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SYNTHESIS AND BIOLOGICAL SCREENING OF SOME NOVEL 1,2,4-TRIAZOLE DERIVATIVES AS ANTIMICROBIAL AGENTS

Haribhai Rabari*, Jigarkumar Damor

Department of Pharmaceutical Chemistry, L. M. College of Pharmacy, Navrangpura, Ahmedabad-380 009.

ABSTRACT

Triazoles constitute an important class of biologically active heterocyclic compounds that have received a great attention since their discovery. A novel series of 3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine has been synthesized from phenyl acetic acid. Synthesized derivatives were characterized by IR, MASS and ^{1}H-NMR spectroscopy. All synthesized compounds were screened for antimicrobial activity using agar plate diffusion method. Synthesized compounds exhibited good antimicrobial activity which was comparable to the standard drug and it can be useful for the further study.

KEYWORDS: Triazoles, Phenyl acetic acid, Antimicrobial activity.

INTRODUCTION

There is significant and continuous concern in the chemistry of five-member N-heterocyclic compounds, mainly tetrazole, triazoles, and their substituted derivatives. Five-membered nitrogen heterocyclic compounds are important structural fragments and considered as biologically active compounds.

1,2,4-Triazole is a five membered heterocyclic system consisting of two carbon atoms and three nitrogen atoms, having wide range of biological activities. Triazole derivatives are showing very promising and excellent therapeutic effectiveness. The major activities exhibited by these derivatives include antibacterial[1-3], antifungal[4], anticonvulsant[5-6], antiviral[7-8] and anti-inflammatory action[9-10].

Triazoles contain three nitrogen atom in five membered ring system at the position on 1,2,4 and 1,2,3. In which 1,2,4-triazole are more important in medicinal and pharmacological profile[11-13]. 1,2,4-triazole nucleus has been found to be potent drug in pharmaceutical industries. It is endowed with a variety of biological activities such as antimicrobial, anti-inflammatory, hypnotic, CNS depressant, anticonvulsant, antitubercular, antitumor, fungicidal etc. Certain 1,2,4-triazoles also find application in preparation of photographic plates, polymers and analytical agents. 1,2,4-triazole nucleus are well known as drugs, *e.g.* fluconazole, itroconazole.

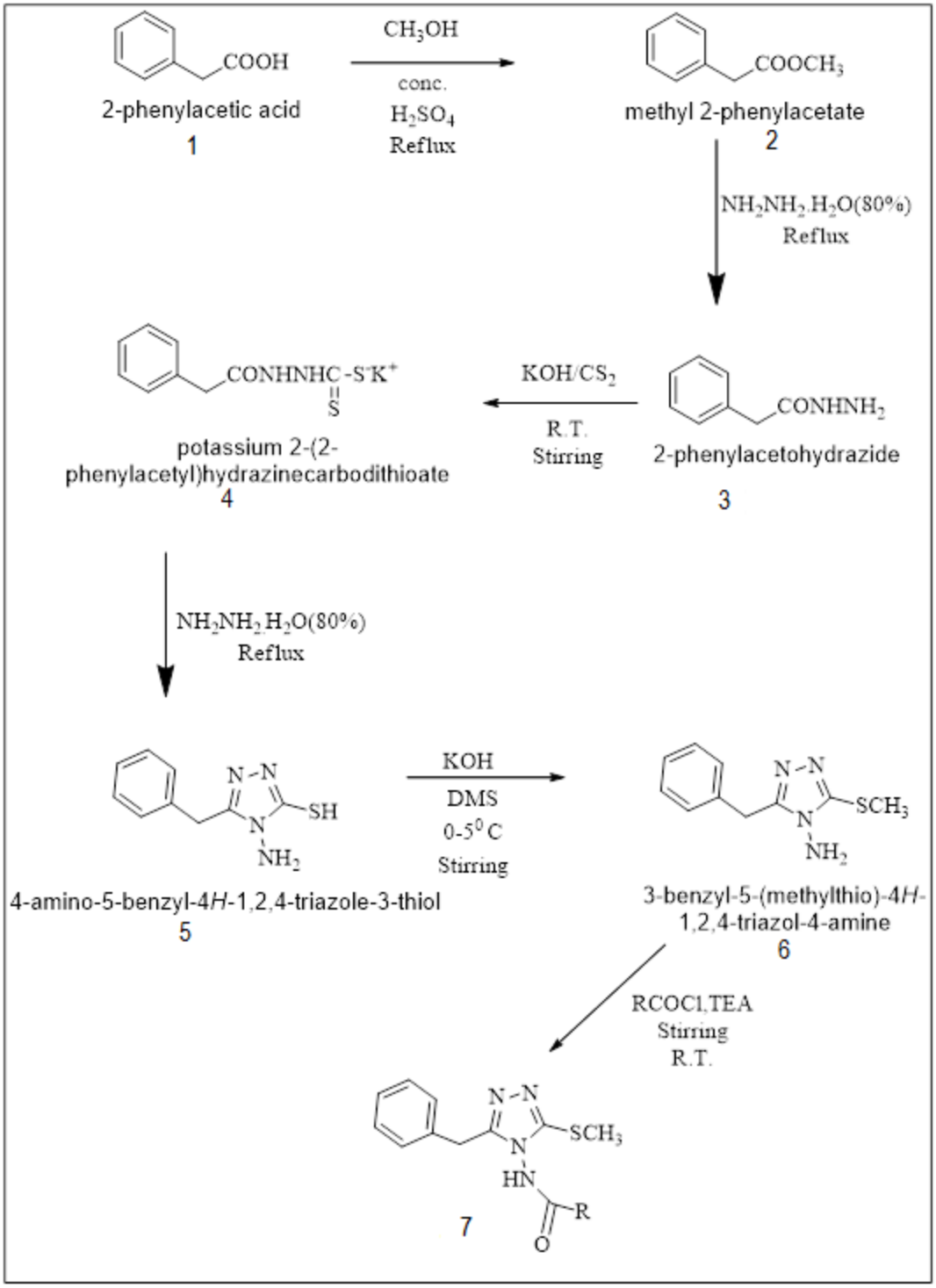
1,2,4- triazole and its derivatives are found to be associated with various biological activities, for example, fluconazole is used as antimicrobial drug, anastrozole is non-steroidal drug used for treatment of cancer and loreclezole is used as anticonvulsant.

In addition to these important biological applications, mercapto-1,2,4-triazoles are also of great utility in preparative organic chemistry, for example, in the presence of various reagents, undergo different types of reactions to yield other heterocyclic compounds, *e.g.* thiazolotriazole, triazolothiadiazoles, triazolothiazines, triazolothiazepines and triazolothiadiazines.

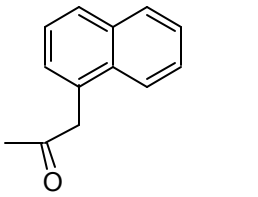
RESULTS AND DISCUSSION

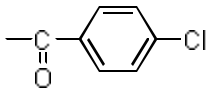
The 2-phenylacetic acid **1** was treated with methanol in the presence of concentrated sulfuric acid to afford methyl-2-phenylacetate **2**. Compounds **2** was treated with hydrazine hydrate in methanol to yield 2-phenylacetohydrazide **3**. Compounds **3** treated with potassium hydroxide in methanol to yield potassium phenylacetyl-hydrazine carbodithioate **4**. Refluxing of compounds **4** with hydrazine hydrate gives 4-amino-5-benzyl-(4*H*)1,2,4-triazole-3-thiol **5**. The compound **5** was added in potsssium hydroxide solution to yield 3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine **6**. Compound **6** was treated with chloro acetyl chloride, 3-chloro propionyl chloride, benzoyl chloride, 4-chloro benzoyl chloride, 3,5-dimethoxy benzoyl chloride to yield substituted trizole derivateves **(7a-e)**.The structures of all the synthesized compounds were confirmed by spectral analysis. The reaction equation is outlined in **Scheme I**.

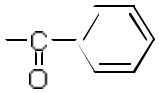
Scheme I for the synthesis of target compounds

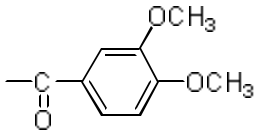


Where, (7a-e) R = -$COCH_2Cl$







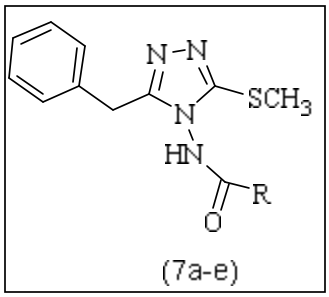


Pharmacological Screening:

Results for antibacterial activity:

All the synthesized compounds were screened for antibacterial activity by Agar diffusion method against the Gram negative bacterial strain *E. coli* and Gram positive bacterial strain *S. aureus*. Potent antibacterial activity was observed against all bacterial strain with MIC value ranging from 50µg/ml to 200µg/ml. The standard drug Ampicillin showed 250µg/ml MIC against bacterial strain *S. aureus* and showed 100µg/ml MIC against bacterial strain *E. coli.* Compound **7b** emerged out as the most potent compound with MIC of 62.2µg/ml against *E. coli.* Compounds **7a** and **7b** emerged out as the most potent compound with MIC of 62.5µg/ml and 100µg/ml respectively against *S. aureus.* Compounds **7c** also have exhibited potent antibacterial activity with MIC of 200µg/ml against *S. aureus*. Compound **7a** and **7b** were found more potent than Ampicillin, which can be a potent promising candidate for antimicrobial agent in future. Other Compounds were found comparatively less potent than standard drugs against all bacterial strains. Results were shown in **Table 1**.

Table 1: The Minimum Inhibitory Concentration (µg/ml) of different Triazole derivatives.



Minimum inhibitory concentration (µg/ml)		
Comp. No.	Gram positive	Gram negative
	S. aureus	*E. coli*
7a	62.5	125
7b	100	62.2
7c	200	100
7d	250	100
7e	240	125
Standard	250	100

EXPERIMENTAL SECTION

Melting points were determined in open glass capillaries and were uncorrected. Thin layer chromatography was performed on microscopic slides coated with Silica-Gel-GF_{254} and spots were visualized by UV radiation and exposure to iodine vapor. IR spectra of all synthesized compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer, using KBr as an internal reference. Mass spectra of all synthesized compounds were recorded on LCMS 2010 EV SHIMADZU Mass Spectrometer. ^{1}H NMR spectra were obtained in $CDCl_3$ on BRUKER Advance-II 400 MHz instrument and chemical shifts were measured as parts per million (δ ppm) downfield from Tetramethylsilane (TMS) as an internal standard.

Synthesis of Methyl 2-phenylacetate (2):[14-17]

A mixture of 2-phenylacetic acid (1 mol) in methanol was refluxed in presence of few drops of concentrated sulfuric acid for 3-5 hours. Reaction progress was monitored by TLC. The reaction mixture was poured into ice-cold water to yield methyl-2-phenylacetate. The crude methyl-2-phenylacetate was filtered and separated. Colorless liquid; Yield 80 %; b.p. 216-218 °C; Rf value 0.7 (*n*-hexane:ethyl acetate::3:7); Mol. formula: $C_9H_{10}O_2$; Mol. Wt.: 150 g/mol. IR: cm^{-1}.

Synthesis of 2-phenylacetohydrazide (3):[14-17]

A mixture of crude methyl-2-phenylacetate (1 mol) and hydrazine hydrate (2.5 mol) in methanol was refluxed for 3-5 hours. The reaction mixture was cooled and poured into ice-water. A crude 2-phenylacetohydrazide was filtered and separated. White solid; Yield 70 %; m.p. 114-116 °C; Rf value 0.45 (*n*-hexane:ethyl acetate::3:7); Mol. formula: $C_8H_{10}N_2O$; Mol. Wt.: 150 g/mol. IR: cm^{-1}.

Synthesis of potassium phenylacetyl-hydrazine carbodithioate (4):[14-17]

Potassium hydroxide (1.68 g, 30 mmol) was dissolved in methanol and stirred for 10 minutes. 2-phenylacetohydrazide (2.72 g, 20 mmol) was added at once to the above reaction mixture. After 15 minutes, carbon disulfide (2.28 g, 30 mmol) was added drop wise and the reaction mixture was stirred continuously and temperature was maintained at 20-25°C. After 3 hours, diethyl ether (100 ml) was added to the reaction mixture to yield crude potassium phenylacetyl-hydrazine carbodithioate. The crude precipitate was filtered and washed repeatedly with diethyl ether. White solid; Yield 75 %; m.p. 288-290 °C; Rf value 0.4 (*n*-hexane:ethyl acetate::3:7); Mol. formula: $C_9H_9K_2N_2OS_2$; Mol. Wt.: 264 g/mol. IR: cm^{-1}.

Synthesis of 4-amino-5-benzyl-(4*H*)1,2,4-triazole-3-thiol (5):[14-17]

The potassium salt of phenylacetyl-hydrazine carbodithioate and hydrazine hydrate (1.68 g, 32 mmol) was dissolved in water. The solution was refluxed for 1 hour until the colour of the solution became clear green. After cooling at room temperature, the solution was poured in ice-cold water and neutralized with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol to yield 4-amino-5-benzyl-(4*H*)1,2,4-triazole-3-thiol. White solid; Yield 65 %; m.p. 144-142 °C; Rf value 0.4 (*n*-hexane:ethyl acetate::7:3); Mol. formula: $C_9H_{10}N_4S$; Mol. Wt.: 206 g/mol.

Synthesis of 3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (6):

Triazole (1 mol) was added in solution of pottasium hydroxide (2 mol) with constant stirring in an ice bath, followed by addition of DMS (2 mol) drop by drop with constant stirring. Reaction mixture was stirred for 1 hour. Reaction mixture was poured in ice-water, filtered and dried to get 3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine. White solid; Yield 80 %; m.p. 125-127 °C; Rf value 0.48 (*n*-hexane:ethyl acetate::7:3); Mol. formula: $C_{10}H_{12}N_4S$; Mol. Wt.: 220 g/mol.

Synthesis of triazole derivatives (7a-e):

Synthesis of N-(3-benzyl-5-(methylthio)-4H-1,2,4-triazole-4-yl)-2-chloroacetamide (7a):

3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (1 mol) and triethyl amine (1.1 mol) was dissolved in 10 ml acetonitrile. Chloro acetyl chloride (1.5 mol) was added to the above reaction

mixture and stirred for 1 hour. Reaction mixture was poured into ice cold water, precipitates was filtered, dried and recrystallized from methanol to yield N-(3-benzyl-5-(methylthio)-4H-1,2,4-triazole-4-yl)-2-chloroacetamide. White solid; Yield 68 %; m.p. 120-124 °C; Rf value 0.52 (*n*-hexane:ethyl acetate::7:3); Mol. formula: $C_{12}H_{13}ClN_4OS$; Mol. Wt.: 296.5 g/mol. IR: 1712.67 (-CO- str) 3249.83 (-NH- str) 2935.46 (-CH_3- str) 767.62 (-Cl str) cm^{-1}; ^{1}H NMR: 3.70 (s,-3H,-S*H*$_3$-), 3.88 (s,-2H,COCH), 4.13 (s,-2H,C*H*$_2$), 7.13-7.29 (t,-5H,Ar-*H*), 8.72 (S,-1H,N*H*) δ ; MS: *m/z* 297.1(M+1), 299.1(M+2).

Synthesis of N-(3-benzyl-5-(methylthio)-4*H*-1,2,4-triazole-4-yl)-2-(napthalen-1-yl) acetamide (7b):

3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (1 mmol) was dissolved in 10 ml glacial acetic acid. 3-chloro propionyl chloride (1.5 mol) was added at once to the above reaction mixture and it was stirred for 1 hour. Reaction mixture was poured into ice cold water, precipitates was filtered, washed with cold water, dried and recrystallized from methanol to get N-(3-benzyl-5-(methylthio)-4*H*-1,2,4-triazole-4-yl)-2-(napthalen-1-yl)acetamide. White solid; Yield 94 %; m.p. 134-136 °C; Rf value 0.31 (*n*-hexane:ethyl acetate::7:3); Mol. formula: $C_{22}H_{20}N_4OS$; Mol. Wt.: 388.49 g/mol. IR: 1701.10 (-CO- str), 3043.46 (-NH- str), 2912.31 (-CH_3-) cm^{-1}; MS: *m/z* 389.3 (M+1).

Synthesis of N-(3-benzyl-5-(methylthio)-4*H*-1,2,4-triazole-4-yl)benzamide (7c):

3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (1 mol) and triethyl amine (1.1 mol) was dissolved in 10 ml acetonitrile. Benzoyl chloride (1 mol) was added at once to the above reaction mixture and it was stirred for 1 hour. Reaction mixture was poured in ice cold water, precipitates was filtered, washed with cold water, dried and recrystallized from methanol to get N-(3-benzyl-5-(methylthio)-4*H*-1,2,4-triazole-4-yl)benzamide. White solid; Yield 86 %; m.p. 208-210 °C; Rf value 0.47 (*n*-hexane:ethyl acetate::7:3); Mol. formula: $C_{17}H_{16}N_4OS$; Mol. Wt.: 324.10 g/mol. IR: 1674.10 (-CO- str), 3230.54 (-NH- str), 2937.83 (-CH_3-str) cm^{-1}; ^{1}H NMR: 3.70 (S,-3H, SC*H*$_3$-), 4.021 (S,-2H,CH_2), 7.21-7.45 (m,-5H,Ar-H), 7.43-7.85 (d,-5H,Ar-*H*), 9.14 (S,-1H,N*H*) δ ; ^{13}CNMR: 31.3(-C*H*$_2$-), 36.9 (S-CH_3), 127.63, 127.89, 128.91, 128.92, 130.30,133.29,133.53,150.76, 166.23, 166.30 δ; MS: *m/z* 325.3(M+1).

Synthesis of N-(3-benzyl-5-(methylthio)-4*H*-1,2,4-triazole-4-yl)-4-chlorobenzamide (7d):

3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (1 mol) and triethyl amine (1.1 mol) was dissolved in 10 ml acetonitrile. 4-chloro benzoyl chloride (1.5 mol) was added at once to the above reaction mixture and it was stirred for 1 hour. Reaction mixture was poured in ice cold water, precipitates was filtered, washed with cold water, dried and recrystallized from methanol

to get N-(3-benzyl-5-(methylthio)-4*H*-1,2,4-triazole-4-yl)-4-chlorobenzamide. White solid; Yield 95 %; m.p. 213-215 °C; Rf value 0.78 (*n*-hexane:ethyl acetate::7:3); Mol. formula: $C_{17}H_{15}ClN_4OS$; Mol. Wt.: 358.85 g/mol. IR: 1718.46 (-CO- str), 3199.69 (-NH- str), 3033.82 (-CH_3- str), 744.47 (-Cl- str) cm^{-1}; MS: *m/z* 358.9 (M+1), 361.4 (M+2).

Synthesis of N-(3-benzyl-5-(methylthio)-4*H*-1,2,4-triazole-4-yl)-3,4-dimethoxybenzamide (7e):

3-benzyl-5-(methylthio)-4*H*-1,2,4-triazol-4-amine (1 mol) and triethyl amine (1.1 mol) was dissolved in 10 ml acetonitrile. 3,5-dimethoxy benzoyl chloride (1 mmol) was added to the above reaction mixture and it was stirred for 1 hour. Reaction mixture was poured in ice cold water, precipitates was filtered, washed with cold water, dried and recrystallized from methanol to get N-(3-benzyl-5-(methylthio)-4*H*-1,2,4-triazole-4-yl)-3,4-dimethoxybenzamide. White solid; Yield 82 %; m.p. 148-150 °C; Rf value 0.45 (*n*-hexane:ethyl acetate::7:3); Mol. formula: $C_{19}H_{20}N_4O_3S$; Mol. Wt.: 384.45 g/mol. IR: 1633.59 (-CO- str), 3330.84 (-NH- str), 2933.53 (-CH_3- str) cm^{-1}; MS: *m/z* 385.3(M+1).

Antimicrobial activity:

Agar diffusion method was used to evaluate the antibacterial activity[18-19]. All the petri dishes were sterilized in oven at 160 °C for 1 hour. Agar media, borer and test solutions were sterilized in autoclave at 121 °C at 15psi. Molten sterile agar was poured in sterile petri dishes aseptically. The agar was allowed to cool and the bacterial suspension was poured into the petri dishes aseptically. Placing the impregnated absorbent paper with solution of the drugs (test and standard) in the agar plate petri dishes aseptically. Petri dishes was incubated at 37 °C for antimicrobial activity for 24 hour and the zone of inhibition was observed.

All the synthesized compounds were screened for antibacterial activity by agar diffusion method against the gram negative bacterial strains *E. coli* and gram positive bacterial strains *S. aureus*. Primary screening was done at the dose of 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml concentrations of the synthesized compounds. The significantly active compound in primary screening was further tested in a second set of dilution against all microorganisms. The minimum inhibitory concentration (MIC) was measured and compared with control and standard drug ampicillin.

CONCLUSION

The results obtained from antimicrobial activity of synthesized compounds is good, which is comparable to the standard drug and it can be useful for the further study.

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