Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2237–2242

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A 2-NITROIMIDAZOLE CARBAMATE PRODRUG OF 5-AMINO-1-(CHLOROMETHYL)-3-[(5,6,7-TRIMETHOXYINDOL-2-YL)CARBONYL]-1,2-DIHYDRO-3*H*-BENZ[E]INDOLE (AMINO-*SECO*-CBI-TMI) FOR USE WITH ADEPT AND GDEPT

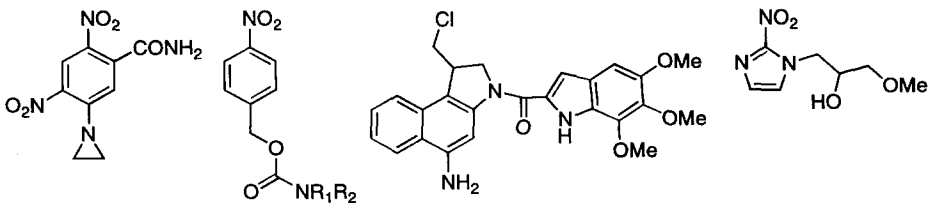
Michael P. Hay,* Bridget M. Sykes, William A. Denny, and William R. Wilson

Auckland Cancer Society Research Centre
University of Auckland School of Medicine
Private Bag 92019, Auckland, New Zealand

Received 31 May 1999; accepted 25 June 1999

Abstract: The synthesis of a 2-nitroimidazol-5-ylmethyl carbamate prodrug **10** of the potent minor groove alkylating agent amino-*seco*-CBI-TMI **3** is described. Chemical, radiolytic, and enzymic reductions of a model 2-nitroimidazol-5-yl carbamate **8** show release of the amine effector upon reduction. Prodrug **10** gives a ten fold increase in cytotoxicity against human ovarian carcinoma SKOV3 cells in the presence of *E. coli* B nitroreductase (NTR) and a 21-fold increase in cytotoxicity against a SKOV3 cell line (SC3.2) transfected with the gene for NTR. The cytotoxicity of **10** increased 15- to 40-fold under hypoxia. Prodrug **10** has significant potential as a prodrug for use in ADEPT and GDEPT applications, and as a hypoxia-selective cytotoxin.

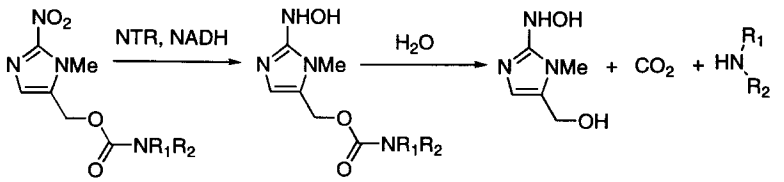
Antibody-directed enzyme prodrug therapy (ADEPT) and gene-directed enzyme prodrug therapy (GDEPT) are new techniques with potential in cancer chemotherapy and have been extensively reviewed.[1-7] Both techniques aim to produce tumour-specific localisation of an enzyme capable of activating a prodrug to release a cytotoxin or other bioactive molecule. One enzyme under evaluation for use in both ADEPT and GDEPT is an aerobic nitroreductase (NTR) from *Escherichia coli* B,[8,9] which in conjunction with NADH or NADPH, reduces certain aromatic nitro groups to the corresponding hydroxylamine.[10] Prodrugs activated by this enzyme have fallen into two classes. The first class is exemplified by 2,4-dinitrobenzamides, e.g., CB 1954 (**1**),[8] 2,4-dinitro and related nitrogen mustards.[11-13] The second class of substrates includes 4-nitrobenzyloxycarbonyl derivatives



1 **2** **3** **4**

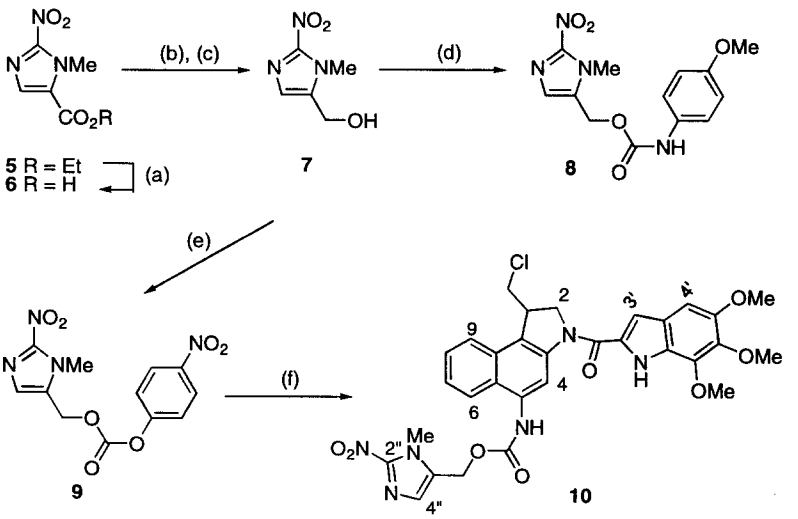
0960-894X/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(99)00381-9

(**2**) of a range of amine-bearing cytotoxins including actinomycin D,[14] mitomycin,[14] enediynes,[15] amino-*seco*-cyclopropylindoline derivatives,[16] and tallimustine analogues.[17] The protection of the amine moiety in the latter class of compounds results in masking of the active drug. Reduction of such nitrobenzyloxycarbamates (**2**) by NTR gives the corresponding 4-hydroxylamino derivative[18] in which increased electron release to the π-system stabilizes the developing positive charge on the benzylic carbon and thereby facilitates fragmentation to release an amine.



Scheme 1

In an effort to extend the range of substrates for NTR we examined the 2-nitroimidazole moiety as a possible substrate which may subsequently fragment and release a drug (Scheme 1). Deactivation of the active drug as a carbamate and fragmentation of the carbamate moiety following reduction, with subsequent release of the cytotoxin are central concepts of this prodrug approach.

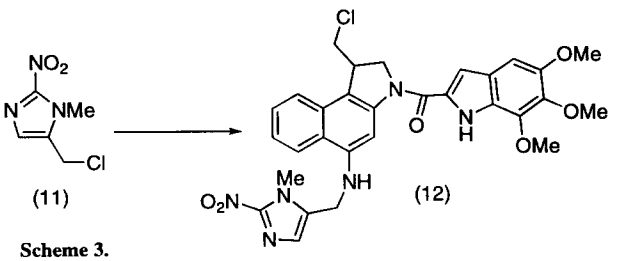


Scheme 2. *Reagents*: (a) NaOH, MeOH; (b) CDI, THF; (c) $NaBH_4$, MeOH; (d) 4-MeOPhNCO, $nBu_2Sn(OAc)_2$, DCM; (e) 4-NO_2PhOCOCl, pyridine; (f) HOBT, DIEA, **3**, 4 A sieves, DMF.

The 2-nitroimidazole unit has been the focus of extensive efforts to develop radiosensitizers and bioreductive drugs to combat regions of radioresistant hypoxic tissue in the radiotherapy of solid tumours,[19,20] and also for

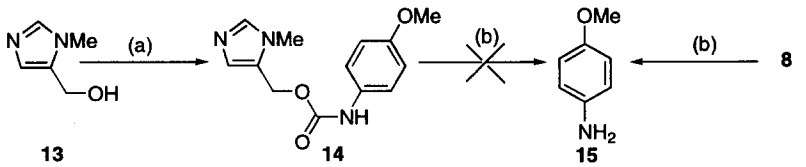
the imaging of tumour hypoxia.[21-23] Misonidazole (**4**) undergoes stepwise reduction to a reactive species, the first step being inhibitable by oxygen, thus providing the basis for hypoxic selectivity.[24] Fragmentation of 5-nitroimidazol-2-ylmethyl carbamates after nitro group reduction has been documented for ronidazole[25] and aniline mustard derivatives.[26,27] However, there has been no report of the bioreductive fragmentation of reduced 2-nitroimidazol-5-ylmethyl carbamates, although a recent report noted fragmentation of a 2-nitroimidazol-5-yl methyl ester of salicylic acid.[28] We have examined nitro-group reduction and carbamate fragmentation of a 2-nitroimidazol-5-ylmethyl carbamate model **8** using chemical, radiolytic, and enzymic methods to validate the concept. The deactivation of the potent minor groove binding alkylating agent amino-*seco*-CBI-TMI **3**[29] as the prodrug **10** and activation by extracellular NTR with NADH has been determined from the *in vitro* cytotoxicity of **3** and **10** against a human ovarian carcinoma cell line (SKOV3). This system is a model for the ADEPT approach. The cytotoxicity of **3** and **10** against a stably-transfected cell line expressing NTR (SC3.2) has been determined to gauge the usefulness of **10** for a GDEPT approach. The potential for **10** to be reduced, in an oxygen-inhibitable manner, by endogenous human one-electron reductases was examined using stirred suspension cultures of SKOV3 cells under aerobic and hypoxic conditions.

The key synthetic intermediate, 2-nitroimidazole-5-methanol (**7**), is readily prepared from the ester **5**[30] in high yield (Scheme 2). Thus, base hydrolysis of the **5** gives acid **6** cleanly. Formation of the imidazolide of **6** with CDI and subsequent reduction with $NaBH_4$[31] gives the alcohol **7** in excellent yield, 68% from the ester **5**. This represents a significant improvement on the $LiBH_4$ reduction[30] of **5** which in our hands proved to be low yielding and unreliable. Coupling of **7** with 4-methoxyphenylisocyanate in the presence of catalytic dibutyltin diacetate gave the carbamate **8** in 84% yield, while reaction with 4-nitrophenylchloroformate gave the carbonate **9** in 80% yield. Reaction of **9** with the amino-*seco*-CBI-TMI **3** was extremely slow due to the low nucleophilicity of **3**. Addition of HOBT[32] to the reaction mixture gave increased reaction with a maximum yield of **10**[33] of 33%. Attempts to form the carbamate **10** using the chloroformate of **7** gave varying and low yields, because of the instability of the intermediate chloroformate. Indeed, the amine **12**, formed by alkylation of **3** by the chloride **11**, was found in all reactions, despite the use of low temperatures and a variety of solvents and bases (Scheme 3).



Scheme 3.

Mild chemical reduction of the model substrate **8** with $NaBH_4$ in the presence of palladium[34] gave anisidine (**15**) in 83% yield, indicating fragmentation of the carbamate moiety following reduction to the amine (Scheme 4). Reaction of the imidazolyl carbamate **14**, prepared by reaction of imidazole-5-methanol[35] (**13**) and 4-

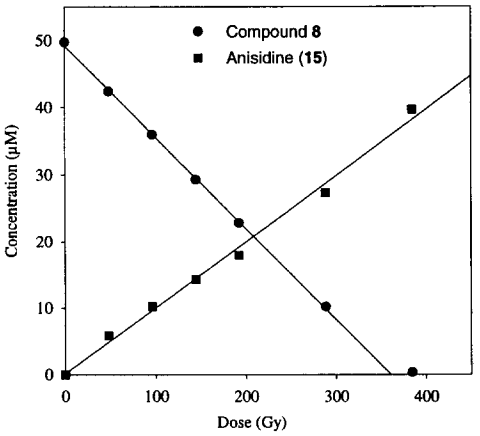


Scheme 4. *Reagents*: (a) 4-MeOPhNCO, toluene; (b) $NaBH_4$, Pd/C, MeOH.

methoxyphenylisocyanate, with $NaBH_4$/Pd in MeOH gave only starting material, confirming the stability of the carbamate linkage under these conditions.

Radiolytic reduction of **8** was carried out using a published procedure.[36] A deaerated aqueous solution of **8** (50 μM) was reduced radiolytically using doses corresponding to the addition of 1- 6 stoichiometric equivalents of reducing isopropanol β radicals and the compositions of the reduced solutions examined by HPLC. The loss of prodrug **8** and production of anisidine (**15**) are shown in Figure 1. Release of **15** mirrors the stoichiometric dependence of reduction, reaching a plateau with ca. four fold stoichiometry ($G_{(total\ reductants)}/G_{(loss\ of\ parent)}$ = 0.52 μmol.J^{-1}/ 0.14 μmol.J^{-1} = 3.7), implying the hydroxylamine intermediate undergoes fragmentation.[37]

Figure 1. Radiolytic reduction of **8** (50 μM) in 10 mM phosphate buffer pH 7.4 4% acetone 4% IPA after 10 min bubbling with nitrogen



The ability of NTR to activate the nitroimidazole moiety was examined by incubating aqueous solutions (20 μM) of **8** and misonidazole (**4**) with NADH (100 μM) and NTR (1.5 μg/mL). HPLC analysis of the solutions confirmed[11] that misonidazole was not a substrate for the NTR, whereas **8** was readily reduced with complete loss of the parent compound and a 45% yield of anisidine by 3 h.

The ability of the NTR to activate prodrug **10** was assayed by determining the cytotoxicity of **3** and **10** to the human ovarian carcinoma cell line SKOV3 using a published protocol.[11] Thus, cells were exposed for 18 h in 96 well plates under aerobic conditions to drug alone, drug and cofactor (1 mM NADH), or drug, cofactor and enzyme (1 μg/mL), and subsequent cell growth measured after 72 h. IC_{50} values were calculated in each case (Table 1). The ability of intracellular NTR to activate **10** was determined using a transfected SKOV3 cell line

SC3.2, exposed to drug for 18 h in 96 well plates under aerobic conditions. The hypoxia-selective cytotoxicity of **10** against the SKOV3 cell line was determined in aerobic and hypoxic stirred suspension cultures.[38] Cytotoxic potency (C_{10}) is quantitated as the product of drug concentration × time required to reduce survival to 10% (Figure 1).

Table 1. In vitro cytotoxicity of **3** and **10** against SKOV3 and SC3.2 cell lines.

Parameter	Cell line	O_2 (%)[a]	Time (h) [b]	Additions	**3**	**10**
IC_{50} [c] (nM)	SKOV3	20	18	-	1.10 ± 0.08[d]	75 ± 7
			18	1 mM NADH	0.96 ± 0.05	57 ± 6
			18	NADH + NTR[e]	1.14 ± 0.11	8.0 ± 1.7
	SC3.2	20	18	-	2.17 ± 0.20	3.5 ± 0.6
C_{10} (nM)	SKOV3	20	1	-	-	2100
			4	-	-	600
		<0.01	1	-	-	135
			4	-	-	15

[a]O_2 in gas phase. [b]Duration of drug exposure. [c]Concentration for 50% inhibition of cell proliferation. [d]Values are mean ± SEM for replicate experiments. [e]1 μg/mL.

The amino-*seco*-CBI-TMI drug **3** is a very potent cytotoxin with an IC_{50} of 1.1 nM against the SKOV3 cell line and 2.2 nM against the SC3.2 cell line. Masking the amino function provides significant (68-fold) deactivation of **3** in the SKOV3 line. Cofactor alone provides little activation of prodrug **10**, while the presence of extracellular NTR and cofactor provides an 11-fold activation of **10**. Intracellular NTR provides a 21-fold increase in cytotoxicity against the SC3.2 cell line. Prodrug **10** is 15- to 40-fold more cytotoxic under hypoxic conditions than under aerobic conditions, indicating **10** is also a substrate for one-electron reductases.

These data suggest that the 2-nitroimidazol-5-ylmethyl carbamates are a new class of NTR substrates with significant potential as prodrugs for use in conjunction with ADEPT and GDEPT strategies. Activity as hypoxia-selective cytotoxins may provide increased tumour selectivity and complement the directed enzyme prodrug strategies.

Acknowledgments: The authors thank Dr Maruta Boyd, Dianne Ferry, Li Fong Leong, and Susan Pullen, for technical assistance, Graham Atwell for providing amino-*seco*-CBI-TMI **3**, and Dr Martin Ford, Glaxo-Wellcome, Stevenage, U.K., for providing the SC3.2 cell line. This work was supported supported by the Marsden Fund of New Zealand (MPH), the U.K. Cancer Research Campaign (BMS), the Auckland Division of the Cancer Society of New Zealand (WAD) and the Health Research Council of New Zealand (WRW).

References and Notes.

1. Jungheim, L. N.; Shepherd, T. A. *Chem. Rev.* **1994**, *94*, 1553.
2. Niculescu-Duvaz, I.; Springer, C. J. *Curr. Med. Chem.* **1995**, *2*, 687.
3. Melton, R. G.; Sherwood, R. F.; *J. Natl Cancer Inst.* **1996**, *88*, 153.
4. Hay, M. P.; Denny, W. A. *Drugs Future* **1996**, *21*, 917.
5. Huber, B. E.; Richards, C. A.; Austin, E. A. *Adv. Drug Deliv. Rev.* **1995**, *17*, 279.
6. Niculescu-Duvaz, I.; Springer, C. J. *Exp. Op. Invest. Drugs*, **1997**, *6*, 685.
7. Denny, W.A. *Curr. Pharmaceut. Design* **1996**, *2*, 289.
8. Anlezark, G. M.; Melton, R. G.; Sherwood, R. F.; Coles, B.; Friedlos, F.; Knox, R. J. *Biochem. Pharmacol.* **1992**, *44*, 2289.

9. Knox, R. J.; Friedlos, F.; Boland, M. P. *Cancer Metastasis Rev.* **1993**, *12*, 195.
10. Knox, R. J.; Friedlos, F.; Sherwood, R. F.; Melton, R. G.; Anlezark, G.M. *Biochem. Pharmacol.* **1992**, *44*, 2297.
11. Anlezark, G. M.; Melton, R. G.; Sherwood, R. F.; Wilson, W. R.; Denny, W. A.; Palmer, B. D.; Friedlos, F.; Williams, A. *Biochem. Pharmacol.* **1995**, *50*, 609.
12. Atwell, G. J.; Boyd, M.; Palmer, B. D.; Anderson, R. F.; Pullen, S. M.; Wilson, W. R.; Denny, W. A. *Anti-Cancer Drug Design* **1996**, *11*, 553.
13. Friedlos, F.; Denny, W.A.; Palmer, B. P.; Springer, C. J. *J. Med. Chem.* **1997**, *40*, 1270.
14. Mauger, A. B.; Burke, P. J.; Somani, H. H.; Friedlos, F.; Knox, R. J. *J. Med. Chem.* **1994**, *37*, 3452.
15. Hay, M. P.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2829.
16. Tercel, M.; Denny, W.A.; Wilson, W.R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2741.
17. Lee, M.; Simpson, Jr, J. E.; Woo, S.; Kaenzig, C.; Anlezark, G. M.; Eno-Amooquaye, E.; Burke, P. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1065.
18. Hay, M. P. Sykes, B. M. O'Connor, C. J.; Denny, W. A. *J. Chem. Soc. Perkin Trans. 1*, submitted for publication.
19. Bremner, J. C. M. *Cancer Metastasis Rev.* **1993**, *12*, 177.
20. Lee, H. H.; Palmer, B. D.; Wilson, W. R.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1741.
21. Hodgkiss, R. J.; Jones, G.; Long, A.; Parrick, J.; Smith, K. A.; Stratford, M. R. L.; Wilson, G.D. *Brit. J. Cancer* **1991**, *63*, 119.
22. Evans, S. M.; Jenkins, W. T.; Joiner, B.; Lord, E. M.; Koch, C. J. *Cancer Res.* **1996**, *56*, 405.
23. Aboagye, E. O.; Maxwell, R. J.; Kelson, A. B.; Tracy, M.; Lewis, A. D.; Graham, M. A.; Horsman, M. R.; Griffiths, J. R.; Workman, P. *Cancer Res.* **1997**, *57*, 3314.
24. Wardman, P.; Clarke, E. D. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 1267.
25. Alvaro, R. F.; Wislocki, P. G.; Miwa, G. T.; Lu, A. Y. H. *Chem.-Biol. Interact.* **1992**, *82*, 21.
26. Sinhababu, A. K.; Thakker, D. R. *Adv. Drug Deliv. Rev.* **1996**, *19*, 241.
27. Prakash, S. R.; Correa, I. D.; Gan, L.-S.; Chism, J. P.; Arrendale, R. F.; Miwa, G. T.; Thakker, D. R. *Synth. Appl. Isotop. Labelled Compd, Proc. 5th Int. Symp., 1994*, Allen, J. Voges, R., Eds.; Wiley, Chicester, U.K. **1995**, 573.
28. Everett, S. A.; Naylor, M. A.; Patel, K. B.; Stratford, M. R. L.; Wardman, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1267.
29. Atwell, G. J.; Tercel, M.; Boyd, M.; Wilson, W. R.; Denny, W. A. *J. Org. Chem.* **1998**, *63*, 9414.
30. Cavalleri, B.; Ballota, R.; Lancini, G. C. *J. Het. Chem.* **1972**, *9*, 979.
31. Sharma, R.; Voynov, G.H.; Ovaska, T. V.; Marquez, V. E. *Synlett* **1995**, 839.
32. Dubrowchik, G.M.; King, H. D.; Pham-Kaplita, K. *Tetrahedron Lett.* **1997**, *38*, 5261.
33. Data for compound **10**: foam, mp 180- 185 °C; IR (KBr) ν 3234, 1730, 1616, 1527, 1453, 1312 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.49 (br s, 1 H, NH), 8.81 (br s, 1 H, OCONH), 7.85 (d, J = 8.5 Hz, 1 H, H 6), 7.78 (d, J = 8.3 Hz, 1 H, H 9), 7.57 (m, 1 H, H 8), 7.43 (m, 1 H, H 7), 7.25 (s, 1 H, H 4"), 7.21 (br s, 1 H, H 4), 7.00 (d, J = 1.6 Hz, 1 H, H 3'), 6.87 (s, 1 H, H 4'), 5.31 (d, J = 13.6 Hz, 1 H, CH_2O), 5.25 (d, J = 13.6 Hz, 1 H, CH_2O), 4.80 (dd, J = 10.7, 1.6 Hz, 1 H, H 2), 4.65 (dd, J = 10.5, 8.7 Hz, 1 H, H 2), 4.13- 4.20 (m, 1 H, H 1), 4.11 (s, 3 H, OCH_3), 4.01 (s, 3 H, NCH_3), 3.94- 3.98 (m, 4 H, CH_2Cl, OCH_3), 3.92 (s, 3 H, OCH_3), 3.47 (dd, J = 10.8, 8.7 Hz, 1 H, CH_2Cl); ^{13}C NMR δ 160.4 (CO), 153.5 (OCONH), 150.2 (C 5'), 146.4 (C 2"), 141.6 (C 3a), 140.7 (C 6'), 138.9 (C 7'), 133.3 (C 5), 132.1 (C 5"), 129.8 (C 4"), 129.7 (C 9a), 19.5 (C 2'), 127.6 (C 8), 125.7 (C 4 and C 7a'), 125.1 (C 7 and C 5a'), 123.6 (C 3a'), 123.2 (C 9), 122.3 (C 6 and C 9 b), 106.6 (C 3'), 97.6 (C 4'), 61.5 (OCH_3), 61.2 (OCH_3), 56.3 (OCH_3), 55.8 (CH_2O), 54.9 (C 2), 45.8 (CH_2Cl), 43.4 (C 1), 34.3 (NCH_3). NMR assignments were determined on the basis of 2D COSY, HSQC and HMBC experiments. FABMS *m/z* 651 ($M^{37}ClH^+$, 1%), 651 ($M^{35}ClH^+$, 2%); HRFABMS calcd for $C_{31}H_{30}{}^{35}ClN_6O_8$ (MH^+) *m/z* 649.1814, found 649.1767; calcd for $C_{31}H_{30}{}^{37}ClN_6O_8$ (MH^+) *m/z* 651.1784, found 651.1819.
34. Berry, J. M.; Watson, C. Y.; Whish, W. J. D.; Threadgill, M. D. *J. Chem. Soc. Perkin Trans. 1* **1997**, 1147.
35. Dener, J. M.; Zhang, L.-H.; Rapoport, H. *J. Org. Chem.* **1993**, *58*, 1159.
36. Sykes, B. M.; Atwell, G. J.; Denny, W. A.; O'Connor, C. J. *J. Phys. Org. Chem.* **1995**, *8*, 587.
37. Wardman, P; Anderson, R. F.; Clarke, E. D.; Jones, N. R.; Minchinton, A. I.; Patel, K. B.; Stratford, M. R. L.; Watts, M. E. *Int. J. Radiat. Oncology Biol. Phys.* **1982**, *8*, 777.
38. Whillans, D. W.; Rauth, A. M. *Radiat. Res.* **1980**, *84*, 97.