Original Research Article

Synthesis and Anti tubercular activity of some new N’-(3-hydroxy-3, 4-dihydroquinoxaline-2-carbonyl)-N-phenylcarbamimidic acid derivatives

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A B S T R A C T

A new series new N’-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl)-N-phenylcarbamimidic acid derivatives were designed and synthesized. The newly synthesized compounds were evaluated for their anti-tb activity. The structure of the synthesized compounds was confirmed by elemental analysis and spectral data (IR, ¹H NMR and Mass). The data obtained from biological screening revealed that; synthesized compounds showed the good to moderate anti-tb activities.

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1. Introduction

Quinoxaline derivatives show a wide range of biological activities, namely antimicrobial,¹–³ cytotoxic, anti-tubercular, anxiolytic, anti-HIV, antioxidant, anti-inflammatory,⁴,⁵ antimalarial, anticancer, antidepressant, antibacterial, and antifungal. Antibiotics, such as echinomycin, levomycin, and actinoleutin, are known to inhibit the growth of Grampositive bacteria and are active against various transplantable tumors,⁵,⁶ as well as rheumatoid arthritis, hemangioma, and Kaposi’s sarcoma. Dihydroxy quinoxaline and their derivatives constitute an important class of organic compounds with diverse agricultural, industrial, and biological activities.⁷,⁸

2. Experimental Section

The chemicals used were standard grade they were used without any further purification. Melting points were determined on a bache apparatus and are uncorrected. And Infrared spectra were recorded on Shimadzu FTIR instrument. NMR spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.00 MHz (1H) with TMS as internal standard. All chemical shifts (δ) were reported in ppm with Tri Methyl Silane as internal standard. The homogeneity of the compounds was checked using precoated TLC plates.

3. Methodology

3.1. General procedure for synthesis of N’-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl) carbamimidic acid

A new series of N’-(3-hydroxy-3, 4-dihydroquinoxaline-2-carbonyl)carbamimidic acid derivatives were designed and synthesized. Starting with ortho-phenylenediamine by its reaction with alloxane with methyl alcohol at room temperature for 10 hrs to afford N’-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl) carbamimidic acid.

3.2. Derivatives of N’-(3-hydroxy-3, 4-dihydroquinoxaline-2-carbonyl) carbamimidic acid

N’-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl)carbamimidic acid (500 mg, 2.45 mmol, 1 eq) in EtOH (10 mL) 2-methylbenzaldehyde (309 mg, 2.57 mmol, 1.05 eq.) and catalytic amount of conc. HCl

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were added at RT and the reaction mixture was stirred at RT for 1 hr. After completion of the reaction (checked by TLC). The mixture was diluted with water, basified with sat. aq. NaHCO₃ and resultant solid was filtered, washed with water and dried. The structure of the compound was confirmed by ¹H NMR and LC-MS data. Other members of this series were also prepared using this procedure. Yield: 600 mg (80%); Yellow solid.

3.3. Scheme

\[
\text{N'-(3-hydroxy-3, 4-dihydroquinoxaline-2-carbonyl) carbamimidic II (a) acid.}
\]
M.p.: 325–327°C, Yield 85%, ¹HNMR (DMSO-d₆, 400 MHz): δ = 8.62 (s, 1H), 8.30 (d, 2H), 8.14 (s, 1H), 7.82 (t, 2H), 5.21 (brs, 2H).

\[
\text{N'-(3-hydroxy-3, 4-dihydroquinoxaline-2-carbonyl) carbamimidic II (b) acid.}
\]
M.p.: 380–383°C, Yield 89% ¹HNMR (DMSO-d₆, 400 MHz): δ = 8.84 (s, 1H), 8.24 (d, 2H), 8.11 (s, 1H), 7.98 (s, 1H), 7.81 (t, 2H), 7.51 (d, 2H), 7.23 (m, 3H).

\[
\text{N'-(3-hydroxy-3, 4-dihydroquinoxaline-2-carbonyl) carbamimidic II (c) acid.}
\]
M.p.: 417–419°C, Yield 89% ¹HNMR (DMSO-d₆, 400 MHz): δ = 8.88 (s, 1H), 8.26 (d, 2H), 8.10 (s, 1H), 7.99 (s, 1H), 7.83 (t, 2H), 7.65–7.80 (m, 4H).

\[
\text{N'-(3-hydroxy-3, 4-dihydroquinoxaline-2-carbonyl) carbamimidic II (d) acid.}
\]
M.p.: 422–425°C, Yield 83% ¹HNMR (DMSO-d₆, 400 MHz): δ = 8.86 (s, 1H), 8.25 (d, 2H), 8.11 (s, 1H), 8.00 (s, 1H), 7.80 (t, 2H), 7.76 (d, 2H) 7.66 (d, 2H).

\[
\text{The antimycobacterial activities of compounds 6(a-h) were assessed against M. tuberculosis ATCC 2729415 using the micro plate Alamar Blue assay (MABA) 16. This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods.}
\]

3.4. Procedure for Anti-TB activity using alamar blue dye

1. The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).
2. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.
3. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
4. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
5. The final drug concentrations tested were 100 to 0.2 µg/ml.
6. Plates were covered and sealed with parafilm and incubated at 37°C for five days.
7. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
8. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
9. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Standard Strain used: Mycobacteria tuberculosis (Vaccine strain, H37 RV strain): ATCC No- 27294.

Standard values for the Anti-Tb test which was performed.

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrazinamide</td>
<td>3.125 µg/mL</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3.125 µg/mL</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>6.25 µg/mL</td>
</tr>
</tbody>
</table>

4. Conclusion

All of the derivatives tested were active against the M. tuberculosis in different concentration, among all the sample the best results were observed in the compounds II (c) (6.25 µg/mL) and 6h (6.25 µg/mL). The compounds II(a) (12.5 µg/mL), II(b), (12.5 µg/mL), II(d) (12.5 µg/mL) and II(f) (12.5 µg/mL) were shown moderate sensitivity. While the compounds II(e) (100 µg/mL) was shown least sensitivity against M. tuberculosis when compared with first line drugs as Pyrazinamide (3.12µg/mL), Ciprofloxacin (3.12 µg/mL) and Streptomycin (6.25 µg/mL).
Table 1:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>100 µg/ml</th>
<th>50 µg/ml</th>
<th>25 µg/ml</th>
<th>12.5 µg/ml</th>
<th>6.25 µg/ml</th>
<th>3.12 µg/ml</th>
<th>1.6 µg/ml</th>
<th>0.8 µg/ml</th>
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<td>S</td>
<td>S</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
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<td>II (b)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
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<td>S</td>
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<tr>
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<td>S</td>
<td>R</td>
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<tr>
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<td>II (e)</td>
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<td>II (f)</td>
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<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
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</table>

It suggests that this class of compounds may be selectively targeted to M. tuberculosis Growth, also considering that they were not cytotoxic to host cells at the same concentration and could be a good starting point to find new lead compounds.

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References


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