Original Research Article

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

Shripad Potadar^{1,*}, Ramaling Kotnal¹¹BLDEA'S SSM College of Pharmacy & Research Centre, BLDE University Campus, Vijayapura, Karnataka, India

ARTICLE INFO

Article history:

Received 10-06-2020

Accepted 19-06-2020

Available online 12-08-2020

Keywords:

Quinoxaline

Orthophenylenediamine

Alloxane and antimycobacterial

ABSTRACT

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.

© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC license (<https://creativecommons.org/licenses/by-nc/4.0/>)

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)lcarbamimidic acid derivatives were designed and synthesized. Starting with ortho-phenylenediamine by its reaction with alloxane with methyl alcohol at room temprecture 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

* Corresponding author.

E-mail address: shripad.potadar@gmail.com (S. Potadar).

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. NaHCO_3 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

$\text{N}'\text{-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl) carbamimidic II (a) acid}$.

M.p.: 325–327°C, Yield 85%, ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.62 (s, 1H), 8.30 (d, 2H), 8.14 (s, 1H), 7.82 (t, 2H), 5.21 (brs, 2H).

$\text{N}'\text{-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl) carbamimidic II (b) acid}$.

M.p.: 380–383°C, Yield 89% ^1H NMR (DMSO- d_6 , 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).

$\text{N}'\text{-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl) carbamimidic II (c) acid}$.

M.p.: 417–419°C, Yield 89% ^1H NMR (DMSO- d_6 , 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).

$\text{N}'\text{-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl) carbamimidic II (d) acid}$.

M.p.: 422–425°C, Yield 83% ^1H NMR (DMSO- d_6 , 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).

$\text{N}'\text{-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl) carbamimidic II (e) acid}$.

M.p.: 397–399°C, Yield 80% ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.88 (s, 1H), 8.26 (d, 2H), 8.11 (s, 1H), 8.00 (s, 1H), 7.82 (t, 2H), 7.78 (m, 2H) 7.68 (d, 2H).

$\text{N}'\text{-(3-hydroxy-3,4-dihydroquinoxaline-2-carbonyl) carbamimidic II (f) acid}$.

M.p.: 390–392°C, Yield 84% ^1H NMR (DMSO- d_6 , 400 MHz): δ = 8.87 (s, 1H), 8.25 (d, 2H), 8.11 (s, 1H), 8.00 (s, 1H), 7.84 (t, 2H), 7.80 (m, 2H) 7.70 (m, 2H).

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, 200 μl 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 $\mu\text{g}/\text{ml}$.
6. Plates were covered and sealed with parafilm and incubated at 37°C for five days.
7. After this time, 25 μl 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.

Pyrazinamide- 3.125 $\mu\text{g}/\text{ml}$

Ciprofloxacin-3.125 $\mu\text{g}/\text{ml}$

Streptomycin- 6.25 $\mu\text{g}/\text{ml}$

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 $\mu\text{g}/\text{mL}$) and 6h (6.25 $\mu\text{g}/\text{mL}$). The compounds II(a) (12.5 $\mu\text{g}/\text{mL}$), II(b), (12.5 $\mu\text{g}/\text{mL}$), II(d) (12.5 $\mu\text{g}/\text{mL}$) and II(f) (12.5 $\mu\text{g}/\text{mL}$) were shown moderate sensitivity. While the compounds II(e) (100 $\mu\text{g}/\text{mL}$) was shown least sensitivity against *M. tuberculosis* when compared with first line drugs as Pyrazinamide (3.12 $\mu\text{g}/\text{mL}$), Ciprofloxacin (3.12 $\mu\text{g}/\text{mL}$) and Streptomycin (6.25 $\mu\text{g}/\text{mL}$).

Table 1:

S. No.	Sample	100 μg/ml	50 μg/ml	25 μg/ml	12.5 μg/ml	6.25 μg/ml	3.12 μg/ml	1.6 μg/ml	0.8 μg/ml
01	II (a)	S	S	S	S	R	R	R	R
02	II (b)	S	S	S	S	R	R	R	R
03	II (c)	S	S	S	S	S	R	R	R
04	II (d)	S	S	S	S	R	R	R	R
05	II (e)	S	R	R	R	R	R	R	R
06	II (f)	S	S	S	S	R	R	R	R

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

5. Acknowledgement

One of the authors Shripad Potadar is thankful to, Management and Principal of BLDEA's SSM College of Pharmacy and research centre and Vice chancellor of BLDE (Deemed to be) University Vijayapur for support and encouragement.

References

1. Das PJ, Sarkar S. An efficient synthesis of quinoxalines in water mediated by tetraethylammonium bromate. *Int J Chem Res.* 2011;3:56–60.
2. Mohammad VS, Saeed B. Sulfonated organic salts: Recyclable green catalysts for the facile and rapid route synthesis of 2, 3-disubstituted quinoxaline derivatives in Water. *World Appl Sci J.* 2013;21:394–401.
3. Antonioti S, Donach E. Direct and catalytic synthesis of quinoxaline derivatives from epoxides and ene-1,2-diamines. *Tetrahedron Lett.* 2002;43(22):3971–3.
4. Bhosale RS, Sarda SR, Ardhapure SS, Jadhav WN, Bhusare SR, Pawar RP. An efficient protocol for the synthesis of quinoxaline derivatives at room temperature using molecular iodine as the catalyst. *Tetrahedron*

Lett. 2005;46(42):7183–6.

5. Arnowski TZ, Kleinrok Z, Turski WA, Czuczwar SJ. 3-dihydroxy-6-nitro-7 sulfamoylbenzo-(F)quinoxaline enhances the protective activity of common antiepileptic drugs against maximal electroshock-induced seizures in mice. *Neuropharmacol.* 1993;2:895–900.
6. Abu-Hashem AA, Gouda MA, Badria FA. Synthesis of some new pyrimido[2',1':2,3]thiazolo[4,5-b]quinoxaline derivatives as anti-inflammatory and analgesic agents. *Eur J Med Chem.* 2010;45(5):1976–81.
7. Wagle S, Adhikari AV, Kumari NS. Synthesis of some new 4-styryltetrazolo[1,5-a]quinoxaline and 1-substituted-4-styryl. *Eur J Med Chem.* 2009;44:1135–43.
8. Husain A, Rashid M, Akhter A, Mishra R, Gupta D. Design, synthesis and pharmacological activities of novel N-1-2-(substituted phenyl)-4-oxo-1,3-thiazolan-3-yl-2,2-diphenyl-acetamides. *Int J Pharm Sci Rev Res.* 2010;5(2):102–6.

Author biography

Shripad Potadar Assistant Professor

Ramaling Kotnal Principal

Cite this article: Potadar S, Kotnal R. Synthesis and Anti tubercular activity of some new N'-(3-hydroxy-3, 4-dihydroquinoxaline-2-carbonyl)-N-phenylcarbamidic acid derivatives. *Int J Pharm Chem Anal* 2020;7(2):66-68.