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Influence of steric factors on the interaction of isomeric phenyloxazoles and phenylthiazoles with microsomal oxidation

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In view of their potential for causing unforeseen drug interactions, it is important to identify classes of compounds which inhibit microsomal drug oxidation and to establish the structural features on which inhibition depends. Such compounds also have potential practical importance for commercial development as insecticide synergists [1]. In recent years attention has been focused on the activity of a variety of nitrogen-containing heterocycles such as the 1- and 4(5)-imidazoles [2, 4], benzimidazoles [5] and 1,2,3-benzothiadiazoles [6]. Many of these compounds are potent inhibitors of microsomal oxidation and with the former, correlations between inhibitory potency and spectral (type II) dissociation constants (K_s), have led to the view that activity results from direct ligand interaction between the ring nitrogen and the heme moiety of cytochrome P-450 [4]. This view is strengthened by the observation that steric hindrance around the nitrogen atoms in substituted imidazoles leads to a dramatic decrease in both inhibitory potency and type II binding [7]. If, indeed, this constitutes their major inhibitory action, a variety of other structurally related nitrogen-containing heterocycles such as oxazoles and thiazoles, known to be inhibitors of microsomal drug oxidation [5], should exhibit similar steric constraints with respect to their inhibitory action. We have accordingly prepared the isomeric phenyloxazoles and phenylthiazoles and evaluated their capacity to bind to cytochrome P450 and to inhibit epoxidase and *N*-demethylase activity in rat liver microsomal and southern armyworm (*Spodoptera eridania*) larval midgut preparations. The related compounds benzoxazole and benzothiazole were also evaluated.

The isomeric phenyloxazoles (I–III) and phenylthiazoles (IV–VI) (Fig. 1) were prepared according to established procedures. The reaction of vinylene carbonate with benzamide in polyphosphoric acid gave 2-phenyloxazole (III) [8]. Treatment of α-aminoacetophenone with refluxing triethyl orthoformate gave 5-phenyloxazole (I) [9], and 4-phenyloxazole(II) was formed in low purified yield by the reaction of α-bromoacetophenone with ammonium formate in dry formic acid[10]. Direct bromination of 2-phenylacetaldehyde [11], followed by treatment with thioformamide [12, 13] in ether, yielded 5-phenylthiazole (IV); 2-phenylthiazole (VI) was formed by refluxing an ethanolic solution of thiobenzamide and bromoacetaldehyde diethyl acetal for 20 hr with a few drops of added piperidine[11, 13]. 4-Phenylthiazole (V) was prepared by the reaction of phenacyl bromide with thioformamide [11, 13]. The physical properties of all compounds agreed closely with reported literature values, and satisfactory elemental analyses and spectroscopic data were obtained for all samples used for testing. Benzoxazole (VII) and benzothiazole (VIII) were purchased from Aldrich Chemical Co., Metuchen, NJ.

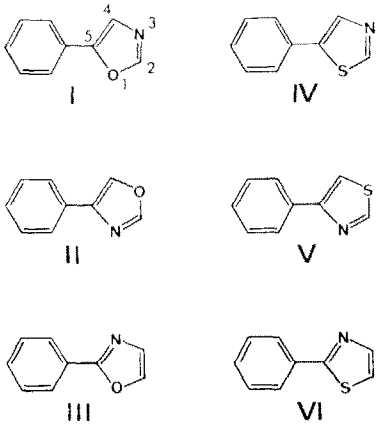

Fig. 1. Isomeric phenyloxazoles and phenylthiazoles.

All enzyme preparations, microsomal oxidase assay procedures and binding measurements were effected as previously described [7, 14].

The results in Table 1 are generally consistent with the steric model developed with the imidazoles [7]. Thus, as predicted, compounds I and IV with the heterocyclic nitrogen atom in the position least hindered by the phenyl substituent exhibit the highest inhibitory activity and low spectral (type II) dissociation constants (K_s) with both rat liver and armyworm midgut preparations. As with many of the imidazoles [3, 14], inhibitory activity in the insect preparation is greater than that in rat liver microsomes. *N*-demethylation with the latter being particularly insensitive to inhibition. In accordance with the fact that 4-phenyloxazole (II) shows only a low affinity (high spectral dissociation constant) and compounds III and V–VIII show little spectral interaction with rat liver microsomes, none of these compounds have any significant inhibitory effects, with the notable exception of 2-phenylthiazole (VI). This latter compound is anomalous in that it has an I_{50} of 4.7×10^{-6} M toward epoxidation in rat liver microsomes. The mechanism by which this occurs in unclear, although the inability of VI to exhibit type II binding characteristics suggests a mode of inhibition different from that of I and IV. Indeed, in armyworm midgut microsomes, compounds III, V and VI exhibited type I optical difference spectra, and Fig. 2 clearly shows the dramatic change in binding associated with moving the phenyl substituent from the 5-position of the thiazole ring (IV, type II) to the 4-position (V, type I). Two related compounds, benzoxazole (VII) and benzothiazole (VIII), showed no significant inhibition and no affinity for rat liver microsomes; indeed VII showed some stimulatory activity towards *N*-demethylation. Other benzoxazoles and benzothiazoles have been reported [5] to stimulate aromatic hydroxylation, but stimulation of *N*-demethylation by such compounds appears to be unusual.

Evidently high type II affinity depends specifically on the presence in the molecule of an unhindered nitrogen atom, and it is likely that any heterocycle meeting this requirement will exhibit this property. Type II affinity *per se*, however, does not necessarily mean that the compound will possess inhibitory properties towards

Table 1. Activity of oxazoles and thiazoles *in vitro*

Compound	I_{50} (M)				Spectral dissociation constant (K_s)	
	N-demethylation		Epoxidation			
	RLM*	AW*	RLM	AW	RLM	AWM*
I	$>10^{-4}$ (43)	1.6×10^{-6}	1.9×10^{-5}	1.5×10^{-5}	$3.83\pm0.50\times10^{-6}$	$1.32\pm0.06\times10^{-6}$
II	$>10^{-4}$ (7)	5.4×10^{-5}	$>10^{-4}$ (14)	$>10^{-4}$ (30)	$1.08\pm0.06\times10^{-4}$	$3.86\pm0.28\times10^{-5}$
III	$>10^{-4}$ (0)	$>10^{-4}$ (40)	$>10^{-4}$ (8)	$>10^{-4}$ (40)	ND†	(type I)‡
IV	$>10^{-4}$ (27)	1.4×10^{-6}	1.6×10^{-5}	5.4×10^{-6}	$5.43\pm0.66\times10^{-6}$	$1.52\pm0.13\times10^{-6}$
V	$>10^{-4}$ (10)	$>10^{-4}$ (24)	$>10^{-4}$ (24)	$>10^{-4}$ (36)	ND	$6.17\pm0.48\times10^{-4}$
VI	$>10^{-4}$ (32)	$>10^{-4}$ (47)	4.7×10^{-6}	$>10^{-4}$ (42)	ND	$3.19\pm0.20\times10^{-4}$
VII	Stimulates	Stimulates	$>10^{-4}$ (0)	$>10^{-4}$ (3)	ND	
VIII	$>10^{-4}$ (5)	$>10^{-4}$ (7)	$>10^{-4}$ (0)	$>10^{-4}$ (7)	ND	

*RLM, rat liver microsomes; AW, armyworm midgut preparation; and AWM, armyworm midgut microsomes. Numbers in parentheses refer to per cent inhibition at 10^{-4} M.

†Not detected at 10^{-4} M.

‡Compound III showed type I binding with AWM; K_s values for V and VI are based on type I spectra.

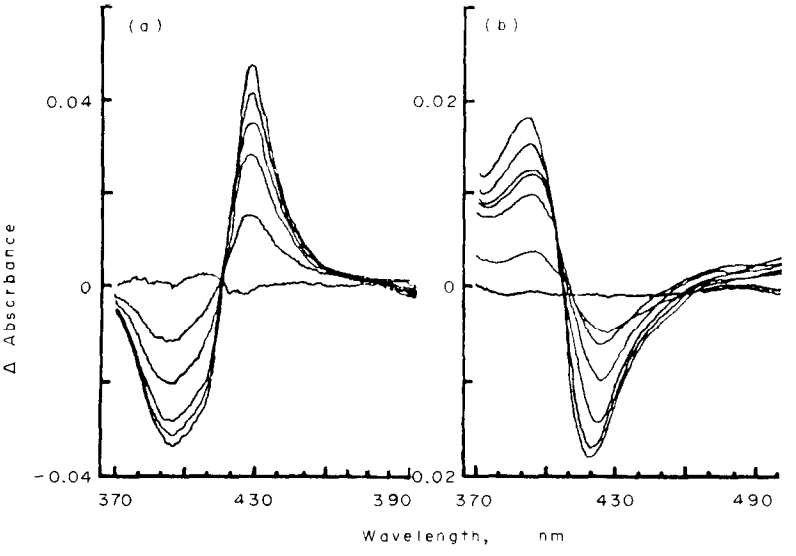

Fig. 2. Optical difference spectra from binding of compounds IV(a) and V(b) to armyworm midgut microsomes. In panel a, 2.0-μl increments of 0.040 mg/ml of ethanolic IV were added to a 4 mg/ml suspension of microsomes in 70 mM phosphate buffer; equal increments of ethanol were added to the reference cuvette. In panel b, the same procedure was followed, using a 4.02 mg/ml ethanolic solution of V.

microsomal oxidation, and even where inhibition is observed, clearly there are differences in the sensitivity of different oxidative reactions [3, 14].

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