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Inhibition of HIV replication by amino-sugar derivatives

George W.J. Fleet*, Abraham Karpas†, Raymond A. Dwek°, Linda E. Fellows, A.S. Tyms×, Sigthor Petursson*°, Sung K. Namgoong*, Nigel G. Ramsden*, Paul W. Smith*, Jong Chan Son*, Francis Wilson*, David R. Witty*, Gary S. Jacob+° and Thomas W. Rademacher°

*Dyson Perrins Laboratory, Department of Organic Chemistry, University of Oxford, South Parks Road, Oxford OX1 3QY, †Clinical School, Department of Haematological Medicine, University of Cambridge, Hills Road, Cambridge CB2 2QL, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond TW9 3DS, ×Division of Virology, Department of Medical Microbiology, St. Mary's Hospital Medical School, London W2 1PG, England, +Department of Molecular and Cell Biology, G.D. Searle & Co., St. Louis, MO 63198, USA and °Glycobiology Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, England

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The plant alkaloids castanospermine, dihydroxymethyldihydroxypyrrolidine and deoxynojirimycin have recently been shown to have potential anti-HIV activity [(1987) Proc. Natl. Acad. Sci. USA 84, 8120–8124; (1987) Nature 330, 74–77; (1987) Lancet i, 1025–1026]. They are thought to act by inhibiting α-glucosidase I, an enzyme involved in the processing of *N*-linked oligosaccharides on glycoproteins. We report here the relative efficacy of a spectrum of amino-sugar derivatives as inhibition of HIV cytopathicity. Several α-glucosidase inhibitors and α-fucosidase inhibitors were found to be active at concentrations which were non-cytotoxic.

AIDS; HIV; Glycosidase inhibitor; Amino-sugar; Glycosylation

1. INTRODUCTION

Polyhydroxylated octahydroindolizines, piperidine and pyrrolidine alkaloids extracted from plants and microorganisms have been found to have a number of biological activities, which in most cases can probably be ascribed to their acting as glycosidase inhibitors. Many of these alkaloids can be considered as analogues of monosaccharides in which the ring oxygen is replaced by nitrogen. Recent reviews [4,5] have dealt with historical and general aspects of these compounds and currently there are about 13 natural alkaloidal glycosidase inhibitors reported [5]. They are being used to study the mechanisms of oligosaccharide processing and may also be of potential clinical value as anti-neoplastic agents [6–9]. Recent studies have suggested that those alkaloids which inhibit α-glucosidase I may have anti-HIV activity [1–3]. In order to confirm and extend these earlier studies we have isolated or synthesized 47 amino-sugar derivatives and screened them for potential anti-HIV activity. To discriminate between specific anti-HIV activity and cytotoxicity we have assessed in parallel their effects on HIV infected and non-infected T lymphocytes.

Correspondence address: G.W.J. Fleet, (*organic syntheses*), Dyson Perrins Laboratory, Department of Organic Chemistry, University of Oxford, South Parks Road, Oxford OX1 3QY, England; or T.W. Rademacher (*reprints*), Glycobiology Unit, Dept of Biochemistry, University of Oxford, South Parks Rd, Oxford OX1 3QU, England

2. MATERIALS AND METHODS

The T-cell line (Karpas 45) was established from a child with acute lymphoblastic leukaemia. HIV-1 and HIV-2 were both isolated in Cambridge, HIV-1 from a British patient with AIDS and HIV-2 from a West African patient with AIDS. Cell-free suspensions of HIV were prepared from infected cultures as reported elsewhere [10]. The concentration of infectious particles (TCID, tissue culture infectious dose) was estimated using an end-point titration where the number of infectious HIV particles in each preparation was determined by the highest dilution

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which contained infectious HIV, as detected by syncytial formation, cytotoxicity and HIV antigen synthesis after 10 days of culture with 10^4 T-45 cells. The efficacy of the compounds used in this study was assessed as reported elsewhere [10].

Stock solutions of all compounds were prepared by dissolving each compound at a concentration of 1 mg/ml in growth medium. These solutions were filtered and sterilised (0.22 μm). Initially, each compound was tested at concentrations of 0.1 mg/ml and 0.5 mg/ml. If a given compound showed inhibition of HIV replication without cytotoxicity the assay was

Table 1

Cytotoxicity and anti-HIV activity

Compound	Abbreviation	Ox no.	Compound dosage (mg/ml)	Cytotoxic activity (% cell death)	Anti-HIV activity (% CPE reduction)
Castanospermine	Cast	16	0.1 , 0.4	0, 40	75, 60
L-1,6-Diepicastanospermine	LDCast	63	0.1 , 0.5	0, 0	0, 50
L-6-Epicastanospermine	LECast	62	0.1 , 0.5	0, 0	0, 0
Deoxynojirimycin	DNJ	55	0.5	0	50
N-Methyl-DNJ	MeDNJ	36	0.1 , 0.5	0, 40	100, 40
N-Ethyl-DNJ	EtDNJ	56	0.1 , 0.5	0, 25	100, 75
N-(*n*-Butyl)-DNJ	BuDNJ	57	0.1 , 0.5	0, 0	100, 100
2,5-Dideoxy-2,5-imino-D-mannitol	DMDP	17	0.32	35	25
N-Methyl-DMDP	MeDMDP	35	0.1	0	25
1,4-Dideoxy-1,4-imino-D-arabinitol	DAB	7	0.17	0	25
N-Methyl-DAB	MeDAB	33	0.3	0	0
1,4-Dideoxy-1,4-imino-L-arabinitol	LAB	38	0.1 , 0.5	0, 0	50, 100
N-Methyl-LAB	MeLAB	34	0.1	0	25
1,4-Dideoxy-1,4-imino-D-glucitol	DIG	11	0.16	0	0
N-Benzyl-1,4-dideoxy-1,4-imino-D-glucitol	BzDIG	51	0.1	0	0
1,4-Dideoxy-1,4-imino-D-mannitol	DIM	32	0.3	0	10
N-Methyl-DIM	MeDIM	31	0.3	0	10
D-Mannonic-1,4-lactam	-	42	0.3	0	0
1,4-Dideoxy-1,4-imino-D-tallitol	DIT	12	0.16	0	25
N-Benzyl-1,4-dideoxy-1,4-imino-D-tallitol	BzDIT	53	0.1	0	0
D-Deoxymannojirimycin	DMJ	5	0.06	25	0
N-Methyl-DMJ	MeDMJ	37	0.3	0	25
L-Deoxymannojirimycin	LDMJ	61	0.1 , 0.5	0, 0	0, 0
N-Methyl-L-DMJ	MeLDMJ	60	0.1 , 0.5	0, 0	0, 0
L-Mannonic-1,5-lactam	-	59	0.1 , 0.5	0, 0	0, 0
Fagomine	FAG	6	0.18	25	0
2-Acetamido-1,5-imino-1,2,5-trideoxy-D-glucitol	AGlu	4	0.19	25	0
2-Acetamido-1,5-imino-1,2,5-trideoxy-D-galactitol	AGal	3	0.19	0	0
L-Fuconic-1,5-lactam	-	2	0.10	25	50
1,5-Dideoxy-1,5-imino-L-fucitol	FT	1	0.15	0	0
N-Methyl-FT	MeFT	45	0.1	0	25
N-Acetyl-FT	AcFT	46	0.3	0	0
N-(5-Carboxymethyl-1-pentyl)	LFT	47	0.10, 0.25	0, 0	75, 90
1,4-Dideoxy-1,4-imino-D-ribitol	DRib	15	0.13	0	50
N-Benzyl-DRib	BzDRib	39	0.1	0	0
N-Methyl-DRib	MeDRib	48	0.1	0	25
1,4-Dideoxy-1,4-imino-L-ribitol	LRib	14	0.13	0	25
N-Benzyl-LRib	BzLRib	40	0.1	0	25
1,4-Dideoxy-1,4-imino-D-galactitol	-	58	0.1 , 0.5	0, 0	0, 0
D-Galactonic-1,4-lactam	-	41	0.3	0	0
1,4-Dideoxy-1,4-imino-D-allitol	DIA	13	0.16	0	0
N-Benzyl-DIA	BzDIA	52	0.1	0	0
2*S*,3*R*,4*R*,5*R*-3,4,5-Trihydroxypipecolic acid	-	8	0.16	25	0
2*S*,3*R*,4*R*-3,4-Dihydroxypipecolic acid	-	9	0.18	25	0
2*S*,3*R*,4*R*-3,4-Dihydroxyproline	-	10	0.15	0	0
Dextran sulfate	Dex	-	0.01, 1.0	0, 0	25, 60

repeated with greater dilutions or, if it showed partial inhibition, with higher concentrations.

Ox16 was isolated from *Castanospermum australe* [11]; Ox13, Ox15, Ox39, Ox52, were synthesized from D-gulonolactone [12]; Ox59 was synthesized from D-gulonolactone [13]; Ox62 and Ox63 were synthesized from D-gulonolactone [14–16]; Ox55 was either isolated [17], purchased from Sigma or synthesized from D-glucose [18]; Ox36, Ox56 and Ox57 were synthesized from deoxynojirimycin [19]; Ox17 was either isolated [17] or also synthesized from D-glucose [20]; Ox35 was synthesized from 2,5-dideoxy-2,5-imino-D-mannitol [19]; Ox7 and Ox38 were synthesized from D-xylose [21]; Ox33 and Ox34 were synthesized from 1,4-dideoxy-1,4-imino-D-arabinitol [19]; Ox11 and Ox51 were synthesized from D-galactonolactone [12]; Ox31 was synthesized from 1,4-dideoxy-1,4-imino-D-mannitol [19]; Ox12, Ox14, Ox53 and Ox40 [25], Ox32 [23], Ox42 [24] and Ox38 [19] were all synthesized from D-mannose; Ox37 was synthesized from D-deoxymannojirimycin [19]; Ox61 was synthesized from L-deoxymannojirimycin [19]; Ox1 [31], Ox2 [26], Ox3 [27], Ox4, Ox5, Ox6 and Ox8 [18], Ox9 and Ox10 [28], Ox15 [18] and Ox41 and Ox58 [24] were all synthesized from glucose; Ox45 and Ox47 were synthesized from 1,5-dideoxy-1,5-imino-L-fucitol [19]; and Ox46 was prepared by peracetylation of 1,5-imino-L-fucitol followed by selective deacylation [24].

3. RESULTS

Table 1 and fig.1 list the polyhydroxylated piperidines, pyrrolidines, and octahydroindolizines and others which were tested in this study and the effect of these compounds on the cytopathic effect (CPE) and their cytotoxicity. The data in table 1 confirmed the inhibition of CPE by the α-glucosidase I inhibitor castanospermine (Ox16) as reported previously [1–3]. In contrast, neither L-6-epicastanospermine (Ox62) nor L-1,6-epicastanospermine (Ox63) was inhibitory. Deoxynojirimycin (Ox55), previously reported to be inhibitory [2,3], was found to have only a marginal effect, however, the activity was greatly increased by *N*-alkylation; thus the *N*-methyl (Ox36), *N*-ethyl (Ox56), and *N*-butyl (Ox57) derivatives were all potent inhibitors of CPE at concentrations which were not cytotoxic. The azafructose analogue (Ox17), a hydroxylated pyrrolidine, was found to have a weak inhibitory effect. However, *N*-methylation of Ox17 to give Ox35 did not change the inhibitory properties of the compound in contrast to the increase in efficacy found after *N*-alkylation of deoxynojirimycin. Both enantiomers of 1,4-dideoxy-1,4-imino-arabinitol (Ox7, Ox38) were previously shown to be glucosidase inhibitors [29,30], although each enantiomer had a different range of glucosidase inhibitor specificity [21]. The D-enantiomer (Ox7) had very little effect on HIV replication, whereas the L-enantiomer (Ox38) had significant inhibitory properties. For both enantiomers, *N*-methylation (Ox33, Ox34) reduced the anti-HIV activity with total loss of activity of Ox33 and only marginal inhibition with Ox34. Neither the azafuranose analogue of glucose (Ox11) nor the *N*-benzyl derivative (Ox51) was found to have an effect on CPE. Similarly, no inhibition activity was observed for fagomine (Ox6), the 2-deoxyglucose analogue.

In contrast to the effect of inhibitors of α-glucosidase I, such as castanospermine, inhibitors of processing mannosidases such as deomannojirimycin were reported to have no effect on HIV replication [2]. Thus the mannosidase inhibitor (Ox32), the *N*-methyl derivative (Ox31) and the corresponding lactam (Ox42) all had no effect on CPE [54]. However, weak inhibition by the mannosidase inhibitor (Ox12) was observed although the *N*-benzyl derivative (Ox53) had no effect. Deoxymannojirimycin (Ox5) was confirmed to have only very weak activity as reported elsewhere [2], but the corresponding *N*-methyl derivative (Ox37) was found to have significant inhibitory activity. Neither of the enantiomers of deoxymannojirimycin (Ox60 and 61) nor the corresponding lactam (Ox59) had any inhibitory effect.

The effects of a number of other known glycosidase inhibitors were also studied. Neither the aza analogue of *N*-acetylglucosamine (Ox4) nor *N*-acetylgalactosamine (Ox3), which are specific hexosaminidase inhibitors [30], had any effect on the CPE. The fuconolactam (Ox2), which is a weak L-fucosidase inhibitor [26], had a significant effect, although the powerful fucosidase inhibitor Ox1 [31] had no effect. However the *N*-methyl (Ox45) and *N*-acetyl (Ox46) derivatives of Ox1 showed weak inhibition. Surprisingly the *N*-pentylcarboxymethyl derivative (Ox47) showed potent inhibition of CPE. The D-ribose derivative (Ox15) and the enantiomeric L-ribose isomer (Ox14) both had modest inhibitory effects as did their alkylated derivatives (Ox48, Ox39, and Ox40). No effects were observed with the iminohexitol derivatives (Ox58, Ox41, Ox13, Ox52) nor the hydroxylated amino acids (Ox8, Ox9, Ox10).

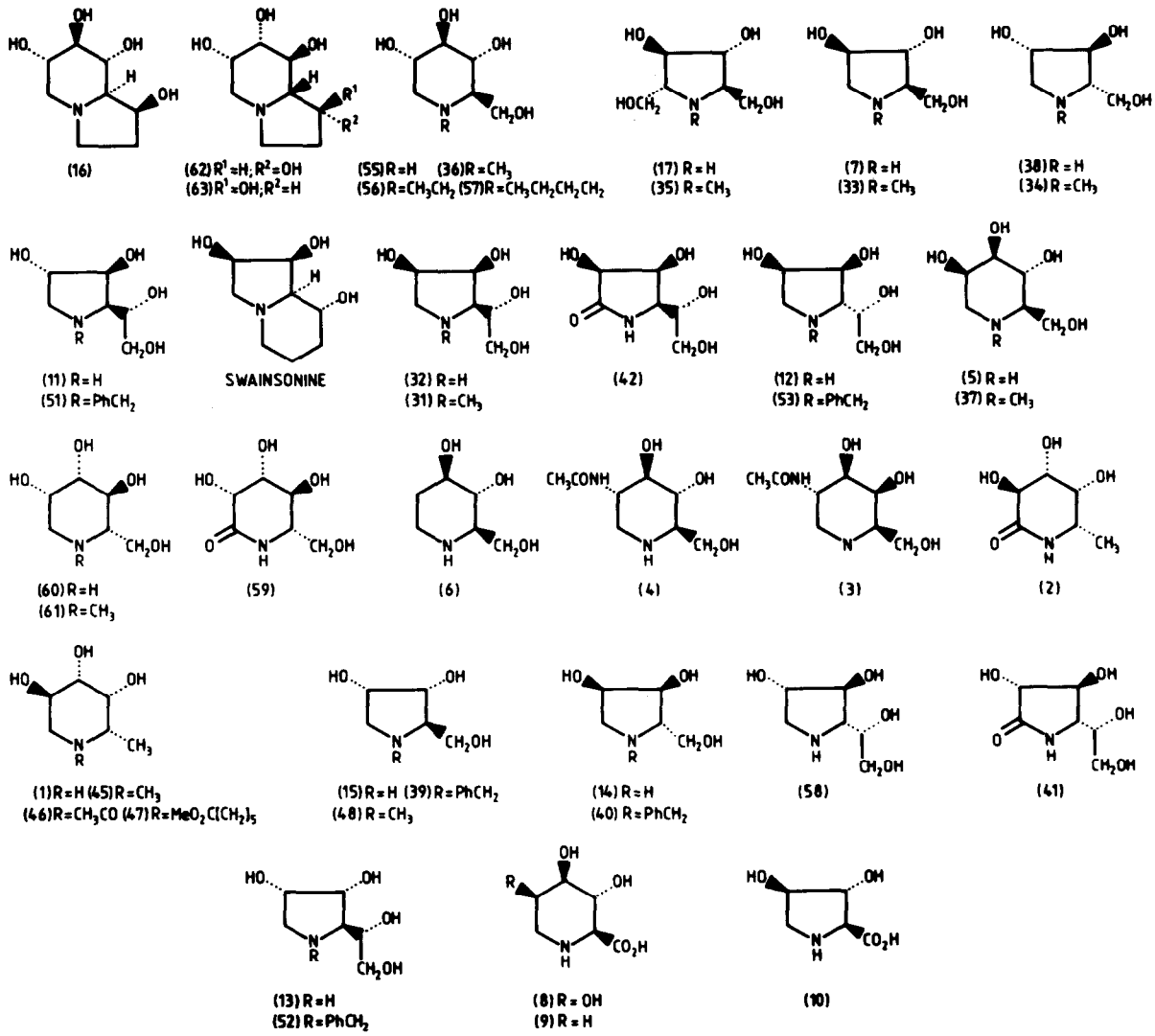

Fig.1. Structural formula of the compounds tested for anti-HIV activity. Table 1 lists the corresponding chemical names and abbreviations. The numbers in parenthesis refer to their Ox registry number and are drawn in the same order as they are discussed in the text. See section 2 for references on their synthesis.

4. DISCUSSION

The data reported here confirm that anti-HIV activity is exhibited by a number of amino-sugar derivatives some of which are known to interfere with oligosaccharide biosynthesis. For example, significant activity (inhibition of CPE) was found for the compounds (Ox16, 36, 38, 47, 56, 57) while compounds 38 and 47 had a lower activity. Of this group (Ox16, Ox36, Ox57) are known to be inhibitors of α-glucosidase I [32,33], however, Ox47 is a potent inhibitor of α-L-fucosidase (unpublished). Ox38 is known to be an α-glucosidase inhibitor, but has yet to be tested for activity against α-glucosidase I [29,35]. Weak to marginal inhibition was found for the compounds Ox62, 63, 16, 55, 17, 34, 2, 45, 48, 14, 40. Of this group (Ox55, Ox17) are known to be α-glucosidase I inhibitors [32–34,36], (Ox7) is an α-glucosidase inhibitor [35] not tested against α-glucosidase I) and

(Ox2, Ox45) are known to be fucosidase inhibitors [33]. No activity was found for the compounds (Ox6, Ox32) although these compounds are known to have α-glycosidase activity [29,35]. The data also show that in some cases *N*-alkylation results in a more active compound (55 → 36) while for other compounds (7 → 33) there is complete loss of activity.

From the data reported above, the optimal structural requirement for suppression of HIV cytopathicity is not obvious. However, these observations add support to the idea that amino-sugar derivatives may be of use in limiting the spread of HIV infections. This possibility is explored experimentally in a parallel publication [10].

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