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Studies on the Toxicity of Analogues of Dapsone In-vitro Using Rat, Human and Heterologously Expressed Metabolizing Systems†

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Abstract

Three metabolizing systems (rat, heterologously expressed CYP3A4 and human liver) were used to evaluate 12 analogues of dapsone (4,4′diaminodiphenylsulphone) in-vitro. Methaemoglobin formation in a two-compartment and cytotoxicity in a single-compartment model were studied using human erythrocytes and neutrophils, respectively, as target cells.

In the two-compartment system using rat microsomes as a generating system and methaemoglobin as an endpoint, the least potent methaemoglobin formers tested were the 2-methyl-4-propylamino (AXDD14), 2-hydroxy-4-4′amino (ABDD5) derivatives and a sulphone/trimethoprim derivative (K-130). Dapsone itself, a 2-methoxy-4-ethylamino (W10) and a 2-hydroxyl-4-ethylamino compound (ABDD39) were the most toxic. In the single-compartment cytotoxicity test using rat microsomes, AXDD14 was again among the least toxic, as was a 2-methyl 4-cyclopentyl derivative (AXDD17) and surprisingly ABDD39. The most cytotoxic compounds again included dapsone itself as well as two 2-trifluoromethyl derivatives. The only significant methaemoglobin formation and cytotoxicity shown with the heterologously expressed human CYP 3A4 was with AXDD14, which was extensively activated. Interestingly, metabolism of dapsone was low using the expressed CYP 3A4.

In the two-compartment system using human liver microsomes, AXDD14, K-130 and ABDD5 were oxidized to a significantly lesser extent compared with dapsone and these preliminary findings indicate that future development of these compounds may be worthwhile.

Inhibition of bacterial and protozoan de-novo synthesis of folate has been one of the oldest and most successful broad-spectrum antibiotic therapeutic approaches. Two enzyme systems in this pathway can be inhibited: dihydropteroate synthetase (DHPS), by analogues of *p*-amino benzoic acid such as dapsone and sulphonamides (Koehler & Hopfinger 1988), as well as dihydrofolate reductase (DHFR), which can be blocked by 2,4 diamino 5-benzyl pyrimidines such as trimethoprim (Roth et al 1961). These compounds have become useful in the chemotherapy of HIV-related *Pneumocystis carinii* pneumonia and toxoplasmosis (Torres et al 1992) and although dapsone and sulphonamides are equipotent (Voeller et al 1994), the sulphone is better tolerated by AIDS patients (Lee et al 1989). Unfortunately, the applications of dapsone are dose-limited due to its hepatic phase I activation to haematologically toxic hydroxylamines (Coleman et al 1989, 1990a). Short-term efforts to reduce dapsone toxicity and improve patient tolerance involving co-administration of cimetidine have been moderately successful (Coleman 1993); however, a number of potentially less toxic but more intrinsically active derivatives of this sulphone have been synthesized (Wiese et al 1987; Pieper et al 1989), where cytochrome P450-mediated metabolism was intended to be reduced by 2-substitution. In addition, increased lipophilicity brought about by 4-amino alkyl substitution, markedly promoted bacterial membrane penetration (Pieper et al 1989) compared with the parent drug.

Preliminary animal studies have indicated that a number of these analogues are far less toxic than dapsone (Pieper et al 1989); therefore, we have toxicologically tested 12 of these analogues in-vitro.

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† Dedicated to the memory of Mark J. Winn, Ph.D.

Materials and Methods

Chemicals

The following dapsone (4,4′ diaminodiphenyl sulphone) analogues were evaluated (Fig. 1): K-130 (2,4-diamino-5-{4-[3-(4″-aminophenyl-4′sulphonylphenylamino)propoxy]-3,5-dimethoxybenzyl}pyrimidine (Kansy et al 1992); AXDD14: 2-methyl-4-propylamino-4′amino-diphenylsulphone; AXDD17: 2-methyl-4-cyclopentylamino-4′amino-diphenylsulphone; ABDD5: 2-hydroxy-4-4′amino-diphenylsulphone; ABDD39: 2-hydroxy methyl-4′ethyl amino-diphenylsulphone; ABDD1: 2-hydroxy-4-propylamino-4′amino-diphenylsulphone; AXDD16: 2-methyl 4-4′hexylamino diphenyl sulphone; W10: 2-methoxy-4-ethylamino-4′amino-diphenyl sulphone; PXDD19: 2-trifluoromethyl-4-ethylamino-4′amino-diphenyl sulphone; PXDD4: 2-trifluoromethyl-4′amino-diphenyl sulphone; W25: 2-amino-4′amino-diphenyl sulphone; W13: 2-nitro-4-amino-4′amino-diphenyl sulphone; (Pieper et al 1989). The relative lipophilicities (log k values) and the Van der Waals volumes (V_w) of the respective compounds (Table 1) are derived from Pieper et al (1989). Dapsone was obtained

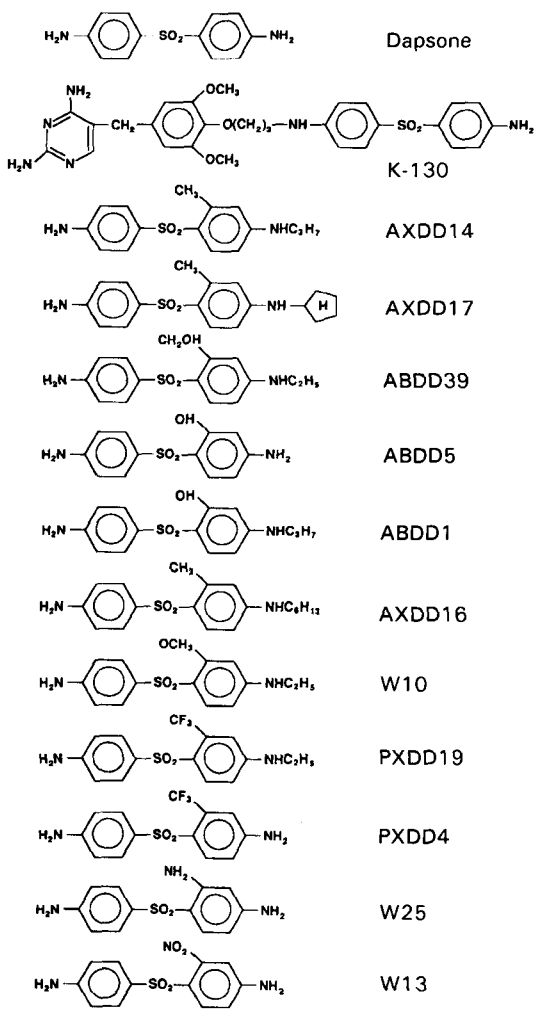

FIG. 1. Structural formulae of dapsone and 12 novel sulphone analogues.

from Aldrich Chemicals (Poole, UK), NADPH was obtained from Fluka Chemicals (UK).

Biological materials

Rat (male Sprague-Dawley) microsomal preparations were prepared according to the methods of Purba et al (1987), cytochrome P450 levels were determined by the method of Omura & Sato (1964) to be 0·64 nmol (mg protein)$^{-1}$. Human cytochrome P450 3A4 was expressed in *Saccharomyces cerevisiae* strain AH22. Cells transformed with the vector pAAH5 incorporating P450 3A4 cDNA (Guengerich et al 1991) downstream of an alcohol dehydrogenase promotor, were maintained on yeast minimal medium (YMM) Difco, Detroit, USA comprising yeast nitrogen base, without amino acids (6·7 g L^{-1} glucose 5 g L^{-1}; histidine 20 mg L^{-1}; agar 20 g L^{-1}) at 30°C. Transformed cells were grown with shaking (120 rev min^{-1}) in liquid YMM at 30°C to an approximate cell density of 5×10^7 mL^{-1}, concentrated by centrifugation, then disrupted by three passages through an ice-cooled French Press. Microsomes were prepared from the yeast using the method of Kenna et al (1989). The total P450 content was measured spectrometrically (156 pmol (mg protein)$^{-1}$) and protein content was determined using Bio-Rad reagents.

Microsomal suspensions of three human liver samples (livers 1, 2 and 3) were kindly provided by Dr Barry Jones, of Pfizer Pharmaceuticals Ltd. These had been ascribed CYP3A4 activities by the use of felodipine and testosterone assays and comparison of the values with 25 other livers in the Pfizer human liver bank. Human liver 1 (P450 ± 6 nmol (mg protein)$^{-1}$) was described as relatively high in activity (felodipine metabolism: 1110·6 ± 145 pmol (mg protein)$^{-1}$ min^{-1}; testosterone metabolism: 9228·6 ± 1592 nmol (mg protein)$^{-1}$ min^{-1}), liver 2 (2·8 nmol (mg protein)$^{-1}$ min^{-1}), medium (felodipine metabolism: 380·7 ± 31·9 pmol (mg protein)$^{-1}$ min^{-1}; testosterone metabolism: 2803·2 ± 478·8 nmol (mg protein)$^{-1}$ min^{-1}) and liver 3 (5·5 nmol (mg protein)$^{-1}$ min^{-1}), low activities (felodipine metabolism: 57·6 ± 3·4 pmol (mg protein)$^{-1}$ min^{-1}; testosterone metabolism: 241·9 ± 21·6

Table 1. Rank orders of toxicity (number 1 is most toxic) of 12 sulphone analogues and dapsone, determined in in-vitro test systems, as well as two physicochemical parameters (log k′ and V_w (Pieper et al 1989)).

Compound	log k′	V_w	Methaemoglobin formation		Cytotoxicity		
			Rat	3A4 Human	Liver Human	Rat	Human 3A4
Dapsone	0.339	3.32	2	6	1	1	11
K-130	2.09	3.3	12	3	2	5	12
AXDD14	2.213	13.67	13	1	3	10	1
AXDD17	3.288	13.67	8	8	nt	13	6
ABDD39	0.616	18.77	4	5	nt	12	7=
ABDD5	−0.0091	8.04	11	7	4	11	4
ABDD1	1.933	8.04	9	10=	nt	6	9
AXDD16	4.04	13.67	10	2	nt	9	5
W10	1.27	16.07	1	10=	nt	4	2
PXDD19	2.45	21.33	7	9=	nt	3	8
PXDD4	1.409	21.33	6	9=	nt	2	7=
W25	−0.096	10.54	3	10=	nt	7	10
W13	0.8543	16.8	5	4	nt	8	3

nt denotes not tested, = denotes equal ranking.

nmol (mg protein)$^{-1}$ min^{-1}). The microsomes also contained cytochromes 2E1, 2C, 2D and 1A2, which were characterized by the use of *p*-nitrophenol, diclofenac, bufurolol and caffeine, respectively. The microsomal suspensions were stored at −70°C until utilized. Washed human erythrocytes were prepared as previously described (Coleman & Jacobus 1993).

Experimental design

The 12 compounds were screened using five assays: the first involved a two-compartment in-vitro system which consists of two Teflon compartments separated by a cellulose (molecular weight cut-off 5000 Da) membrane (Tingle et al 1990). Compartment A contained 2 mg rat liver microsomes, 1 mM NADPH and 5 μL DMSO solution of a test analogue (final concentration 100 μM). This concentration of analogue was employed in previous dapsone analogue studies (Coleman et al 1991). Vehicle controls contained 1% DMSO and buffer and microsomal controls contained all the above except for NADPH. Compartment B contained 500 μL washed (50% haematocrit) human erythrocytes. Incubations were carried out in triplicate. In all cases, no methaemoglobin was generated in NADPH-free incubations before determination of methaemoglobin formation using an IL-482 CO-oximeter.

The second test system involved the use of a cytotoxicity assay (Spielberg 1984), where human mononuclear leucocytes isolated according to the method of Boyum (1976) from blood donated by normal volunteers, were exposed to a metabolite-generating system (rat microsomes, as above). The final incubation volume in plastic screw-capped 10-mL tubes (LIP, Shipley, UK) was 1 mL. These incubations of human mononuclear leucocytes (1 million cells per incubation) were maintained in a shaking water bath for 1 h and then removed from the bath and centrifuged at 1100 rev min^{-1} and the supernatant discarded. The cells were then resuspended in HEPES buffer containing 5 mg mL^{-1} bovine serum albumin and maintained overnight at 37°C. Cell death was determined by trypan blue exclusion 18 h later.

The third test system involved the two-compartment system where the metabolizing system (in compartment A) involved the use of microsomes produced from heterologously expressed human 3A4. Compartment one contained 13 mg microsomal protein, NADPH (1 mM) as well as the compounds at 100 μM concentration. As P450 concentrations in the heterologously expressed protein were much lower compared with those of the rat, almost five times as much heterologously expressed protein was necessary to produce the equivalent methaemoglobin level in the washed human erythrocytes in compartment B.

The fourth test system was as above, except compartment B contained human mononucleocytes (1 million cells per incubation). The fifth and final test system involved the use of the two-compartment system which contained (compartment A) human liver microsomal suspension (3 mg protein per incubation) as well as NADPH (1 mM). Due to the restricted availability of human liver microsomes and on the basis of the results of the first four studies, only three analogues were selected for testing (AXDD14, ABDD5 and K-130). Three assays were carried out, where dapsone was used as a positive control with each of the three liver suspensions. Compartment B contained 500 μL washed erythrocytes as above.

Vehicle controls contained 1% DMSO and cells, microsome controls containing cells and DMSO (1%) were also run parallel to the test incubations. Samples were placed in a water bath at 37°C and incubated for 1 h. Two physicochemical parameters log k′ and V_w have been included in Table 1 and these parameters correspond to relative lipophilicity and molecular size (Pieper et al 1989). All statistical analysis was by Student's *t*-test and all data are represented as mean ± s.d. Where more than one comparison was made with the same data, the Bonferroni correction (Elashoff 1981) was employed, where the acceptable level of significance was reduced to 0·05/k (where k is the number of tests) to compensate for the increased likelihood of reaching $P < 0.05$ during multiple testing.

Results and Discussion

The results of all experiments in this study are summarized in Tables 1, 2 and 3.

Two generating systems have been employed in these studies to examine the toxicity of 12 dapsone derivatives and to select the least toxic compounds for further evaluation using using human liver microsomes. Rat liver microsomes are a reasonable preliminary test substitute for human liver microsomes as the CYP2C sub-family represents about 50% of rat and 20% of human liver cytochrome P450 (Nedelcheva & Gut 1994). In addition, rat and human forms of CYP 2E1 are also similar (Soucek & Gut 1992). As CYP 3A4 is the major human P450 (Nedelcheva & Gut 1994), we have used heterologously expressed 3A4 to determine the extent of the metabolism of these analogues in the absence of competing cytochromes P450.

Previous studies have been unable to detect any ring-hydroxylated metabolites of dapsone (Israili et al 1973; Tingle et al 1990), so it is highly likely that the structural analogues of dapsone under study in this report are also oxidized as the parent drug is, to hydroxylamines, which in turn may be cytotoxic as well as generators of methaemoglobin (Coleman et al 1989, 1991). Methaemoglobin formation is the most common side-effect which limits dapsone's effectiveness in patients (Manfredi et al 1979) and is thus valuable in the study of sulphone analogue toxicity (Coleman et al 1991; Tingle et al 1991). In general, sulphone-derived hydroxylamines react with oxyhaemoglobin, which is thought to result in the formation of a short-lived nitroso derivative and methaemoglobin (Kiese et al 1950). The nitroso derivative is reduced by erythrocytic reductases and glutathione to the hydroxylamine and is thus able to oxidize another oxyhaemoglobin molecule (Kramer et al 1972). Mononucleocyte cytotoxicity has been used successfully as a bioassay for the formation of sulphone hydroxylamines, which undergo auto-oxidation to cytotoxic nitroso derivatives; stabilization of the hydroxylamines with ascorbate prevents nitroso formation and thus cytotoxicity is reduced (Coleman et al 1989). In the present study, dapsone itself was used as a positive control in terms of both methaemoglobin formation and cytotoxicity (Table 2) mediated by all three metabolite-generating systems.

AXDD14 (2-methyl-4-propylamino), ABDD5 (2-hydroxy-4-amino) and AXDD16 (2-methyl-4-hexylamino) were the least toxic compounds in both the methaemoglobin and cytotoxicity assays. Only dapsone itself and W10 (2-methoxy-4-propylamino) were shown to be the most toxic in both systems.

Table 2. In-vitro toxicity caused by microsomal oxidation of dapsone and 12 sulphone analogues.

Sulphone	Rat microsomes		Heterologous human 3A4 microsomes	
	Methaemoglobin (%)	Cell death (%)	Methaemoglobin (%)	Cell death (%)
Control	0.7 ± 0.2	2.2 ± 1.1	0.8 ± 0.2	4.2 ± 3.1
Dapsone	34.3 ± 1.2**	24.7 ± 10.2***	1.3 ± 0.2*	4.7 ± 1.2
K-130	12.5 ± 1.3**††	10.5 ± 3.6**	2.6 ± 1.3*	3.3 ± 1.9
AXDD14	4.0 ± 0.4**††††	7.2 ± 3.8*	33.2 ± 1.7****	13.0 ± 4.4**
AXDD17	18.9 ± 1.3**††	2.1 ± 1.0	0.9 ± 0.2	6.9 ± 1.0
ABDD39	31.6 ± 6.6**	3.6 ± 1.3	1.6 ± 0.6	6.0 ± 1.3
ABDD5	12.4 ± 1.1**††	4.1 ± 3.1	1.1 ± 0.1	8.3 ± 5.7
ABDD1	18.0 ± 2.3**††	10.1 ± 3.2**	0.5 ± 0.2	5.6 ± 3.2
AXDD16	17.9 ± 2.1**††	8.8 ± 2.8**	3.4 ± 1.7*	8.0 ± 2.8
W10	34.5 ± 2.0**	13.7 ± 11.1	0.5 ± 0.1	10.1 ± 6.0
PXDD19	22.9 ± 2.5**††	14.2 ± 4.7***	0.6 ± 0.1	5.7 ± 4.7
PXDD4	28.2 ± 3.3**	17.0 ± 4.5***	0.6 ± 0.3	6.0 ± 4.5
W25	33.4 ± 1.0**	9.2 ± 2.5**	0.5 ± 0.2	5.0 ± 2.5
W13	28.4 ± 0.7**	9.1 ± 3.25**	1.7 ± 0.7	9.1 ± 3.25

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ significantly greater compared with control. †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$, ††††$P < 0.0001$ significantly greater compared with positive control (dapsone). All values are mean ± s.d. For the methaemoglobin experiments all incubations were carried out in triplicate. For the mononucleocyte cell-death experiments n = 4 per incubation.

Table 3. Methaemoglobin (%) formed in the two-compartment system, where compartment A contained either of three human liver suspensions (3.0 mg protein) plus NADPH (1 mM) and one of three analogues, each compared with dapsone at the same concentration (100 μM) in the same assay. Compartment B contained 500 μL of washed human erythrocytes.

Assay	Human liver 1		Human liver 2		Human liver 3	
A	Dapsone	AXDD14	Dapsone	AXDD14	Dapsone	AXDD14
	6.7 ± 0.7	4.2 ± 0.5**	4.7 ± 0.37	4.0 ± 0.15*	4.1 ± 0.3	3.4 ± 0.1*
B	Dapsone	K-130	Dapsone	K-130	Dapsone	K-130
	4.6 ± 0.2	3.4 ± 0.15*	3.2 ± 0.3	2.0 ± 0.11*	3.0 ± 0.5	0.75 ± 0.1**
C	Dapsone	ABDD5	Dapsone	ABDD5	Dapsone	ABDD5
	5.3 ± 0.4	2.1 ± 0.47**	4.0 ± 0.2	1.9 ± 0.2**	3.1 ± 0.9	1.6 ± 0.5**

Values are mean ± s.d., n = 3. *$P < 0.05$, **$P < 0.01$ significantly different compared with dapsone.

These observations could be accounted for in terms of the likelihood of rat P450s to oxidize the compounds to methaemoglobin-forming hydroxylamines. With the 2-substituted analogues, the electron-donating nature of the 2-methyl (AXDD14, AXDD16 and AXDD17) and hydroxy (depending on degree of ionization; ABDD1, ABDD5) groups (Ceppi et al 1973) may have been a factor in the comparative lack of activation of these analogues compared with dapsone. Further substitution at the 4-amino group of a propyl derivative provided optimum lack of activation (AXDD14) while the bulkier AXDD16 (hexyl) and AXDD17 (cyclopentyl) were more toxic. With increasing length, the 4-alkyl groups may have facilitated binding to the P450's active sites.

Concerning the 4-ethyl derivatives, substitution in the 2 position of methoxy (W10) or hydroxymethyl groups (ABDD39) led to toxicity on a par with dapsone itself, although the 2-fluoromethyl-substituted PXDD19 was less toxic. As the trifluoromethyl groups are unlikely to hydrogen-bond, the methoxy and hydroxymethyl groups of W10 and ABDD39 may have facilitated catalysis possibly through hydrogen bonding to the cytochrome P450s. However, compared with the 2-methyl AXDD14, the 2-trifluoromethyl substituted PXDD19 and PXDD4 were themselves cytotoxic and considerably active methaemoglobin formers. This was in spite of the similar ionic radius of a trifluoromethyl to a methyl group. The electron withdrawing character of the fluoromethyl groups (Ceppi et al 1973) may have increased the vulnerability to metabolism of these analogues compared with 2-methyl derivatives.

4-Amino substitution was originally conceived to increase the lipophilicity of the compounds to aid penetration of bacterial membranes (Wiese et al 1987). Toxicologically, the effect of increased hydrophobicity was mixed. The least toxic derivatives, AXDD14, K-130 and AXDD16, were highly lipophilic, while the four most toxic compounds, W10, dapsone, W25 and ABDD39, were all relatively hydrophilic. The exceptions included ABDD5, which was low in toxicity and lipophilicity and one of the 2-hydroxy compounds ABDD1 (4-propylamino). Compared with the more hydrophilic 4-amino substituted ABDD5, ABDD1 was more toxic in both cytotoxicity and methaemoglobin systems. In the rat microsomes in this study, the toxicity shown by the less lipophilic compounds suggests that these were likely to have been metabolized by cytochromes P450 of the 2C series, as these enzymes are known to metabolize dapsone itself (Coleman et al 1990b; Vage & Svensson 1994). There was no clear correllation

between Van der Waals radii and methaemoglobin formation in the rat.

K-130 is effectively trimethoprim and dapsone combined and exerts a therapeutic action at the DHPS as well as the DHFR stage of bacterial DNA synthesis (Kansy et al 1992). In this report, K-130 was a comparatively poor methaemoglobin former and was also less than half as cytotoxic as dapsone. This may have been due to its hydrophobicity, as well as the bulk of the trimethoprim moeity and its steric hindrance to the *N*-hydroxylation of one of the amine groups of the sulphone. Trimethoprim itself is extensively metabolized in the rat (Schwartz et al 1970), and it is likely that oxidation of this moeity to 1- and 3-*N*-oxides, would partially divert K-130 from hydroxylamine formation.

Some compounds which showed substantial methaemoglobin-forming capability, such as AXDD17 (2-methyl 4-cyclopentylamino), ABDD39 (2-hydroxymethyl-4-ethylamino), PXDD19 (2-trifluoromethyl-4-ethylamino) and W25 (2-4-amino) were not as correspondingly cytotoxic to the mononuclear leucocytes. As well as the respective structural predispositions of the analogues to hydroxylation, the toxic effects of the hydroxylamines thus formed will also depend on adjacent structural moieties of the molecule. In general, hydroxylamines formed from sulphonamides are less stable and are less likely to form methaemoglobin compared with those of sulphones (Pirmohamed et al 1991). Dapsone hydroxylamine appears to be mid-way in stability, as it is both cytotoxic and a considerable methaemoglobin former. In the present study, low toxicity in both systems corresponded to a lack of activation, while potent methaemoglobin formers and poor cytotoxics were probably hydroxylated to comparatively stable hydroxylamines, which did not readily auto-oxidize to the more cytotoxic nitroso derivatives.

Dapsone has been proposed as a marker for cytochrome P450 3A4 in man (Fleming et al 1994) and given that approximately 60% of the P450 in a typical human liver is 3A4 (Gillam et al 1993), it was unexpected that there was such a marked disparity in this work between the rat–human microsome-mediated pattern of metabolism compared with that of the heterologously expressed 3A4 system. Human and rat cytochromes vary widely and overlap in specificities, affinities and activities (Nedelcheva & Gut 1994), so direct comparison of the performance of a single cytochrome P450 with a group of isoenzymes could be problematic. However, we found dapsone to be poorly metabolized (< 1·2% methaemoglobia formed) by the heterologously expressed cytochrome P450 3A4 at the concentration range (0·1–7·5 mM) selected in this study, while the 2-methyl-4-propylamino analogue was extensively oxidized at only 0·1 mM. Indeed, concerning dapsone, AXDD14, ABDD5 and K-130, the rat microsomes showed a closer approximation in metabolic profile to human liver microsomes than did the heterologously expressed microsomes. These results may have been due in part to the nature of the variant of the 3A4 enzyme derived from that particular human cDNA. In addition, P450 3A4 is said to vary widely in catalytic activity in-vivo and in-vitro (Watkins 1994) and apparently exists in a number of distinct conformers, which account for its capacity to bind such a wide range of substrates (Koley et al 1995). In marked contrast to this apparent broad specificity, the heterologously expressed 3A4 in this work could distinguish between AXDD17 and AXDD14, which only differed at the 4-position by a cyclopentyl compared with a propyl moeity. Overall, our studies agree with previous work (Coleman et al 1990b; Mitra et al 1993, 1994; Vage & Svensson 1994), in that dapsone and its analogues may undergo considerable metabolism mediated by a number of P450s and the role of 3A4 in the metabolism of dapsone related compounds in man is much smaller than was originally envisaged.

Although microsomes from only three human livers were available for this study, all three compounds (AXDD14, ABDD5 and K-130, Table 3) selected to be tested with human liver microsomes, were less toxic than dapsone in their capability for methaemoglobin generation. Although the human livers used were known to contain P450s which are similar to those of the rat (2E1 and 2C) as well as P450 3A4, an isoenzyme not found in rodents, AXDD14 and ABDD5 generated considerable methaemoglobin in comparison with dapsone, in contrast to the rat liver. Clearly even minor differences between the respective species P450s may markedly alter the net toxicity profile of a test compound. The electron-withdrawing effect of the methyl group of AXDD14 might have contributed to less activation in the rat microsomes, but in the human cytochrome 3A4 and to some extent in the human microsomes, this benefit was not so apparent. However, the benefits of shielding of one of K-130's amine groups and 2-substitution of the methyl or hydroxyl groups seen in the rat system were to some extent carried through to human liver metabolism of these derivatives. Moreover, previous in-vitro studies involving human and rat microsomal-generating systems (Coleman et al 1990b, 1991; Tingle et al 1990, 1991) have been shown to be comparatively faithful predictors of possible in-vivo
sulphone toxicity. It is possible that these compounds might show less methaemoglobin formation compared with dapsone in-vivo.

K-130, AXDD14 and ABDD5 have been previously shown to be far superior to dapsone as antibacterial and antiprotozoan agents (Pieper et al 1989) and in this preliminary study these compounds have displayed some potential in comparison with dapsone in terms of lower haematological toxicity in-vitro. These derivatives are promising candidates for further in-vitro and in-vivo testing.

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