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Synthesis and antimicrobial evaluation of some 1,2,4-triazole heterocyclic compounds

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ABSTRACT

In the present work, cinnamic acid was modified to prepare 1,2,4-triazole-thiol analogs were synthesized and evaluated for anti-bacterial activity using disc diffusion/cup and plate method. Spectral analyses (IR and NMR) of the compounds satisfactorily supported the structures of the synthesized compounds. The FT-IR spectrum of compounds exhibited sharp peaks at 3200-2700 cm^{-1} (aliphatic C-H), 1400-1700 cm^{-1} (aromatic ring) and 2400-2600 cm^{-1} (S-H str.), in all compounds while vibrations of NO_2 (1300-1400 cm^{-1}), C-Cl (850-550 cm^{-1}) were also found in the corresponding compounds. The ^1H NMR spectra exhibit chemical shifts at δ 6.5-7.9 (aromatic protons), 1.25-2.56 (CH_2) and 0.8 (CH_3) and 8.0-8.4 (=CH) in corresponding compounds. The antibacterial activity of the synthesized compounds was evaluated at four different concentrations and the zone of inhibition was measured against gram negative and gram positive bacteria. Only one compound (**CT4**) was found to contain a significant antibacterial action against the tested microorganisms. Also the action was better against gram positive bacteria in comparison to the gram negative bacteria.

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Introduction

The most common heteroatoms present in heterocyclic compounds are nitrogen (N), oxygen (O) and sulphur (S).[1] Triazole, also known as pyrrodiazole, is one of the classes of organic heterocyclic compounds containing a five membered di-unsaturated ring structure composed of three nitrogen atoms and two carbon atoms at non-adjacent positions.[2] Several triazole containing compounds have been clinically significant and are used worldwide to cure various diseases.[3] Literature reveals the importance of the the 1,2,4-triazole nucleus pharmacologically.[4-8]

In the past years, health-care associated infections have become an important cause of morbidity and mortality, whilst the incidence of antibiotic-resistant bacteria has increased dramatically and become a serious threat. In fact, the management of bacterial infections is getting increasingly tough due to augmented prevalence of MDR pathogens, which represent a major challenge to antimicrobial therapy. Microbial resistance is now frequently confronted to common antibiotics being used in clinical settings, and there is an imperative demand for newer anti-infective agents to overcome emerging multi-drug resistance. Today, the need for novel antimicrobials has been greater than ever in the face of increasing resistance to the older ones and increasingly tough management of bacterial infections. In spite of urge for such agents, the scientific progression in terms of antimicrobial research and discovery of new antibacterial molecules has declined dramatically in the past few years. Hence in the present study it has been attempted to synthesize a few 1,2,4-triazole based compounds and evaluate their anti-microbial activity.

Material and Methods

Cinnamic acid was purchased from Loba, ethanol was obtained from Sigma Aldrich, hydrazine hydrate, sulfuric acid, potassium hydroxide, hydrochloric acid, benzaldehyde, 3-nitrobenzaldehyde, 4-nitrobenzaldehyde, chlorobenzaldehyde, hydroxybenzaldehyde, and all other chemicals used were of analytical or synthetic grade and purchased from CDH. All the chemicals were used as received without any further purification. Digital weighing balance (Wensar), Heating mantle (BioTechnics), melting point apparatus (BioTechnics) were used during the study.

The experimental scheme was adapted from the report of Kumari *et al.*[9] and optimized to the conditions of the laboratory (Figure 1).

Synthesis of cinnamohydrazide

The cinnamohydrazide was prepared from cinnamic acid in two consecutive steps involving activation of the carboxyl group and conversion of the intermediate to hydrazide. A mixture of cinnamic acid (1.48 g, 0.01 mol), ethanol (50 ml) and conc. sulphuric acid (0.5 ml) was taken in a round bottom flask and the mixture was refluxed for 12 h. On completion of the reaction the reaction mixture was cooled to 5 °C using an ice bath. The solution was extracted with diethyl ether and the organic layer was concentrated to obtain the ethyl cinnamate[10]. To a solution of ethyl cinnamate (1.76 g, 0.01 mol) in ethanol (30 ml), hydrazine hydrate (0.64 g, 0.02 mol) was added and the mixture was refluxed for 6 h. The reaction mixture was allowed to cool in an ice bath and the solid product obtained was filtered and recrystallized from ethanol[10].

Synthesis of 1,2,4-triazole-3-thiol

The traizole derivative from cinnamic acid was synthesized in two steps involving formation of oxadiazole-thione followed by its reaction with hydrazine hydrate to form triazole-thiol. To a solution of potassium hydroxide (1.12 g, 0.02 mol) in ethanol (30 ml) was added cinnamohydrazide (1.62 g, 0.01 mol) and dissolved. To the mixture was then slowly added (0.76 g, 0.01 mol) carbon disulfide with stirring. The reaction mixture was then refluxed for 10–12 h, cooled at room temperature hydrochloric acid was added to the mixture for neutralization of product. The precipitated solid was filtered, washed with ethanol, dried and recrystallized from ethanol. An ethanolic (30 ml) solution of the above oxadiazole thione intermediate (1.40 g, 0.01 mol) and hydrazine hydrate (0.38 g, 0.01 mol) was heated under reflux for 3 h. The mixture was poured over crushed ice and the solid product that precipitated out was filtered, washed and recrystallized from ethanol[11].

General method for synthesis of benzylidene amino derivative

To a reaction mixture of 1,2,4-triazole-3-thiol (2.18 g, 0.01 mol) and aromatic aldehyde (0.01 mol) in ethanol was added a few drops of sulfuric acid and the mixture was refluxed for an appropriate time. The completion of the reaction was monitored by thin layer chromatography. After completion of reaction, the reaction mixture was poured in crushed ice and the solid obtained was filtered, washed with cold water and finally recrystallized from ethanol[11].

Evaluation of antibacterial action[12,13]

The antibacterial action of the synthesized compounds was evaluated against one gram positive (*Bacillus subtilis*) and one gram negative bacteria (*Escherichia coli*).

Preparation of test solutions

The synthesized triazole compounds were dissolved in dimethyl sulfoxide (DMSO) and the further dilutions of the test compounds were prepared at the required quantities of 1000 µg/mL concentrations with nutrient broth medium.

Determination of zone of inhibition

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method.[36]

Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the triazole compounds (50, 75, 100 & 150 µg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters. Norfloxacin (gift sample from Medriech Pharmaceuticals) was used as the standard.

Results and Discussion

Five 1,2,4-triazole derivatives were synthesized and characterized by using TLC, IR, and NMR analysis (Table 1).

The spectral characteristics were used to confirm the structures of the synthesized compounds. The purity and homogeneity of the synthesized compounds was confirmed by TLC. Spectral analyses (IR and NMR) of the compounds satisfactorily supported the structures of the synthesized compounds. The FT-IR spectrum of compounds exhibited sharp peaks at 3200-2700 cm^{-1} (aliphatic C-H), 1400-1700 cm^{-1} (aromatic ring) and 2400-2600 cm^{-1} (S-H str.) , in all compounds while vibrations of NO_2 (1300-1400 cm^{-1}), C-Cl (850-550 cm^{-1}) were also found in the corresponding compounds.

The ^1H NMR spectra exhibit chemical shifts at δ 6.5-7.9 (aromatic protons), 1.25-2.56 (CH_2) and 0.8 (CH_3) and 8.0-8.4 (=CH) in corresponding compounds.

4-((Z)-benzylideneamino)-5-((E)-styryl)-4H-1,2,4-triazole-3-thiol, ***CT1***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 1.24–2.45 (CH_2), 0.84 (CH_3), 7.41–7.93 (Ar–H), 3.37 (SH); IR (KBr): 2925 (C–H str., aliphatic), 1625 (C=N str.), 3100 (C=C str., aromatic), 1719 (C–H str., aromatic.), 2568 (S–H str.)

4-((Z)-(4-nitrobenzylidene)amino)-5-((E)-styryl)-4H-1,2,4-triazole-3-thiol, ***CT2***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 1.24–2.56 (CH_2), 0.86 (CH_3), 8.31 (=CH), 6.90–7.76 (Ar–H), 3.07 (SH); IR (KBr): 2880 (C–H str., aliphatic), 1647 (C=N str.), 1498 (C=C str., aromatic), 3076 (C–H str., aromatic), 1520 (NO2 str.), 2572(S–H str.)

4-((Z)-(3-nitrobenzylidene)amino)-5-((E)-styryl)-4H-1,2,4-triazole-3-thiol, ***CT3***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 1.29–2.51 (CH_2), 0.87 (CH_3), 8.01 (=CH), 7.51–7.96 (Ar–H), 3.37 (SH); IR (KBr): 2878 (C–H str., aliphatic), 1648 (C=N str.), 1646 (C=C str., aromatic), 3045 (C–H str., aromatic), 1424 (NO2 str.), 2572 (S–H str.)

4-((Z)-(3-chlorobenzylidene)amino)-5-((E)-styryl)-4H-1,2,4-triazole-3-thiol, ***CT4***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 1.26–2.56 (CH_2), 0.87 (CH_3), 8.01 (=CH), 6.90–7.68 (Ar–H), 3.2 (SH); IR (KBr): 2916 (C–H str., aliphatic), 1650 (C=N str.), 1635 (C=C str., aromatic), 3105 (C–H str., aromatic), 767 (C–Cl str.), 2572 (S–H str.)

3-((Z)-((3-mercapto-5-((E)-styryl)-4H-1,2,4-triazol-4-yl)imino)methyl)phenol, ***CT5***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 1.28–2.53 (CH_2), 0.88 (CH_3), 8.10 (=CH), 6.43–7.49 (Ar–H), 3.37 (SH), 5.01 (OH); IR (KBr): 2919 (C–H str., aliphatic), 1686 (C=N str.), 3483 (O–H, str.), 1665 (C=C str., aromatic), 3098 (C–H str., aromatic), 2572 (S–H str.)

Antibacterial Activity

The antibacterial activity of the synthesized compounds was evaluated at four different concentrations and the zone of inhibition was measured against gram negative and gram positive bacteria (Table 2).

Only one compound (**CT4**) was found to contain a significant antibacterial action against the tested microorganisms. Also the action was better against gram positive bacteria in comparison to the gram negative bacteria.

The antimicrobial potential of the synthesized compounds has been evaluated by determining the zone of inhibition by cup and plate method. The results obtained indicate that the presence of a chloro group on the phenyl ring directly attached to the imine carbon was beneficial for the antibacterial activity of the compounds.

Conclusion

In the present study, 1,2,4-triazole derivatives were prepared in a multistep reaction starting with cinnamic acid, followed by hydrazination

and subsequent cyclization and reaction with aldehydes to yield imine linked compounds. The compounds were found to be of good purity and yield. One of the synthesized compounds exhibited antibacterial potential against both gram negative and gram positive bacterium tested.

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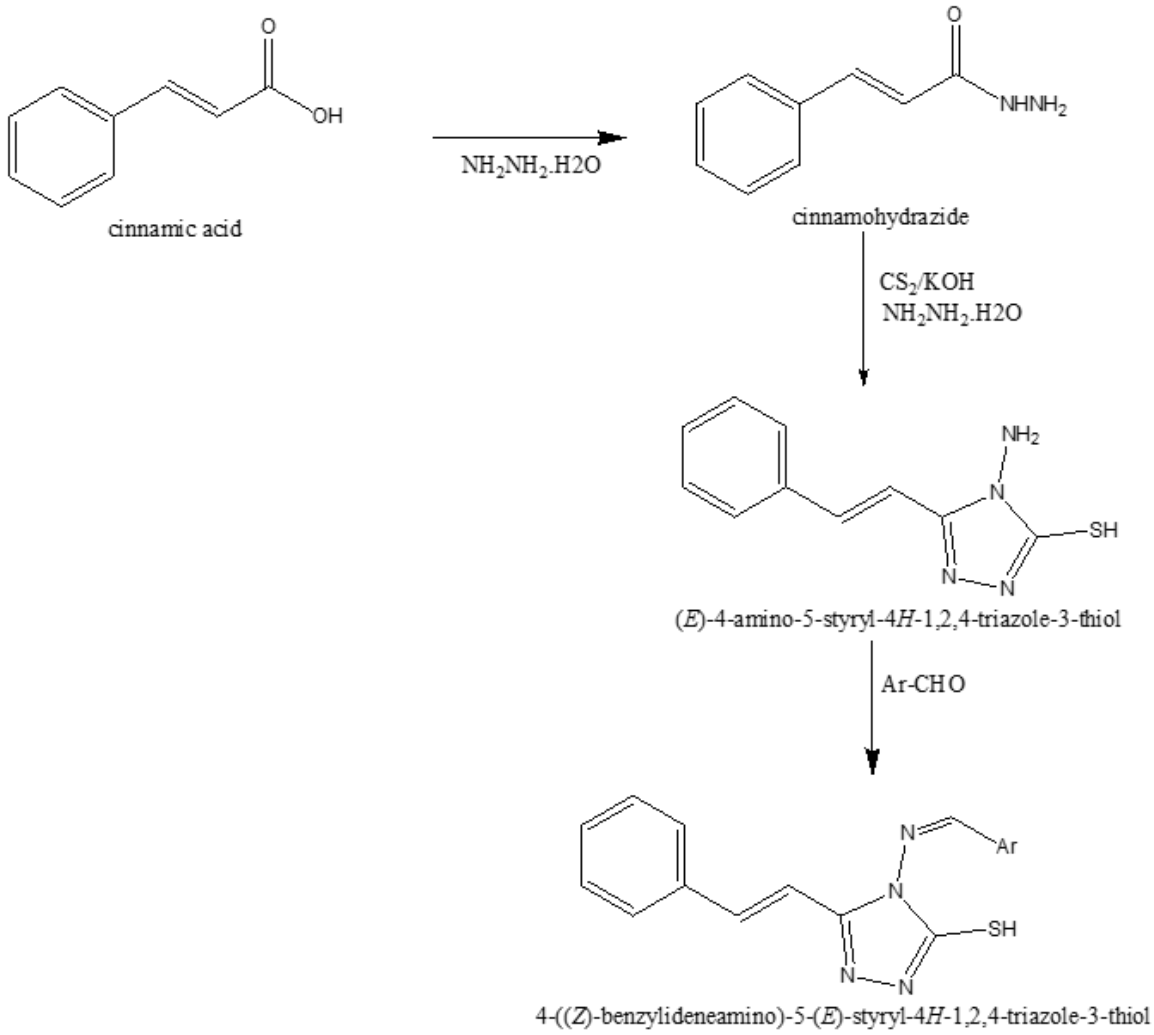
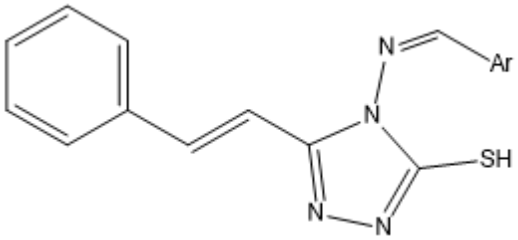


Figure 1 Scheme for synthesis of 1,2,4-triazole compounds

Table 1 Properties of the synthesized compounds



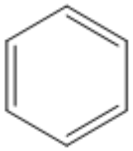
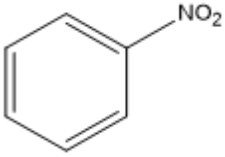
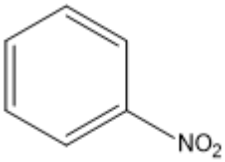
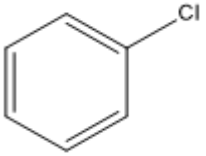
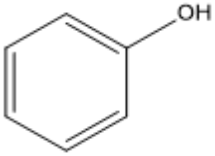
Code	Ar	Yield (%)	Color	Melting point
CT1		62	Pale Yellow	223-225
CT2		69	Light Brown	232-234
CT3		67	Off white	236-238
CT4		64	Pale Yellow	218-220
CT5		69	Pale Yellow	222-224

Table 2 Zone of inhibition of the synthesized compounds

Compound Code	Zone of Inhibition (mm)*							
	S. auerus				*E.coli*			
	25µg	50µg	100µg	150µg	25µg	50µg	100µg	150µg
CT1	-	-	13	17	-	-	-	13
CT2	-	-	12	13	-	-	11	12
CT3	-	-	13	13	-	-	11	12
CT4	-	-	16	**23**	-	-	13	17
CT5	-	-	14	16	-	-	12	14
Norfloxacin	**22**	**-**	**-**	**-**	**23**	**-**	**-**	**-**

Table 1 Preformulation characters of mesalamine

S. No	Parameter	Observation
1	Physical appearance	Off-white powder
2	Odour	Odourless
3	Melting Point	276-280°C
4	Taste	Bitter
5	Partition coefficient	1.8
6	LOD	0.32 %
7	Solubility	Insoluble in water, soluble in ethanol, DMSO

***In vitro* release of mesalamine from PA-Se@Mes**

Time (h)	% Cumulative release	
	Absence of rat caecal content	Presence of rat caecal content
0	0	0
1	4.81	6.48
2	14.82	16.88
4	17.88	23.49
6	22.62	31.63
8	29.49	37.23
10	35.36	43.36
12	41.03	48.37
16	45.03	60.64
20	55.5	70.18
24	69.31	85.19