Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(3):368-373

ISSN No: 0975-7384
CODEN(USA): JCPRC5

In-vitro screening of quinoxaline-2-one derivatives for antitubercular activity

Gopal Krishna Rao[a], Ramaling B. Kotnal*and PN Sanjay Pai[b]

[a]Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Hosur Road, Near Lalbagh Main Gate, Bangalore
[b]Department of Quality Assurance, Al-Ameen College of Pharmacy, Hosur Road, near Lalbagh Main Gate, Bangalore

ABSTRACT

A series of ethyl 2-[(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetyl]-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylate derivatives were prepared and evaluated for their antitubercular activities. All compounds were screened for *in vitro* antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB). Minimum inhibitory concentrations were determined and interpreted for *Mycobacterium tuberculosis* $H_{37}Rv$ according to the procedure of the approved macro dilution reference method of antimicrobial susceptibility testing. Among all the compounds synthesized, (GKR 4b, 4c, and 4f) were found to be the most active compounds against MTB with MIC (25 µg/mL).

Key words: Schiff's bases, antitubercular activity, minimum inhibitory concentration (MIC).

INTRODUCTION

Tuberculosis (TB), a highly infectious disease primarily caused by *Mycobacterium tuberculosis*, is one of the oldest recorded human afflictions and remains today a leading cause of impoverishment, human suffering and death [1-4]. The majority of deaths resulting from TB infection occur in poverty stricken regions of the developing world. While TB in humans is primarily due to infection with *M. tuberculosis*, several other species of the *M. tuberculosis* complex, namely *M. bovis*, *M. africanum*, *M. microti* and *M. canettii* may also cause the disease [5]. Recently two additional species have been suggested as belonging to the *M. tuberculosis* complex: *M. caprae* and *M. pinnipedii* [6].

M. tuberculosis, like most other mycobacteria, is a Gram-positive, non-motile rod-shaped bacterium. These rod-shaped bacilli measure 1-4 μm in length and 0.3-0.6 μm in diameter. *M. tuberculosis* is slow growing with a generation time of 12-20 hours. Mycobacteria possess a unique cell wall of high lipid content, which includes mycolic acids and other glycolipids. Mycolic acids of the mycobacterial cell wall are one of the specific features of bacteria belonging to this genus. However, these fatty acids can also be found in the cell walls of certain genera of aerobic *Actinomycetes* and in the majority of species of the genus *Corynebacterium*[6]. This unique cell wall is thought to be responsible for certain features of mycobacteria, including low cell wall permeability and pathogenicity. It is the low permeability of the mycobacterial cell wall which is believed to play a significant role in the antibiotic resistance of *M. tuberculosis* [7,8].

TB is an airborne disease that spreads much like the common cold, with the circulation of *M. tuberculosis* containing aerosols into the air caused by the coughing, sneezing, talking or spitting of a person with infectious pulmonary TB. Inhalation of only a small number of these TB bacilli by a person will result in their infection with the disease. Once the *M. tuberculosis* containing aerosols enter the pulmonary alveoli, a healthy immune system will respond by "walling-off" the TB bacilli, resulting in their protection with a thick waxy coat that allows the TB bacilli to lay dormant for years (latent TB) [9,10]. TB usually affects the lungs (pulmonary TB) of an infected person, but the disease can also affect other areas of the body including kidneys, bones, joints and lymph nodes (extra pulmonary TB). A person infected with latent TB will exhibit none of the obvious symptoms of TB, which include a fever, fatigue, weight loss, a persistent cough and sputum production which will contain blood at an advanced stage of the disease. While a person with latent TB is not infectious, they may develop the active form of the disease if their immune system is compromised.

It is estimated that one person in every 10 of the two billion people infected worldwide will develop infectious TB. A number of factors have led to the reduction in successful treatment of the disease. These include a lengthy (6-9 months) multi-drug treatment program, the misuse or mismanagement of which can lead to the development of multi or extensive drug resistant TB (MDRTB or XDRTB) and the co-infection of TB with the human immunodeficiency virus (HIV) [11]. A new anti-TB drug needs to show the well pharmacokinetic distribution and permeation into lung tissue and cells. Furthermore, it is also desired that the novel candidate exhibits the potent bactericidal activity both against exponential and stable phase of *M. tuberculosis in vivo*. In addition, it is ideal that the novel agent possesses narrow anti-microbial spectrum specialized only against Mycobacterial species. Among the various classes of nitrogen heterocyclic compounds, quinoxaline derivatives display a broad spectrum of biological activities. Some analogs are synthesized and evaluated for antimicrobial activity and many possess diverse biological activities such as insecticidal, fungicidal, herbicidal, anthelmintic and antiviral [12].

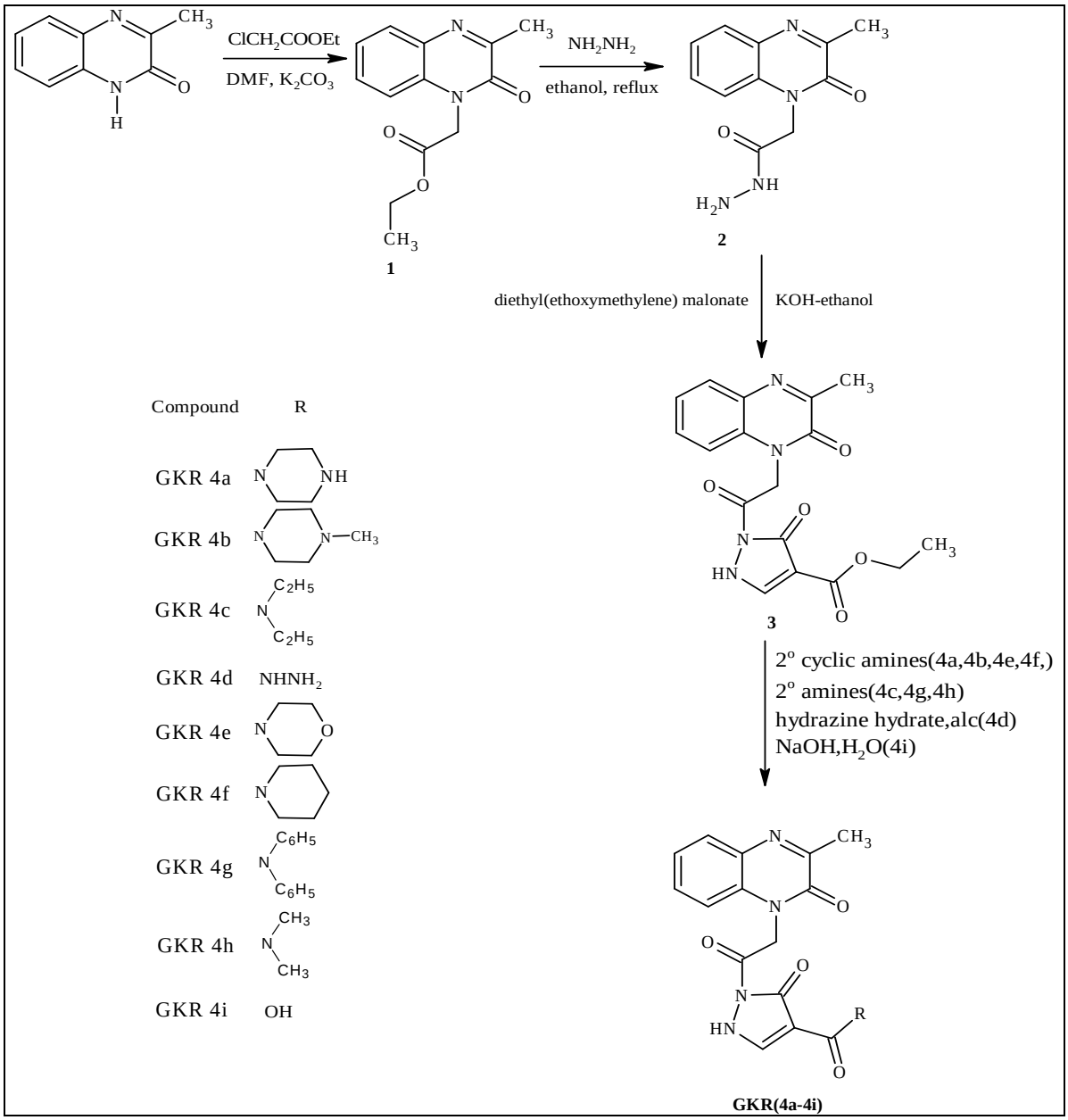
EXPERIMENTAL SECTION

***Synthesis of compounds* GKR4a-c & e-h.** A mixture of compound **(3)** (1.0 g, 0.0028 mol), secondary amine (0.0028mol) was refluxed in presence of sodium methoxide for a 3.5-7.0 h. The reaction mixture cooled and diluted with cold water. Resulting solution was acidified with concentrated aqueous hydrochloric acid to pH 5.0, refrigerated over night. The crystalline solid was collected by filtration and washed with cold water.

***Synthesis of compound* GKR4d.** To a solution of compound **3** (1.0 g, 0.0028 mol) in ethanol (15 mL) was added hydrazine hydrate (0.161 g, 0.0032 mol). The resulting mixture was refluxed for 5 h. The precipitate formed was collected by filtration and washed with cold ethanol. The solid was recrystalised from ethanol to give GKR 4d (0.391 g, yield 43%)

***Synthesis of compound* GKR4i.** A solution of compound **(3)** (1.0 g, 0.0028 mol) in 5% sodium hydroxide (10 mL) was heated at temperature 110 °C for 1 h. The resulting mixture was cooled and acidified to pH 5.0 with dilute hydrochloric acid. The obtained precipitate was filtered and washed with cold water. The compound was recrystalised from ethanol to give GKR 4i (0.432 g, yield 47%). All Compounds were prepared according to procedure mentioned in literature [13-15]

Scheme



Spectral data for selected compound

GKR 4a: Yield 48%; mp 220 °C; ^{1}H NMR (400 MHz, DMSO): δ 7.34-7.79 (m, 4H), 5.0 (s, 2H), 2.49-2.51 (m, 8H), 2.08 (s, 3H); LCMS m/z= 397 $(M)^+$; IR (KBr) (υ_{max}/cm^{-1}): 3416, 1710, 1643, 1592, 1469, 1271, 766. **GKR 4c:** Yield 43%; mp 232 °C; ^{1}H NMR (200 MHz, DMSO): δ 7.32-7.78 (m, 4H), 5.08 (s, 2H), 3.36 (br, s, 1H), 2.46 (q, 2H) (j value), 1.23 (t, 3H) (j value) ; ^{13}C NMR (200 MHz, DMSO): δ 21.3, 38.6-41.1, 43.8, 114.8, 123.8, 129.2, 130.1, 132.2, 132.8, 154.5, 157.8, 169.1; IR (KBr) (υ_{max}/cm^{-1}): 3417, 3268, 1709, 1643, 1592,1469, 1433, 1229, 766.

Table 1: Characterization data of compounds (GKR-3, 4a-4i)

Compound	Mol Formula	Mol. weight	M.P. ^{o}C	% yield	R_f^*
GKR-3	$C_{17}H_{16}N_4O_5$	356	210	65	0.69
GKR-4a	$C_{19}H_{20}N_6O_4$	396	220	48	0.23
GKR-4b	$C_{20}H_{22}N_6O_4$	410	226	42	0.20
GKR-4c	$C_{19}H_{21}N_5O_4$	383	222	43	0.33
GKR-4d	$C_{15}H_{14}N_6O_3$	326	214	43	0.84
GKR-4e	$C_{19}H_{19}N_5O_5$	397	224	43	0.50
GKR-4f	$C_{20}H_{21}N_5O_4$	395	222	61	0.19
GKR-4g	$C_{27}H_{21}N_5O_4$	479	178	40	0.40
GKR-4h	$C_{17}H_{17}N_5O_4$	355	228	46	0.20
GKR-4i	$C_{15}H_{12}N_4O_5$	328	220	47	0.45

TLC solvents: Chloroform: Methanol (9:1)

Table 2: Inhibitory concentrations of quinoxaline-2-one derivatives

Compounds	*Mycobacterium tuberculosis* H_{37}Rv Concentration				*c*log*P*
	25 μg/mL	50 μg/mL	100 μg/mL	> 100 μg/mL	
GKR-3	x	√	√	√	-1.92
GKR-4a	x	x	√	√	-2.86
GKR-4b	√	√	√	√	-2.57
GKR-4c	√	√	√	√	-1.74
GKR-4d	x	x	√	√	-3.04
GKR-4e	x	x	x	√	-1.72
GKR-4f	√	√	√	√	-0.57
GKR-4g	x	x	√	√	-0.77
GKR-4h	x	√	√	√	-0.64
GKR-4i	x	√	√	√	-0.84
Pyrazinamide	10 μg/ml				-1.09
Streptomycin	7.5 μg/ml				-1.22

√ = *growth of Mycobacterium tuberculosis inhibited*
x = no growth inhibition

Antitubercular activity

Test compounds were evaluated for *in vitro* antimycobacterial activity. MICs were determined and interpreted for *Mycobacterium tuberculosis* $H_{37}Rv$ according to the procedure of the approved macro dilution reference method of antimicrobial susceptibility testing [16]. Compounds were taken at concentrations of 100 µg/mL, 50 µg/mL and 25 µg/mL in DMF. *M. tuberculosis* $H_{37}Rv$ strain was used in Middle brook 7H-9 broth which was inoculated with standard as well test compounds and incubated at 37°C for 4 weeks. The bottles were inspected for growth twice a week for a period of three weeks. Readings were taken at the end of 4 weeks. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain.

RESULTS AND DISCUSSION

All compounds were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ by the broth dilution method according to recommended procedure by the National Committee for Clinical Laboratory Standards for the determination of minimum inhibitory concentration (MIC). Compounds **GKR- 4b, 4c** and **4f** were active against *M. tuberculosis* $H_{37}Rv$ (MIC < 25 µg/ml) (Table 1). The lipophilicity of the drug is well known to play an important role in the penetration of these compounds into bacterial cells. The clog*P* values of the synthesized compounds were calculated using ACD labs software -3.04 to -0.57 and they are having drug-like character as reference drugs, pyrazinamide (-1.09) and streptomycin (-1.22).

CONCLUSION

The results of antimicrobial testing revealed the compounds **GKR-4b, 4c** and **4f** have shown promising anti-tubercular activity. Therefore, this work would be fruitful matrix for the development of novel class of antimicrobial/antimycobacterial agents. It is convincing that, derivatives showing significant antibacterial and antifungal activities can be further modified to exhibit better potency as that of standard drugs.

Acknowledgement

Authors are thankful to Principal, Al-Ameen College of Pharmacy, Bangalore for providing necessary facility. One of the authors Mr.R.B.Kotnal is thankful to the Principal, BLDEA's College of Pharmacy, Bijapur for support.

REFERENCES

[1] The Global Plan to Stop TB, 2006-2015. http://www.stoptb.org /globalplan/assets/ documents/ GlobalPlanFinal.pdf(date accessed 21st May 2010),
[2] I Smith. *Clin. Microbiol. Rev.,* **2003,** 16, 463-496.
[3] NW Schluger. *Am. J. Respir. Cell Mol. Biol.*, **2005,** 32, 251-256.
[4] P Tiruviluamala; LB Reichman; *Annu. Rev. Public Health,* **2002,** 23, 403- 426.
[5] A Somoskovi; J Dormandy; LM Parsons; M Kaswa; KS Goh; Rastogi N; Salfinger M. *J. Clin. Microbiol.*, **2007,** 45, 595-599.
[6] F Boulahbal; L Heifets. 'Bacteriology of Tuberculosis'. In *Reichman and Hershfield's Tuberculosis,* Third (Part A) ed.; MC Raviglione,., Ed. Informa Healthcare USA, Inc.: New York, 2006; Vol. 219, pp 29-47.
[7] M Rezwan; MA Laneelle; P Sander; M Daffe. *J. Microbiol. Methods,* **2007,** 68, 32-39.

[8] D Chatterjee. *Curr. Opin. Chem. Biol.*, **1997,** 1, 579-588.
[9] Tuberculosis: Infection and Transmission. http://www.who.int/mediacentre/ factsheets/fs104/en/index.html (date accessed 21st May 2010),
[10] MC Tsai; S Chakravarty; G Zhu; J Xu; K Tanaka; C Koch; J Tufariello; J Flynn; J Chan. *Cell Microbiol.,*. **2006,** 8, 218-232.
[11] BR Copp; AN Pearce. *Nat. Prod. Rep.,* **2007,** 24, 278-297.
[12] MM Ali; F MM Ismail.; MSA El-Gaby; MA YA Zahran; Ammar. *Molecules*, **2000**, 5(6), 864-873.
[13] DA Vyas; NA Chauhan; AR Parikh. *Indian J. Chem*., **2007**, 46(B), 1699-1702
[14] DS Lawrence; JE Copper; CD Smith. *J. Med. Chem*., **2001**, 44, 594-601.
[15] R Harada; Y Kinoshita. *Bull. Chem. Soc. Jap*.**, 1967,** 40(11), 2706-2708.
[16] Andrews JM. *J Antimicrob Chemother* **2001,** 48(sppl*)*: 5-16.