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Original Article

Synthesis and Biological Activity of Certain Mannich Bases Derivatives from 1, 2, 4-Triazoles

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Abstract

Substituted 1, 2, 4- Triazoles have shown multiple biological activities such as anti-inflammatory, anti fungal, etc. 5-mercapto triazoles were prepared from the potassium dithiocarbazinates. These triazoles were used for preparation of different derivatives by two different schemes. In the first scheme the Mannich bases were prepared from 5- marcapto-s triazole Quinazolines. The 5-Marcato-s-Triazole Quinazolines were synthesized from 5-Marcapto-s-Trizole by reacting with the Quinazoline benzoic acid or acid chlorides. In the second scheme, the synthesis of Quinazolyl Triazolo Thiadiazole Derivatives were prepared by the reaction of Quinazoline benzoic acid and 5-Marcapto-s –Triazoles in $POCl_3$.All the newly synthesized compound structures were elucidated using various spectral techniques viz. IR, ^{1}HNMR and screened for *in vitro* antibacterial and anti fungal activities. Anti bacterial activity was carried out against organisms Bacillus pumilus, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli as well as anti fungal activity were carried out against A.Nigier and C.Albicans. The results substantiated that the synthesized compounds were effective against bacteria, fungi.

*Keywords***:** Antibacterial activity, Antifungal activity, Antimicrobial, Mannich bases, Quinazoline,Triazole,

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1. Introduction

Since few decades, the chemistry of 1, 2, 4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. For instance, a large number of 1,2,4-triazole-containing ring systems have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants, sedatives, antianxiety, antimicrobial agents [1,2] and antimycotic age nts such as fluconazole, itraconazole, voriconazole [3,4]. Apart from this, there are known drugs containing the 1, 2, 4- triazole group e.g. Triazolam [5], Alprazolam [6], Etizolam [7], Furacylin[8] *etc*. Moreover, heterocycles containing sulphur represent an important group of sulphur compounds that are promising for use in practical applications. Among these heterocycles, the mercapto- and thione-substituted 1,2,4-triazole ring systems have been well studied and a variety of biological activities have been reported for a large number of their derivatives, such as antibacterial [9-12], antifungal [13,14], antitubercular[15], antimycobacterial [16], anticancer [17,18], diuretic [19,20],hypoglycemic [21] properties etc. The Mannich base is an end product of the Mannich reaction, i.e. nucleophilic addition reaction of a non-enolizable aldehyde and any primary or secondary amine to produce resonance stabilized imine.

This initiated the authors to make a sincere attempt to develop new biologically active compounds, as Mannich bases derived from triazole, through a convenient synthesis unreported as of now.

2. Materials and Methods

2.1. Experimental

Synthesized compounds were subjected to MP, FTIR and ^{1}HNMR spectral studies. Melting point of compounds was determined in open capillary tube. The IR spectra of the samples were recorded in KBr on a Jasco FT/IR-460 plus Fourier transforms infrared spectrophotometer. ^{1}H NMR study was carried out on EM 390 MHz spectrometer (DMSO-d6) using TMS as internal standard (Chemical shifts are expressed in δ ppm). All the compounds showed satisfactory micro analytical results for C, H and N. Samples were also checked by TLC using n-hexane: ethyl acetate (4:1) and

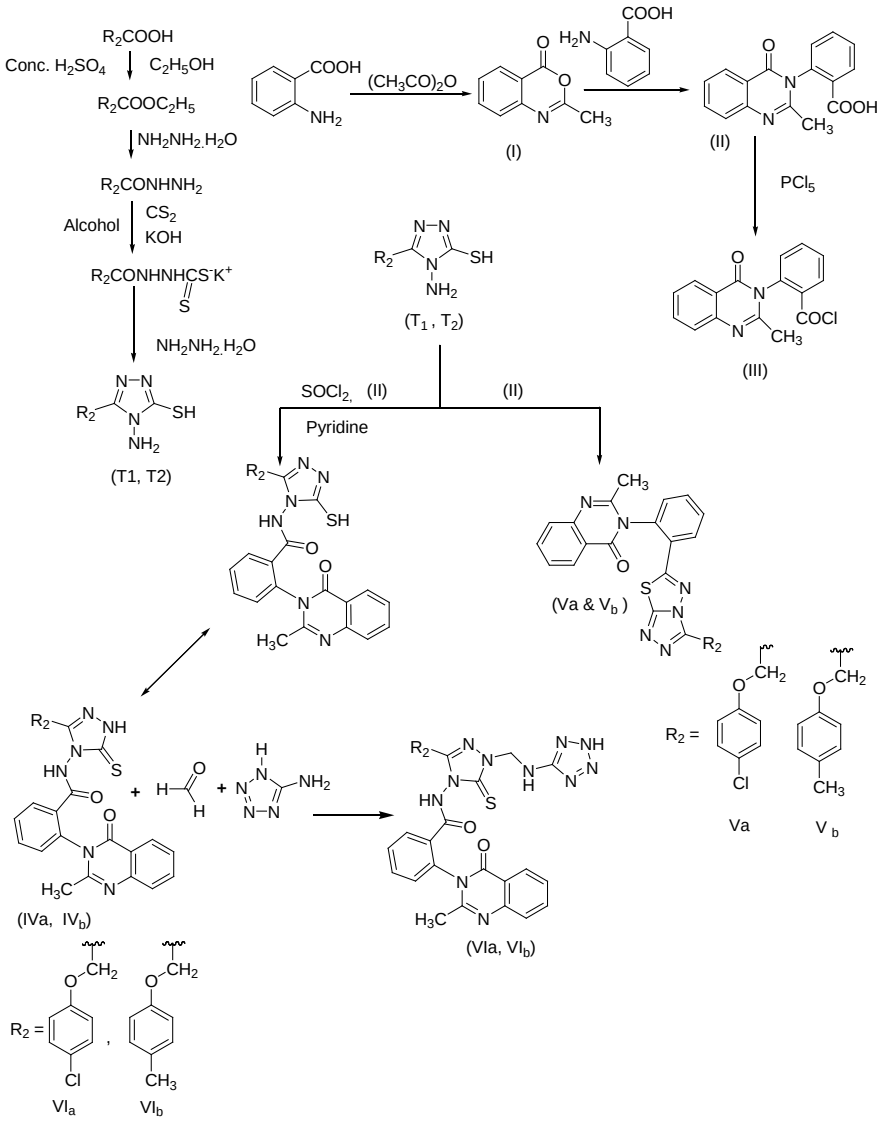

Figure 1. Scheme for the synthesis of different triazoles and Mannich bases.

chloroform: methanol(4:1) as mobile phase and silica gel G as the stationary phase.

2.2. General Synthesis Procedure (Figure 1)

2.2.1. Synthesis of hydrazides

Appropriate quantities of acid (0.1mol) and ethanol (50 ml) was introduced into a clean and dry round-bottomed flask and stirred well for 10 min. To the above mixture, few drops of concentrated sulfuric acid were added and the reaction mixture was refluxed for 6 hours. The reaction mixture was concentrated by distilling the excess ethanol under reduced pressure and treated with saturated solution of sodium bi-carbonate. The ester formed in the reaction was used for the preparation of hydrazides

directly. The appropriate ester (0.1mol) was dissolved in 50 ml of ethanol in a clean dry round-bottomed flask and to this hydrazine hydrate (0.1mol) was added. The reaction mixture was then refluxed for a period of 15 to 18 hours. The excess ethanol was distilled off under reduced pressure. The resultant mixture was then poured into ice cold water and the obtained solid was filtered, recrystallized from ethanol.

2.2.2. Synthesis of Potassium Dithiocarbazinates

An appropriate quantity of acid hydrazide (0.01mol) was taken in a clean and dry round bottomed flask. Carbon disulphide and alcoholic potassium hydroxide (1.5mol) was introduced in to the round bottomed flask containing acid hydrazide. The reaction mixture was refluxed for a period of 3-4 hours. After cooling, the separated product was collected by filtration, washed with water and dried. These compounds were used directly for the next step.

2.2.3. Synthesis of 5–Mercapto-s-Triazoles (T_1-T_2)

The appropriate Potassium Dithiocarbazinate salt (0.01mol), Hydrazine hydrate (0.01mol) and water (5 ml) was transferred in to a clean and dry round bottomed flask and the reaction mixture was refluxed for 6 hours till profuse H_2S gas evolves. The reaction mixture was further boiled for 30 minutes at the same temperature. After reflux the reaction mixture was cooled and the solid material was separated by filtration. The solid was washed with dil HCl (50%, 25 ml) and cold water (3 X 25 ml) and dried. The resultant compounds (T_1-T_2) were recrystallized by using ethanol as solvent.

2.2.4. Synthesis of Benzoxazine-4-one (I)

Required quantities of anthranilic acid (1 mol) and acetic anhydride (2mol) were introduced in to a clean and dry round bottomed flask. 25 ml of ethanol was added to the above mixture. The contents were refluxed for 2 hours. The excess ethanol was distilled off under reduced pressure. The reaction mixture was cooled and the resultant solid was collected by filtration and recrystallized by using ethanol.

2.2.5. Synthesis of Quinazoline benzoic acid (II)

A mixture of benzoxazin-4-one **(I)** (0.05 mol) and primary amines (2 or 4-amino benzoic acid) (0.05 mol) were taken in to a clean and dry round bottomed flask and glacial acetic acid (25 ml) was added. The contents of the round bottomed flask were refluxed for 4 hours. After reflux the reaction mixture was then allowed to cool and poured into ice-cold water with continuous stirring and was kept in the

refrigerator overnight. The resultant solid was filtered, washed with water, dried and recrystallized by using ethanol.

2.2.6. Synthesis of Quinazolines benzoyl chloride (III)

A mixture of appropriate quantities of quinazoline benzoic acid (0.10 mol) and phosphorus penta chloride (0.12 mol) was placed in a round bottomed flask and the reaction mixture was refluxed in an oil bath at 120-130^{o}c for 2-3 hours. The reaction mixture was then allowed to cool and the phosphorus oxychloride was removed by distillation. Then the temperature of an oil bath was raised again to 110^{o}c and the residual Quinazoline benzoyl chloride was solidified on cooling.

2.2.7. Synthesis of Quinazolyl Triazolo Thiadiazole (Va & Vb)

A mixture of Quinazoline benzoic acid (II) (0.01mol) 5–Mercapto-s-Triazoles (T_1-T_5) (0.01mol) and $POCl_3$ (10 ml) was refluxed for 4 hours. The reaction mixture was sufficiently cooled and poured into a beaker containing crushed ice with vigorous stirring. The residue thus obtained was filtered and recrystallised by using aqueous ethanol.

Va:(2-methyl-3(2-(3-((p-tolyloxy)methyl)-methyl)-1,2,4] triazolo[3,4-b][1,3,4]thiadiazol- 6-yl) phenyl) quinazolin-4(3H)-one) ^{1}HNMR, signal appeared at 6.7-7.8 ppm (Aromatic-12H), 4.8ppm singlet (2H of -OCH_2-), 1.4ppm Singlet (3H of CH_3). IR spectra shows following peaks: 2946 cm^{-1} (C-H stretching, Ar-H), 2834 cm^{-1}(C-H Stretching, Ar-OCH_2-), 1597 cm^{-1}(C=N Stretching), 1690 cm^{-1} (C= O Stretching), 1321 cm^{-1}(C-H Bending, Ar-CH_3). 1311 cm^{-1}(C-H bending, Ar-H), 739 cm^{-1} (C-Cl Stretching, Ar-Cl). m.p 250^{o}C. Yield 82 %.

Vb: (3-(2-(3-((4-chlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b]1,3,4]thiadiazole-6-yl) phenyl)-2-methylquinazolin-4(3H) one) ^{1}HNMR, signal appeared at 6.6-7.8 ppm(Aromatic-12H), 4.7ppm singlet (2H of -OCH_2-), 1.4ppm Singlet (3H of CH_3). IR spectra shows following peaks: 3010 cm^{-1} (C-H stretching, Ar-H), 2841 cm^{-1}(C-H Stretching, Ar-OCH_2-), 1590 cm^{-1}(C=N Stretching), 1690 cm^{-1}(C= O Stretching), 1293 cm^{-1} (C-H Bending, Ar-CH_3). 1311 cm^{-1}(C-H bending, Ar-H),.m.p 120^{o}C.Yield 65 %.

2.2.8. Synthesis of Triazolyl Quinazolines (IV)

Quinazoline benzoic acid (II) (0.001mol) and 20 ml of dry benzene were taken in to a round bottomed flask and stirred to dissolve the solids. To the above solution thionyl chloride (0.001) was added slowly drop wise with continuous stirring. After complete addition of thionyl chloride, the contents of the flask were refluxed for 3

hours. Cooled and distilled off the excess benzene. Fresh benzene 20 ml was added, distilled and the same procedure was repeated with another 20 ml of fresh benzene. After distillation, the 5–Mercapto-s-Triazole (T_1-T_2) (0.001mol) and 10 ml of pyridine was added to the above residue. The contents of the flask were refluxed for 12 hours. The reaction mixture was cooled and poured into an ice water. The separated solid was collected by filtration and recrystallized by means of suitable solvent.

2.2.9. Synthesis of Mannich bases (VIa and VIb)

Triazolyl Quinazoline (IVa and IVb) derivative (0.001mol) was dissolved in sufficient quantity of ethanol in a clean and dry round bottomed flask. To this solution formaldehyde (0.4 ml, 40%) was added drop wise with constant stirring. After few minutes, 2- amino 1, 2, 3, 4- tetrazole (0.001 mol) in ethanol was added drop wise with constant stirring. The stirring was continued for another 1 hr at room temperature. After 1 hr, 5-6 drops of concentrated hydrochloric acid was added and the stirring was continued for 4-6 hours. After this the solution found to be viscous and the solid product was separated by filtration, and was dried, recrystallized by using ethanol.

VI a: (N-(1-(((2H-tetrazole-5-yl) amino) methyl-3((4-chlorophenoxy)-5-thioxo-1, 5-dihydro-4H-1,2,4-triazol-4-yl)-2-(2-methyl-4-oxoquinazoline-3(4H)-yl) benzamide.

^{1}HNMR, signal appeared at 8.3ppm Singlet1H (sec. amide), 4.1ppm Singlet1H (H of CO-NH-), 6.77-7.93ppm (Aromatic-12H), 5.22ppm (2H of -CH_2-NH-), 4.3ppm singlet (2H of -OCH_2-), 4.1ppm Singlet (-CH_2-NH), 1.2ppm Singlet (3H of CH_3). IR spectra shows following peaks: 2948 cm^{-1}(C-H stretching, Ar-H), 2832 cm^{-1}(C-H Stretching, Ar-OCH_2-), 1590 cm^{-1}(C=N Stretching), 1720 cm^{-1}(C= O Stretching), 1327 cm^{-1}(C-H Bending, Ar-CH_3). 1320 cm^{-1} (C-H bending, Ar-H), 735 cm^{-1} (C-Cl Stretching, Ar-Cl).M.P 270^{o}C.Yield 60 %.

VI b: N-(1-(((2H-tetrazole-5-yl) amino) methyl)-5-thioxo-3((p-tolyloxy) methyl) 1, 5-dihydro-4H-1, 2, 4-triazole-4-yl)-2-(2-methyl-4-oxoquinazolin-3(4H)-yl) banzamide.

^{1}HNMR, signal appeared at 8.2ppm Singlet1H (sec. amide), 4.3ppm Singlet1H (H of CO-NH-), 6.65-7.95ppm(Aromatic-12H), 5.3ppmSinglet (2H of -CH_2-NH-), 4.2ppm singlet (2H of -OCH_2-), 4.1ppm Singlet(-CH_2-NH), 1.2ppm Singlet(3H of CH_3-Quinazolin), 2.3ppm singlet(3H of CH_3). IR spectra shows following peaks: 2940 cm^{-1} (C-H stretching, Ar-H), 2834 cm^{-1}(C-H Stretching, Ar-OCH_2-), 1592 cm^{-1} (C=N Stretching), 1720 cm^{-1} (C= O Stretching), 1421 cm^{-1} (C-H Bending, Ar-CH_3). 1339 cm^{-1}(C-H bending, Ar-H). M.P 245^{o}C. Yield 65 %.

2.2.10. Antimicrobial activity

All the synthesized compounds [(Va, Vb) and (VIa, VIb)] have been screened for *in Vitro* antibacterial activity against the organism Bacillus pumilus, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli and was assessed by disc-diffusion method on nutrient agar media at 50 and 100 µg/mL concentration. DMF was used as solvent control. Ciprofloxacin was used as standard drug.

The compounds [(Va, Vb) and (VIa, VIb)] were also tested for antifungal activity against the fungal organism Aspergillus Niger, Candida albicans on potato dextrose agar medium and clotrimazole 50 and 100 µg/mL was used as a standard and solvent DMF was used as control.

The solutions of 50 and 100 µg/mL conc. were prepared by diluting with sterile distilled water. The discs of 6-7 mm in diameter were punched with sterile cork borer of same size. These discs were sterilized at 140 ^{0}C for 60 minutes in hot air oven. Then the standard and test solutions were added to each disc and the discs were air-dried. The sterilized media was cooled to 45 ^{0}C with gentle shaking, and inoculated for 18-24 hrs in old culture under aseptic conditions, mixed well by gentle shaking. This was poured into sterile petri dishes (properly labeled) and allowed the medium to settle. After solidification, all the petri dishes were transferred into laminar air flow unit. The prepared discs were carefully placed on solidified media by using previously sterilized forceps. These petri dishes were kept aside for one-hour diffusion at room temperature. And then the petri dishes were kept at 37^0 c for 24 hours in an incubator. The area of inhibition was measured after 24 hours and was expressed in mm as zone of inhibition.

3. Results and Discussion

The acid hydrazides were prepared from corresponding esters by reacting with hydrazine hydrate using known method .The hydrazides then converted into their corresponding potassium dithiocarbazinates by reacting with carbon disulphide in presence of alcoholic potassium hydroxide. Excellent yields of required triazoles were observed in one pot reaction between mixture of potassium dithiocarbazinates and hydrazine hydrate for 6-8 hr. Quinoxoline acid chloride was prepared and fused with triazole to get the N- bridge compounds and further reacted with formic acid and NH_2 drug moiety to get the corresponding Mannich bases. And the other side Prepared Quinoxoline acid reacted with triazole in presence of $POCl_3$ to get the Quinazolyl Triazolo Thiadiazoles.

Table 2. In vitro Antifungal activity of synthesized compounds.

Compound	*Inhibition zone diameter in mm			
	A.Nigier		C.Albicans	
	50µg	100µg	50µg	100µg
Va	6±0.03	9±0.01	5±0.05	8±0.06
Vb	4±0.04	8±0.03	6±0.02	9±0.04
VIa	5±0.01	12±0.06	5±0.02	11±0.05
VIb	4±0.02	12±0.04	6±0.05	12±0.03
Clotrimazole	9±0.04	14±0.03	8±0.01	13±0.01
DMF	-	-	-	-

*Average triplicate ± Standard deviation
Note: - "– "denote no activity

Antimicrobial activity results indicated that few Quinazolyl Triazolo Thiadiazoles and Triazoles with Mannich bases posses significant activity , and were found to be less or more equal to the standards at given concentration level. The compounds like **Va** and **Vb** were found to posses' good activity against both gram positive and gram negative bacteria (Relative inhibition 55-70%). And the compounds VIa and VIb were found to posses' significant activity against selected fungi. (Table 1 & 2)

4. Conclusion

The two moieties, i.e. Triazole and Quinazoline independently are anti-fungal and antibacterial agents. In the present research, the two moieties were combined, which resulted in bi heterocyclic compound and was converted as Mannich base, screened for antimicrobial activity. The result showed a significant broad spectrum of antibacterial and antifungal activity. The above results established the fact that Triazolyl Quinazolines can be a rich source for further utilization.

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