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INDOLEQUINONE ANTITUMOR AGENTS: RELATIONSHIP BETWEEN QUINONE STRUCTURE AND RATE OF METABOLISM BY RECOMBINANT HUMAN NQO1†

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Abstract: A series of indolequinones bearing various functional groups has been synthesized, and the effects of substituents on the metabolism of the quinones by recombinant human NAD(P)H:quinone oxidoreductase (NQO1), and on the toxicity toward nonsmall cell lung cancer cells with either high NQO1 activity (H460) or with no detectable activity (H596) were studied.

NAD(P)H:Quinone oxidoreductase (NQO1, EC 1.6.99.2), also known as DT-diaphorase, is an obligate 2-electron reductase that is characterized by its ability to use either NADH or NADPH as cofactor.[1,2] The enzyme, a flavoprotein, exists as a homodimer with one mol of FAD per mol of NQO1,[3] and the human enzyme has been cloned and sequenced.[4] The crystal structure of the rat liver enzyme, which shows about 85% homology with human NQO1, has been determined to 2.1 Å resolution.[5] NQO1 catalyzes the 2-electron reduction of quinones and can protect cells against the toxic effects of quinones. However NQO1 is also involved in the reductive activation of anticancer agents such as mitomycin C (MMC) **1** which operate by the so-called bioreductive mechanism,[6–8] and although other enzymes have also been implicated in the reductive bioactivation process,[8] NQO1 has generated considerable interest because of its elevated levels in many tumors and tumor cell lines.[7,9]

The role of NQO1 in the bioactivation of MMC **1** is somewhat controversial, although recently a clear correlation between NQO1 activity and MMC sensitivity in human lung and breast cancer cell lines has been demonstrated.[10] On the other hand, the importance of NQO1 in the bioactivation of other cytotoxic quinones such as EO9 **2**,[11–13] aziridinylbenzoquinones such as MeDZQ **3**,[10,14] streptonigrin,[15] and the novel cyclopropamitosenes **4**[16] has been established. However, there has been no systematic attempt to correlate quinone structure with rate of metabolism by NQO1 and toxicity towards human tumor cell lines, and therefore we report the preliminary results of such a study in a series of indolequinones **5**.

The synthesis of the indolequinone ring system **5** has already been developed in our laboratory, and the preparation of compounds **5a–5c**, **5e**, **5n**, and **5o** has been described previously.[17,18] Several new compounds were included in the present study with the aim of investigating the substituent effects at the 5- and 3-positions. Hence a range of compounds containing a greater variety of amine substituents at C-5 was prepared, and the C-3

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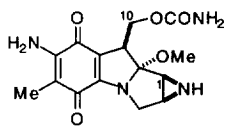
position was unsubstituted or substituted with hydroxymethyl of carbamoyloxymethyl groups. Compounds containing electron withdrawing groups (CHO or CO_2Et) were also included in the study. Of the new compounds, the indolequinone ester **5d** was obtained from commercially available ethyl 5-hydroxy-2-methylindole-3-carboxylate,[19] and a range of indolequinones containing amine substituents was prepared by reaction of the corresponding methoxy quinone with the appropriate amine, a substitution reaction widely used in the mitomycin series.[20,21] Thus indolequinones **5f–5m** were obtained from the 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione **5e** by reaction with the amine in DMF. Compounds **5e**, **5i** and **5j** have also been studied independently by another research group with a view to establishing their activity under hypoxic conditions.[22]



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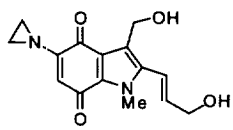

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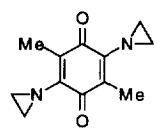

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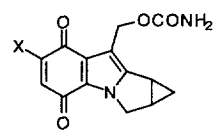

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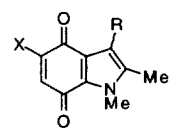

5

Metabolism of the indolequinones **5** (Table) by recombinant human NQO1[23] was studied using an HPLC system designed to quantify both NADH oxidation and quinone reduction. NADH oxidation is irreversible in this assay and is therefore used for comparison of reduction velocities. In the indolequinone series **5**, measurable NADH oxidation was observed for compounds **5a–5k** and **5m**. Compounds **5b** and **5i**, which have an aziridinyl substituent at the 5-position, were better substrates for human NQO1 than the corresponding compounds **5a** and **5e**, which have a methoxy group at the 5-position. Compound **5j**, the methylaziridinyl analogue of **5i**, was reduced at a slightly slower rate than the aziridine **5i**, though it was still a better substrate than the methoxy compound **5e**. Compounds containing a basic nitrogen substituent at C-5 were poor substrates for human NQO1, and some (e.g., **5l**) were not metabolised at all. Reduction rates for those compounds with hydroxymethyl substituents at the 3-position, for example, **5e** and **5i**, were somewhat lower than the corresponding unsubstituted derivatives, **5a** and **5b**. The 3-carbamoyloxymethyl derivatives, **5n** and **5o**, were not substrates for human NQO1. Studies with recombinant rat NQO1 have shown that carbamates are NADH-dependent inactivators of the enzyme (data not shown). However compounds containing an electron-withdrawing group such as an ester at C-3 were excellent substrates; within the 5-methoxyindolequinones the rate of metabolism by the enzyme decreased as the 3-substituent was altered in the order: $CO_2Et > CHO > H > CH_2OH > CH_2OCONH_2$. Quinone reduction was undetectable for all of the compounds suggesting that the reduced quinones (hydroquinones) reoxidize rapidly in air to regenerate the parent quinones.

It is clear that the C-5 substituent on the quinone ring has a marked effect on the rate of metabolism of the indolequinones by NQO1. In general those compounds bearing amine substituents (other than aziridines) at C-5 are not good substrates for the enzyme, either as a result of steric effects (in the case of substituents such as pyrrolidine or morpholine) or because such substituents render the quinone more difficult to reduce by virtue of the donation of the nitrogen lone pair into the quinone.[18] Conversely the indolequinones that are easiest to reduce,[24] i.e. those bearing an aziridine substituent, are the best substrates for NQO1, with the methylaziridine and methoxy compounds being somewhat less efficient. The aziridine ring, because of its inability to donate electron density into the quinone, which would involve an unfavorable flattening of the aziridine nitrogen, has a similar electronic effect to the methoxy group.

Table. Metabolism of indolequinones **5** by recombinant human NQO1 and cytotoxicity towards non-small cell lung cancer cell lines

Compound	R	X	Metabolism (μmol/min/mg) NADH oxidation	Cytotoxicity IC_{50} (μM) H460	Cytotoxicity IC_{50} (μM) H596	Selectivity ratio
5a	H	OMe	5.31 ± 0.93	>25	>25	1
5b	H	aziridinyl	11.6 ± 0.5	0.96 ± 0.24	>25	> 26
5c	CHO	OMe	8.78 ± 1.91			
5d	CO_2Et	OMe	14.3 ± 4.9			
5e	CH_2OH	OMe	1.25 ± 0.03	>25	>25	1
5f	CH_2OH	NHMe	0.49 ± 0.06			
5g	CH_2OH	NMe_2	0.46 ± 0.04			
5h	CH_2OH	$NHCH_2CH_2OMe$	0.10 ± 0.01			
5i	CH_2OH	aziridinyl	3.35 ± 0.65	0.018 ± 0.004	9.22 ± 1.51	510
5j	CH_2OH	methylaziridinyl	2.01 ± 0.43	0.11 ± 0.02	>25	> 220
5k	CH_2OH	azetidinyl	0.22 ± 0.03			
5l	CH_2OH	pyrrolidinyl	not detected			
5m	CH_2OH	morpholinyl	0.69 ± 0.08			
5n	CH_2OCONH_2	OMe	not detected	7.45 ± 0.37	2.16 ± 0.47	0.3
5o	CH_2OCONH_2	aziridinyl	not detected	0.15 ± 0.01	0.24 ± 0.03	1.6

The substituent at the indole-3-position also has a considerable effect on metabolism by NQO1. The carbamates are generally not substrates, although this is possibly due to inactivation of the enzyme by these compounds. Compounds with an electron withdrawing substituent at this position are the best substrates of those indolequinones studied, although the 3-unsubstituted compounds and the hydroxymethyl derivatives are also efficiently metabolized. However, being an efficient substrate for the enzyme does not necessarily mean that the quinone will be cytotoxic, and therefore a number of the compounds were tested in vitro against tumor cell lines.

Cytotoxicity (cell survival) was measured using the MTT colorimetric assay.[25] Two nonsmall cell lung cancer (NSCLC) cell lines were used; H460 with high NQO1 activity (1360 nmol min^{-1} mg^{-1}) and H596, which has no measurable NQO1 activity due to a polymorphism in the NQO1 gene.[26] IC_{50} values (concentration at which cell survival equals 50% of control) are reported for the indolequinones **5** (Table). A compound with an IC_{50} value which is lower in the H460 cells than in the H596 cells suggests a compound which is selectively toxic to the cell line with elevated NQO1 activity. The compounds with the highest selectivity ratio were the 1,2-dimethylindolequinones **5b**, **5i**, and **5j** each with greater than a 25-fold difference in IC_{50} values between the two cell lines; in the case of **5i** this selectivity was > 500-fold. All three compounds were good substrates for human NQO1, and each has either an aziridinyl substituent or a methylaziridinyl substituent at the 5-position.

In conclusion, in studying these indolequinones we have established for the first time a relationship between quinone structure and the rate of metabolism by human NQO1; further studies are in progress.

Acknowledgements

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