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IN VITRO METABOLISM OF AZASQUALENE DERIVATIVES AND THEIR EFFECTS ON AMINOPYRINE *N*-DEMETHYLASE ACTIVITY IN RAT LIVER MICROSOMES

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Abstract—The metabolism of squalene dimethylamine (I), a potent inhibitor of 2,3-oxidosqualene (SO) cyclase, and of sixteen other squalene derivatives was investigated in rat liver microsomes. N-oxidation was the only metabolic pathway observed, squalene dimethylamine *N*-oxide being the only metabolite isolated from incubation of I. The azasqualane and quaternary ammonium derivatives did not form *N*-oxides during their metabolism. The inhibition of aminopyrine *N*-demethylase activity was also studied and the IC_{50}, for compound I, which shows weak competitive inhibition, was determined. At 1 mM concentration the oth · ·qualene derivatives showed a range of inhibition activity possibly due to their different lipophilicity.

For many years our laboratory has been concerned with the synthesis and biological activity of a number of new inhibitors I–XVI (Fig. 1) of SO cyclase, a key enzyme in the biosynthesis of sterols [1–3]. Our purpose in studying new inhibitors of ergosterol, cholesterol, and phytosterol biosynthesis was twofold: to search for new antifungal, hypocholesterolemic or phytotoxic drugs [4], and to characterize the structural differences shown by the target enzyme belonging to animals, fungi or higher plants [5].

The prototype inhibitor of this group, squalene dimethylamine I, strongly inhibited the SO cyclase from rat liver [6], higher plants [7, 8] and yeast [9]. The most promising aspect of these compounds is the development of new hypocholesterolemic agents, since they selectively block the biosynthesis of cholesterol in 3T3 fibroblast cells [10].

These substances could be a good alternative to the well known inhibitors of HMGCoA reductase, such as compactine [11]. Among the derivatives of azasqualene, the *N*-oxide shows the highest activity in inhibiting rat liver SO cyclase [12] or inhibiting more rapidly cholesterol biosynthesis *in vivo* [10].

Though these compounds show poor selectivity [13], nevertheless their antifungal properties are also of interest, since they inhibit the growth of many yeasts and fungi [9, 13, 14].

It is known that N-oxidation is an usual metabolic pathway and *N*-oxides of drugs do not show an increase in biological activity in comparison with the parent tertiary amines [15, 16]. In rare cases, *N*-oxides may exhibit pharmacological activity greater than the parent molecule and these are currently used as drugs [17, 18].

Moreover, a large number of tertiary amine drugs may behave as inhibitors of liver drug metabolizing enzymes, and this could increase the intensity and duration of action of the drugs as well as increase liver toxicity [19–21].

Because of these findings and in view of future pharmacological studies of these SO cyclase inhibitors in animals, it would be interesting to determine: (a) the *in vitro* metabolism of azasqualene derivatives using rat liver microsomal preparations; (b) the inhibition by azasqualene derivatives of rat liver cytochrome P-450 activity; and (c) the structure–activity relationship concerning both metabolic transformation and possibly the effectiveness of inhibition.

In the present work we found that N-oxidation (probably catalysed by the Ziegler enzyme) is the sole metabolic route for azasqualene and its derivatives in rat liver microsomes, and that these compounds are generally weak competitive inhibitors of rat liver cytochrome P-450, causing type I spectral changes.

MATERIALS AND METHODS

Chemicals. Squalene aldehyde, squalene dimethylamine, squalene diethylamine, squalene trimethylammonium iodide, squalene dimethylamine *N*-oxide, squalene diethylamine *N*-oxide, squalane dimethylamine, squalane diethylamine, squalane diethylmethylammonium iodide, squalane dimethylamine *N*-oxide, squalane diethylamine *N*-oxide, squalene bis-diethylamine, squalene bis-diethylmethylammonium iodide, squalene bis-diethylamine *N*-oxide, squalane bis-diethylamine, squalane bis-diethylmethylammonium iodide, squalane bis-diethylamine *N*-oxide, were synthesized as reported in [14] and squalene amine and squalene methylamine were synthesized as reported in [6]. Aminopyrine, chromic anhydride, dimethylamine and Titriplex, were from E. Merck (Darmstadt, West Germany). NADP, glucose 6-phosphate and glucose 6-phosphate dehydrogenase were from Boehringer Biochemia Robin (Milano, Italy). SKF 525-A was a generous gift from Smith Kline & French (Milano, Italy).

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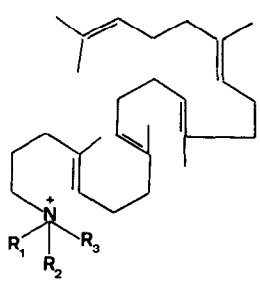
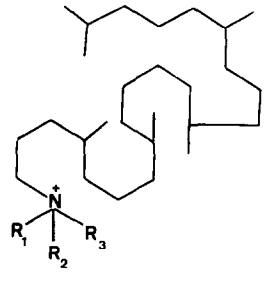
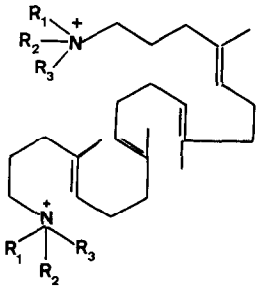
Abbreviations used: SO, 2,3-oxidosqualene; APDM, aminopyrine-*N*-demethylase.



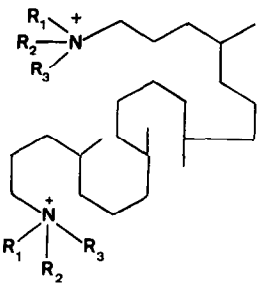
I $R_1=R_2=CH_3$ $R_3=H$
II $R_1=R_2=CH_2-CH_3$ $R_3=H$
III $R_1=R_2=CH_3$ $R_3=O^-$
IV $R_1=R_2=CH_2-CH_3$ $R_3=O^-$
V $R_1=R_2=R_3=CH_3$



VI $R_1=R_2=CH_3$ $R_3=H$
VII $R_1=R_2=CH_2-CH_3$ $R_3=H$
VIII $R_1=R_2=CH_3$ $R_3=O^-$
IX $R_1=R_2=CH_2-CH_3$ $R_3=O^-$
X $R_1=R_2=CH_2-CH_3$ $R_3=CH_3$



XI $R_1=R_2=CH_2-CH_3$ $R_3=H$
XII $R_1=R_2=CH_2-CH_3$ $R_3=O^-$
XIII $R_1=R_2=CH_2-CH_3$ $R_3=CH_3$



XIV $R_1=R_2=CH_2-CH_3$ $R_3=H$
XV $R_1=R_2=CH_2-CH_3$ $R_3=O^-$
XVI $R_1=R_2=CH_2-CH_3$ $R_3=CH_3$

Fig. 1. Azasqualene and azasqualane derivatives.

Synthesis of [1-^{3}H]squalene dimethylamine. The chemicals and solvents used in this synthesis were treated as follows: methanol was dried over magnesium shavings, refluxed for 2 hr and distilled. Dichloromethane was purified by shaking with concentrated H_2SO_4, washing with H_2O, then saturated brine, drying with $LiAlH_4$ and distilling.

Pyridine was dried by distillation from NaOH and stored over NaOH pastilles. CrO_3 was dried at 140° for 4 hr and dessicated over P_2O_5, under vacuum for one day.

[1-^{3}H]-1,1′,2-tris-nor-squalene alcohol: [1-^{3}H]-4E, 8E, 12E, 16E, 20E-4, 8, 13, 17, 21-pentamethyl-4,8,12,16,20-docosapentaen-1-ol.

Freshly prepared 1,1′,2-tris-nor-squalene aldehyde (12 mg, 0.0312 mmol) in anhydrous methanol (3 ml) was added to [^{3}H]$NaBH_4$ (total activity 100 mCi; sp. act. 5–10 Ci/mmol; 0.01–0.02 mmol), and stirred for 45 min. Then $NaBH_4$ (3 eq; 3.5 mg, 0.0936 mmol) was added to complete the reduction, and the reaction mixture was stirred for a further 2 hr. The solvent was evaporated under nitrogen, and 10 ml anhydrous CH_2Cl_2 were added; the suspension was filtered to separate solids, washed with CH_2Cl_2 (3 × 10 ml) and dried over anhydrous sodium sulphate. After evaporation to dryness *in vacuo*, the crude oil was purified by TLC ($CHCl_3$/CH_3OH 99.2:0.8), to give labelled squalene alcohol (11 mg, 8.1 10^{10} dpm), as a colourless oil.

[1-^{3}H]-1,1′,2-tris-nor-squalene aldehyde: [1-^{3}H]-4E, 8E, 12E, 16E, 20E-4, 8, 13, 17, 21-pentamethyl-4,8,12,16,20-docosapentaenal.

CrO_3 (28 mg, 0.28 mmol) was added to a stirred solution of pyridine (44 mg; 0.56 mmol) in anhydrous CH_2Cl_2 (0.7 ml) under argon and stirred for 15 min at room temperature [22]. A solution of labelled squalene alcohol (11 mg, 8.1 10^{10} dpm) in CH_2Cl_2 (0.3 ml) was then added, and a tar-like, black deposit separated immediately. After further stirring for 15 min at room temperature under argon, the solu-

tion was decanted, and the residue was washed with 5% aqueous NaOH (2 × 10 ml), 5% aqueous HCl (2 × 10 ml), 5% aqueous $NaHCO_3$ (2 × 10 ml) and saturated brine (2 × 10 ml), dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. The crude oil was purified by TLC (cyclohexane/ethyl acetate 85:15) to give labelled squalene aldehyde (10 mg, 3.8 10^{10} dpm) as a colourless oil.

[1-^{3}H]squalene dimethylamine: [1-^{3}H]-4E, 8E, 12E, 16E, 20E-*N*,*N*-dimethyl-4, 8, 13, 17, 21-pentamethyl-4,8,12,16,20-docosapentaenylamine.

To a solution of dimethylamine in anhydrous methanol (great excess, 30% solution, 1 ml) an appropriate vol. of HCl in methanol was added until the pH reached 5 upon cooling at 0°, under stirring. Labelled squalene aldehyde (10 mg, 3.8 10^{10} dpm) in methanol (0.5 ml) was added, followed by $NaBH_3CN$ (6.2 mg, 0.10 mmol). After further addition of $NaBH_3CN$ until the pH reached 5, the mixture was allowed to react at room temperature for 24 hr, under stirring.

After addition of 20 ml water, the mixture was extracted with CH_2Cl_2 (20 ml × 3); the organic phases were dried over anhydrous sodium sulphate and evaporated to dryness *in vacuo*. The resulting oil was purified by TLC using methanol as eluant to give labelled squalene dimethylamine (6 mg, 8.2 10^9 dpm), as a colourless oil.

Pretreatment of animals. Male Wistar NOS-rats weighing 250–300 g (from Morini S.Polo d'Enza, Reggio Emilia, Italy) were used throughout and were allowed food and water *ad lib*.

Phenobarbitone pretreatment consisted of one daily i.p. injection of 80 mg/kg body wt in saline, for three consecutive days. Control animals received saline only.

Preparation of microsomes. The animals were decapitated on the fourth day. The livers were perfused *in situ* with ice-cold 1.15% (w/v) KCl solution, and then homogenized in a Potter–Elvehjem glass–Teflon homogenizer. The homogenates were centrifuged for 20 min at 10,000 *g* and the supernatants recentrifuged for 60 min at 100,000 *g* in a Beckman L5-50 ultracentrifuge.

The microsomal fractions recovered were washed by resuspension in KCl, resedimented for 35 min at 100,000 *g* and finally resuspended in 1.5 mM Titriplex/phosphate buffer (0.1 M pH 7.4) mixture.

All operations were performed at 4°. Metabolic assays were carried out with either fresh or frozen microsomes stored in phosphate buffer at −30° for up to 4 weeks; no difference in activity was observed.

Microsomal proteins were assayed by the method of Lowry as modified by Schacterle and Pollack [23].

Inhibition of aminopyrine N-demethylase (APDM) activity. For determination of APDM activity, we used incubations containing 2 mM aminopyrine (0.4, 1.0, 2.0 mM for the kinetic studies) magnesium chloride (0.8 mg), NADP (0.9 mg), glucose 6-phosphate (3.3 mg), glucose 6-phosphate dehydrogenase (1.2 units) in 0.1 M phosphate buffer (pH 7.4) containing 1.5 mM Titriplex to a final vol. of 3 ml.

An appropriate vol. of microsomal suspension was added to yield a final protein concentration of 2 mg/ml. When the assays were done with preincubation, the substrate, aminopyrine, was added 10 min after the start of the experiment with an additional amount of NADPH generating system.

The tested compounds were added as organic solutions, together with Tween 80 in acetone (final concentration of Tween 0.2 mg/ml). After evaporation of the solvent under a stream of nitrogen, the products were dissolved by shaking in the incubation medium. Squalene dimethylamine was used at 0.25, 0.5, 0.75 and 1.0 mM for the kinetic studies.

All other azasqualene derivatives were tested at a concentration of 1 mM. After 20 min at 37° under air, the incubations were stopped by adding 1ml 15% ZnSO4 and 1 ml saturated $Ba(OH)_2$. The precipitate was removed by centrifugation and the formaldehyde formed was measured by the Nash reaction [24] in 2 ml of supernatant.

The amount of formaldehyde was calculated relative to standard curves containing the same microsomal protein concentration. Previous experiments showed no interference between azasqualene and the Nash reagent.

Addition of semicarbazide was found to be unnecessary; no difference was found between assays done with or without it. Linear conditions were observed over the time and concentration range used.

In the kinetic studies, the K_i value was obtained graphically from initial velocities determined at different substrate and inhibition concentrations according to the method of Dixon [25]. Cornish-Bowden plots were used to establish the inhibition pattern [26].

Linear regression analysis was employed to estimate the fit of the experimental data to theoretical straight lines in both Dixon and Cornish-Bowden plots. In the Cornish-Bowden plots, parallelism was tested by the method of Snedecor and Cochran [27].

Metabolic studies. The incubations were carried out in 0.1 M phosphate buffer (pH 7.4, final vol. 3 ml) containing the same NADPH-generating system as described above. In the study with labelled squalene dimethylamine the reaction mixture contained squalene dimethylamine (3000 cpm diluted with cold squalene dimethylamine to a final concentration 0.2 mM).

After 20 min at 37° under air, the incubations were stopped by adding CH_2Cl_2. The mixture was extracted twice with an equivalent vol. of CH_2Cl_2; the combined extracts were evaporated to dryness under reduced pressure, and the residues were applied to silica TLC plates and developed in methanol.

To visualize the bands the plates were exposed to iodine vapours. The areas stained were scraped and counted for radioactivity in a Beckman LS100 after careful elimination of iodine.

Isolation and purification of metabolite. To obtain sufficient amounts of metabolite for structure determination, fifty incubations (25 ml final vol.) were done with microsomes from untreated rat liver. After 1 hr at 37° the incubations were stopped by addition of CH_2Cl_2. The metabolite(s) were extracted with CH_2Cl_2 (500 ml × 3).

The combined organic organic phases were dried over sodium sulphate and the solvent evaporated under reduced pressure.

Table 1. NMR and MS spectral data of squalene dimethylamine (A) and squalene diethylamine (B) metabolites

A

^{1}H-NMR 270 MHz δ*($CDCl^3$):1.60(m,18H,allylic-CH_3)1.68(m,2H,CH_2-CH_2-N)1.98–2.10(m,18H,allylic-CH_2-)-3.30(m,8H,CH_2-$NO(CH_3)_2$)5.14(m,5H,vinylic-CH-).
MS(EI) m/z 429(M^+,1),413(30),276(13),208(14),140;30),100(22),58(100).

B

^{1}H-NMR 270 MHz δ ($CDCl_3$):1.33(t,6H,(CH_3-CH_2)$_2$N).1.57-1.67(m,18H,allylic CH_3 and CH_2-CH_2-N)1.95-2.09(m,18H,allylic-CH_2-)3.23(t,2H,CH_2-CH_2-N)3.39(q,4H,(CH_3-CH_2)$_2$N)5.10–5.21(m,5H,vinylic-CH-).
MS(EI) m/z:457(M^+,2),441(28),276(4),168(30),140(29),112(20),86(100).

* NMR spectra were recorded with tetramethylsilane as internal standard.

The crude oil obtained was purified by flash-column chromatography using 50:50 CH_3OH/CH_2Cl_2, 50:50 as eluant, and then by preparative TLC with CH_3OH as eluant.

Spectral studies. Cytochrome P-450 was determined by the method of Omura and Sato [28] assuming an extinction coefficient of 91 $mM^{-1}cm^{-1}$. Difference spectra were recorded at 25° using a Beckman DU-7 UV-VIS spectrophotometer. Phenobarbitone rat liver microsomal suspension (1 ml) containing 1 mg protein/ml in phosphate buffer, pH 7.4, was added to a cuvette and a baseline was established between 350–500 nm. An appropriate amount of the tested compound was then added to a final concentration of 1 mM and difference spectra were recorded.

RESULTS

Identification of metabolites

Incubation of squalene dimethylamine (I) (Fig. 1) with rat liver microsomes fractions gave rise only to metabolite III, which was characterized by MS and NMR analysis (Table 1).

Metabolite III showed an abundant molecular ion at m/z 429 accompanied by a mass fragment at m/z 413 corresponding to loss of oxygen; its NMR spectrum showing the typical signal at $\delta = 3.30$ (*N*-methyl and *N*-methylene) of *N*,*N*-dimethylamino groups, confirmed the structure as the *N*-oxide derivative of squalene dimethylamine. The final identification of III was obtained by comparing it (TLC, NMR and MS) with a synthetic sample.

The control experiments with heat-inactivated enzyme preparation gave no N-oxidation products, the substrate being recovered unalterated.

The N-oxidation rate of I, as measured by incubating in rat liver microsomes, ^{3}[H]squalene dimethylamine, was slightly affected by SKF-525-A(0.4 mM), since the *N*-oxide metabolite was present at 92 ± 2% of the amount of control incubation. By using ^{3}[H]squalene dimethylamine, as a radiotracer, it was also possible to verify the co-occurrence of some other potential metabolites, such as those arising from the oxidative N-demethylation of tertiary amines. Radioscanning the TLC plates developed after extraction and chromatography of the metabolic mixture, revealed two radioactive peaks corresponding with the squalene dimethylamine and its *N*-oxide derivative reference standards.

No radioactivity was detected in other regions of the chromatogram, notably not in the locations corresponding to authentic samples of squalene methylamine, squalene amine and squalene aldehyde.

Finally, formaldehyde, a product frequently found in *in vitro* N-demethylation experiments [29, 30], was not produced during rat liver microsomal incubation with squalene dimethylamine.

To determine the metabolic fate of a series of compounds structurally related to I, squalene diethylamine (II), the bisquaternary ammonium salt (XIII), squalane dimethylamine (VI) and squalane diethylamine (VII) were also incubated with rat liver microsomes. The results showed that, while the diethyl analog of I was actively transformed into the corresponding *N*-oxide IV (identified by MS, NMR, and comparison with synthetic sample) (Table 1), the more lipophilic azasqualane derivatives, VI and VII, and the more polar bisquaternary ammonium product, XIII, were not metabolized at all.

Inhibition of APDM activity

Inhibition of APDM activity by sixteen azasqualene and azasqualane-related molecules was tested in liver microsomes from phenobarbitone-induced rats; the results are shown in Table 2.

By comparing the inhibitory potency of the azasqualene series with that of azasqualane derivatives, we found that inhibition increased by introducing an additional tertiary amino, or *N*-oxide group to the basic squalene carrier (compare XI with II or XII with IV). In contrast, hydrogenation of the azasqualene (i.e. azasqualane series) greatly reduced inhibitory activity, which was completely lost in the case of VI or VII.

Interestingly, both the parent tertiary amines I, II and the *N*-oxide metabolic products III, and IV inhibited the APDM activity to nearly the same degree.

The inhibition kinetics of the compound I was

Table 2. Inhibition of rat liver aminopyrine *N*-demethylase activity following incubation with azasqualene or azasqualane derivatives

Compound*	% Inhibition†	Compound	% Inhibition
I	51.2 ± 4.1	IX	19 ± 3.9
II	58.5 ± 4.7	X	64 ± 4.3
III	43 ± 3.8	XI	89.7 ± 2.0
IV	37 ± 5.7	XII	83 ± 11.2
V	62.6 ± 3.5	XIII	89 ± 2.8
VI	ND	XIV	19.2 ± 3.2
VII	ND	XV	52 ± 3.6
VIII	19.5 ± 2.3	XVI	75 ± 0.8

ND = Not detected.

* Aminopyrine concentration was 2 mM and the compounds were tested at 1 mM.

† Means ± SD of duplicate assays of two different experiments. Average rates of formation of formaldehyde in control incubations containing no inhibitors was 2.2 nmol HCHO/mg prot min.

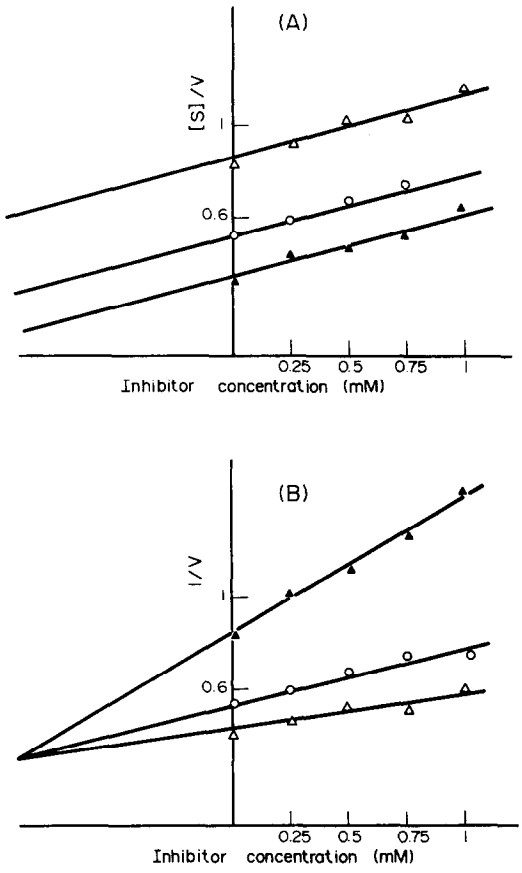


Fig. 2. Cornish-Bowden (A) and Dixon (B) analysis of inhibition by squalene dimethylamine on rat liver microsomal aminopyrine *N*-demethylase activity, determined over a range of inhibitor concentrations, with three different concentrations: △2 mM; ○ 1 mM; ▲ 0.4 mM of substrate (aminopyrine). Points are means of three different experiments, each with duplicate incubations. * V = nmol HCHO/mg prot min.

assessed on rat liver microsomes, comparing Dixon plots with Cornish-Bowden plots (Fig. 2), at different substrate concentrations. Compound I behaved as a competitive inhibitor with K_i of 0.93 mM.

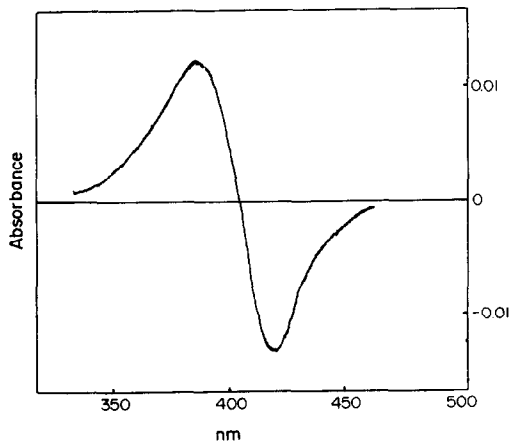


Fig. 3. Type I difference spectrum elicited by 1 mM squalene dimethylamine in phenobarbitone-induced microsomal fractions (1 mg/ml protein concentration).

When the microsomes were preincubated 10 min with I in the presence of the NADPH-generating system before addition of aminopyrine, the observed inhibition was still competitive. Squalene dimethylamine, at 1mM concentration, elicited a characteristic type I optical difference spectrum, (absorption maximum near 385 nm and minimum near 420 nm (Fig. 3) in phenobarbitone induced rat liver microsomes. Moreover, no difference either in type of interaction or in amount of cytochrome P-450 was detected after 10 min of incubation.

DISCUSSION

Since the azasqualene *N*-oxide derivatives proved to be better inhibitors of animal, yeast and higher plant sterol biosynthesis than the parent azasqualene compounds [4, 14], we tested the ability of rat liver microsomes to carry out the N-oxidation of these tertiary amines. It is known that virtually all *N,N*-disubstituted alkyl and aryl amine groups are converted to the corresponding *N*-oxides and that the extent to which N-oxidation occurs depends on sev-

eral factors including lipophilicity, steric hindrance and stereochemistry [31, 32].

In general, *N,N*-dimethyl substituents are preferred substrates over other alkyl groups. For example, the antioestrogenic drug tamoxifen (an *N,N*-dimethylamino derivative) forms *in vitro* the *N*-oxide derivative, while the more sterically hindered *N,N*-diethyl analog enclominphene does not [33, 34].

The data obtained in this work could be of pharmacological interest, since the major metabolite of squalene dimethylamine (I) and squalene diethylamine (II) are the *N*-oxide derivatives III and IV, the compounds showing the highest hypocholesterolemic activities.

The conversion of azasqualene derivatives to the corresponding *N*-oxides seems primarily catalysed by the flavoprotein mixed function oxidase which is considered to be the enzyme responsible for N-oxidation of aliphatic and lipophilic tertiary amines [35, 36]. Indeed, when squalene dimethylamine (I) was incubated in rat liver microsomes in the presence of SKF-525A, a well-known inhibitor of cytochrome P-450 monooxygenases, the rate of N-oxidation was unaffected.

The most striking feature in the *in vitro* biotransformation of azasqualene derivatives was the complete lack of metabolites in addition to those generated by N-oxidation. For example, the cytochrome P-450 dependent monooxygenases mediate the N-dealkylation of a variety of tertiary amine xenobiotics whose common feature is their basicity and lipophilic character [32, 37, 38]. Nevertheless, the azasqualene derivatives were poor substrates for the cytochrome P-450-dependent N-dealkylation. This biochemical behaviour led us to the suggestion that compound I could act as an inhibitor of these enzymes.

Our studies indicate that azasqualene-related compounds moderately inhibit cytochrome P-450 dependent N-dealkylation. The kinetic studies related to the mechanism of inhibition of APDM activity by the azasqualene compound I agreed with a competitive inhibition model.

In order to account for the different degree of inhibition of ADPM activity by azasqualene derivatives, it is important to note that the bis-functionalized derivatives XI, XII, and XIII were more active than the mono-functionalized parent compounds II, IV and V, and in homologous series the squalene derivatives are better inhibitors than the squalane counterparts.

In previous work [10] we demonstrated that azasqualenes bearing a double tertiary amine or quaternary ammonium group, behave as amphipilic molecules, and so may induce changes in lipid membranes. As cytochrome P-450 is closely associated with the endoplasmic reticulum [39, 40] it is likely that compounds such as the quaternary ammonium derivatives, V, X, XIII and XVI, show an increased inhibitory activity as they may disrupt phospholipid organisation.

The mechanism of inhibition by azasqualenes is probably of the "alternate substrate inhibition" type (i.e. simple competition for binding to the lipophilic domain of the active site). Compounds acting by alternate substrate inhibition do not present defined structural features such as a particular functional group (i.e. tertiary amine, *N*-oxide or quarternary ammonium) but they show rather general molecular properties, mainly a certain degree of lipophilicity. Indeed compound I, when added to a microsomal preparation of rat liver, elicited a typical type I difference spectrum due to binding with the lipophilic domain of the enzyme. In contrast, inhibitors that bind to the lipophilic region of proteins and simultaneously to the prosthetic heme iron, i.e. "ligand binding" inhibitors (usually nitrogen containing aliphatic and aromatic compounds) [41, 42], are also enzymically oxidized to yield metabolic intermediates. These form stable complexes with the cytochrome P-450 heme and are quasi-irreversible inhibitors [43–45].

Many of these compounds, when added to native microsomes give a type I difference spectrum and show competitive inhibition. However, after preincubation the kinetics of inhibition are of a noncompetitive or a mixed type [46] and the difference spectrum shows a peak due to formation of the cytochrome P-450-metabolic intermediate complex.

Change of the inhibition kinetics to the noncompetitive type was not observed after preincubation with squalene dimethylamine; this, in addition to the observed type I difference spectrum, confirms that the inhibition of the APDM activity is due to a lipophilic interaction of the squalene skeleton with catalytic site of P-450 dependent monooxygenases.

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