SYNTHESIS OF SOME NOVEL PERYLENE DI IMIDES AND EVALUATION OF THEIR ANTI-CANCER ACTIVITY

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ABSTRACT

Objective: The main objective of the study is to synthesis some novel perylene di imides and to evaluate for antioxidant activity and anticancer activity.

Methods: Antioxidant assay was carried out to study the reducing activity of the compounds. The cytotoxicity assay was studied to find the best potent compound among the synthesized compound by using the HCT-116, a colon cancer cell line. Synthesized substituted amine derivatives of perylene di imides. From the evaluation study Compound, A shows potent activity when compared with the standard drug 5-Fluorouracil.

Results: The results of the total antioxidant capacity assay of perylene compounds are evaluated by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) method and nitric oxide scavenging method. All the synthesized compounds are evaluated for their antioxidant power. For the results of DPPH and nitric oxide scavenging assay, Compound A, B, C and D showed potent activity when compared with the standard. For further evaluation of cell line studies, based upon the IC₅₀ values, Compound A, B and C were taken for study. The molecular modeling data's are exactly correlated with the in vitro studies. We have used 5-Fluorouracil and PIPER as a standard for in vitro study and molecular modeling study respectively.

Conclusions: From the results, Compound A will be efficient to inhibit telomerase enzyme and the Compound A will be effective for anti-cancer therapy.

Keywords: Perylene derivatives, QSAR plus, G-Quadruplex ligand database, Docking, Antioxidant study, Cell line study.

INTRODUCTION

G-Quartets are square planar arrangements of four guanine bases, which can form extraordinarily stable stacks when present in nucleic acid sequences [1,2]. Guanine-rich nucleic acids are well known for their ability to adopt non-Watson–Crick hydrogen-bonded structures [3,4]. These structures are well known as G-Quadruplexes and share the common feature of stacked guanine tetrads as basic motif [5]. G-Quadruplexes are four-stranded guanine-rich DNA structure found at the ends of the eukaryotic telomeres. Human telomeric DNA is usually 4–14-kilo bases long and is comprised of TTAGGG tandem repeats. Up-regulated telomerase activity in cancer cells maintains the length of telomeres after cell division, conferring immortality. The role of G-Quadruplex and the telomerase activity has been studied since the early 1990s [6]. G-Quadruplex stabilization leads to inhibition of the telomerase activity, which induce the apoptosis. The compounds like perylene diimide (PDI), naphthalene diimide, oxazole, are the possible intercalators acting on the G-Quartet structure and stabilize it. For example, cationic porphyrin, quindoline and berberine, and tri-substituted are O-alloxazines have been demonstrated to interfere with the oncogenic transcription in vitro. Quarfloxin, developed by cylene pharmaceuticals, entered clinical trials due to its ability to interact with G-Quadruplexes in vivo [7-10].

QSAR study has been carried out by V Life MDS Software-QSAR Plus Module to predict and compare the biological activity of standard and newly designed compounds. QSAR has been done by developing variable regression methods. The compounds are divided into training and test set compounds by manual selection, random and sphere exclusion methods. The models are developed and based on regression values; we selected eight equations to design new compounds of perylene di imides. Out of 497 compounds, 59 compounds possess better biological activity when compared with the standard compounds.

These 59 selected compounds from QSAR study has been chosen for docking study. Docking has been done by G-Quadruplex Ligand Database (G4LDB). This is an online database which was having in built tools and performed by open babel 2.3.0 to predict the binding affinity with the targets. The targets (1LIH, 1NZM, 3CES, 3SC8 and 2HR1) for the docking are selected based upon the literature survey and the selected compounds are docked. The results are visualized by Discovery studio Visualizer 4.1 Visualizer. From the results, 9 compounds are selected and the results of these compounds are visualized using Discovery Studio 4.1 Visualizer. To narrow down the results, 3 best compounds are selected and the
compounds are Compound 11, Compound 20 and Compound 48. The hydrogen bond interactions and binding free energy levels, $pK_i$ values are compared with the standard PIPER compound.

From the results of G4LDB Database, the 9 compounds are docked with the specific protein (PDB ID: 4B18), a telomerase protein. Docking has been done by Auto Dock 4.2. Finally, from the AutoDock results, Compound 11 shows good binding energy when compared with the standard PIPER compound. The study states as from the G4LDB Database 2 compounds possess good binding affinity, and from the Auto Dock Compound 11 possess good binding energy. As in our previous study from the in silico studies we studied structure activity relationship for the compounds and predicted biological activity, and by docking studies concluded the scaffold for the synthesis [11-13]. Molecular Docking Analysis was done by the output file of the docking that was generated after the study. The binding energy, Inhibition Constant, and the number of hydrogen bonds were considered for the analysis. Compound 11, 20 and 48 showed potent binding interactions with the protein 4B18 and compared with the standard PIPER.

The best compounds are compared with the standard PIPER and the binding energy values are showing more potent than the standard PIPER. The binding energy values for the compound 11, 20, 48 and PIPER are −6.23, −6.42, −6.59 and −5.59 kcal/mol respectively. From the scaffold, we selected Compound 11 as a scaffold moiety and sketched a scheme for the synthesis of Perylene di imides targeting telomerase for anti-cancer activity.

METHODS
Starting materials are all commercially available reagents and solvents used as received except for statements. All solvents were purified using standard procedures. Reactions were monitored by thin-layer chromatography on pre-coated silica gel plates and visualized using ultraviolet irradiation (254 nm). Column chromatography was performed on silica gel (100–200 Mesh).

Synthetic scheme

Procedure
Step 1: Bromination and condensation of PTCDA [14,15]
Preparation of dibromo-N,N-diamine substituted-3,4,9,10 perylene tetra carboxylic Diimides (2a,b,c and 2d)
To a solution of PTCDA compound (1.0 g, 20 mmol) was added concentrated sulfuric acid (200 mL) which was then stirred for 24 h at room temperature. The mixture was warmed to 55–60°C and iodine (0.12 g, 2.5 mmol) was added. After 5 h, bromine (3.3 mL, 62.5 mmol) was added to the mixture slowly which was then warmed to 80°C and stirred for 8 h. The mixture was quenched with ice water and filtered under reduced pressure to give bromo compound. The crude Bromo Compound (500 mg, 0.9 mmol), amines (R$_1$, R$_2$, R$_3$, and R$_4$) and acetic acid (272 mg, 4.5 mmol) in N-methyl-2-pyrrolidinone (NMP; 15 mL) were stirred at 85°C under N$_2$ for 12 h. After cooling the mixture to room temperature and pouring into aqueous HCl (10 vol.-%, 100 mL), the precipitate was separated by suction filtration, washed with deionized water until pH 7, washed and dried under vacuum. The crude product was purified by silica gel column Hexane-ethylacetate 4:1, $\nu$/c, $R_f$ = 0.71 - Compound 2a, $R_f$ = 0.61 - Compound 2b, $R_f$ = 0.42 - Compound 2c, $R_f$ = 0.61 - Compound 2d as eluent and Compound 2a, 2b 2c and 2d was obtained after evaporation of the solvent as a red powder.

Step 2: Alkylation of substituted and condensed dibromo PTCDA
Preparation of 1,7-Bis(n-diethanolamino)-N,N-disubstituted amines-3,4,9,10-perylenetetracarboxylic diimides (3a,b,c and 3d)
1,7-dibromo PDI (2ab cd and $d$ 0.1 mmol) was dissolved into 5 mL of dimethylformamide. To which diethylacetamide (0.5 mmol) and potassium carbonate (K$_2$CO$_3$, 0.5 mmol) was added. The resulted mixture was then allowed reacted at 80°C for 15 h. The reaction mixture was then powered into 15 mL water and the red solid was then re-dissolved in 20 mL dichloromethane (DCM) and washed with 1N hydrochloric acid and then water each for 3 times. Then, DCM layer was dried over Na$_2$SO$_4$. After removal of DCM, the residue was applied to chromatography with CH$_2$Cl$_2$/ethyl acetate (100:0–100:2) as eluents to afford the desired products 3a, 3b, 3c and 3d. The final synthesized compounds are tabulated in Table 1.

All the compounds are Characterize by IR, $^1$H nuclear magnetic resonance (NMR), $^{13}$CNMR and high resolution-mass spectrometry HRMS (electrospray ionization [ESI]). The results are as following.

Spectral results-Compound A Step 2: 1,7-bis(n-diethanolamino)-N,N-dibutyl-3,4,9,10-perylenetetracarboxylic diimides-Compound A

Result analysis
IR (KBr, cm$^{-1}$) Hydroxyl group (3335 cm$^{-1}$), Amide carbonyl (1710, 1700, 1620 cm$^{-1}$), Aromatic CH (3063, 2957 cm$^{-1}$), Aliphatic CH (2870 cm$^{-1}$), C=C (1591 cm$^{-1}$).
**1H NMR spectra-3a (Compound A)**

Result analysis

1H NMR (400 MHz, CDCl₃) δ 9.52 (d, J=15Hz, 2H), 8.95 (s, 2H), 8.74 (d, J=10Hz, 2H), 4.23 (t, J=10Hz, 4H), 3.89 (t, J=10Hz, 4H), 3.65 (s, 4H), 3.19 (t, J=10Hz,4H), 1.61-0.83 (m, 12H) ppm;

**13C NMR Spectra- 3a (Compound A)**

Result analysis

13C NMR (100 MHz, CDCl₃) δ 163.29, 154.93, 135.01, 131.01, 130.43, 129.06, 129.01, 127.18, 127.16, 119.41, 116.23, 61.18, 58.83, 41.85, 29.69, 19.30, 14.12 ppm;

**HRMS (ESI) spectra-3a (Compound A)**

Result analysis


Spectral Results - Compound B Step 2: 1,7-Bis(n-diethanolamino)-N,N-dinapthylamine-3,4,9,10-perylenetetracarboxylic diimides - Compound B.

**IR Spectra - 3b**

Result analysis

IR (KBr, cm⁻¹); Hydroxyl group (3336 cm⁻¹), Amide carbonyl (1712, 1696 cm⁻¹), Aromatic CH (3114, 3064, 2953 cm⁻¹), Aliphatic CH (2885 cm⁻¹), C=C (1592 cm⁻¹);

**1H NMR Spectra - 3b (Compound B)**

Result analysis

1H NMR (500 MHz, CDCl₃) δ 9.55 (d, J=15Hz, 2H), 8.88(s, 2H), 8.68 (d, J=15Hz, 2H), 7.64–7.13 (m, 14H), 4.28 (t, J=10Hz, 4H), 3.75 (t, J=5Hz, 4H), 3.63 (s, 4H) ppm;
C NMR (100 MHz, CDCl$_3$) δ 162.98, 155.52, 147.38, 141.90, 135.01, 133.58, 130.88, 128.95, 128.88, 127.91, 127.68, 127.13, 127.11, 126.50, 125.91, 123.72, 119.36, 118.20, 117.07, 62.99, 57.37 ppm;

HRMS (ESI) Spectra - 3b (Compound B)

**Result analysis**

HRMS (ESI): Anal. Calcd. for (C$_{52}$H$_{40}$N$_4$O$_8$) (M$^+$): 848.2846, Found: 848.2261.

Spectral Results=Compound C Step 2: 5,12-bis(bis(2-hydroxyethyl) amino)-2,9dihexylanthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone - Compound C.

R$_f$=0.68 [H: EtOAc/1:1];
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Result analysis

$\text{^{13}C NMR (100 MHz, CDCl}_3 \delta 162.65, 144.84, 140.82, 139.11, 137.73, 131.14, 129.25, 128.81, 127.71, 126.15, 125.68, 125.49, 124.22, 123.02, 119.53, 109.54, 61.72, 58.47, 41.58, 39.36, 32.74, 29.74, 27.11, 22.90, 14.40 ppm;}

HRMS (ESI) Spectra - 3c (Compound C)

Result analysis

HRMS (ESI): Anal. Calcd. for (C$_{32}$H$_{28}$N$_4$O$_{10}$) (M$^+$): 628.1805, Found: 628.7116.

Spectral results

Compound D Step 2: 1,7-Bis(n-diethanolamino)-N,N-dihydroxylamine-3,4,9,10-perylenetetracarboxylic diimides (3d)-Compound D.

R$_f$=0.70 (H: EtOAc/1:1);

IR Spectra - 3d

Result analysis

IR (KBr, cm$^{-1}$): Hydroxyl group (3390 cm$^{-1}$), Amide carbonyl (1727,1667 cm$^{-1}$), Aromatic CH (3057, 2956 cm$^{-1}$), Aliphatic CH (2857 cm$^{-1}$), C=C (1593 cm$^{-1}$);

$\text{^1H NMR Spectra - 3d (Compound D)}$

Result analysis

$\text{^1H NMR (400 MHz, CDCl}_3 \delta$ ppm; $\delta$ 9.55 (d, $J=10$Hz, 2H), 8.90 (s, 2H), 8.81 (d, $J=10$Hz, 2H), 4.52 (t, $J=10$Hz, 8H), 3.91 (t, $J=5$Hz, 8H), 3.67 (s, 4H), 3.38 (t, $J=10$Hz, 4H).

$\text{^{13}CNMR Spectra - 3d (Compound D)}$

Result analysis

$\text{^{13}C NMR (100 MHz, CDCl}_3 \delta$ ppm; $\delta$ 9.51 (d, $J=10$Hz, 2H), 8.94 (s, 2H), 8.73 (d, $J=10$Hz, 2), 4.22 (t, $J=10$Hz, 8H), 3.73 (t, $J=10$Hz, 8H), 3.49 (s, 4H) ppm;

HRMS (ESI) Spectra - 3d (Compound D)

Result analysis

HRMS (ESI): Anal. Calcd. for (C$_{32}$H$_{28}$N$_4$O$_{10}$) (M$^+$): 628.1805, Found: 628.7116.

429
**In vitro studies**

**Anti oxidant assay:** Free radical scavenging ability by the use of a stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (DPPH)

The effect of the perylene di imides on DPPH radical was estimated according to the procedure described by Von Gadow et al. [20]. The DPPH solution (10 mg/mL) was incubated with various concentrations of the compounds A, B, C and Standard drug 5-Fluorouracil. The percentage of cell inhibition at the highest concentration was found to be 94.53%. The standard compound 5-Fluorouracil showed 26.93 µM IC50 value of cell inhibition at the highest concentration was found to be 91.79%. Four different amines exhibited considerable Nitric oxide scavenging activity of IC50 values and this has been shown in (Table 2).

**MTT assay**

**Cytotoxicity assay**

The assay was carried out using MTT. The assay is a method to determine the cell viability in a dose-response manner. 5-Fluorouracil was taken as a standard drug for this study, by the use of a stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (DPPH). The compound was found to be 35.67 µM. The percentage of cell inhibition at the highest concentration was found to be 91.79%. The standard compound 5-Fluorouracil showed 26.93 µM IC50 value of cell inhibition at the highest concentration was found to be 94.53%. The percentage of cell inhibition at the highest concentration was found to be 94.53%. The standard compound 5-Fluorouracil showed 26.93 µM IC50 value of cell inhibition at the highest concentration was found to be 94.53%. The percentage of cell inhibition at the highest concentration was found to be 94.53%. The standard compound 5-Fluorouracil showed 26.93 µM IC50 value of cell inhibition at the highest concentration was found to be 94.53%. The percentage of cell inhibition at the highest concentration was found to be 94.53%. The standard compound 5-Fluorouracil showed 26.93 µM IC50 value of cell inhibition at the highest concentration was found to be 94.53%. The percentage of cell inhibition at the highest concentration was found to be 94.53%. The standard compound 5-Fluorouracil showed 26.93 µM IC50 value of cell inhibition at the highest concentration was found to be 94.53%.
perylene di-imides were used as a G-Quadruplex stabilizer, which can be stabilized and inactivate telomerase protection towards telomere, which promotes apoptosis. QSAR studies have been carried out using standard perylene compounds targeting telomerase by QSAR PLUS Module- Vlife Software. Around 491 compounds have been drawn using accelrys draw and studies. From the results, 59 compounds are selected for docking using G4LDB, patch Dock Server, and AutoDock by using selective G-Quadruplex targets (1NZM, 3CE5, 1LIH and 4B18).

9 compounds are selected from the three docking results based upon the binding free energy, pKi, complementary shape analysis. These 9 compounds are compared with the standard drug PIPER and the interactions are visualized by Discovery studio Visualizer 4.1. Among the 9 compounds, 2 compounds (Compound 11 and 20) possess best interactions with the receptor and may have potent inhibitory effect against the telomerase enzyme for anti-cancer activity. From the 2 compounds Compound 11 was chosen as a scaffold for synthesis. The

Table 1: Structure of novel perylene di-imides-final target molecules

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
</table>

Table 2: DPPH free radical scavenging assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control</th>
<th>200</th>
<th>400</th>
<th>600</th>
<th>800</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OD (%)</td>
<td>0.629 (0)</td>
<td>0.56 (10.97)</td>
<td>0.554 (11.92)</td>
<td>0.546 (13.2)</td>
<td>0.533 (15.26)</td>
<td>0.522 (17.01)</td>
</tr>
<tr>
<td>B</td>
<td>0.629 (0)</td>
<td>0.56 (29.09)</td>
<td>0.554 (38.79)</td>
<td>0.546 (39.9)</td>
<td>0.533 (42.77)</td>
<td>0.522 (44.2)</td>
</tr>
<tr>
<td>C</td>
<td>0.629 (0)</td>
<td>0.56 (0.16)</td>
<td>0.554 (8.43)</td>
<td>0.546 (15.58)</td>
<td>0.533 (21.14)</td>
<td>0.522 (24.96)</td>
</tr>
<tr>
<td>D</td>
<td>0.629 (0)</td>
<td>0.56 (16.22)</td>
<td>0.554 (16.69)</td>
<td>0.546 (19.55)</td>
<td>0.533 (20.67)</td>
<td>0.522 (28.93)</td>
</tr>
<tr>
<td>Std-Ascorbic Acid</td>
<td>0.169 (0)</td>
<td>0.561 (76.85)</td>
<td>0.582 (79.73)</td>
<td>0.626 (85.75)</td>
<td>0.658 (90.14)</td>
<td>0.711 (97.40)</td>
</tr>
</tbody>
</table>

DPPH: 1,1-Diphenyl-2-picryl hydrazyl

Table 3: Nitric oxide scavenging assay

<table>
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<tr>
<th>Compound</th>
<th>Control</th>
<th>200</th>
<th>400</th>
<th>600</th>
<th>800</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OD (%)</td>
<td>0.390 (0)</td>
<td>0.282 (27.69)</td>
<td>0.26 (33.33)</td>
<td>0.251 (35.64)</td>
<td>0.242 (37.95)</td>
<td>0.225 (42.31)</td>
</tr>
<tr>
<td>B</td>
<td>0.390 (0.00)</td>
<td>0.34 (12.82)</td>
<td>0.292 (25.13)</td>
<td>0.28 (28.21)</td>
<td>0.266 (31.79)</td>
<td>0.245 (37.18)</td>
</tr>
<tr>
<td>C</td>
<td>0.390 (0)</td>
<td>0.385 (1.28)</td>
<td>0.355 (8.97)</td>
<td>0.334 (14.36)</td>
<td>0.315 (19.23)</td>
<td>0.285 (26.92)</td>
</tr>
<tr>
<td>D</td>
<td>0.390 (0)</td>
<td>0.389 (0.26)</td>
<td>0.382 (2.05)</td>
<td>0.371 (4.87)</td>
<td>0.361 (7.44)</td>
<td>0.35 (10.26)</td>
</tr>
<tr>
<td>Std-Quercetin</td>
<td>0.584 (0)</td>
<td>0.464 (20.55)</td>
<td>0.324 (44.52)</td>
<td>0.236 (59.59)</td>
<td>0.157 (73.12)</td>
<td>0.082 (85.96)</td>
</tr>
</tbody>
</table>
synthesis has been designed as bromination of Perylene Di imides at position 1\textsuperscript{st} and 7\textsuperscript{th} position and then condensed with amines, finally alkylation with Diethanolamine to remove the bromine as HBr. We have synthesized four PDI prototypes and characterized by FT-IR, \textsuperscript{1}HNMR, ESI-Mass and also purified by column chromatography with a combination of solvents. We have taken a colon cancer cell line i.e. HCT-116 to screen our three ligands (Compound A, B and C) by MTT assay for understanding the cell cytotoxicity towards the cancer cells. Ligands were chosen based on the elaborate molecular modeling study to prove our hypothesis. The molecular modeling data’s are exactly correlated with the \textit{in vitro} studies. We have used 5-Fluorouracil and PIPER as a standard for \textit{in vitro} study and molecular modeling study respectively. In \textit{in vitro} study revealed that Compound A and Compound B were remarkably able to kill the cancer cells as per the hypothesis being made earlier. Compound A and Compound B showed significant activity with respect to 5-Fluorouracil. Molecular modeling study helps to open future de novo modeling of a new compound to treat cancer. Other compounds were failed to show promising cytotoxicity towards cell line. The compounds will be exposed to other human cancer cell lines to understand the molecular mechanism of the ligands for the future prototypes.

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![Table 4: Consolidated table of concentration of drugs versus cell viability % - normal Vero cells](image)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (µM)</th>
<th>Compound A</th>
<th>Compound B</th>
<th>Compound C</th>
<th>Standard Drug</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>95.13</td>
<td>98.63</td>
<td>96.86</td>
<td>98.11</td>
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<tr>
<td>2</td>
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<td>4</td>
<td>50</td>
<td>83.46</td>
<td>84.95</td>
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<tr>
<td>5</td>
<td>100</td>
<td>80.50</td>
<td>82.21</td>
<td>78.68</td>
<td>78.21</td>
</tr>
</tbody>
</table>

![Table 5: Consolidated table of concentration of drugs versus cell inhibition % - HCT-116 colon cancer cells](image)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (µM)</th>
<th>Compound A</th>
<th>Compound B</th>
<th>Compound C</th>
<th>Standard Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>6.95</td>
<td>1.30</td>
<td>9.05</td>
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<td>77.13</td>
<td>74.95</td>
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<tr>
<td>5</td>
<td>100</td>
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<td>91.79</td>
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<td>98.97</td>
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<td>6</td>
<td>IC50</td>
<td>32.37 µM</td>
<td>35.67 µM</td>
<td>57.40 µM</td>
<td>26.93 µM</td>
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</table>

The authors are thankful to Vels Institute of Science, Technology and Advanced Studies (VISTAS) and its management for providing research facilities and encouragement. The author is obliged to...
**Authors Contribution**

The authors equally contributed to the research work and preparing the manuscript.

**Conflicts of Interests**

The authors declare no conflict of interest.

**References**