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Synthesis and Antitubercular activity of Isoxazole incorporated 1, 2, 3-Triazole Derivatives.

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ABSTRACT

Cyclization of 1-azido-4-methoxy benzene (**1**) with acetyl acetone in presence of sodium ethoxide gave 1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazole-4-yl)ethanone(**2**). Claisen-Schmidt condensation of compound **2** with different aromatic and heretocyclic aldehydes afforded triazolyl Chalcones **(3a-h)** which on refluxing with hydroxylamine hydrochloride in glacial acetic acid gave 4-(5-(4-substituted phenyl)isoxazol-3-yl)-1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazoles **4(a-h)** in good yields. In antitubercular screening against H_{37}RV and DUK 156, compounds 4a, 4d and 4e exhibited good activity and the results are comparable with the standard drug, Isoniazid

Keywords: Triazole, Isoniazid, M. tuberculosis, Antitubercular

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INTRODUCTION

1, 2, 3-triazole is considered as an important chemical moiety because of its various physiological and pharmacological activities like anti-microbial[1], anti-malarial[2], analgesic & anti-inflammatory[3], anti-tubercular[4,5], anti-viral[6], and anticancer[7] etc., Compounds possessing isoxazole moiety are endowed with anti-bacterial[8], antiviral[9], anti-fungal[10], anti-tumour, anti-inflammatory[11], and anti-convulsant[12] activities. The combination of structural features of both of these heterocycles into a single scaffold is expected to provide new chemical entities with synergistic pharmacological properties. In the present study, eight new 4-(5-(4-substituted phenyl)isoxazol-3-yl)-1-(4-methoxyphenyl)-5-methyl- 1*H*-1,2,3-triazole derivatives (4a-h) were synthesized and characterized by physical and spectral data and were screened for anti tubercular activity.

EXPERIMENTAL

All the chemicals and solvents used were of synthetic grade from Sd fine chemicals Ltd., (Mumbai, India) and Aldrich chemicals. The purity of the compounds was checked by a single spot in TLC and solvent system for TLC was determined on trial and error basis. Melting points were determined in open capillary tubes using ANALAB melting point apparatus and are uncorrected. All the ^{1}H NMR spectra were recorded on AVANCE 300 MHz spectrometer using DMSO-d_6 as solvent and tetra methyl silane(TMS) as an internal standard. Chemical shift values are listed in δ scale. The IR spectra were recorded on Schimadzu FTIR spectrophotometer by using 1**%** KBr discs. Mass spectra of the compounds were recorded on mass spectrometer of Agilent 6430 triple quadruple LC-MS system.

Synthesis of chalcones 3(a-h)

Equimolar quantities of 1-1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)ethanone and substituted aromatic/heterocyclic aldehyde were dissolved in 15 - 20 ml of methanol and to that 3 to 5 ml of NaOH (10%) was added and stirred to obtain the precipitate. The reaction was monitored by TLC and after the completion of the reaction, the precipitate was filtered and washed thoroughly with water and recrystallized from aq. methanol.

Synthesis of 4-(5-(4-substituted phenyl)isoxazol-3-yl)-1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazoles 4(a-h)

To the chalcone (0.01 M), three times of hydroxyl amine hyrdrochloride (0.03 M) was added followed by 1g of sodium acetate and 10-15 ml of acetic acid contained in a RB flask and allowed to reflux for 14 –16 h at 120 ^{0}C. The completion of the reaction was monitored by TLC. The reaction mixture was poured into crushed ice to obtain the solid product. Then the precipitate was filtered under suction, washed thoroughly with water and recrystallised from aq. methanol. The structural confirmation and purity of the compound was done with the help of FTIR, Mass, ^{1}HNMR spectra and Melting point.

(4a) : IR: (KBr)cm^{-1}:2926 (C-H of CH_3),1612 (C=N), 1600 (C=C), 1343 (N-O), 1093 (C-O), 756(C-Cl).; ^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6):2.5 (s,3H, CH_3), 3.85 (s,3H, OCH_3), 7.2-8.1 (9H, Ar-H); Mass : m/z :367 (M+1);[Found :C, 62.05; H, 3.98; N, 15.09 $C_{19}H_{15}N_4O_2Cl$ calculated C,62.22; H,4.12; N,15.27 %].

(4b): IR: (KBr)cm^{-1}: 2980 (C-H of CH_3), 1612 (C=N), 1550 (C=C), 1343 (N-O), 1093 (C-O), 753(C-Cl); ^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6): 2.2 (s, 3H, CH_3), 3.55(s,3H, OCH_3), 7.4-8.2 (9H, Ar-H); Mass : m/z :367 (M^+1);[Found :C, 62.05; H, 3.98; N, 15.09 $C_{19}H_{15}N_4O_2Cl$ calculated C,62.22; H,4.12; N,15.27 %].

(4c): IR: (KBr)cm^{-1}: 2980 (C-H of CH_3),1604 (C=N), 1580 (C=C), 1431 (N-O), 1093(C-O), 810 (C-F); ^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6): 2.4 (s, 3H, CH_3) , 3.7(s, 3H, OCH_3) 7.1 – 8.0 (9H, Ar-H); Mass : m/z :351 (M^+1);[Found :C, 64.95; H, 4.18; N, 15.89 $C_{19}H_{15}N_4O_2F$ calculated C,65.14; H,4.32; N,15.99 %].

(4d): IR: (KBr)cm^{-1}:2850 (C-H of CH_3), 1606(C=N), 1575 (C=C), 1343 (N-O),1160 (C-O), 810 (C-NO_2); ^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6): 2.3 (s, 3 H, CH_3) ,3.75(s,3H, OCH_3) 7.3 – 8.1 (9H, Ar-H); Mass : m/z :378 (M+1);[Found :C, 60.35; H, 3.88; N, 18.49 $C_{19}H_{15}N_5O_4$ calculated C,60.48; H,4.01; N,18.56 %].

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(4e): IR: (KBr)cm^{-1}:2855 (C-H of CH_3), 1616(C=N), 1575 (C=C), 1343 (N-O),1160 (C-O), 810 (C-NO_2);^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6): 2.2 (s, 3H, CH_3) ,3.81(s, 3H, OCH_3) 7.2 – 8.0 (9H, Ar-H); Mass : m/z :378 (M+1);[Found :C, 60.35; H, 3.88; N, 18.49 $C_{19}H_{15}N_5O_4$ calculated C,60.48; H,4.01; N,18.56 %].

(4f): IR: (KBr)cm^{-1}: 2992 (C-H of CH_3), 1608 (C=N), 1558 (C=C), 1303 (N-O),1170 (C-O); ^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6): 2.5 (3H s, CH_3) ,3.72 (s,3H, OCH_3) 7.3 – 8.1 (9H, Ar-H); Mass : m/z :323 (M+1);[Found :C, 63.15; H, 4.09; N, 17.29 $C_{17}H_{14}N_4O_3$ calculated C,63.35; H,4.38; N,17.38 %].

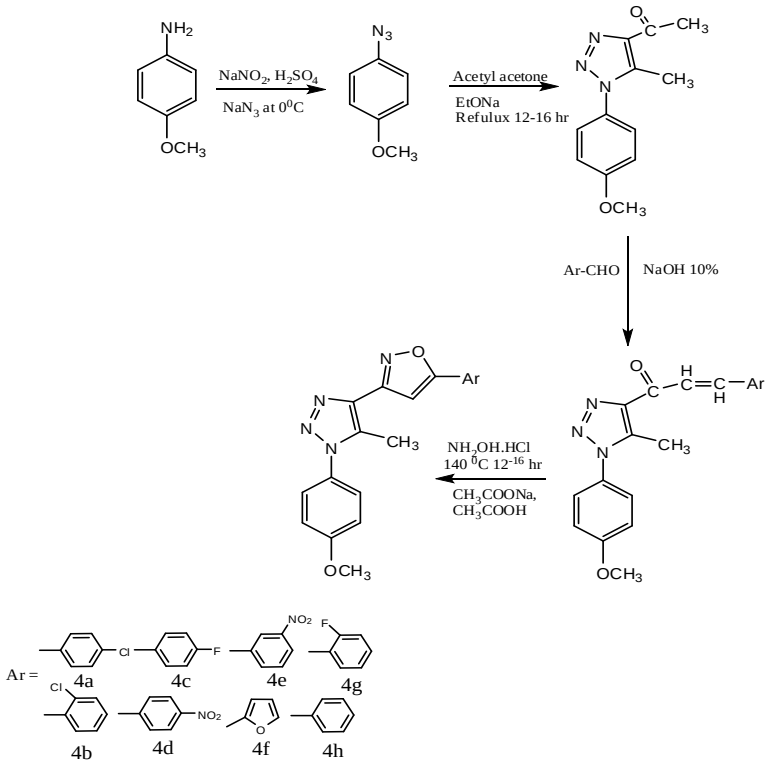
(4g): IR: (KBr)cm^{-1}: 2980 (C-H of CH_3),1604 (C=N), 1580 (C=C), 1431 (N-O), 1093(C-O), 810 (C-F); ^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6): 2.3 (s, 3H, CH_3) ,3.68(s,3H, OCH_3) 7.2 – 8.1 (9H, Ar-H); Mass : m/z :351 (M+1);[Found :C, 64.95; H, 4.18; N, 15.89 $C_{19}H_{15}N_4O_2F$ calculated C,65.14; H,4.32; N,15.99 %].

(4h): IR: (KBr)cm^{-1}: (KBr)cm^{-1}:2916 (C-H of CH_3),1614 (C=N), 1600 (C=C), 1343 (N-O), 1093 (C-O), ^{1}H NMR (300MHz, $CDCl_3$ +DMSO d_6): 2.3 (s,3H, CH_3) ,3.82(s,3H, OCH_3) 7.4 – 8.2 (9H, Ar-H); Mass : m/z :331 (M+1);[Found :C, 68.55; H, 4.78; N, 16.79 $C_{19}H_{15}N_4O_2$ calculated C,68.66; H,4.85; N,16.86 %].`

ANTI-TUBERCULAR ACTIVITY

The anti-tubercular screening of synthesized compounds was performed against INH sensitive strain H_{37}Rv and multidrug resistant strain DKU 156. The MIC of each compound was determined by broth dilution assay[13]. In the assay, compounds were prepared in two fold dilutions from 10 µg/ml to 0.3125 µg/ml in DMSO. Then the inoculated cultures were incubated at 37 ±2 ^{0}C for three weeks. The appearance of turbidity was considered as growth and indicated resistance to the compound. Growth was compared against negative control (without drug and inoculum) positive control (without drug), and with standard isoniazid by visualization.

RESULTS AND DISCUSSION



Scheme 1

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Isooxazole incorporated 1,2,3-triazole derivatives were synthesized as per scheme 1 from 1-azido-4-methoxy benzene(1). Cyclization of compound 1 with acetyl acetone in presence of sodium ethoxide gave 1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazole-4-yl)ethanone(2). From compound 2, different Chalcones (3a-h) were synthesized by Claisen-Schdmit condensation using different aromatic and heretocyclic aldehydes. The structures of resulting chalcones were confirmed on the basis of FTIR and Mass spectral data. Further, the chalcones on heating with hydroxylamine hydrochloride in acetic acid at 120^0 C for 14-16 h gave isoxazolyl triazole derivatives (4a-h) in good yields. The structural confirmation of all the synthesized compounds was done with the help of FTIR, Mass, ^{1}H NMR spectra and physical data. The physical data of synthesized compounds are presented in Table 1. In IR spectra, the disappearance of carbonyl absorptions at 1660 cm^{-1} in chalcone and appearance of a new absorption peaks around 1600 cm^{-1} due to C=N stretching clearly indicated the formation of final compounds. Further proton NMR spectra of compounds showed singlet around δ2.5 due to three protons of methyl group, a singlet around δ3.85 due to three methoxy protons and aromatic protons appeared in the range of δ7.2-8.1. In mass spectra, all the compounds gave molecular ion peaks of 100% intensity corresponding to their molecular weights gave further confirmation.

Table 1: Physical data of synthesized compounds (4a-h)

S.NO	Compound	Molecular formula	Molecular weight	Melting point (^{0}C)	Yield %
1	4a	$C_{19}H_{15}N_4O_2Cl$	366	128-131	78
2	4b	$C_{19}H_{15}N_4O_2Cl$	366	130-132	70
3	4c	$C_{19}H_{15}N_4O_2F$	350	127-129	74
4	4d	$C_{19}H_{15}N_5O_4$	377	132-135	73
5	4e	$C_{19}H_{15}N_5O_4$	377	134-135	70
6	4f	$C_{17}H_{14}N_4O_3$	322	135-137	68
7	4g	$C_{19}H_{15}N_4O_2F$	350	126-128	72
8	4h	$C_{19}H_{15}N_4O_2$	330	122	67

ANTI TUBERCULAR ACTIVITY

All the synthesized compounds (4a-h) were screened for anti-tubercular activity against *Mycobacterium tuberculosis* H_{37}Rv and DKU 156 strains using broth dilution assay method [13]. The MIC values of synthesized compounds are given in Table 2. In screening, among eight derivatives, except 4b, others have shown excellent activity against both the strains at tested concentrations. Particularly, Compounds 4d and 4e showed activity almost equivalent to Isoniazid, while compound 4a has slightly lower activity than the standard. However, compounds 4c, 4f, 4g and 4h are moderately active. All the tested compounds, except 4b and 4h exhibited better activity against DKU 156 strain compared to Isoniazid.

Table 2: Anti-tubercular activity of 4-(5-(4-substituted phenyl)isoxazol-3-yl)-1-(4-methoxyphenyl)-5-methyl-1*H*-1,2,3-triazole derivatives (4a-h)

Compound	MIC values (µg/mL)	
	H_{37}Rv	***DKU 156***
4a	**1.25**	2.5
4b	>10	>10
4c	5	5
4d	**0.625**	1.25
4e	**0.625**	1.25
4f	2.5	5
4g	2.5	5
4h	2.5	10
INH	0.625	10

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