DNA BINDING AFFINITY AND MCF-7 BREAST TUMOUR CYTOTOXICITY OF ANTHRAPYRAZOLES: COMPARISON WITH DOXORUBICIN AND MITOXANTRONE

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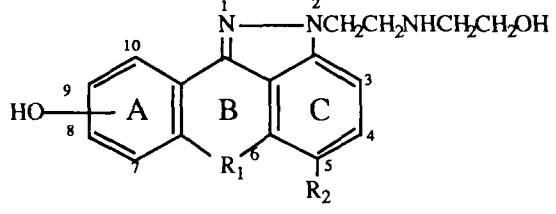
Anthrapyrazoles (anthra[1,9-cd]pyrazol-6(2H)-ones) are a novel group of antitumour agents developed by the Warner Lambert Company to circumvent the cardiotoxic side effects produced by anthracyclines, including doxorubicin. The anthrapyrazole CI941 is presently undergoing Phase I/II clinical trials against advanced breast cancer. We present data from studies on DNA-binding affinities of anthrapyrazoles and related benzothiopyranoindazole containing hydroxy-substitution in ring A of the nucleus, and have correlated these with cytotoxicity against cultured MCF-7 breast tumour cells; these data are compared with similar data for the clinically important antitumour agents doxorubicin and mitoxantrone.



COMPOUND	OH	R_1	R_2
CI941	7	C = O	$NH(CH_2)_2NH(CH_2)_2OH$
PD 111815	7,10	C = O	$NH(CH_2)_3NH_2$
PD 113309	7,10	C = O	$NH(CH_2)_2NHCH_3$
PD 114254	7,8,10	C = O	$NH(CH_2)_2NH(CH_2)_2OH$
PD 118484	8	S	$NH(CH_2)_3NH_2$

Drug solutions (5 x 10^{-5}M) were prepared in isotonic Tris HCl buffer pH 7.4 and tested for affinity for calf-thymus DNA (2.9 x 10^{-3}M); techniques used included spectrophotometric titration, ethidium bromide (2 x 10^{-6}M) displacement, fluorescence polarization, and Scatchard plot studies as described elsewhere(Plumbridge et al., 1978; Islam et al., 1985). Results for DNA binding and cytotoxicity studies using 10^6 MCF-7 cells ml^{-1} for doxorubicin, mitoxantrone, anthrapyrazole and benzothioindazole agents are given in Table 1. These data show that DNA binding constants (K) and associated binding numbers (n) in combination with the ethidium bromide displacement data are consistent with an intercalative mode of binding. Drug uptake into 10^6 MCF-7 cells over 1 hr at 37°C was determined, and the results showed there was no significant difference in % uptake between these antitumour agents (data not shown).

COMPOUND	Scatchard Plots		Fluorescence Polarization C50 (uM)	Ethidium Bromide Displacement C50 (uM)	Cytotoxicity IC50 (uM)
	K (uM)	n			
Doxorubicin	5.45	0.20	0.81	0.63	0.30
Mitoxantrone	0.93	0.24	1.21	1.07	0.05
CI941	1.01	0.24	1.02	0.75	0.01
PD111815	0.66	0.24	1.61	1.16	9.00
PD113309	0.62	0.25	1.81	1.73	6.00
PD114254	0.47	0.22	4.70	3.30	inactive
PD118484	0.55	0.26	3.25	2.20	inactive

Table 1 Effects of DNA Binding Affinity, and Cytotoxicity against MCF-7 cells, of Anthrapyrazoles and Related Antitumour Agents (C50 = drug concentration displacing 50% ethidium bromide)

The cytotoxicity results confirm the high potency of doxorubicin, mitoxantrone and CI941 against cultured MCF-7 breast tumour cells. Other agents tested were either considerably less active, or inactive. PD114254 is unstable at 37°C and this may account for its lack of cytotoxic activity. These results show that mechanisms other than DNA binding are likely to contribute to anthrapyrazole-mediated cytotoxicity.

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Plumbridge T.W., Aarons L.J., Brown J.R. (1978), J.Pharm.Pharmacol. 30: 69-74