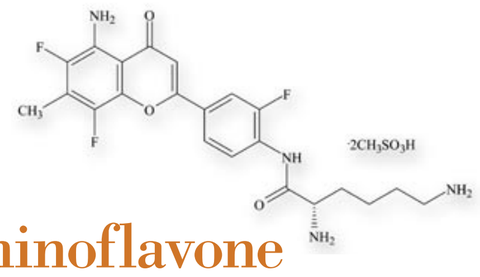
Aminoflavone Prodrug vs. Phortress
A Go–No Go Study

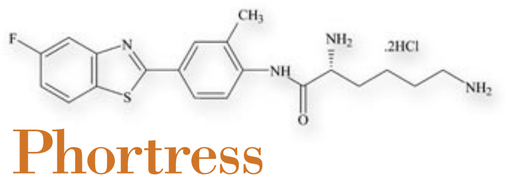
SUCCESS STORY

AMINOFLAVONE PRODRUG (NSC 710464).....PHORTRESS (NSC 710305)

Developmental Therapeutics Program of the National Cancer Institute



Aminoflavone



Phortress

Structure and 60 Cell Line Activity

Both drugs have unique structures and are active in 60 cell assay.

GI 50 Mean Graph for Compound 710464
NCI Cancer Screen Current Data, August 2004
Average GI_{50} over all cell lines is 2.97E-6

Cell Panel	Cell Line	Log GI_{50}
Leukemia	CCRF-CEM	-5.5
	HL-60(TB)	-5.6
	K-562	-6.6
	MOLT-4	-5.9
	RPMI-8226	-6.0
	SR	-5.6
Non-Small Cell Lung	A549/ATCC	-6.2
	EKVX	-5.2
	HOP-62	-5.1
	HOP-92	-4.7
	NCI-H226	-7.4
	NCI-H23	-4.8
	NCI-H322M	-5.1
	NCI-H460	-7.0
	NCI-H522	-5.3
Colon	COLO 205	-5.2
	HCC-2998	-6.1
	HCT-116	-5.5
	HCT-15	-4.9
	HT29	-4.9
	KM12	-5.0
	SW-620	-5.8
Central Nervous System	SF-268	-4.9
	SF-295	-4.8
	SF-539	-5.2
	SNB-19	-4.7
	SNB-75	-5.3
	U251	-6.1
Melanoma	LOX IMVI	-5.1
	MALME-3M	-5.0
	M14	-4.8
	SK-MEL-2,10	-4.7
	SK-MEL-28	-4.9
	Melanoma,SK-MEL-5	-5.6
	Melanoma,UACC-257	-6.6
Ovarian	IGROV1	-7.4
	OVCAR-3	-6.6
	OVCAR-4	-6.2
	OVCAR-5	-6.6
	OVCAR-8	-5.0
	SK-OV-3	-4.8
Renal	786-0	-5.0
	A498,9	-7.3
	ACHN	-4.9
	CAKI-1	-6.4
	RXF 393	-4.7
	SN12C	-4.8
	TK-10	-6.9
	UO-31	-5.1
Prostate	PC-3	-4.8
	DU-145	-4.9
Breast	MCF7	-7.5
	NCI/ADR-RES	-4.7
	MDA-MB-231/ATCC	-4.9
	HS 578T	-4.9
	MDA-MB-435	-5.1
	MDA-N	-5.1
	BT-549	-4.6
	T-47D	-6.8

GI 50 Mean Graph for Compound 710305
NCI Cancer Screen Current Data, August 2004
Average GI_{50} over all cell lines is 2.1E-6

Cell Panel	Cell Line	Log GI_{50}
Leukemia	HL-60(TB)	-5.7
	K-562	-5.7
	MOLT-4	-5.9
	RPMI-8226	-5.7
	SR	-6.1
Non-Small Cell Lung	A549/ATCC	-5.8
	EKVX	-5.7
	HOP-62	-5.5
	HOP-92	-4.9
	NCI-H226	-6.9
	NCI-H23	-5.8
	NCI-H322M	-5.8
	NCI-H460	-6.9
	NCI-H522	-5.3
Colon	COLO 205	-5.6
	HCC-2998	-6.1
	HCT-116	-5.7
	HCT-15	-5.4
	HT29	-5.6
	KM12	-5.9
	SW-620	-5.6
Central Nervous System	SF-268	-5.2
	SF-295	-5.6
	SF-539	-5.3
	SNB-19	-5.0
	SNB-75	-5.7
	U251	-5.7
Melanoma	LOX IMVI	-5.7
	MALME-3M	-4.8
	M14	-5.7
	SK-MEL-2	-5.5
	SK-MEL-28	-4.7
	SK-MEL-5	-4.9
	UACC-257	-5.2
	UACC-62	-5.0
Ovarian	IGROV1	-7.9
	OVCAR-3	-5.2
	OVCAR-4	-5.7
	OVCAR-5	-6.9
	OVCAR-8	-5.3
	SK-OV-3	-5.1
Renal	786-0	-5.6
	A498	-5.0
	ACHN	-5.2
	CAKI-1	-6.2
	RXF 393	-5.1
	SN12C	-4.9
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GO

Mechanism of Action

Induction of P450 enzyme CYP1A1—entry into cells is via the arylhydrocarbon (AHR) receptor. Cell line and tumor sensitivity correlate well with CYP1A1 and CYP1A2 expression.	Pattern of sensitivity of cell lines correlates well with induction of CYP1A1 and CYP1B1; entry into cells is via AHR.
Unique mechanism of action confirmed in laboratory studies.	
GO	

In Vivo Activity

Active in 2 renal (A498; CAKI-1) and 1 breast (MCF7) xenograft tumor models. Tumor-free animals in renal tumors.	Active against estrogen positive and negative human breast tumors.
Both drugs have activity in multiple xenografts.	
GO	

Bulk Synthesis and Formulation

Manageable 11-step synthesis of prodrug. Prodrug has significantly improved aqueous solubility over parent (> 5,000 fold).	Manageable 9-step synthesis to produce prodrug. Poor solubility of parent benzothiazole was overcome by synthesis of Lysyl prodrug.
Both drugs can be produced in a positive cost-efficient manner. Formulation is clinically acceptable.	
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Pharmacokinetics

I.v. administration of prodrug maintains steady state of parent aminoflavone in plasma for 3 hours in monkeys and dogs.	I.v. administration of Phortress results in plasma levels of parent sustained for 4–6 hours in monkeys and dogs.
Both drugs achieve plasma levels at doses shown to be efficacious in *in vitro* studies.	
GO	

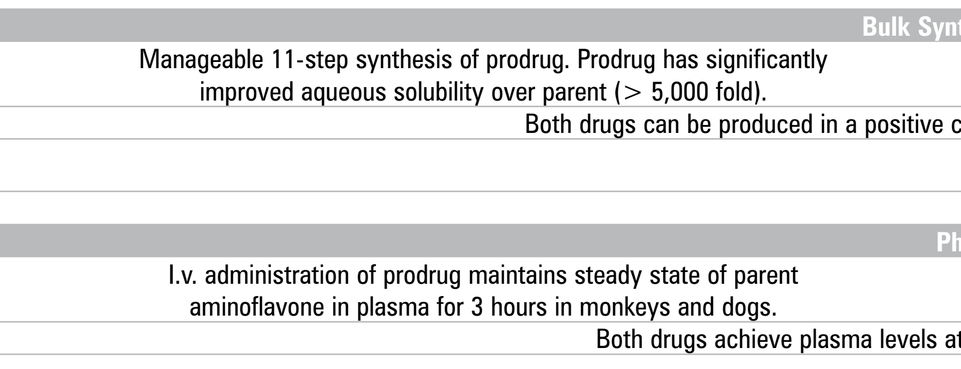
Pharmacodynamics

Mechanism of action is induction of P450 enzyme CYP1A1; entry into cells is via the arylhydrocarbon receptor, which translocates to the nucleus. Cell line and tumor sensitivity correlate well with CYP1A1 and CYP1A2 expression. Cell line sensitivity was correlated directly with induction of apoptosis using an ELISA assay.	Following binding to AHR and dimerization, the drug-AHR entity complexes with the *cyp1a1* promoter and activates gene transcription. The subsequent induction of CYP1A1-catalyzed metabolism of Phortress results in DNA-adduct formation in sensitive cells only. MCF7 has been found to be sensitive to Phortress while MDA-MB-435 is not. (Mol Cancer Ther 2004; 3(12). December 2004)
Examination of resistant (8) and sensitive (5) renal cell isolates from patients showed a trend for increased binding of aminoflavone to sensitive cell lines over resistant cell lines. It might be possible to use this as a test to select patients for phase I trials.	
GO	

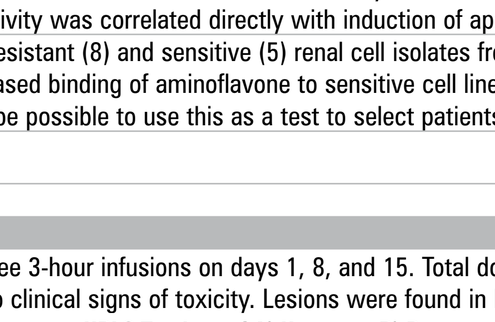
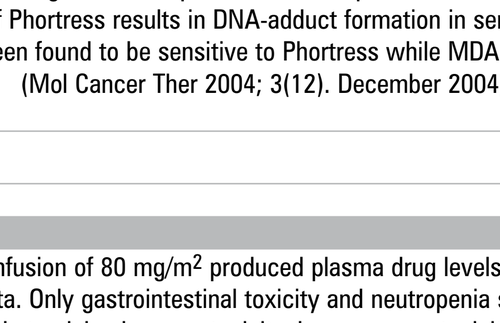
Toxicology

Dogs given three 3-hour infusions on days 1, 8, and 15. Total dose of 684 mg/m^2. No clinical signs of toxicity. Lesions were found in lungs.	Dogs given a 1-hour infusion of 80 mg/m^2 produced plasma drug levels within efficacious range based on *in vitro* data. Only gastrointestinal toxicity and neutropenia seen. A dose 3.5 times higher produced substantial pulmonary toxicity, bone marrow toxicity, and hepatotoxicity.
	One-hour infusions of 60 or 80 mg/m^2 in non-human primates produced no evidence of pulmonary toxicity at the 60 mg/m^2 dose, but produced severe pulmonary toxicity at the higher dose with rapid death (< 24 hours later).
Lung toxicity may be problematic. Additional studies performed.	

HPLC Tracings of A) Human or B) Dog Microsomal Incubations with 100 μM AF for 60 Minutes



Pulmonary Function Study

Diffusion Constant of Carbon Monoxide for Aminoflavone (NSC 710464)	**Diffusion Constant of Carbon Monoxide for Phortress (NSC 710305)**
	
Results show a distinct change in CO diffusion over time and a clear delineation between the control, low-dose, and high-dose animals.	Results do not show clear distinction between control, low-dose, and high-dose animals. Variability in study animals also made interpretation of study results difficult.
Study results indicate that a pulmonary function test in patients can be used to evaluate drug toxicity.	Study results indicate no value in monitoring pulmonary function in patients as an indicator of drug toxicity. Hepatotoxicity must be monitored as a surrogate for pulmonary toxicity.
GO	**NO GO**
Phase I trials to commence in US.	**Drug on hold pending results of trials in UK.**

TURNING MOLECULES INTO medicines FOR THE public health