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# Correlations between the Rate Constant of Singlet Oxygen Quenching by Imidazole Derivatives and Anti-inflammatory Activity in Rats

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## SUMMARY

The second-order rate constants, $k_\Delta$, for quenching of molecular singlet oxygen $O_2$ ($^1\Delta_g$) by nonsteroidal anti-inflammatory imidazole drugs have been determined using time-resolved phosphorescence detection of singlet oxygen. A linear correlation was observed between log $k_\Delta$ (ranging from 7.90 to 8.50) and the anti-inflammatory activity of these compounds (ranging from $ED_{50}$ = 15 to 300 mg/kg), as measured in rats by Jørgensen and Dyrsting [United States Patent 4,424,229 (1984)]. The correlation between this physico-chemical parameter measured *in vitro* and a biological activity measured *in vivo* might be useful in screening other types of candidate anti-inflammatory drugs. The rate constant ($k_\Delta$) can be considered as a quantitative expression of the electron-donating power of the imidazole drug, as suggested by a correlation of log $k_\Delta$ (ranging from 6.02 to 7.45) with Hammett substituent parameters observed in the case of 2-substituted imidazoles.

The anti-inflammatory activity of a series of fluorine-containing 2,4,5-triphenylimidazoles has been measured and expressed as $ED_{50}$ (mg/kg) by Jørgensen and Dyrsting (1), using the carrageenin test of Winter *et al.* (2). The aim of our work has been to seek a possible correlation of this anti-inflammatory activity with a physico-chemical parameter, the rate constant ($k_\Delta$) of quenching of singlet oxygen $O_2$ ($^1\Delta_g$), using laser flash photolysis. This rate constant can be considered as a quantitative expression of the relative electron-donating power of the molecule interacting with $^1O_2$. This is suggested by the correlation of $k_\Delta$ with Hammett substituent parameters, observed in the case of 2-substituted imidazoles.

## Materials and Methods

**Imidazole derivatives.** The imidazole anti-inflammatory derivatives under investigation were fluorine-containing 2,4,5-triphenylimidazoles and were prepared by Jørgensen and Dyrsting (1). The structures of these compounds are shown in Fig. 1. The imidazoles substituted at their 2-position with either H, $NO_2$, CHO, $C_6H_5$, SH, or $CH_3$ were purchased from Aldrich.

**Other reagents.** The *meso*-tetra(4-*N*-methylpyridyl)porphyrin tosylate was purchased from Porphyrin Products (Logan, UT). Perdeuterated methanol (99.8% $CD_3OD$) was purchased from Commissariat à l'Energie Atomique (Saclay, France).

**Production and detection of singlet oxygen.** The sensitized photoproduction of singlet oxygen was carried out by transfer of energy to ground state triplet oxygen, $^3O_2$, from a porphyrin excited to its triplet state, $^3P$, by the frequency-doubled line (532 nm) of a 6-nsec pulse emitted by a Quantel Nd/Yag laser. The energy of the unfocused 8-mm laser pulse incident upon the aerated solutions in a 10- × 10-mm Spectrosil quartz cell was between 1 and 10 mJ. The laser pulse was passed through BG39 and KG5 filters to remove small amounts of the fundamental light at 1.065 μm. The laser energy was measured by deflecting approximately 10% of the exciting light onto a pyroelectric energy meter (RjP-735; Laser Precision Corp.). The porphyrin absorbance ranged between 0.4 and 0.8 at 532 nm in a 1-cm cell. The characteristic $^1O_2$ phosphorescence with $\lambda_{max}$ at 1.27 μm was detected at 90° to the laser pulse by a 3-mm-diameter Judson J16 germanium diode, and the photocurrent was passed through a load resistance of 2 kΩ. Response time of the $^1O_2$ detector was 1 μsec at the load resistance of 2 kΩ. The resulting voltage signal was then applied to a Judson amplifier, whose output was fed to a Tektronix 7912 AD digitizer interfaced with a Hewlett Packard 9816 computer. A long-pass silicon filter ($\lambda > 1.1$ μm) placed between the irradiation cell and the Judson diode isolated the $^1O_2$ phosphorescence from the porphyrin fluorescence and scattered laser light. The intrinsic lifetime of singlet oxygen was measured in $CD_3OD$ and was determined to be approximately 223 μsec. This value is consistent with that reported by Gorman and Rodgers (3) and permits accurate determination of the second-order rate constant for quenching of singlet oxygen by the different imidazole derivatives added.

**Inhibition of carrageenin-induced paw edema in rat.** Tests for antiinflammatory activity were performed by Jørgensen and Dyrsting (1). The imidazole test substance was given by gavage 1 hr before injection of a carrageenin suspension into the rat paw. The increase in paw volume was measured 3 hr after injection of the suspension, and inhibition of edema was evaluated. Table 1 shows the dose-related anti-edema effects, expressed as $ED_{50}$.

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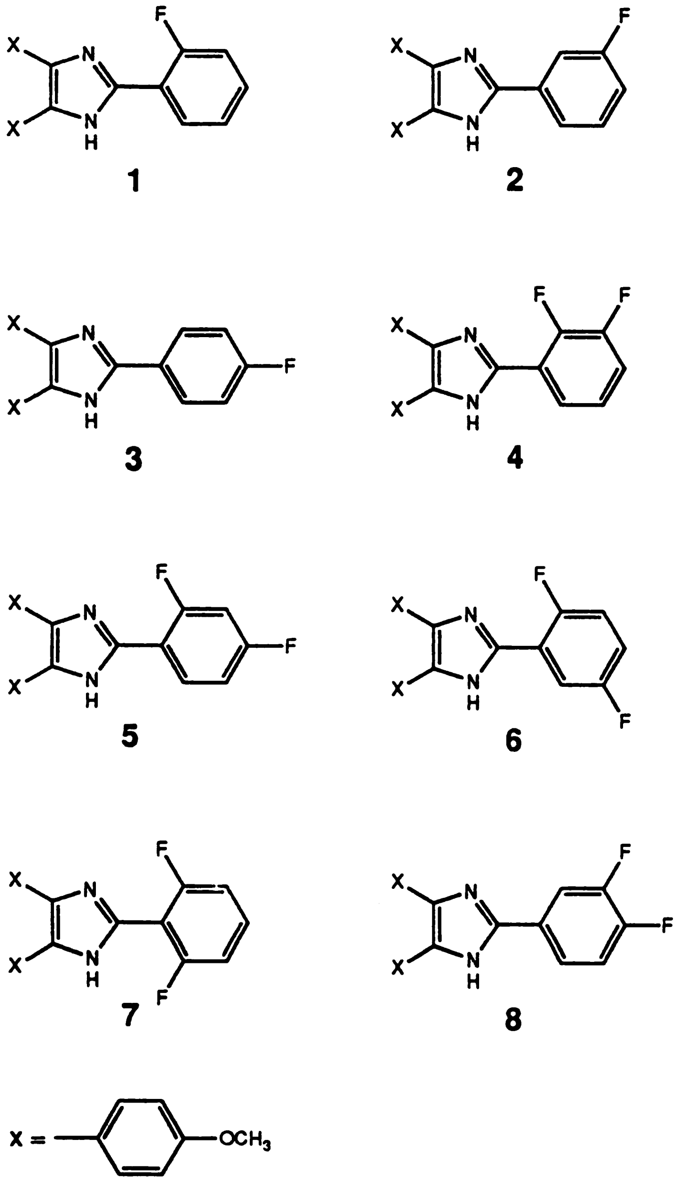

**Fig. 1.** Structures of the fluorine-containing 2,4,5-triphenylimidazoles.

TABLE 1

**Anti-inflammatory activity of the fluorine-containing 2,4,5-triphenylimidazoles and their rate constants of singlet oxygen deactivation, $k\Delta$**

| Anti-inflammatory drug[a] | $ED_{50}$[b] | $k_\Delta$ | log $k_\Delta$ |
|---|---|---|---|
| | *mg/kg* | *$10^8$ dm³/mol/sec* | |
| 1 | 130 | 1.65 | 8.21 |
| 2 | 38 | 2.15 | 8.33 |
| 3 | 14 | 3.20 | 8.50 |
| 4 | 120 | 1.53 | 8.18 |
| 5 | 47 | 1.98 | 8.32 |
| 6 | 110 | 1.22 | 8.09 |
| 7 | 300 | 7.97 | 7.90 |
| 8 | 15 | 2.15 | 8.33 |

[a] Numbers correspond to the compounds shown in Fig. 1.

[b] Measured in rats (specific pathogen-free males of the strain Sprague-Dawley, weighing ≈150 g) by Jörgensen and Dyrsting (1).

## Results

**Singlet oxygen quenching by the fluorine-containing 2,4,5-triphenylimidazoles.** A simplified scheme of the different decays of $^1O_2$ is as follows:

$$^1O_2 \xrightarrow{k_d} {}^3O_2$$

$$^1O_2 + Q \xrightarrow{k_q} {}^3O_2 + Q$$

$$^1O_2 + Q \xrightarrow{k_r} \text{oxidation products}$$

$k_d$ is the intrinsic first-order rate constant of singlet oxygen decay in the solvent and $k_\Delta = (k_q + k_r)$ is the overall second-order rate constant for the quenching of singlet oxygen by the imidazole derivatives Q and may be expressed as the sum of the chemical ($k_r$) and the physical ($k_q$) rate constants. After 2 $\mu$sec, the formation of $^1O_2$ in aerated solutions via energy transfer is complete, and the singlet oxygen decay is given by:

$$-d[^1O_2]/dt = \{k_d + k_\Delta\,[Q]\}\,[^1O_2] \qquad (1)$$

Because Q is in excess and can be considered as a constant during the reaction, integration of eq. 1 yields:

$$[^1O_2] = [^1O_2]_0 \exp(-k_{obs}\,t)$$

where $k_{obs} = k_d + k_\Delta\,[Q]$

The second-order rate constant of deactivation ($k_\Delta$) by the different imidazole derivatives was determined via measurement of the pseudo-first-order rate constant ($k_{obs}$) of decay of singlet oxygen in the presence of differing concentrations of Q, which fell within the range of $6.6 \times 10^{-6}$ to $2.85 \times 10^{-4}$ mol/dm³. Each concentration of Q was studied independently in a fresh solution. The average value of $k_d$ in $CD_3OD$ from eight experiments was $4.48 \times 10^3$ sec$^{-1}$, corresponding to a lifetime in $CD_3OD$ of 223 $\mu$sec, in excellent agreement with the value of 227 $\mu$sec reported by Gorman and Rodgers (3). The value of $k_\Delta$ was determined from the slope of $k_{obs}$ versus [Q]. An example of such a plot is presented in Fig. 2. Similar plots were constructed for each of the other compounds and displayed satisfactory linear correlations.

All the fluorine-containing 2,4,5-triphenylimidazoles were found to be rather good quenchers of $^1O_2$. The values of $k_\Delta$ reported in Table 1 range from $8.0 \times 10^7$ to $3.2 \times 10^8$ dm³/mol/sec. These values characterize the triphenyl imidazoles as attractive reaction partners for $^1O_2$, in line with well known scavengers like 1,3-diphenylisobenzofuran and 2,5-dimethylfuran ($k_\Delta = 8 \times 10^8$ and $4 \times 10^8$ dm³/mol/sec, respectively) (4). A plot of log $k_\Delta$ versus the $ED_{50}$ obtained for each compound by Jørgensen and Dyrsting (1) reveals a linear correlation between these two parameters (Fig. 3). The parameter $ED_{50}$ expresses the anti-inflammatory efficiency of the various compounds and ranges from 14 (compound **3**) to 300 (compound **7**) mg/kg (Fig. 3; Table 1). The variation of log $k_\Delta$ spans a range of <1 order of magnitude, whereas values of $ED_{50}$ span a range between 1 and 2 orders of magnitude. This correlation indicates that, as the rate constant of reaction of an imidazole with $^1O_2$ increases, so does the anti-inflammatory efficiency of the imidazole compound. It should be noted that no correlation was found between log $k_\Delta$ and the other pharmacological properties of these imidazoles that Jørgensen and Dyrsting (1) measured, including ulcerogenic activity, inhibition of prostaglandin synthetase, and inhibition of platelet aggregation.

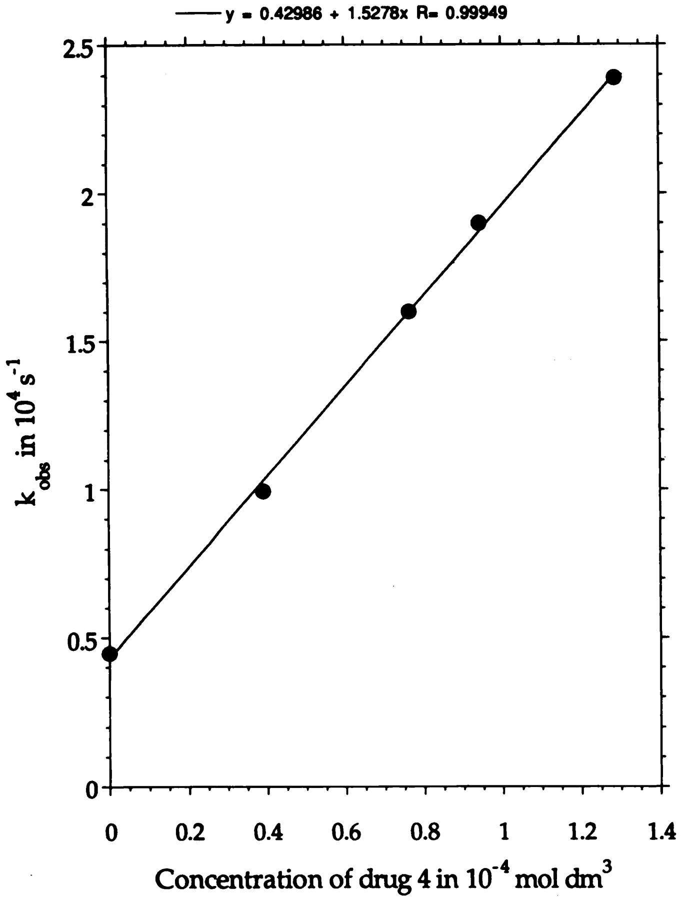

**Fig. 2.** Plot of $k_{obs}$, determined in $CD_3OD$, versus concentration of compound **4**, with a slope giving the second-order rate constant of singlet oxygen quenching ($k_\Delta$) of 1.53 × $10^8$ $dm^3$/mol/sec and a linear correlation coefficient of 0.999.

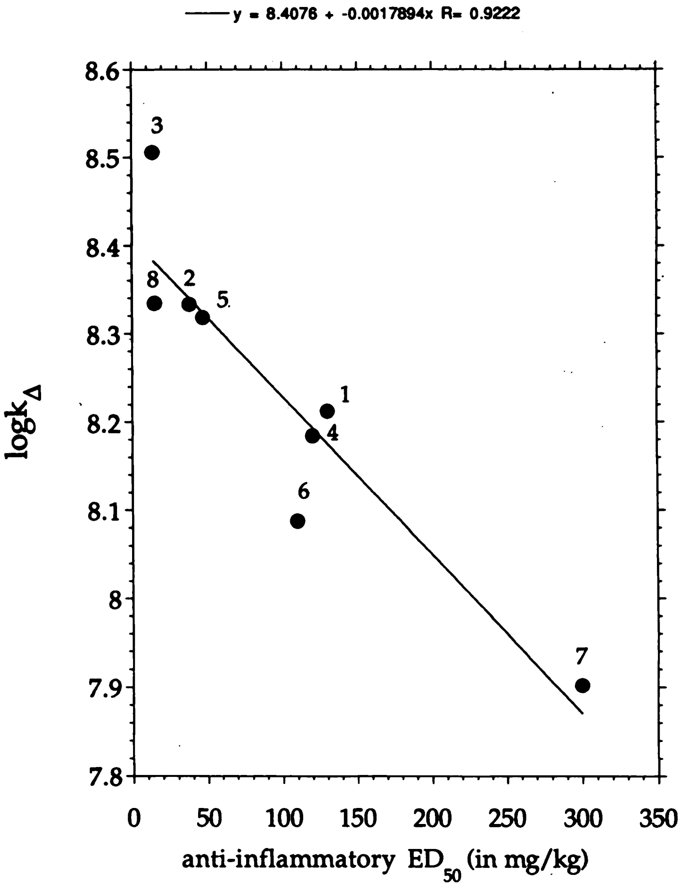

**Fig. 3.** Plot of log $k_\Delta$ of the nonsteroidal anti-inflammatory imidazoles versus their anti-inflammatory activity $ED_{50}$, tested in rats by Jørgensen and Dyrsting (1), with a linear correlation coefficient of 0.922.

TABLE 2

**Rate constants of singlet oxygen quenching by 2-substituted imidazoles, $k_\Delta$, and Hammett constants ($\sigma_m$, $\sigma_p$) of their substituents**

| Substituent | log $k_\Delta$ | log $k_i/k_H$[a] | $\sigma_m$[b] | $\sigma_p$[b] |
|---|---|---|---|---|
| H | 6.84 | | | |
| $CH_3$ | 7.45 | 0.61 | −0.07 | −0.17 |
| $C_6H_5$ | 7.23 | 0.40 | 0.06 | −0.01 |
| SH | 7.23 | 0.40 | 0.25 | 0.15 |
| CHO | 6.36 | −0.36 | 0.35 | 0.42 |
| $NO_2$ | 6.02 | −0.82 | 0.71 | 0.78 |

[a] $k_i$ is the overall singlet oxygen-quenching rate constant for the 2-substituted imidazoles and $k_H$ corresponds to the rate constant for the unsubstituted molecule.

[b] $\sigma_m$ and $\sigma_p$ are the Hammett substituent constants reported by Hansch *et al.* (7).

The products and intermediates (hydroperoxides, endoperoxides, and dioxetanes) most commonly formed by a photooxidation of imidazoles with molecular singlet oxygen have been previously determined (5, 6).

**Singlet oxygen quenching by the 2-substituted imidazoles.** In order to assess the possibility of an electron excess or deficiency in the transition state of the interaction between singlet oxygen and imidazoles, we have studied simpler imidazoles, for which substituent effects are more easily expressed than for the imidazole drugs. The rate constant of singlet oxygen quenching by a series of 2-substituted imidazoles was measured using the method described above for the anti-inflammatory compounds. The substituents in position 2 were the following, listed in order of their electron-withdrawing power (lowest to highest): $CH_3$, $C_6H_5$, SH, CHO, and $NO_2$. The rate constant of singlet oxygen quenching is higher for molecules with an electron-donating substituent such as $CH_3$ and lower for those with an electron-withdrawing substituent such as $NO_2$. Table 2 reports the variation of log $k_\Delta$ as a function of the Hammett substituent parameters ($\sigma_m$ and $\sigma_p$) taken from the survey of Hansch *et al.* (7). Straight lines are observed for the Hammett plots of Figs. 4 and 5. These straight lines can be expressed as log $k_i/k_H = \rho\sigma_m$ (or $\rho\sigma_p$) + Cte, with $k_i$ being the overall $^1O_2$ deactivation rate constant for the 2-substituted compounds and $k_H$ corresponding to that for the unsubstituted imidazole. The two Hammett parameters, related to six-carbon rings, are strongly correlated for the substituents of our imidazole derivatives, and both appear appropriate for the five-membered-ring imidazoles. The $\rho$ values obtained for the $\sigma_m$ and $\sigma_p$ parameters are −1.91 and −1.59, respectively. They are in fair agreement with the values obtained for the reaction between $^1O_2$ and *N,N*-dimethylanilines (−1.54) by Saito *et al.* (8) and for phenols (−1.72) by Thomas and Foote (9). These negative $\rho$ values obtained for the imidazoles suggest a significant charge-transfer character in the intermediate involved in the deactivation reaction of singlet oxygen. This is consistent with the increase of the anti-inflammatory power of the more complex fluorine-containing 2,4,5-triphenyl imidazoles being correlated with a decrease of the electron-withdrawing power of the substituents.

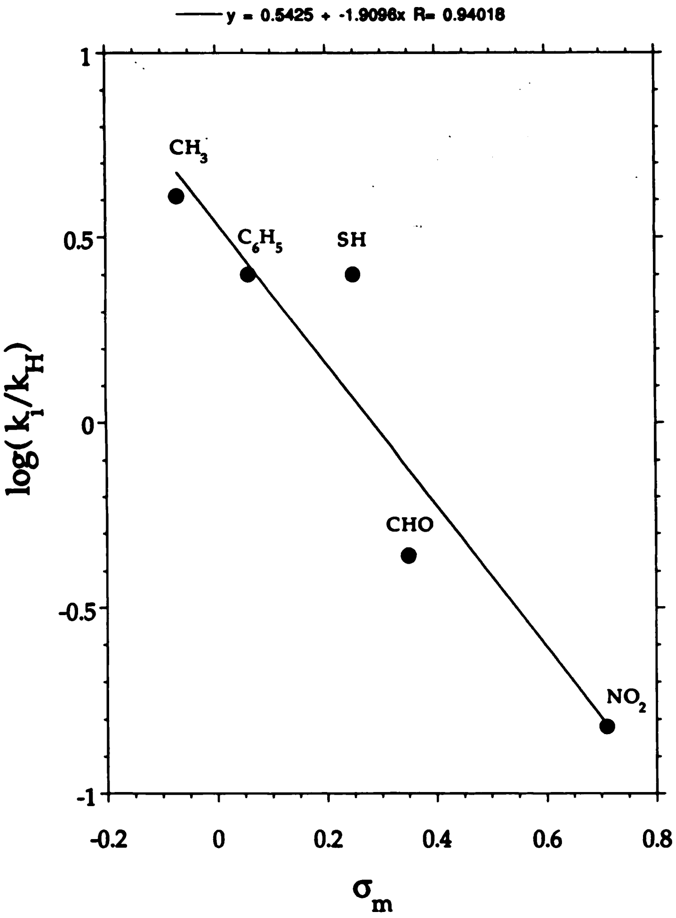

**Fig. 4.** Plot of log $k_i/k_H$ of the 2-substituted imidazoles versus the Hammett $\sigma_m$ parameter taken from the study of Hansch *et al.* (7), with a linear correlation coefficient of 0.940.

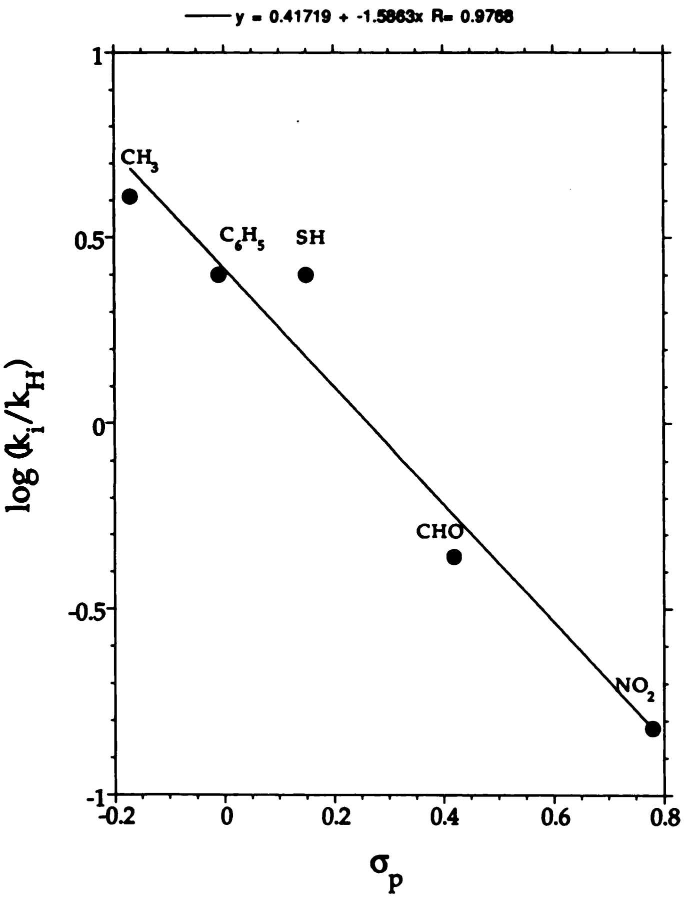

**Fig. 5.** Plot of log $k_i/k_H$ of the 2-substituted imidazoles versus the Hammett $\sigma_p$ parameter taken from the study of Hansch *et al.* (7), with a linear correlation coefficient of 0.977.

The rate constant $k_\Delta$ for the 2-mercaptoimidazole deviates significantly from the line in Figs. 4 and 5. This might be due to a different mode of reaction, which, at least in part, does not involve a charge-transfer complex, in contrast to the other imidazoles.

## Conclusion

For a series of fluorine-containing triphenylimidazoles, the correlation between the rate constant of singlet oxygen quenching, a physico-chemical parameter, and anti-inflammatory efficiency, a multiparametric biological property, is rather striking. This correlation could be valid for other types of compounds and may be useful in providing a screening method for candidate anti-inflammatory drugs. Although our study demonstrates that singlet oxygen quenching is a useful tool for the scaling of the antioxidant and anti-inflammatory efficiency of the imidazoles, our results do not imply that singlet oxygen is the main toxic inflammatory species *in vivo*. During inflammation, this activated form of oxygen can be produced by the recombination of peroxyl radicals generated by lipid peroxidation (10) via a Russell mechanism (11). However, the imidazole anti-inflammatory activity is certainly not due to a scavenging of singlet oxygen. The complexity of the biochemical events associated with inflammation *in vivo* (12) does not permit the anti-inflammatory effects of these drugs to be attributed to a single, simple, bimolecular reaction. Rather, our results imply that the redox properties of the drugs might be involved in their biological efficiency.

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