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Methadone: a potent inhibitor of rat liver aldehyde oxidase

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Abstract—Several drugs with structural similarities to SKF-525A were tested for their ability to inhibit rat liver aldehyde oxidase using the experimental antitumour agent *N*-[(2′-dimethylamino)ethyl]acridine-4-carboxamide (AC; NSC 601316; acridine carboxamide) as substrate. The antihistamine D-chlorpheniramine, and the antiarrhythmics disopyramide, procainamide and lignocaine were ineffective in inhibiting this reaction. The antihistamines diphenhydramine, pheniramine, doxylamine, orphenadrine, methapyrilene and pyrilamine, gave IC_{50} values of 100–500 μM. The narcotic analgesics D-propoxyphene and, in particular, methadone were potent inhibitors of acridone formation with IC_{50} values of 15.5 and 0.31 μM, respectively. Further analysis indicates mixed non-competitive type inhibition by methadone with inhibition constants (K_{is} and K_{ii}, respectively) of 0.03 ± 0.01 (SE) and 0.57 ± 0.12 μM.

We have recently shown the classical cytochrome P450 inhibitor SKF-525A to be a potent mixed inhibitor of aldehyde oxidase (aldehyde: O_2 oxidoreductase, EC 1.2.3.1) mediated 9(10*H*)acridone formation from the experimental anti-tumour agent acridine carboxamide (AC; NSC 601316; *N*-[(2′-dimethylamino)ethyl]acridine-4-carboxamide), using an enriched enzyme preparation from rat liver [1]. A number of drugs have a similar primary structure to that of SKF-525A, some of which have also been shown to inhibit cytochrome P450 [2–4]. The antihistamines D-chlorpheniramine, diphenhydramine, pheniramine, doxylamine, orphenadrine, methapyrilene and pyrilamine, the antiarrhythmics disopyramide, procainamide and lignocaine, and the narcotic analgesics D-propoxyphene and methadone were therefore compared with SKF-525A for their ability to inhibit aldehyde oxidase in this system.

Materials and Methods

Materials. SKF-525A (proadifen HCl) was kindly provided by SmithKline Beecham (Sydney, Australia). Methadone HCl, diphenhydramine HCl, D-propoxyphene HCl, D-chlorpheniramine maleate, pheniramine hydrogen maleate, disopyramide phosphate, doxylamine succinate, orphenadrine citrate, procainamide HCl, lignocaine HCl, and methapyrilene HCl were obtained from the standards repository of the New Zealand Institute of Environmental Health and Forensic Sciences, and pyrilamine maleate from the Sigma Chemical Co. (St Louis, MO, U.S.A.). The structures are shown in Table 1. The compounds were used as supplied and were formulated immediately before use: orphenadrine citrate in dimethylacetamide, with subsequent dilution in Milli-Q water (the final concentration of dimethylacetamide in the incubations was 1%) and the remaining compounds in water. All other reagents and solvents were of analytical or HPLC grade.

Assay of inhibition of aldehyde oxidase activity. Aldehyde oxidase activity was determined using AC as substrate. The enrichment of aldehyde oxidase from rat liver cytosol, incubation conditions, HPLC analysis of AC-9(10*H*)-acridone formation, determination of IC_{50} values (concentration of inhibitor needed to reduce acridone formation by 50%) and the Michaelis–Menten kinetic parameters have been described [1, 5]. The maximum inhibitor concentration tested was 200 μM and SKF-525A was included as a positive control in each experiment.

Results

The effects of the compounds on acridone formation are summarized as the IC_{50} values in Table 1. Methadone and D-propoxyphene, in addition to SKF-525A, significantly inhibited acridone formation. Of the remaining compounds only diphenhydramine, pheniramine, doxylamine, orphenadrine, methapyrilene and pyrilamine, gave IC_{50} values of 100–500 μM. D-chlorpheniramine, disopyramide, procainamide and lignocaine were ineffective in inhibiting this reaction.

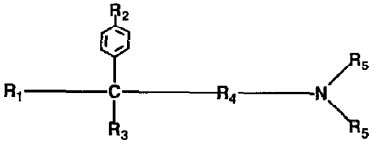
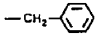
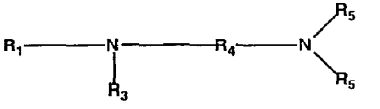
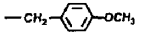
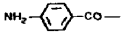
A more detailed kinetic analysis, over a range of inhibitor and substrate concentrations, was made of the inhibition of acridone formation by methadone. The best fit, as judged by the Schwarz criterion and log likelihood [6], using non-linear regression analysis indicated mixed non-competitive inhibition with inhibition constants (K_{is} and K_{ii}, respectively) of 0.03 ± 0.01 (SE) and 0.57 ± 0.12 μM. A double reciprocal plot of these data is shown in Fig. 1. Calculation of the IC_{50} value for methadone can be made from these values [7] and the calculated value (0.15 μM; at 50 μM AC and K_m of 5 μM) is in reasonable agreement with the direct determination shown in Table 1 (0.31 ± 0.04).

Discussion

SKF-525A and related analogues are well known as inhibitors of cytochrome P450 [2–4]. We have shown that SKF-525A is also a potent inhibitor of aldehyde oxidase [1] and have now extended this finding to methadone (approximately 8-fold more potent than SKF-525A) and D-propoxyphene (approximately 6-fold less potent than SKF-525A). An apparent K_{is} value of 0.03 μM has been estimated for methadone. This is comparable to the K_i of 0.06 μM estimated for the inhibition of rabbit liver aldehyde oxidase by amsacrine [8] although we have obtained a higher value (1.4 μM) (Robertson *et al.* manuscript in preparation) for amsacrine with the rat liver enzyme fraction used here. Further, the methadone used in these experiments is the racemic mixture. Significant differences in the toxicity and effectiveness of the enantiomers have been observed [9] and the potency of one of these may thus be even greater than that observed for the racemate. Methadone is thus one of the most potent inhibitors of aldehyde oxidase found to date.

The active site of aldehyde oxidase is thought to include an extended lipophilic site [10] which appears to be able to accommodate the acridine nucleus of AC and the diphenyl ring systems of methadone, SKF-525A and D-propoxyphene in particular. These and the remaining compounds tested also have some structural similarity to AC in containing an alkylamino side chain with a tertiary amine group. This group would be charged at physiological pH and may be involved in the interaction of these inhibitors with the enzyme. The finding that the inhibition

Table 1. The structural relationship, and the inhibition of AC-9(10*H*)acridone formation, of narcotic and other drugs related to SKF-525A

Compound	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)*
						
Methadone HCl	$-COCH_2CH_3$	—H		$-CH_2CH(CH_3)-$	$-CH_3$	0.31 ± 0.04
SKF-525A HCl	$-CH_2CH_2CH_3$	—H		$-COOCH_2CH_2-$	$-CH_2CH_3$	2.6 ± 0.4
D-Propoxyphene HCl	$-OCOCH_2CH_3$	—H		$-CH(CH_3)CH_2-$	$-CH_3$	15.5 ± 2.6
Orphenadrine citrate	—H	—H		$-OCH_2CH_2-$	$-CH_3$	115 ± 32
Doxylamine succinate	$-CH_3$	—H		$-OCH_2CH_2-$	$-CH_3$	167 ± 60
Diphenhydramine HCl	—H	—H		$-OCH_2CH_2-$	$-CH_3$	212 ± 45
Pheniramine hydr. maleate	—H	—H		$-CH_2CH_2-$	$-CH_3$	216 ± 39
Disopyramide phosphate	$-CONH_2$	—H		$-CH_2CH_2-$	$-CH(CH_3)_2$	1060 ± 420
D-Chlorpheniramine maleate	—H	—Cl		$-CH_2CH_2-$	$-CH_3$	>2000
						
Methapyrilene HCl				$-CH_2CH_2-$	$-CH_3$	116 ± 32
Pyrilamine maleate				$-CH_2CH_2-$	$-CH_3$	510 ± 200
Procainamide HCl			—H	$-CH_2CH_2-$	$-CH_2CH_3$	>2000
Lignocaine HCl	—H			$-COCH_2-$	$-CH_2CH_3$	>2000

* Values are the means ± SE of the model estimation.

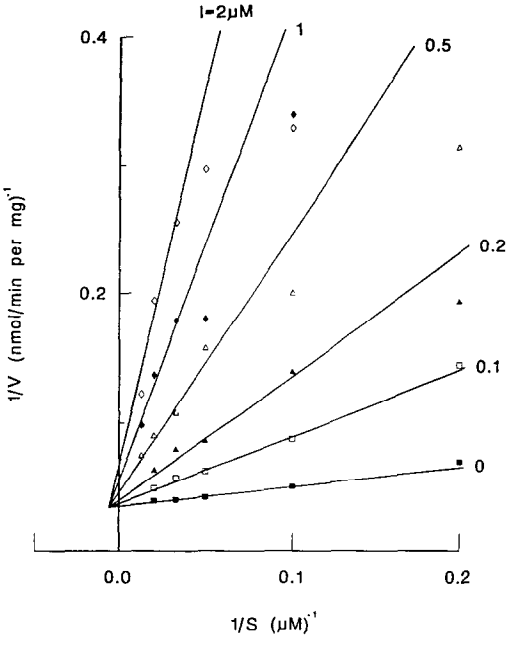


Fig. 1. A double reciprocal plot of inhibition by methadone of 9(10*H*)acridone formation from AC using an enriched enzyme fraction from rat liver cytosol. Values are the means from the combined results of duplicates from two separate experiments (N = 4).

constant for binding of methadone to the enzyme–substrate complex (K_{ii}) is approximately 16-fold greater than that for binding to the free enzyme (K_{is}) indicates that there may be considerable overlap between the substrate and inhibitor binding sites. The importance of an alkylamino side chain is also supported by the greatly increased affinity of AC (K_m of 11 μM, [5]) for the rat liver enzyme compared with acridine (K_m of 0.5 mM, [10]).

The primary structures of methadone, SKF-525A and D-propoxyphene differ in several respects. It is difficult to make any clear conclusions on the structural features affecting interaction of these compounds with the enzyme without detailed modelling studies. However, these compounds can be clearly distinguished from a second group comprising orphenadrine, doxylamine and diphenhydramine which have a common ether-linked tertiary amine side chain but have only H or a methyl group in the R_1 position (Table 1). This may indicate the importance of a side chain in this position. Conversely, alteration in the R_3 ring appears to be well tolerated. Moderate inhibitory potency is also maintained when the ether linkage is removed (pheniramine). Similarly, replacement of both the central carbon with nitrogen and a phenyl group with a (2-thienyl)methyl group is well tolerated (methapyrilene). However, the presence of a 4-methoxy substituted benzyl group or a 4-chlorophenyl substitution (pyrilamine and D-chlorpheniramine, respectively) significantly reduced or abolished the ability to inhibit acridone formation.

A partial competitive mode of inhibition also implies that methadone might be a substrate for aldehyde oxidase. Preliminary examination reveals no significant metabolism of these compounds by aldehyde oxidase. Metabolic-intermediate complex formation has been demonstrated for orphenadrine, diphenhydramine and pyrilamine as wells as SKF-525A and methadone [2–4] although the relative rate of complex formation for methadone, SKF-525A and D-propoxyphene [11] is inversely related to the potency of inhibition of aldehyde oxidase observed here. Further, competitive inhibition in the absence of metabolism does also occur with SKF-525A [3]. However, the extent of metabolism and thus the possible formation of a metabolic–intermediate complex remains to be determined. Similarly, it would be of interest to examine the effectiveness of the secondary and primary amine derivatives in inhibiting this enzyme.

Methadone is typically used in treatment of narcotic addiction but may be used for the long-term treatment of cancer pain [12, 13]. However, the clinical significance of inhibition of aldehyde oxidase by methadone is likely to be small. Although the half-life of elimination of methadone is 24–60 hr there is unlikely to be any overlap between long-term management of pain and chemotherapy in cancer patients.

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