NOVEL QUINOXALINE DERIVATIVES: SYNTHESIS, THERMAL, PHOTO PHYSICAL STUDIES AND BIOLOGICAL ACTIVITY

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ABSTRACT
Several studies have shown that derivatives of Isatin, Phenazine and Phenanthrenedione have a broad spectrum of biological activity. A large number of pharmacology active species can be synthesized from these precursors. Some of its derivatives are molecules with planar structure, a condition that displays the good capacity to interact with DNA by intercalation. Based on this information, we are designing synthesized N-Heteroacene derivatives and testing potential drugs as antimicrobial, antifungal and anticancer agents. The synthesized N-Heteroacene derivatives are characterized by IR, NMR, Mass spectrum, thermal and photophysical studies.

Keywords: N-Heteroacene, UV, NMR, Quinoxaline, Thermal and Biological Activity

INTRODUCTION
The chemistry of heterocyclic compounds has been a fascinating field of study for a decade. Among them, Quinoxaline, which is belonged to nitrogen-containing heteroarene compounds, which exhibits an excellent spectrum of biological activities and pharmacological applications. Important biological agents can be derived from a wide variety of substituted quinoxaline. Significant research effort has been directed towards these heterocyclic compounds, containing pharmacophore, which generally possesses biological activity and exhibits excellent clinical and therapeutic properties.

Functionalized quinoxaline moiety is present in a number of antibiotics which also work as active drugs to treat tuberculosis. A wide variety of heterocyclic quinoxaline compounds needs to be synthesized for drug delivery to cure various diseases and should possess viral and fungal activities for developing pharmacologically important derivatives.\(^1\) Both natural and synthetic compounds which possess Quinoxaline moieties constitute basic skeleton in different antibiotic drugs such as actinoleutin and levomycin, which are known to retard the growth of Gram +ve bacteria\(^2\) and act as specific drugs against various transplantable tumors.\(^3\) These derivatives possess some similarities with important antitubercular drugs and as well a wide spectrum of antibiotic drugs. In precise, they are applied to use as antitumor, antibacterial, antifungal\(^4\), antiviral\(^5\), anti-inflammatory\(^6\), anticonvulsant\(^7\), antineoplastic.\(^8\) Some of these compounds also have antimalarial and anti-HIV, anti-oxidant, antiamoebic, anthelmintics, vascular smooth muscle cell proliferation inhibitor and potent, selective activities such as AMPA antagonist, 5HT3 receptor antagonist. So, quinoxaline derivative constitutes synthetic libraries for drug discovery.\(^9\)

A novel quinoxaline compounds such as dihydro-(1H)-pyrazole analogues synthesized by “Asuncion B and performance evaluation done for biological activity. The compounds shows inhibition against anti-inflammatory and anti-oxidant activity.\(^10\) Ethyl 3-(aryl ethynyl) quinoxaline-2-carboxylates derivatives has synthesized by Hajiri et al, which exhibited anti-proliferative activities against both human non-small cell lung carcinoma and GBM cell lines.\(^11\)

New sugar conjugates of quinoxalines synthesized by Ramalingam et al and carried out in vitro studies against Mycobacterium tuberculosis and pathogenic bacteria.\(^12\) N.Ramalakshmi et al synthesized and evaluated in vitro antitubercular activities of a series of different quinoxaline derivatives.\(^13\) Reddy et al synthesized a novel quinoxaline derivative N-(4-fluorophenyl)-2-(3-oxo-3, 4-dihydroquinoxalin-2-yl) hydrazine carbothioamide were used as an anti-diabetic agent for the treatment of high sugar level.\(^14\)

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Nowadays, quinoxaline derivatives tested against HIV virus. Diphenyl quinoxaline synthesized and screened by selvam et al activity against vitro against the aids virus HIV-1. From the SAR studies, it was believed that quinoxaline analogs were always expected to show antitubercular activity. Many of the insecticides, fungicides, herbicides are constituted with quinoxaline moiety, which is important in for biological applications.

Number of reactions can easily produce substituted quinoxalines. But, conventional method of generation is the condensation of 1, 2-dicarbonyls with 1, 2 diamines in presence of suitable catalyst using various solvent systems for 24 hours and producing yields up to 85%.

**EXPERIMENTAL**

**Materials**

The following Solvents and chemicals were purchased and used as such without purification. 2,3-Diamino phenazine, Dibromo phenanthrene dione, 6-bromo-1-hexylindoline-2,3-dione (DBD), Ethanol, Acetic acid, Diethyl ether, DMF, THF, Hexane.

**Instrumental Methods**

UV-Vis spectroscopy was taken from LMSP-UV 1900 S double beam UV-Vis spectrophotometer. The wavelength scanned from 190 nm to 1100 nm. FI-IR spectroscopy was recorded from ITRacer-100-Fourier-Transform infrared spectrometer. NMR spectrum was recorded using Brucker Avance-3-500MHz. Chemical shifts are measured in Dimethyl sulphoxide-d6 and tetramethylsilane is used as an internal standard and the signals were recorded as s (singlet), d (doublet), m (multiplet). Mass spectral analysis were carried out by Mass-LC-MS-ESI-(2020) Shimadzu, Japan. Thermo gravimetric analysis was done on a REGULUS-TGA-DSC thermal analyser instrument under nitrogen atmosphere at heating rate of 10° C/min from room temperature to 700°C with samples mounted on a alumina sample holder.

**Synthesis of 2,7-dibromodibenzo[a,c]quinoxalino[2,3-i]phenazine(A)**

2, 7-dibromophenanthrene-9, 10-Dione and substituted 2,3 phenazinediamine were taken in equimolar quantities in 250ml round bottom flask and refluxed in acetic acid for 24 hrs at 90°C. Finally, the reaction mixture was filtered, washed with water and dried. The product appeared as a dark brown color powder. Molecular formula C_{26}H_{12}Br_{2}N_{4}, Yield 90%.

**Scheme-1**


6-bromo-1-hexylindoline-2,3-dione and 2,3 Phenazinediamine were taken in equimolar quantities in 250ml R.B flask and refluxed in acetic acid for 24 hrs at 90°C. Finally, the reaction mixture were filtered, washed with water and dried. The Product appeared as a brown color powder. Molecular formula C_{28}H_{22}BrN_{5}, Yield 87%.

**Minimum Inhibitory Concentration**

The MIC of the quinoxaline derivatives was assessed using the standard broth microdilution method. In this procedure, the highest concentration used was 5mM, as suggested by the results from the disc diffusion method. The concentration was reduced in a gradient fashion such as 5mM, 2.5mM, 1.25mM, 0.625mM and 0.312mM using a serial dilution of the test compound. 50ul of the bacterial suspension at 1x10^7 cfu/ml
was added with 50ul of the test compound and the total volume was made up to 200 µl with LB broth. The microtiter plate was incubated at 37°C for 48 hours. The bacterial growth inhibition was assessed by adding 40 µl of INT dye and again the plate was incubated at 37°C for 30 minutes until the formazan crystals were formed which was diluted using DMSO. The results were analysed by observing the plate at 570nm for enumeration of the bacterial cells that are inhibited based on the colour of the INT.

\[ \text{Reflux, 80°C} \]

\[ \text{6-bromo-1-hexylindoline-2,3-dione} \]

\[ \text{phenazine-2,3-diamine} \]

\[ \text{3-bromo-5-hexyl-5H-indolo[2',3':5,6]pyrazino[2,3-b]phenazine} \]

Scheme-2

RESULTS AND DISCUSSION

Characterization Techniques: UV-Vis Spectroscopy

The UV-Vis spectrum of the synthesized quinoxalines in Fig.-1 was measured in the tetrahyrafuran. From the UV-Visible spectrum of both A and B it is clearly seen that both compounds absorb strongly in the UV-Visible portion of the spectrum. Figure-1 shows UV-Visible spectrums of the as-synthesized compounds A and B. In the case, the compound A, the absorption maximum for compound A is around 252 nm, because of \( \pi-\pi^* \) transitions.

\[ \text{Fig.-1: UV-Vis Spectrum of (a) Compound A; (b) Compound B} \]

The peak around 442 nm is because of \( n-\pi^* \) transitions in which the lone pair of electrons in the nitrogen atom is orthogonal to the planar pi-electron system. For compound B, the same \( \pi-\pi^* \) transition was observed at 252 nm and absorption maximum observed at 397nm, due to the \( n-\pi^* \) transition from the extended conjugation of the planar molecular backbone.

FTIR Spectroscopy

Table-1: The Stretching Frequency for the Different Functional Group are tabulated form the IR Spectra of Compounds A and B

<table>
<thead>
<tr>
<th>Functional Groups</th>
<th>Frequencies, Cm(^{-1})(A)</th>
<th>Frequencies, Cm(^{-1})(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>=C-H str</td>
<td>3091</td>
<td>2925</td>
</tr>
<tr>
<td>-C=C- str</td>
<td>1671</td>
<td>1598</td>
</tr>
<tr>
<td>=C-H ben</td>
<td>1458</td>
<td>1457</td>
</tr>
<tr>
<td>-C=N ben</td>
<td>1077</td>
<td>1124</td>
</tr>
<tr>
<td>=C-Br ben</td>
<td>705</td>
<td>754</td>
</tr>
</tbody>
</table>

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NMR Spectroscopy

Figure-3 displays the $^1$HNMR spectrum of compounds A and B. In the case of compound A, many peaks appeared as a multiplets in the range δ 7.67-8.82, because of polycyclic aromatic protons. The absence of other peaks from δ 5.0-1.0 indicates that no aliphatic protons are present in the compound and confirmed the expected structure. In Compound B, aromatic proton produce peaks in the range δ 7.34-8.52. The peaks appeared around δ 1.0-5.0, because of aliphatic protons present in the side, which are appeared as multiplets due to coupling between the adjacent protons.

Mass Spectral Data Analysis

Mass spectrums of both compounds in Fig.-4 and 5 clearly indicates the molecular mass of the synthesized compounds A and B and it is consistent with the expected molecular weight. Further, proves that both are bromine substituted Compounds which produce (M+2) peaks, in which compound A is capable to produce more fragment ions. When compared to Compound A, B is provide intense molecular ion peak.
Thermal Analysis
The results indicated that both the derivatives show nice thermal stability. Compound A is started to decompose around 120°C. After that, sudden loss in weight will be expected, due to removal of quinoxaline moiety and above the temperature of 200°C only slight change in the loss of weight. Compared with compound A, B shows excellent thermal stability up to 250°C. Further decrease in weight is expected due to removal of indole moiety from compound B.
Antibacterial Activity
By using disc diffusion method, the anti-bacterial activity of the synthesized quinoxaline derivatives with Gentamycin as the reference antibiotic (40μg/ml). The synthesized compounds were tested against a Gram +ve bacterial strain Staphylococcus aureus. The outcome of the results is shown in Table-1. It is evident from the results that, the derivatives exhibited the highest activity against the tested bacteria when compared to the positive control of the assay.

Table-2: The Antibacterial Activity of Compound A and B are Tabulated with the Staphylococcus aureus.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mM</td>
<td>0.325</td>
<td>0.465</td>
</tr>
<tr>
<td>2.5mM</td>
<td>0.453</td>
<td>0.488</td>
</tr>
<tr>
<td>1.25mM</td>
<td>0.495</td>
<td>0.495</td>
</tr>
<tr>
<td>0.62mM</td>
<td>1.32</td>
<td>1.246</td>
</tr>
<tr>
<td>0.31mM</td>
<td>1.336</td>
<td>1.356</td>
</tr>
</tbody>
</table>

Table-3: The Zone of Inhibition of Compound A and B with Positive Control are Tabulated

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>32±1.3</td>
</tr>
<tr>
<td>A</td>
<td>28±1.2</td>
</tr>
<tr>
<td>B</td>
<td>29±0.35</td>
</tr>
</tbody>
</table>

Fig.-7: Zone of inhibition against Staphylococcus aureus.

MIC
It is defined as the least concentration required to complete suppression of bacterial growth. The MIC values of all the samples were found to be 1.25mM, at which the samples inhibited the bacterial growth lower than the initial inoculum. The bacterial growth seemed to have resumed their growth after 1.25mM concentration which suggests that both the derivatives are bacteriastically active against the bacterial test strain.

Fig.-8: MIC of Quinoxaline Derivatives against Staphylococcus aureus
Cytotoxic Assay

Fig.-9: The Plot of % of Cell Viability of Compounds A and B

Table-4: The Cell Viability Results are tabulated for Compounds A and B against the HeLa Cancer Cell Lines.

<table>
<thead>
<tr>
<th>Conc (mM)</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>20.31</td>
<td>17.23</td>
</tr>
<tr>
<td>5</td>
<td>24.68</td>
<td>20.87</td>
</tr>
<tr>
<td>2.5</td>
<td>26.97</td>
<td>25.16</td>
</tr>
<tr>
<td>1.25</td>
<td>31.54</td>
<td>27.09</td>
</tr>
<tr>
<td>0.62</td>
<td>38.76</td>
<td>28.13</td>
</tr>
<tr>
<td>0.31</td>
<td>41.91</td>
<td>30.61</td>
</tr>
<tr>
<td>0.15</td>
<td>42.67</td>
<td>35.67</td>
</tr>
<tr>
<td>0.07</td>
<td>43.65</td>
<td>37.28</td>
</tr>
</tbody>
</table>

CONCLUSION

We have successfully synthesized two novel Quinoxaline based N-heteroacene derivatives via the simple condensation reaction of diketones with dicarbonyl compounds and confirmed in both NMR and Mass data analysis. These compounds showed excellent photophysical and thermal properties. The antibacterial activity of these compounds was evaluated then the minimum inhibitory concentration (MIC) was determined. The results showed that the two compounds showed a concentration-dependent decrease in cell viability. Both compounds A and B tested against HeLa cancer cell lines. Compound A showed the highest viability, followed by compound B. The presence of two bromine atoms along with four nitrogen atoms is more advantageous. Further, this makes the compound more electron-deficient in nature than B, which is expected to be the reason behind this outcome.

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