tal cortex binding sites [1], is markedly more potent as an inhibitor of 5HT-induced shape change than of 5HT uptake. However, the compound is less specific for 5HT-induced shape change than for active uptake when compared with methysergide which in brain receptor binding studies acts on $5HT_1$ and $5HT_2$ receptors [1].

In agreement with earlier observations [23], therefore, the present study does not provide unequivocal evidence of an analogy between the types of brain receptor for 5HT and those involved in functional platelet changes induced by 5HT.

Acknowledgements—This work was supported by the Italian National Research Council, CNR-CT 81.00183.04. Ketanserin was obtained from Janssen Pharmaceutica, Beerse, Belgium, through the courtesy of Dr. F. De Clerck. We thank Professor P. A. J. Janssen and Dr. F. De Clerck for helpful discussion. Judith Baggott, Anna Mancini and Antonella Bottazzi helped prepare the manuscript.

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Biochemical Pharmacology, Vol. 31, No. 18, pp. 3002–3005, 1982.
Printed in Great Britain.

0006-2952/82/183002–04 $03.00/0

Inhibition and enhancement of mixed-function oxidases by nitrogen heterocycles

(*Received* 21 *December* 1981; *accepted* 12 *March* 1982)

Mixed-function oxidases (MFO) are inhibited, both *in vitro* and *in vivo*, by several classes of organic compounds. Many nitrogen heterocycles, including imidazoles [1–3], benzimidazoles [4], and pyrroles [5], are effective inhibitors of MFO activities.

Aromatic hydroxylations, particularly the *para*-hydroxylation of aniline, are capable of being enhanced by a range of xenobiotic molecules. Aniline *p*-hydroxylase activity is enhanced in the presence of ethyl isocyanide [6], acetone and butanone [7], 2,2′-dipyridyl [8], and acetophenone [9]. Even though several ketones and pyridine-containing compounds enhance microsomal MFO activity, no generalisations regarding the structural requirements for enhancement have been made.

The present study was undertaken to examine further if any particular classes of compounds could be categorised as enhancers of aniline *p*-hydroxylation and to examine whether any relationships exist between the inhibition of aminopyrine *N*-demethylase (APDM) and the enhancement of aniline *p*-hydroxylase (APH).

5(6)-Benzoylbenzimidazole (I) was synthesised by the reaction of 4-benzoyl-*o*-phenylenediamine with formic acid. The yield was 20%, and the compound melted at 143–145° (microanalysis: $C_{14}H_{10}N_2O$; calc. C 75.7%, H 4.5%, N 12.6%; found C 75.5%, H 4.7%, N 12.5%). 6-Ethoxy-2-methylbenzoxazole (III) was synthesised from *m*-ethoxyphenol after nitration at 25–35° in acetic acid/nitric acid (1:1) and reduction in a Parr hydrogenation apparatus with Raney nickel and acetic anhydride/acetic acid solvent. Cyclisation was effected in 10% acetic anhy-

Table 1. Inhibition and enhancement of mixed-function oxidations by nitrogen heterocycles*

Compound No.	Name	Inhibition of APDM ($I_{50} \times 10^{-5}$ M)	Enhancement of APH at 2×10^{-4} M (% of control)
I	5(6)-Benzoylbenzimidazole	2.0	118
II	2,5-Diphenyloxazole	8.2	220
III	6-Ethoxy-2-methylbenzoxazole	21	149
IV	5-Methylisoxazole	178	108
V	2-Aminothiazole	NI†	102
VI	6-Methoxy-2-methylbenzothiazole	9.3	151
VII	5-Nitroindazole	32	133
VIII	1*H*-Imidazolo(4,5-*b*)pyridine	NI	116
IX	1*H*-Imidazolo(4,5-*c*)pyridine	NI	100
X	2,2'-Dipyridyl	30	136
XI	1,10-Phenanthroline	29	109
XII	2,2'-Biquinoline	NI	180

* Control values ± standard errors (N = 20) were 4.9 ± 0.9 nmoles formaldehyde formed per mg protein per min for APDM activity and 0.9 ± 0.2 nmole *p*-aminophenol formed per mg protein per min for APH activity.

† NI = no inhibition observed below 300×10^{-5} M.

dride in acetic acid after a 12-hr reflux. Compound III melted at 20° (lit: 20.5° [10]). 5-Nitroindazole was synthesised as reported previously [11]. The isomeric imidazolopyridines (VIII and IX) were synthesised from the appropriate diaminopyridine with formic acid to effect cyclisation [12, 13].

Compounds VI and XII were obtained from the Aldrich Chemical Co., Inc., Milwaukee, WI, U.S.A., and compounds IV and V were obtained from Fluka AG, Zurich, Switzerland. Compounds II and XI were obtained from the Ajax Co., Sydney, Australia, and compound X was obtained from BDH Inc., Sydney, Australia.

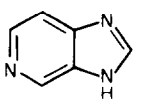
Biochemicals were obtained from Boehringer Mannheim, Sydney, Australia. All other chemicals were of analytical grade.

Microsomal fractions were prepared as described previously [4]. Protein was determined by the biuret method of Robinson and Hogden [14] with bovine serum albumin as the standard.

APDM activity was detected with the colorimetric procedure of Nash [15]. Incubations contained 1.61 μmoles aminopyrine, 10 μmoles magnesium chloride, 15.6 μmoles glucose-6-phosphate, 1 unit glucose-6-phosphate dehydrogenase, 1.4 μmoles $NADP^+$, 25 μmoles semicarbazide hydrochloride, and 5.6 mg of microsomal protein, in potassium phosphate buffer (0.1 M, pH 7.4 to 3.0 ml). I_{50} values were determined with seven inhibitor concentrations and were replicable to within ±10% of the stated mean values.

APH activity was assayed as described previously [4]. Incubations contained 15 μmoles aniline, 7.5 μmoles magnesium chloride, 13.4 μmoles glucose-6-phosphate, 2.5 units glucose-6-phosphate dehydrogenase, 2.7 μmoles $NADP^+$, and 9.2 mg of microsomal protein, in potassium phosphate buffer (0.1 M, pH 7.4 to 3.5 ml). Enhancement profiles were determined from the mean values of duplicate experiments that varied by less than ±10%.

APDM and APH activities were determined after 15 and 12 min of incubation respectively. Compounds were added in either dimethylsulfoxide (100 μl) or 0.07 M HCl (100 μl) whilst solvent alone was added to control flasks. The I_{50} values were the same when either solvent was used.



IX

Fig. 1. Structure of 1*H*-imidazolo(4,5-*c*)pyridine (IX).

Table 1 shows the effect of twelve nitrogen heterocycles on rat hepatic microsomal APDM and APH activities. APDM was most effectively inhibited by 5(6)-benzoylbenzimidazole ($I_{50} = 2.0 \times 10^{-5}$ M) and 2,5-diphenyloxazole ($I_{50} = 8.2 \times 10^{-5}$ M). Benzimidazoles are, in general, efficient inhibitors of APDM [4], but oxazoles have not been examined in detail. However, 5-phenyloxazole is a good inhibitor of rat hepatic aldrin epoxidase [16]. Two other oxazoles were examined in the present study, namely 6-ethoxy-2-methylbenzoxazole (APDM $I_{50} = 21 \times 10^{-5}$ M) and 5-methylisoxazole (APDM $I_{50} = 178 \times 10^{-5}$ M). The latter compound is probably a weak inhibitor of APDM, compared to the other oxazoles tested, due to its low hydrophobic character (calculated log P = 0.64). Hydrophobicity has been found to be a major factor in the inhibitory action of imidazoles [3] and benzimidazoles ([4] and *).

The three oxazoles (II–IV) examined in this study enhanced APH activity (Table 1). Log P values were calculated for compounds II–IV (with methods outlined in Ref. 17) and are 4.13, 2.53 and 0.64 respectively. Even though compound IV, at a concentration of 2×10^{-4} M, only enhanced APH by 8% over control activity, the order of potency of APH enhancement by II–IV was the same as the order of hydrophobicity. It is possible that compound hydrophobicity, at least in the case of oxazoles, can influence enhancing potency.

Compounds V and VI are thiazole derivatives, which are more hydrophobic than the structurally similar oxazoles due to the replacement of the ring oxygen with a sulphur atom. 2-Aminothiazole (V) did not inhibit APDM activity and produced only minimal enhancement of APH activity, whilst the benzothiazole (VI) was a potent inhibitor of APDM ($I_{50} = 9.3 \times 10^{-5}$ M) and enhancer of APH (151% of control at a concentration of 2×10^{-4} M).

5-Nitroindazole was a moderately potent inhibitor of APDM and enhancer of APH compared to other compounds in Table 1. Interestingly, this compound had an almost identical I_{50} against APDM as did 5(6)-nitrobenzimidazole ($I_{50} = 31 \times 10^{-5}$ M*), but whereas the benzimidazole inhibited APH with an I_{50} of 145×10^{-5} M, the indazole enhanced the activity. The two structures are obviously identical in terms of fulfilling the requirements

* M. Murray, A. J. Ryan and P. J. Little, unpublished results.

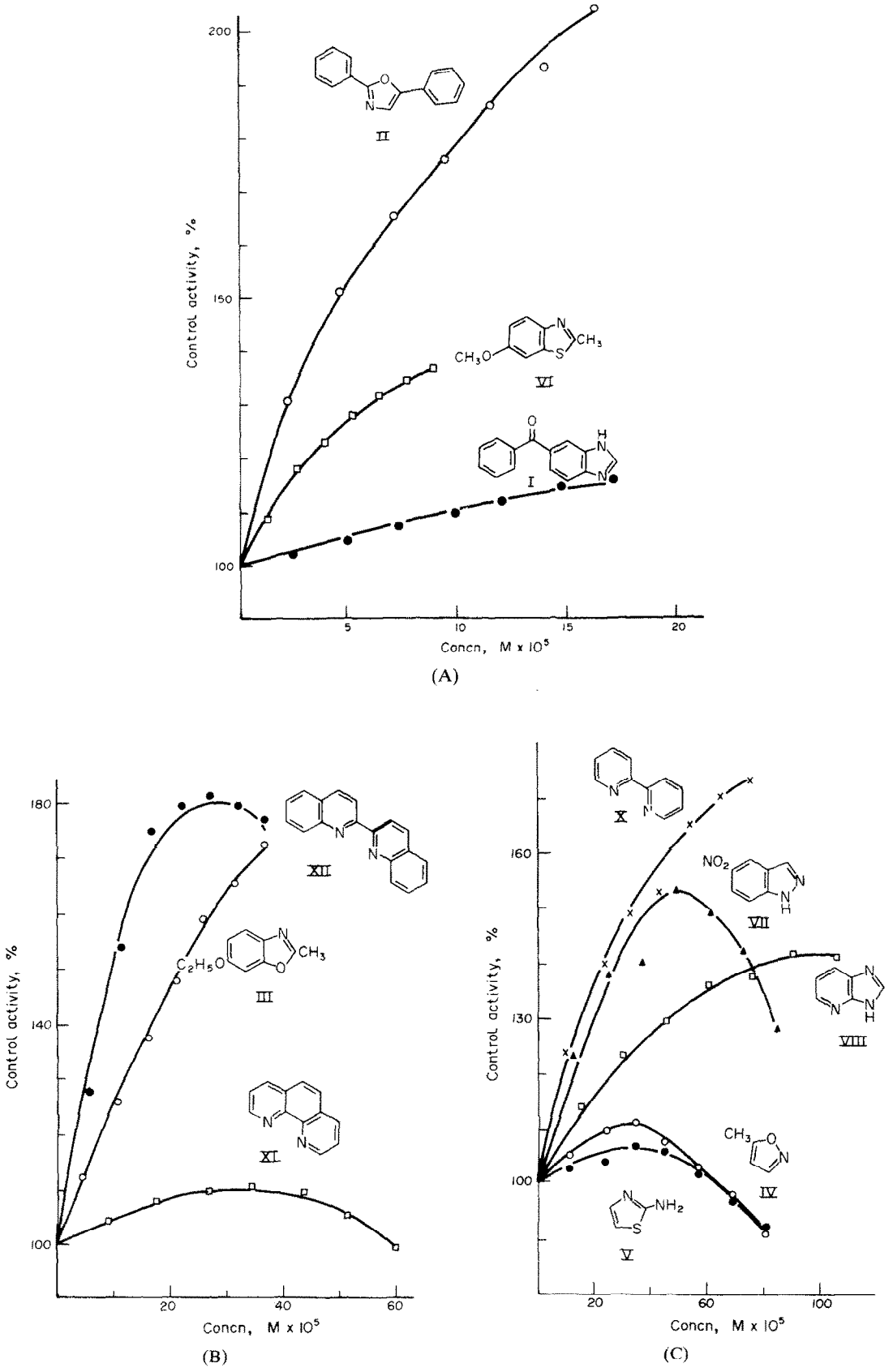


Fig. 2. Enhancement of APH activity with (A) compounds I, II and VI; (B) compounds III, XI and XII; and (C) compounds IV, V, VII, VIII and X.

for APDM inhibition, but are totally different with respect to their influence on APH activity. The body of data regarding indazoles and MFO inhibition or enhancement is small and a more substantial study is necessary to establish whether the structural factors which determine APH inhibition and enhancement can be differentiated from those which determine APDM inhibition.

Compound VIII [1*H*-imidazolo(4,5-*b*)pyridine] was inactive as an inhibitor of APDM activity, but produced a 16% enhancement of APH over control levels at a concentration of 2×10^{-4} M. The isomeric compound IX (Fig. 1) neither inhibited APDM nor enhanced APH.

Compounds X–XII are structurally similar compounds, and both X and XI have been observed previously to stimulate APH activity [8]. They are included in this study as reference compounds. Against rat hepatic APDM activity, both compounds X and XI are essentially equipotent which infers that, as 1,10-phenanthroline is a rigid molecule, 2,2′-dipyridyl is probably interacting in a similar fashion with cytochrome P-450 to produce inhibition of APDM activity. That is, the ability of compound X to rotate about the bond linking the two pyridine nuclei does not alter its capacity to inhibit APDM. Why compound XII (2,2′-biquinoline) is not an inhibitor of APDM is not clear, but this observation may reflect a size limitation of the cytochrome P-450 active site. Perhaps the bulky XII is not able to penetrate into and bind at the P-450 catalytic site. Another observation that is difficult to account for is the extremely high potency of compound XII as an enhancer of APH activity (180% of control at 2×10^{-4} M). A possible explanation is that, if a compound such as XII cannot reach the active site of cytochrome P-450 to effect APDM inhibition but is still able to produce APH enhancement, perhaps APH enhancement is not linked to direct binding to the cytochrome P-450 which metabolises aniline.

Figure 2 (panels A–C) shows the profiles obtained by plotting percent of control aniline *p*-hydroxylation versus enhancer concentration. Several compounds (IV, V, VII, XI and XII) were found to produce maximal enhancement at a specific concentration and then a decline in enhancement occurred beyond that concentration. The reason for this effect is not clear, but it is possible that inhibition was occurring beyond the maximal enhancing concentration. The other enhancers (I–III, VI, VIII and X) did not show a decrease in enhancement beyond a certain concentration, at least within the range of concentrations examined.

MFO inhibition may result from several types of interactions with cytochrome P-450. Among these interactions are mixed alternate substrate and irreversible binding behaviour [18], haem binding with the inhibitor acting as an axial ligand [3, 19], and displacement of substrate from its binding site with cytochrome P-450 by compounds which elicit a reverse type I spectral change [20] in oxidised microsomes. The mechanism by which APH is stimulated is not clear, and, indeed, much of the work to date has consisted of descriptions of the biphasic effects of compounds such as ethyl isocyanide [21]. Several theories to account for APH enhancement have appeared including facilitated breakdown of the enzyme–substrate complex [22], modification of the binding of aniline at the cytochrome P-450 catalytic site [23], and the postulation of the existence of an equilibrium between forms of cytochrome P-450 with differing affinities for enhancers [24]. Anders and Gander [25], following studies with cumene hydroperoxide, have suggested that acetone may enhance APH by influencing the formation of activated oxygen or its insertion into substrate. While all of these postulates have some evidence to support them, the mechanism of enhancement remains unclear.

Since subtle structural changes can result in an enhancer instead of an inhibitor, e.g. 5-nitroindazole compared to 5(6)-nitrobenzimidazole, further data should be obtained relating enhancing potency to structure and to differentiate between the factors which facilitate inhibition and enhancement. One important finding, which has emerged from the present study, is that groups of structurally similar compounds (oxazoles and thiazoles) consistently enhance APH activity. Previously, it has not been possible to consistently assign enhancing ability to a single structural type.

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