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Synthesis and antitubercular activity of some novel N-methyl triazolone derivatives

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ABSTRACT

The N-methyl Triazolone is prepared by methylation of triazole-5-one which was further converted in to the respective acids by reacting with p-amino benzoic acid (Mannich base condensation). The same was converted to ester and then to hydrazides. From this the various Schiff bases with aldehydes were prepared. The structures of the synthesized Compounds were evaluated by IR, 1H NMR spectra Mass Spectroscopy and elemental method of analysis. All the synthesized compounds were screened for their anti-tubercular activities. Anti-tubercular activity was carried out by using middle brook 7H9 broth base (M198) medium against Mycobacterium tuberculosis. Compounds P4 and P5 showed very significant activity while P1, P2 & P3 showed moderate antitubercular activity. Streptomycin and Pyrizinamide were used as standard drugs.

Keywords: N-methyl-triazolone, anti-tubercular activity, Mannich Base, Schiff base, Mycobacterium tuberculosis.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, a slow growing bacterium, evolved from soil bacterium. Tuberculosis is a disease that has been known from the earliest of recorded history [1]. The insidious nature of the infection and its association with some of the brightest minds invested the disease with a mystical quality [2]. The present first line drugs like INH, Pyrazinamide, Ethambutol, and Rifampicin are potent antitubercular agent. They act by inhibition of mycolic acid and RNA / DNA synthesis [3]. They possess numerous adverse reactions so to avoid these effects it seemed promising to look for more selective compounds, at other targets to suppress the activity [4].Lots of modifications have been made during last decades on Triazolone nucleus and their derivatives have been studied extensively for their biological activities. A survey of literature revealed that these Triazolone derivatives possess different types of potential biological activities that include Antioxidant, Antifungal, Antihaemostatic and Antibacterial. [5, 6, 7, 8]

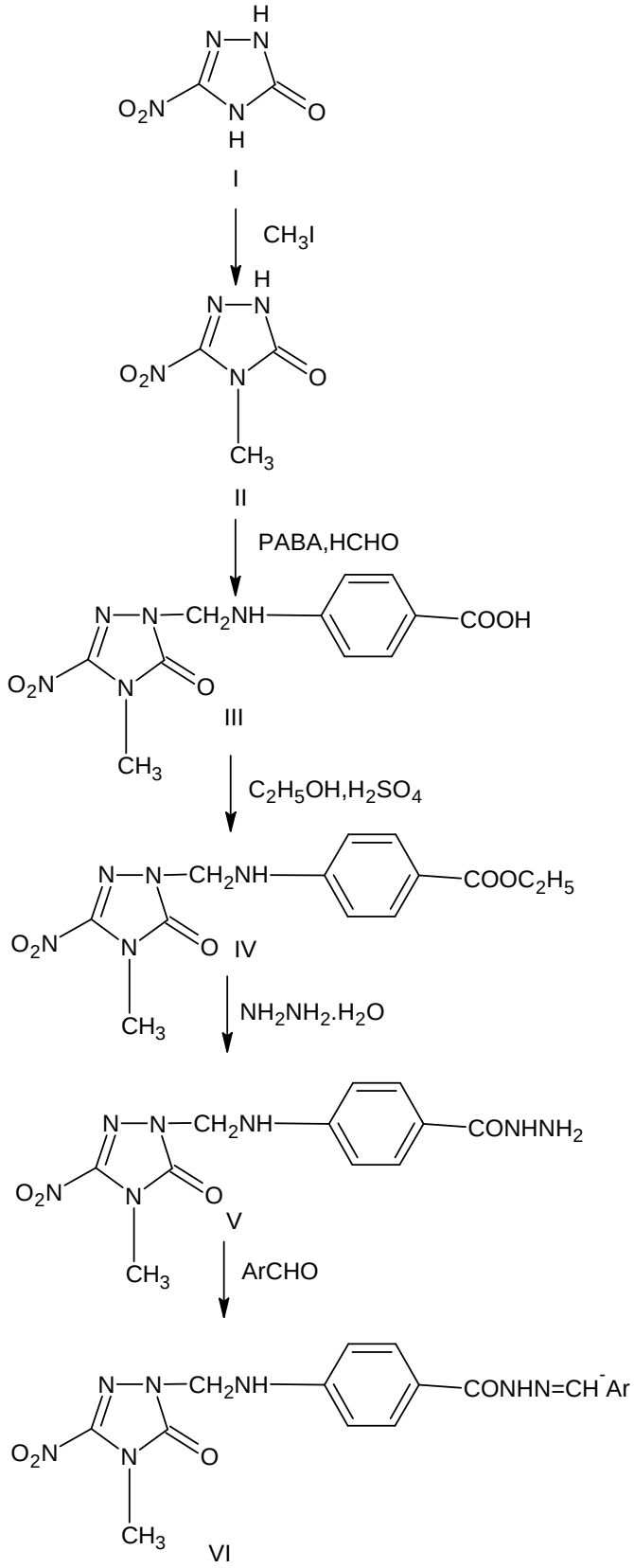
Substituted aromatic aldehyde moiety with Triazolone nucleus for the first time has been associated to be designed for biological activity. As Triazolone nucleus moiety possesses potent anti-tubercular activity [8]. From all the above forgoing facts it was thought and considered to be very interesting to synthesis new series of Triazolone derivatives fused with aromatic aldehyde for anti-tubercular activity.

MATERIALS AND METHODS

The melting point of compounds were determined by open capillary tube method and are uncorrected. Synthesized compounds were subjected to ^{1}HNMR. IR spectra were recorded on spectrophotometer using KBr disc method. All solvents used were analytical grade.

Synthesis of 1, 2, 4,-triazolin-5-one: A mixture of semicarbazide hydrochloride. (10g, 0.90 mol), trimethyl orthoformate (21.22g, 2.0 mol) and methanol (10 ml.) was stirred at 20°C for 3 days. the solvent was then removed under reduced pressure and toluene (10 ml.) was added and the slurry was concentrated further to remove residual methanol the mixture was then cooled to 0°C and filtered to afford 1,2,4,-triazolin-5-one as a white solid.[9]

Scheme for Synthesis



Synthesis of 5-nitro-2,4-dihydro-3H-1,2,4-triazol-3-one (nitration): Nitric acid (90ml) was added to the (10g) of triazolone marinating the temperature between 0°C to 5°C. The mixture was heated to 60-70°C with constant stirring .The reaction was exothermic and brown fumes were evolved. After sometime mixture was chilled (0 to5°C) in an ice-bath, filtered and washed with water to remove excess nitric acid. Pure nitrotriazolone was crystallized by water. Yield 80% [10].

Synthesis of N-Methylation of 5-nitro-2,4-dihydro-3H-1,2,4-triazol-3-one: The mixture of 3-Nitro-1,2,4-Triazol-5-one in acetone and Dry Potassium carbonate and Methyl Iodide in Acetone were taken in the RBF in proportion of 1: 1. The mixture was Refluxed for 10 hrs. After Refluxation acetone was removed and recrystallised from water.

General procedure for Mannich base condensation: A mixture of corresponding compound (II) (0.01mol.) in ethanol (15ml), formaldehyde (0.02mol.) and aromatic amine (0.02mol.) was added. The reaction mixture was refluxed for a period 2-6-hours, the solvent was poured into ice water. Resulting solid was filtered off dried and recrystallized using appropriate solvent. [11]

Esterification: Ethylchloroacetate (0.01mol.) was added to a corresponding compound (III) (0.01mol.) in dry acetone (20ml).to that solution potassium bicarbonate (1gm) was added and the mixture was refluxed for 10 hours, Acetone was removed after completion of the reaction and the residue was crystallized from ethanol. [12]

4-{[(4-methyl-3-nitro-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]amino}benzohydrazide:
A solution of corresponding compound (IV) (0.01mol.) in n-butanol was refluxed with hydrazine hydrate (0.025mol.) for 4 hours .After cooling to room temperature a solid was appeared and this was recrystallized from an ethanol to afford the desired product [13].

Synthesis of N'-(4-aldehyde)-4-{[(4-methyl-3-nitro-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1 yl) methyl] amino} benzohydrazide: A solution of the corresponding compound (III) (0.01 mol) in ethanol was refluxed with appropriate aldehyde (0.01 mol) for 3 h. after cooling to room temperature, a white solid appeared. This crude product was recrystallized from an ethanol to afford the desired product. [14]

IUPAC names of all synthesized compounds are mentioned in Table No 03. The antitubercular screening was carried out by Middle brook 7H9 agar medium against H37Rv Strain. Middle brook 7H9 agar medium containing different derivatives (P1 – P5), standard drug as well as control, Middle brook 7H9 agar medium was inoculated with Mycobacterium tuberculosis of H37Rv Strain. The inoculated bottles were incubated for 37°C for four weeks. After four weeks they were checked for growth. [15 & 16]

Table No. 01: List of Substituent's

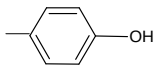
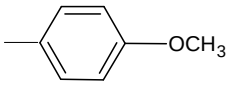
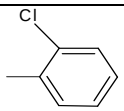
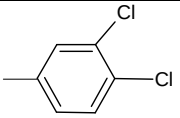
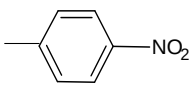
List of Substitutions in Scheme 01 Where -Ar- denotes






Table No 02: Analytical data of the synthesized compounds

Comp.	Mol. Formula	Mol. Wt.	m.p ^{0}C	Yield %	Elemental analyses Calculated (%)			Rf Value
					C	N	H	
P_1	$C_{18}H_{19}N_7O_3$	381.89	122	71	56.69	25.71	5.02	0.6
P_2	$C_{19}H_{21}N_7O_3$	395.42	135	63	57.71	24.80	5.35	0.5
P_3	$C_{18}H_{18}ClN_7O_2$	399.83	110	60	54.07	24.52	4.54	0.4
P_4	$C_{18}H_{17}Cl_2N_7O_2$	433.28	118	65	49.78	22.28	9.95	0.6
P_5	$C_{18}H_{18}N_8O_4$	410.39	125	65	52.68	27.30	4.42	0.5

Table No. 03: IUPAC names of the synthesized compounds

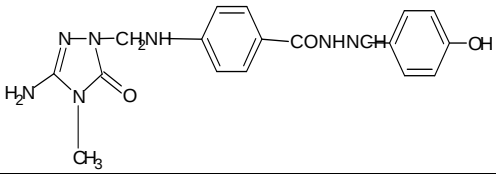
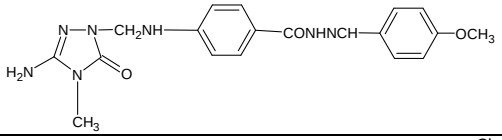
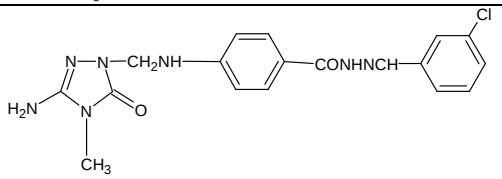
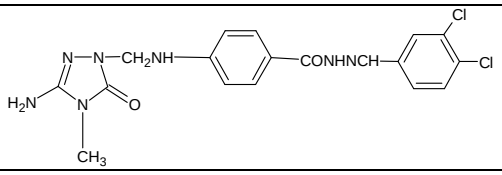
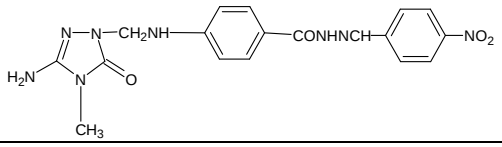
Compounds	Structures	IUPAC Names
P_1		N'-(4-hydroxybenzylidene)-4-{[(4-methyl-3-nitro-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]amino}benzohydrazide
P_2		N'-(4-methoxybenzylidene)-4-{[(4-methyl-3-nitro-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]amino}benzohydrazide
P_3		N'-(3-chlorobenzylidene)-4-{[(4-methyl-3-nitro-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]amino}benzohydrazide
P_4		N'-(3,4-dichlorobenzylidene)-4-{[(4-methyl-3-nitro-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]amino}benzohydrazide
P_5		4-{[(4-methyl-3-nitro-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]amino}-N'-(4-nitrobenzylidene)benzohydrazide

Table No. 04: Spectral data (IR) of synthesized compounds

Compound Code	IR Frequency in cm-1	Type of vibrations
NT	1631 1561	-C=O -NO_2 Str
NMT	1678 1548 3211	-C=O -NO_2 Str =NH Str
P_3	650 1700 1530 3050 3300 1475	Ar-Cl Str -C=O -NO_2 Str = CH Str =NH Str = CN Str
P_4	753 1631 1593 3039 3359 1473	Ar-Cl Str -C=O -NO_2 Str = CH Str =NH Str = CN Str
P_5	1632 1594 3217 3360 1444	-C=O -NO_2 Str = CH Str =NH Str = CN Str

Table No. 05: Spectral data (^{1}H NMR) of synthesized compounds.

Compound Code	No. of protons	δ values in ppm
NMT	3H of CH_3 1H of NH	3.42 12.83
P_5	3H of CH_3 1H of N-Aro. ring Aro. ring Enolic NH Enolic CH	2.00 3.01 6.8 7.3 7.8 8.2

Table No. 06: Microbial Data

Compounds(mcg/ml)	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
P_1	S	S	R	R	R	R	R	R	R	R
P_2	S	S	S	R	R	R	R	R	R	R
P_3	S	S	S	S	S	R	R	R	R	R
P_4	S	S	S	S	S	S	R	R	R	R
P_5	S	S	S	S	S	S	R	R	R	R
Pyrizinamide	S	S	S	S	S	S	R	R	R	R
Streptomycin	S	S	S	S	S	R	R	R	R	R

***R**- Resistance and **S**- Sensitive.*

RESULTS AND DISCUSSION

Present work intended to synthesize the N-methyl triazolone derivatives associated with p-amino benzoic acid.The N-methyl Triazolone is prepared by methylation of triazole-5-one which was further converted in to the respective acids by reacting with p-amino benzoic acid (Mannich base condensation). The same was converted to ester and then to hydrazide. From this the various Schiff bases with aldehydes were prepared. Structure of compounds was confirmed by IR, NMR and Mass spectra (Table No: 04 & 05). The synthesized compounds were screened for antitubercular activity. In the antitubercular activity studies, of these compounds P4 and P5 showed very significant activity and P1, P2, P3 showed moderate antitubercular activity (Table No: 06). Middlebrook 7H9 broth was used. Streptomycin and Pyrizinamide were used as standard drugs for comparison. All the compounds were found to be very good antitubercular agents and present synthesized compounds can definitely act as lead compound for future molecular manipulation studies.

CONCLUSION

From the above result it is evident that suitable molecular manipulation can still bring about compounds which could prove equal or better activity compared to the standard drug.

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