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INDUCTION OF RAT LIVER DRUG-METABOLIZING ENZYMES BY HETEROCYCLE-CONTAINING MONO-, DI-, TRI- AND TETRA-ARYLMETHANES

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Abstract—The effect of a nitrogen heterocycle constituent on the ability of arylmethanes to induce phase I and phase II drug-metabolizing enzymes has been examined. Rats were treated with tetra-, tri-, di- or monoarylmethane compounds daily for 3 days at a dose of 75 mg/kg. Induction of UDP-glucuronosyltransferase(morphine) activity was seen with twelve of the eighteen compounds investigated, and for three compounds it occurred independent of any induction of cytochrome P450. Induction of glutathione *S*-transferase activity was seen with ten of the compounds and was generally paralleled by changes in overall cytochrome P450 concentration and in both pentoxyresorufin and erythromycin dealkylase activities. Major induction of ethoxyresorufin deethylase activity was only apparent with two diarylmethanes that contained a 1-substituted imidazole moiety. UDP-glucuronosyltransferase(1-naphthol) activity was coinduced by these two compounds. A third compound, diphenyl-4-pyridylmethane, induced UDP-glucuronosyltransferase(1-naphthol) activity without increasing ethoxyresorufin deethylase activity. Cytosolic sulfotransferase activity was not induced by the administration of any compound in this study. Among arylmethane derivatives, the presence of two aryl groups appeared to be a minimum requirement for induction of drug-metabolizing enzymes. If one of the aryl groups was not a heterocycle, or if the nitrogen atom of the heterocycle was sterically hindered, major induction of cytochrome P450 did not occur. With triarylmethanes, induction was independent of whether the heterocycle was imidazole, pyridine or pyrimidine.

The influence of the chemical structure of aryl substituted nitrogen heterocycles on the induction of drug-metabolizing enzymes has generated considerable interest since studies showed that clotrimazole [1–5] and 1-benzylimidazole [6, 7] elicit divergent induction profiles. Both are efficacious inducers of cytochrome P450 (P450†) but differ as to the major isozymes that each induces. Clotrimazole treatment induces P450IIB and P450IIIA isozymes, while the most prominent induction with 1-benzylimidazole is in the P450IA isozyme(s). Both compounds are also major inducers of UDP-glucuronosyltransferase (UGT) and glutathione *S*-transferase (GST) activities but differ in the breadth of the UGT response. 1-Benzylimidazole induces both UGT(1-naphthol) and UGT(morphine) activities, but clotrimazole induces only the latter [6–8]. A change in the heterocyclic ring from imidazole to pyridine (1-benzylimidazole to 4-benzylpyridine) results in a selective loss of the ability to induce the P450IA isozyme [9]. A change in the steric hindrance around the nitrogen of the heterocycle (4-benzyl- to 2-benzylpyridine) results in the complete loss of P450-inducing ability but induction of phase II enzymes is retained. In addition, when two compounds lacking a heterocyclic ring (biphenyl and *trans*-stilbene) are compared with their heterocyclic analogs (phenylpyridines and 1,2-bis(pyridyl)-ethylenes), the ability to induce either phase I or phase II drug-metabolizing enzymes is seen to be lost entirely [9].

In a recent study in which only P450 changes were investigated, it was determined that 1-tritylimidazole, a non-halogenated analog of clotrimazole, is also a high magnitude inducing agent [10]. Induction also occurs if the heterocyclic ring is an azole but not if it is a pyrrole. The substitution on the heterocyclic ring does not have to be a triphenylmethane group for induction to occur. 1-Diphenylmethylimidazole, diphenyl-4-pyridylmethane and 4-benzylpyridine all induce P450 to the same extent (100% increase) [10]. Two compounds lacking a heterocyclic ring (tri- and diphenylmethane) do not induce P450. However, three other compounds not containing a heterocyclic ring, benzhydrol, triphenylmethanol and "monochlorotriphenylmethanol," elicit a slight (15–20%) increase in P450 [10]. Thus, the limits of the requirement for the presence of a nitrogen heterocycle for P450 induction to occur are unclear.

Although the nature of the group attached to the nitrogen heterocycle appears to be a relatively unimportant feature with respect to major P450 induction [10], these observations contrast with the differences that are known to exist in the nature and extent of P450 induction between various N-

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† Abbreviations: P450, cytochrome P450; UGT, UDP-glucuronosyltransferase; GST, glutathione *S*-transferase; EROD, ethoxyresorufin *O*-deethylase; PROD, pentoxyresorufin *O*-dependentylase; and EMND, erythromycin *N*-demethylase.

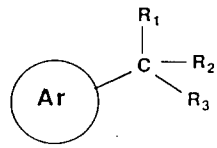
substituted imidazole antifungal agents. Large differences in response have been shown in rats [8, 11, 12] and, more recently, in human hepatocytes in primary culture [13]. Closer examination of the structure–activity relationships of the antifungal agents reveals that P450 induction is less if the imidazole and the closest aryl ring are separated by more than one carbon atom. Additional evidence for the possible importance of the presence of a heterocycle-methyl-aryl moiety as a contributing factor to high magnitude P450 induction can be found in a study with a non-steroidal aromatase inhibitor, 5-bis(4-chlorophenyl)methylpyrimidine, where a 10 mg/kg dose given orally to rats for 14 days increases P450 concentration over 3-fold [14].

Induction of phase II or conjugation enzymes by N-substituted imidazole antifungal agents has received much less attention than induction of P450. Although evidence cited above for clotrimazole and 1-benzylimidazole would indicate that UGT induction often accompanies increases in P450, the two are not always closely linked. Tioconazole was only a weak inducer of P450 but elicited large increases in UGT and GST activities [8]. This present study was undertaken in an attempt to clarify the importance of the presence and nature of the nitrogenous heterocycle in the induction of either phase I or phase II drug-metabolizing enzymes. In addition, the influence of the structure (with emphasis on the number of aryl groups and position of the substitution on the heterocyclic ring) on the induction response was also examined. Based on observations discussed above, compounds in which the aryl groups are separated from the heterocyclic group by a single carbon atom were chosen for investigation.

MATERIALS AND METHODS

Unless specified below, compounds employed in this study were purchased from the Aldrich Chemical Co. (Milwaukee, WI), and were used as supplied. Clotrimazole was purchased from the Sigma Chemical Co. (St. Louis, MO), 1-(2-naphthylmethyl)imidazole was a gift from Syntex Research, (Palo Alto, CA), and 5-bis(4-chlorophenyl)-methylpyrimidine) was a gift from Eli Lilly & Co. (Indianapolis, IN). 1-Tritylimidazole and 1-diphenylmethylimidazole were synthesized and characterized in the Department of Medicinal Chemistry, University of Utah. The compounds were synthesized from imidazole and triphenylmethanol or benzhydrol, respectively, by refluxing with boron trifluoride etherate (for 6.5 and 70 hr, respectively), neutralizing with sodium bicarbonate and recrystallizing the solid five times from ethyl acetate (Dr. C. Whitehead). Mono substitution was assured from the correct molecular ion by GC/MS (Dr. J. McCloskey) and substitution of the N-1 position was confirmed by the non-equivalence of protons 4 and 5 by NMR (Dr. A. Broom). Treatment compounds were either solubilized in an equivalent molarity of dilute hydrochloric acid immediately prior to administration or given as suspensions in 1% methyl cellulose or 30% polyethylene glycol 400. The compounds (Table 1) were administered daily for 3 days (75 mg/kg, i.g.), and the animals were euthanized 48 hr after the last dose. The 2-day time period has been established previously as allowing clearance of residual inducing agent from the liver without major losses of induced enzymes [2, 8]. Untreated rats served as experimental controls. Rats

Table 1. Compounds examined for their ability to induce hepatic drug-metabolizing enzymes



Ar	R_1	R_2	R_3	Compound
Tetra-arylmethanes				
Imidazole	C_6H_5	C_6H_5	C_6H_4Cl	Clotrimazole
Imidazole	C_6H_5	C_6H_5	C_6H_5	1-Tritylimidazole
Triarylmethanes				
Imidazole	H	C_6H_5	C_6H_5	1-Diphenylmethylimidazole
Pyridine	H	C_6H_5	C_6H_5	Diphenyl-(2-, 3-, and 4-)pyridylmethane
Pyridine	OH	C_6H_5	C_6H_5	α'-(4-Pyridyl)benzhydrol
Pyrimidine	H	C_6H_4Cl	C_6H_4Cl	5-Bis(4-chlorophenyl)methylpyrimidine
Phenyl	H	C_6H_5	C_6H_5	Triphenylmethane
Phenyl	OH	C_6H_5	C_6H_5	Triphenylmethanol
Diarylmethanes				
Imidazole	H	H	C_6H_5	1-Benzylimidazole
Imidazole	H	H	$C_{10}H_7$	1-(2-Naphthylmethyl)imidazole
Pyridine	H	H	C_6H_4Cl	4-(4-Chlorobenzyl)pyridine
Pyridine	H	H	C_6H_5	3-Benzylpyridine
Monoarylmethanes				
Pyridine	H	C_4H_9	C_4H_9	4-(1-Butylpentyl)pyridine
Pyridine	H	H	H	2-, 3-, and 4-Picoline

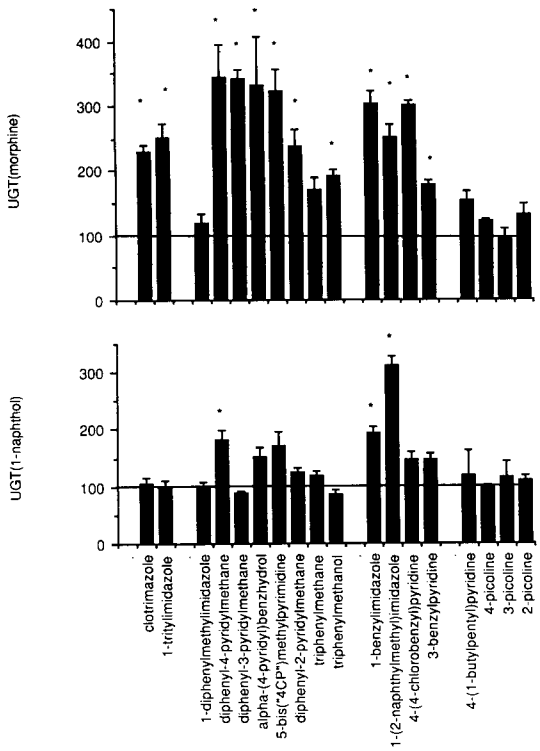


Fig. 1. Effect of animal treatment with arylmethanes (75 mg/kg, i.g., daily for 3 days) on hepatic microsomal UGT(morphine) and UGT(1-naphthol) activities. Values are means ± SEM, expressed as percent of concomitant controls. The number of animals in each treatment group was 7 for clotrimazole, 6 for 1-tritylimidazole, 3 for 1-diphenylmethylimidazole, 5-bis(4-chlorophenyl)methylpyrimidine, triphenylmethane, triphenylmethanol, 1-benzylimidazole and 4-, 3- and 2-picoline, and 2 for 1-(2-naphthylmethyl)imidazole. (In the latter case, the values are given as means ± range.) All other treatment groups were comprised of 4 animals. The abbreviation "4CP" = 4-chlorophenyl. The mean control values were 7.05 ± 0.62 and 47.3 ± 4.9 nmol/mg microsomal protein/min for UGT(morphine) and UGT(1-naphthol) activities, respectively. Asterisks (*) indicate significant ($P < 0.05$) increases from control.

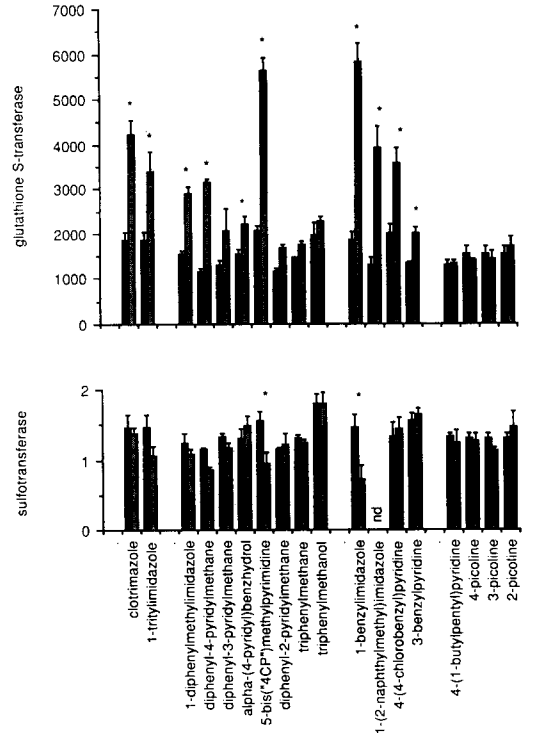


Fig. 2. Effect of animal treatment with arylmethanes (75 mg/kg, i.g., daily for 3 days) on hepatic cytosolic glutathione *S*-transferase and sulfotransferase activities. Values are means ± SEM (nmol/mg cytosolic protein/min), with control values shown as solid columns and treated values as shaded columns. The number of animals in each treatment group was 7 for clotrimazole, 6 for 1-tritylimidazole, 3 for 1-diphenylmethylimidazole, 5-bis(4-chlorophenyl)methylpyrimidine, triphenylmethane, triphenylmethanol, 1-benzylimidazole and 4-, 3- and 2-picoline, and 2 for 1-(2-naphthylmethyl)imidazole. (In the latter case, the values are given as means ± range.) All other treatment groups were comprised of 4 animals. Abbreviations: "4CP", 4-chlorophenyl; nd, not determined. Asterisks (*) indicate significant ($P < 0.05$) increases from control.

(male, Sprague–Dawley, 150–250 g) were purchased from Simonsen Laboratories (Gilroy, CA), and all animals were maintained under a 12-hr light–dark cycle in a temperature- and humidity-controlled environment and allowed free access to food and water. Weight-matched control and treatment groups were selected from the same batch of rats. All subcellular fractionations and enzyme assays on control and treated groups of each batch were performed concurrently.

Hepatic microsomal and cytosolic fractions were prepared as described previously [15]. Protein concentrations were assayed according to the method of Lowry *et al.* [16], and all enzyme activities were reported on this basis. Reverse-phase high pressure liquid chromatography was employed for the direct quantification of glucuronide formation from 1-naphthol and morphine [17]. Cytosolic GST activity towards 1-chloro-2,4-dinitrobenzene was analyzed spectrophotometrically according to Habig and Jakoby [18], and cytosolic sulfotransferase activity was determined from the disappearance of 4-nitrophenol [8]. P450 was determined by the method of Omura and Sato [19], 4-nitroanisole demethylase activity from the rate of *p*-nitrophenol generation [20], and ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin *O*-dependentylase (PROD) activities were measured from the rate of resorufin formation [21, 22]. Erythromycin demethylase activity was determined from the formaldehyde produced [23]. Statistical analyses were first made using ANOVA and differences between the original weight-matched treated and control groups assessed by the Scheffé F-test. Differences were considered significant at P values of less than 0.05.

RESULTS

UGT(morphine) activity was increased following

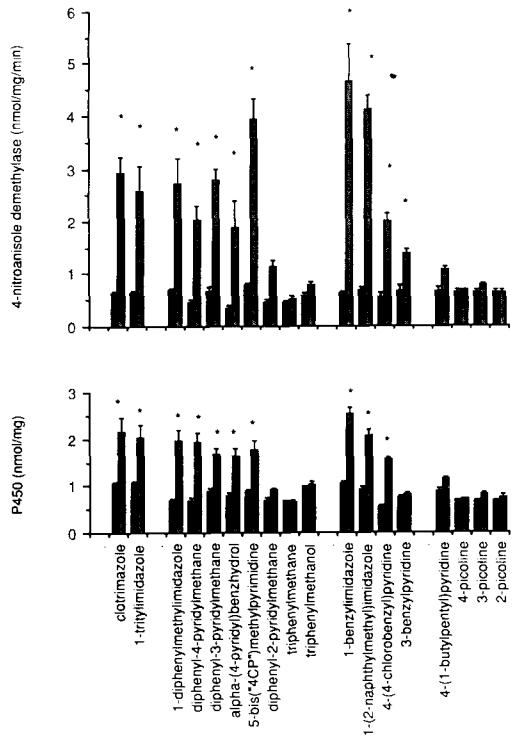


Fig. 3. Effect of animal treatment with arylmethanes (75 mg/kg, i.g., daily for 3 days) on hepatic microsomal 4-nitroanisole demethylase activity and P450 concentration. Values are means ± SEM, with control values shown as solid columns and treated values as shaded columns. Both parameters are expressed on the basis of microsomal protein. The number of animals in each treatment group was 7 for clotrimazole, 6 for 1-tritylimidazole, 3 for 1-diphenylmethylimidazole, 5-bis(4-chlorophenyl)methylpyrimidine, triphenylmethane, triphenylmethanol, 1-benzylimidazole and 4-, 3- and 2-picoline, and 9 for 1-(2-naphthylmethyl)imidazole. All other treatment groups were comprised of 4 animals. The abbreviation "4CP" = 4-chlorophenyl. Asterisks (*) indicate significant ($P < 0.05$) increases from control.

animal treatment with both of the tetra-arylmethanes investigated, six of eight triarylmethanes investigated, and all four diarylmethanes investigated (Fig. 1, top). With triarylmethanes, induction occurred when the heterocyclic ring was pyridine or pyrimidine but the imidazole derivative examined failed to elicit an increase. Induction was also observed with one of the two triarylmethanes (triphenylmethanol), which does not contain a heterocyclic ring. None of the four monoarylmethanes investigated, all of which contain pyridine as the aryl group, increased UGT(morphine) activity. In most instances, the changes in UGT(morphine) activity were not accompanied by responses of UGT(1-naphthol) activity (Fig. 2, bottom). UGT(1-naphthol) activity was only induced by a single triarylmethane (diphenyl-4-pyridylmethane) and by two of the four diarylmethanes investigated. Both of these diarylmethanes were 1-substituted imidazoles. Neither tetra-arylmethane investigated significantly altered this activity, despite also being 1-substituted imidazoles. The monoarylmethanes were the only compounds, taken as a class, where the response was the same for the two UGT activities, but the common response for the class was one of no induction.

Changes in a cytosolic conjugation reaction, GST (Fig. 2, top), were similar to those seen for microsomal UGT(morphine) for the tetra-arylmethane, diarylmethane and monoarylmethane compounds but the profile differed for the triarylmethane compounds. Within the triarylmethanes, three compounds that showed significant induction of UGT(morphine) activity (diphenyl-3-pyridylmethane, diphenyl-2-pyridylmethane and triphenylmethanol) failed to induce GST activity. GST activity was increased by 1-diphenylmethylimidazole, a compound that failed to induce UGT activities. The changes in GST activity were not seen in another cytosolic conjugation enzyme, sulfotransferase. Cytosolic 4-nitrophenol sulfotransferase activity was not induced by the administration of any compound in the entire study (Fig. 2, bottom). It was decreased by the two compounds showing the greatest induction of GST activity. This decrease may be attributable to a dilution effect since activities are calculated on a per mg protein basis and a greater proportion of the cytosolic protein is now accounted for by GST enzymes.

The changes in P450 concentration (Fig. 3, bottom) and 4-nitroanisole demethylase activity (Fig. 3, top) most closely followed responses in GST activity. Both of the tetra-arylmethanes and, with the single exception of 3-benzylpyridine which failed to increase overall P450 concentration, all four diarylmethanes investigated induced these parameters. As with GST activity changes among the triarylmethanes, there was no induction with the compounds that either lacked a heterocyclic ring (triphenylmethane and triphenylmethanol) or in which the nitrogen atom of the heterocyclic ring was sterically hindered (diphenyl-2-pyridylmethane). Identical to effects on all conjugation activities examined, monooxygenase parameters were also not induced by the monoarylmethanes.

The induction of P450-dependent EROD activity (Table 2) paralleled changes in UGT(1-naphthol) by the diarylmethanes (induction by the same two of four), and tetra- and monoarylmethanes (no induction). However, there was a reversal among the triarylmethanes, where diphenyl-4-pyridylmethane, which induced UGT(1-naphthol), did not induce EROD activity, and where 1-diphenylmethylimidazole induced EROD activity but not UGT(1-naphthol) activity. With two exceptions, a lack of PROD induction by 1-(2-naphthylmethyl)imidazole and a slight induction of erythromycin *N*-demethylase (EMND) by triphenylmethanol, changes in PROD and EMND activities (Table 2) over the whole range of compounds investigated paralleled the induction of overall P450 concentration (Fig. 3, bottom).

DISCUSSION

This report expands the range of heterocyclic

Table 2. Effect of animal treatment with arylmethanes on hepatic microsomal monoxygenase activities

Treatment	Monooxygenase activity (nmol/mg/min)		
	EROD	PROD	EMND
None (51)	0.03 ± 0.00	0.02 ± 0.01	0.54 ± 0.06
Clotrimazole (7)	0.13 ± 0.02	0.19 ± 0.06*	3.77 ± 0.99*
1-Tritylimidazole (6)	0.05 ± 0.02	0.14 ± 0.03*	2.69 ± 0.81*
1-Diphenylmethylimidazole (3)	0.36 ± 0.11*	0.31 ± 0.13*	2.55 ± 0.21*
Diphenyl-4-pyridylmethane (4)	0.08 ± 0.02	0.12 ± 0.03*	3.16 ± 0.68*
Diphenyl-3-pyridylmethane (4)	0.04 ± 0.01	0.22 ± 0.08*	2.13 ± 0.24*
α-(4-Pyridyl)benzhydrol (4)	0.03 ± 0.02	0.29 ± 0.04*	0.95 ± 0.17*
5-Bis(4-chlorophenyl)methylpyrimidine (3)	0.07 ± 0.03	0.58 ± 0.12*	3.12 ± 0.26*
Diphenyl-2-pyridylmethane (4)	0.01 ± 0.01	0.03 ± 0.01	0.53 ± 0.08
Triphenylmethane (3)	0.02 ± 0.01	0.01 ± 0.01	0.60 ± 0.06
Triphenylmethanol (3)	0.07 ± 0.01	0.04 ± 0.02	0.66 ± 0.03*
1-Benzylimidazole (3)	2.20 ± 0.69*	0.23 ± 0.04*	3.09 ± 0.13*
1-(2-Naphthylmethyl)imidazole (5)	1.81 ± 0.11*	0.04 ± 0.01	0.84 ± 0.07*
4-(4-Chlorobenzyl)pyridine (4)	0.19 ± 0.05	0.09 ± 0.01*	0.97 ± 0.14*
3-Benzylpyridine (4)	0.07 ± 0.02	0.02 ± 0.01	0.52 ± 0.09
4-(1-Butylpentyl)pyridine (4)	0.07 ± 0.01	0.01 ± 0.00	0.89 ± 0.20
4-Picoline (3)	0.10 ± 0.03	0.02 ± 0.01	0.51 ± 0.10
3-Picoline (3)	0.10 ± 0.03	0.02 ± 0.00	0.75 ± 0.02
2-Picoline (3)	0.09 ± 0.04	0.01 ± 0.00	0.61 ± 0.07

The numbers in parentheses following the treatment compound indicate the number of microsomal preparations evaluated. Monooxygenase values are means ± SEM.

Abbreviations: EROD, ethoxyresorufin *O*-deethylase; PROD, pentoxyresorufin *O*-dependentylase; and EMND, erythromycin *N*-demethylase.

* Significantly different from control ($P < 0.095$).

compounds structurally related to clotrimazole and other N-substituted imidazole antifungal agents that have been investigated for their ability to induce hepatic drug-metabolizing enzymes. Among the tetra-, tri-, di-, and monoarylmethanes investigated, only the monoarylmethanes did not contain any inducing agents. This provides further support for a previously derived conclusion that at least two aromatic ring systems appear to be necessary for major induction. A previous conclusion that has been compromised by the current study is the requirement of the presence of a heterocyclic ring for the induction of any drug-metabolizing reactions. In this study, one of the two non-heterocyclic compounds investigated, triphenylmethanol, increased a phase II activity, UGT(morphine). However, a heterocyclic ring may still be a requirement for significant P450 induction since the present study was unable to show the previously reported ability of triphenylmethanol to induce carbon monoxide-detectable P450 [10]. The study did substantiate the inability of triphenylmethane to produce a net increase in P450 concentration [10]. In addition to the heterocyclic ring requirement, it appears that the nitrogen atom in the heterocyclic ring must also be sterically unhindered if net P450 induction is to occur.

1-Substituted imidazole compounds were present in three of the four classes of arylmethanes investigated, specifically those containing four, three, and two aryl groups. In the tetra-arylmethanes , the absence of a chlorine atom present in a phenyl ring of clotrimazole did not affect the extent or nature of the induction response; 1-tritylimidazole produced essentially similar changes to clotrimazole in both phase II and phase I drug-metabolizing enzymes. A reduction in the number of phenyl groups from three to two (1-tritylimidazole to 1-diphenylmethylimidazole) markedly decreased the ability to induce UGT(morphine) activity. There was a slight but significant increase in EROD activity, but no induction of UGT(1-naphthol) was apparent. The large changes in ability to induce these two activities came when there was only one phenyl group (i.e. 1-benzylimidazole). The ability to induce EROD and UGT(1-naphthol) activities did not result in a loss of the ability to also elicit major induction of UGT(morphine) and GST activities.

For the compounds examined, the most ubiquitous induction response was of UGT(morphine) activity, a microsomal phase II enzyme. After this UGT response the next most often seen response was in GST activity, a cytosolic phase II enzyme. The induction of this activity was closely paralleled by the induction of P450 and, based on PROD and EMND activities, resulted at least in part from changes in P450IIB and P450IIIA isozymes. Major (10-fold) induction of P450IA isozymes, as determined from EROD activity, was a property of only two compounds, both diarylmethanes containing a 1-substituted imidazole. As noted above, major EROD induction was not seen with triarylmethane compounds containing a 1-substituted imidazole, nor was it seen with triarylmethanes containing other heterocyclic rings. The major changes in EROD activity were accompanied by significant induction of UGT(1-naphthol) activity. However, a third compound (diphenyl-4-pyridylmethane) induced

UGT(1-naphthol) activity without increasing EROD activity. There was an even greater deviation from a coordinated induction of UGT(morphine) and PROD activities following treatment with at least five of the fourteen compounds that elicited any inductive response. These two pairs of UGT and P450 activities are often considered as coordinately regulated based on changes elicited by prototypic inducing agents, such as β-naphthoflavone and phenobarbital.

The abilities of all four triarylmethane derivatives containing two phenyl groups and a heterocycle with an unhindered nitrogen (1-imidazole, 3- or 4-pyridines, 5-pyrimidine) to cause major increases in P450 concentration and 4-nitroanisole *O*-demethylase, EMND and PROD activities were similar to each other. However, 1-diphenylmethylimidazole was alone among the triarylmethanes in not eliciting a major induction of UGT(morphine) and also different from the others in inducing EROD activity to a low, but significant extent. No definitive conclusions about the relative ability of imidazole, pyridine or pyrimidine rings to influence the induction of GST activity could be made although the induction by the pyrimidine derivative was the only one of sufficient magnitude to produce the presumed dilution effect (i.e. decrease) in sulfotransferase activity that was also seen with 1-benzylimidazole.

Among the diarylmethanes investigated, all induced P450 concentration, 4-nitroanisole demethylase, UGT(morphine) and GST activities, the two imidazole derivatives generally inducing to a greater extent than the two pyridine derivatives. The imidazole but not pyridine derivatives significantly induced EROD and UGT(1-naphthol) activities. Compared to 4-benzylpyridine [9], 4-(4-chlorobenzyl)pyridine elicited a greater induction of P450 and UGT(morphine) while the induction of GST activity remained the same. The changes elicited by 3-benzylpyridine, with the exception of the extent of P450 concentration induction, were similar to those reported previously for 4-benzylpyridine and thus different from those seen with 2-benzylpyridine [9], an isomer in which the pyridine nitrogen is sterically hindered.

In this study the objective was to investigate the nature and not necessarily the maximum magnitude of the response of rat hepatic drug-metabolizing enzymes to a wide range of nitrogen heterocycle containing compounds. The treatment regimen used was based on exhaustive previous studies with imidazole and pyridine containing compounds in which effects of dose and time were considered [2, 6, 8, 24–26]. This single dosage regimen approach has made possible the screening of a wide range of compounds for their possible inductive effects, thus facilitating the selection of unique compounds for more intense investigation of their mechanism(s) of induction. Based on previous studies it is unlikely that induction effects have been overlooked although it should be recognized that additional experiments optimizing individual dosage regimens may alter the relative magnitude of the enzyme changes. Even so, it should be noted that the magnitude of most of the changes seen in this single dose evaluation was comparable with those elicited by the "classical" inducing agents [2, 6, 8].

Over the range of compounds investigated in this study, it has been found that induction of UGT(morphine) activity is a more ubiquitous response than induction of either P450 or any individual P450 isozymes. UGT(morphine) induction can occur independent of the induction of P450IIB isozymes, and another transferase activity, UGT(1-naphthol), can be induced independent of P450IA isozymes. Among arylmethane derivatives, the presence of two aryl groups appears to be a minimum requirement for induction of rat hepatic drug-metabolizing enzymes. If one of the aryl groups is not a heterocycle, or if the nitrogen atom of the heterocycle is sterically hindered, induction of P450 does not occur although the ability to induce UGT(morphine) may be retained.

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