} s^{-1}) do not noticeably retard the rate of styrene autoxidation at normally achievable concentrations. If it is suspected that a compound that does not retard the autoxidation of styrene actually is a CBA, its ability to retard the rate of autoxidation of cumene should be determined. This is because the propagation rate constant, k_{11}, for cumene is small (0.18 M^{-1} s^{-1} at 30 °C) (*29*, *32*) and even weak CBAs can noticeably reduce its rate of oxidation.

$$\mathrm{LOO^\bullet + PhCHMe_2 \xrightarrow{k_{11}} LOOH + PhC(^\bullet)Me_2} \qquad (11)$$

Under standard experimental conditions (viz., [styrene] = 4.35 M in chlorobenzene, [AIBN] = 2.12×10^{-2} M, XH (inhibitor) concentration = 6.6×10^{-6} M, 1 atm of air, 30 °C), the rate of oxygen uptake was reduced most strongly by PMHC, less strongly by DHAC, and even less strongly by *N*-deuterio-DHAC; see Figure 1. The lengths of time that the rates of oxidation are suppressed, i.e., the induction periods, for PMHC and DHAC are equal (ca. 5700 s, see intersections of the dashed lines in Figure 1). This means that these two inhibitors trap equal numbers of peroxyl radicals. Because PMHC is known to trap ca. 2 peroxyls per molecule under these conditions (*20*), the stoichiometric factor, *n*, for DHAC must also be ca. 2. This value of *n* is consistent with our product studies that showed that DHAC was quantitatively converted into acridine, reaction 12

[2] In alcohols, many phenols are partially ionized and the monitored kinetics reflect the fast electron transfer between the small concentration of phenoxide anion and the dpph$^\bullet$. This process formally amounts to a H-atom transfer from the phenol to the dpph$^\bullet$. This mechanism has been dubbed Sequential Proton Loss Electron Transfer (SPLET) (*26b*).



(12)

The CBA-inhibited rate of styrene oxidation can be converted into the rate constant for reaction of the peroxyl radicals with the CBA, k_9, according to:

$$\frac{-d[O_2]}{dt} = \frac{k_{10}[PhCH{=}CH_2]R_i}{nk_9^S[XH]}$$

where n is the stoichiometric factor for the inhibitor, XH (usually $n = 2$; see eqs 3 and 4). Values of k_9^S could be obtained for DHAC and *N*-deuterio-DHAC, while k_9^S values obtained under the same experimental conditions for PMHC and *O*-deuterio-PMHC are available in the literature (*20*) (see Table 2). Neither the Hantsch esters **1a** and **1b** nor the *N*-Me-DHAC produced any significant reduction in the rate of autoxidation of styrene under standard conditions. These compounds are, therefore, very poor CBAs. However, these compounds did retard the oxidation of cumene when employed at concentrations significantly greater than the "standard" ~6 μM concentrations used in styrene (see Figure 2). Values of k_9^S were calculated from the rates of cumene oxidation retarded by **1a**, **1b**, and *N*-Me-DHAC with the assumption that $n = 2$ for all three compounds. These "inhibition" rate constants are also given in Table 2, together with the known rate constant for the moderately weak CBA, 2,6-di-*tert*-butyl-4-methylphenol, BHT (*30*). Both the deuterium kinetic isotope effect, DKIE, for DHAC and a comparison of the rate constants for DHAC and *N*-Me-DHAC indicate that the k_9^S value for DHAC (Table 2) relates primarily to H-atom abstraction from the NH group in this compound. Abstraction by LOO• from the NH group of the two Hantzsch esters is also to be expected (and is demonstrated for abstraction by dpph•

Table 2. Rate Constants, k_9^S (M^{-1} s^{-1}), for Hydrogen Atom Abstraction by Peroxyl at 30 °C

compound[a]	LH[b]	$k_{9,H}^S$	$k_{9,D}^S$	$k_{9,H}^S/k_{9,D}^S$	$\alpha_2^{H_c}$	$k_{9,H}^0$ [d]
1a (nimodipine)	C	3.7×10^1	2.8×10^1	1.3	0.50	1.2×10^2
1b	C	6.8×10^1	4.5×10^1	1.5	0.44	1.9×10^2
N-Me-DHAC	C	2.4×10^2			0[e]	2.4×10^2
BHT	S	1.4×10^4[f]	2.0×10^3[g,h]	6.8[i]	0.22[j]	2.3×10^4
DHAC	S	5.5×10^5[k]	2.6×10^5[g]	2.1[k]	0.33	1.2×10^6
PMHC	S	3.8×10^6[f]	5.9×10^5[g,h]	5.1[i]	0.37[l]	8.9×10^6

[a] **1a** ($R_2 = R_6 =$ Me, $R_3 =$ *i*-Pr, $R_5 = CH_2CH_2OMe$, $R_4 =$ 3-NO_2-phenyl); **1b** ($R_2 = R_4 = R_6 =$ Me, $R_3 = R_5 =$ Et); *N*-Me-DHAC, *N*-methyl-9,10-dihydroacridine; BHT, 2,6-di-*tert*-butyl-4-methylphenol; DHAC, 9,10-dihydroacridine; and PMHC, 2,2,5,7,8-pentamethyl-6-hydroxy-chroman. [b] LH, oxidized substrate; C, cumene; S, styrene. These substrates were oxidized in the presence of one-third the volume of chlorobenzene. [c] Relative hydrogen bond donating ability of XH; see ref *33*. Unless otherwise noted, these values were obtained from a nonlinear fit obeying: $\alpha_2^H = 23.41(pK_a)^{-1.32}$, with $r^2 = 0.984$, comprising the α_2^H values (*33*) for aniline, 4-NO_2-aniline, diphenylamine, and carbazole, and their corresponding pK_a values (*34*) in DMSO. For **1a** and **1c** (Table 3), the pK_a values were assumed to be 18.3, equal to the pK_a of **1** ($R_2 = R_6 =$ Me, $R_3 = R_5 =$ Et, $R_4 =$ 4-CN-phenyl), and for **1b**, the pK_a was assumed to be 20.3, equal to the pK_a of **1** ($R_2 = R_6 =$ Me, $R_3 = R_5 =$ Et, $R_4 =$ *i*-Pr) (*35*). For DHAC, the pK_a was assumed to be 22.5, equal to the pK_a of iminobibenzyl (*34*). [d] This is the rate constant in a nonhydrogen bond accepting solvent calculated from the equation: $\log[k_9^S\ (M^{-1}\ s^{-1})] = \log[k_9^0\ (M^{-1}\ s^{-1})] - 8.3\alpha_2^H\beta_2^H$ (*36*) where β_2^H is the relative hydrogen bond accepting ability of the solvent (*37*), estimated as 0.12 in these systems. [e] The C−H group from which an H-atom is abstracted will not form a hydrogen bond with the solvent. [f] Ref *30*. [g] In the presence of a small amount of D_2O. [h] Based on the DKIE reported in ref *20*. [i] Ref *20*. [j] Mean value; see ref *26a*. [k] In the presence of a small amount of H_2O, the rate of oxygen uptake decreased slightly. [l] Assumed equal to the value reported in ref *36* for the structurally related α-tocopherol.

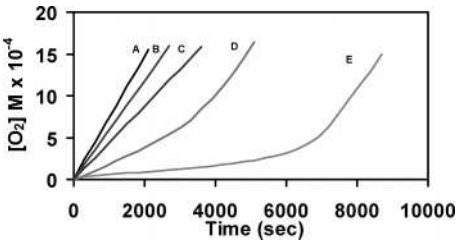


Figure 2. Oxygen uptake profiles for the inhibited autoxidation of cumene, 5.35 M, in chlorobenzene initiated by AIBN, 2.12×10^{-2} M, in a sample volume of 2.0 mL at 30 °C: curve A, uninhibited, not affected by **1a**, 6.37 μM, or by **1b**, 6.02 μM; curve B, effect of **1a**, 59.6 μM; curve C, effect of **1b**, 59.8 μM; curve D, inhibited by *N*-Me-DHAC, 14.4 μM; and curve E, inhibited by 2,6-di-*tert*-butyl-4-methylphenol, BHT, 11.6 μM.

Table 3. Rate Constants for Hydrogen Atom Abstraction, k_{13}^S (M^{-1} s^{-1}), by dpph• in Toluene at 25 °C

compound[a]	$k_{13,H}^S$[b]	$k_{13,D}^S$[c]	$k_{13,H}^S/k_{13,D}^S$	$\alpha_2^{H_d}$	$k_{13,H}^0$[e]
1b	8.7×10^{-2}	4.8×10^{-2}	1.8	0.44	2.8×10^{-1}
1c (nifedipine)	5.7×10^{-2}	2.4×10^{-2}	2.4	0.50	2.2×10^{-1}
N-Me-DHAC	6.6×10^{-1}			0	6.6×10^{-1}
BHT	1.9×10^1	1.2×10^1	1.6	0.22	3.4×10^1
DHAC	1.3×10^2	7.5×10^1	1.7	0.33	3.0×10^2
PMHC[f]	1.8×10^3[g]	7.5×10^2	2.4	0.37	4.8×10^3

[a] See footnote *a*, Table 2; **1c** ($R_2 = R_3 = R_5 = R_6 =$ Me, $R_4 =$ 2-NO_2-phenyl). [b] In the presence of trace H_2O. [c] In the presence of trace D_2O. [d] See footnote *c*, Table 2. [e] See footnote *d*, Table 2, β_2^H (toluene) = β_2^H (benzene) = 0.14 (*37*). [f] With benzene as the solvent including traces of H_2O or D_2O. [g] In neat benzene, this rate constant has been reported to be 2.75×10^3 M^{-1} s^{-1} (*36*).

by the observation of a DKIE when the reactions were run in the presence of D_2O, which exchanges only the NH hydrogen to deuterium; see below). Because of hydrogen bonding between the N−H solutes and the solvent, the measured rate constants will be lower than the rate constants, k_9^0, in a nonhydrogen bond-accepting solvent such as heptane. This nonconvoluted (i.e., characteristic) rate constant, k_9^0, for each reaction can be calculated from the equation: $\log[k_9^S\ (M^{-1}\ s^{-1})] = \log[k_9^0\ (M^{-1}\ s^{-1})] - 8.3\alpha_2^H\beta_2^H$ (*36*), where α_2^H and β_2^H refer to Abraham's scales of relative hydrogen bond donor activity (*33*) of the H-atom donor and relative hydrogen bond acceptor activity of the solvent (*37*). The α_2^H values, when not available from experiment, can be estimated using an empirical relationship between the α_2^H and the pK_a in DMSO that has been found for structurally related compounds (see footnote *c* in Table 2). From this table, it can be seen that the hydrogen bond donor activities of Hantzsch esters are larger than for DHAC. Presumably, this is due mainly to the electron-withdrawing carboxyl groups at the 3- and 5-positions of **1**.

Hydrogen Atom Abstraction by dpph•. The rate constants, k_{13}^S, have been determined for hydrogen atom abstraction by the dpph• radical, reaction 13, from two Hantzsch esters **1b** and **1c** ($R_2 = R_3 = R_5 = R_6 =$ Me, $R_4 =$ 2-NO_2-phenyl, the pharmaceutically active Nifedipine), *N*-Me-DHAC, BHT, DHAC, and PMHC. The kinetic measurements were carried out at 25 °C as described previously (*26a*). Toluene was used as the solvent. The results, summarized in Table 3

$$dpph^\bullet + XH \xrightarrow{k_{13}^S} dpph\text{-}H + X^\bullet \qquad (13)$$

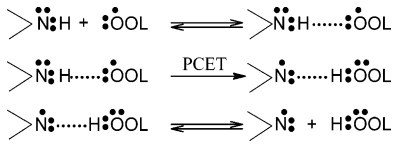
include experiments performed in the presence of very small amounts of H_2O or D_2O (to convert the *N*-H to *N*-D) and allow the deuterium kinetic isotope effects (DKIEs), $k_{13,H}^S/k_{13,D}^S$, to be determined. Comparison of the rate constants for DHAC and

N-Me-DHAC, plus the DKIEs for **1b**, **1c**, and DHAC of 1.7−2.4, indicate that the k_{13}^{S} values for **1b**, **1c**, and DHAC (Table 3) relate primarily to H-atom abstraction from the NH group in these compounds by dpph•. When comparing the results presented in Tables 2 and 3, it is gratifying to note that the trends in the peroxyl and dpph• rate constants are quite similar.

Discussion

The results presented in Table 2 demonstrate that Hantzsch esters are very poor H-atom donors to peroxyl radicals and do not function as CBAs. Their very poor H-atom-donating ability is further confirmed by the rate constants measured for their reactions with dpph• (Table 3). The validity of the experimental procedures that we have employed is attested to by the rather good H-atom donor activity found for 9,10-dihydroacridine, DHAC, toward both peroxyl and dpph• radicals. DHAC is roughly 1/10 as reactive toward these two radicals as the outstanding CBA and H-atom donor, PMHC, but it is several orders of magnitude more reactive than the Hantzsch esters. The reactions of the peroxyl and dpph• radicals with the Hantzsch esters are much slower than the reactions with the structurally related DHAC. Presumably, this is due to the two electron-withdrawing carboxyl groups in **1**, which will increase the N−H BDEs and hence decrease the H-atom donor capability (*38*). Our results are fully consistent with those reported by Ursini and co-workers (*11*) using $^{+}$AOO• radicals and the crocin bleaching assay (see Table 1). Hantzsch esters cannot act as peroxyl radicals trapping, CBAs despite a claim of the contrary (*12*).

N−H vs C−H Abstraction. Rate constants for the reactions of DHAC with peroxyl radicals and dpph• are orders of magnitudes greater than for the corresponding reactions with *N*-Me-DHAC. Thus, in DHAC H-atom transfer from N−H is, as we expected, strongly preferred over H-atom transfer from CH_2 despite the fact that the N−H BDE is calculated to be ca. 11 kcal/mol stronger than the C−H BDE (*27*). We attribute this preference for N−H abstraction to the occurrence of proton-coupled electron transfer (PCET) (*39*).[3] We use PCET to describe a hydrogen atom transfer between two heteroatoms in which the hydrogen transferred participates in a hydrogen bond and moves as a proton between two lone pairs of electrons accompanied by an electron from a nonbonding orbital, which may (*41*) or may not (*42*) be perpendicular to the local molecular framework, e.g.,



With *N*-Me-DHAC, the hydrogen atom transfer can take place only from a carbon atom. The retardation of the oxidation of cumene by *N*-Me-DHAC must mean that its carbon-centered radical either does not react with dioxygen or that its reaction with dioxygen is reversible with an equilibrium that lies on the alkyl radical side. This alkyl radical can then trap a second LOO• radical.[4] Hydrocarbons that retard autoxidations by this mechanism are a well-recognized class of CBAs (*43*).

[3] Zavitsas (*40a*) has proposed an alternative explanation for the much greater ease of H-atom abstraction from an NH group than from a CH group having a similar BDE. This involves differences in the antibonding energies of the two transition states. However, the validity of this approach has recently been questioned (*40b*).

Materials and Methods

Chemicals. The Hantzsch esters **1a** (nimodipine), **1b** (di-ethyl-1,4-dihydro-2,4,6-trimethyl-3,5-pyridinecarboxylate), and **1c** (nifidipine), and BHT and PMHC were used as received. DHAC (9,10-dihydroacridine) and *N*-Me-DHAC (*N*-methyl-9,10-dihydroacridine) were synthesized as described below.

General Methods. The syntheses were conducted under N_2 atmospheres. All NMR experiments (^{1}H, ^{13}C) were recorded on an AC-Brüker instrument (400 MHz). Unless otherwise noted, proton and carbon chemical shifts are reported in ppm using residual $CHCl_3$ as an internal standard at 7.26 and 77.0 ppm, respectively. Analysis by electrospray mass spectrometry was performed on a VG Quattro I (Micromass) mass spectrometer equipped with a pneumatically assisted electrospray ionization source, operating in positive mode. HRMS analysis was performed on a JEOL JMS-AX505H mass spectrometer. GC-MS samples were run on a HP 5890 Gas Chromatograph instrument with a 5970 Series Mass Selective Detector.

9,10-Dihydroacridine (DHAC). To a yellow solution of acridine (1.79 g, 10.0 mmol) in anhydrous THF (40 mL) was added BF_3•OEt_2 (2.53 mL, 20.0 mmol) in one portion at room temperature; the solution first turned red and then dark brown. Sodium cyanoborohydride (1.26 g, 20.0 mmol) was then added in portions for a period of 5 min, the reaction mixture turned orange-red, and bubbles of hydrogen evolved. The solution was stirred at room temperature for 2 h and then heated to reflux for 8 h, and the red-brown color gradually faded to almost colorless. The reaction mixture was cooled to room temperature, stirred for 4 h, and was then poured into ice water (200 mL) and extracted with EtOAc (3 × 40 mL), and the combined organics were washed with brine, dried, and concentrated to give the crude product as a green solid. The crude product was dissolved in boiling EtOH, cooled to room temperature, and crystallized at −20 °C to yield 1.60 g (88%) of pure product as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ_H 4.11 (s, 2H), 5.98 (s, 1H), 6.69−6.71 (m, 2H), 6.89−6.93 (m, 2H), 7.11−7.16 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm): δ_C 31.81, 113.89, 120.47, 121.07, 127.44, 129.05, 140.55.

***N*-Methyl-9,10-Dihydroacridine (*N*-Me-DHAC).** The above synthesized 9,10-dihydroacridine (0.50 g, 2.76 mmol) was dissolved in anhydrous THF (40 mL) and cooled to 0 °C, *n*-butyllithium (2.0 M solution in hexanes,1.52 mL, 3.04 mmol) was added dropwise for a period of 10 min, and the light yellow solution first turned green and then brown near the end of the addition. The reaction was stirred at 0 °C for 1.5 h. Methyl iodide (0.172 mL, 2.76 mmol) was then added dropwise via syringe at 0 °C, and the brown color changed instantly to yellow. The reaction mixture was stirred at 0 °C for 2 h, at room temperature overnight, and was then was poured into ice water (100 mL) and extracted with EtOAc (3 × 20 mL), and the combined organics were washed with brine, dried, and concentrated to give crude product as a yellow-brownish solid. The crude product was recrystallized from EtOH at −20 °C to yield 0.48 g (89%) of pure product as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ_H 3.42 (s, 3H), 3.94 (s, 2H), 6.92−6.99 (m, 4H), 7.20−7.27 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm): δ_C 33.55, 33.66, 112.29, 121.01, 124.74, 127.27, 127.94, 144.09.

Autoxidation/Inhibition Procedures. Autoxidation experiments were carried out at 30 °C under 760 Torr of air in a dual channel oxygen uptake apparatus equipped with a sensitive pressure transducer, as described previously (*30*, *45*). Styrene was separated from a commercial inhibitor by rapid bulb-to-bulb distillation on

[4] The reactivity towards dioxygen of the benzylic carbon-centered radical (L•) derived from *N*-Me-DHAC has been briefly explored. A solution of *N*-Me-DHAC (1 mM) in benzene/di-*tert*-butyl peroxide (v/v = 1:2) was subjected to 355 nm laser flash photolysis. The *tert*-butoxyl radicals generated will predominantly abstract a benzylic hydrogen from *N*-Me-DHAC and produce L•. The UV spectrum of the transient L• has been reported before (*44*). In both nitrogen-saturated and dioxygen-saturated solutions, L• decayed, monitored at λ = 370 nm, with second-order kinetics (L• + L• → products), which demonstrates that L• has little or no reactivity towards dioxygen.

the vacuum line and passed through a short column of alumina just before use. In the experiments using *N*-deuterio compounds (prepared by adding 50 μL of D_2O to the reaction mixture with a volume of 2 mL), the air was passed into the apparatus through moisture-removing silica gel and calcium chloride and the styrene and chlorobenzene were dried just before use with molecular sieves and calcium chloride. Profiles of oxygen uptake were recorded for the inhibited oxidation of styrene (4.35 M in chlorobenzene) initiated by AIBN, 2.12×10^{-2} M. Cumene was passed through a column of silica gel just before use. Profiles of the oxygen uptake were recorded for the inhibited oxidation of cumene (5.35 M in chlorobenzene), initiated by AIBN, 2.12×10^{-2} M. As has been emphasized (*30*), it is necessary to ensure that there is an appreciable kinetic chain length during the inhibition periods.

Kinetic Measurements with dpph•. These were made following the procedure described previously (*26a*). Decays of dpph• (initial concentrations $3-8 \times 10^{-5}$ M) in the presence of excess of known concentrations of the substrate in toluene (for PMHC in benzene) were monitored at 517 nm on an Applied Photophysics stopped-flow spectrophotometer, SX 18 MV, equipped with a 150 W xenon lamp at ambient temperature. The rate constants presented in Table 3 are mean values from at least two independent sets of measurements of several pseudo-first-order rate constants, k_{exptl}. Values of k_{13}^S M^{-1} s^{-1}, were calculated from the linear least-squares slopes derived from plots of k_{exptl} vs [substrate] according to $k_{exptl} = \text{const} + k_{13}^S[\text{substrate}]$.

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