Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2195–2198

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NOVEL QUINOLINEQUINONE ANTITUMOR AGENTS: STRUCTURE-METABOLISM STUDIES WITH NAD(P)H:QUINONE OXIDOREDUCTASE (NQO1)†

Tara Fryatt,[a] Dean T. Goroski,[b] Zachary D. Nilson,[b]
Christopher J. Moody,[a*] and Howard D. Beall[b*]

[a]*School of Chemistry, University of Exeter, Stocker Road, Exeter EX4 4QD, Devon, U.K.*
[b]*Department of Pharmaceutical Sciences, The University of Montana, Missoula, MT 59812, U.S.A.*

Received 21 May 1999; accepted 21 June 1999

Abstract: The effects of functional group changes on the metabolism of novel quinolinequinones by recombinant human NAD(P)H:quinone oxidoreductase (NQO1) are described. Overall, the quinolinequinones were much better substrates for NQO1 than analogous indolequinones, with compounds containing heterocyclic substituents at C-2 being among the best substrates.

NAD(P)H:quinone oxidoreductase (NQO1) is a cytosolic (>90%), two-electron reductase that is characterized by its capacity for utilizing either NADH or NADPH as reducing cofactors and by its inhibition by dicumarol.[1] NQO1 is generally categorized as a detoxification enzyme, and it can protect the cell from a broad range of chemically reactive metabolites.[2] NQO1 reduces quinones to hydroquinones bypassing the potentially toxic semiquinone radical intermediates.[3]

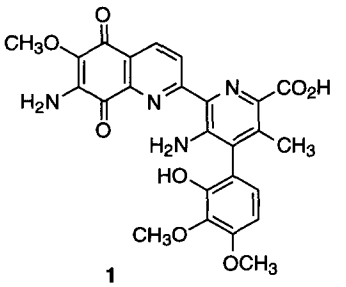
NQO1 can also function as an activating enzyme for the reductive activation of antitumor quinones and other cytotoxic bioreductive agents. NQO1 has been found to be markedly overexpressed in tumors of the colon, breast and lung relative to normal tissues.[4] This suggests that bioreductive agents which are activated by NQO1 may be selectively toxic to those tumors. We have recently examined the relationship between the structure of several series of novel indolequinones, their metabolism by recombinant human NQO1 and their toxicity to a non-small cell lung cancer (NSCLC) cell line with high NQO1 activity (H460) versus a tumor cell line with undetectable NQO1 activity (H596).[5] As expected, the best substrates for NQO1 were also the most toxic to the high NQO1 cell line when compared to the NQO1-deficient cell line.[5]

Streptonigrin (SN) **1** is a quinolinequinone antitumor antibiotic which has activity against a broad range of tumors.[6] SN was studied clinically in the 1960s and 1970s as an antitumor agent, but its use was limited by reports of delayed myelotoxicity.[7] Nevertheless, positive results were reported for SN both as a single agent[8] and in combination chemotherapy.[9] Hydroxyl radical (OH˙) production following reduction of the quinolinequinone moiety of SN leads to DNA degradation and cytotoxicity, and an SN-metal-DNA complex is thought to be involved.[10] SN is an excellent substrate for NQO1, and we have previously shown that it is selectively toxic to colon[11] and lung[12] cell lines with elevated NQO1. This report is the first to examine the

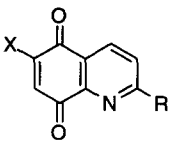
† *Preliminary results presented at 1999 Society of Toxicology Annual Meeting.*
* *Corresponding authors for chemistry (CJM) and biology (HDB).*

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00369-8

effects of functional group substitutions on the metabolism of a range of novel quinolinequinones **2** by recombinant human NQO1.

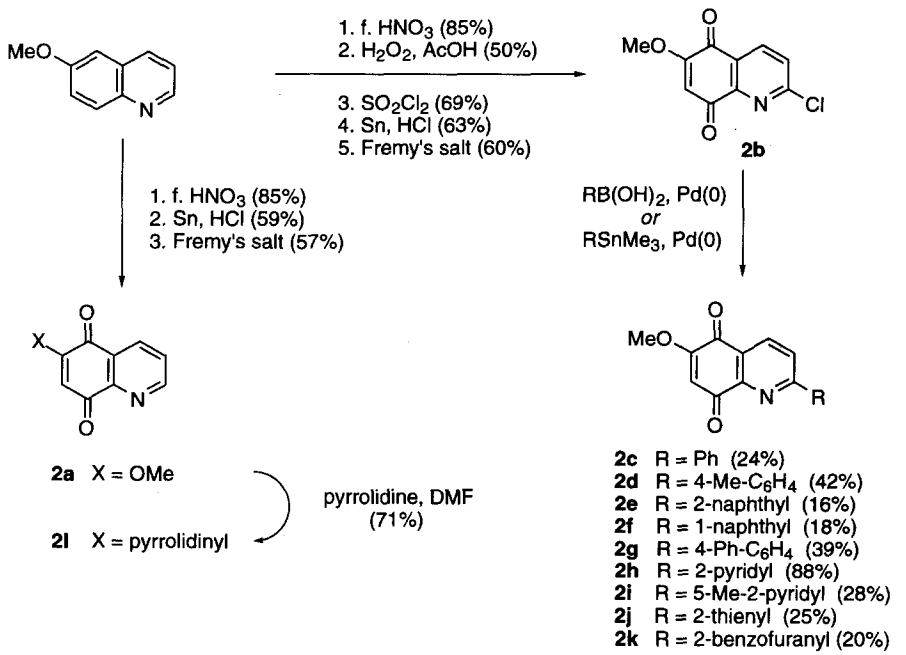


1



2

The starting material for the synthesis of the quinolinequinones **2** was the commercially available 6-methoxyquinoline. Thus nitration at C-5, followed by reduction of the nitro group, and oxidation of the resulting 5-aminoquinoline with Fremy's salt gave the 6-methoxyquinoline-5,8-quinone **2a** (Scheme).



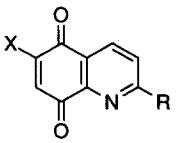
Scheme.

Subsequent displacement of the methoxy group with pyrrolidine gave the quinone **2l**. The 2-substituted quinolinequinones **2** were prepared from the 2-chloro compound **2b**, itself obtained from 6-methoxyquinoline as outlined in the Scheme. Palladium(0) catalysed coupling of the 2-chloroquinolinequinone **2b** with a range of

arylboronic acids or arylstannanes under standard Suzuki or Stille conditions gave the desired 2-substituted quinolinequinones **2** in modest yield.

Metabolism of the quinolinequinones **2** (Table) by purified recombinant human NQO1[13] was studied by reversed-phase HPLC using a standard assay.[5] The system is designed to quantify both NADH oxidation and quinone reduction. The reduced quinolinequinones (hydroquinones) react efficiently with molecular oxygen to regenerate the parent quinone so quinone reduction was undetectable. On the other hand, NADH oxidation is irreversible in this assay and was used for comparison of reduction velocities.

Table. Metabolism of quinolinequinones **2** by recombinant human NQO1.



Compound	X	R	Metabolism (μmol/min/mg) NADH oxidation
1			47.4±11.5
2a	OMe	H	58.7±18.5
2b	OMe	chloro	100±32
2c	OMe	phenyl	28.8±7.9
2d	OMe	4'-methylphenyl	19.2±7.7
2e	OMe	naphth-2-yl	14.0±5.2
2f	OMe	naphth-1-yl	25.1±5.1
2g	OMe	4'-phenyl-C_6H_4	4.75±1.01
2h	OMe	pyrid-2-yl	50.5±10.6
2i	OMe	5'-methylpyrid-2-yl	45.7±13.4
2j	OMe	thien-2-yl	32.1±7.8
2k	OMe	benzofuran-2-yl	33.3±16.8
2l	pyrrolidin-1-yl	H	26.2±1.1

The novel quinolinequinones were much better substrates for recombinant human NQO1 than similar indolequinones (*e.g.* **2a** was reduced at a rate of 58.7±18.5 μmol/min/mg whereas 5-methoxy-1,2-dimethylindole-4,7-dione[5] was reduced at a rate of 5.31±0.93 μmol/min/mg). Substitution at the 2-position with phenyl **2c**, methylphenyl **2d**, naphthyl **2e** and **2f** or biphenyl **2g** yielded the lowest reduction rates, while heterocyclic substituents such as pyridyl **2h**, methylpyridyl **2i**, thienyl **2j** and benzofuranyl **2k** were much better substrates. The 2-chloro compound **2b** produced the highest reduction rate. Surprisingly, the 6-

pyrrolidinyl compound **21** was a good substrate for NQO1 despite the fact that the amine substituent has been shown to deactivate the quinone ring in other series.[5]

In conclusion, we have established that quinolinequinones are excellent substrates for recombinant human NQO1. The quinolinequinone structure appears to be a good starting point for the design of NQO1-directed bioreductive antitumor drugs. Further studies are underway.

Acknowledgments:

We acknowledge support from the Cancer Research Campaign, the University of Exeter, USPHS NIH Grant R15 CA78232 and The University of Montana.

References:

1. Ernster, L. *Meth. Enzymol.* **1967**, *10*, 309.
2. Riley, R. J; Workman, P. *Biochem. Pharmacol.* **1992**, *43*, 1657.
3. Thor, H; Smith, M. T.; Hartzell, P.; Bellomo, G.; Jewell, S. A.; Orrenius, S. *J. Biol. Chem.* **1982**, *257*, 12419; Lind, C.; Hochstein, P; Ernster, L. *Arch. Biochem. Biophys.* **1982**, *216*, 178.
4. Ross, D.; Beall, H. D.; Siegel, D.; Traver, R. D.; Gustafson, D. L. *Br. J. Cancer* **1996**, *74(Suppl. XXVII)*, S1.
5. Beall, H. D.; Winski, S.; Swann, E.; Hudnott, A. R.; Cotterill, A. S.; O'Sullivan, N.; Green, S. J.; Bien, R.; Siegel, D.; Ross, D.; Moody, C. J. *J. Med. Chem.* **1998**, *41*, 4755.
6. Oleson, J. J.; Calderella, L. A.; Mjos, K. J.; Reith, A. R.; Thie, R. S.; Toplin, I. *Antibiot. Chemother.* **1961**, *11*, 158; Teller, M. N.; Wagshul, S. F.; Woolley, G. W. *Antibiot. Chemother.* **1961**, *11*, 165; Reilly, H. C.; Sigura, K. *Antibiot. Chemother.* **1961**, *11*, 174.
7. Hackethal, C. A.; Golbey, R. B.; Tan, C. T. C.; Karnofsky, D. A.; Burchenal, J. H. Antibiot. Chemother. **1961**, *11*, 178; Humphrey, E. W.; Blank, N. *Cancer Chemother. Rep.* **1961**, *12*, 99; Rivers, S. L.; Whittington, R. M.; Medrek, T. J. *Cancer* **1966**, *19*, 1377.
8. Kuang, D. T.; Whittington, R. M.; Spencer, H. H.; Patno, M. E. Cancer **1969**, *23*, 597; Kaung, D. T.; Whittington, R. M.; Spencer, H.; Patno M. E. *Cancer* **1969**, *23*, 1280.
9. Banzet, P.; Jacquillat, C.; Civatte, J.; Puissant, A.; Maral, J.; Chastang, C.; Israel, L.; Belaich, S.; Jourdain, J.-C.; Weil, M.; Auclerc, G. *Cancer* **1978**, *41*, 1240; Forcier, R. J.; McIntyre, O. R.; Nissen, N. I.; Pajak, T. F.; Glidewell, O.; Holland, J. F. *Med. Pediatr. Oncol.* **1978**, *4*, 351.
10. For a review, see: Harding, M. M.; Long, G. V. *Curr. Med. Chem.*, **1997**, *4*, 405.
11. Beall, H. D.; Liu, Y.; Siegel, D.; Bolton, E. M.; Gibson, N. W.; Ross, D. *Biochem. Pharmacol.* **1996**, *51*, 645.
12. Beall, H. D.; Murphy, A. M.; Siegel, D.; Hargreaves, R. H. J.; Butler, J.; Ross, D. *Mol. Pharmacol.* **1995**, *48*, 499.
13. Beall, H. D.; Mulcahy, R. T.; Siegel, D.; Traver, R. D.; Gibson, N. W.; Ross, D. *Cancer Res.* **1994**, *54*, 3196.