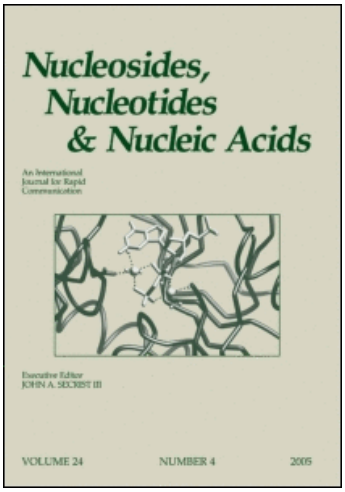

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M. Bretner[ab]; S. Schalinski[c]; P. Borowski[c]; T. Kulikowski[a]
[a] Laboratory of Antimetabolites, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland [b] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland [c] Abteilung für Virologie, Bernhard-Nocht-Institut für Tropenmedizin, Hamburg, Germany

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5′-O-Fluorosulfonylbenzoyl Esters of Purine Nucleosides as Potential Inhibitors of NTPase/Helicase and Polymerase of *Flaviviridae* Viruses

M. Bretner,[1,*] S. Schalinski,[2] P. Borowski,[2] and T. Kulikowski[1]

[1]Laboratory of Antimetabolites, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland
[2]Abteilung für Virologie, Bernhard-Nocht-Institut für Tropenmedizin, Hamburg, Germany

ABSTRACT

Synthesis and interactions of guanosine, inosine and ribavirin 5′-fluorosulfonylbenzoyl esters with hepatitis C virus (HCV) and *Flaviviruses* NTPase/helicase and polymerase are described.

Key Word: Flaviviridae analogue 5′-O-(4-fluorosulfonylbenzoyl)-adenosine (FSBA).

The adenosine analogue 5′-O-(4-fluorosulfonylbenzoyl)-adenosine (FSBA), has been found to react irreversibly or in a limited manner with many enzymes, which use nucleoside 5′-triphosphates (NTP) as substrates. NTPase/helicases are capable of enzymatically unwind duplex RNA or DNA. The helicase activity is

*Correspondence: M. Bretner, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland; E-mail: mbretner@hotmail.com.

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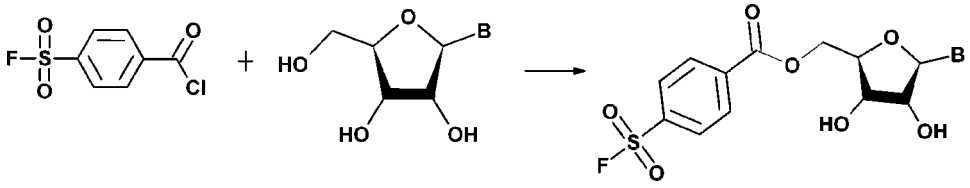
dependent on the energy produced in course of the NTP hydrolysis. Nonhydrolyzable ATP analogues do not substitute for NTP in the RNA-unwinding reaction.[1] It was pointed out that, using FSBA, only a low level or inhibition of unwinding activity of NTPase/helicase of hepatitis C virus (HCV) was detected.[2] Therefore it was of interest to synthesise new analogues of FSBA such as derivatives of guanosine (FSBG), inosine (FSBI) and ribavirin (FSBR), and to examine their activity against *Flaviviridae* viruses NTPase/helicase and polymerase.

FSBG, FSBI and FSBR were synthesized by acylation of the 5′-OH of guanosine, inosine and ribavirin with p-(fluorosulfonyl)benzoyl chloride in HMPA at room temperature to give after 12 h 50–70% yield of 5′-FSB-esters.



FSBG B = Guanine

FSBI B = Hypoxanthine

FSBR B = 1,2,4-triazole-3-carboxamide

When the helicase activity of the HCV NTPase/helicase was tested as function of increasing concentrations of the FSB-derivatives, under standard reaction conditions,[3] an effective inhibition of the unwinding activity was obtained with FSBI (IC_{50} 200 μM). In the case of FSBR and FSBA only modest inhibition with an $IC_{50} > 1$ mM was measured and FSBG acted even stimulating on the helicase activity. After the pre-incubation of the NTPase/helicase with the FSB-derivatives, that is necessary for covalent blockade of the nucleotide binding site(s) of the enzyme, all the investigated compounds displayed an inhibitory activity. The extend of the inhibition was strongly dependent on the time of exposition of the enzymes to the compounds. Thus, after 90 min pre-incubation, a significant enhancing of the inhibitory potential of the FSB-derivatives was obtained.

Comparative studies were performed with the NTPase/helicases of related *Flaviviruses*: West Nile virus (WNV), Japanese encephalitis virus (JEV), and Dengue virus (DENV). When the assay was performed with a not-pre-incubated enzyme no significant inhibition could be measured. However, after the pre-incubation of the NTPase/helicases with the FSB-derivatives dramatic reduction (10 to 100-fold) and even abolition of the helicase activity was seen.

Using various NTPs and polymerases of HCV and WNV we could demonstrate that, similar to the NTPase/helicase, the inhibition of the enzymatic activity depends strongly on the enzyme and substrate used. The preincubation of the enzymes with the FSB-derivatives prior to start of the reaction did not, however, increase the extend of the inhibition (Table 1).

Generally it was observed that the enzymes of the *Flaviviruses* are more susceptible to the FSB-derivatives mediated inhibition than that of HCV. Moreover, even

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Table 1. Inhibitory effect of FSB-derivatives against polymerase activity of HCV and WNV using [α-^{32}P]UTP or [α-^{32}P]GTP as a substrate.[a]

	HCV		WNV	
	[α-^{32}P] UTP	[α-^{32}P] GTP	[α-^{32}P] UTP	[α-^{32}P] GTP
FSBA	>1 mM (> 1 mM)	>1 mM	>1 mM (>1 mM)	>1 mM
FSBG	>1 mM (>1 mM)	>1 mM	>1 mM (600 μM)	>1 mM
FSBI	280 μM (400 μM)	80 μM	70 μM (30 μM)	600 μM
FSBR	>1 mM (> 370 μM)	>1 mM	400 μM (550 μM)	330 μM

[a]The polymerase activity was determined as function of increasing concentrations of FSBA, FSBG, FSBI and FSBR. The inhibitory potential of the compounds was expressed as the inhibitor concentration at which 50% activity was measured. The values given in parenthesis correspond to IC_{50} values measured with enzyme that was pre-incubated with the compound (90 min at 30°C).

within the group of the closely related NTPase/helicases, the extend of the inhibition was enzyme specific. The mechanism of the inhibition remains to be elucidated.

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