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NEW HETEROCYCLIC MODIFIERS OF OXIDATIVE DRUG METABOLISM—I

6-SUBSTITUTED-2-AMINOBENZOTHIAZOLES

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Abstract—A series of 6-substituted-2-aminobenzothiazoles(2-AB) was synthesized and evaluated as *in vitro* inhibitors of microsomal mixed-function oxidase activity (as aminopyrine *N*-demethylase) from phenobarbitone-induced rat liver. Using physiochemical parameters and multiple regression analysis a quantitative structure–activity relationship (QSAR) was derived in which 82% of the data variance was accounted for in terms of the hydrophobic character of the inhibitor and the molar refractivity of the 2-AB 6-substituent. In contrast, literature equations derived from earlier studies with heterocyclic systems possessing non-polar substitutents underestimated by up to an order of magnitude the potency of the present compounds. Kinetic studies revealed that 6-*n*-propoxy-2-AB, one of the more potent compounds, was a pure competitive inhibitor of aminopyrine *N*-demethylase activity (K_i = 60 μM from Dixon analysis), suggesting that the binding of substrate and inhibitor is mutually exclusive at the cytochrome P-450 active site.

Binding studies indicated that most 2-AB derivatives elicited mixed type I/reverse type I optical difference spectra in phenobarbitone-induced microsomes. The overlap of these components resulted in non-linear double reciprocal plots of the spectral titrations and precluded the determination of binding parameters. In contrast, the more potent inhibitors (the 6-propoxy and 6-butoxy derivatives of 2-AB) were type I ligands with quite high affinity for ferric cytochrome P-450. Although no quantitative relationship was apparent between inhibition and spectral binding affinity a good correlation (r = 0.93) was observed between inhibition potency (I_{50}) and the capacity of ten 2-AB derivatives to prevent substrate(aminopyrine) binding to cytochrome P-450. These findings suggest that 2-AB derivatives may inhibit microsomal oxidation via a direct competitive effect on substrate binding to cytochrome P-450. The present study also demonstrates that substitution of heterocyclic systems with hydrophilic groups does not necessarily produce weak inhibitors of mixed-function oxidase activity, and that extrapolation of existing QSAR equations to new inhibitor series must be interpreted with caution.

Cytochrome P-450 is the principal catalytic component of the mammalian hepatic microsomal mixed-function oxidase (MFO) system that is active in the oxidative metabolism of endogenous and exogenous compounds. The MFO system is susceptible to inhibition by a range of compounds, including the benzodioxole derivatives [1–3], phenolic ketones [4], SKF 525-A and congeners [5, 6], and nitrogen heterocycles [7–17]. Nitrogen heterocycles, including the imidazoles [7–9], benzimidazoles [10–13], ellipticines [14, 15] and quinolines [16, 17], constitute a large group of particularly potent inhibitors. Several recent studies have been directed towards the evaluation of structural features of apparent importance to effective MFO inhibition.

It is now apparent that lipophilicity is the physicochemical parameter of fundamental importance in the interactions of imidazoles [8] and benzimidazoles [10, 11] with MFO enzymes, although steric factors may also be important [9, 11]. Certainly, the majority of structure–activity studies to date have concentrated on inhibitory trends observed in several series of alkyl-substituted heteroaromatics (imidazoles [8], benzimidazoles [10, 11], and benzoxazoles [18]). Few studies have evaluated the effect on inhibition potency of substitution of the heterocyclic ring with polar groups. Therefore it was of considerable interest to evaluate a series of amino-substituted nitrogen heterocycles as inhibitors of microsomal MFO activity from rat liver. The benzothiazole nucleus was selected for structural modification as it possesses a greater hydrophobic character than the more widely studied benzimidazole [19]. Accordingly, the unfavourable effect of amino substitution, that would be expected from the findings of previous QSAR studies, was partially offset.

MATERIALS AND METHODS

Chemicals. 2-Aminobenzothiazole (2-AB; compound 1) was synthesized by the following procedure: 2-aminothiophenol (0.05 moles) was refluxed for 2 hr with *S*-methylisothiourea sulphate (0.035 moles) in water containing sodium acetate (0.07 moles). After cooling, the precipitated product was collected and recrystallized from water. Yield 70%, m.p. 130.5° (lit. 130° [20]).

6-Substituted-2-AB derivatives, with the exception of 2-amino-6-thiocyanatobenzothiazole (com-

pound 15), were synthesized by the following general method. The appropriate 4-substituted aniline derivative was stirred with 2 molar equivalents of ammonium thiocyanate in glacial acetic acid at 15–19°, and one equivalent of bromine in acetic acid was added dropwise over 1.5 hr. After stirring overnight at room temperature the reaction mixture was diluted with 10 vol. of distilled water and neutralized with concentrated ammonia solution.

6-Thiocyanato-2-AB (compound 15) was synthesized in a similar fashion except that four equivalents of ammonium thiocyanate and 2 equivalents of bromine were employed.

Crude products were recrystallized at least twice from aqueous ethanol or benzene and final yields were in the range 52–90% of the theoretical. Purity of compounds was assessed by comparison with literature m.p. data and by thin-layer chromatography. Spectroscopic data (^{1}H-NMR and CH_4-chemical ionisation MS) were consistent with assigned structures.

Biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, and all other reagents and solvents were of at least analytical reagent grade.

Animal treatment. Phenobarbitone was administered intraperitoneally to male Wistar rats (200–250 g) in normal saline at a dose of 100 mg/kg once daily for 3 days. Animals were sacrificed 48 hr after the final treatment with inducer.

Microsomal fraction preparation. Washed microsomal fractions were prepared as previously described [21]. Protein was determined by the method of Lowry *et al.* [22] using bovine serum albumin as standard.

Mixed-function oxidase activities. Aminopyrine *N*-demethylase(APDM) activity was determined at 37° as described previously [17]. Inhibitors were incorporated into reaction incubations in 0.1 ml of 50% propylene glycol in 0.05 M HCl; solvent alone was added to control incubations. I_{50} values were obtained from plots of percent inhibition versus log inhibitor concentration. Each plot was constructed from the mean percent inhibition at each of four different inhibitor concentrations in 2–4 separate determinations. The mean standard error of I_{50} determination was found to be 8%.

7-Ethoxycoumarin *O*-deethylase activity was measured in an Aminco SPF-125 spectrofluorometer according to the method of Prough *et al.* [23], aniline *p*-hydroxylase as described by Murray and Ryan [24], and *p*-chloro-*N*-methylaniline *N*-demethylase as described by Farrell and Correia [25]. Inhibition of these three MFO activities was studied at a single concentration of the 2-AB derivative (0.1 mM) added in 50 μl of dimethylformamide (per 3 ml of incubation volume).

Optical difference spectroscopy. Difference spectra were measured at 37° in an Aminco-Chance DW-2a spectrophotometer using 1-cm cuvettes containing 1-ml aliquots of microsomal suspensions (1.87 mg microsomal protein per ml was selected so that spectral studies would more closely reflect the findings obtained in inhibition studies) in potassium phosphate buffer (0.1 M, pH 7.4). Test compounds were added to the sample cuvette in microlitre quantities of absolute ethanol and the difference spectra were recorded between 380 and 500 nm; an equal volume of solvent was added to the reference cuvette.

In other experiments the effect of several 2-aminobenzothiazole derivatives on the type I interaction of aminopyrine with phenobarbitone-induced rat liver microsomes was studied. Here, the test compound was added in 5 μl of dimethylformamide (to a final concentration of 100 μM) to the sample and reference cuvettes, and a baseline of zero light absorbance was established. Aminopyrine was then added to the sample cuvette (to a final concentration of 0.54 mM) and the optical difference spectrum between 380 and 500 nm was recorded.

Cytochrome P-450 was recorded by the spectrophotometric method of Omura and Sato [26] using an extinction coefficient of 91 $mM^{-1}cm^{-1}$ for the ferrocytochrome P-450-carbonyl complex.

Physicochemical parameters. Log *P* values for substituted 2-AB derivatives were taken or derived from the literature using the additivity principle [19]. Molar refractivity values were also taken from the literature [19] and were scaled by 0.1 to yield more manageable coefficients. The indicator variable I_{MR} was assigned the value 1.0 when the molar refractivity of the 6-substituent [19] was greater than 10.0; $I_{MR} = 0.0$ when the substituent molar refractivity was smaller than 10.0.

Computing and statistics. Multiple regression equations were derived using a Hewlett-Packard 86 B computer with the General Statistics Pac and MLR option. An appropriate F test was used to assess the statistical significance of equations and the significance of parameter inclusion.

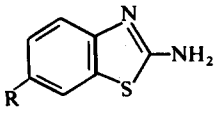
RESULTS AND DISCUSSION

Quantitative structure–activity relationship of the inhibition of aminopyrine N-demethylase activity by 2-aminobenzothiazole derivatives

2-AB derivatives, with the exception of the parent structure (compound 1) and the 6-hydroxy derivative (compound 16) were found to be inhibitors of rat hepatic microsomal aminopyrine *N*-demethylase (Table 1). I_{50}s were observed in the range 6.8×10^{-5} M (the 6-*n*-butoxy derivative, compound 4, Table 1) to 29×10^{-5} M (the 6-fluoro derivative, compound 9, Table 1) so that these compounds are moderately potent inhibitors of phenobarbitone-induced APDM activity. Several regression equations were evaluated in attempts to correlate the observed inhibition potency with physicochemical parameters describing the hydrophobic, steric and electronic character of the differently substituted 2-AB derivatives.

The fundamental dependence of pI_{50} ($-\log_{10}I_{50}$) on hydrophobicity is demonstrated by equation (2) in Table 2. Although the linear relationship between pI_{50} and log P only had a correlation coefficient of 0.41 (equation 1, Table 2), the parabolic relationship in log *P* showed a statistically significant ($P < 0.05$) improvement (equation 2, Table 2; $r = 0.69$, $r^2 = 48\%$). The inclusion of steric parameters was found to improve significantly the regression equations in log *P* alone. Eighty-two per cent of the data variance was accounted for by equation (3) (Table 2 and Fig. 1) after the inclusion of MR (molar refractivity of

Table 1. Inhibition of aminopyrine *N*-demethylase activity by 2-aminobenzothiazole derivatives and parameters used in the derivation of regression equations



Compound number	R	I_{50} ($M \times 10^5$)	log *P**	MR*	I_{MR}*
1	H	—†			
2	C_2H_5O	9.5	1.28	1.247	1.0
3	*n*-C_3H_7O	8.0	1.95	1.706	1.0
4	*n*-C_4H_9O	6.8	2.45	2.166	1.0
5	*n*-$C_5H_{11}O$	13	2.95	2.626	1.0
6	*n*-$C_7H_{15}O$	18	3.95	3.086	1.0
7	*i*-C_3H_7O	7.9	1.93	1.706	1.0
8	*i*-C_4H_9O	8.2	2.43	2.166	1.0
9	F	29	1.04	0.092	0.0
10	Cl	17	1.61	0.603	0.0
11	Br	14	1.76	0.888	0.0
12	NO_2	16	0.62	0.736	0.0
13	CH_3	17	1.46	0.565	0.0
14	CH_3CONH	27	−0.07	1.493	1.0
15	SCN	12	2.05	1.724	1.0
16	OH	—‡			

* log P and MR (scaled by 0.1) data taken from ref. 19. I_{MR} data derived as mentioned in Materials and Methods.

† 34% inhibition observed at a concentration of 30×10^{-5} M.

‡ 30% inhibition observed at a concentration of 50×10^{-5} M.

the 2-AB 6-substituent), whereas 76% of the variance was explained by equation 4 which included I_{MR}, an indicator variable related to the molar refractivity of the 6-substituent. Squared partial correlation matrices for equations 3 and 4 were constructed (Table 3) and clearly demonstrated a significant overlap between log *P* and $(\log P)^2$. On the other hand minimal overlap between log *P* or $(\log P)^2$ and either MR or I_{MR} was observed.

Several additional physiochemical descriptors including electronic parameters (σ), connectivity indices (χ), Verloop parameters (B and L) and other indicator variables were included in regression analyses. Although some of these descriptors, notably $\Sigma\sigma$ (the sum of all substituent electronic effects), increased the overall correlation, the improvement was not statistically significant. In equations (3) and (4) the inclusion of all parameters was statistically justified and therefore these equations demonstrated the highest correlations obtained with the present data set.

Differentiation of equations (2)–(4) (in Table 2) with respect to log *P* yields optimal hydrophobicity ($\log P_0$) values of between 1.61 and 2.44. This suggests that the substitution of 2-AB with an *n*-propyl or *n*-butyl group in the 6-position would produce a derivative with optimal hydrophobicity for APDM inhibition. Clearly, a number of other appropriately-substituted 2-AB derivatives could be proposed as "optimal" inhibitors based on available regression equations.

Little and Ryan [18], in a study of APDM inhi-

Table 2. Parameter coefficients of regression equations for aminopyrine *N*-demethylase inhibition by 2-aminobenzothiazoles*

Equation	log *P*	$(\log P)^2$	MR	I_{MR}	Intercept	*r*	s	*F*	r^2(%)
1	0.081				3.734	0.405	1.89	47	16
	(0.061)								
2	0.381	−0.078			3.518	0.691	0.15	10	48
	(0.016)	(0.001)							
3	0.422	−0.131	0.246		3.298	0.908	0.10	47	82
	(0.006)	(0.000)	(0.003)						
4	0.397	−0.095		0.237	3.406	0.873	0.11	33	76
	(0.008)	(0.000)		(0.005)					

* Numbers in parentheses are variances of parameter estimates.

s = standard deviation from the regression; *r* = (multiple) correlation coefficient; *F* = *F* ratio of the regression; r^2 = percent data variance explained by equation.

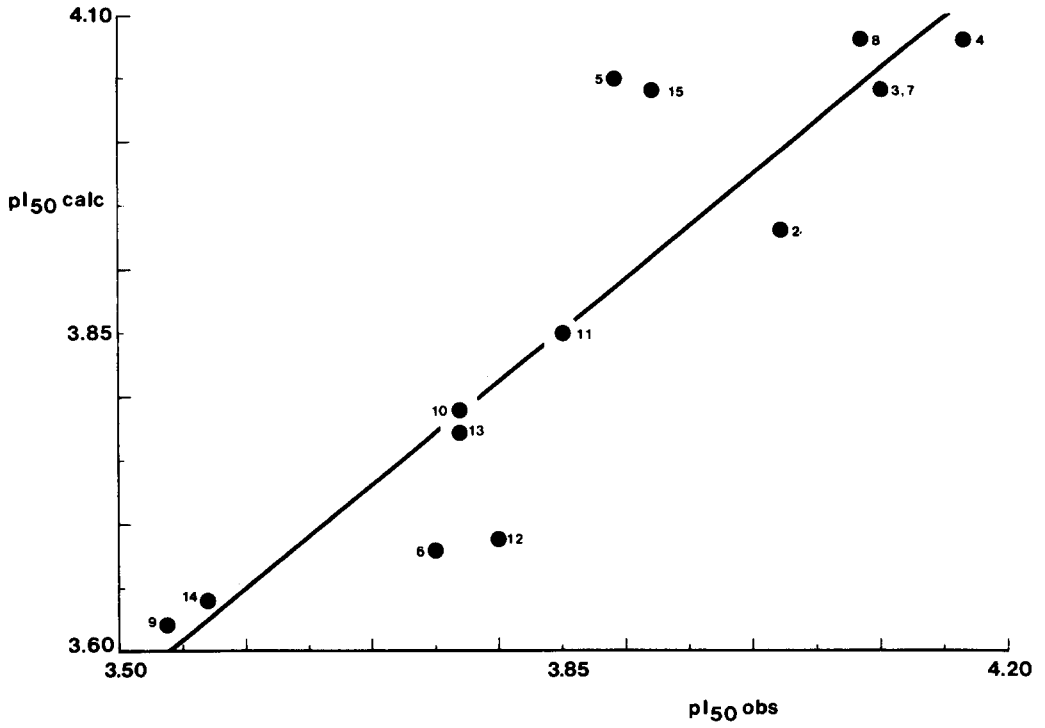


Fig. 1. Relationship between pI_{50} observed and pI_{50} calculated (using equation 3 in Table 2).

bition by a series of 2-*n*-alkylbenzoxazoles (the heterocyclic sulphur atom in benzothiazole replaced by an oxygen atom), noted that optimal potency was attained with the 2-*n*-heptyl analogue ($I_{50} = 5.8 \times 10^{-5}$ M). In contrast, the less lipophilic 2-*n*-heptylbenzimidazole (the heterocyclic sulphur atom in benzothiazole replaced by an NH group) was more potent ($I_{50} = 2.6 \times 10^{-5}$ M) and the apparent pI_{50} optimum was observed with the 2-*n*-undecyl compound ($I_{50} = 1.5 \times 10^{-5}$ M) [27]. Although the full significance of these findings is not clear it appears that a simple increase in the hydrophobicity of the parent heterocycle does not necessarily result in a more potent inhibitor. A parallel may be drawn between apparent optimal chain length in three sets of inhibitors: in the 6-substituted-2-AB series the most potent inhibitor was the 6-*n*-butoxy compound (approximately equilipophilic with an *n*-propyl substituted congener), in the 2-*n*-alkylbenzoxazole series the optimal inhibitor was the 2-*n*-heptyl compound [18] and, in the 2-*n*-alkylbenzimidazole series, the most potent homologue was 2-*n*-undecyl [27]. Thus, as the lipophilicity of the parent ring system is increased, so the length of the optimal side chain for optimal potency is decreased. A likely explanation for this phenomenon is that the inhibitor binding site within the cytochrome P-450 catalytic centre has only a limited capacity for hydrophobic interaction. More hydrophobic side chains than those optimal in each inhibitor series must therefore exceed the capacity of the binding site and decrease the inhibition potency. It also appears that the side chain is a major determinant of MFO inhibition. Thus, although the side chain length for optimal inhibition decreases in the following order: benzimidazole, benzoxazole and benzothiazole, the potency of the most inhibitory compound in each series decreases in the same order. It could be argued therefore that increasing the hydrophobicity of the parent heterocycle is, in fact, detrimental to the intrinsic potency of the system. Instead, relatively hydrophilic parent systems substituted with bulky, hydrophobic groupings may be improved inhibitors. Indeed it is perhaps for these reasons that the alkyl- and arylimidazoles are the most potent inhibitors yet described [7–9, 28–30].

Table 3. Squared partial correlation matrices (r^2) for colinearity among variables used in regression analysis

Equation 3			
	log *P*	$(\log P)^2$	MR
log *P*	1.000	0.725	0.015
$(\log P)^2$		1.000	0.287
MR			1.000
Equation 4			
	log *P*	$(\log P)^2$	I_{MR}
log *P*	1.000	0.857	0.003
$(\log P)^2$		1.000	0.047
I_{MR}			1.000

Table 4 compares three series of pI_{50} values calculated with different regression equations. Column A contains those values calculated with equation 3 from Table 2 (in the present study) and it was found that these values correlated well with the observed pI_{50} data ($r = 0.91$, average deviation from observed $pI_{50} = 0.07$, and Fig. 1). However, observed pI_{50}s were poorly predicted by equations derived using

Table 4. Observed and calculated pI_{50} values for aminopyrine *N*-demethylase inhibition by 2-aminobenzothiazoles

Compound number	pI_{50} observed	pI_{50} calculated A*	B†	C‡
2	4.02	3.93	2.89	2.55
3	4.10	4.04	3.10	3.12
4	4.17	4.08	3.23	3.47
5	3.89	4.05	3.34	3.75
6	3.75	3.68	3.50	4.13
7	4.10	4.04	3.09	3.10
8	4.09	4.08	3.22	3.46
9	3.54	3.62	2.93	2.31
10	3.77	3.79	3.18	2.84
11	3.85	3.85	3.23	2.97
12	3.80	3.69	3.06	1.87
13	3.77	3.77	2.98	2.71
14	3.57	3.64	2.46	1.04
15	3.92	4.04	3.43	3.19

* pI_{50} values in column A calculated with equation (3), Table 2; average deviation = 0.07, correlation with pI_{50} observed = 0.91.

† pI_{50} values in column B calculated with equation (5) in ref. 11; average deviation = 0.76, correlation with pI_{50} observed = 0.38.

‡ pI_{50} values in column C calculated with equation (4) in ref. 18; average deviation = 1.04, correlation with pI_{50} observed = 0.53.

data of APDM-inhibition by benzimidazoles (column B) and benzoxazoles (column C). In those cases the correlations were quite low ($r = 0.38$ and 0.53, respectively) and the average deviation from observed pI_{50} values was about one log-unit. Thus published equations underpredicted the present data by almost an order of magnitude, a finding that implies that the 2-AB skeleton confers a greater intrinsic inhibition potency than anticipated.

Comparative inhibition of mixed-function oxidase activities by derivatives of 2-aminobenzothiazole

Several previous studies have established that not all MFO activities are equally susceptible to inhibition by nitrogen heterocycles [8–10, 12, 13, 29]. Consequently four of the 2-AB derivatives were also tested as inhibitors of other phenobarbitone-induced

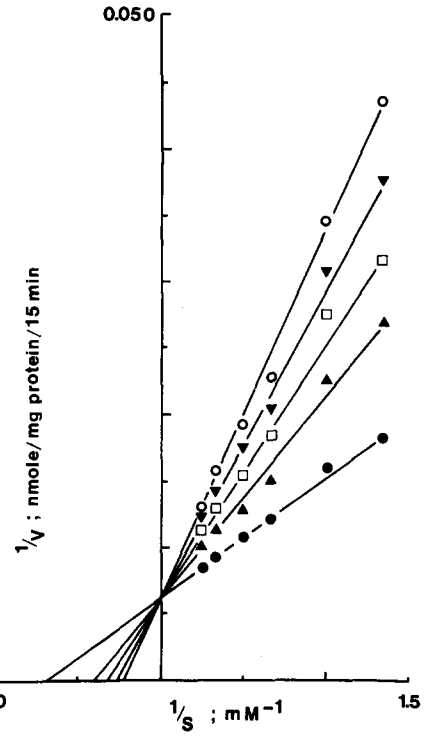


Fig. 2. Lineweaver–Burk plot of the inhibition of aminopyrine *N*-demethylase activity by 6-*n*-propoxy-2-aminobenzothiazole. Inhibitor concentrations: 0, 30, 60, 90, 120 μm.

MFO activities. Compounds 1 (2-AB), 3 (6-*n*-propoxy-2-AB), 8 (6-*iso*-butoxy-2-AB) and 9 (6-fluoro-2-AB) were selected as examples of relatively potent (compounds 3 and 8) and non-potent (compounds 1 and 9) inhibitors. In general, the compounds were similarly potent against 7-ethoxycoumarin *O*-deethylase and aminopyrine *N*-demethylase, but were surprisingly inactive against both *p*-chloro-*N*-methylaniline *N*-demethylase and aniline *p*-hydroxylase (Table 5). Thus it is apparent that, like many other series of heterocyclic compounds, derivatives of 2-AB are not equally active as inhibitors of all MFO activities.

Table 5. Comparative potency of some 2-aminobenzothiazole derivatives against four mixed function oxidase activities in phenobarbitone-induced rat liver microsomes

Compound number*	Percent inhibition Mixed function oxidase activity† ECOD	APDM	APH	CMADM
1	29	33	0	1
3	68	55	3	0
8	84	57	9	0
9	33	29	9	6

* Compounds were tested at a concentration of 0.1 mM. Data are means of duplicate estimates that did not vary by more than 5%.

† Control activities were: ECOD, (7-ethoxycoumarin *O*-deethylase): 3.1 nmole umbelliferone/min/mg protein; APDM, (aminopyrine *N*-demethylase): 6.3 nmole formaldehyde/min/mg protein; APH, (aniline *p*-hydroxylase): 1.2 nmole *p*-aminophenol/min/mg protein; CMADM, (*p*-chloro-*N*-methylaniline *N*-demethylase): 2.5 nmole formaldehyde/min/mg protein.

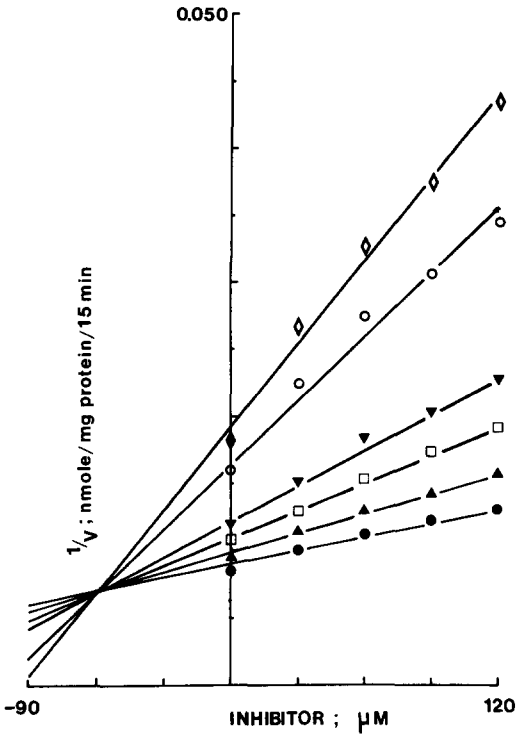


Fig. 3. Dixon plot of the inhibition of aminopyrine *N*-demethylase activity by 6-*n*-propoxy-2-aminobenzothiazole. Substrate concentrations: 0.75, 1.0, 1.5, 2.0, 3.0, 4.0 mM.

Kinetics of the inhibition of aminopyrine N-*demethylase activity by* 6-n-*propoxy-2-aminobenzothiazole*

A kinetic analysis of the inhibition of APDM by 6-*n*-propoxy-2-AB was undertaken. The initial Lineweaver–Burk plot of the data (Fig. 2) suggested that this compound is essentially a simple competitive inhibitor of microsomal APDM activity. From the Dixon plot (Fig. 3), a value of 60 μM was estimated for the equilibrium dissociation constant (K_i) of the enzyme–inhibitor complex. The Dixon plot primary replot (Dixon plot slopes versus reciprocal substrate concentration; Fig. 4) was a straight line that intercepted the origin, a finding that is consistent with competitive inhibition [31]. These observations suggest that at least the more potent inhibitors in the 2-AB series affect APDM activity via a direct interaction with substrate for binding within the cytochrome P-450 active centre.

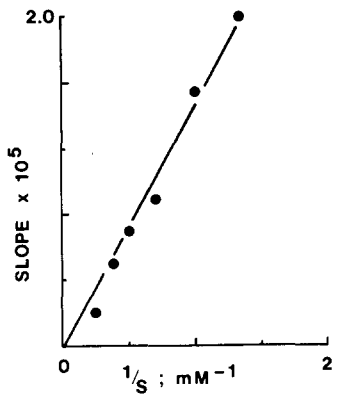


Fig. 4. Primary replot of the Dixon plot inhibition data.

Preincubation of a number of 2-AB derivatives, including 6-alkoxy congeners, with NADPH-fortified microsomes prior to the addition of substrate was found not to affect the capacity of inhibitors to decrease APDM activity. This finding strongly suggests that although certain 2-AB derivatives may be competitive inhibitors of this MFO activity, they are not alternate substrates.

Spectral binding interactions of 2-aminobenzothiazole derivatives in microsomal fractions

Unlike many potent inhibitors of MFO activity, including imidazoles [7–9, 28–30] and analogues of metyrapone [32–34], the 2-AB series did not elicit type II binding spectra in oxidised liver microsomes. 2-AB itself elicited a weak reverse type I optical difference spectrum when tested at a concentration of 0.1 mM; at higher concentrations the difference spectrum appeared to be a mixed type I/reverse type I interaction. The significant overlap of the two spectral components precluded the determination of values for the spectral dissociation constant (K_s) and maximal absorbance change (ΔA_{max}) from the usual plots of reciprocal ligand concentration versus reciprocal absorbance change. Most of the other 2-AB derivatives also behaved in this fashion although the concentration at which the reverse type I change altered to mixed I/reverse type I varied somewhat. The 6-*n*-pentoxy and 6-chloro derivatives of 2-AB (compounds 5 and 10) were reverse type I ligands up to approximately 20 μM whereas the 6-ethoxy, 6-*n*-heptoxy, 6-fluoro and 6-thiocyanato derivatives (compounds 2, 6, 9, and 15, respectively) were reverse type I ligands up to a concentration of about 40 μM. The 6-bromo derivative (compound 11) elicited the mixed type I/reverse type I change at a concentration of about 0.1 mM.

Several 2-AB derivatives did elicit recognisable optical difference spectra that were readily analysed by the conventional double reciprocal plot approach [35]. The binding parameters of these 2-AB congeners are presented in Table 6 and from these data it is apparent that, of the type I compounds, only the 6-*n*-butoxy derivative elicited a high affinity interaction with ferricytochrome P-450. The other type I compounds were relatively poor type I ligands for the cytochrome compared with many of the longer chain 2-*n*-alkyl- and 2-arylalkylbenzimidazoles [13, 36]. The 6-acetamido derivative of 2-AB was the only pure reverse type I ligand in the present data set. This compound underwent a high affinity binding interaction with oxidised cytochrome P-450 despite being a poor inhibitor of APDM activity. 6-Hydroxy-2-AB (compound 16) did not elicit any apparent binding interaction in oxidised microsomes and the 6-nitro derivative (compound 12) possessed a strong absorbance in the 380–500 nm region of the visible spectrum. These studies illustrated that, for the most part, quantification of the parameters for the binding of 2-AB derivatives to oxidised cytochrome P-450 was not possible. Most compounds were mixed type ligands for the cytochrome and the significant overlap

Table 6. Spectral binding characteristics of 2-aminobenzothiazole derivatives in phenobarbitone-induced rat liver microsomes

Compound number	Spectral type	Spectral dissociation constant, K_s (μM)	Maximal absorbance change, $\Delta A_{max} \times 10^3$ (abs. units/nmole P-450)
1	mixed RI/I	—	—
2	mixed RI/I	—	—
3	I	52	8.0
4	I	8.2	9.1
5	mixed RI/I	—	—
6	mixed RI/I	—	—
7	I	28	11.0
8	I	160	9.4
9	mixed RI/I	—	—
10	mixed RI/I	—	—
11	mixed RI/I	—	—
12	—*	—	—
13	I	400	18.0
14	RI	12	10.6
15	mixed RI/I	—	—
16	—†	—	—

Mixed RI/I = mixed reverse type I/type I spectral change.
Data are means of at least 2 estimates that did not vary by more than 10%.
* Intense absorption in the 380–500 nm wavelength region.
† No interaction observed.

of the individual binding components over a narrow concentration range no doubt contributed to the problems. An additional series of experiments was required to develop a relationship between binding to microsomal cytochrome P-450 and inhibition of APDM activity.

Competition between substrate and inhibitors for binding to microsomal cytochrome P-450

Table 7 presents the results of experiments in which the capacity of ten 2-AB derivatives to affect the subsequent type I spectral change elicited by aminopyrine was evaluated. Four 6-alkoxy derivatives (compounds 3, 4, 7 and 8) totally abolished the type I change produced by 0.54 mM aminopyrine, which appears to be consistent with their inhibition potency towards APDM activity.

Three other compounds, including the 6-ethoxy derivative (compound 2), the 6-*n*-heptoxy derivative (compound 6) and the 6-methyl derivative (compound 13) reduced the aminopyrine binding interaction to 15–39 percent of control and three further analogues including the 6-fluoro, 6-acetamido and 6-hydroxy derivatives (compounds 9, 14 and 16, respectively) exerted only slight effects on aminopyrine binding in oxidised microsomes. Thus a good correlation ($r = 0.93$, Fig. 5) was observed between the capacity to inhibit aminopyrine binding to ferric cytochrome P-450 and the inhibition of microsomal APDM activity.

Table 7. Effect of 2-aminobenzothiazole derivatives on the aminopyrine type I spectral interaction in phenobarbitone-induced rat liver microsomes

Compound number	Aminopyrine type I change (percent of control interaction)*
2	31 ± 2
3	0
4	0
6	15 ± 3
7	0
8	0
9	94 ± 5
13	39 ± 3
14	103 ± 2
16	75 ± 4

* Data are mean ± S.D. of at least three determinations.
The benzothiazole derivative was added to the sample and reference cuvettes prior to aminopyrine addition, and the type I spectral change elicited by the latter was then computed as a percentage of the control type I change (observed in the absence of any benzothiazole derivative).

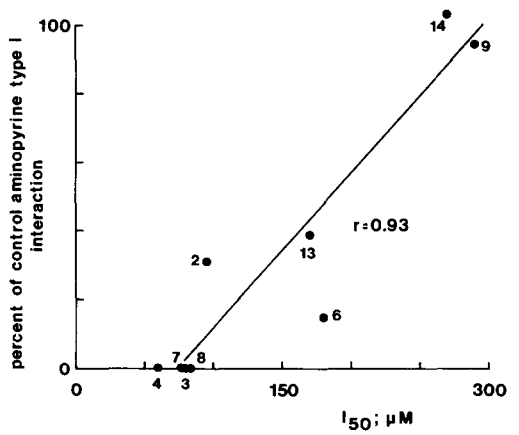


Fig. 5. Relationship between I_{50} against aminopyrine *N*-demethylase activity and the percent of the control Aminopyrine Type I spectral change in the presence of inhibitor (data from Table 7).

General discussion

A number of studies have established the potency of benzimidazole derivatives as inhibitors of hepatic microsomal MFO activities [10–13]. These studies have all stressed the fundamental importance of lipophilicity to inhibitory potency but attempts to improve potency by altering the nature of the heterocyclic system have not always succeeded.

The benzothiazole ring system in the present series of compounds has greater lipophilic character (log P = 2.13 [19]) than the benzimidazole system (log P = 1.38 [19]). However, amino substitution of the benzothiazole system significantly decreases lipophilicity ($\Pi NH_2 = -1.23$). Despite this fact, several 6-substituted-2-AB derivatives were quite potent inhibitors of APDM and 7-ethoxycoumarin *O*-deethylase activities (although little potency towards *p*-chloro-*N*-methylaniline *N*-demethylase and aniline *p*-hydroxylase was noted). Thus it appears that substitution with lipophilic or bulky groups in the 6-position produces inhibitors of greater potency towards this MFO.

Most 2-AB derivatives in this study elicited mixed spectral changes. For example, higher members of the 6-*n*-alkoxy subseries elicited low capacity reverse type I interactions around 20–40 μM and type I interactions (superimposed over the reverse type I change) at higher concentrations. Neither of these spectral perturbations is as well understood as the type II change but it is widely thought that the type I change involves an interaction between a lipophilic substance and an apoprotein binding site near the haem iron [37]. The origin of the reverse type I change is more contentious but it is thought to involve the displacement of a substrate (or high spin ligand) from the active region of the cytochrome by lipophilic compounds (such as some of the 2-AB derivatives in the present study) [38]. Previous studies have indicated that certain benzimidazoles elicit type I and reverse type I spectral changes [10, 12, 13, 36, 39] and that the binding spectrum may well be related to the size and hydrophobicity of the substituents. In the 2-*n*-alkyl- and 2-arylalkylbenzimidazole series higher homologues elicited type I spectral changes of high affinity, but very low capacity, that were readily quantified by the standard technique of optical titration. Concentrations of ligand several-fold in excess of the K_s concentration produced a decrease in the magnitude of the type I change and a conversion to reverse type I behaviour [13, 36]. In the 2-AB series this conversion occurred over a narrow concentration range and produced non-linear double reciprocal plots. This phenomenon may well indicate the involvement of more than one functional group in a substituted 2-AB congener in binding interactions at the cytochrome P-450 active site. Interestingly, the weakly inhibitory 6-acetamido derivative was a pure reverse type I ligand which may reflect the inability of the acetamido side chain to interact in type I fashion with cytochrome P-450. In view of the complexity of these data additional mechanistic studies were undertaken to determine the role of inhibitor-substrate competition for microsomal binding sites. The data presented in Table 7 and Fig. 5 clearly demonstrate the capacity of both type I and reverse type I compounds from the present study to inhibit the binding of substrate to oxidised cytochrome P-450. As the substrate binding interaction of aminopyrine is considered to be the initial event in the cytochrome P-450 reaction cycle it is not surprising that modulation of substrate binding affects the rate of oxidative drug metabolism. A finding of particular interest in the present study was the observation that the 2-AB derivatives were considerably more potent as inhibitors of microsomal APDM activity than is predicted by regression equations that have appeared in the literature. The preparation of additional polar-substituted nitrogen heterocycles may lead to a greater understanding as to why these compounds are more potent inhibitors than predicted. It may eventually be possible to account for the differences in potency between different series of inhibitors in terms of specific physiochemical properties.

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