Biochimica et Biophysica Acta, 1099 (1992) 262–266

BBABIO 13881

The acridones, new inhibitors of mitochondrial NADH : ubiquinone oxidoreductase (complex I)

Walter Oettmeier, Klaus Masson and Michael Soll

Lehrstuhl Biochemie der Pflanzen, Ruhr-Universität, Bochum (Germany)

(Received 23 September 1991)

Key words: Acridone; NADH : ubiquinone reductase; Complex I; QSAR; (Bovine heart); (Mitochondria)

Acridones (9-azaanthracen-10-ones) were found to be powerful inhibitors of mitochondrial NADH : ubiquinone oxidoreductase. Their inhibitory activity was best if an alkyl or alkyloxy substituent resided in the 4-position. Biological activity reached a maximum at a chain length of 9–10 Å. Halogen substitution in position 7, but not in positions 6 and 7, further enhanced activity. 2-Alkylacridones were much less active. Inhibitory activity in a Quantitative Structure-Activity Relationship (QSAR) could be correlated to Verloop's STERIMOL parameters L and L^2 (Verloop, A., Hoogenstraten, W. and Tipker, J. (1976) in Drug Design (Ariens, E. J., ed.), Vol. 7, pp. 165–207, Academic Press, New York). The QSAR could be further improved by inclusion of the lipophilicity parameter π.

Introduction

In the mitochondrial respiratory chain, the initial step is the oxidation of NADH. This reaction is catalyzed by the NADH : ubiquinone oxidoreductase (complex I). Among the proton pumping membrane complexes, complex I with approx. 30 protein subunits is the largest and most complex (for recent reviews see Refs. 1, 2).

In general, inhibitors can serve as useful probes to elucidate structural and mechanistic aspects of enzyme reactions. Piericidins, amytal and rotenone are well-known, naturally occurring inhibitors of NADH oxidation of complex I at the ubiquinone binding site [1, 2]. Also the ubicidins, compounds with piericidin ring structure and ubiquinone side chain were found to be potent inhibitors of NADH-oxidation [3]. More recently, inhibition of complex I was reported for capsaicin, the pungent principle of red pepper [4]. Furthermore, inhibition of NADH-oxidation was found for analogues of the dopaminergic neurotoxin 1-methyl-4-phenylpyridinium [5–7], *N*-methyl-β-carbolines [8], quinolinium compounds [9], 4-hydroxypyridines and 4-hydroxyquinolines [10] and hydroxyflavones [11].

We wish to report here that acridones (9-azaanthracen-10-ones) (Fig. 1) are powerful inhibitors of NADH : ubiquinone dehydrogenase. Biological activity is best if an alkyl or alkyloxy group resides in position 4. The maximum of inhibition is achieved at a chain length of 8 carbon atoms. For the first time (to our knowledge) we present a Quantitative Structure-Activity Relationship (QSAR) for inhibitors of mitochondrial NADH-oxidation. In this QSAR inhibitory activity is correlated in a parabolic equation to Verloop's STERIMOL parameters L and L^2 [12] The QSAR can be further enhanced by addition of the lipophilicity constant π.

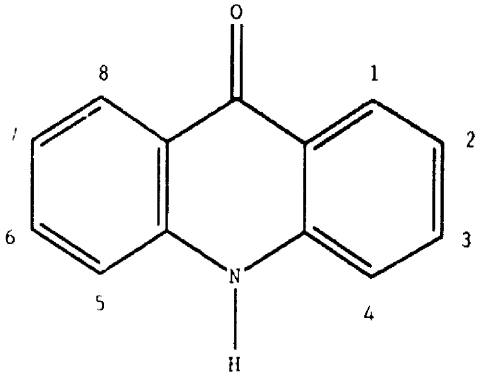

Fig. 1. Structural formula of acridone.

Dedicated to Joseph J. Katz on the occasion of his 80th birthday.

Correspondence: W. Oettmeier, Fakultät für Biologie, Ruhr Universität, Postfach 102148, D-4630 Bochum 1, Germany.

Materials and Methods

Chemicals

Synthesis of acridones (Fig. 1) started by reaction of suitably substituted 2-chlorobenzoic acids with substituted anilines to yield 2-carboxydiphenylamines according to standard procedures [13]. Ring closure to acridones was achieved by heating the diphenylamine at 120°C in conc. sulphuric acid [13] or at 100°C in polyphosphoric acid [14]. The 7-azido-4-*sec*-butylacridone was synthesized via reduction of the corresponding nitro-compound by $SnCl_2$ to the aminoacridone, diazotation and exchange of the diazo group by sodium azide [15]. The identity of the acridones was confirmed by elemental analysis and/or mass spectroscopy.

Biochemical methods

Mitochondria from beef heart were isolated according to Smith [16] and submitochondrial particles according to Thierbach and Reichenbach [17]. NADH-oxidation by submitochondrial particles was measured at 30 ° in a Clark-type oxygen electrode. The assay medium contained in a volume of 2 ml 75 mM phosphate buffer (pH 7.4); 1 mM EDTA; 1 mM $MgCl_2$ and 0.5 mM NADH. If the activity of complex I alone was assayed, the medium contained in addition 10 mM KCN, 3 μM antimycin and 0.1 mM ubiquinone-1 as artificial electron acceptor. In this case, NADH oxidation was measured spectrophotometrically at 340 nm.

Binding and displacement experiments have been performed using 4-*sec*-butylacridone, because this compound is highly fluorescent (excitation maximum 403 nm; emission maximum 427 nm). In a typical assay, beef heart mitochondria (0.5 mg protein) in a total volume of 2 ml in the presence of 0.25 M sucrose, 50 mM Tris buffer (pH 7.5), 10 mM $MgCl_2$ and 1 mM EDTA were incubated at various concentrations of 4-*sec*-butylacridone for 10 min. Samples were centrifuged at $10000 \times g$ and assayed in a Shimadzu spectrofluorometer RF-510 for their fluorescence.

QSAR calculations

QSAR calculations were performed on an MS-DOS personal computer using the software QSAR-PC by BIOSOFT, Cambridge, UK.

Results and Discussion

Table I lists pI_{50} values ($-\log$ of IC_{50}, the concentration where 50% inhibition is achieved) for a variety of acridones in two different mitochondrial electron transport systems. In the system NADH > UQ-1, ubiquinone-1 is used as an artificial electron acceptor and complexes III and IV are inhibited by antimycin or KCN, respectively. In this case, only complex I is assayed. In the system NADH > O_2, electrons are transported from NADH through the entire mitochondrial electron transport chain to the terminal electron acceptor oxygen. We note that in this system the pI_{50} values of the acridones are generally higher as compared to complex I. This is specifically true for 2-sub-

TABLE I

pI_{50} values for inhibition of NADH oxidation in submitochondrial particles by various substituted acridones

No.		pI_{50} value	
		NADH > UQ-1	NADH > O_2
(a) 4-Substituted acridones			
1	4-*n*-octadecyloxy	< 3.5	4.22
2	4-methoxy-2-nitro	< 3.5	4.58
3	5,7-dichloro-4-*sec*-butyl	< 3.5	4.92
4	6-chloro-4-methoxy	< 3.5	5.86
5	.....	3.91	5.49
6	7-chloro-4-phenyl	3.99	5.21
7	4-hydroxy	3.98	5.04
8	4-methyl	4.00	5.06
9	4-chloro	4.02	4.62
10	4-methoxy	4.62	5.67
11	4-phenyl	4.84	5.27
12	4-ethoxy	4.96	6.09
13	4-ethyl	5.10	5.68
14	7-amino-4-*s*-butyl	5.33	5.84
15	4-*i*-propyl	5.37	5.58
16	4-*s*-butyl-7-nitro	5.38	5.85
17	4-*t*-butyl	5.42	5.44
18	4-*n*-dodecyloxy	5.42	6.29
19	4-phenyloxy	5.60	6.04
20	4-*n*-propyl	5.61	5.82
21	4-*s*-butyl	5.65	6.10
22	4-*n*-pentyloxy	5.75	5.97
23	7-chloro-4-*s*-butyl	5.95	6.58
24	7-azido-4-*s*-butyl	6.00	6.75
25	4-*n*-decyloxy	6.26	6.58
26	4-*n*-octyloxy	6.38	7.15
27	7-bromo-4-*s*-butyl	6.56	7.11
28	7-chloro-4-*n*-octyloxy	6.67	7.55
(b) 2-Substituted acridones			
29	6-chloro-2-*s*-butyl	< 3.5	3.80
30	2-*n*-decyloxy	< 3.5	3.81
31	2-*n*-dodecyl	< 3.5	4.06
32	2-*n*-octyloxy	< 3.5	4.40
33	2-*t*-butyl	< 3.5	4.68
34	2-*n*-decyl	< 3.5	4.70
35	2-fluoro-4-methyl	< 3.5	5.11
36	2-*n*-propyl	< 3.5	5.15
37	2-*s*-butyl	< 3.5	5.28
38	2-*n*-butyl	3.62	5.54
39	2-methyl	4.06	5.47
40	2-ethyl	4.16	5.12
41	2-*i*-propyl	4.29	5.05
42	2-*t*-butyl-5-methyl	4.29	5.10
43	2-*n*-pentyloxy	< 3.5	5.57
44	2-*n*-pentyl	< 3.5	5.81
45	2-*n*-hexyl	< 3.5	5.89
46	2-*n*-hexyloxy	< 3.5	5.99
47	2-*n*-heptyl	< 3.5	6.27
48	2-*n*-octyl	< 3.5	6.33

stituted acridones (see Table I, compounds 43–48). This behaviour is attributed to a second inhibition site of acridones, located at the ubiquinol cytochrome *c* oxido-reductase (Oettmeier, W., Masson, K. and Soll, M., unpublished results).

At complex I, unsubstituted acridone is a moderate inhibitor, exhibiting a pI_{50} value of 3.91 (Table I, cpd. 5). Substitution in the 4 position greatly enhances inhibitory activity. It rises with the length of the alkyl side-chain from methyl (pI_{50} value 4.00; cpd. 8) ethyl (5.10; 13), *i*-prop (5.37; 15), *n*-prop (5.61; 20), *t*-butyl (5.42; 17) to *s*-butyl (5.65; 21). Acridones with higher alkyl substituents could not be synthesized due to the lack of suitable starting material.

A similar increase in inhibitory potency is observed if an alkoxy substituent is present in the 4-position. Inhibitory activity increases from methoxy (4.62; 10), ethoxy (4.96; 12), *n*-pentyloxy (5.75; 22) to *n*-octyloxy (6.38; 26). Further increase in the length of the alkoxy side chain diminishes the activity. It drops from *n*-decyloxy (6.26; 25) to *n*-octadecyloxy (< 3.5; 1). A suitable substituent in the 4-position is also a phenyl (4.84; 11) or even better a phenyloxy group (5.60; 19).

Unfavourable for biological activity is an additional substitution in the 5 or 6 position (compare 21 and 3 or 10 and 4; Table I). Contrary, halogen substitution in the 7-position enhances biological activity of the acridones. 7-chloro- (5.95; 23) and 7-bromo-4-*s*-butylacridone (6.56; 27) are more active than 4-*s*-butylacridone (5.65; 21). 7-Chloro-4-*n*-octyloxyacridone (6.67; 28) is the most potent inhibitor of the acridone type found so far. Note also the high activity of 1-azido-4-*s*-butylacridone (6.00; 24), which after a radioactive synthesis may serve as a suitable photoaffinity reagent for identification of the acridone binding site in complex I.

Substitution in the 2-position in general leads to a decreased activity of the acridones; however; 2-alkyl- and 2-alkyloxyacridones are inhibitors of complex III (43–48, Table I) as mentioned above.

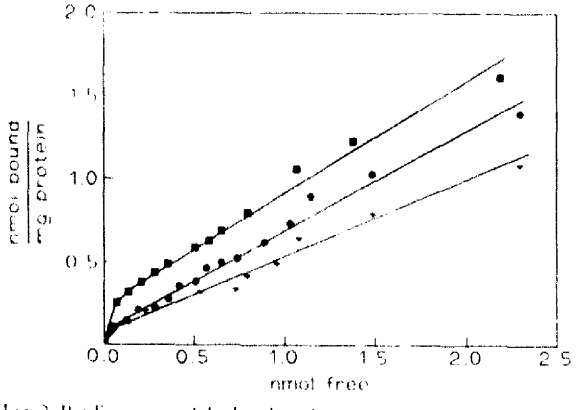

Fig. 2. Binding curve of 4-*s*-butylacridone to beef heart mitochondria in the absence (■) and in the presence of $5 \cdot 10^{-9}$ M (●) or $1 \cdot 10^{-8}$ M (▼) rotenone.

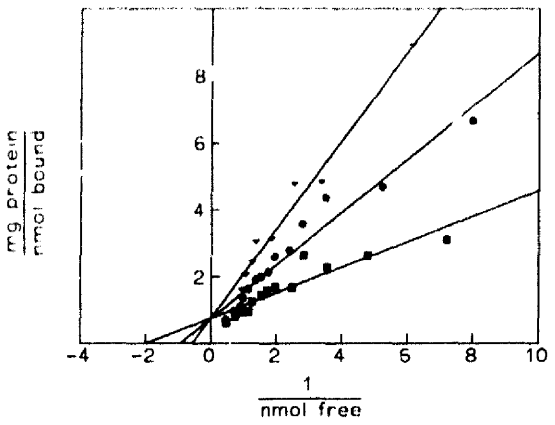

Fig. 3. Lineweaver-Burk plot of the binding data from Fig. 2.

The binding behaviour of 4-*s*-butylacridone (cpd. 21, Table I; pI_{50} value 6.10; $K_i = 0.79$ μM) was analyzed in more detail by a fluorescence assay. 4-*s*-butylacridone exhibits a biphasic binding behaviour, which is characterized by specific (high affinity) and unspecific (low affinity) binding (Fig. 2). The latter is linearly dependent on the concentration of the acridone and presumably associated with binding to lipids. A similar biphasic binding is observed for photosystem II herbicides, which compete with the native plastoquinone at the photosystem II Q_B binding site [18, 19]. From the Lineweaver-Burk plot of the binding data (Fig. 3), a binding constant K_b of 0.25 μM (corresponding to a pK_b of 6.60) and a number of binding sites x_i of 1.35 mmoles/mg protein could be calculated. The pK_b of 6.60 correlates relatively good with the pI_{50} value of 6.47, extrapolated to zero protein concentration (data not shown).

Rotenone can displace 4-*s*-butylacridone from its binding site (Fig. 2). As is evident from the regression lines in the Lineweaver-Burk plot (Fig. 3), they share a common *y*-intercept (x_i), but different *x*-intercepts (K_b). This indicates a competitive displacement mechanism [20]. Consequently, rotenone and 4-*s*-butylacridone have an identical binding site in complex I.

It has already been stressed that the inhibitory activity of 4-alkoxyacridones peaks at a chain length of 8 carbon atoms. It was, therefore, attempted to treat the biological data for 4-substituted acridones by a QSAR. The length of a substituent is described by Verloop's STERIMOL parameter *L* [12]. Since the inhibitory activity reached a maximum at a certain carbon chain length, a multiple linear regression between pI_{50} and *L* and L^2 was performed. The following parabolic equation was obtained:

$$pI_{50} = 0.953\,L - 0.051\,L^2 + 1.85 \qquad (1)$$

$n = 17,\ s = 0.335,\ r = 0.917,\ F = 36.88$

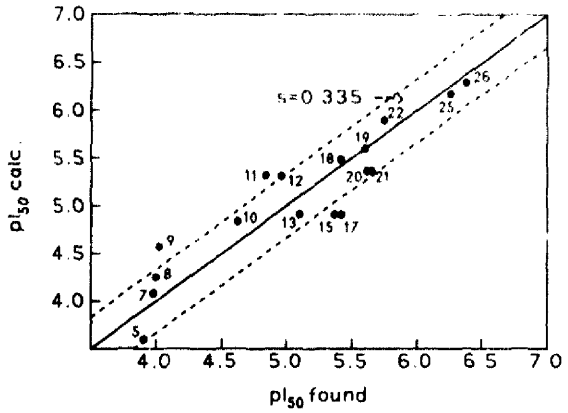

Fig. 4. Plot of pI_{50} values of 4-substituted acridones found versus calculated (according to Eqn. 1). The standard deviation is indicated by dashed lines. The numbering is in accordance with Table I.

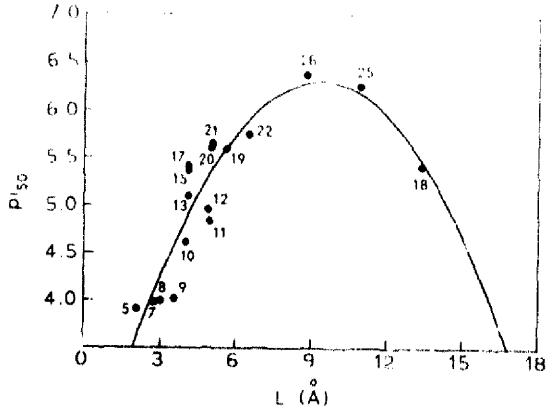

Fig. 5. Plot of Verloop's STERIMOL parameter L of the 4-substituent versus pI_{50} values of 4-substituted acridones. The numbering is in accordance with Table I.

The values of L and the pI_{50} values found and calculated according to this equation are given in Table II and a plot of pI_{50} values found versus calculated is shown in Fig. 4. Fig. 5 demonstrates the dependence of the pI_{50} value from the length L of the substituent in 4-position. It is evident that the inhibitory activity reaches its maximum at a substituent length of 9–10 Å. This indicates that the hydrophobic binding pocket of the inhibitor binding protein can accommodate maximally a side-chain 9–10 Å long. Smaller and greater substituents will not fit well and exhibit a decreased activity.

Further inspection of the data base for the physicochemical parameters of the acridones revealed that in the correlation matrix in addition to L and L^2 the lipophilicity parameter π had a high correlation coefficient of 0.93. Other parameters, electronic or steric, exhibited much lower values. Therefore, π was included as an additional parameter, which substantially improved the QSAR. The following equation is obtained:

$$pI_{50} = 0.765\,L - 0.048\,L^2 + 0.281\,\pi + 2.29 \qquad (2)$$

$$n = 17,\ s = 0.302,\ r = 0.938,\ F = 31.69$$

Values of the lipophilicity parameter π together with the pI_{50} values calculated according to this equation are also given in Table II. A plot of pI_{50} values found versus calculated is presented in Fig. 6.

In conclusion, in the acridones some new and efficient inhibitors of mitochondrial NADH:ubiquinone dehydrogenase were found. When the synthesis of the

TABLE II

Verloop's STERIMOL parameter L, lipophilicity π, pI_{50} values found and calculated for various 4-substituted acridones

Subst.	No.	L	π	pI_{50} found	pI_{50} calc. (L, L^2)	Diff.	pI_{50} calc. (L, L^2, π)	Diff.
H	5	2.06	0	3.91	3.60	0.31	3.67	0.24
OH	7	2.74	−0.67	3.98	4.08	−0.10	3.84	0.14
CH_3	8	3.00	0.56	4.00	4.25	−0.25	4.32	−0.32
Cl	9	3.52	0.71	4.02	4.58	−0.56	4.59	−0.57
OCH_3	10	3.98	−0.02	4.62	4.84	−0.22	4.58	0.04
C_6H_5	11	4.95	1.96	4.84	5.32	−0.48	5.46	−0.62
OC_2H_5	12	4.92	0.38	4.96	5.31	−0.35	5.01	−0.05
C_2H_5	13	4.11	1.02	5.10	4.91	0.19	4.92	0.18
i-C_3H_7	15	4.11	1.53	5.37	4.91	0.46	5.06	0.31
t-C_4H_9	17	4.11	1.98	5.42	4.91	0.51	5.19	0.23
n-$OC_{12}H_{25}$	18	13.40	5.33	5.42	5.49	−0.07	5.50	−0.08
OC_6H_5	19	5.62	2.08	5.60	5.60	0	5.67	−0.07
n-C_3H_7	20	5.02	1.55	5.61	5.37	0.24	5.37	0.24
s-C_4H_9	21	5.05	2.04	5.65	5.37	0.28	5.51	0.14
n-OC_5H_{11}	22	6.51	1.87	5.75	5.90	−0.15	5.78	−0.03
n-$OC_{10}H_{21}$	25	10.90	4.34	6.26	6.18	0.09	6.17	0.09
n-OC_8H_{17}	26	8.76	3.35	6.38	6.29	0.09	6.27	0.11

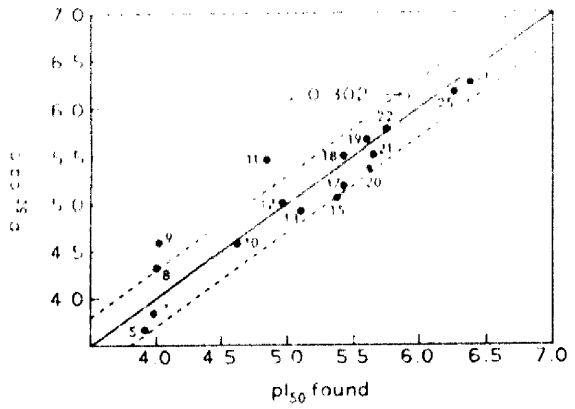

Fig. 6. Plot of pI_{50} values of 4-substituted acridones found versus calculated (according to Eqn. 2). The standard deviation is indicated by dashed lines. The numbering is in accordance with Table I.

radioactively labelled azidoacridone has been completed, it may be possible to identify the acridone binding protein within complex I and possibly also individual amino acids participating in acridone binding.

Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft.

References

1 Ragan, C.I. (1987) Curr. Topics Bioenerg. 15, 1–36.
2 Weiss, H., Friedrich, T., Hofhaus, G. and Preis, D. (1991) Eur. J. Biochem. 197, 563–576.
3 Gutman, M. and Kliatchko, S. (1976) FEBS Lett. 67, 348–353.
4 Yagi, T. (1990) Arch. Biochem. Biophys. 281, 305–311.
5 Ehses, M. and Werner, S. (1988) Biochem. Pharm. 37, 2551–2558.
6 Savic, I.M., Singh, M.P., Arora, P.K., Wang, F., McPeak, R.J. and Hoppel, C.L. (1990) Arch. Biochem. Biophys. 280, 274–283.
7 Krueger, M.J., Singer, T.P., Casida, J.E. and Ramsay, R.R. (1990) Biochem. Biophys. Res. Commun. 169, 123–128.
8 Albores, R., Neafsey, E.J., Drucker, G., Fields, J.Z. and Collins, M.A. (1990) Proc. Natl. Acad. Sci. USA 87, 9368–9372.
9 Anderson, W.M., Patheja, H.S., Delinck, D.L., Baldwin, W.W., Smiley, S.T. and Chen, L.B. (1989) Biochem. Int. 19, 673–685.
10 Chung, K.H., Cho, K.Y., Asami, Y., Takahashi, N. and Yoshida, S. (1989) Z. Naturforsch. 44c, 609–616.
11 Ravanel, P., Creuzet, S. and Tissut, M. (1990) Phytochem. 29, 441–445.
12 Verloop, A., Hoogenstraten, W. and Tipker, J. (1976) in Drug Design (Ariens, E.J., ed.), Vol. 7, pp. 165–207, Academic Press, New York.
13 Ullmann, F. (1907) Liebigs Ann. 355, 312–371.
14 Brockmann, H. and Musfeldt, H. (1956) Chem. Ber. 89, 1397–1402.
15 Oettmeier, W., Masson, K. and Johanningmeier, U. (1980) FEBS Lett. 118, 267–270.
16 Smith, A.L. (1967) Methods Enzymol. 10, 81–86.
17 Thierbach, G. and Reichenbach, H. (1981) Biochim. Biophys. Acta 638, 282–289.
18 Oettmeier, W., Masson, K., Fedtke, C., Konze, J. and Schmidt, R.R. (1982) Pestic. Biochem. Physiol. 18, 357–367.
19 Laasch, H., Pfister, K. and Urbach, W. (1982) Z. Naturforsch. 37c, 620–631.
20 Segel, I.H. (1976) Biochemical Calculations, 2nd Edn. pp.246–273, John Wiley & Sons, New York.