Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
PubMed (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2018: 88.52
Volume 8, Issue 6: November-December: 2019, 118-122

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

The Synthesis and Evaluation of anti-tubercular activity for some new 4-{4-[(hydrazinylmethoxy)(hydroxy)methyl]phenyl}-3-methyl-3,4dihydro quinoxalin -2(1*H*)-one novel Derivatives.

Shripad Potadar* & Ramling Kotnal
BLDEA'S SSM College of Pharmacy & Research Centre, BLDE University Vijayapur-586101

Article Info: Received 22 November 2019; Accepted 28 December. 2019
DOI: https://doi.org/10.32553/jbpr.v8i6.703
Corresponding Author: Shripad Potadar
Conflict of interest statement: No conflict of interest

ABSTRACT:
A new series of 4-{4-[(hydrazinylmethoxy)(hydroxy)methyl]phenyl}-3-methyl-3,4dihydro quinoxalin -2(1*H*)-one were designed and synthesized in order to evaluate 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.
Keywords: Quinoxaline, ortho-phenylenediamine and hydrazinehydrate.

Introduction:

Heterocycles compounds are used in many various industries[1]. However most of hetero cycle compounds aren't extracted from nature source, but are synthesized. Almost all alkaloids that are used as drugs are formed from hetero aromatic molecules. Because these compounds cause to cancer, these chemicals must be removed from output materials of smokestack in factories[2-3]. Quinoxaline derivatives are an important class of compounds that find use in medicinal chemistry[4-5]. For example, quinoxaline is a part of various antibiotics such as echinomycin, levomycin, and actinoleutin that are known to inhibit growth of gram positive bacteria[6], and are active against various transplantable tumors[7].

Numerous methods are available for the synthesis of quinoxaline derivatives which involve condensation of 1,2-diamines with α-diketones[8], 1,4-addition of 1,2- diamines to diazenylbutenes, cyclization-oxidation of phenacyl bromides and oxidative coupling of epoxides with ene-1,2-diamines[09]. 2,3-Disubstituted quinoxalines have also been prepared via the Suzuki- Miyaura coupling reaction[10], condensation of *o*-phenylenediamines with 1,2-dicarbonyl compounds in MeOH/AcOH under microwave irradiation[11], and iodine catalyzed cyclocondensation of 1,2-dicarbonyl.

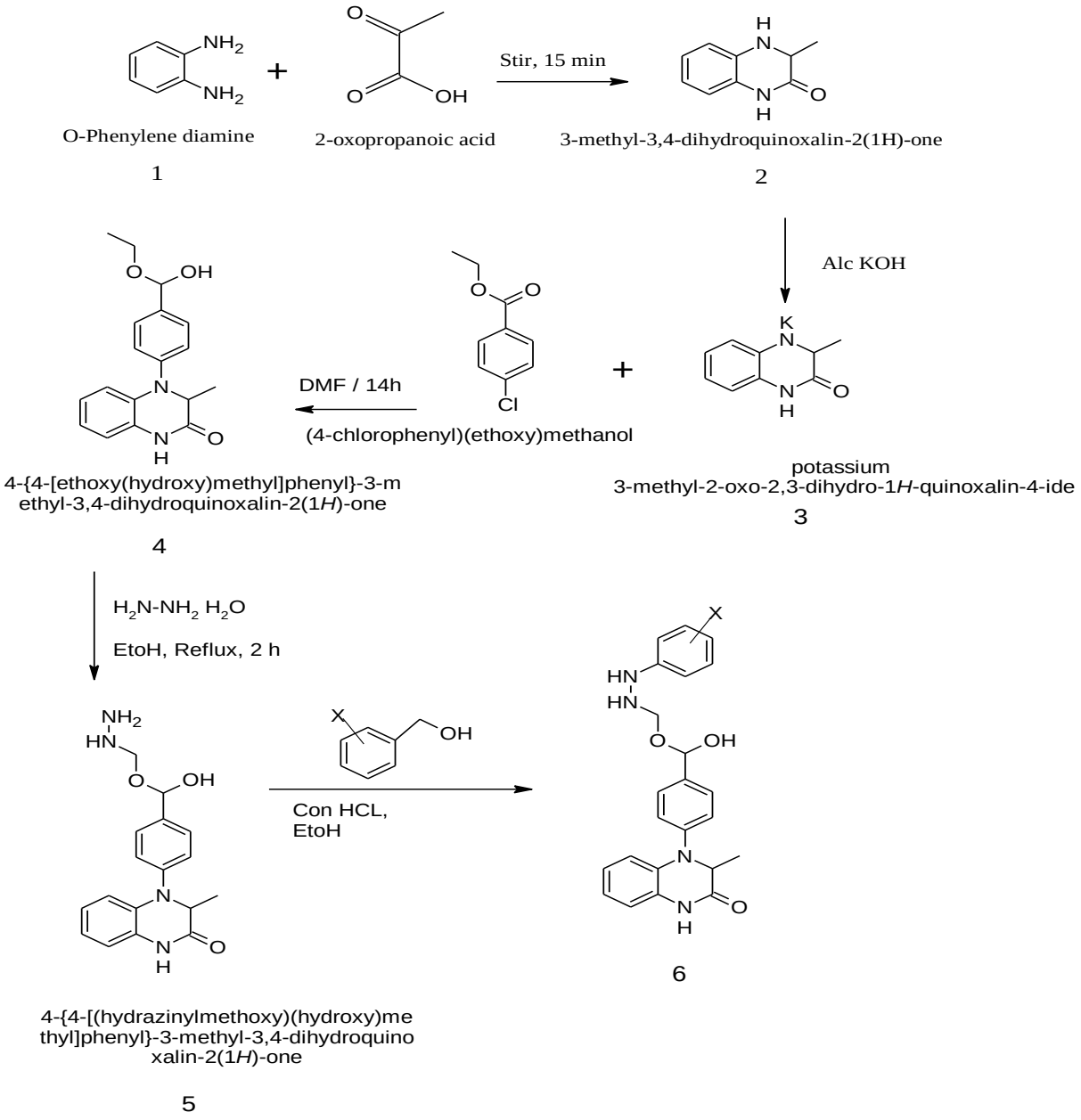
Experimental Section

The chemicals used were standard grade they were used without any further purification. Melting points were determined on a Buchi 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.

Methodology

A new series of 4-{4-[(hydrazinylmethoxy)(hydroxy)methyl]phenyl}-3-methyl-3,4dihydro quinoxalin -2(1*H*) were designed and synthesized starting with ortho-phenylenediamine by its reaction with 2 oxopropanoic acid to afford 3-methylquinoxalin-2(1H)-one, following the reported procedures,[10] which was then treated with alcoholic potassium hydroxide to afford the corresponding potassium salt. Heating of the obtained potassium salt with ethyl-4-(2-chloroacetamido)-benzoate afforded the corresponding ethyl ester (4). The reaction of (4) with hydrazine hydrate afforded the intermediate compound N-(4-(hydrazinecarbonyl) phenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide (5) to this different aromatic aldehydes were attached .

SCHEME



Potassium 3-methyl-2-oxo-2,3-dihydro-1*H*-quinoxalin-4-ide its potassium salt were obtained according to the reported procedures[12].

Method of prepration of 4-{4-[ethoxy (hydroxy) methyl] phenyl}-3-methyl-3,4-dihydroquinoxalin-2(1*H*)-one.

A mixture of the potassium salt of 3-methylquinoxalin-2(1H)-one (19.80 g, 0.1 mol) and ethyl 4-(2-chloroacetamido)benzoate (24.1 g, 0.01 mol) in DMF (50 ml) was heated on a water-bath for 14 h. After cooling to room temperature, the reaction mixture was poured onto ice-water (500 ml) and stirred for 30 min. The formed precipitate was filtered, washed with water and crystallized from ethanol to give white crystals.

Yield, 75%;

Melting Point: 213–214 °C

IR (KBr, cm_1): 3278 (NH), 3050 (C–H aromatic), 2985 (C–H aliphatic), 1740 (C=O ester), 1667 (C=O amide), 1601 (C=O quinoxaline).

1H NMR (CDCl3, ppm): 1.36 (t, 3H, CH2CH3, J= 6.8 Hz), 2.61 (s, 3H, CH3-quinox.), 4.32 (q, 2H, CH2CH3,

J = 6.8 Hz), 5.03 (s, 2H, CH2), 7.26–8.03 (m, 8H, Ar–H), 9.04 (s, 1H, NH), (D2O exchangeable).
MS (m/z): 365 (M+, 5.12%), 321 (6.34%), 201 (80.12%), 173 (20.33%) 145 (100%, base beak).

Method of prepration of 4-{4-[(hydrazinylmethoxy)(hydroxy)methyl]phenyl}-3-methyl-3,4-dihydroquinoxalin-2(1*H*)-one.

A mixture of ester (3.65 g, 0.01 mol) and hydrazine hydrate (10 ml, 85%) in ethanol (20 ml) was stirred well and refluxedfor 6 h. The reaction mixture was cooled and the crude product was collected by filtration, washed with water and recrystallized from ethanol.
Yield, 74%;
Melting Point :- 295–297 °C.
IR (KBr, cm_1): 3342, 3300 (NH– NH2), 3250 (NH), 3040 (C–H aromatic), 2975 (C–H aliphatic), 1672 (C=O NHNH2), 1626 (C= O amide), 1600 (C=O quinox.).
1H NMR (DMSO-d6, ppm): 2.50 (s, 3H, CH3,) 4.44 (s, 2H, NH2) (D2O exchangeable), 5.14 (s, 2H, CH2), 7.36– 7.80 (m, 8H, Ar–H), 9.63 (s, 1H, NH–NH2), (D2O exchangeable), 10.63 (s, 1H, NH-phenyl) (D2O exchangeable).
MS (m/ z): 351(M+, 2.01%), 320 (7.23%), 201 (20.43%), 159 (3.25%), 145 (96.20%), 131 (13.57%), 119 (100%, base peak).

Method of prepration of different derivatives of 4-{4-[(hydrazinylmethoxy) (hydroxy)methyl]phenyl}-3-methyl-3,4-dihydroquinoxalin-2(1*H*)-one

A mixture of equimolar quantities of the acid hydrazide (2) (0.70 g, 0.002 mol) and the appropriate substitutes ware heated under reflux at 110_C for 6 h. The reaction mixture was cooled to room temperature, poured carefully onto an ice-water (300 ml), and then neutralized with solid sodium bicarbonate. The formed precipitate, after standing for 1 h, was filtered, washed with water, dried and crystallized from ethanol to afford compounds (6a–g), respectively.

Compound 4a.

Yield, 60%
Melting Point: 260–262 °C
IR (KBr, cm_1): 3240 (NH), 3053 (C–H aromatic), 2923 (C–H aliphatic), 1651 (C=O amide), 1611 (C=O quinox.).
MS (m/z): 437 (M+, 2.13%), 377 (2.02%), 236 (5.32%), 201 (27.82%), 173 (5.48%), 145 (100% base beak), 105 (75.70%).

The antimycobacterial activities of compounds 6(a-h) were assessed against *M. tuberculosis* ATTC 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.

Procedure fot Anti-TB activity using Alamar Blue Dye

- The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).
- This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.
- 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.
- The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
- The final drug concentrations tested were 100 to 0.2 µg/ml.
- Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
- 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.
- A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
- 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- 3.125µg/ml
Ciprofloxacin-3.125µg/ml
Streptomycin- 6.25µg/ml

Table 1: Results of antimycobacterial activity.

Sl. 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	**6a**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**
02	**6b**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**
03	**6c**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**
04	**6d**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**
05	**6e**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
06	**6f**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**
07	**6g**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
08	**6h**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**

NOTE: S – Sensitive R- Resistant

Table 2: The MIC of different samples is as follows

S/NO	Compound Code	MIC In µg/ml
	6a	**12.5**
	6b	**12.5**
	6c	**6.25**
	6d	**12.5**
	6e	**100**
	6f	**12.5**
	6g	**100**
	6h	**6.25**

Table 2: The MIC of different standards used is as follows

S/NO	Standard Drug Name	MIC In µg/ml
	Pyrazinamide	**3.12**
	Ciprofloxacin	**3.12**
	Streptomycin	**6.25**

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 6c (6.25 µg/mL) and 6h (6.25 µg/mL) .The compounds 6a (12.5 µg/mL), 6b, (12.5 µg/mL),6d (12.5 µg/mL) and 6f (12.5 µg/mL) were shown moderate sensitivity. While the compounds 6e (100 µg/mL) and 6g (100 µg/mL) were 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).

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

Acknowledgement

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

References

1. Venkatesh, S Z Mohamad Shamsuddin and N M Mubarak .*Indian J.Chem* 2016 :55(B) ; 843-850.
2. Ramalingam Peraman,Rajendran Kuppusamy,Sunil Kumar Killi,and Y Padmanabha Reddy. *Int J of Med Chemistry*.2016:1;8-13
3. Jaso A, Zarranz B, Aldana I & Monge A, *J Med Chem*, 48, 2005, 2019;
4. Carta A, Paglietti G, Nikookar M E R, Sanna P, Sechi L & Zanetti S, *Eur J Med Chem*, 37, 2002, 355
5. Aparicio D, Attanasi O A, Filippone P, Ignacio R, Lillini S, Mantellini F, Palacios F & Santos J M, *J Org Chem*, 71, 2006, 5897
6. S K, Gupta P, Duggineni S & Kundu B, *Synlett*, 2003, 2147. Antoniotti S & Donach E, *Tetrahedron Lett*, 43, 2002, 3971
7. Bhosale R S, Sarda S R, Ardhapure S S, Jadhav W N, Bhusare S R & Pawar R P, *Tetrahedron Lett*, 46, 2005, 7183
8. Zhao Z, Wisnoski D D, Wolkenberg S E, Leister W H, Wang Y & Lindsley C W, *Tetrahedron Lett*, 45, 2004, 4873
9. . Elhelby AA, Ayyad RR, Zayed MF. Synthesis and biological evaluation of some novel quinoxaline derivatives as anticonvulsant agents. Arzneimittelforschung 2011;61(7):379–81
10. Synthesis of a set of ethyl1-carbamoyl-3-oxo-quinoxaline-2-carboxyl atesand of their constrained analogue imidazo1,5-aquinoxaline-1,3,4-trionesasglycine/NMDA receptor antagonists. Eur J Med Chem 2001;36:203–9.
11. . Bailly C, Echepare S, Gago F & Waring M, *J Anti-*

Cancer Drug Des, 15, 1999, 291

12. Ibrahim MK, Abdel-Rahman AA, Ayyad RRA, El-Adl K, Elsherbeny F, Rashed M. Design and synthesis of some novel Nphthalimide derivatives with potential anticonvulsant activity. Al- Azhar J Pharm Sci 2010;42:305–22.