Supplementary Tables and Figures for:

Exploring the Potential of Iminosugars as Antivirals for Crimean-Congo Haemorrhagic Fever Virus, Using the Surrogate Hazara Virus: Liquid-Chromatography-Based Mapping of Viral N-Glycosylation and In Vitro Antiviral Assays

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Supplementary Table S1. O-glycosylation prediction of HAZV and CCHFV. Scores from the NetOGlyc 4.0 Server (DTU Bioinformatics, University of Denmark). A total of 14 sites on HAZV and 90 sites on CCHFV had O-glycosylation potentials above a threshold of 0.7. The gradation of colours relate to the O-glycosylation score where the highest scores are shown in darker green, lowest scores are in darker red and scores in between are in yellow. In both viruses, the O-glycosylation potential is highest in the mucin-like variable region.

Chain	HAZV		CCHFV	
	Position	Score	Position	Score
Mucin-like variable region	22	0.827772	23	0.931262
	24	0.829553	27	0.972228
	28	0.959723	32	0.966068
	29	0.969206	34	0.952545
	33	0.975653	36	0.965209
	34	0.918613	37	0.988626
			43	0.977169
			44	0.980107
			49	0.965769
			50	0.939262
			53	0.875142
			55	0.968651
			59	0.992849
			60	0.978914
			62	0.987548
			64	0.986252
			65	0.994089
			68	0.93942
			73	0.905363
			78	0.959212
			79	0.974422
			83	0.971223
			84	0.956699
			86	0.927088
			90	0.982535
			92	0.974401
			93	0.978733
			99	0.989353
			100	0.977282
			102	0.903175
			104	0.939893
			106	0.973813
			113	0.98995
			114	0.943063
			116	0.988119
			120	0.988506
			121	0.993504

				124	0.996035
				127	0.97703
				128	0.981646
				132	0.993158
				134	0.99748
				135	0.981392
				137	0.99253
				138	0.985115
				142	0.973401
				151	0.932734
				153	0.976887
				154	0.990018
				160	0.975784
				161	0.96955
				162	0.977372
				164	0.976607
				166	0.924413
				170	0.987979
				171	0.963666
				173	0.970329
				174	0.978832
				175	0.982323
				180	0.995965
				185	0.993082
				189	0.972056
				194	0.981983
				198	0.939476
				202	0.979987
				207	0.942452
				209	0.930992
				211	0.987156
				213	0.9466
				218	0.913611
				219	0.977484
				221	0.9736
				228	0.990185
				230	0.982063
				233	0.939943
				237	0.970974
				240	0.973973
				242	0.982473
GP38	113	0.738189		323	0.771324
				326	0.933441
				329	0.938859
				330	0.949821
				332	0.983169

			333	0.94856
			339	0.759312
			378	0.726875
			381	0.936315
			382	0.876265
			387	0.823809
Non-Structural protein M	633	0.856664		
	638	0.76701		
	679	0.903456		
	683	0.911063		
	685	0.828454		
	698	0.756311		
Gc	894	0.875082	1399	0.771154

CCHFV, Crimean-Congo haemorrhagic fever virus; HAZV, Hazara virus.

Supplementary Table S2. Sites predicted to be N-glycosylated in the HAZV glycoprotein (UniProtKB accession number A6XIP3) using GlycoEP (N-linked glycosylation prediction based on binary profile of patterns using default SVM 0.0 thresholds. The darker the green colour for the score cells, the higher the score. N41 and N159 were excluded due to the prolines (P) in the sequons and since the scores were very low.

Position	Sequon	Score
41	NPS	0.189
97	NSS	1.069
159	NPS	0.33
346	NGS	0.637
639	NQS	1.23
1081	NST	0.919
1299	NET	0.801

HAZV, Hazara virus.

Supplementary Figure S1. N-glycosylation prediction of HAZV. Modified output from the NetNGlyc 1.0 Server (DTU Bioinformatics, University of Denmark). Default settings were used where predictions were done only on the Asn-Xaa-Ser/Thr sequons. All Asn-Pro-Ser/Thr sequons were excluded and the remaining sequons were included for sequence alignment. A total of eight Asn-Xaa-Ser/Thr sequons were identified (highlighted in blue). Three of these were Asn-Pro-Ser/Thr sequons (Asn shown in red at N41, N159 and N930) which were excluded. Two sequons (Asn shown in orange at N639 and N1299) were below the N-glycosylation potential threshold of 0.5 (at at 0.4717 and 0.4993 respectively) with long tryptic peptides and therefore were not considered for analysis by mass spectrometry but were included in the sequence alignment to see if CCHFV has potential N-glycosylation sites which align with these sequons of HAZV. The remaining three sequons (Asn shown in green at N97, N346 and N1081) were considered to have potential N-glycosylation sites and were selected for further analysis by mass spectrometry.

Asn-Xaa-Ser/Thr sequons in the sequence output below are highlighted in **blue**.

Asparagines predicted to be N-glycosylated are highlighted in **green**.

Output for HAZV glycoprotein sequence (Uniprot accession number A6XIP3)

```
Name:  HAZV glycoprotein      Length:  1421
MEGSYWWLSLLALLAWGANGESTSPAETSPAPTTPNPPVVNPSLRRKIVNQRILSAMGMDSDPSNEALNGVCQSIHSNGC     80
NANELKLRLADFFIDTNSSQCYDEILVKKPCSSLTPAHNSHWVPRGLDKSEVDKIFDTKLKLFFSQSRKVTCLSASALNP    160
SQFVKHFQVKIQETSGPAKQSLRSLHCVNLVWSHSHKGEKEVVHVLQSAVPVKLKNCLAMLNFRQCYYNQQSEGPVVVPS    240
YQHNGEKWVTGAYTMTVEVDKHADGPCEISTTCITEGSEIKPGVHSLRGFKTTLVIHGKRNTGRRLLSSSNARQECSSGT    320
FLGEGGSAQVVGPKNDGPGDHITFCNGSVVTKIRLGQEHGCYTVRRIKTYRNCRPEEGSSACEVDDELKPCGAQKCMNVH    400
LSVKGLVKTSRGSNVQVHSCDKDCLIQIPEGFGDIQIDCPGGTQHYLESNVLDVDCPMYNRLGGLMLYFCRMSHRPRTCL    480
ALFIWLGAGYGITCIAGYMVYYAILALSMLTRCLKRKYMVKGDFCLKCEQKCVTSLDQTLHDESCSYNICPYCGNRLPEE    560
GLRRHVPSCPKRKQRLEEIDLYLDYLLVPCPLHFALSTAVKLGTLLKRLSWVTVFLCLFLTAIAPVQGQVTTSPVLPSNQ    640
STECTLLPPPVFLIFSAVLMSKTLKRMGPVNKVGAAGHSARRTNSPKNLYKSKQIANTKSGPREPRRRVVVKALLILTAS    720
SALQSIHLAQAFDSGSLPEGAWEEEMQLVQGCNQECSLEEDECSCPDGQSMTRKLLFFKGLNSAASKMASSHRLLTSVSI    800
DTPWGAIKVESTYKPRLASSNIQLAWNSIEEQGDKVILSGKSTSIIKLEEKTGMQWSLGSESAAEEKRLLVSILDYTQVY    880
SSTFQYITGDRTVSEWPKATCTGDCPDRCGCSTSSCLYKSWPHSRNWRCNPTWCWGVGTGCTCCGVDILRPFNKYFVTKW    960
TTEYVRTDVLVCVELTDQERHCDVVEAGSQFVIGPVRVVVSDPQNVQTKLPSEILTIQKLEGNQVVDIMHATSIVSAKNA   1040
CKLQSCTHGSPGDMQILHTDNLIQHSHDGGLNLADLNPLVNSTWMSWEGCDLDYYCTTGSWPSCTYTGINSENTESFDNL   1120
LNTESNLCERFHFHSKRISASGSTLQMDLKGRPNSGGGELSVLVDVKGLELHSKKISLKGLSFKTLSCSGCYACSSGLSC   1200
TVEVRIERPDEFTVHLRSVSPDIAVAEGSIIARRMTGGPLSRLRAFAVRKVKKICFEIVEKSYCKDCKNEDTTKCIEVEL   1280
QPPKDILLEHKGTIIKRQNETCVSGLQCWTESASSFVSGVGSFFRNYLGSITLGIVLTLLPVAVVLLFFCYGDKLFKLCS   1360
CFRCCRGLSRGKVRKELDEDELRNKLKKFSKEGELFGKEKKDARTIALLLSGKGKNYKELV
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..............................................................................  80
.....................N..........................................................  160
..............................................................................  240
..............................................................................  320
........................N.......................................................  400
..............................................................................  480
..............................................................................  560
..............................................................................  640
..............................................................................  720
..............................................................................  800
..............................................................................  880
..............................................................................  960
.............................................................................. 1040
.................................N............................................. 1120
.............................................................................. 1200
.............................................................................. 1280
.............................................................................. 1360
.................................................................. 1440
```

(Threshold=0.5)

SeqName	Position	Potential	Jury agreement	N-Glyc result	
HAZV	41 NPSL	0.6857	(9/9)	++	WARNING: PRO-X1.
HAZV	97 NSSQ	0.6163	(8/9)	+	
HAZV	159 NPSQ	0.7756	(9/9)	+++	WARNING: PRO-X1.
HAZV	346 NGSV	0.5580	(7/9)	+	
HAZV	639 NQST	0.4717	(7/9)	−	
HAZV	930 NPTW	0.5820	(8/9)	+	WARNING: PRO-X1.
HAZV	1081 NSTW	0.5839	(8/9)	+	
HAZV	1299 NETC	0.4993	(5/9)	−	

NetNGlyc 1.0: predicted N-glycosylation sites in Sequence
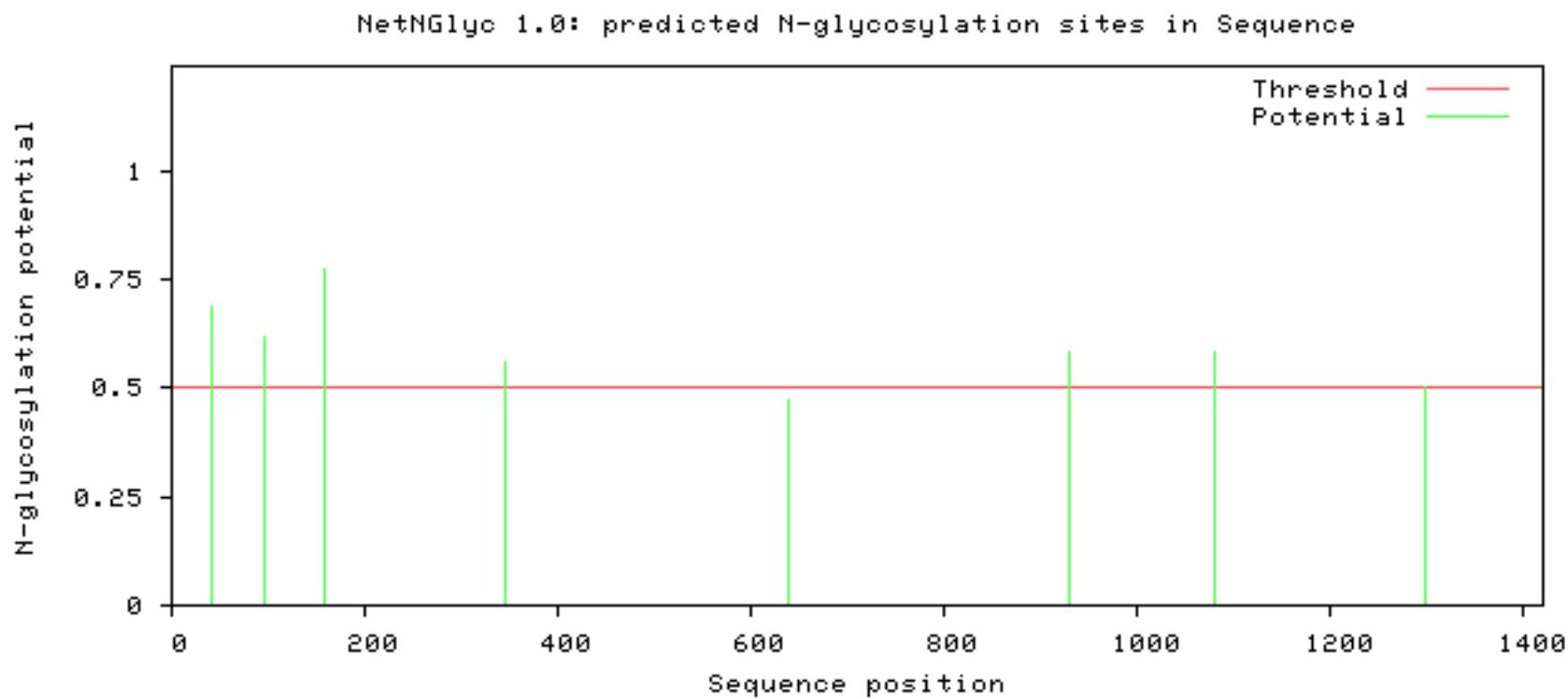
CCHFV, Crimean-Congo haemorrhagic fever virus; HAZV, Hazara virus.

Supplementary Figure S2. Sequence alignment of CCHFV (strain Nigeria / IbAr10200 / 1970; Uniprot Q8JSZ3) with HAZV glycoprotein (Uniprot A6XIP3). Chains are as currently shown on Uniprot (Release 12-Oct-2022). All HAZV glycoprotein peptides identified by mass spectrometry are shown in bold.

Mucin-like variable region

GP38

Glycoprotein N

Non-Structural protein M

Glycoprotein C

Propeptide

N = Potential N-glycosylation sites (as shown in Uniprot for CCHF/HAZV and as predicted by NetNGlyc 1.0 and GlycoEP for HAZV).

Underlined = Tryptic peptides covering N-glycosylation sites.

Bold in blue = Peptides identified in 84 kDa band.

Bold in green = Peptides identified in 45 kDa band.

Bold in yellow = Peptides identified in 30 kDa band.

HAZARA = Hazara virus glycoprotein (Uniprot A6XIP3)

CCHFV = Crimean-Congo hemorrhagic fever virus Envelopment polyprotein strain Nigeria / IbAr10200 / 1970 (Uniprot Q8JSZ3)

```
                                               10        20
CCHFV                          MHISLMYAILCLQLCGLGETHGSHNE

          30        40        50        60        70        80
CCHFV   TRHNKTDTMTTPGDNPSSEPPVSTALSITLDPSTVTPTTPASGLEGSGEVYTSPPITTGS

          90        100       110       120       130       140
CCHFV   LPLSETTPELPVTTGTDTLSAGDVDPSTQTAGGTSAPTVRTSLPNSPSTPSTPQDTHHPV

          150       160       170       180       190       200
CCHFV   RNLLSVTSPGPDETSTPSGTGKESSATSSPHPVSNRPPTPPATAQGPTENDSHNATEHPE

          210       220       230       240       250       260
CCHFV   SLTQSATPGLMTSPTQIVHPQSATPITVQDTHPSPTNRSKRNLKMEIILTLSQGLKKYYG
                       :                                    :     :
HAZARA        MEGSYWWLSLLALLAWGANGESTSPAETSPAPTTPNPPVVNPSLRRKIVNQ
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                     10         20         30         40         50

              270        280        290        300        310        320
CCHFV   KILRLLQLTLEEDTEGLLEWCKRNLGLDCDDTFFQKRIEEFFITGEGH--FNEVLQFRTP
        .:: .   .       ::.   :  .  :.   .. :.  .::: .:    ..:.  .
HAZARA  RILSAMGMDSDPSNEALNGVCQSIHSNGCNANELKLRLADFFIDTNSSQCYDEILVKKPC
                60         70         80         90        100        110

              330        340        350        360        370        380
CCHFV   GTLSTTEST---PAGLPTAEPFKSYFAKGFLSIDSGYYSAKCYSGTSNSGLQLINITRHS
        .:. ...    : ::   .: :  : .: : .:  : .  :. :::. .... . .
HAZARA  SSLTPAHNSHWVPRGLDKSEVDKIFDTKLKLFF-SQSRKVTCLSASALNPSQFVK--HFQ
                120        130        140        150        160

              390        400        410        420        430        440
CCHFV   TRIVDTPGPKITNLKTINCINLKASIFKEHREVEINVLLPQVAVNLSNCHVVIKSHVCDY
        .: .: ::    .:..:.:: :  .. ..  ..:: . : :.:.:: .... .  .:
HAZARA  VKIQETSGPAKQSLRSLHCVNLVWSHSHKGEKEVVHVLQSAVPVKLKNCLAMLNFRQCYY
          170        180        190        200        210        220

              450        460        470        480        490        500
CCHFV   SLDIDGAVRLPHIYHEGV-FIPGTYKIVIDKKNKLNDRCTLFTDCVIKGREVRKGQSVLR
        . .   .: :  .:  :.:  .. :.: .... ..  : . :: .:. :  :     ::
HAZARA  NQQSEGPVVVPSYQHNGEKWVTGAYTMTVEVDKHADGPCEISTTCITEGSEIKPGVHSLR
          230        240        250        260        270        280

              510        520        530        540        550
CCHFV   QYKTEIRI-GKASTGSRRLL-SEEPSDDCISRTQLLRTETAEIHGDNYGGPGDKITICNGS
        .:: . . :: .:: :::: .  .. :.: . .  ... :   :::::::.:::CNGS
HAZARA  GFKTTLVIHGKRNTG-RRLLSSSNARQECSSGTFLGEGGSAQVVGPKNDGPGDHITFCNGS
          290        300        310        320        330        340

         560        570        580        590        600        610
CCHFV   TIVDQRLGSELGCYTINRVRSFKLCENSATGKNCEIDSVPVKCRQGYCLRITQEGRGHVK
        ...  :::.: ::::. :..... .:    .. ::.:.  :   :  :. .   .: ::
HAZARA  VVTKIRLGQEHGCYTVRRIKTYRNCRPEEGSSACEVDDELKPCGAQKCMNVHLSVKGLVK
          350        360        370        380        390        400

         620        630        640        650        660        670
CCHFV   LSRGSEVVLDACDTSCEIMIPKGTGDILVDCSGGQQHFLKDNLIDLGCPKIPLLGKMAIY
        :::::. .:  .: ::.:.:::.:: ::: :: ::.::.::..:.. ::     ::  .:
HAZARA  TSRGSNVQVHSCDKDCLIQIPEGFGDIQIDCPGGTQHYLESNVLDVDCPMYNRLGGLMLY
          410        420        430        440        450        460

         680        690        700        710        720        730
CCHFV   ICRMSNHPKTTMAFLFWFSFGYVITCILCKAIFYLLIIVGTLGKRLKQYRELKPQTCTIC
        .::::.  .:.:.::::..:: :: ::::..: :. ... .:. :: .   .: .:  :
HAZARA  FCRMSHRPRTCLALFIWLGAGYGITCIAGYMVYYAILALSMLTRCLKRKYMVKGDFCLKC
          470        480        490        500        510        520

         740        750        760        770        780        790
CCHFV   ETTPVNAIDAEMHDLNCSYNICPYCASRLTSDGLARHVIQCPKRKEKVEETELYLNLERI
        :  .....  .:. :.::::::::::::. .:  .:: .:: .::.::::.::.. .
HAZARA  EQKCVTSLDQTLHDESCSYNICPYCGNRLPEEGLRRHVPSCPKRKQRLEEIDLYLDYLLV
          530        540        550        560        570        580
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         800       810       820       830       840       850
CCHFV   PWVVRKLLQVSESTGVALKRSSWLIVLLVLFTVSLSPVQSAPIGQGKTIEAYRAREGYTS
         :   ..    .:.  .:.  ::: ::.  :.: ::  ...::      ::  :   .   ..
HAZARA  PCPLHFALSTAVKLGTLLKRLSWVTVFLCLFLTAIAPVQ----GQVTTSPVLPSNQ--ST
         590       600       610       620       630       640

         860       870       880       890       900       910
CCHFV   ICLFVLGSILFIVSC-LMKGLVDSVGNSFFPGLSICKTCSISSINGFEIESHKCYCSLFC
         :  ..     ...: :   ::.   .    .:             .:       .:.
HAZARA  ECTLLPPPVFLIFSAVLMSKTLKRMG---------------PVNKVGAAGHSA------
               650       660                        670       680

         920       930       940       950       960       970
CCHFV   CPYCRHCSTDKEIHKLHLSICKKRKKGSNVMLAVCKLMCFRATMEVSNRALFIRSIINTT
           :.  .. :...:         :.  .:.           .  .  :      :   .
HAZARA  ----RRTNSPKNLYK--------SKQIANT--------------KSGPREPRRRVVVKAL
             690                                   700       710

         980       990      1000      1010      1020      1030
CCHFV   FVLCILILAVCVVSTSAVEMENLPAGTWEREEDLTNFCHQECQVTETECLCPYEALVLRK
         ..:         .    .:   .::  ::::  .:.  .::.: : :: ::     .  ::
HAZARA  LILTASSALQSIHLAQAFDSGSLPEGAWEEEMQLVQGCNQECSLEEDECSCPDGQSMTRK
         720       730       740       750       760       770

        1040      1050      1060      1070      1080      1090
CCHFV   PLFLDSTAKGMKNLLNSTSLETSLSIEAPWGAINVQSTYKPTVSTANIALSWSSVEHRGN
         ::.  ..  ... .:    :  ::.::.:::::.:::::.   ...:: :.:.:.:.:.
HAZARA  LLFFKGLNSAASKMASSHRLLTSVSIDTPWGAIKVESTYKPRLASSNIQLAWNSIEEQGD
         780       790       800       810       820       830

        1100      1110      1120      1130      1140      1150
CCHFV   KILVSGRSESIMKLEERTGISWDLGVEDASESKLLTVSVMDLSQMYSPVFEYLSGDRQVG
         :...::.: ::.::.::.:.::.::  :  :  :.::.  :.::.. :.:. :.:.:... ..
HAZARA  KVILSGKSTSIIKLEEKTGMQWSLGSESAAEEKRLLVSILDYTQVYSSTFQYITGDRTVS
         840       850       860       870       880       890

        1160      1170      1180      1190      1200      1210
CCHFV   EWPKATCTGDCPERCGCTSSTCLHKEWPHSRNWRCNPTWCWGVGTGCTCCGLDVKDLFTD
         :::::::::::::.: ::.:::::.: ::::::::::::::::::::::::::.  .:.
HAZARA  EWPKATCTGDCPDRCGCSTSSCLYKSWPHSRNWRCNPTWCWGVGTGCTCCGVDILRPFNK
         900       910       920       930       940       950

        1220      1230      1240      1250      1260      1270
CCHFV   YMFVKWKVEYIKTEAIVCVELTSQERQCSLIEAGTRFNLGPVTITLSEPRNIQQKLPPEI
         :.  .::  .::.:.::.:::::::.::::.:  .::.: .::: .:..:.:.: ::: ::
HAZARA  YFVTKWTTEYVRTDVLVCVELTDQERHCDVVEAGSQFVIGPVRVVVSDPQNVQTKLPSEI
         960       970       980       990      1000      1010

        1280      1290      1300      1310      1320      1330
CCHFV   ITLHPRIEEGFFDLMHVQKVLSASTVCKLQSCTHGVPGDLQVYHIGNLLKGDKVNGHLIH
         .::.     .   :.::: .::::.:.::::::::::: .::: :  : :::.   .. .:  .
HAZARA  LTIQKLEGNQVVDIMHATSIVSAKNACKLQSCTHGSPGDMQILHTDNLIQHSHDGGLNLA
        1020      1030      1040      1050      1060      1070

        1340      1350      1360      1370      1380      1390
CCHFV   KIEPHFNTSWMSWDGCDLDYYCNMGDWPSCTYTGVTQHNHASFVNLLNIETDYTKNFHFH
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                  ..:   :..::::.:::::::. :.:.:::::::...: :: :::: :..  . ::::
HAZARA  DLNPLVNSTWMSWEGCDLDYYCTTGSWPSCTYTGINSENTESFDNLLNTESNLCERFHFH
           1080      1090      1100      1110      1120      1130

          1400      1410      1420      1430      1440      1450
CCHFV   SKRVTAHGDTPQLDLKARPTYGAGEITVLVEVADMELHTKKIEISGLKFASLACTGCYAC
        ::::..: :.: :.:::.::. :.::..:::.:  .:::.::: ..:::.: .:.:.::::::
HAZARA  SKRISASGSTLQMDLKGRPNSGGGELSVLVDVKGLELHSKKISLKGLSFKTLSCSGCYAC
           1140      1150      1160      1170      1180      1190

          1460      1470      1480      1490      1500      1510
CCHFV   SSGISCKVRIHVDEPDELTVHVKSDDPDVVAASSSLMARKLEFGTDSTFKAFSAMPKTSL
        ::::.:: :.....::....::.::.: .::...: .:...:.  :  :  ..:::.    ..
HAZARA  SSGLSCTVEVRIERPDEFTVHLRSVSPDIAVAEGSIIARRMTGGPLSRLRAFAVRKVKKI
           1200      1210      1220      1230      1240      1250

          1520      1530      1540      1550      1560      1570
CCHFV   CFYIVEREHCKSCSEEDTKKCVNTKLEQPQSILIEHKGTIIGKQNSTCTAKASCWLESVK
        :: :::. .::.:.:::: ::...:. :.::.::.::::::. .: ::.   .:: ::..
HAZARA  CFEIVEKSYCKDCKNEDTTKCIEVELQPPKDILLEHKGTIIKRQNETCVSGLQCWTESAS
           1260      1270      1280      1290      1300      1310

          1580      1590      1600      1610      1620      1630
CCHFV   SFFYGLKNMLSGIFGNVFMGIFLFLAPFILLILFFMFG---WRILFCFKCCRRTRGLFKY
        :: :. ... . .:.. .:: : : ...:: .:  ... ::.:: :::: .
HAZARA  SFVSGVGSFFRNYLGSITLGIVLTLLPVAVVLLFFCYGDKLFKLCSCFRCC---RGLSRG
           1320      1330      1340      1350      1360      1370

          1640      1650      1660      1670      1680
CCHFV   RHLKDDEETGYRRIIEKLNNKKGKNKLLDGERLADRRIAELFSTKTHIG
        . .:. .:  :  ..:.. :.:. :. :.   : :: :.: :
HAZARA  KVRKELDEDELRNKLKKFS-KEGE--LFGKEKKDARTIALLLSGKGKNYKELV
            1380      1390      1400      1410      1420
```

CCHFV, Crimean-Congo haemorrhagic fever virus; HAZV, Hazara virus.

Supplementary Figure S3. Example of MS/MS spectrum (from Mascot) of peptide NDGPGDHITFCNGSVVTK (m/z 640.291500, 3+) with deamidation on N346 confirming that this site is occupied in HAZV.

Gn.MS/MS Fragmentation of **NDGPGDHITFCNGSVVTK**
Found in **gi|111154390** in **NCBInr**, glycoprotein precursor [Hazara virus]
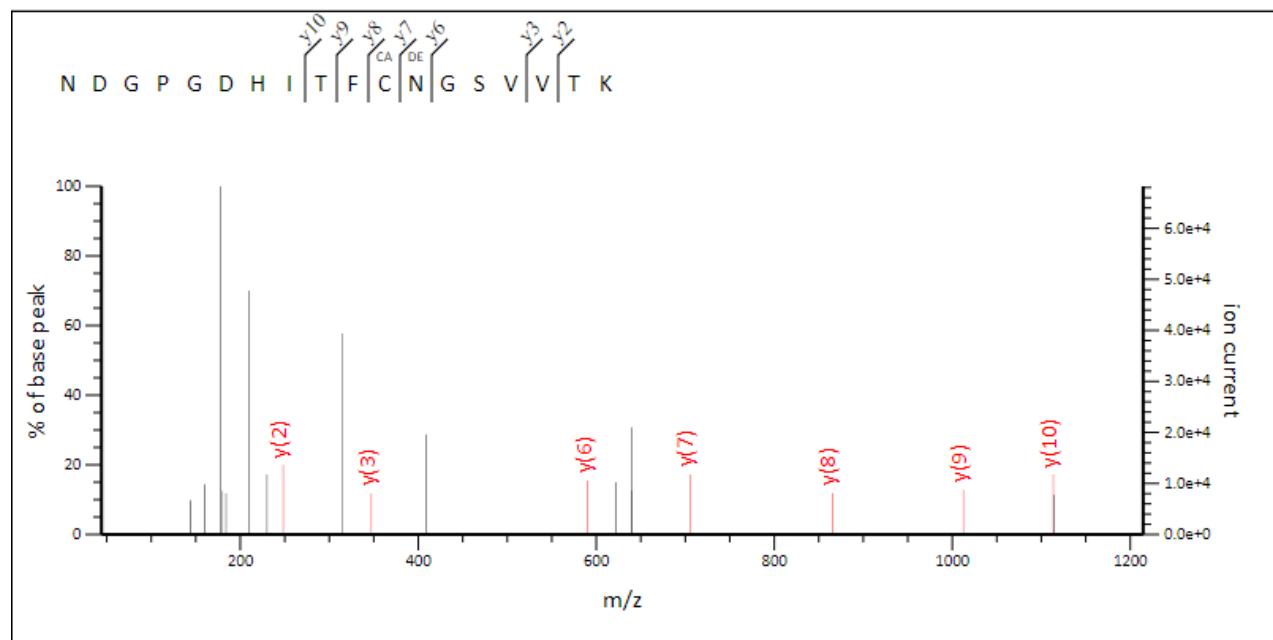
Monoisotopic mass of neutral peptide Mr(calc): 1917.8527
**Variable modifications:**
C11 : Carbamidomethyl (C)
N12 : Deamidated (N)**Ions Score:** 39 **Expect:** 0.00021
Matches : 7/252 fragment ions using 13 most intense peaks

Score	Mr(calc)	Delta	Sequence
39.2	1917.8527	-0.0001	NDGPGDHITFCNGSVVTK

HAZV, Hazara virus; MS, mass spectrometry.

Supplementary Figure S4. Uninfected and HAZV-infected SW13 cells are susceptible to iminosugar-mediated ER α-glucosidase inhibition. SW13 cells (assayed in technical duplicate) were left untreated or treated with 100 µM 2THO-DNJ, NN-DNJ, NN-DGJ or ribavirin, or 316 µM NB-DNJ or NB-DGJ for 3 days (**A**, **B**) or 6 days (**C**, **D**). In (**A**, **B**), cells were either mock- or HAZV-infected on day 0, immediately prior to drug treatment. In (**C**, **D**), cells were drug-treated for 3 days prior to mock- or HAZV-infection, after which the same drug treatment was continued for 3 days. At the end of the culture period, cells were lysed and FOS species purified and detected by NP-HPLC.
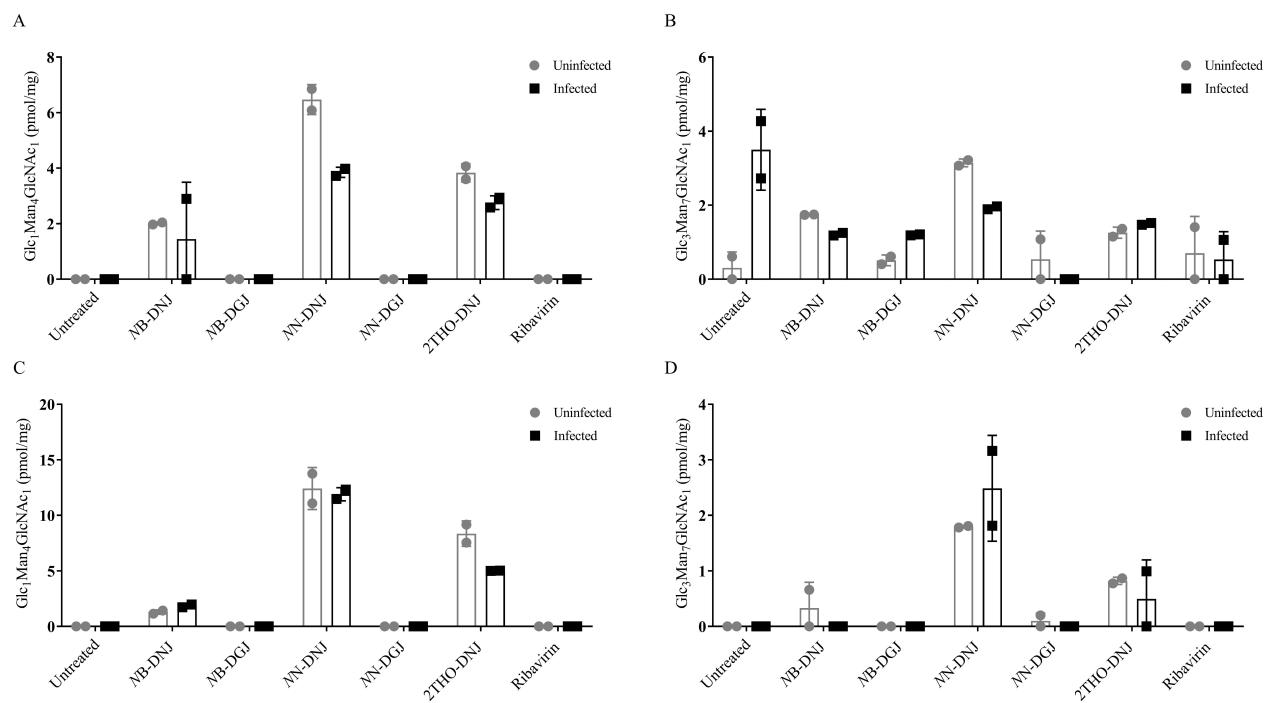
2THO-DNJ, N-8′-(2″-tetrahydrofuranyl)-octyl-deoxynojirimycin; ER, endoplasmic reticulum; FOS, free oligosaccharide; HAZV, Hazara virus; NB-DGJ, N-butyl-deoxygalactojirimycin; NB-DNJ, N-butyl-deoxynojirimycin; NN-DGJ, NN-deoxygalactonojirimycin; NN-DNJ, N-nonyl-deoxynojirimycin; NP-HPLC, normal phase-high performance liquid chromatography.

Supplementary Figure S5. Cytotoxicity of iminosugars and ribavirin in SW13 (**A**) and Vero cells (**B**). SW13 and Vero cells (assayed in technical quadruplicate) were treated with drug as indicated for 3 days prior to cell viability assessment by MTS assay. Data were normalised to untreated and represented as mean ± standard deviation.

A

B
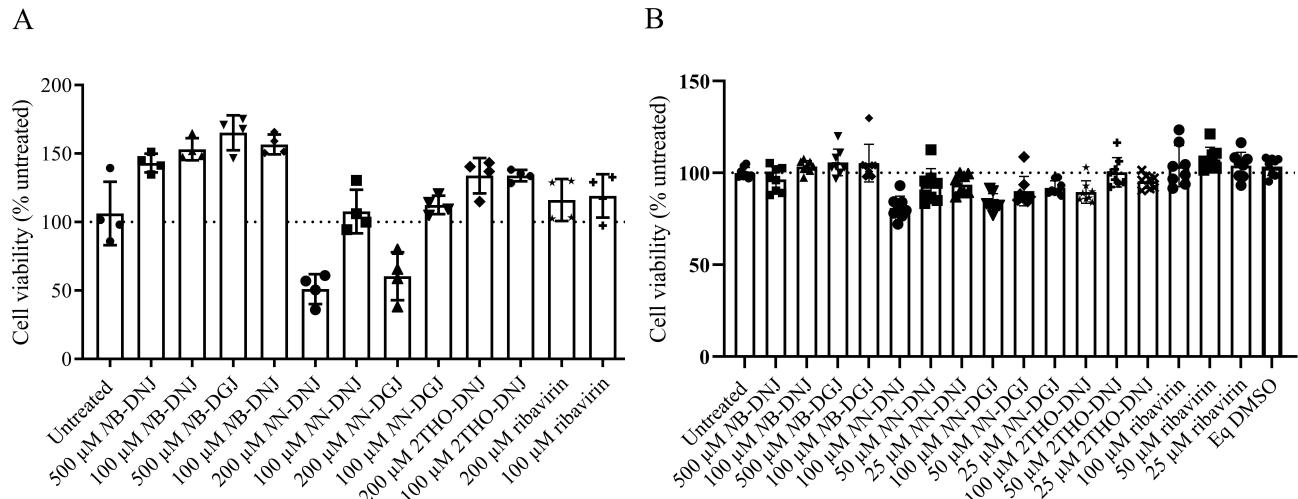
2THO-DNJ, N-8′-(2″-tetrahydrofuranyl)-octyl-deoxynojirimycin; MTS, 3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NB-DGJ, N-butyl-deoxygalactojirimycin; NB-DNJ, N-butyl-deoxynojirimycin; NN-DGJ, NN-deoxygalactonojirimycin; NN-DNJ, N-nonyl-deoxynojirimycin.

Supplementary Figure S6. Uninfected Vero cells are susceptible to iminosugar-mediated ER α-glucosidase inhibition. Vero cells (assayed in technical triplicate) were left untreated or treated with 316 μM *N*B-DNJ, *N*B-DGJ or 100 μM ribavirin for 3 days. At the end of the culture period, cells were lysed and FOS species purified and measured by NP-HPLC. (**A**) $Glc_1Man_4GlcNAc_1$ and (**B**) $Glc_3Man_7GlcNAc_2$ FOS species were detected: these are diagnostic for ER α-glucosidase II and I inhibition, respectively. These were normalised to total protein content and plotted as mean ± standard deviation.
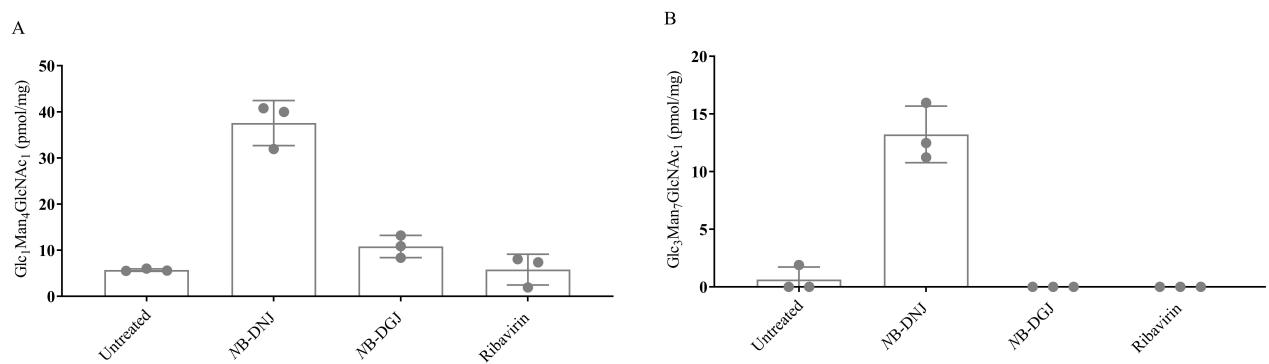
ER, endoplasmic reticulum; FOS, free oligosaccharide; *N*B-DGJ, *N*-butyl-deoxygalactojirimycin; *N*B-DNJ, *N*-butyl-deoxynojirimycin; NP-HPLC, normal phase-high performance liquid chromatography.