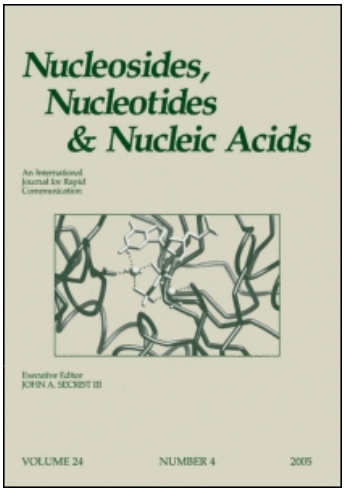

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An In Situ Pig Liver Esterase Assay as a Useful Predictive Tool for the Likely In Vitro Anti Viral Activity of Phosphoramidate Pro-Drugs

Carlo Ballatore[a]; Christopher McGuigan[a]; Erik De Clercq[b]; Jan Balzarini[b]
[a] Welsh School of Pharmacy, Cardiff University, Cardiff, UK [b] Rega Institute for Medical Research, Leuven, Belgium

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AN *IN SITU* PIG LIVER ESTERASE ASSAY AS A USEFUL PREDICTIVE TOOL FOR THE LIKELY *IN VITRO* ANTI VIRAL ACTIVITY OF PHOSPHORAMIDATE PRO-DRUGS.

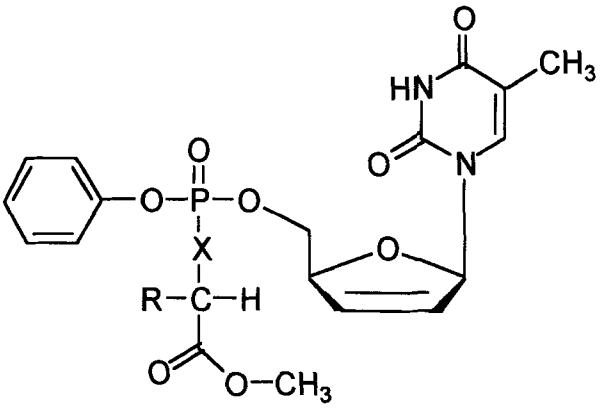
Carlo Ballatore,[1]* Christopher McGuigan,[1] Erik De Clercq,[2] and Jan Balzarini.[2]

1. Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF1 3XF, UK. 2. Rega Institute for Medical Research, Minderbroederstraat 10, Leuven B-3000, BELGIUM.

ABSTRACT: The pig liver esterase (PLE) assay has been designed to «reproduce» *in vitro* the first step of the metabolism of phosphoramidate pro-drugs that generates the free amino acyl phosphoramidate intermediate which has been described as a key metabolite *necessary* but not *sufficient* for the biological activity. The method could be used as a predictive tool for the likely *in vitro* biological activity as well as for Structure - Activity Relationship establishment (SAR).

Among the different masked nucleoside monophosphates as pro-drugs for anti viral nucleosides, the phosphoramidates have been extensively studied and some of these have shown higher anti viral potency and lower cytotoxicity when compared to the parent nucleosides[1]. These are typified by the phenyl methoxy alaninyl phosphoramidate of d4T (So324)[2] (**FIG. 1**). The mechanism of action consists in the delivery of the nucleoside monophosphate (NMP) directly into the cell avoiding the dependence on thymidine kinase - mediated first phosphorylation, which has been described to be the rate limiting step[2]. The metabolic pathways that lead to the drug's activation have been investigated and two possible enzymatic steps have been suggested[3]: the first is the carboxy esterase mediated hydrolysis of the carboxylic ester function; the second is the enzymatic cleavage of the P-N bond. The PLE assay has been designed to «reproduce» *in vitro* the first enzymatic step involved in the generation of the amino acyl phosphoramidate intermediate which has been described as a key intermediate[4]. The method should tell whether a certain phosphoramidate is a good substrate for the enzyme and whether it is able to generate the

FIG. 1



Cpd	X	R
So324	N	CH_3 (L)
1	N	CH_3 (D)
2	N	H
3	O	CH_3 (L)
4	O	H

TABLE 1[§]

Cpd	EC_{50} HIV-1 CEM Cell	EC_{50} HIV-2 CEM Cell	EC_{50} HIV-2 CEM TK-	CC_{50} CEM	PLE $t_{1/2}$ (h)
So324	0.075	0.075	0.075	>100	301
1	3	2	2.5	>250	*
2	6	6	7	>250	16.7***
3	40	50	>250	>250	33.44***
4	27.5	50	>250	>250	**

§ All data are expressed in μmol

* No reaction is detectable after 48 hrs.

** Compound **4** is processed by the enzyme however it does not generate the charged phosphate intermediate.

***Compounds **2** and **3**, despite their quick ezymatic-mediated hydrolysis, are yet far less active than the So324 and this could be due to a further step of the metabolism.

free amino acyl phosphoramidate intermediate[5]. As a result the assay could be useful as a predictive tool for the *in vitro* biological activity as well as for studies of Structure - Activity Relationship. An additional important feature of the PLE assay is its simplicity, in fact it can be run directly in a NMR tube and monitored by ^{31}P NMR. Typically 9μmol of substrate and 10 mg PLE (E.C.3.1.1.1 Sigma UK; activity 15 units/mg) are dissolved in 1 ml of buffer solution (0.5M Trizma buffer, pH 7.6) made up in D_2O. The mixture is then maintained at 37 °C for 75 hours and the reaction followed by ^{31}P NMR at regular intervals (every two hours). The kinetics can be followed plotting the - logarithm of the percentage of the remaining starting material (obtained by integration of the ^{31}P NMR spectra) against time. On the other hand, the main limit of the assay is that it reproduces the first step only of the metabolism of phosphoramidates and this is the reason why the production of the free amino acyl phosphoramidate intermediate, obtained *in vitro* via PLE, seems to be clearly *necessary* for the biological activity but not *sufficient* itself. In **TAB. 1**, the *in vitro* biological activity of compounds 1-4 and So324 are listed along with their half lives obtained with the pig liver esterase (PLE) assay.

REFERENCES

1. McGuigan, C.; Pathirana, R. N.; Balzarini, J.; De Clercq, E.; *J. Med. Chem.*, **1993**, 36, 8, 1048-1052
2. McGuigan, C.; Cahard, D.; Sheeka, H.M.; De Clercq, E.; Balzarini, J.; *Bioorg. Med. Chem. Lett.*, **1996**, 6, 1183-1186
3. Valette, G.; Pompon, A.; Girardet, J.; Cappellacci, L.; Franchetti, P.; Grifantini, M.; La Colla, P.; Loi, A.G.; Perigaud, C.; Gosselin, G.; Imbach, J-L.; *J. Med. Chem.*, **1996**, 39, 10, 1981-1990
4. Balzarini, J.; Karlsson, A.; Perno, C.F.; Cahard, D.; Naesens, L.; De Clercq, E.; McGuigan, C.; *Proc. Natl. Acad. Sci. USA*, **1996**, 93, 7295-7299
5. McGuigan, C.; Tsang, H.-W.; Sutton, P.; De Clercq, E.; Balzarini, J.; *Antiviral Chem. Chemother.*, **1998**, 9, 109-115