Research Article

Synthesis and Evaluation of Antibacterial Activity of -4,5- Substituted thiophene - Sulphonamide Derivatives

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ABSTRACT

Drug resistance to bacterial infection is one of the fastest growing challenge in field of drug development. Reasons behind this are environmental factors as well as physiological factors. Now a days there are various strain of the bacteria are resistant to convectional anti-bacterial drugs like penicillin, ciprofloxacin. Many of the drugs exert the anti-bacterial and anti- fungal activity. As known by us that sulphonamide exert antibacterial activity. Limitations of these compound are as they have low spectrum of for the bacterial infection known and therefore it is necessary to develop new antibacterial agents. Thiophene ring is becoming versatile scaffold because of ability of this moiety to deliver variety of therapeutic actions. Derivatives containing substituted thiophene have shown promising activities like anti-cancer, anti-tuburcular, antibacterial activity. In this study we have made an attempt to synthesize derivatives which have substituted thiophene ring as core moiety. Various ketones are used as a source of substituted thiophene ring. This study also involves synthesis of different thiophene derivative by using gewald synthesis. Also, the sulphonamide was obtained by sulphonyl chloride Derivatives synthesized were characterized by IR, NMR and mass spectrometric techniques. Total eight derivatives have been synthesized and evaluated for their antibacterial activity. substitution on 4,5 position of 2- amino 3-carbetoxy thiophene ring proved beneficial and have shown good antibacterial activity on bacterial culture when compared with sulfathiazole and gentamycin as standard.

Keywords: Thiophene, Gewald reaction, Sulphonamide Derivatives.

INTRODUCTION

The importance of this field of heterocyclic chemistry gave impetus to the present study, where the data on synthesis, reactivity and application of variously substituted 2-aminothiophenes are systematized and analysed. Emphasis is given to the recent studies published, in which the most general approaches to the synthesis of basic 2-aminothiophenes via the Gewald reaction and other target structures were considered. Data of the utilization of 2-aminothiophenes in the synthesis of novel type of fused heterocycles and their application are included. Highly substituted thiophene derivatives are important heterocycles found in numerous biologically active and natural compounds. The interest in this kind of heterocycles has spread from dye chemistry. Traditionally, polysubstituted -2-aminothiophenes with an electron-withdrawing group such as cyano, ethoxycarbonyl or aminocarbonyl in the 3-position and alkyl was been synthesized. Particular attention is given to studies published in the previous 15-20 years.[1]

So far various new thiophenes substituted derivatives at different position of lead moiety thiophene have been synthesized and screened in our laboratories for antimicrobial activity. The enthusiastic results prompted us to continue the investigation. So, an attempt was made to synthesize some new substituted thiophenes as antimicrobial agent adapting Gewald reaction. Hence the synthesis of "2-amino-3-(4,5,6,7)tetra hydro benzo(b)thiophene" is achieved. The different derivatives of the parent compound were achieved by using different aryl aldehydes to obtain a series of active compound.

Substituted Thiophene and its derivatives constitute one of the major classes in heterocyclic chemistry. They have been shown to have interesting biological properties such as[2] Anticancer, Antitumor, Anti-inflammatory, Antimicrobial.

The aim of the study is Synthesis and Evaluation of antibacterial activity of - 4, 5 - substituted thiophene – sulphonamide derivatives.

MATERIALS AND METHODS

Chemicals

Acetanilide, Chlorsulphonic acid, DMF, EtOH, Ethyl cynoacetate, Diethyl-amine, Triethyl amine, Etc .

Substituted ketone

Butanone, Butaraldehyde, Ethylacetoacetate, Cyclohexanone, Substituted benzene. Etc.

Agar medium

Beef infusion: 300 g

Acid hydrolysate of casein: 17.5 g

Starch: 1.5 g

Agar: 17 g

Distilled water: 1 Lit.

Instruments:

1. UV-Visible spectrophotometer
2. IR or ATR spectrophotometer
3. NMR spectrophotometer
4. Hot air oven
5. Magnetic stirrer
6. Digital melting point apparatus
7. Heating mantle
8. UV cabinet

1. General procedure for synthesis of different Gewald's /derivative

In a 250-ml of RBF ketones (0.05 mol), sulfur 1.6 g (0.05 mol), ethyl cyanoacetate 5.8 g(0.05 mol), and diethylamine 3.65 g (0.05 mol) were mixed together then added 10 ml of Ethanol and Stirred it for 3 hours. On chilling, the reaction mixture produced the desire product. which get filtered, washed with chilled aq. ethanol, and dry at room temperature.[15,16]

Table 1: Synthesis of different Gewalds

S.no	Ketone	Compound name	R_1	R_2
1	Cyclohexanone	2-Amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(b)thiophene	$-(CH_2)_4$	
2	Ethyl methyl ketone	2-Amino-3-carbethoxy-4,5-dimethylthiophene	CH_3	CH_3
3	Ethyl acetoacetate	2-Amino-3,5-dicarbethoxy-4-methylthiophene	CH_3	COC_2H_5
4	Butaraldehyde	Synthesis of 2-amino-3-carbethoxy-5-ethylthiophene	H	C_2H_5

1.a. Synthesis of 2-amino 3-carbethoxy - 4,5,6,7 - tetrahydro benzothiophene:

In 250 ml of RBF take Cyclohexanone 9.8 g (0.1 mole), sulfur 3.2g (0.1 mole), ethyl cyanoacetate 11.3 g (0.1 mole) and 20 ml ethanol were mixed and stirred together. To this well stirred mixture diethylamine 9.14 g (0.125 mole) was added dropwise and stirring continued for about 3 hours at ambient temperature.

The reaction mixture was kept in refrigerator overnight. On Next day the solid separated was filtered and washed with 20 ml chilled 50% aqueous methanol. The product was characterized as 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(b)thinophene.[16]

1.b. Synthesis of 2-Amino-3-carbethoxy-4,5-dimethylthiophene

In a 250 ml of RBF take2-Butanone(Ethylmethylketone) 7.21g (0.1mole), sulphur 3.2g (0.1mole), Ethylcyanoacetate 11.3g(0.1 mole) and 20 ml of ethanol were mixed and stirred together. To this well stirred mixture diethylamine 9.14 g (0.125 mole) was added dropwise stirring continued for about 3 hours at ambient temperature.

The reaction mixture was kept in refrigerator overnight. On Next day the solid separated was filtered and washed with 20 ml chilled 50% aqueous methanol. The product was characterized as 2-amino-3-carbethoxy-4,5-dimethylthiophene.[16]

1.c. Synthesis of 2-Amino-3,5-dicarbethoxy-4-methylthiophene

In 250 ml RBF take Ethyl acetoacetate 4.3g (0.033 mole), sulfur 1.1g (0.033 mole)ethyl cyanoacetate 3.8g (0.03mole), and 20 ml ethanol were mixed and stirred together. To this well stirred mixture diethylamine9.14 g (0.125 mole) was added drop wise and stirred at 30-40 0C about 12 hrs.

The reaction mixture was kept in refrigerator overnight. On Next day the solid separated was filtered and washed with 20 ml chilled 50% aqueous methanol. The product was characterized as 2-amino-3,5-dicarbethoxy-4-methyl thiophene. [16]

1.d. Synthesis of 2-amino-3-carbethoxy-5-ethylthiophene

In 250 ml RBF take Butyraldehyde 7.2 g (0.1 mole), sulfur 3.2g (0.1 mole)ethylcyanoacetate11.3g (0.1 mole), 15.2 ml dimethylformamide and 20 ml ethanol were mixed and stirred to that mixture7.5ml of Triethylamine was added drop wise and stirred together about overnight maintained temperature around 50°C

The reaction mixture was kept in refrigerator overnight. On Next day the solid separated was filtered and washed with 20 ml chilled 50% aqueous methanol. The product was characterized as 2-amino-3-carbethoxy-5-ethyl thiophene.[16]

2. Synthesis of p- acetamido benzene sulfonyl chloride:

Procedure for Synthesis of p- acetamido benzene sulfonyl chloride:

Dry acetanilide 20gm was placed in flask to it chlorosulfonic acid 50ml was added in a small portion and the flask was shaken from time to time when addition been completed the flask was heated on water bath for 1hr the flask was allowed to cool an oil mixture was produced.

Then the cooled oil mixture is poured in crushed ice slowly. And stirred with glass rod time to time then the desired product was obtained as a white precipitate.[17]

3. Synthesis of thiophene - sulphonamide derivative:

General Procedure -

In a 250 ml take RBF fitted with reflux condenser the mixture of substituted thiophene (0.01 M) and p-acetamido benzene sulphonyl chloride (0.01M) in 15 ml pyridine was heated under refluxed condition for 22 hr. then cooled at room temperature.[18]

3.a. Synthesis of 2-(4-acetamidobenzene sulphonylamide)-3-carbethoxy - 4,5,6,7 - tetrahydro benzo(b) thiophene(C_a).

In a 250 ml RBF fitted with reflux condenser the mixture of 2-amino 3-carbethoxy 4,5,6,7 - tetrahydro benzothiophene (0.01 M) and p-acetamido benzene sulphonyl chloride (0.01M) in 15 ml pyridine was heated under refluxed condition for 22 hr. Then cooled at room temperature. Then the reaction mixture was poured into crushed ice. The product so obtained was recrystallized from ethanol.[18]

3.b. Synthesis of 2- (4-acetamidobenzene sulphonyl amide)-3-carbethoxy-4,5-dimethylthiophene(C_b).

In a 250 ml RBF fitted with reflux condenser the mixture of 2-Amino-3-carbethoxy-4,5-dimethylthiophene (0.01 M) and p-acetamido benzene sulphonyl chloride (0.01M)in 15 ml pyridine was heated under refluxed condition for 22 hr. then cool at room temperature.

Then the reaction mixture was poured into crushed ice. The product obtained was recrystallized from ethanol.[18]

3.c. Synthesis of 2- (4-acetamidobenzene sulphonyl amide)-3,5-dicarbethoxy-4-methylthiophene(C_c).

In a 250 ml RBF fitted with reflux condenser the mixture of 2-amino -3,5- dicarbethoyl thiophene (0.01 M) and p-acetamido benzene sulphonyl chloride (0.01M) in 15 ml pyridine was heated under refluxed condition for 22 hr. then cool at room temperature.

Then the reaction mixture was poured into crushed ice. The product obtained was recrystallized from ethanol.[18]

3.d. Synthesis of 2- (4-acetamidobenzene sulphonyl amide)-3-carbethoxy-5-ethylthiophene(C_d).

In a 250 ml RBF fitted with reflux condenser the mixture of 2-amino 3-carbethoxy-5-ethylthiophene (0.01 M) and p-acetamido benzene sulphonyl chloride (0.01M) in 15 ml pyridine was heated under refluxed condition for 22. Then cool at room temperature.

Then the reaction mixture was poured into crushed ice. The product obtained was recrystallized from ethanol.[18]

Structural analysis

Table 2: IR data for compound C_a, C_b, C_c, C_d

SR. No.	Std. frequency	Obs. frequency For Ca	Obs. frequency For Cb	Obs. frequency For Cc	Obs. frequency For Cd	
1	650-780	779	771	783	771	C-S stretch
2	1140-1780	1157	1157	1165	1157	SO_2 sy^m streching
3	1375-1450	1481	1419	1492	1481	CH_3 Bend
4	1480-1515	1589	1481	1597	1589	Benzene ring
4	1565-1615	3170	1581	1647	1651	C=O (NH pr. amide)
5	1590 -1620	3294	1589	3167	3170	Ring strech
6	2870-3030	1589	3170	3305	3294	Aromatic CH stretch

2. NMR Data:

Table 3: NMR data for compound

SR. No.	δ Value (PPM)	Splitting Pattern	Assignment of Hydrogen
1	1.	Multiplet	8(H)CH_2 (cyclo thiophene)
2	2.5	Multiplet	3(H) CH_3(carbethoxy)
3	4.2	Singlet	1(H) NH(Thiophene)
4	4.4	Singlet	1(H) NH (Benzene)
5	7.3	Doublet	4(H)CH(Benzene)
6	7.8	Singlet	3(H) CH_3 (Acetamido gr.)
7	8	Singlet	1(H) NH(Thiophene)

Antimicrobial Susceptibility Testing

The standardized disc agar diffusion method was followed to determine the activity of the synthesized compounds against the sensitive organisms *Staphylococcus aureus* as a Gram positive bacterium*, P.aerugenosa* and *E. coli* as Gram negative bacteria and *Candida albicans* as a fungus strain. The antibiotic gentamycin and sulphathiazol were used in concentration 100 µg mL-1, as references for antibacterial and antifungal agents. The Antimicrobial Susceptibility Testing was carried out using Mueller-Hinton broth medium.[19]

Method

The bacteriostatic property of the compounds was tested by disc diffusion method as described by Bauer kirby's method.

[A] Preparation of Mueller-Hinton agar

(1) Beef infusion : 300 g

(2) Acid hydrolysate of casein : 17.5 g

(3) Starch : 1.5 g

(4) Agar : 17 g

(5) Distilled water : 1 Lit.

The above constituents were weighed and dissolved in water. The mixture was warmed on water bath till agar dissolved. This was then sterilized in an autoclave at 15 lbs pressure and 121 °C for fifteen minutes. The sterilized medium (20 ml) was poured in sterilized Petri dishes under aseptic condition, allowing them to solidify on a plane table.

[B] Preparation of Antibacterial Solution

All the compounds were dissolved in dimethyl formamide (DMF). Proper drug controls were used. Compound was taken at concentration of 100 µg/ml for testing antibacterial activity. The compound diffused into the medium produced a concentration gradient. After the incubation period, the zones of inhibition were measured in mm. The tabulated results represent the actual readings control.

[C] Test cultures

Following common standard strains were used for screening of antibacterial and antifungal activities:

I. *E. coli [Gram negative bacteria]*

II. *P. aerugenosa [Gram negative bacteria]*

III. *Staphylococcus aureus [Gram positive bacterium]*

IV. *Candida albicans [Fungus]*

[D] Swabs preparation

A supply of cotton wool swabs on wooden applicator sticks was prepared. They were sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.

[E] Experimental procedure

1) The plates were inoculated by dipping a sterile swab into inoculums. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid.

2) The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60 °C after each application. Finally the swab was passed round the edge of the agar surface. The inoculation was dried for a few minutes, at room temperature, with the lid closed.

3) Ditch the bore in plate. Add compounds solution in bore.

4) The plates were placed in an incubator at 37 °C within 30 minutes of preparation for bacteria and 22 °C for fungal.

5) After 48 hrs incubation for bacteria and 7-days for fungal, the diameter of zone (including the diameter disc) was measured and recorded in mm. The measurements were taken with a ruler, from the bottom of the plate, without opening the lid.

I] Activity of compounds against *E. coli* strain

Table 4: Antimicrobial activity against *E. coli* strain

Sr. no.	Concentration in µg/ml zone of inhibition in mm					
	1	10	20	50	100	MIC
1	0.00	0.00	21.74	22.57	24.69	20
2	0.00	0.00	25.55	26.72	26.75	20
3	0.00	0.00	0.00	12.49	1343	50
4	0.00	0.00	19.92	21.65	22.85	20
Sulphathiazol	0.00	0.00	22.57	24.73	25.53	20
Gentamycin	0.00	0.00	29.63	30.47	32.27	20

II] Activity of compounds against *S.aureus* strain

Table 5: Antimicrobial activity against *S.aureus* strain

Sr. no.	Concentration in μg/ml zone of inhibition in mm					
	1	10	20	50	100	MIC
1	0.00	0.00	0.00	15.57	26.74	50
2	0.00	0.00	20.55	22.72	24.75	20
3	0.00	0.00	0.00	9.36	11.94	50
4	0.00	0.00	14.72	16.59	18.98	20
sulphathiazol	0.00	0.00	24.93	25.35	27.56	20
Gentamycin	0.00	0.00	19.45	21.0.	22.67	20

III] Activity of compound against *P. aerugenosa* strain

Table 6: Antimicrobial activity against *P. aerugenosa* strain

Sr. no.	Concentration in μg/ml zone of inhibition in mm					
	1	10	20	50	100	MIC
1	0.00	0.00	0.00	8.92	10.44	50
2	0.00	0.00	0.00	13.22	14.75	50
3	0.00	0.00	17.84	19.57	20.83	20
4	0.00	0.00	0.00	16.59	18.98	50
Sulphathiazol	0.00	0.00	18.75	20.65	21.22	20
Gentamycin	0.00	0.00	24.29	25.17	27.93	20

IV] Activity of compound against *Candida albican* strain

Table 7: Antimicrobial activity against *Candida albican* strain

Sr. no.	Concentration in μg/ml zone of inhibition in mm					
	1	10	20	50	100	MIC
1	0.00	0.00	0.00	7.45	8.57	50
2	0.00	0.00	0.00	9.73	9.93	50
3	0.00	0.00	6.63	8.35	10.53	20
4	0.00	0.00	10.72	11.35	13.15	20
Sulphathiazol	0.00	0.00	15.74	16.53	18.83	20
Gentamycin	0.00	0.00	0.00	0.00	0.00	00

Table 8: Zone of inhibition of synthesized compound

Compound No.	Diameter of inhibition zone (mm), Conc. (100 μg ml^{-1})			
	E. coli (Gram –Ve)	*S.aureus* (Gram +ve)	*P.aerugenosa* (Gram –ve)	*C.albicans* (Fungal strain)
C_a	20	50	50	50
C_b	20	20	50	50
C_c	50	50	20	20
C_d	20	20	50	20
Sulphathiazol	20	20	20	20
Gentamycin	20	20	20	00

RESULTS AND DISCUSSION

An attempt was made to synthesized eight derivatives of thiophene-sulphonamide and evaluated them for their antibacterial activity. The study was planned as per literature survey.

The investigations were planned in the following manner.

- Synthesis of 2-amino 3-carbethoxy thiophene derivatives.
- Synthesis of p-acetamido benzene sulphonyl chloride.
- Synthesis of thiophene -sulphonamide derivatives.
- Establishment of structures of targeted compounds on the basis of Infra-red spectra, NMR spectra & Mass spectrum.
- Evaluation of targeted compounds for their antibacterial activity.

Antibacterial activity

Eight compounds were screened in vitro for their antibacterial activities against Gram positive bacteria – *Staphylococcus aureus,* Gram negative bacterium - *Escherichia coli* and *P.erugenosa* and fungus strain *C.albicans.* Their antibacterial activity was compared with Gentamycin and Sulphathizole as control drugs for activity respectively. By inspection of the experimental results of the antimicrobial activity of the synthesized compounds (table 8), the following structural activity relationship assumptions are suggested.

I] compound C_a, C_b, C_d have higher antibacterial activities towards *E. coli.* With MIC 20.

II] compound C_b, C_d have higher antibacterial activities towards *S. Ausaureus* With MIC 20.

III] compound C_c, have higher antibacterial activities towards *P.aerugenosa* With MIC 20.

IV] compound C_c, C_d, have higher antibacterial activities towards *C.albicans* With MIC 20.

CONCLUSION

In conclusion the antimicrobial activity of synthesized compounds. thiophenes are a group of heterocyclic compounds currently being of great interest. For this reason, the isolation and the structural characterisation of novel derivatives, together with the development of new synthetic methods and antibacterial properties, are topics of growing interest for a great number of research groups. substituted thiophene-sulphonamide derivatives were synthesized and their in vitro antibacterial activity was evaluated against four representative microorganisms. The results of antibacterial study indicated that the presence of in aromatic ring improved antibacterial activity, whereas the presence of nitro group improved antibacterial activity of substituted thiophene. To understand the relationship between physicochemical parameters and antimicrobial activity of substituted thiophene -sulphonamide derivatives in describing Thiophene nucleus, which is a useful structure for research and development of new pharmaceutical molecules, has received much attention in the last decade. Due to their antimicrobial activities, new thiophene - sulphonamide derivatives have been synthesized and investigated for medical applications. As resistance to antimicrobial drugs is widespread, there is an increase necessity for the identification of novel structures which could lead to the design of new, potent and less toxic antimicrobial agents.

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