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# 4′,6-Dichloroflavan (BW683C), a new anti-rhinovirus compound


**D. J. Bauer, J. W. T. Selway*, J. F. Batchelor, Margaret Tisdale, Ian C. Caldwell & D. A. B. Young**

Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, UK



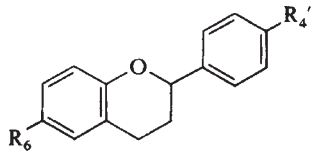
**Derivatives of flavan have been synthesized as chemical intermediates, but the only reported biological action is the ability of certain alkyl and alkoxy derivatives to lower blood cholesterol concentrations[1]. It was therefore surprising to discover that flavan itself (Table 1) is a highly effective inhibitor of the replication of certain serotypes of rhinovirus, and that a simple derivative, BW683C (4′,6-dichloroflavan), is the most potent antiviral compound yet reported. The present work examines the antiviral activity of flavan derivatives with a view to selecting the compound most suitable for trial in volunteers infected with a common cold virus.**


4′,6-Dichloroflavan, which is new to the chemical literature, has been prepared by methods used for the synthesis of substituted flavans[2–5]. It is a colourless crystalline solid, m.p. 101 °C, soluble in water only to the extent of 1 mg $l^{-1}$ at room temperature.

Antiviral activity was detected *in vitro* by means of plaque inhibition tests[6,7] with monolayers of M-HeLa cells[8,9] infected with rhinovirus 1B. Activity was measured by plaque reduction assays in which doubling concentrations of compound were incorporated into the overlay medium. Plaque counts, expressed as a percentage of the control value, were plotted against the logarithm of the compound concentration, to yield a dose–response line from which the $IC_{50}$ value could be determined. The $IC_{50}$ values for BW683C and several analogues are shown in Table 1. Flavan ($R_6 = R_{4'} = H$), with an $IC_{50}$ of 0.046 μM, is one of the least active of the compounds tested. The activity is generally increased by the presence of a single halogen substituent, and more so by the presence of two chlorine atoms, with the most active compound tested being BW683C which, with an $IC_{50}$ of 0.007 μM, is some six times more potent than the parent compound. The $IC_{90}$ of BW683C was 0.02 μM.

* To whom correspondence should be addressed.

**Table 1** Structure–activity relationship of a selection of halogen-substituted flavans against rhinovirus type 1B




| $R_6$ | $R_4'$ | $IC_{50}$(μM) |
|---|---|---|
| H | H | 0.046 |
| F | H | 0.020 |
| Cl | H | 0.050 |
| Br | H | 0.019 |
| H | F | 0.018 |
| H | Cl | 0.039 |
| H | Br | 0.036 |
| F | F | 0.068 |
| Cl | Cl | 0.007 |
| Br | Br | 0.010 |
| I | I | 0.043 |

For any agent to be effective in the prophylaxis or treatment of the common cold, it must be active against a high proportion of rhinovirus serotypes. There are at least 89 serotypes, the most prevalent being 1A, 1B, 2, 4, 15, 29, 30 and 31 (ref. 10). $IC_{50}$ values were obtained for BW683C against 19 serotypes (Table 2). Seven of the eight most prevalent serotypes were inhibited, although they varied considerably in sensitivity. The sensitivity of the other 11 serotypes was also variable, but was sufficient to suggest that the compound may be clinically useful.

In tissue culture tests 4′,6-dichloroflavan did not inhibit the replication of other RNA viruses, including bunyavirus, coronavirus, equine rhinovirus, influenza virus (NWS strain), measles virus, poliovirus (Sabin 1), Semliki Forest virus, Sindbis virus and respiratory syncytial virus. It also failed to inhibit the DNA viruses adenovirus type 5 and herpesvirus type 1.

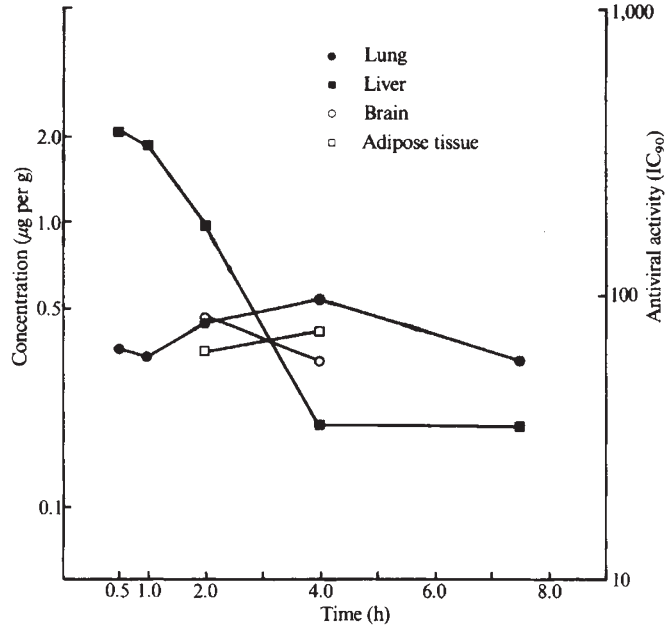



**Fig. 1** Tissue concentrations of BW683C determined by gas–liquid chromatography. Tissue homogenate (1 ml) was mixed with 0.5 ml ethylene glycol/water/2 M citric acid (2:2:1) and 5 ml hexane. The mixture was shaken for 30 min and centrifuged. The hexane layer was collected and mixed with 1 ml of a mixture of ethylene glycol/1 M $Na_2CO_3$ (1:9), shaken again and centrifuged. The upper layer was collected, dried in a stream of $N_2$ and the residue was dissolved in a small volume of toluene.

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**Table 2** Sensitivity of rhinoviruses to BW683C

| Serotype | $IC_{50}$ (μM) | Serotype | $IC_{50}$ (μM) |
|---|---|---|---|
| 1A* | 0.013 | 13 | 0.66 |
| 1B* | 0.007 | 14 | Inactive |
| 2* | 0.04 | 15* | 0.17 |
| 3 | 10.10 | 16 | 0.02 |
| 4* | Inactive | 18 | 0.29 |
| 5 | Inactive | 19 | 0.81 |
| 8 | 8.00 | 21 | 1.70 |
| 9 | 0.011 | 29* | 0.008 |
| 12 | 0.15 | 30* | 52.00 |
| | | 31* | 0.013 |

* Serotypes described by Roebuck[10] as common.

Preliminary studies were carried out on the absorption and tissue distribution of 4',6-dichloroflavan in rats after its administration at 5 mg per kg by gavage as a suspension of finely ground particles in aqueous methylcellulose. Samples of plasma and selected tissues were collected at intervals up to 7.5 h after dosing, and unchanged compound was determined by analysis of tissue extracts by gas–liquid chromatography with electron capture detection or by specific ion monitoring using a mass spectrometer. Highest concentrations were found in liver (Fig. 1); peak concentrations of ~7.3 μM, some 350 times the $IC_{90}$, were observed at 0.5 h, and these declined rapidly. Concentrations of compound well in excess of the $IC_{90}$ value were also detected in plasma, lung, brain and adipose tissue, and the time of peak compound concentrations in lung samples seemed to occur between 2 and 4 h after dosing; compound concentrations in excess of the $IC_{90}$ level could still be found in lung 7.5 h after dosing. These results indicate that 4',6-dichloroflavan is adequately absorbed from the gastrointestinal tract, and that compound concentrations well above those required for maximum antiviral activity *in vitro* can be attained at doses as low as 5 mg per kg. The apparent persistence of this compound implies that infrequent administration may suffice to maintain effective antiviral concentrations *in vivo*. The above findings were supported by autoradiography of rats, which received $^3$H-BW683C as an aqueous suspension by gavage, and were killed 2 or 4 h later.

When BW683C was added to HeLa cells in culture no cytotoxic effects were observed at drug concentrations up to 3.6 μM, the limit of aqueous solubility and many times the $IC_{90}$ and $IC_{50}$ values determined with rhinovirus 1B. In the rat, the $LD_{50}$ after subcutaneous administration was ~300 mg per kg; animals showed no ill effects after oral administration at doses up to 1 g per kg or intraperitoneal administration at doses up to 700 mg per kg. BW683C showed no evidence of mutagenicity when tested in the presence or absence of a liver microsome preparation by the method of Ames[11].

Preliminary studies showed that BW683C interacted directly with virus, reducing infectivity by about 0.5 log unit, but it was not a typical contact inactivator because inactivation did not increase with time. The possible binding of compound to virus particles was investigated by mixing tritiated compound with virus and centrifuging the mixture through a 15–45% sucrose gradient; radioactively labelled compound was found in the virus peak.

In a time-of-addition study, the compound was added at hourly intervals throughout a single growth cycle of rhinovirus 1B in M-HeLa cells. Maximum antiviral activity was obtained when the compound was added to the culture at the same time as the virus. Although some antiviral activity was observed when addition was delayed for 1 h or longer, it was no greater than that obtained by adding compound at the end of the cycle.

Further experiments with $^3$H-uridine-labelled virus indicated that the compound does not interfere with adsorption of the virus to the cell, nor with the uncoating or entry of the viral RNA into the cell. However, viral RNA synthesis was inhibited by the presence of the compound. BW683C therefore seems to bind to the virus and to inhibit some stage of viral replication immediately following entry of the viral RNA into the cell.

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# Mutation of gene encoding regulatory polypeptide of aspartate carbamoyltransferase

**André Feller*†, André Piérard*†, Nicolas Glansdorff†‡, Daniel Charlier†‡ & Marjolène Crabeel†‡**

* Laboratoire de Microbiologie, Faculté des Sciences, Université Libre de Bruxelles, 1, avenue E. Gryson, B-1070 Bruxelles, Belgium
† Institut de Recherches du CERIA, 1, avenue E. Gryson, B-1070 Bruxelles, Belgium
‡ Erfelijkheidsleer en Microbiologie, Vrije Universiteit Brussel, E. Grysonlaan, 1-1070 Brussel, Belgium

**In our studies on the regulation of the expression of the genes for the biosynthesis of arginine and pyrimidines in *Escherichia coli*, we discovered that an aspartate carbamoyltransferase (ATCase) synthesized *in vitro* from our λ transducing phage λ0TC3 lacked substrate cooperative interactions and was insensitive to feedback inhibition by CTP. We show here that these abnormal properties result from a mutation in the gene for the regulatory polypeptide chain of ATCase. We believe this to be the first report of a mutation in the gene for this regulatory chain.**

ATCase catalyses the first reaction specific to pyrimidine biosynthesis, the formation of *N*-carbamoyl-L-aspartate from L-aspartate and carbamoylphosphate. In *E. coli*, ATCase activity is modulated by feedback inhibition by CTP, the end product of the pyrimidine pathway, and activation by ATP, product of the parallel purine pathway[1]. Because *E. coli* ATCase exhibits the various types of regulatory interactions characteristic of allosteric proteins, it has been considered a model system and has become the most extensively investigated regulatory enzyme[2,3]. It differs, however, from most other allosteric proteins in that its catalytic and regulatory functions can be physically separated. Native ATCase, with a molecular weight (MW) of 300,000, consists of two trimeric subunits which possess catalytic activity but are insensitive to the allosteric effectors and three dimeric subunits which are catalytically inactive but bear the effector binding sites[4–6]. Thus, the completely different catalytic (C) and regulatory (R) chains (respective MWs 34,000 and 17,000) form a $2C_3:3R_2$ structure. $Zn^{2+}$ ions, one per regulatory chain, are required to stabilize this quaternary structure[7–9].

The zinc-binding domains on the regulatory subunits participate in the R:C bonding domains essential for the allosteric interactions[10]. The gene *pyrB* coding for the catalytic chain of ATCase lies at 96 min on the *E. coli* chromosome[11,12], but the localization of the gene for the regulatory chain has been hampered by the failure to obtain mutations affecting this gene. However, a thorough investigation of their biosynthesis[13,14]

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