Original Article

Pharmacological Modulation of Flavivirus Methyltransferase Activity of Dengue Virus (DEV)

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ABSTRACT

Background: The occurrence of dengue fever plagues has expanded significantly in the course of the most recent couple of decades. Be that as it may, no immunization or antiviral treatments are accessible. In this manner, the requirement for protected and successful antiviral medications have turned out to be essential. **Aim:** Methyltransferases, with their altered enzymatic action, have been found associated with several diseases or infections. It has been observed that the dengue virus infection is correlated with the hyperactivity of methyltransferase. In the current study, we proposed to investigate the inhibitory effects of commercially available compounds on the methyltransferase activity. **Materials and Methods:** Evaluation of methyltransferase activity of the purified enzyme and their inhibitors was carried out using Methyltransferase Activity Assay Kit (Colorimetric). **Results:** Among the screened components, we identify two effective compounds that revealed significant effect against flavivirus methyltransferase activity. Further, time-dependent inhibition profiles for both compounds revealed that preincubation of fractions of Azacitidine and Zebularine for different time windows showed time-dependent inactivation of methyltransferase as the degree of inhibition increased with time. **Conclusion:** We conclude that both Azacitidine and Zebularine may be emerging as possible suitable drugs for the treatment of dengue virus infection.

Key words: Methyltransferase, Dengue virus, Inhibitor, Concentration, Time course.

INTRODUCTION

Dengue is a mosquito-borne viral illness that has turned into a noteworthy general wellbeing concern worldwide as of late. Every year, 100 million instances of dengue fever and 500,000 cases of dengue hemorrhagic fever happen.[1] At present, dengue is endemic in 112 nations around the globe.[2] Be that as it may, there is no immunization or treatment other than vector control and steady restorative consideration. The advancement of protected and successful therapeutics is subsequently direly needed. The genomic RNA encodes a solitary polyprotein that is co-and posttranslational handled by both viral and cell proteases into three essential proteins, the capsid (C), pre-membrane (prM) and envelope (E) proteins and seven non-structural proteins, the NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 proteins.[3] Since the mixes framework has appeared potential for the advancement of new atoms with anti-viral action, we chose to test five related accessible related mixes. Here we depict the screening and time subordinate anti-viral movement of two new compounds against the dengue infections (DENV) *in vitro*.[4] Pharmacological balance of MTases by little particles speaks to a novel approach to remedial mediation in malignancy and different infections.[5] Notwithstanding, because the center spaces of different MTases are saved,

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structuring inhibitors that explicitly obstruct the malady related MTase without influencing other MTases, has been testing. The capacity to objectively structure, what is more, produce specific inhibitors would have significant ramifications for improvement of new meds for some methyltransferase-intervened disease.

These outcomes are significant as a beginning stage for further explanation of the exact instruments of the antiviral action and to pick up the expected information to additionally grow new, progressively powerful and safe medications to diminish the diseases brought about by this pertinent pathogen.[6]

MATERIALS AND METHODS

Materials

Azacitidine (4-Amino-1-(β-D-ribofuranosyl)-1,3,5-triazin-2(1*H*)-one),[7] Genistein (5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), Decitabine,[8] Zebularine (2-Pyrimidone-1-β-D-riboside)[9] and Netropsin were procured from Sigma Aldrich.[10] Methyltransferase Activity Assay Kit (Colorimetric) was purchased from BioVision California, USA.[11]

Methods

In the current study, we have screened 5 compounds, namely Azacitidine, Genistein, Decitabine, Zebularine and Netropsin dihydrochloride against the activity of Flavivirus MTase of Dengue virus (DEV). The activity of methyltransferase was assayed by using Methyltransferase Activity Assay Kit (Colorimetric). This assessment is based on the principle of "kinetic evaluation of methyltransferase activity of purified enzymes and their inhibitors. The transfer of a methyl group from S-Adenosyl Methionine (SAM) cofactor to a corresponding substrate generates S-Adenosyl Homocysteine (SAH) as a product. SAH is detected by coupling the methyl transfer reaction to a multi-step enzymatic cascade, resulting in the generation of an intermediate that reacts with OxiRed™ probe. The reaction product exhibits a strong absorbance at 570 nm". All the five compounds (Azacitidine, Genistein, Decitabine, Zebularine and Netropsin) were screened at Eight different concentration (0, 10, 20, 30, 40, 80, 100, 120 μM/ml) against the activity of flavivirus methyltransferase. Further based on screening results, we carried out the time-dependent (0, 10, 20, 30, 60 and 90 mins) inhibition assay of two compounds using 50 μM/ml concentration. Screening, as well as time-dependent inhibition experimental assays, were carried out in triplicates.

Statistical analysis

All the data was processed and analyzed in GraphPad Prism and SPSS (Version 22.0). One-way ANOVA was employed to compare the average mean values of triplicates of eight different concentrations. The statistical value $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Effect of various compounds on MTase activity

Inhibition profiles for five compounds (Azacitidine, Genistein, Decitabine, Zebularine and Netropsin) Figure 1 (A,B,C,D and E) at various concentrations (0, 10, 20, 30, 40, 80, 100, 120 μM/ml) are represented in Figure 2A. Values represent the average of triplicate experiments. From the screening results, we observed that two compounds, namely Azacitidine and Zebularine, showed a more than 70% inhibition (Figure 2A). Pharmacological balance of MTase by little particles speaks to a novel approach to remedial mediation in malignancy and different infections.[5,7a,12] Our results corroborate with the results of other studies that have also highlighted the inhibitory effects of several commercially available compounds.[11,13] Other studies have revealed that inhibition of methyltransferases has a significant effect on the dengue virus infection.[13,14] In order to validate the differences between eight different concentrations, we employed the ANOVA test. Results of ANOVA showed statistically significant differences across eight different concentrations for both Azacitidine and Zebularine (Figure 2B and 2C). Similar kind of findings was reported by other Yalcin and Bayraktar.[13,15]

Time-dependent inhibition

After screening five compounds, we identify two effective compounds that revealed significant effect against flavivirus methyltransferase the activity. Time-dependent inhibition profiles for both compounds namely Azacitidine and Zebularine are represented in Figure 3A and B. The results revealed that preincubation of

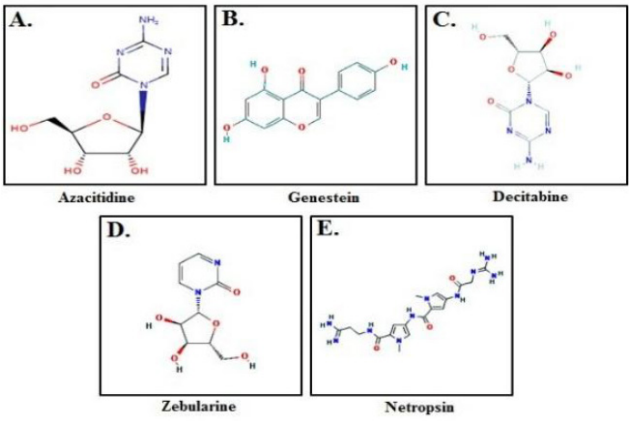

Figure 1: Chemical structure of compounds. (A) Azacitidine, (B) Genestein, (C) Decitabine, (D) Zebularine and (E) Netropsin.

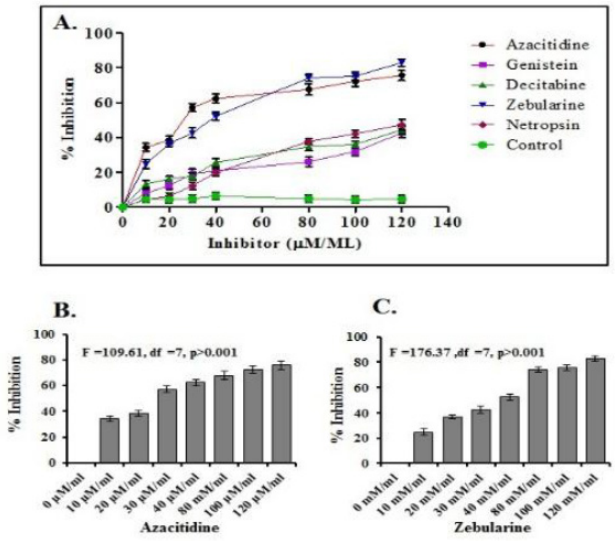

Figure 2A-C: Effect of various compounds on methyltransferase activity. Figure 2A shows the screening of compounds (Azacitidine, Genistein, Decitabine, Zebularine and Netropsin) against the activity of methyltransferase. In the graph, X-axis represents relative inhibition in percent and Y-axis shows eight different concentrations for corresponding compounds. Figure 2B and 2C represent results of one-way ANOVA; showing means differences across different concentrations.

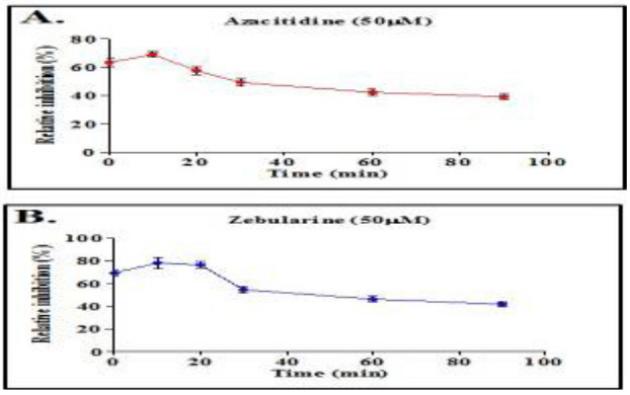

Figure 3A and 3B: Time-dependent inhibition profile of Azacitidine and Zebularine. In each graph (3A and B) X-axis represent relative inhibition in percent and Y-axis shows time course in min for corresponding compounds.

fractions of Azacitidine and Zebularine for 0, 10, 20, 30, 60 and 90 mins showed time-dependent inactivation of methyltransferase as the degree of inhibition increased with time (Figure 3A and B). Other authors such as Yalcin and Bayraktar also reported time-dependent inactivation of methyltransferase as the degree of inhibition increased with time.[11-13,15,16]

CONCLUSION

These outcomes are significant as a beginning stage for further illustration of the specific components of the antiviral movement and to pick up the expected information to additionally grow new, increasingly intense and safe medications to decrease the diseases brought about by this pertinent pathogen. The two compounds were found as the most potent inhibitors among the five different screened compounds. The inhibition performance of both compounds was significant. The relatively high concentration Azacitidine and Zebularine was thought to be responsible for the observed inhibitory effect. As a result of this study, it was observed that commercially available compounds such as Azacitidine and Zebularine could be an alternative source of medicine in the treatment of Dengue virus infection.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

DEV: Dengue virus; **C:** Capsid; **prM:** Pre-membrane; **E:** Envelope; **SAM:** S-Adenosyl Methionine **SAH:** S-Adenosyl Homocysteine.

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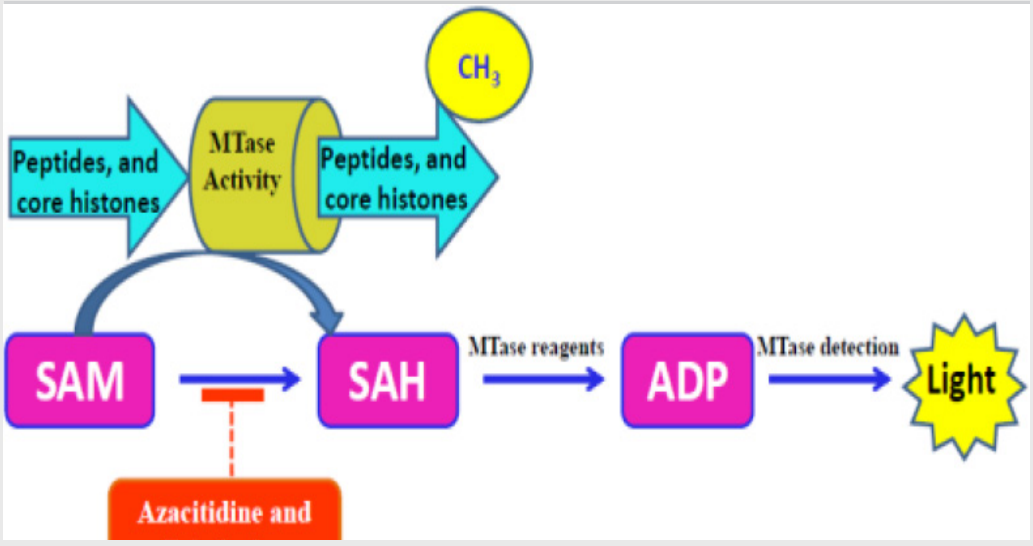
PICTORIAL ABSTRACT



SUMMARY

This investigation, clearly shows that Azacitidine and Zebularine could be an elective second, possible, of a drug in the treatment of Dengue (DENV) infection.

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