A novel N-oxide drug, AQ4N, demonstrates in vitro cytotoxicity on solid tumor and hematopoietic tumor cell lines

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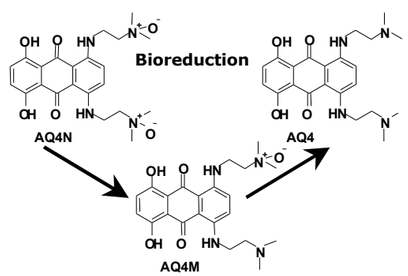
Abstract

AQ4N (1,4 bis{2-(dimethylamino)ethylamino}-5,8-hydroxyanthracene-9,10-dione bis N-oxide) is designed to have little or no toxicity until selectively bioreduced in vivo by hypoxic cells to AQ4 (reduced AQ4N), a highly potent DNA topoisomerase II inhibitor. Cytotoxicity of AQ4 has been investigated by MTT assay on a panel of solid and hematopoietic tumor cell lines. The IC50 of AQ4 ranged from 0.4-1.6 μM on the pancreatic tumor lines; the IC50 of 0.4 μM on Panc-1 was four-fold lower than that of the standard agent, gemcitabine. On the colon carcinoma line, HT-29, AQ4 demonstrated an IC50 of 0.3 μM, comparable to SN38, and ten-fold better than oxaliplatin. On glioma lines U118, U251, U87, IC50's of AQ4 were 0.3, 0.6, 1.6 μM respectively. AQ4 also demonstrated potent cytotoxicity on a panel of lymphoma and leukemia tumor cell lines. The IC50 of AQ4 was 0.2 nM on Namalwa human lymphoma, 10 μM and 1.5 μM on HL60 and Molt-4 human leukemia, and 1.2 nM and 10 nM on L1210 and P388 murine leukemia; on each of these tumor lines cytotoxicity was greater than that seen with doxorubicin. AQ4N had significant activity in Namalwa, MOLT-4, KGa1, K562, L1210 and P388, suggesting a non-hypoxia-related mechanism of bioreduction in these hematologic lines. Potent AQ4 activity was also seen on the human multiple myeloma lines, RPMI8226 and ARH-77. The potent in vitro activity of AQ4 is currently being followed up with in vivo efficacy studies of AQ4N.

Background

- AQ4N is a N-oxide prodrug designed to be bioreduced under hypoxic conditions
- Previous studies have shown that AQ4N undergoes two consecutive 2 electron reductions to yield AQ4, a potent topoisomerase II inhibitor (see Figure; Patterson, Cancer Metastasis Rev 1993; 12:119-34)
- Bioreduction of AQ4N is catalyzed by cytochrome P450 enzymes 1A1, 1B1, and 3A4 (Patterson, Drug Metab Rev 2002; 34: 581-92)
- Work is ongoing to investigate the potential roles of other enzymes in the bioreduction of AQ4N
- AQ4N is currently being studied in several human Phase I studies in the US and Europe



Cytotoxicity Assessment

Tumor Line	Type	AQ4 (IC50)	AQ4N (IC50)*	Standard	STD/AQ4***
Daudi	Burkitt Lymph	4.6 nM	NA**	0.5 nM Dox	0.109
Raji	" "	200 nM	NA	0.9 nM Dox	0.005
Ramos	" "	8.0 nM	NA	1.8 nM Dox	0.150
Namalwa	" "	0.2 nM	400 nM	7.4 nM Dox	37.0
MOLT-4	ALL (human)	2 nM	700 nM	7.5 nM Dox	3.75
HL-60	AML (human)	10 nM	NA	100 nM Dox	10.0
KG1a	AML (human)	50 nM	43 μM	0.6 μM Dox	12.0
K562	CML (human)	400 nM	1.0 μM	0.2 μM Dox	0.500
P388	CLL (mouse)	10 nM	31.5 μM	0.1 μM Dox	10.0
L1210	ALL (mouse)	1.2 nM	0.6 μM	50 nM Dox	41.7
CCRF-CEM	T-ALL	0.62 μM	237.5 μM	10 nM VLB	0.016
CCRF-CEM/VLB	T-ALL	0.34 μM	310 μM	1041 μM VLB	3062
L5178Y	mouse lymph	30 nM	300 nM	50 nM Dox	1.67
RPMI8226	multiple myel.	0.2 μM	NA	0.1 μM Dox	0.5
RPMI8226/Dox	" "	1.1 μM	NA	54.7 μM Dox	49.7
ARH177	" "	0.2 μM	NA	--	--
BXPC-3	Pancreatic	1.6 μM	3.6 μM	59.5 nM Gem	0.037
MiaPaCa	" "	1.6 μM	NA	23 nM Gem	0.014
Panc-1	" "	.4 μM	NA	1.7 μM Gem	4.25
HT-29	Colon	0.7 μM	101.5 μM	220 nM SN38	0.314
HCT116	" "	3.9 μM	NA	803 nM SN38	0.204
LoVo	" "	0.2 μM	49.9 μM	81 nM SN38	0.386
LS174T	" "	0.95 μM	14.4 μM	235 nM SN38	0.247
U87MG	Glioma	0.9 μM	NA	0.4 μM Dox	0.444
U118MG	" "	1.3 μM	NA	0.03 μM Dox	0.023
U251	" "	0.6 μM	NA	0.03 μM Dox	0.050
FaDu	Pharynx SCC	0.3 μM	64.7 μM	6.5 μM Taxol	21.7
KB	Mouth EC	0.6 μM	13.4 μM	2.0 μM Taxol	3.33
KB-3	" rad.resist.	3.3 μM	NA	19.1 μM Taxol	5.79
Hep3B2.1-7	Hepatocell.	0.8 μM	NA	13.7 μM Taxol	17.1
A375-SM	Melanoma(hu)	3.3 μM	NA	None	--
B16-F10	Melanoma(mu)	0.02 μM	NA	None	--

Blue = AQ4N activity and/or AQ4 more active than standard

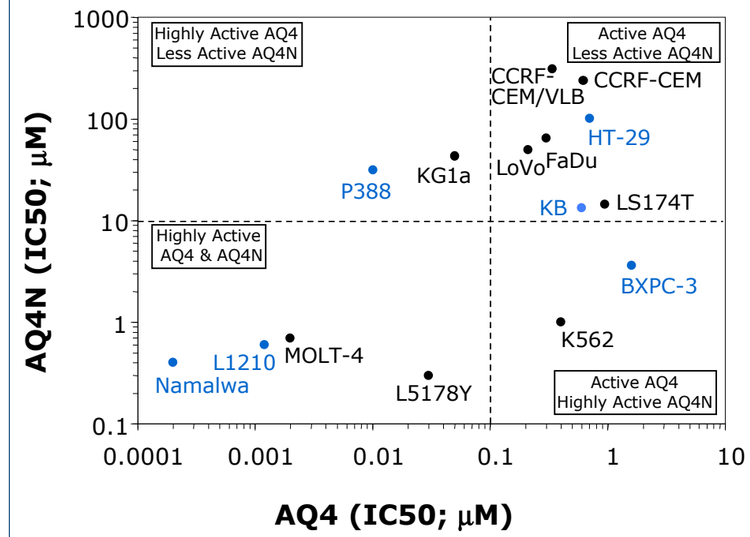
All results represent 24 hr drug exposure followed 48 hrs later by MTS assay for live cells (range nM to μM)

* Study done under normoxic conditions only

** NA = not active; IC_{50} > 100 μM

*** Ratio of IC50 values for Standard and AQ4 (Larger value indicates AQ4 more potent than standard under these conditions)

Relative Potency AQ4N/AQ4



Blue = In Vivo Activity

Summary

- AQ4N is cytotoxic under normoxic conditions
- Some cell lines are significantly more sensitive to AQ4N than others, even when accounting for their sensitivity to AQ4
- AQ4 cytotoxicity is greater than doxorubicin in many of the hematological tumor lines and some of the solid tumor lines
- The mechanism of AQ4N cytotoxicity under normoxic conditions is unknown
- The broad spectrum of activity observed for AQ4N and AQ4 is supportive of AQ4N monotherapy in several oncology indications.

Ongoing Studies:

- AQ4N in vivo activity in solid and hematopoietic tumor models (AACR 2005)
- Mechanism of action studies with enzyme inhibitors
- AQ4N biodistribution in solid tumor model (AACR 2005)