Vulpinic acids inhibit influenza (RNA) viruses but not herpes (DNA) viruses

Luay J. Rashan, Mikdad T. Ayoub, Layla Al-Omar and Ramzia Al-Khayatt

Three synthetic vulpinic acids inhibited two influenza RNA viruses, type A (Philippine) and B (Paraha), in tissue culture with ID₅₀ values ranging from 3.9 to 15.5 µg/ml. They had no activity against a third influenza virus or against two herpes viruses.

Trois acides vulpiniques de synthèse inhibent deux virus à RNA de l'influenza, types A (Philippine) et B (Paraha), en culture tissulaire avec des valeurs d'ID₅₀ s'étalant de 3.9 à 15.5 µg/ml. Ces acides vulpiniques ne présentent d'activité ni contre un troisième virus de l'influenza, ni contre deux virus de l'herpes.

Luay J. Rashan is with the Department of Biology, College of Education, and Mikdad T. Ayoub is with the Department of Chemistry, College of Science, of the University of Mosul, Mosul, Iraq; Layla Al-Omar is with the Department of Microbiology, College of Medicine, University of Baghdad, Iraq; and Ramzia Al-Khayatt is with the Virology Section, Central Public Laboratory, Baghdad, Iraq. Luay J. Rashan is the corresponding author.

Despite the considerable efforts of many investigators in recent years toward the discovery of new antiviral drugs, few have been approved for clinical use (Prusoff et al. 1986). The search for safe and effective drugs is therefore still continuing, especially for anti-influenza compounds.

The potent pharmacological activity of some but-2-enoloides (Khezerlu 1971) has generated much interest in their synthesis and several new naturally-occurring compounds of this type have been isolated (Pelter et al. 1987). For example, Patulin (I) is a fungal toxin found in both food (Scott & Kennedy 1973) and plants (Ellis & McCalla 1973).

Several synthetic methods for the preparation of vulpinic acids (III) have been described (Knight & Pattenden 1979; Ayoub & Gussab Bashi 1985) and a general synthetic route to vulpinic acids via Reformatsky-type reactions with aryl methoxymaleic anhydride has been developed by Pattenden et al. (1986). Thus, having repeated the experiment in the literature (Foden et al. 1975; Ayoub & Gussab Bashi 1985), we synthesized symmetrically substituted vulpinic acids from benzyl cyanides and diethyl oxalate (II) by the route first described by Volhard and later modified by Edwards et al. (1968).

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The present study was to investigate the *in vitro* antiviral activity as a part of our interest in the biological activities of these compounds.

**Materials and Methods**

*General Procedure for Preparation of Vulpinic Acids (IIIa-c) (Ayoub & Gussab Bashi 1985)*

The vulpinic acids (IIIa-c) were prepared according to Ayoub & Gussab Bashi (1985) using diethyl oxalate (II above) and aryl cyanides.

The structures of these acids were confirmed by elemental analysis, chemical properties and spectral data (i.r. and NMR) and found to be in agreement with that mentioned in literature (Ayoub & Gussab Bashi 1985).

**Antiviral Screening**

(a) *Influenza viruses.* Two strains of influenza type A viruses and one strain of influenza type B virus were kindly supplied by WHO, Geneva. These were influenza viruses A/Philippine/82 (H3N2) and A/Chilli/83 (H1N1) and influenza virus B/Paraha/85. The viruses were propagated and pools from each strain were grown in embryonated hens’ eggs. The viral titre in allantoic fluid were performed according to de Fazekas & White (1958), except the culture media used was LS of Leibowitz (1963).

The antiviral activity of these compounds were examined using two-fold concentration starting from 62 to 1.6 μg/ml. The compounds were dissolved in dimethyl sulphoxide and stock solutions were prepared in phosphate buffer/saline. The Allantois-On-Shell (AOS) cultures and the compounds were charged with 0.05 ml of ten-fold serial dilutions of the virus (10⁻² to 10⁻⁶) per incubation well. The haemagglutination tests were carried out at 72 h using a standard method. The inhibitory concentration of vulpinic acid which inhibited virus replication by 50% (ID₅₀) was then calculated.

(b) *Herpes viruses hominis HSV-1 (strain F) and HSV-2 (strain 333).* The preparation of the viruses, stocks and titration were carried out in the Virology Department, Medical School, Hallamshire Hospital, Sheffield, England, as described previously by Barton *et al.* (1982). Stock concentrations of 10 μg of each compound/ml were prepared in phosphate buffer/saline. Plaque reduction assays were prepared in triplicate on Vero cells. The concentrations of the test compounds necessary to reduce the number of plaques by 50% were compared with the untreated control.

**Results and Discussion**

The physical and spectral data (proton and ¹³C NMR) of vulpinic acids, IIIa-c, were identical in all respects with those already published (Ayoub & Gussab Bashi 1985). Vulpinic acids (IIIa-c) had an antiviral activity on influenza virus type A/Philippine and influenza virus type B/Paraha but not against type A/Chilli (Table 1). The ID₅₀ values ranged from 3.9 to 15.5 μg/ml (see Table 1). No antiviral activity against viruses HSV-1 and HSV-2 was detected at any concentration used.

The discovery of synthetic compounds which exert a demonstrable effect against RNA-viruses is most significant. In the present study, vulpinic acids (IIIa-c) were highly active against two influenza viruses but not against a third. Vulpinic acids appear to be as effective as other antiviral agents when tested *in vitro* (Swallow & Kampfner 1985) though *in vitro* activity cannot be used as a major criterion to judge activity (Buthala 1965; Sidwell *et al.* 1968).

The mode of action of vulpinic acids was not investigated in the present study, though these compounds are effective against the RNA-containing influenza