Biochemical Pharmacology, Vol. 37, No. 9, pp. 1849–1850, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

SELECTIVE MODIFICATION OF THYMIDINE BY DANSYL GROUP AND BIOLOGICAL EFFECTS OF THE DANSYL DERIVATIVES

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INTRODUCTION

The use of fluorescent nucleosides could represent a useful tool for studying some of the biological properties of enzymes involved in nucleic acid metabolism (1), in addition of being an alternative to radioactive labels.

We have been able to introduce selectively the dansyl (DNS: 1-dimethylaminonaphtalene-5-sulphonyl-) group (2) on the sugar moiety of thymidine (dT). The compounds obtained have been used as fluorescent competitive inhibitors of HSV-1 thymidine kinase.

MATERIALS AND METHODS

3'- and 5'-DNS-dT were synthesized by reacting thymidine with a 10% excess of dansyl chloride in pyridine, in the presence of dimethylaminopyridine as catalyst. After 48 hours, the reaction mixture was purified by preparative thin-layer chromatography (Silica Gel 60 F254; layer thickness: 2.0 mm; solvent system: chloroform/ethanol = 9/1). Two main yellow fluorescent spots, corresponding to the two dansylated thymidines, were separately collected. Characterization of the products was achieved by: i) UV spectroscopy of a 10^{-4} M solution in MeOH and DMSO/H_2O = 5/95 (Perkin Elmer Lambda 5); ii) fluorescence spectroscopy of a 10^{-5} M solution in MeOH and DMSO/H_2O = 5/95 (Perkin Elmer MPF 66); iii) ^1H NMR spectroscopy of a 10^{-3} M solution in DMSO-d6 (Bruker WP200SY). Viral thymidine kinase (TK) was obtained from L TK^- $HPRGT^-$ cells infected at a multiplicity of 20 PFU/cell. The enzyme was recovered from the high speed supernatant fraction of disrupted cells and assayed as reported before (3). Initial rate kinetics of TK were measured in the presence of different concentrations of thymidine and inhibitors. Ki values were derived directly from the Dixon plots.

RESULTS AND DISCUSSION

Our synthetic approach clearly indicate the possibility of obtaining a selective introduction of the dansyl group at the 3'- and 5'-position of thymidine. Spectral behaviours of the resulting compounds are in agreement with the presence of one DNS residue on the sugar moiety of the nucleoside. The UV spectra in MeOH are characterized by three peaks centered at 215 nm, 255 nm and 347 nm (dansyl absorption); in DMSO/H_2O the two major peaks are superimposed (250 nm, $\varepsilon \simeq 20.000$) with a slight blue shift for the dansyl peak (336 nm). The fluorescence emission spectra (excitation at 345 nm) (Fig. 1) revealed a maximum at 550 nm in MeOH and at 585 nm in DMSO/H_2O, with quantum yield lower in more polar solvent. ^1H NMR spectra in DMSO-d6 are characterized by chemical shifts of OH, H-3'

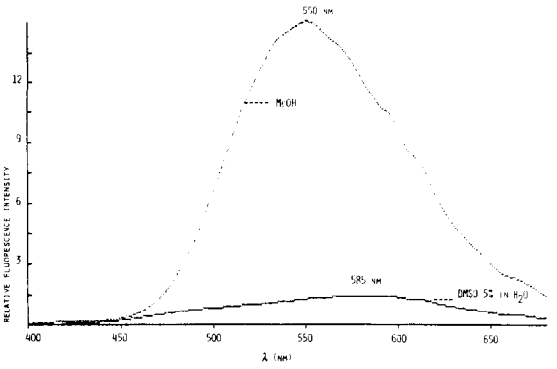

Fig. 1 Fluorescence emission spectra of 3'-DNS-dT.

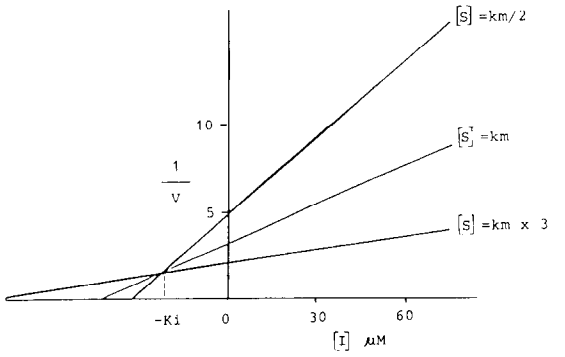

Fig. 2 Dixon plot for determination of Ki of thymidine kinase by 3'-DNS-dT.

and H-5' in agreement with the selective substitution and chemical purity.

Both dansyl derivatives were able to inhibit HSV-1 encoded TK by a competitive type of mechanism as evident from the graphical analysis of the data. An example is reported in Fig. 2 which depicts the Dixon plot of 3'-DNS compound. The apparent Ki value which is derived from this experiment is approximately 20 uM. Instead, a quite higher constant of inhibition (- 70 uM) is obtained for 5'-DNS derivative. The different ability of the two drugs to compete with thymidine for HSV-1 TK may be explained when considering that 3'-DNS-dT has a free hydroxyl group at position 5' (which represents the natural site of phosphorylation in nucleosides), while its 5'-DNS congener is blocked at the same site.

In conclusion, the reaction of thymidine with dansyl chloride enabled us to obtain two new fluorescent nucleoside derivatives. These products act as competitive inhibitors of HSV-1 thymidine kinase and might be therefore endowed with antiviral activity. Furthermore, they can be considered as useful reagents for studies which deal with specific mechanism of substrate recognition.

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ACKNOWLEDGEMENTS

We wish to thank Mr. M. Guida for technical assistance and greatly acknowledge support from CNR (grant N. 85.00865.52) and MPI.