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Synthesis and dengue inhibition potential of new uridine derivatives: The DENV 2 inhibitors

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Abstract: Dengue is an important arboviral infection worldwide for which presently there is no specific medicine. Evidence suggests there are four serotypes of dengue virus (DENV1-4), of which DENV 2 is considered to cause the most sever dengue. Therefore, this study was aimed to develop the new uridine derivatives (NUDs) against dengue virus (DENV 2). In current study 2-(3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-((substituted cyclohexa-2,5-dienylidene)methyl)-1,2,4-triazine-3,5(2H,4H)-dione (2a-f), were obtained via reaction of substituted uridine (1) and different aromatic aldehydes separately. Synthesized NUDs were further characterized using FTIR, 1H & 13C-NMR, mass, and element analysis data. Characterized NUDs were assessed for their inhibition potential against DENV 2. Synthesized NUDs were also evaluated for their cytotoxicity towards Vero cells by MTT assay method. This investigation successfully synthesized NUDs 2a-f and reported their high inhibitory activity against DENV 2. The synthesized NUDs exhibited negligible cytotoxicity. High anti-viral activity against DENV 2 serotype and least/no cytotoxicity of NUDs suggests their importance in the treatment of dengue. Present study recommends that in future these NUDs must be investigated for their clinical importance to establish them as a choice for dengue treatment.

Keywords: Dengue, DENV 2, nucleoside, antiviral, uridine, enamines.

INTRODUCTION

Today, dengue fever is considered as major warning for half of the world's population health (Patigo *et al*., 2022). World health organization (WHO) 2023 facts sheet reported 0.1-0.4 billion cases per year with 75% of mild and asymptomatic cases (Alagasamy *et al*., 2023). Evidence suggests in humans this dengue infection occurs through flavivirus known as dengue or DENV (Uno *et al*., 2018). There exists 4 distinct serotypes of dengue such as DENV 1, 2, 3 and 4. Moreover the patients can be infected by the serotypes several times (Rimal *et al*., 2023). Among these four serotypes DENV 2 is considered to cause the most severe dengue (Fried *et al*., 2010). Fact suggests severe dengue infection as the reason for serious ailment and related deaths in some Latin American and Asian countries. Study suggests association of DENV 2 with dengue hemorrhagic fever (DHF) or severe dengue. Irony is that for dengue / severe dengue no specific treatment is available (Fried *et al*., 2010). Currently, dengue treatment is limited to fluid therapy and supportive care, which is resulting in severe economic burden of around 8.9 billion USD per annum (Shepard *et al*., 2016). Hence, there is a high need to search a potent and safer anti-dengue agent that can inhibit viral replication and in turn reduce the hospitalization and mortality rate (Lim *et al*., 2013). Although currently several efficacious anti-dengue drugs are tested in humans and are at different development stages; however, yet no drug is clinically approved for the treatment of dengue (Tayal *et al*., 2023). Some studies showed evaluation of various of nucleoside inhibitors for dengue treatment, however the safety of those drugs was a limitation for the investigators (Chen *et al*., 2015; Roche *et al*., 2013; Wiwanitkit *et al*., 2010; Chen *et al*., 2010). A study reported promising results of enamines against *Aedes aegypti* (Oliveira *et al*., 2002). Another previous study highlighted the safety of some new synthesized

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nucleosides, but the IC_{50} of new compounds in comparison to 6-azaurdine was a limitation (Alagasamy *et al*, 2023). Hence, based on the severity of dengue, problems associated with available treatment approaches, and anti-viral response of enamines and inhibitors of nucleosides, present investigation was designed to carry out synthesis, characterization and cytotoxicity and anti-dengue studies on novel uridine derivatives (NUDs). Current study offered new compounds with much higher antidengue activity and safety when compared with 6-azauridine.

MATERIALS AND METHODS

General information

For present study the reagents and chemicals were acquired from HmbG® Chemicals, Merck KGaA, Qrec Chemicals, Sigma-Aldrich and Friendemann Schmidt Chemicals. Filtration process involved use of Whattmans' filter paper No. 1. The NUDs characterization was based on the 1H-NMR and 13C-NMR (Bruker) peaks over scale of δ value in ppm using tetra methyl silane as standard and deuterated DMSO as solvent. The FTIR spectra of NUDs were scanned using Jasco 6700 spectrometer in a range from 400-4000cm^{-1}. Mass data of NUDs were noted on quadrupole orbitrap mass analyzer (Thermo Scientific). The elemental analysis was carried out on Elemental analyzer of Perkin-Elmer. The NUDs purity, was assessed by melting point utilizing SMP11 Analogue apparatus. The reactions monitoring was done with TLC (Merck Millipore) and chloroform: Methanol (0.5:9.5) under UV viewing cabinet CM-26 (Spectroline).

General Procedure for the Synthesis of 2-(3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-((substituted cyclohexa-2,5-dienylidene)methyl)-1,2,4-triazine-3,5(2H, 4H)-dione (2a-f)

The NUDs 2a-f were synthesized following the standard protocol with minor modification (Sa'ad *et al*., 2022; Aiyelabola *et al*., 2020; Thomas *et al*., 2007), briefly, an equimolar concentration of compound 1 and 3-phenoxy benzaldehyde (0.001 mol) were weighed and dissolved in 200mL of dried ethanol. Mixture was subjected to reflux 2 after addition of drops of sulfuric acid at 55°C for 8h. Obtained crude was filtered and subjected to purification by recrystallization to yield compound (2a), followed by spectrometric characterization. The scheme of synthesis for compound 2a is presented in the fig. 1. Reaction was carried out in anhydrous condition and NUDs were recrystallized through methanol and charcoal. Similarly, other compounds 2b-f were also synthesized using different substituted aromatic aldehydes, purified and characterized.

Biological activity

Cytotoxicity analysis

The cytotoxicity of synthesized NUDs 2a-f was done using MTT assay method (Zandi *et al*., 2012). Briefly, seeding of Vero cells C1008 (ATCC) was done in 96-wells culture micro plate (with density of 10000 cells per well) using 5% FBS supplemented DMEM and subjected to overnight incubation maintained with humidification at 37°C and 5% CO_2. The NUDs 2a-f were serially diluted using DMEM, followed by transfer to each well to reach a concentration ranged from 12.5µg/mL - 400µg/mL. Next, micro plates were allowed to incubate at 37°C, with 5% CO_2 for three days. After incubation, MTT solution (10 µL) was poured in the wells and further incubation in dark was done for 4h at 37°C. Next, after 4h incubation, the contents of wells were pipetted, and to respective well DMSO (100µL) was added, followed by absorbance measurement on 490 nm using 750 nm as standard wavelength and determination of percent cell viability (%) using equation (1).

$$\text{Cell viability percentage (\%)} = \frac{\text{Absorbance of treated well}}{\text{Absorbance of untreated well}} \times 100$$

In vitro anti-dengue assay of compounds 2a-f

In vitro anti-dengue potential of NUDs 2a-f against active DENV 2 was based on the standard procedure (Panda *et al*., 2021; Maryam *et al*., 2020; Okuno *et al*., 1979). Briefly, overnight incubated 24 wells plate seeded with Vero cells (having 50000 cells density per well) was infected by DENV 2 serotype (infection multiplicity = 0.1). After 3 hours of infection, the NUDs 2a-f in various concentrations were added in triplicate to the micro wells, followed by rinsing of NUDs treated cells (twice with PBS), and incubation of micro plates for three days at 37°C and 5% CO_2. After incubation, the micro plates were exposed to the two freeze-thaw cycles, followed by collection and storage of supernatants at -80 °C for further experimentation. The DENV 2 viral load was evaluated using quantitative real time polymerase chain reaction (qRTPCR) method (Low *et al*., 2021). The extraction of DENV 2 viral RNA from supernatant was done with RNA extraction kit (Favorgen Biotech) and further stored at -80°C. Next, qPCR with dye of SYBR green (Biorad) and primers for DENV 2 was developed. Sequence of forwards primer for DENV 2 was 5'-AGTTGTTAGTCT ACGTGGACCGACA whereas sequence of reverse primer was 5'-CGCCACAAGGGCCATGAACAG (251bp size). This study was done by Bio-rad CFX 96 with given conditions, such as: Activation for 15min at 95°C, 35 cycles at 95°C of 30s, 30s at 60°C, 1min at 72°C and final elongation at 72°C upto 10min. The recording of standard curves was done with the aid of qRTPCR with serial dilutions for known copies number of pure amplified product of DENV2 (10^8 to 10^3 PFU/mL). Obtained results were subjected to determination of IC_{50} of NUDs 2a-f.

STATISTICAL ANALYSIS

The resultant data was analysed using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test with multiple comparison to determine significant difference between the control group and test group using

GraphPad Prism software version 5 (GraphPad Software, Inc., CA). Data is expressed as mean ± standard deviation of the mean. Statistical significance is indicated by *p < 0.05, **p < 0.01; ***p < 0.001, ****p < 0.0001).

RESULTS

Chemistry

Experimental protocol of present investigation offered NUDs 2a-f. The resultant physical and characterization data of NUDs is given as follows.

2-(3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-((3-phenoxycyclohexa-2,5-dienylidene)methyl)-1,2,4-triazine-3,5(2H,4H)-dione (2a)

Light-brown crystal (Yield 78%, m.p. 134 ˚C); IR (cm^{-1}): 3250-3100 (hydroxyl), 3014 (Alkenyl =C-H), 2862 (Alkyl -C-H), 1694 (carbonyl), 1584 (imine); 1H-NMR (ppm) δ: 2.496 (d, 2H, H-4''), 3.355 (q, 1H, H-3'), 3.400 (m, 2H, H-5'), 3.497 (m, 1H, H-4'), 3.773 (m, 1H, H-2'), 4.007 (brs, 1H, OH-5'), 4.225 (brs, 1H, OH-2'), 4.653 (brs, 1H, OH-3'), 5.042 (s, 1H, H-6''), 5.266(q, 1H, H-3''), 5.885 (d, 1H, H-1'), 6.168 (d, 1H, H-2''), 7.061-7.706 (m, 5H, Ar'-H) 9.960 (s, 1H, =CH-N), 12.24 (s, 1H, =CH-N-N); and 13C–NMR (DMSO, ppm) δ: 29.264 (C4''), 61.970 (C5'), 70.307 (C2'), 72.257 (C3'), 84.570 (C4'), 89.309 (C1'), 105.626 (C1''), 109.112 (C6''), 115.003 (=C-N), 117.352 (C2'''&6'''), 117.907 (=CH), 119.371 (C2''), 124.070 (C4'''), 130.279 (C3'''&C5'''), 132.671 (C3''), 136.331 (C6),148.372 (C5''), 155.786 (C1'''), 166.694 (C3), 192.690 (C5); and Mass (m/z): 427. Analysis calculated for C21H21N3O7: C, 59.01; H, 4.95; N, 9.83%; Found: C, 58.98; H, 3.89; N, 9.79%.

2-(3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-((4-hydroxycyclohexa-2,5-dienylidene)methyl)-1,2,4-triazine-3,5(2H,4H)-dione (2b)

White crystals (Yield 82%, m.p. 149˚C); IR (cm^{-1}): 3250-3100 (hydroxyl), 3079 (Alkenyl =C-H), 2867 (Alkyl -C-H), 1664 (carbonyl), 1586 (imine); 1H-NMR (ppm) δ: 3.363 (m, 1H, H-3'), 3.492 (m, 2H, H-5'), 3.773 (m, 1H, H-4'), 3.995 (m, 1H, H-2'), 4.214 (m, 1H, H-4''), 4.655 (brs, 1H, OH-5'), 5.044 (brs, 1H, OH-2'), 5.268 (brs, 1H, OH-3'), 5.646 (t, 2H, H-3''&H-5''), 5.884 (d, 1H, H-1'), 6.916 (d, H, H-2''&H-6''), 7.560 (brs, 1H, OH''), 10.881 (s, 1H, =CH-N), 12.032 (s, 1H, =CH-N-N); 13C–NMR (DMSO, ppm) δ: 61.980 (C5'), 70.320 (C2'), 72.273 (C3'), 74.965 (C4''), 84.579 (C4'), 89.325 (C1'), 108.212 (C1''), 115.679 (=C-N), 119.259 (C2'''&6'''), 128.458 (C3''&C5''), 136.343 (C6), 163.354 (C3), 190.993 (C5); and Mass (m/z): 351. A Analysis calculated for C15H17N3O7: C, 51.28; H, 4.88; N, 11.96; Found: C, 51.32; 4.91; N, 12.01%.

2-(3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-((4-(dimethylamino)cyclohexa-2,5-dienylidene)methyl)-1,2,4-triazine-3,5(2H,4H)-dione (2c)

Brown (Yield 72%, m.p. 158˚C); IR (cm^{-1}): 3250-3100 (hydroxyl), 3079 (Alkenyl =C-H), 2865 (Alkyl -C-H), 1677 (carbonyl), 1569 (imine); 1H-NMR (ppm) δ: 2.496 (s, 6H, (CH3)2), 3.036 (m, 1H, H-4''), 3.373 (m, 1H, H-3'), 3.402 (m, 2H, H-5'), 3.488 (m, 1H, H-4'), 3.776 (m, 1H, H-2'), 3.990 (t, 1H, H-4''), 4.004 (brs, 1H, OH-5'), 4.641 (brs, 1H, OH-2'), 5.036 (brs, 1H, OH-3'), 5.888 (d, 1H, H-1'), 6.768 (t, 2H, H-3''&H-5''), 7.662 (d, 2H, H-2''&H-6''), 9.663 (s, 1H, =CH-N), 12.241 (s, 1H, =CH-N-N); 13C-NMR (DMSO, ppm) δ: 44.294 (NCH3), 61.970 (C5'), 62.258 (C4''), 70.320 (C2'), 72.273 (C3'), 84.579 (C4'), 89.325 (C1'), 111.078 (C1''), 115.681 (=C-N), 119.262 (C2'''&6'''), 124.536 (C3''&C5''), 136.324 (C6), 163.338(C3), 189.878 (C5) and Mass (m/z): 378. Analysis calculated for C17H22N4O6: C, 53.96; H, 5.86; N, 14.81; Found: C, 54.01; H, 5.82; N, 14.77%.

2-(3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-((4-chlorocyclohexa-2,5-dienylidene)methyl)- -1,2,4-triazine-3,5(2H,4H)-dione (2d)

White (Yield 85%, m.p. 190˚C); IR (cm^{-1}): 3250-3100 (hydroxyl), 3079 (Alkenyl =C-H), 2865 (Alkyl -C-H), 1677 (carbonyl), 1568 (imine); 1H-NMR (ppm) δ: 3.365 (m, 1H, H-3'), 3.494 (m, 2H, H-5'), 3.774 (m, 1H, H-4'), 3.996 (m, 1H, H-2'), 4.218 (m, 1H, H-4''), 4.655 (brs, 1H, OH-5'), 5.046 (brs, 1H, OH-2'), 5.269 (brs, 1H, OH-3'), 5.648 (t, 2H, H3''&H-5''), 5.888 (d, 1H, H-1'), 6.918 (d, H, H-2''&H-6''), 10.882 (s, 1H, =CH-N), 12.036 (s, 1H, =CH-N-N); 13C–NMR (DMSO, ppm) δ: 54.668 (C4''), 61.958 (C5'), 70.295 (C2'), 72.242 (C3'), 84.557 (C4'), 89.295 (C1'), 106.989 (C1''), 115.679 (=C-N), 121.116 (C2'''&6'''), 128.458 (C3''&C5''), 136.330 (C6), 163.327 (C3), 189.882 (C5); 13C-NMR (DMSO, ppm) δ: 44.294 (NCH3), 61.970 (C5'), 62.258 (C4''), 70.320 (C2'), 72.273 (C3'), 84.579 (C4'), 89.325 (C1'), 111.078 (C1''), 115.681 (=C-N), 119.262 (C2'''&6'''), 124.536 (C3''&C5''), 136.324 (C6), 163.338(C3), 189.878 (C5); Mass (m/z): 369; Analysis calculated for C15H16ClN3O6: C, 48.72; H, 4.36; Cl, 9.59; N, 11.36; Found: C, 48.75; H, 4.39; N, 11.29%.

2-(3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-((2-nitrocyclohexa-2,5-dienylidene)methyl)-1,2,4-triazine-3,5(2H,4H)-dione (2e)

Yellow (Yield 76%, m.p. 155˚C); IR (cm^{-1}): 3250-3102 (hydroxyl), 3079 (Alkenyl =C-H), 2863 (Alkyl -C-H), 1694 (carbonyl), 1607 (imine); 1H-NMR (ppm) δ: 2.496 (t, 2H, H-4''), 3.335 (m, 1H, H-3'), 3.396 (m, 2H, H-5'), 3.482 (m, 1H, H-4'), 3.769 (m, 1H, H-2'), 3.983 (brs, 1H, OH-5'), 4.204 (brs, 1H, OH-2'), 4.635 (brs, 1H, OH-3'), 5.031 (t, 1H, H-5''), 5.256 (d, 1H, H-6''), 5.881 (d, 1H, H-1'), 7.875 (t, 1H, H-3''), 10.241 (s, 1H, =CH-N), 12.240 (s, 1H, =CH-N-N); 13C-NMR (DMSO, ppm) δ: 29.458 (C4''), 61.965 (C5'), 70.302 (C2'), 72.248 (C3'), 84.565 (C4'), 89.299 (C1'), 115.679 (=C-N), 120.608 (C6''), 124.004 (C1''), 126.256 (C3''), 134.199 (C5''), 148.367 (C2''), 136.326 (C6), 163.331 (C3), 189.938

(C5); Mass (m/z): 380; Analysis calculated for C15H16N4O8: C, 47.37; H, 4.24; N, 14.73; Found: C, 47.41; H, 4.28; N, 14.66%.

2-(3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-((4-nitrocyclohexa-2,5-dienylidene)methyl)-1,2,4-triazine-3,5(2H,4H)-dione (2f)

Brown (Yield 72%, m.p. 151˚C); IR (cm^{-1}): 3250-3100 (hydroxyl), 3043 (Alkenyl =C-H), 2849 (Alkyl -C-H), 1704 (carbonyl), 1605 (imine); 1H-NMR (ppm) δ: 3.349 (m, 1H, H-3‘), 3.396 (m, 2H, H-5‘), 3.483 (m, 1H, H-4‘), 3.770 (m, 1H, H-2‘), 3.998 (m, 1H, H-4’’), 4.216 (brs, 1H, OH-5‘), 4.640 (brs, 1H, OH-2‘), 5.036 (brs, 1H, OH-3‘), 5.699 (d, 2H, H-3’’&H-5’’), 5.882 (d, 1H, H-1’), 7.562 (d, 2H, H-2’’&H-6’’), 10.154 (s, 1H, =CH-N), 12.240 (s, 1H, =CH-N-N); 13C–NMR (DMSO, ppm) δ: 61.967 (C5’), 70.307 (C2’), 72.260 (C3’), 84.565 (C4’), 86.894 (C4’’), 89.300 (C1’), 115.679 (=C-N), 130.669 (C2’’&C6’’), 124.307(C1’’), 126.963 (C3’’&C5’’), 136.334 (C6), 163.326 (C3), 192.370 (C5); Mass (m/z): 380; Analysis calculated for C15H16N4O8: C, 47.37; H, 4.24; N, 14.73; Found: C, 47.29; H, 4.21; N, 14.78%.

Biological Activity

The characterized NUDs were further evaluated for their cytotoxicity and invitro anti-dengue potential. Table 1, fig. 2 and table 2 present the cytotoxicity (cell viability) and invitro anti-dengue activity data of NUDs 2a-f.

DISCUSSION

Chemistry

The NUDs 2a-f were obtained by following the Schiffs’ reaction of 6-azauridine, with aromatic aldehydes such as: 3-phenoxy, 4-hydroxy, 4-dimethylamino, 4-chloro, 2-nitro and 4-nitro benzaldehyde respectively. Scheme given in fig. 1 shows the synthetic route to synthesize NUDs 2a-f.

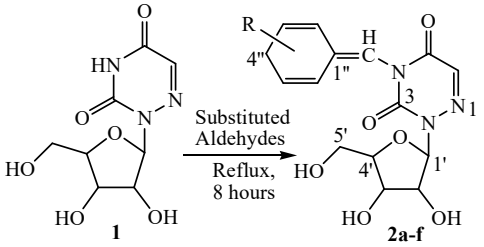

Fig. 1: Scheme for synthesis of NUD 2a-f

Synthetic scheme offered substituted enamines 2a-f in good yield. The schemes for the syntheses of enamines were based on the protocols given in the literature (Sa’ad *et al*., 2022; Aiyelabola *et al*., 2020; Thomas, 2007). The purities of NUDs 2a-f were assessed by elemental analyses, TLC and melting points. Elemental analyses data determined that in NUDs the C, H and N elements were in ±0.4% range of estimated values. NUDs 2a-f synthesis was proven with the presence of characteristic IR signal between 1704-1664 (N-C=O), 1568-1607 (C=N) and 3014-3079 (=CH); ^{1}H-NMR peaks at 9.660-10.882 (s, 1H, =CH-N); & ^{13}C-NMR peaks at 115.003-115.681 (=C-N). The proposed structures of NUDs were found in agreement with IR, 1H & 13C-NMR and mass spectra and were elucidated as per the literary facts (Sharma *et al*., 2021; Fuloria *et al*., 2013).

Biological activity

Cytotoxicity of NUDs 2a-f on Vero cells was assessed using MTT assay on 96-well micro plate. The related percent cell viability was analysed with Graph Pad Prism 9.51. Protocol for the experiment was based on the standard procedure given in the literature (Zandi *et al*., 2012). Results of the cytotoxicity study reveals the nontoxic property of NUDs 2a-f in comparison to 6-azaudridine (standard). This is based on result that on addition of NUDs 2a-f (at 12.5µg/mL dose) to Vero cells offered more than 89% cell viability, that was much better than standard (table 1 and fig. 1). The experimental results are stated as mean ± standard deviation (SD) and reperformed 3 times. Experiment data was statistically analysed using Dunnett’s test and one-way analysis of variance to test the level of significance. Statistical results are presented as the mean ± SD and significance level is indicated by p value.

Resultant cyto-toxicity data indicates high safety of NUDs 2a-f, also the present results are also in agreement with the other investigations such as study of Park research team highlighted 6-azauridine to offer cytotoxicity (Alagasamy *et al*, 2023; Park *et al*., 2020). Amongst NUDs 2a-f, the NUD 2b offered highest safety (96.7-104.5% cell viability) at all concentrations. Comparison of cytotoxicity data of NUDs 2a-f and compound 1 (standard) realized that substituting a strong electron donating group such as: Hydroxy group in cyclohexa-2,5-dienylidene) methyl group at position 4 of compound 2b offers maximum anti dengue activity and safety. This is based on a fact that during transfer of electrons from electron donating groups, the electron transport chain (ETC) flushes protons into inter membranous spaces, which generates a gradient on internal membrane of mitochondria which enzymatically results in ATP synthesis. This fueling of ETC by electron donating groups supports numerous processes required for cell viability. For example, production of reactive oxygen species, redox state, potential of membrane of mitochondria and import of protein of mitochondria etc. The anti-dengue potential of synthesized NUDs 2a-f was performed taking 6-azauridine or compound 1 as the standard (Alagasamy *et al*, 2023; Low *et al*., 2021; Hariono *et al*., 2019) and the Vero cells infected with DENV 2 serotype were exposed to different dilutions of NUDs 2a-f. From all NUDs, compound 2c offered least half minimal inhibitor concentration (IC_{50}) in comparison to standard. IC_{50} data of all synthesized NUDs is presented in table 2.

Table 1: Cytotoxicity (Cell Viability) of Vero cells after treatment of NUDs 2a-f

Concentration in µg/mL	2a	2b	2c	2d	2e	2f	Standard (compound 1)
400.0	96.8±2.39***	96.7±1.54***	93.5±4.22**	90.8±1.85	97.8±0.82***	98.7±1.99****	86.1±2.13
200.0	92.8±7.30	100.0±4.58*	96.0±1.60	88.9±1.78	96.4±8.10	94.1±6.07	83.1±8.63
100.0	99.2±1.09*	103.2±2.15**	101.0±8.24*	91.6±7.38	93.1±3.34	99.5±1.45*	86.3±5.16
50.0	90.1±2.15	104.5±6.55***	92.7±2.62	88.3±6.23	94.1±6.52	96.0±4.38*	81.7±6.27
25.0	94.3±5.09	99.4±13.48*	94.7±6.27	90.7±8.52	95.2±2.11	91.6±11.35	77.8±4.46
12.5	96.1±2.55****	103.7±4.35***	89.0±5.20****	90.8±2.43****	90.3±4.04****	91.2±3.63****	65.1±3.68

Data stated as mean ± SD with each experiment were commenced three times (n = 3). The values with superscript '*' indicate ****p<0.0001, ***p<0.001; **p<0.01 and *p<0.05.

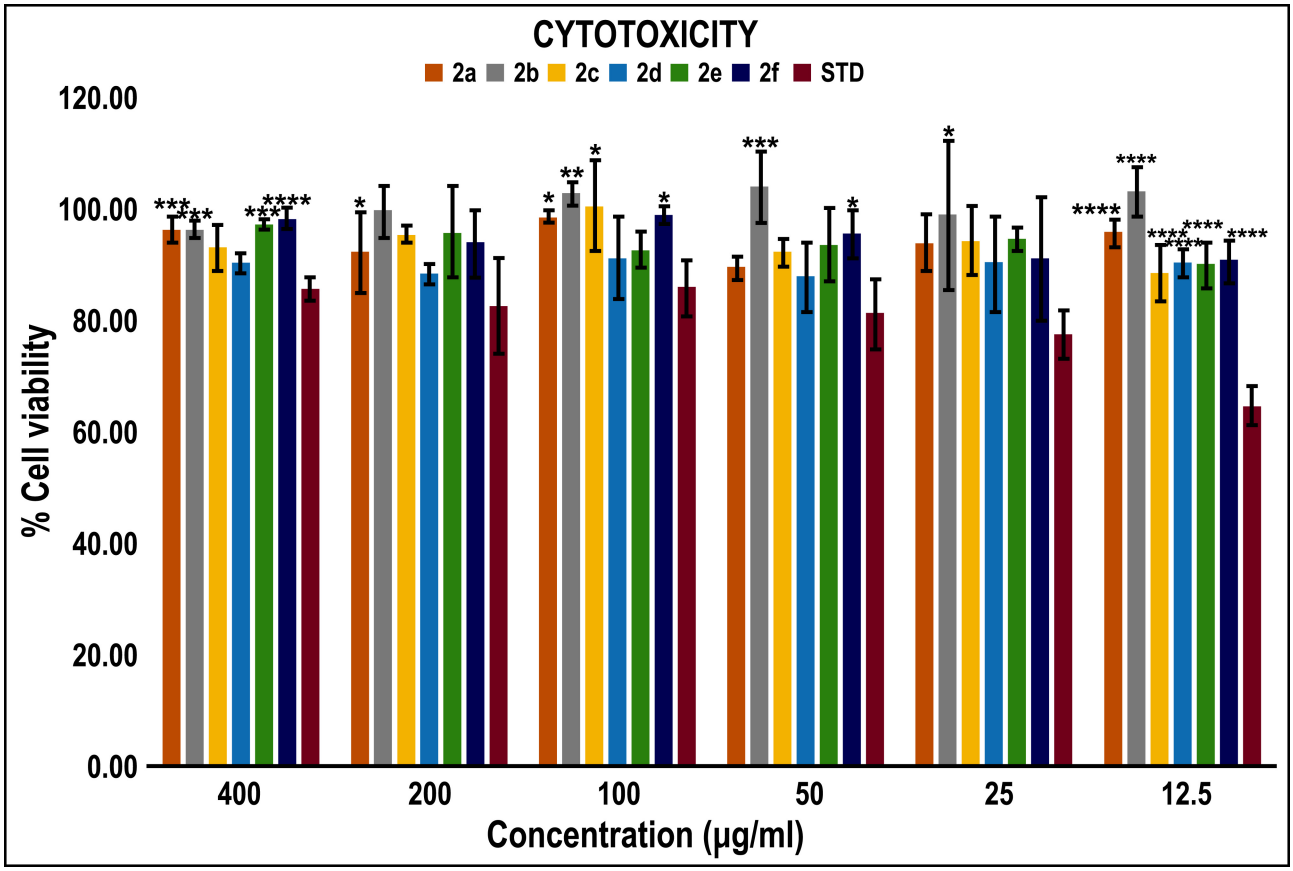

Fig. 2: Cell viability of Vero cells (C1008) after the treatment of synthesized NUDs. Where, superscript '*' indicate ****p<0.0001, ***p<0.001; **p<0.01 and *p<0.05.

Table 2: Anti-dengue activity of NUD 2a-f

NUD	IC_{50} in µg	% IC_{50}
2a	7.73	92.27
2b	65.85	34.15
2c	4.87	95.13
2d	36.74	63.26
2e	48.53	51.47
2f	65.19	34.81
6-azauridine (Standard)	6.25	93.75

Experimental data of the anti-dengue activity of NUDs 2a-f realized that substitution of electron-donating group which is dimethyl amino group at 4th position of the cyclo-hexyl ring in NUD 2c chemical structure exhibits highest DENV2 inhibition. The resultant data of both cytotoxicity analysis and anti-dengue activity over NUDs 2a-f suggests their greater anti dengue potential and safety. However, in future these NUDs must be tested for their clinical applications.

CONCLUSION

Present study successfully synthesized and characterized new uridine derivatives (NUDs) 2a-f. The *in vitro* cyto-toxicity analysis and dengue inhibition potential of NUDs concludes NUDs 2a-f to cause significant inhibition of DENV2 with an IC_{50} between 4.87-65.85µg/mL. This study claims that NUD 2c possesses better IC_{50} and safety profile when compared to 6-azauridine the standard. The high antiviral potential of NUDs against DENV2 suggests their importance plication in the dengue treatment. Study recommends that, in future further clinical in vivo experiments are required to establish these facts.

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