Synthesis and Biological Evaluation of 2-(3-Methyl-2-oxoquinoxalin-1(2*H*)-yl)-*N'*-(substituted phenyl-methylenedene/ethylidene)acetohydrazides

GOPAL KRISHNA RAO, R. B. KOTNAL and P. N. SANJAY PAI*

Department of Pharmaceutical Chemistry,

*Department of Quality Assurance, Al-Ameen College of Pharmacy,
Opp. Lalbagh Main Gate, Hosur Road Bangalore-560 027, India.

rbkotnal@gmail.com

Received 15 January 2010; Accepted 10 March 2010

Abstract: A series of quinoxaline derivatives was prepared and evaluated for antitubercular, antibacterial and antifungal activities. The title compounds were prepared by condensation of substituted aromatic aldehydes and substituted acetophenones with 2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl) acetohydrazide. Structures of all these compounds were confirmed by their spectral studies. Among synthesized compounds (**4r**, **4t**, **4u**, **4w** and **4x**) have shown good anti tubercular activity (25 $\mu\text{g mL}^{-1}$) when compared to reference drugs pyrazinamide (10 $\mu\text{g mL}^{-1}$) and streptomycin (7.5 $\mu\text{g mL}^{-1}$). In this study, few derivatives showed broad spectrum of antimicrobial activity at low concentration. The MICs (Minimum inhibitory concentration) of some compounds are 2-4 $\mu\text{g mL}^{-1}$.

Keywords: Antimicrobial activity, Antitubercular activity, Minimum inhibitory concentration (MIC).

Introduction

Tuberculosis (TB) is still the greatest single infectious cause of mortality worldwide. However, powerful new anti-TB drugs with new mechanisms of action have not been developed in the last over thirty years^{1, 2}. It is expected that development of the new effective anti-TB drug will bring us various outcomes such as shortening the total duration of treatment, improvement of the treatment completion ratio, prevention and treatment of the multiple drug resistant tuberculosis (MDR-TB) and reducing the total medical expenditure.

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^{3,4}. Schiff bases are also known

for their antibacterial and antitubercular activity⁵⁻⁷. Quinoxalines constitutes an important class of compounds; some analogs are synthesized and evaluated for antimicrobial activity and many possess diverse biological activity such as insecticidal, fungicidal, herbicidal and anthelmintic⁸.

Experimental

Melting points were determined in open capillaries on a Thermoionic melting point apparatus and were uncorrected. Infra-red spectra were recorded on Fourier Transform IR spectrophotometer (Shimadzu FT-IR 8700) using KBr disc method ($\nu_{\max}$ in cm^{-1}). ^1H NMR spectra were recorded in CDCl_3 or DMSO on Bruker-200 NMR spectrophotometer using TMS as internal reference standard (chemical shifts in δ ppm); and LCMS recorded on Shimadzu 2010A spectrophotometer.

Antimicrobial activity

All the synthesized compounds were tested for antimicrobial activity by tube dilution method⁹⁻¹¹. Four bacteria, two fungal and a *Mycobacterium* strains were used as the antimicrobial test strains namely: *Staphylococcus aureus* ATCC 12598, *Enterococcus faecalis* ATCC 35550, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 29665, *Candida albicans* ATCC 2091, *Aspergillus fumigates* ATCC 13073 and *Mycobacterium tuberculosis* H₃₇Rv. Ciprofloxacin and fluconazole were used as standard drugs against bacteria and fungi respectively. Pyrazinamide and Streptomycin were used as standard drugs against *Mycobacterium*. In all determinations tests were performed to find out minimum inhibitory concentration (MIC) at two fold concentration from $1\ \mu\text{g mL}^{-1}$ - $500\ \mu\text{g mL}^{-1}$. Results are presented in Table 1.

Table 1. Antitubercular, antibacterial and antifungal activities of compounds (**4k-4q** & **4s-4x**) (MIC in $\mu\text{g mL}^{-1}$)

Compound	<i>K.pneumoniae</i>	<i>E. coli</i>	<i>S.aureus</i>	<i>E.fecalis</i>	<i>C.albicans</i>	<i>A.fumigates</i>	<i>M.tuberculosis</i> H ₃₇ Rv
4k	250	>500	16.12	125	>500	31.25	100
4l	125	>500	8.0	62.5	>500	8.0	100
4m	4.0	>500	1.0	1.0	>500	16.12	50
4n	2.0	>500	8.0	16.12	>500	>500	50
4o	31.25	>500	8.0	31.25	>500	8.0	>100
4p	4.0	>500	4.0	8.0	>500	16.12	25
4q	2.0	>500	31.25	16.12	>500	2.0	25
4s	125	125	250	31.25	16.06	4	100
4t	125	125	125	16.06	16.06	1	25
4u	125	125	62.5	31.25	250	8.06	25
4v	125	125	125	31.25	16.06	4	50
4w	125	125	250	16.06	4	4	25
4x	125	125	250	16.06	16.06	8.06	25
Pyrazinamide	--	--	--	--	--	--	10
Streptomycin	--	--	--	--	--	--	7.5
Ciprofloxacin	1	1	2	1	--	--	--
Fluconazole	--	--	--	--	16	8	--

Synthesis of ethyl (3-methyl-2-oxoquinoxalin-1(2H)-yl) acetate (**2**)

A solution of compound (**1**) (2.32 g, 0.01 mol), ethyl chloroacetate (1.22 g, 0.01 mol), anhydrous K_2CO_3 (1.38 g, 0.01 mol) in dimethylformamide (15 mL) was stirred at room temperature for 8 h. The reaction mixture was diluted with ice-cold water. The separated

solid was filtered, washed with water and recrystallised from cyclohexane to obtain colorless needles. Yield 2.49 g (70%), m.p. 128–30°.

2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)acetohydrazide (3)

A solution of compound (2) (2.46 g, 0.01 mol) and hydrazine hydrate (0.75 g, 0.015 mol) in ethanol (15 mL) was refluxed for 4 h. The reaction mixture was cooled and poured onto ice-cold water with stirring. The separated solid was filtered, washed with water and recrystallised from ethanol to afford (3). Yield 1.9 g (81.8%), m.p. 218°.

General procedure for synthesis of compounds (4k–4q & 4s–4x)

A solution of substituted aromatic aldehydes or acetophenones in DMF was added drop wise to a solution of compound (3) in DMF. The mixture was refluxed for 3–5 h in presence of catalytic amount of glacial acetic acid, cooled and poured onto water containing crushed ice. The precipitate thus obtained was filtered, washed with cold water and recrystallised from alcohol / DMSO-water mixture to obtain (4k–4q & 4s–4x). Results are tabulated in Table 2.

Table 2. Characterization data of compounds (4k–4q & 4s–4x)

Compd	R ₁	R ₂	Mol. formula	Mol. Wt.	m.p.°C	Yield, %
4k	<i>m</i> -NO ₂ (C ₆ H ₅)	H	C ₁₈ H ₁₅ N ₅ O ₄	365	282	80
4l	<i>m</i> -Cl(C ₆ H ₅)	H	C ₁₈ H ₁₅ N ₅ O ₂ Cl	354	284	65
4m	<i>p</i> -Cl(C ₆ H ₅)	H	C ₁₈ H ₁₅ N ₅ O ₂ Cl	354	270	78
4n	2,3(OCH ₃) ₂ (C ₆ H ₅)	H	C ₂₀ H ₂₀ N ₄ O ₄	380	230	87
4o	Furfuraldehyde	H	C ₁₆ H ₁₄ N ₄ O ₃	310	238	53
4p	Cinnamaldehyde	H	C ₂₀ H ₁₈ N ₄ O ₂	346	270	68
4q	Acetaldehyde	H	C ₁₃ H ₁₄ N ₄ O ₂	234	215	65
4s	(C ₆ H ₅)	CH ₃	C ₁₉ H ₁₈ N ₄ O ₂	334	214	52
4t	<i>p</i> -NH ₂ (C ₆ H ₅)	CH ₃	C ₁₉ H ₁₉ N ₅ O ₂	349	242	30
4u	<i>p</i> -OCH ₃ (C ₆ H ₅)	CH ₃	C ₂₀ H ₂₀ N ₄ O ₃	364	190	32
4v	<i>p</i> -Br(C ₆ H ₅)	CH ₃	C ₁₉ H ₁₇ N ₄ O ₂ Br	413	238	42
4w	<i>p</i> -OH(C ₆ H ₅)	CH ₃	C ₁₉ H ₁₈ N ₄ O ₃	350	250	55
4x	3-pyridyl	CH ₃	C ₁₈ H ₁₇ N ₅ O ₂	335	270	51

Results and Discussion

It was observed that a strong nucleophile can attack directly, without help from an acid catalyst. For a weak nucleophile such as amine (-NH₂) an acid catalyst is needed so that the carbonyl carbon is prepared to share a pair of electrons as a new covalent bond. The experiments proved that the use of catalytic amount of acid not only fasten the reaction but also improves the product yield.

Antimicrobial activity

Antimicrobial activity of all the compounds was shown in Table 2. Among the series of synthesized compounds, **4r**, **4t**, **4u**, **4w** and **4x** have shown considerable antitubercular activity at 25 µg mL⁻¹ in comparison to pyrazinamide 10 µg mL⁻¹. The Schiff bases modified at N=CH-, the replacement of H with CH₃ has resulted in better activity. The electron releasing nature of CH₃ may also be accounted for this fact. Compounds **4r–4x** have shown significant antifungal activity even at lower concentrations (1–4 µg mL⁻¹) than standard drug fluconazole (8 µg mL⁻¹). The substitutions on the aromatic ring influenced quantitatively, on biological activities. Compounds **4k** (*m*-NO₂) and **4l** (*p*-NO₂) contains nitro group which is known for its antibacterial activity.

Spectral data of selected compounds

Compound 2: ^1H NMR (CDCl_3): δ ppm 1.28 (t, 3H, CH_3), 1.61 (s, 3H, ring CH_3), 4.27 (q, 2H, CH_2), 5.02 (s, 2H, N- CH_2), 7.04-7.85 (m, 4H, Ar-H); IR (KBr, ν_{max} in cm^{-1}): 1649 (ester $\text{C}=\text{O}$), 1641 (ring $\text{C}=\text{O}$), 1602 ($\text{C}=\text{N}$); LCMS, m/z : 245 (M^+ , 80%), 231(5), 217(15), 199(10) and 159(100).

Compound 3: ^1H NMR (DMSO): δ ppm 2.44 (br, 2H, NH_2), 3.42 (s, 3H, ring CH_3), 4.95 (s, 2H, N- CH_2), 7.21-7.78 (m, 4H, Ar-H), 9.37 (s, 1H, CONH); IR (KBr, ν_{max} in cm^{-1}): 3332, 3209, 3053 (- NHNH_2 , str), 1685 (ring $\text{C}=\text{O}$), 1654 (CONH), 1600 ($\text{C}=\text{N}$); LCMS, m/z : 233 ($\text{M}+1$, 50%), 203(100), 175(60), 160(40) and 145(20).

Compound 4k: ^1H NMR (DMSO): δ ppm 2.50 (s, 3H, CH_3), 5.07 (s, 1H, CONH), 5.45 (s, 2H, N- CH_2), 7.33- 8.21 (m, 8H, Ar-H), 8.47 (s, 1H, $\text{N}=\text{CH}$); IR (KBr) cm^{-1} : 3199 (Ar-H, str), 1677 (ring $\text{C}=\text{O}$), 1645 ($\text{C}=\text{O}$ str. of CONH), 1600 (N-H, bend), 1535, 1334 ($\text{N}=\text{O}$ str.).

Compound 4v: ^1H NMR (DMSO): δ ppm 2.11 (s, 3H, ring CH_3), 2.76 (s, 3H, CH_3 of acetophenone), 5.54 (s, 2H, N- CH_2), 7.08-9.34 (m, 8H, Ar-H), 10.12 (s, 1H, NH); IR (KBr) cm^{-1} : 3202 (C-H, Ar), 2921 (C-H, CH_3), 1682 ($\text{C}=\text{O}$, ring), 1658 ($\text{C}=\text{O}$, CONH), 1601 ($\text{C}=\text{N}$), 826 (w, Br).

Scheme 1

(4k-4q and 4s-4x)

Conclusion

In conclusion, the target compounds have been synthesized and are purified. The results of antimicrobial testing revealed the compounds to possess broad spectrum *in vitro* activity. Therefore, this work would be fruitful matrix for the development of novel class of antimicrobial agents. It is convincing that, derivatives showing significant antibacterial and antifungal activities can further be modified to exhibit better potency as that of standard drugs.

Acknowledgment

Authors are thankful to Principal Al-Ameen College of Pharmacy, Bangalore. One of the authors (R.B. Kotnal) is grateful to Principal B.L.D.E. A's College of Pharmacy, Bijapur for their support.

References

1. Zhang Y and Amzel L M, *Current Drug Targets*, 2002, **3**(2), 131-154.
2. Heravi M M, Baghernejad B and Oskooie H A, *Tetrahedron Lett.*, 2009, **50**(7), 767-769.
3. Rafaat H M, Moneer A A and Khalil O M, *Arch Pharm Res.*, 2004, **27**(11), 1093-1098.
4. Krishnan V S H, Chowdary K S, Dubey P K and Vijaya S, *Indian J Chem.*, 2001, **40B**, 565-573.
5. Gopal Krishna Rao, Venugopala K N and Sanjay Pai P N, *J Pharmacol Toxicol.*, 2007, **2**(5), 481-488.
6. Venugopala K N, Rao G K and Sanjay Pai P N, *J Pharmacol Toxicol.*, 2007, **2**(3), 248-255.
7. Abul-FAdl T A, Hamid Mohammed F A and Hasssan E A, *Arch Pharm Res.*, 2003, **26** (10), 778-784.
8. Ali M M, Ismail M M F, El-Gaby M S A, Zahran M A and Ammar Y A, *Molecules*, 2000, **5**(6), 864-873.
9. Andrews J M, *J Antimicrob Chemother.*, 2001, **48**, 5-16.
10. Reimer L G, Stratton C W and Reller L B, *Antimicrob Agents Chemother.*, 1981, **19**(6), 1050-1055.
11. Stratton C W and Cooksey R C, *Susceptibility tests*; 5th Ed., *Manual of Clinical Microbiology*; American Society for Microbiology: Washington DC, 1990, 1153.