DOI 10.5012/bkcs.2011.32.8.2657

Synthesis of Triazoloquinoxalines as Antitubercular Agents

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Received February 21, 2011, Accepted June 27, 2011

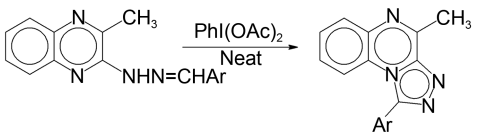
1,2,4-Triazoles and quinoxalines were found to display various pharmacological activities. Hence a series of 1-aryl-4-methyl-1,2,4-triazolo[4,3-*a*]quinoxalines were synthesized. Due to various advantages of organic reactions under solvent-free conditions these compounds were developed using iodobenzene diacetate under solvent-free conditions. The synthesized compounds were characterized by elemental microanalysis, infrared spectroscopy, ^{1}H NMR, ^{13}C NMR and HRMS. All the synthesized compounds were investigated for their antitubercular activity and **5g** was found to the most active compound.

Key Words : Triazolo quinoxalines, Antitubercular, Iodobenzene diacetate

Introduction

Tuberculosis (TB) is a contagious and deadly disease that spreads through the air, which has reached pandemic proportions. According to World Health Organization (WHO), 9.2 million new cases and 1.7 million deaths from TB have been reported.[1] With the emergence of HIV, TB has become the most common opportunistic infection afflicting patients living with AIDS. There are 0.7 million HIV-positive people infected with TB, contributing to 0.2 million deaths worldwide.[1] Poor chemotherapeutics and the inadequate administration of drugs have led to the development of multidrug resistant TB (MDR-TB),[2] the treatment of which requires administration of more expensive, second line antibiotics for up to 2 years. In addition, even more alarming cases of extensively drug resistant strains of TB that are resistant to both first and second line drugs have been reported.[3] Recent findings by WHO from 2000 to 2004 suggested that 4% of MDR-TB cases meet the criteria for drug resistant strains of TB. Consequently, there is a pressing need for the development of novel TB drugs that are effective against both drug sensitive and resistant *Mycobacterium tuberculosis* strains.

Triazoles are an important class of heterocyclic compounds and specifically the 1,2,4-triazole nucleus has been found to be an integral part of therapeutically interesting compounds that display significant antibacterial, CNS stimulative, sedative, antifungal, antitubercular and antitumor activities.[4-8] Consequently, the synthesis of this heterocyclic nucleus has gained great importance in organic synthesis. Quinoxaline derivatives were also found to possess antimycobacterial activity.[9-13] Generally, syntheses of 1,2,4-triazoles are accomplished by the condensation of 2-hydrazinoderivatives with carboxylic acids at elevated temperature,[14] 1,3-dipolar cycloaddition reaction of aromatic nitriles in presence of strong base and followed by hydrogen elimination,[15] photolysis of triazole-3-thiones,[16] and oxidation of arylhydrazones.[17,18] However, these methods involve multi-step, harsh reaction conditions, toxic reagents and require longer reaction time. A variety of 1,2,4-triazolo[4,3-*a*] quinoxalines have been reported till date with various pharmacological activities.[19-22] Recently, organic reactions under solvent-free conditions have received much attention due to several advantages over the conventional methods in terms of time, yields and relatively benign conditions.[23-28] In view of our initial successes on the utilization of hypervalent iodine reagents for the synthesis of various heterocyclic compounds under solvent-free conditions,[29,30] and because of the biological significance of 1,2,4-triazole derivatives, we decided to develop an efficient and environmentally benign synthesis of 1,2,4-triazoles that proceeds under solvent-free conditions. Herein, we report our results on oxidative transformation of arenecarbaldehyde 3-methylquinoxalin-2-yl-hydrazones to 1-aryl-4-methyl-1,2,4-triazolo[4,3-a]quinoxalines (Scheme 1) with iodobenzene diacetate that leads to the expeditious formation of 1-aryl-4-methyl-1,2,4-triazolo[4,3-a]quinoxalines in fairly good yields. This oxidative conversion simply involves a thorough mixing of substrates with iodobenzene diacetate at room temperature (slightly warming in some cases) *via* an exothermic reaction. Hydrazones form a yellowish eutectic melt with iodobenzene diacetate upon mixing prior to the occurrence of a mildly exothermic reaction. This is in accord with the postulated model for such solid-solid reactions.[23]



Ar = *p*-$CH_3C_6H_4$, *p*-$OCH_3C_6H_4$, *p*-$NO_2C_6H_4$, *m*-$NO_2C_6H_4$, *p*-$N(CH_3)_2C_6H_4$, C_6H_5, *p*-ClC_6H_4, *m*-OCH_3H_4

Scheme 1. Synthesis of target compounds **(5a-h)**.

Experimental

Melting points were recorded on Buchi R-535 apparatus and are uncorrected. Infra red (IR) spectra were recorded in KBr pellets on Schimadzu IR Prestige-21 FT-IR spectrophotometer (ν_{max} in cm^{-1}). ^1H, ^{13}C NMR spectra were recorded on a Bruker 400 M Hz spectrometer in $CDCl_3$ using TMS as internal standard, elemental analysis on a Carla Erba 1108 elemental analyzer and mass spectra on a VG-70-S mass spectrometer. All the arenecarbaldehyde 3-methylquinoxalin-2-yl-hydrazones (**4a-h**) were prepared according to the literature procedure.[17,18] Iodobenzene diacetate was purchased from Aldrich.

General Procedure.

Synthesis of 1-Aryl-4-methyl-1, 2,4-triazolo [4,3-*a*]-quinoxalines: A mixture of 3-methylquinoxalin-2-yl-hydrazones[17,18] (1 mM) and iodobenzene diacetate (1.2 mM) was ground thoroughly in a pestle and mortar. After 2-3 minutes an exothermic reaction ensued while in some cases slightly warming to ~40 °C for 2 min was required to initiate the reaction. The residue was washed with hexane and then recrystallized to afford pure products. The yields obtained along with the melting points and antimycobacterial activity of final compounds is tabulated in Table 1.

Spectral Data of Title Compounds.

4-Methyl-1-[(*p*-(methylphenyl)]-1,2,4-triazolo[4,3-*a*]-quinoxaline (5a): ^1H NMR ($CDCl_3$): δ 2.50 (s, 3H, CH_3), 3.04 (s, 3H, CH_3), 7.32 (dd, 1H, *J* = 1.0, 8.0 Hz, H-7), 7.40 (d, 2H, *J* = 7.0 Hz, H-3', H-5'), 7.51-7.58 (m, 4H, H-aromatic), 8.02 (dd, 1H, *J* = 1.0, 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 21.2, 21.7, 116, 125.2, 126, 127.5, 128.3, 129.9, 130, 136.4, 141.5, 144.90 150.4, 153. HRMS (FAB) Calc. for $C_{17}H_{14}N_4$: 274.1218, Found: 274.1209.

4-Methyl-1-[(*p*-(methoxyphenyl)]-1,2,4-triazolo[4,3-*a*]-quinoxaline (5b): ^1H NMR ($CDCl_3$): δ 3.03(s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 7.10 (d, 2H, *J* = 8.0 Hz, H-3', -5'), 7.32 (dd, 1H, *J* = 1.0, 8.0 Hz, H-7), 7.51-7.62 (m, 4H, H-2', -6', -8, -9), 8.01 (dd, 1H, *J* = 1.0 & 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 21.28, 55.57, 114.70, 115.85, 120.15, 125.98, 127.58, 128.17, 130.12, 131.51, 136.62, 145.00, 150.11, 152.96, 161.67. HRMS (FAB) Calc. For $C_{17}H_{14}N_4O$: 290.1168, Found: 290.1161.

4-Methyl-1-[(*p*-(nitrophenyl)]-1,2,4-triazolo[4,3-*a*]quinoxaline (5c): ^1H NMR ($CDCl_3$): δ 3.07 (s, 3H, CH_3), 7.14 (d, 2H, *J* = 8.0 Hz, H-3', -5'), 7.39 (dd, 1H, *J* = 1.0, 8.0 Hz, H-7), 7.49-7.56 (m, 4H, H-2', -6', -8, -9), 8.04 (dd, 1H, *J* = 1.0 & 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 20.82, 112.66, 113.89, 121.51, 125.98, 127.58, 128.19, 130.09, 131.51, 135.69, 144.86, 151.19, 153.87, 160.79. HRMS (FAB) Calc. for $C_{16}H_{11}N_5O_2$: 305.0913, Found: 305.1008.

4-Methyl-1-[(*m*-(nitrophenyl)]-1,2,4-triazolo[4,3-*a*]quinoxaline (5d): ^1H NMR ($CDCl_3$): δ 3.01 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 7.03 (dd, 1H, *J* = 1.0, 8.0 Hz, H-3'), 7.15-7.24 (m, 1H, H-5'), 7.37-7.41 (m, 2H, H-7, -8), 7.54-7.59 (m, 1H, H-4'), 7.67-7.69 (m, 2H, H-6', -9), 8.08 (dd, 1H, *J* = 1.0, 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 20.96, 111.19, 116.53, 118.84, 123.25, 126.28, 127.33, 128.08, 129.58, 132.12, 132.91, 136.25, 144.81, 147.60, 152.84, 156.08. HRMS (FAB) Calc. for $C_{16}H_{11}N_5O_2$: 305.0913, Found: 305.0911.

1-[(*p*-(*N,N*-Dimethylphenyl)]-4-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline (5e): ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, CH_3), 3.10 (s, 6H, $N(CH_3)_2$), 6.86 (d, 2H, *J* = 9.0 Hz, H-3', -5'), 7.34 (dd, 1H, *J* = 1.0, 8.0 Hz, H-7), 7.51-7.58 (m, 3H, H-aromatic), 7.75 (dd, 1H, *J* = 1.0, 8.0 Hz, H-9), 8.01 (dd, 1H, *J* = 1.0, 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 19.46, 38.54, 110.25, 114.29, 124.48, 125.57, 126.24, 127.71, 128.13, 129.14, 134.84, 143.17, 149.25, 150.01, 151.23. HRMS (FAB) Calc. For $C_{18}H_{17}N_5$: 303.1484, Found: 303.1479.

4-Methyl-1-phenyl-1,2,4-triazolo[4,3-*a*]quinoxaline (5f): ^1H NMR ($CDCl_3$): δ 2.48 (s, 3H, CH_3), 7.39 (dd, 1H, *J* = 1.0, 8.0 Hz, H-7), 7.50-7.56 (m, 5H, H-aromatic), 7.67 (d, 2H, *J* = 7.0 Hz, H-3', H-5'), 8.09 (dd, 1H, *J* = 1.0, 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 21.2, 21.7, 116, 125.2, 126, 127.5, 128.3, 129.9, 130, 136.4, 141.5, 144.90 150.4, 153. HRMS (FAB) Calc. for $C_{16}H_{12}N_4$: 260.1062, Found: 260.1069.

1-[(*p*-(Chlorophenyl)]-4-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline (5g): ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, CH_3), 7.35 (dd, 1H, *J* = 1.0, 8.0 Hz, H-7), 7.50-7.66 (m, 6H, H-aromatic), 8.04 (dd, 1H, *J* = 1.0 & 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 21.68, 116.09, 126.04, 127.08, 128.25, 128.73, 130.04, 130.74, 131.80, 137.02, 137.88, 145.52, 149.45, 153.33. HRMS (FAB) Calc. For $C_{16}H_{11}N_4Cl$: 294.0672,

Table 1. Physical Characteristics of Title Compounds

S.No	Compd	mp (°C)	Yield (%)	Molecular Formula[a]	% inhibition[b] against *Mycobacterium tuberculosis* H37 Rv
5a	*p*-$CH_3C_6H_4$	212-213	76	$C_{17}H_{14}N_4$	58
5b	*p*-$OCH_3C_6H_4$	186-187	74	$C_{17}H_{14}N_4O$	43
5c	*p*-$NO_2C_6H_4$	248-250	61	$C_{16}H_{11}N_5O_2$	22
5d	*m*-$NO_2C_6H_4$	201-203	60	$C_{16}H_{11}N_5O_2$	19
5e	*p*-$N(CH_3)_2C_6H_4$	257-259	69	$C_{18}H_{17}N_5$	62
5f	C_6H_5	205-206 (203[14])	65	$C_{16}H_{12}N_4$	02
5g	*p*-ClC_6H_4	220-222	75	$C_{16}H_{11}N_4Cl$	74
5h	*m*-$OCH_3C_6H_4$	168-170	72	$C_{17}H_{14}N_4O$	67
INH		-	-	-	95
Rifampin		-	-	-	98

[a]Elemental analyses for C, H, N are within ± 0.4% of the theoretical values. [b]Compounds were tested at a single concentration of 6.25 μg/mL.

Found: 294.0666.

4-Methyl-1-[(*m*-(methoxyphenyl)]-1,2,4-triazolo[4,3-*a*]-quinoxaline (5h): ^{1}H NMR ($CDCl_3$): δ 3.05 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 7.07 (dd, 1H, J = 1.0, 8.0 Hz, H-3'), 7.17-7.21 (m, 1H, H-5'), 7.30-7.39 (m, 2H, H-7, -8), 7.50-7.54 (m, 1H, H-4'), 7.61-7.65 (m, 2H, H-6', -9), 8.01 (dd, 1H, J = 1.0, 8.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 21.22, 55.44, 111.14, 115.53, 117.48, 121.28, 126.22, 127.33, 128.08, 129.58, 132.12, 132.91, 136.25, 144.98, 147.56, 152.84, 158.08. HRMS (FAB) Calc. For $C_{17}H_{14}N_4O$: 290.1168, Found: 290.1162.

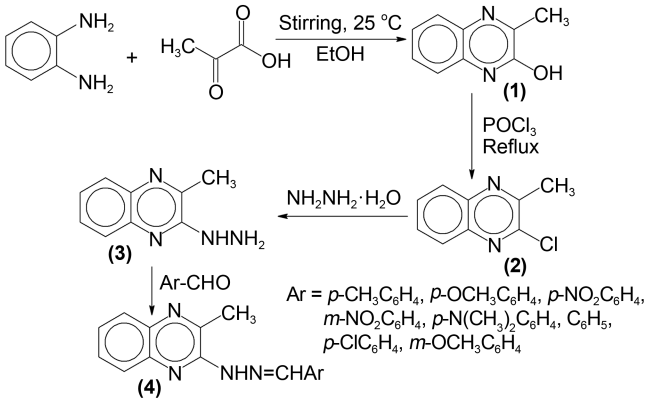
***In vitro* Antimycobacterial Screening:** The synthesized compounds were tested for their antimycobacterial activity *in vitro* against *Mycobacterium tuberculosis* $H_{37}R_v$ using the BACTEC 460 radiometric system.[31] Stock solutions of test compounds were prepared in DMSO at 1 mg/mL and sterilized by passage through 0.22 mm PFTE filters (Millex-FG, Millipore, Bedford, MA). Controls received 50 mL DMSO. Rifampin (Sigma Chemical Co, St. Louis, MO) was included as a positive drug control. Assays were usually completed in 5-8 days and percent inhibition was measured as (1-GI of test sample/GI of control) × 100 where GI represents growth index.

Results and Discussion

We report herein synthesis of triazolo quinoxalines under solvent free conditions. 3-methylquinoxalin-2-yl-hydrazones, arenecarbaldehyde and iodobenzene diacetate were triturated in a pestle and mortar for about 2-3 minutes with slight warming in some cases to afford the title compounds. 3-methylquinoxalin-2-yl-hydrazones, were prepared as per the literature protocol (Scheme 2).[17,18]

All the synthesized compounds (**5a-h**) were characterized by elemental analysis (CHN) and spectral (IR, ^{1}H NMR and MS) data. Formation of hydrazones (**4a-h**) was confirmed by previously reported melting points[17,18] and the absence of primary amino group at 3350 cm^{-1} in IR spectra. An important characteristic feature in the ^{1}H NMR spectra of **5a-h** was the disappearance of the signals at δ 8.5 and 9.3 for aldehydic H and for NH, respectively, which were present in the spectra of the intermediate hydrazones (**4a-h**). In ^{1}H NMR spectra, peak at δ 7.66-8.33 and δ 6.35-8.07 indicates the presence of quinoxaline ring and phenyl ring respectively. Elemental (CHN) analysis indicated that calculated and observed values were within the acceptable limits (± 0.4%).

All the synthesized compounds were screened for their antitubercular activity against *Mycobacterium tuberculosis* $H_{37}R_v$. Rapid glance to the obtained results revealed that the synthesized compounds did not show any improvement in antimycobacterial activity compared to the parent drug. A comparison of the substitution pattern in the 1,2,4-triazolo nucleus demonstrated that the order of activity among the substituents was *p*-Cl > *m*-OCH_3 > *p*-$N(CH_3)_2$ > *p*-CH_3 > *p*-OCH_3 > *p*-NO_2 > *m*-NO_2. Unsubstituted derivative was found be the least active compound. Interestingly the *ortho*, *para* directors were found to exhibit better activity than the *meta* directors followed by the unsubstituted derivative. Overall, among the newer derivatives, **5g** was found to be most active one with percentage inhibition of 74%.



Scheme 2. Synthesis of 3-methylquinoxalin-2-yl-hydrazones[17,18] **(4a-h)**.

Conclusion

A rapid, solvent-free and environmentally benign method was developed for the synthesis of 1-aryl-4-methyl-1,2,4-triazolo[4,3-*a*]quinoxalines and these compounds were tested for their antitubercular activity. Among the tested compounds **5g** was found to be the most active one.

Acknowledgments. The authors are thankful to Southern Research Institute, Birmingham, Alabama, USA, for the *in vitro* evaluation of antimycobacterial activity and RSIC-CDRI, Lucknow for analytical support.

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