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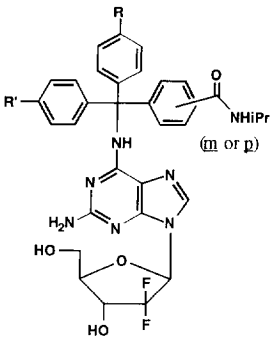
TRITYLATED ONCOLYTICS AS PRODRUGS

Vinod F. Patel*, Julie N. Hardin, Gerald B. Grindey and Richard M. Schultz
Lilly Research Laboratories, Eli Lilly & Company,
Indianapolis, Indiana 46285

Abstract: A series of tritylated difluorodeoxynucleosides represent novel prodrugs in which there exists a predictable correlation between the electronic nature of the aromatic substituents on the trityl moiety and the acid mediated dissociation to free drug in *ex vivo, in vitro and in vivo* systems.

The application of antineoplastic drugs in cancer therapy is often restricted by the non-selective toxicity of these agents to normal cells.[1] Many strategies, such as drug targeting using tumor selective monoclonal antibodies,[2] have been invoked to address this fundamental problem. Improving biological properties of drugs by chemically transforming them to prodrugs is a well established strategy.[3] In essence, the parent drug is produced either spontaneously or enzymatically from the prodrug at the target site. In cancer, the increased acidity of the tumor and its environment[4] relative to normal tissue presents an opportunity to design acid labile prodrugs which are selectively converted to drug at the tumor.



(1)

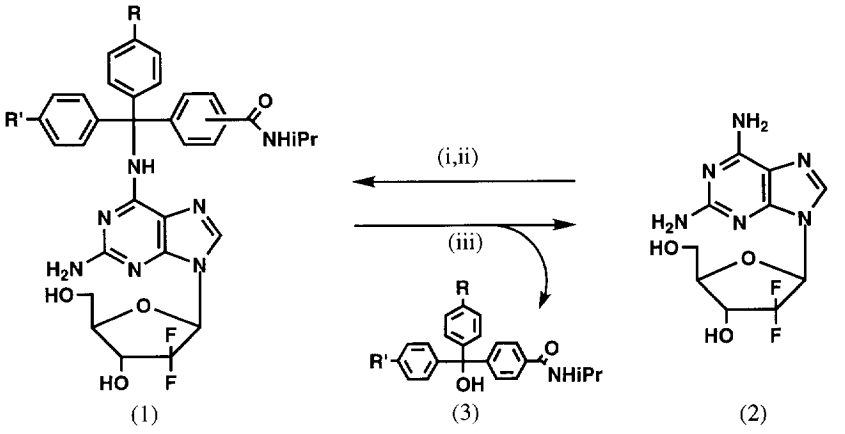
Table 1: N6-Trityl-207702-iPA

(1)		R	R'
(a)	mDMT	OMe	OMe
(b)	pDMT	OMe	OMe
(c)	pMMeT	Me	OMe
(d)	pMMT	H	OMe
(e)	pMeT	Me	H
(f)	pT	H	H

LY207702 (2) is a member of the **deoxydifluororibofuranosyl purines** (DFP), a novel class of nucleoside antimetabolites, which exhibits excellent *in vivo* antitumor activity in a panel of human tumor xenograft models.[5] However, evaluation of nucleoside (2) in preclinical toxicology studies revealed a drug related cardiotoxicity.[5] This communication describes the modification of DFP(2) into an acid-labile prodrug in an attempt to enhance the concentration of drug at the tumor site and increase its therapeutic index. To achieve site selective delivery of drug to the tumor cells, it is necessary to develop a prodrug which remains intact under physiological conditions (37°C, pH7.4) but dissociates under the acidic conditions provided by the tumor. Consequently, the central feature in the design of the prodrug focused on the ability to control the rate of dissociation of drug from the prodrug.

The trityl moiety has long served as a useful acid labile protecting group in organic chemistry. It is well known that the aromatic substituents on a trityl moiety govern its stability through the electronic resonance stabilization of the intermediate trityl carbocation. We believed that the three aromatic rings would provide the possibility for a diversity of substitution and thereby allow a high degree of control in the rate of dissociation. Thus, derivatization of drugs with the trityl group should lead to prodrugs with defined and predictable release characteristics. Accordingly, a series of N6-Trityl-207702 amides (1a,b,d,f) was initally prepared[6] by reacting the corresponding trityl chloride (TrCl) with LY207702 (2), according to the previously described procedure

Scheme1



(i) TrCl, Ref.7 (ii) $iPrNH_2$, CH_2Cl_2, r.t. (iii) ~6mM in 0.1M KH_2PO_4-D_2O Buffer / d_4-MeOD, 37°C

Graph 1: Dissociation of N6-Trityl -207702-iPA @ pH5.4/37°C

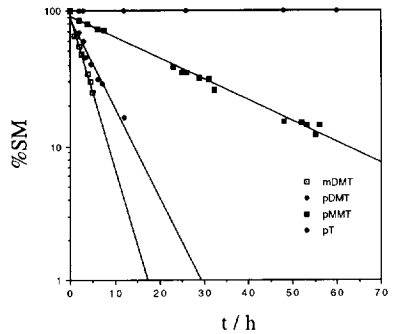


Table 2: $t_{1/2}$ (h) for acid mediated dissociation of N6-Trityl-207702-iPA prodrugs

pH	mDMT	pDMT	pMMT	pT
5.4	2.35	3.71	16.7	#
6.4	23.3	14.8	547	#
7.4	133.3*	146.3	800*	#

* Extrapolated values
\# No dissociation up to day 33

(Table 1).[7] Stability studies were performed on prodrugs (1a,b,d,f) to determine relative release rates of LY207702 (2) under varying pH conditions at 37°C (Scheme 1). Proton NMR spectra of both prodrug (1) and free drug (2) showed an unhindered singlet proton resonance for H8 at δ 7.95 and 7.83 ppm, respectively, which conveniently allowed the two species to be readily quantified by integration.Thus, when a ~6mM solution of amide (1a,b,d,f) in deuteromethanolic buffer adjusted to pH 5.4, 6.4 and 7.4 was heated at 37°C in an NMR tube, prodrug (1) disappeared with concomitant formation of free drug (2) and corresponding trityl alcohol (3) as observed by NMR spectroscopy and confimed by tlc analysis. A logarithmic plot of % starting material (SM) (1) versus time (t) gave linear relationships, examples of which are shown in Graph 1 for prodrug (1d) at pH 5.40, indicating that the dissociation of prodrugs (1a,b,d,f) exhibit pseudo-first-order kinetics. Subsequently, the half-lives ($t_{1/2}$) of prodrugs (1) at pH 5.4, 6.4 and 7.4 were determined from the graphs, the results of which are summarized in Table 2. The study indicates that the rate of release of free drug (2) from the prodrug (1) can be ranked in the order (a) for trityl group: m**Di**M**e**thoxy**T**rityl = p**Di**M**e**thoxy**T**rityl> p**M**ono**M**ethoxy **T**rityl >> p**T**rityl and (b) at pH: 5.4 >6.4 >7.4. This data confirms the correlation between the degree of electronic (i.e.

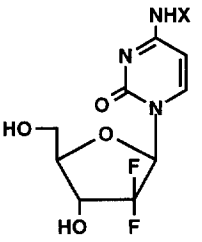
Table 3: Cytotoxicity of N6-Trityl-207702-iPA Prodrugs in a CCRF-CEM assay

N6-Trityl-207702-iPA		IC_{50} (ug/ml)
(2)	-	0.024
(1a)	mDMT	0.084
(1b)	pDMT	0.078
(1c)	pMMeT	0.1
(1d)	pMMT	0.1
(1e)	pMeT	0.8
(1f)	pT	1.7

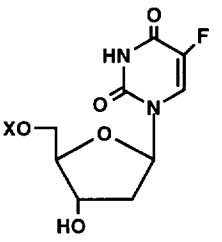
electron donating) stabilization of the tritylcation by substituents R and R' and the rate of dissociation. The predictive value of the correlation was tested in a biological system by preparing two further analogues, p**M**ethoxy**M**ethyl**T**rityl (1c) and p**M**ethyl**T**rityl (1e)[8] and then evaluating the rate of release of free drug (2) from prodrugs (1a-f) as antitumor activity in an *in vitro* cytotoxicity assay.[9] Indeed, the cytotoxicity data showed that the order of potency of the prodrugs (1a-f), pDMT~mDMT>pMMeT=pMMT>>pMeT>>pT, is consistent with the electronic arguments.

In preliminary *in vivo* studies against the HC-1 human colon xenograft,[10] prodrugs (1b-f) were administered intraperitoneally (ip) to C3H nude mice on days 1,4,7 and 10, after a 14d delay from implanting the tumor subcutaneously in the axillary region by trocar. Tumor growth inhibition was then determined following therapy.[11] The first measurements, the day after the last dose, showed that maximal inhibition (>95%) of tumor growth was achieved at all doses by prodrugs (1b,c,d), however, later measurements (weekly) displayed both a dose and trityl group dependant regrowth of tumor (data not shown). It was found that the rate of tumor regrowth in the prodrugs (1) was (1b)<(1c)<(1d). In the case of prodrug (1e), a dose dependent inhibition of tumor growth was observed on the first measurement with an ED_{50} of 30mg/kg, while prodrug (1f) was inactive. These antitumor data suggest that trityl prodrugs (1b-f) possess a relative ranking in activity of pDMT>pMMeT> pMMT>> pMeT>>> pT under *in vivo* conditions. These results are consistent with the findings in both the stability studies and the *in vitro* determinations. In similar study, when parent drug (2) was dosed at 20, 37.5, 50, and 75mg/kg on days 1,3,5,7 and 9, complete (>95%) tumor inhibition resulted, however more frequent dosing (daily x 10) at 30mg/kg led to drug related toxicity. Since no toxicity was found in the initial studies described with the prodrugs (1), further investigations will be necessary to determine the maximum tolerated dose and the therapeutic index.

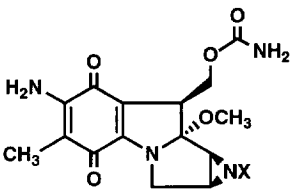
Figure 1



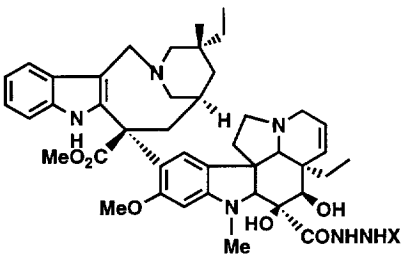
(4)



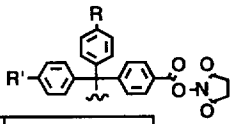
(5)



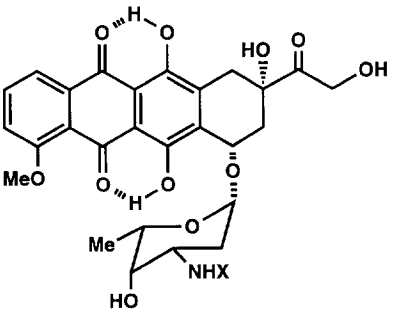
(6)



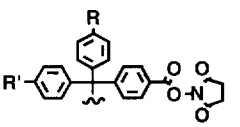
(7)



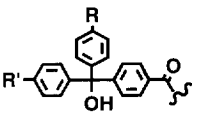
	X		X
(4a)	H	(4b)	MMT
(5a)	H	(5b)	MMT
(6a)	H	(6b)	MMT
(7a)	H	(7b)	MeT



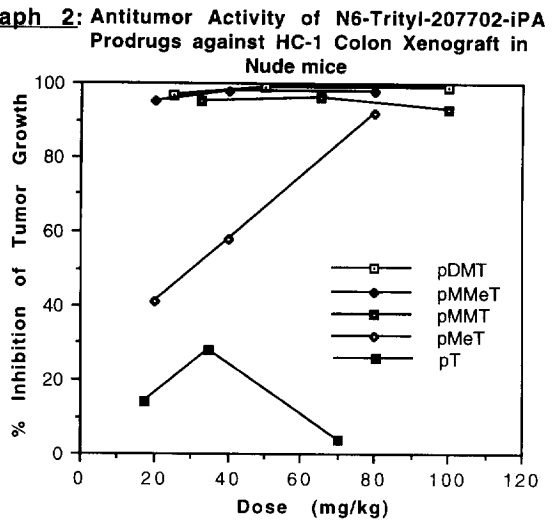
(8) (8a) X=H



	X
(8b)	DMT
(8c)	MMT
(8d)	T



	X
(8e)	DMT
(8d)	MMT
(8f)	T



The general application of converting known oncolytics into trityl prodrugs may prove of wider use in the treatment of a variety of solid tumors. Accordingly, tritylation, under standard conditions, of several unprotected, structurally diverse oncolytics was performed. In this manner, nucleoside (4a) was regioselectively tritylated at N4 to give prodrug precursor (4b) as a white solid in 60% yield. Furthermore, 5FdUr (5a) was efficiently converted to the 5'O derivative (5b) and Mitomycin C (6a) to (6b).[12] Alkylation of the more complex vinca acyl hydrazide (7a) using MeTCl gave (7b), albeit in a low yield. Limitations in the synthesis were realized when doxorubicin (8a) was reacted with an equivalent of trityl chloride reagent to give a mixture of the desired alkylated product (8b,c,d) and acylated product (8e,f,g,). These facile chemical transformations give access to N-hydroxy esters (4b-7b, 8a-d) which are versatile precursors for introducing functional groups to obtain desired physiochemical properties (e.g. water solubility) without altering the release characteristics of the trityl group. Evaluation of the release characteristics and biological properties of oncoloytic prodrugs (4-8) has been initiated.

In summary, tritylated LY207702 (1) serve as prodrugs in which substituents R and R' on the aromatic rings modulate, in a predictable fashion, the relative rate of release of parent drug (2) and the antitumor activity of the prodrug.

Acknowledgment: The authors are grateful for the technical assistance provided by S.Andis, P. Rutherford, T. D. Dudley, T. Self, J. Bewley, P. Seymour, and D. Marlow.

References and Notes:

1. For example 5-FdUr (intestinal toxicity-diarrhea): Tominaga T., Kitamura M., Hayashi K., Takahashi I., and Kosaki G., *Jpn. J. Cancer Chemother.*, **1985**,*12*, 2172-2178; Doxorubicin (cardiac toxicity): Olson R.D. and Mushlin P.S., *The FASEB Journal*, **1990**,*4*, 3076.
2. (a) Baldwin R.W., Monoclonal Antibodies Targeting of Anticancer Agents:Muhlbock Memorial Lecture *Eur.J.Cancer Clin. Oncol.*, **1985**, *21(11)*,1281-1285 (b) see preceding communication.
3. (a) Bundgaard H. (ed.), *Design of Prodrugs*, **1985**, Elsevier Science, Amsterdam (b) Roche E.B. (ed.), *Design of Biopharmaceutical Properties through Prodrugs and Analogues*, 1977, American Pharmaceutical Asscociation, Washington (c) Stella V.J., Mikkelson T.J., Pipkin J.D., *Drug Delivery Systems*, Juliano R.L. (ed.), **1980**, Oxford University Press, Oxford, p112 (d) Stella V.J., Charman W.N.A., Naringrekar V.H.,*Drugs*, **1985**, *29*,455 (e) Ninomiya Y., Miwa M., Hiroyuki E., et.al.,*Jpn. J. Cancer Res.*, **1990**, *81*,188-195 (f)Saari W.S., Schwering J.E., Lyle P.A. et.al. ,*J.Med. Chem.*, **1990**, *33(9)*, 2590-2595.
4. Lavie, E.; Hirschberg, D.L.; Screiber, G.; Thor, G.; Hill, L.; Hellstrom, I.; Hellstrom, K-E. *Cancer Immunol. Immunother.*, **1991**, *33*, 223-230.

5. Andis, S.L.; Bewley J.R.; Boder G.B.; et al, Medicinal Chemistry of Difluoropurines,*Seminars in Oncology,* in press.
6. All compounds were fully characterized by spectroscopic methods.
^{1}H NMR data:

(1a): (d6-acetone) δ 8.03 (s,ArH), 7.83 (s,H8), 7.65 (d,J8,ArH), 7.52 (d,J8,ArH), 7.48 (d,J9,ArH), 7.26 (d,J8,4ArH), 6.80 (d,J8,4ArH), 6.35 (s,NH), 6.20 (brs,NH2), 5.76-5.71 (m,H1'), 5.55 (brs, 3'OH), 4.82 (brs,5'OH), 4.62-4.57 (m,H3'),4.20-4.12 (m,CH), 3.93-3.74 (m,3H,H4'5'5'), 3.74 (s,6H,2OMe), 1.14 (J6.6,6H,2Me) ppm

(1b): ($CDCl_3$/MeOD) δ 7.83 (s,H8), 7.62 (d,J8,2ArH), 7.40 (d,J8,2ArH), 7.19 (d,J8,4ArH), 6.74 (d,J8,4ArH), 5.53- 5.45 (m,H1'), 4.65-4.55 (m,2H,CH,H3'), 4.35-4.15 (m,3H,H4'5'5'), 3.74 (s,6H,2OMe),1.19 (d,J7,6H,2Me) ppm

(1d): (d6-acetone) δ 7.80 (s,H8),7.72 (d,J6,2ArH), 7.46 (d,J6,2ArH),7.42-7.36 (m,2ArH), 7.32-7.14 (m,5ArH), 6.82 (d,J8,2ArH), 6.28 (s,NH), 6.10 (brs, NH2), 5.80-5.65 (m,H1'), 5.42 (d,J6,OH), 4.72-4.50 (m,2H,OH,H3'), 4.28- 4.10 (m,CH), 3.86-3.70 (m,3H,H4'5'5'), 3.76 (s,OMe), 1.21 (d,J6,6H,2Me) ppm

(1f): (d6-acetone) δ 7.80 (s,H8), 7.73 (d,J8,2ArH), 7.48 (d,J8,2ArH), 7.42-7.37 (m,4ArH), 7.32-7.15 (m,6ArH), 6.36 (s,NH), 6.12 (brs,NH2), 5.78-5.64 (m,H1'), 5.43 (d,J8,OH), 4.74-4.52 (m,2H,OH,H3'),4.26-4.16 (m,CH), 3.96-3.72 (m,3H,H4'5'5'), 1.20 (d,J6,6H,2Me) ppm.
7. Patel V.F., Hardin J.N., Starling J.J. and Mastro J.M., *Bioorg. Med. Chem. Lett.*, preceding communication.
8. ^{1}H NMR data:

(1c):(d4-MeOD)δ 7.95 (s,H8), 7.67 (d,J8,2ArH), 7.45 (d,J8,2ArH), 7.22 (t,J8,4ArH), 7.07 (d,J6,2ArH), 6.79 (dd,J2and8,2ArH), 5.60-5.47 (m,H1'), 4.40-4.27 (m,H3'), 4.23-4.10 (m,CH), 3.90-3.70 ppm.

(1d): (d4-MeOD) δ 7.88 (s,H8), 7.67 (d,J8,2ArH), 7.46 (d,J10,2ArH), 7.34 (d,J8,2ArH), 7.26-7.12 (m,5ArH), 7.05 (d, J7,2ArH), 5.60-5.46 (m,H1'), 4.41-4.24 (m,H3'), 4.23-4.10 (m,CH), 3.92-3.64 (m,3H,H4'5'5'), 2.28 (s,Me), 1.22 (d,J6,2Me) ppm.
9. CCRF-CEM, a human leukemia cell line, was incubated with test compound in 0.5% final volume DMSO for 72h at 37°C and then cell numbers counted.The concentration of test compound that inhibited 50% of cell growth was determined (IC_{50}).
10. On day 11 (where on day 1 the 1st dose was given) two-dimensional tumor measurements (width and length) were taken and converted to tumor weight using the following formula:
Tumor Weight (mg) = Tumor Length (mm) x Tumor Width (mm)^2/ 2
11. Houghton J.A. and Taylor D.M., *Br. J. Cancer* ,**1978**, *37*, 199.
12. Representive 1H NMR data:

(4b): (d6-DMSO) δ 8.07 (d,J9,2ArH), 7.72 (d,J9,2ArH), 7.59 (d,J8,H6), 7.44-7.11 (m,7ArH), 6.95 (dd,J8 and 1,2ArH), 6.30 (brd,J6,NH_{ex}),6.20 (t,J8,H1'), 5.70 (dd,J8 and 1,H5), 4.37-4.17 (m,H3'), 4.05-3.97 (m,H4'), 3.76 (s,OMe), 3.45-3.26 (m,H5'5'), 2.88 (s,4H,2CH2) ppm.

(5b): (d6-DMSO) δ 11.86 (d,J6,NH), 8.05 (d,J9,2ArH), 7.89 (d,J6,H6), 7.71 (d,J8,2ArH), 7.45-7.11 (m,7ArH), 6.94 (d,J8,2ArH), 6.16 (t,J6,H1'), 5.33 (d,J6,3'OH), 4.30-4.20 (m, H3'), 3.93-3.87 (m, H4'), 3.76 (s, OMe), 3.34-3.23 (m,H5'), 3.20-3.13 (m, H5'), 2.88 (s,4H,2CH2), 2.30-2.20 (m,H2'), 2.20-2.08 (m,H2') ppm.

(6b): (d6-DMSO) δ 7.91 (d,J10,ArH), 7.86 (d,J10,ArH), 7.40-7.15 (m,10H,NH2,8ArH), 6.84 (d,J8, 2ArH), 6.41 (brs,2H,OCONH2), 4.86 (d,J10,H10b), 4.51 (brd,J10,H10a), 3.79 (dd,J12 and 2,H3b), 3.77 (s,OMe), 3.73 (s,OMe), 3.63 (dd,J12 and 2,H9), 3.40-3.30 (m, H3a),3.06 (s,OMe), 2.88 (s,4H,2CH2), 2.30 (d,J4,H1), 1.91 (d,J4,H2), 1.80 (s, Me) ppm.

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