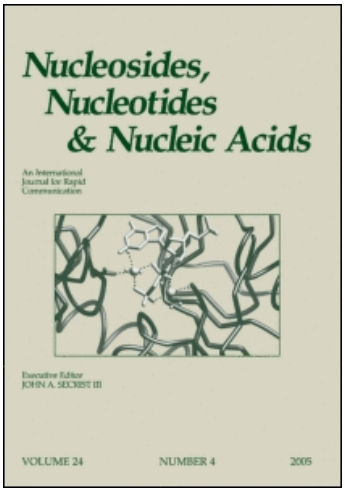

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Adema, A. D. , Radi, M. , Daft, J. , Narayanasamy, J. , Hoebe, E. K. , Alexander, L. E. , Chu, C. K. and Peters, G. J.(2007) 'Troxacitabine Prodrugs for Pancreatic Cancer', Nucleosides, Nucleotides and Nucleic Acids, 26: 8, 1073 — 1077
To link to this Article: DOI: 10.1080/15257770701515591
URL: http://dx.doi.org/10.1080/15257770701515591

Nucleosides, Nucleotides, and Nucleic Acids, 26:1073–1077, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701515591

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TROXACITABINE PRODRUGS FOR PANCREATIC CANCER

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□ *Troxacitabine is a cytotoxic deoxycytidine analogue with an unnatural L-configuration, which is activated by deoxycytidine kinase (dCK). The configuration is responsible for differences in the uptake and metabolism of troxacitabine compared to other deoxynucleoside analogues. The main drawback in the use of most nucleoside anticancer agents originates from their hydrophilic nature, which property requires a high and frequent dosage for an intravenous administration. To overcome this problem several troxacitabine prodrugs modified in the aminogroup with a linear aliphatic chain with a higher lipophilicity were developed. To determine whether these prodrugs have an advantage over Troxacitabine pancreatic cancer cell lines were exposed to Troxacitabine and the lipophilic prodrugs. The addition of linear aliphatic chains to troxacitabine increased sensitivity of pancreatic cancer cell lines to the drug > 100-fold, possibly due to a better uptake and retention of the drug.*

Keywords Troxacitabine; lipophilic prodrugs; pancreatic cancer

INTRODUCTION

Troxacitabine is a cytotoxic deoxycytidine analogue with an unnatural L-configuration (Figure 1). This configuration is responsible for differences in the uptake and metabolism of troxacitabine compared to other deoxynucleoside analogs.[1–3] In contrast to other deoxynucleoside analogues the influx of troxacitabine into the cell might not be mediated by the human

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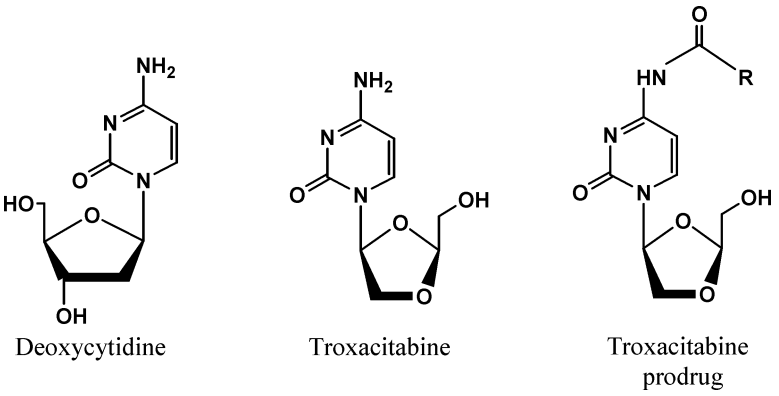


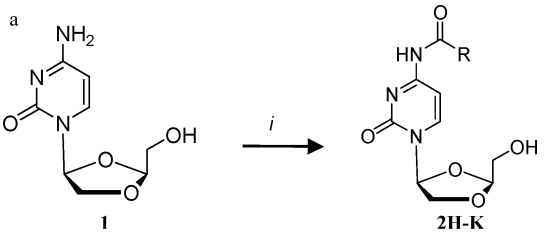
FIGURE 1 Structures of deoxycytidine, troxacitabine and the general structure of the troxacitabine analogs.

equilibrative nucleoside transporter (hENT) and human concentrative nucleoside transporter (hCNT) and might (partially) enter the cell by passive diffusion.[4] Because of the stereospecificity of cytidine deaminase (CDA), troxacitabine cannot be inactivated by deamination.[1] Like gemcitabine and cytarabine, troxacitabine needs to be phosphorylated to its monophosphorylated form by deoxycytidine kinase (dCK) thereby making this the rate-limiting step in the intracellular activation of troxacitabine.[1] Due to the lack of the hydroxyl group in the sugar ring, incorporation of troxacitabine into DNA leads to an immediate chain termination.[5] Damage introduced by troxacitabine is repaired by apurinic/apyrimidinic endonuclease (APE1).[6,7] Gemcitabine is used in the treatment of pancreatic and cancer troxacitabine was found to be more effective then gemcitabine in mouse xenograft models.[8] Troxacitabine also showed promising results in clinical trials as a single agent in a Phase II trial.[9] While troxacitabine showed relatively longer intracellular retention and low systemic clearance, pharmacokinetic studies indicated that it slowly accumulated in cancer cells in comparison to other carrier-transported nucleosides.[4] Troxacitabine, like most other anticancer nucleosides, is a hydrophillic agent and must be administered intravenously in a frequent dosage schedule, which may result in greater toxicity than the single dose schedule.[10] To overcome the problems related to the hydrophilicity of troxacitabine several lipophilic prodrugs of troxacitabine were developed.

MATERIALS AND METHODS

A small library of troxacitabine prodrugs was synthesised using a straightforward parallel solution-phase approach. Troxacitabine 1, synthesized starting from L-gulose according to a well-known procedure,[11] was dissolved

a



b

Analog	R	Yield (%)	LogP
Trox	–	–	-0.66
H	$(CH_2)_7CH_3$	72	2.43
I	$(CH_2)_8CH_3$	66	2.85
J	$(CH_2)_{10}CH_3$	54	3.68
K	$(CH_2)_{14}CH_3$	23	5.35

FIGURE 2 a) Synthesis of troxacitabine prodrugs. Reagents: i. $(RCO)_2O$, MeOH, 55°C, 6 hours. b) Structure of the aliphatic side chains attached to troxacitabine and the lipophilicity of the compounds (LogP), LogP values were estimated using Chemdraw 8.0 ultra.

in anhydrous methanol and treated, in an Argonaut Quest 210 organic synthesizer with different acid anhydrides. After 6 hours at 55°C, the reaction mixtures were simply filtered, and then purified on a small silica flash column with gradient elution (hexanes : ethyl acetate), from which, troxacitabine prodrugs **2H-K** were obtained in a white solid state (Figure 2).

Sensitivity to four prodrugs with linear aliphatic chains of different length was determined by the SRB cytotoxicity assay,[12] and the IC_{50} value of the drug was determined in the different cell lines by interpolating the growth inhibition curves. The tests were performed on the BxPC-3 and Panc-02 pancreatic cancer cell lines.

RESULTS AND DISCUSSION

Increasing the lipophilicity of troxacitabine by adding an aliphatic chain significantly enhances the sensitivity of pancreatic cancer cell lines to the drugs. The troxacitabine analogs **I**, **J**, and **K** showed the greatest modulation compared to troxacitabine, in BxPC-3 analog **J** enhanced the sensitivity 160 fold and in Panc-02 analog I enhanced the sensitivity 1,400-fold (Figure 3). It seems that increasing the lipophilicity decreases the IC_{50} to an optimum level at about $(CH_2)_8$, further increasing the lipophilicity does not seem to have a positive effect on the sensitivity in these cell lines. The effect of increased lipophilicity might be explained by increased influx further bypassing the nucleoside transporters or by an increased retention of the prodrugs

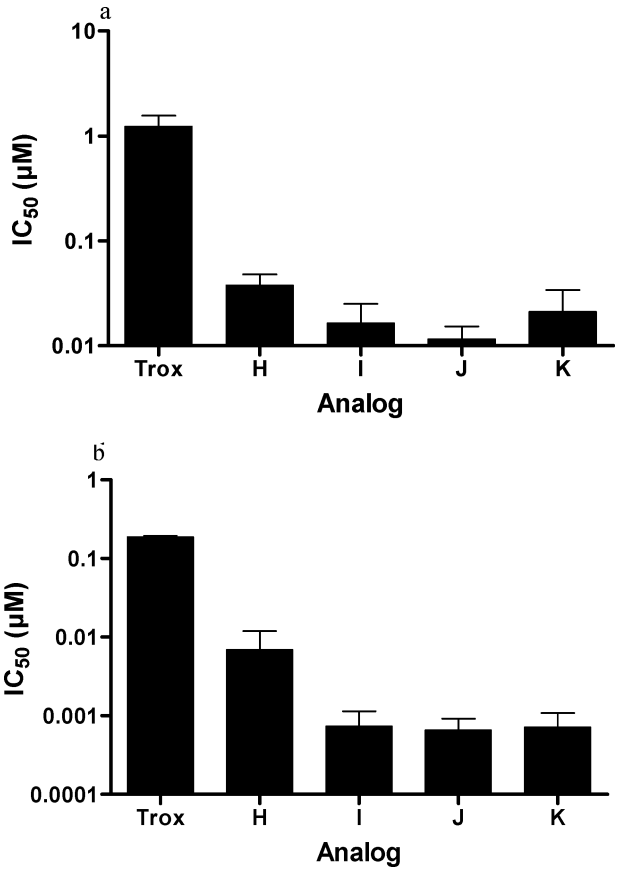


FIGURE 3 Sensitivity of the [a]BxPC-3 and [b]Panc-02 pancreatic cancer cell lines to troxacitabine and the lipophilic analogs **H**, **I**, **J**, and **K**.

in the cells and in non-small cell lung cancer (NSCLC) cell lines,[13] shorter side chains (<6) also showed a lower activity in NSCLC than H,[13] and were therefore not tested in these pancreatic cancer cell lines. Prodrugs of Ara-C also containing an aliphatic side chain showed an increased activity in leukemic cell lines resistant to Ara-C. The aliphatic side chain length and to a lesser extent the amount of double bonds determined the activity of the compound, the compound with the shortest side chain (chain length: 16) and one double bond showed the best activity.[14] Another lipophilic prodrug of Ara-C, NOAC, containing a C_{18} aliphatic side chain, also showed increased activity in a xenograft model against leukemias and solid tumors.[15] It has been shown that the speed of desorption from the membrane is related to the chain length of the fatty acid.[16] This might explain why there seems to be an optimal lipophilicity after which no further enhancement of the drugs was observed. The exact mechanism of entry and retention in the cell of compounds containing aliphatic side chains should be investigated further.

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