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Current Research in Pharmaceutical Sciences

Available online at www.crpsonline.com

ISSN: 2250 – 2688

Received: 19/03/2021
Revised: 30/03/2021
Accepted: 31/03/2021
Published: 08/04/2021

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DOI: 10.24092/CRPS.2021.110103

Website: www.crpsonline.com

Quick Response Code:

Synthesis, Characterization and Antimicrobial Evaluation of some 1,2,4-Triazolo-5-Thione Derivatives

Gurdeep Singh, Neetesh K Jain and Kiran Sahu

Abstract

Due to this increasing predicament of antibiotic confrontation, the lot of distinct antibiotics available is dwindling and there are only smatterings of new antibiotics in the drug development channel. Therefore, an intense necessitate for new antimicrobial drugs. In current study, substituted 1,2,4-triazolo-5-thione derivatives were synthesized from substituted aromatic aldehydes, succinic acids and the structure of synthesized compounds were established by physicochemical (Melting Point, Rf value) and spectral analysis (IR, NMR & Mass). The title compounds were then evaluated for their antimicrobial activity against ciprofloxacin as a standard drug using agar plate method. Among all synthesized derivatives A_5 and A_6 found were found to possess very promising antimicrobial activity.

Keywords: 1,2,4-triazolo-5-thione, anti-microbial, antibiotic, thione, Ciprofloxacin

1. INTRODUCTION

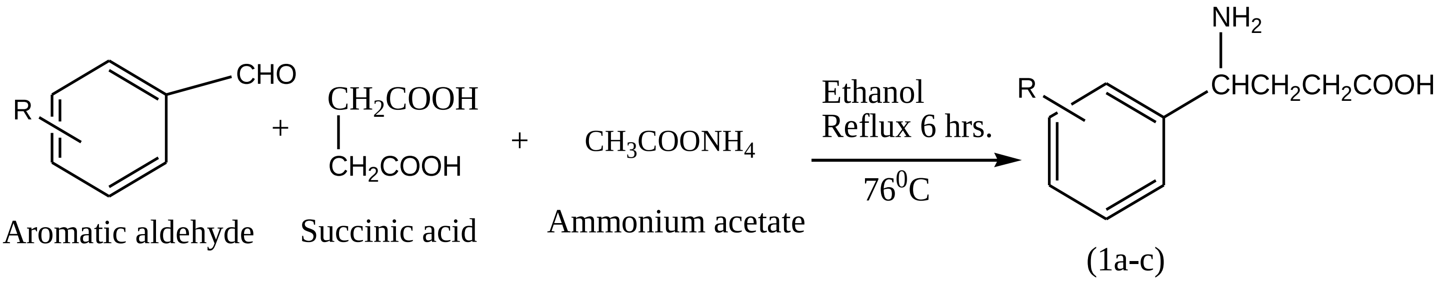
Microorganisms that resist antimicrobial drugs are a complex problem affecting the health of people all over the world. More than 1 million people die from microbial infections every year, and the number of deaths is expected to increase as antimicrobial drug resistance increases. [1] Innovation must be strengthened in research activities related to effective antimicrobial and antifungal drugs.[2] Triazoles are the class of heterocyclic compounds.[3] A number of sulphur containing and heterocyclic ring substituted [1,2,4]-triazole are associated with good biological as well as pharmacological activities like anticancer [4], antibacterial [5], antitubercular [6], antifungal [7], antiviral [8], analgesic [9], anti-inflammatory [10], and tubulin inhibitors [11] and others.

2. MATERIALS AND METHODS:

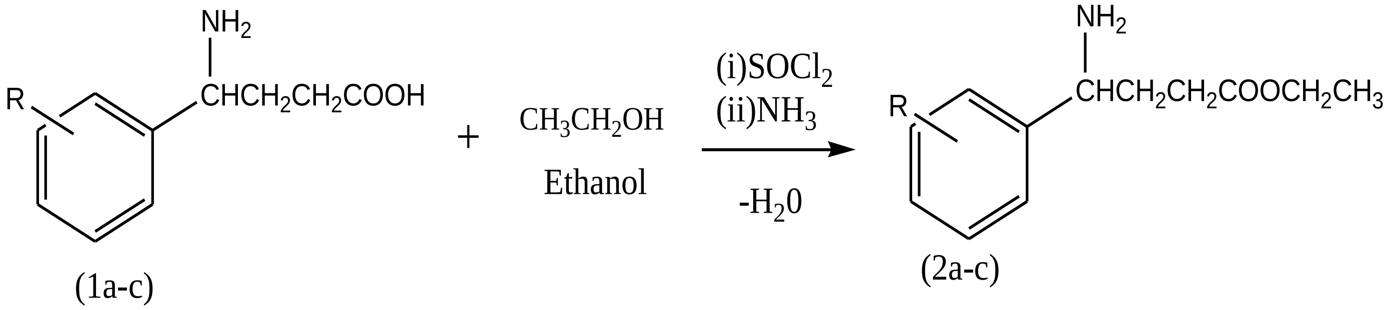
The work aim to synthesized some [1,2,4]-triazole-5-thiones derivatives, which have their own anti-microbial activity.

SCHEME OF WORK

The synthetic scheme includes following step:-



Step I



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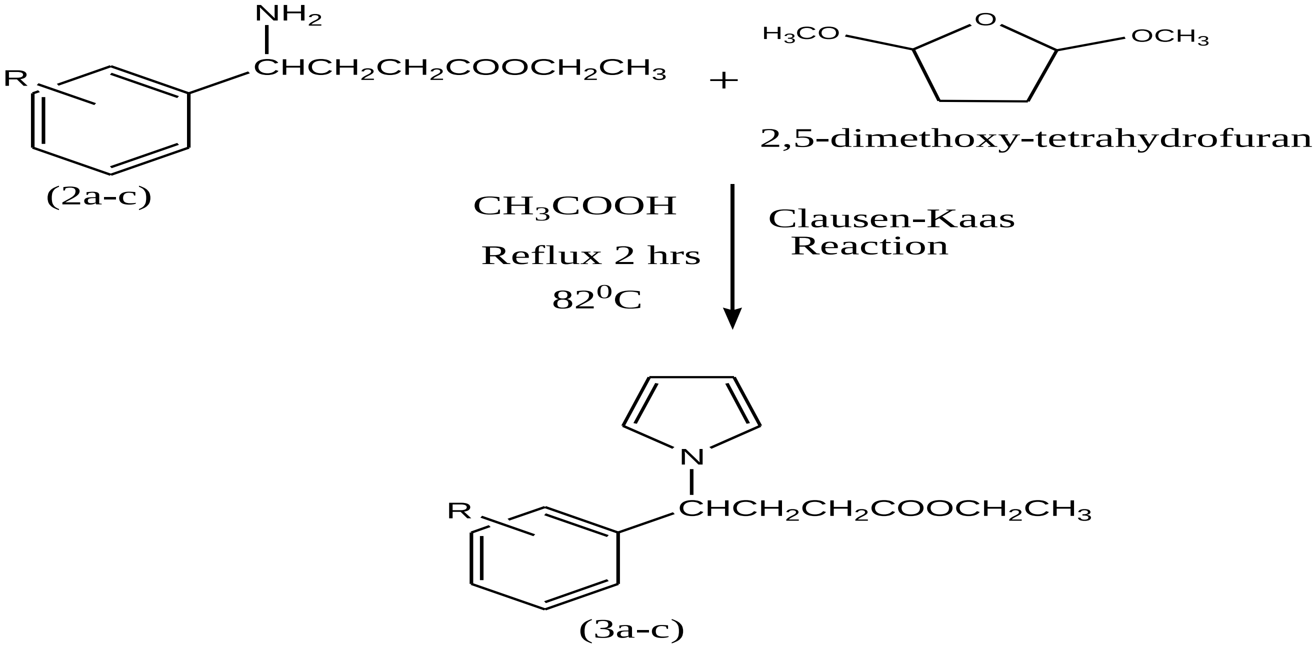

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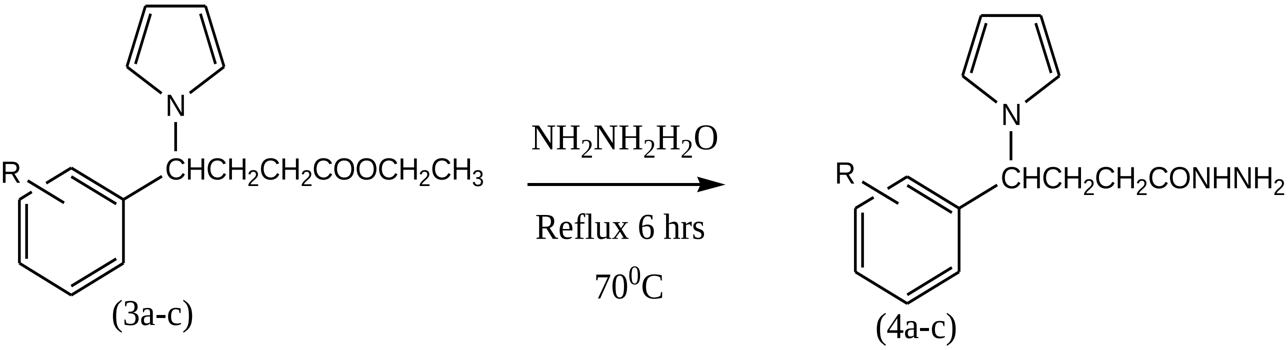

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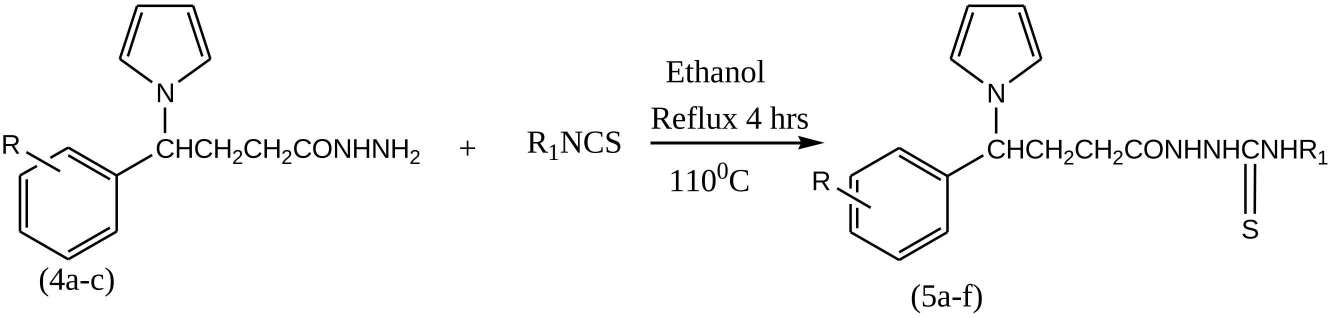

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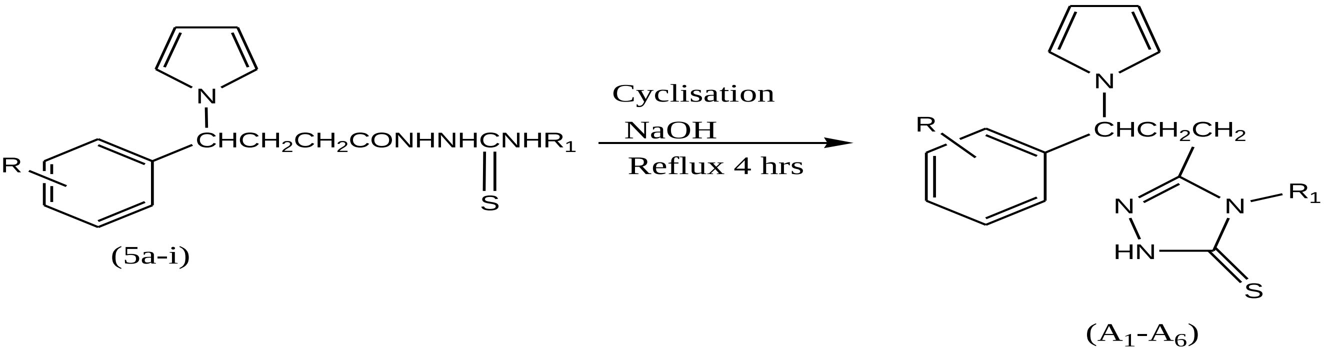

Step VI

S. No.	R	R_1
A_1	-4 NO_2	-C_6H_5
A_2	-4 NO_2	-C_2H_5
A_3	-4 NO_2	-CH_3
A_4	-3 NO_2	-C_6H_5
A_5	-3 NO_2	-C_2H_5
A_6	-3 NO_2	-CH_3

Table 1: List of Substituent's

2.1 Synthesis of Butanoic Acid Derivatives [1a-c]

To a solution of substituted benzaldehyde (0.1 mol) in ethanol (200 ml) succinic acid (0.1mol) and ammonium acetate (0.2 mol) were added. The reaction mixture was refluxed for 6 hrs, after that mixture was cooled and filtered the precipitate, washed with hot ethanol and dried to give solid which was used in the next step without purification.

2.2 Synthesis of [2a-c]

To a stirred suspension of 1a-c (0.050 mol) in ethanol was added dropwise into thionyl chloride (0.055 mol). Stirring was maintained for 30 min at 50°C and the solution was then evaporated to dryness. The solid residue was triturated in ether, filtered and dissolved in a mixture of ether (300 ml) and water (30 ml). The reaction mixture was then bubbled for 2 min at room temperature with an ammonia gas flow. The organic layer was separated and the solvent was evaporated under reduced pressure. The residue obtained was recrystallized with ethanol.

2.3 Synthesis of [3a-c]

To a solution of 3a-c (0.45 mol) in acetic acid (100 ml), 2,5-dimethoxytetrahydrofuran (5.9 ml, 0.045 mol) was added. The reaction mixture was refluxed for 2 hrs and then evaporated to dryness. The residue was taken up in the ether (100 ml) and filtered. The precipitate was collected and recrystallized with methanol.

2.4 Synthesis of [4a-c]

To the substituted ester 3a-c (0.1 M), hydrazine hydrate (80%) (0.1 M) in ethanol was added and refluxed for 6 hrs. The resulting solution was left overnight, filtered; precipitate was washed with water and recyrastallized from methanol.

2.5 Synthesis of [5a-f]

Compounds 4a-c (0.018 mol) were dissolved in boiling ethanol and equimolar amounts of methyl/ethyl/phenyl-isothiocyanate in ethanol were added and refluxed for 4 hrs. The flask content was allowed to cool and concentrated. The precipitated product was filtered, dried and recyrastallized from ethanol.

2.6 Synthesis of 3'-substituted-[1,2,4-triazolo]-3-thiones derivatives [A1-6]

To the compounds in previous step 5a-f (0.01 mol) alcoholic sodium hydroxide (4N, 4ml) was added and refluxed for 4 hrs cooled and filtered. The filtrate was adjusted to pH 4-6 with hydrochloric acid. The mixture was kept a side for 1 h and the crystal produced were filtered, dried and recyrastallized from methanol.

3. RESULTS

The synthesis of the triazole ring was carried out by starting from substituted benzaldehyde with succinic acid on treatment with ammonium acetate according to Rodionow-Johnson reaction to give δ- amino butanoic acid derivative 1a-c and esterification of carboxylic acid group was carried out, without protection of amino group, through a treatment with thionyl chloride in ethanol followed by displacement of the intermediary salt with ammonia in ether to give substituted ethyl 4-amino butanoate 2a-c. This is subjected to Clauson-Kaas reaction using 2,5-dimethoxytetrahydrofuran in acetic acid to yield the ethyl aryl-pyrrolyl-butanoates 3a-c and hydrazinolysis of ester group by using equimolar amount of hydrazine hydrate in ethanol gave aryl-pyrrolyl-butanehydrazide 4a-c which was then reacted with alkyl and aryl- isothiocyanate to yield substituted thiosemicarbazides 5a-f as described. Cyclocondensation of 5a-f in aqueous alkaline medium gives substituted-[1'-(aryl-pyrrol-1H-yl)-3'-(1,2,4-triazolo-5-thione)}]-propane derivatives A1-6.

3.1 Physical Characterization

All synthesized compounds were recrystallized with ethanol and identified by TLC using ethyl acetate : petroleum ether as solvent system in 1:1. Spots were visualized through the iodine chamber and the R_f value was calculated and were found to be in the range of 0.69-0.88.

The melting points of the synthesized compounds were determined by open capillary tube and are uncorrected and were found to be in the range of 198-314^{o}C. All the synthesized compounds were freely soluble in all polar solvent.

3.2 Spectral Analysis

3.2.1 IR Spectra

IR spectra were recorded on Perkin Elmer FTIR spectrometer using KBr. The compounds 1a-c show the presence of C=O stretching at 1754-1693 cm^{-1} and OH stretching at 3434-3334 cm^{-1} and free NH stretching at 3312-3399 cm^{-1} may be due to formation of acid. Compounds 1a-c undergo esterification reaction and gets converted into ester because compound 2a-c show characteristic stretching at 1711-1724 cm^{-1} which decrease from 1a-c compounds it may be due to ester formation and absence of OH stretching in compound 2a-c confirms the formation of ester. In step III pyrrole ring is formed through Clauson-Kaas reaction is confirmed by absence of NH stretching in compounds 3a-c. In compounds 4a-c NH stretching at 3407-3369 cm^{-1} and absence of ester stretching and decrease of C=O stretching in the range 1711-1694 cm^{-1} may be due to hydrazide formation. The presence of C=S stretching at 1200-1050 cm^{-1} in compounds 5a-f may be due formation of thiosemicarbazide from hydrazide by using different aryl/alkyl isothiocynate. In all compounds A1-6, the NH stretching is shifted towards the lower wave number due to triazole formation and C=S stretching in the range of 1191-1049 cm^{-1} show the formation of thione and the absence of absorption due to C=O group established that all thiosemicarbazide had converted to triazole by cycloaddition reaction. In general all compounds show C-H stretching in the range 2978-3100 cm^{-1} is may be due to propane group and C-N stretching in the range of 1340-1250 cm^{-1} and ArC-H bending 750-700 cm^{-1}.

3.2.2 ^{1}HNMR Spectra

^{1}H-NMR spectra were recorded on Mitz-FTNMR by using Bruker Advance II 400 MHz NMR spectrometer. The chemical shift's were reported as parts per million downfield from tetra methyl silane by using DMSO as a solvent. A triplet in the range 1.0-1.4 ppm is due to CH_2 proton and quartet 2.1-2.6 ppm is due to CH_2 group of propane and triplet in the range of 4.5-5.3 ppm is due to CH proton and singlet between 7.0-7.3 ppm that confirms the presence of NH group in triazole nucleus and CH proton of pyrrole ring show triplet in the range of 6.1-6.6 ppm, multiplet in the range 7.1-8.9 ppm showed the presence of aromatic proton. All aromatic protons showed characteristic downfield. All compounds showed respective downfields that supported the structure of various synthesized triazole-5-thiones. In compound A_1, A_4 showed the extra aromatic proton may be due to phenyl group which present in the 4th position of triazole nucleus and compound A_2, A_5 shows triplet may be due to methyl proton in range of 1.0 ppm and methylene proton shows quartet at 3.6-3.8 ppm which present in the 4th position. In A_3, A_6 singlet at 2.6-2.7 ppm is due to methyl group in the 4th position.

3.2.3 Mass spectroscopy

Mass spectroscopy was performed on LC-MS-Tranp-SL2010A SHIMADZU using Dimethyl-sulfoxide as solvent. All the compounds showed characteristic molecular ion (m, m+1) peak and base peak. All the Mass spectra further confirm the structure of synthesized compounds.

3.3 Anti-Microbial Evaluation

3.3.1 Preparation of media

The media used in this method must be Mueller-Hinton agar at only 4 mm deep, poured into either 100 mm or 150 mm Petri dishes. The pH level of the agar must be between 7.2 and 7.4.

3.3.2 Incubation procedure

1. Using an aseptic technique, place a sterile swab into the broth culture of a specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube.
2. Using the swab, streak the Mueller-Hinton agar plate to form a bacterial lawn.
3. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction.
4. Repeat this rotation 3 times.
5. Allow the plate to dry for approximately 5 minutes.
6. Use an antibiotic disc dispenser to dispense discs containing specific antibiotics onto the plate.
7. Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar.
8. Plates should be incubated overnight at an incubation temperature of 37 °C (98.6 °F).

Table 2: Antimicrobial activity of synthesized compounds

Compound Code	**Zone of inhibition(mm)**			
	Gram Positive bacteria		**Gram Negative bacteria**	
	B. subtilis	***S.aureus***	***E.coli***	***P. aeruginosa***
A_1	04	07	05	06
A_2	08	09	04	04
A_3	12	10	09	12
A_4	08	11	06	09
A_5	19	19	19	20
A_6	20	21	18	19
Ciprofloxacin (Std)	24	25	24	26

potent antimicrobial activity against gram positive and gram negative bacteria's respectively as compared to Ciprofloxacin as a reference.

4. CONCLUSION

In the present study, some 1,2,4-triazole-5-thiones derivatives have been synthesized which contain pyrrole nucleus and screened for their antimicrobial activity using agar plate method. The synthesis was based on the conversion of ester to hydrazide derivative which on treatment with different isothiocynate gets converted into thiosemicarbazides. Further on cyclocondensation in alkaline condition gave triazole nucleus having thione group and pyrrole ring is formed by Clauson-Kaas reaction on Ethyl 4-amino-4-(substituted phenyl) butanoate. Six derivatives of 1,2,4-triazole-5-thiones were synthesized and confirmed by physical and spectral analysis. The synthesized compounds were characterized by IR, ^{1}HNMR and Mass spectral data.

All the synthesized compounds show characteristic absorption peaks in IR and NMR spectra. Expected molecular ion peak (M^+) fragments were observed for the entire compounds in mass spectra. The presence of phenyl group in 4th position of triazole nucleus gives a better active compound. Overall it can be concluded that the presence of bulky substitution in 3rd and 4th position of triazole nucleus may give a better active compound.

5. ACKNOWLEDGEMENT

The authors are thankful to Oriental College of Pharmacy and Research, Indore (M.P.) for providing the necessary facilities and requirements for completing the work.

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