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RESEARCH ARTICLE

Antimicrobial Activity of 2-Aminothiophene Derivatives

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ABSTRACT

Heterocyclic compounds offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents. Essential diet ingredients such as thiamine, riboflavin, nicotinamide, pyridoxine, and ascorbic acid are heterocyclic compounds. Thiophene is the backbone of several important products, including pharmaceuticals, dyes, and agrochemicals. In addition, this S-heterocyclic core is present in many natural products, several of which show antibacterial, antifungal, antiamoebic, antioxidant, antitumor, anticoagulant, and antithrombotic activities. 2-Aminothiophenes are important five-membered heterocyclic building blocks in organic synthesis and the chemistry of these small molecules is still developing based on the discovery of cyclization by Gewald. The most convergent and well-established approach for the preparation of 2-aminothiophenes is the Gewald method, which involves the three-component reaction of a ketone, an activated nitrile and elemental sulfur in the presence of morpholine as catalyst. In the continuation of our studies toward the development of new methodologies under green chemistry approaches, herein we reported a mild, efficient, and simple "one pot" Gewald Synthesis of tetra substituted 2-aminothiophene derivatives.

Keywords: Aminothiophene, Antimicrobial, Heterocyclic

INTRODUCTION

Heterocyclic compounds offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents. Many heterocyclic compounds are found as key components in biological processes. Essential diet ingredients such as Thiamine (Vitamin B1), Riboflavin (Vitamin B2), Nicotinamide (Vitamin B3), Pyridoxal (Vitamin B6), and Ascorbic acid (Vitamin C) are heterocyclic compounds. Two of the essential amino acids tryptophan and histidine are also heterocycles. Nucleic acids, hemoglobin, chlorophyll, and many enzymes are also containing important heterocyclic nucleus.

Amongst the heterocyclic compounds triazoles, thiadiazoles, pyrazoles, and oxadiazoles, attracted a tremendous attention, as they are full of many ramifications especially in the biological and industrial applications. In view of the general observation that the biological activities are invariably associated with a large variety of heterocyclic systems such as 1,2,4-Traizoes, Pyrazoles, 1,3,4-Thaidaizole, and 1,3,4-Oxadiazoles a large number of their new

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derivatives have been synthesized and extensively studied for various pharmacological properties.

Thiophene is the backbone of several important products, including pharmaceuticals, dyes, and agrochemicals. In addition, this S-heterocyclic core is present in many natural products, several of which show antibacterial, antifungal, antiamoebic, antioxidant, antitumor, anticoagulant, and antithrombotic activities. Within this family, the 2-aminothiophenes occupy a special position as important intermediates in synthesis because they provide building blocks for several types of heterocyclic systems.

The most convergent and well-established approach for the preparation of 2-aminothiophenes is the Gewald method, which involves the three-component reaction of a ketone, an activated nitrile and elemental sulfur in the presence of morpholine as catalyst. Solvent-free reactions are an interesting alternative approach, mainly when these conditions eliminate the use of a solid support or solvent from the reaction. Solid supported reactions do not entirely meet the definition of solvent-free; however, because an appreciable amount of solvent is sometimes necessary to promote the absorption of the reactants and is always required for the extraction of products at the workup.

Thus far, little research has been reported on the Gewald reaction under solvent-free conditions, many reports use a microwave-assisted synthesis and solid support instead. The application of microwave irradiation in chemical reactions is useful because it enhances the reaction rates and, in many cases, the selectivity. These technologies are used in the field of green chemistry because they avoid organic solvents, but their drawbacks are that they require energy and specialized equipment. One of the principles of green chemistry is that reactions at room temperature are preferred because they minimize the environmental impact of productive activities due to energy matrices. In the continuation of our studies toward the development of new methodologies under green chemistry approaches, herein we reported a mild, efficient and simple "one pot" Gewald Synthesis of tetrasubstituted 2-aminothiophene derivatives.

MATERIALS AND METHODS

Materials and reagents were obtained from Honeywell grade and were used for process**.** The melting points were recorded by electrical melting point apparatus.

Chemistry of 2-Aminothiophenes

Chemistry of 2-aminothiophenes is arguably one of the most extensive and dynamic field of present-day thiophene research. Since 1961 when first report on the Gewald reaction was reported it became a universal method for synthesis of substituted 2-aminothiophenes and has gained prominence in recent times. The availability of reagents and the mild reaction conditions all contribute to the versatility of this reaction.

2-Aminothiophenes are important five-membered heterocyclic building blocks in organic synthesis, and the chemistry of these small molecules is still developing based on the discovery of cyclization by Gewald. Another attractive feature of 2-aminothiophene scaffolds is their ability to act as synthons for the synthesis of biological active thiophene-containing heterocycles, conjugates, and hybrids. At present, the biological actions of 2-aminothiophenes or their 2-*N*-substituted analogues are still being investigated because of their various mechanisms of action (e.g., pharmacophore and pharmacokinetic properties).

Likewise, the 2-aminothiophene family is used as diverse promising selective inhibitors, receptors, and modulators in medicinal chemistry, and these compounds even exhibit effective pharmacological properties in the various clinical phases of appropriate diseases.

2-Aminothiophene derivatives have been used in a number of applications in pesticides, dyes, and pharmaceuticals. The synthesis and properties of these compounds were reviewed in 1999 by Sabinis *et al*. and more recently by Puterová *et al*. In particular, substituted 2-aminothiophenes with alkyl or cycloalkyl substituents in positions 4 and 5 are active.

2-Aminothiophenes are important five-membered heterocyclic building blocks in organic synthesis and the chemistry of these small molecules

is still developing based on the discovery of cyclization by Gewald *et al*.[1,2] Thiophene is the backbone of several important products, including pharmaceuticals,[3] dyes, and agrochemicals.[4] Another attractive feature of 2-aminothiophene scaffolds is their ability to act as synthons for the synthesis of biological active thiophene-containing heterocycles conjugates and hybrids. At present, the biological actions of 2-aminothiophenes or their 2-*N*-substituted analogs are still being investigated because of their various mechanisms of action.

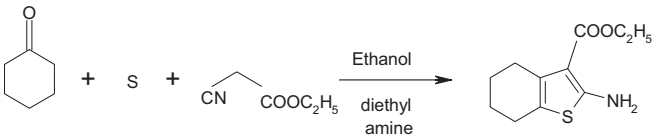
2-Aminothiophene and its derivatives demonstrate diverse array of biological/pharmacological profiles and broad spectrum of applications with remarkable potency.[5]

Various biological activities of 2-aminothiophene derivatives are

- Analgesic[6]
- Anti-inflammatory[7]
- Antioxidant and antibacterial[8]
- Antiproliferative[9]
- Antimicrobial[10]
- Anti-leishmanial[11]
- Anticonvulsant.[12]

Experimental work

Synthesis of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b] thiophene 3-carboxylate



Procedure

A mixture of cyclohexanone (0.1 mole), sulfur (0.1 mole), ethylcyanoacetate (0.1 mole) and ethanol (20 ml) were taken in the round-bottom flask (RBF) and subjected for stirring, to this well stirred mixture diethylamine (0.125 mole) was added drop wise until sulfur went into the solution and continue the stirring for 3 h and kept in refrigerator for 24 h. The obtained precipitate was filtered and dried recrystallized using ethanol.[13-34]

Thin-layer chromatography studies of the compounds

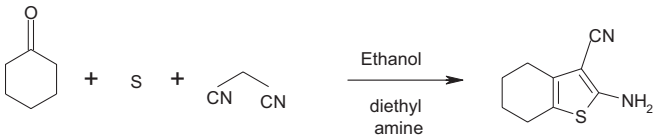
Solvent system: Petroleum ether: Ethyl acetate.
R_f values: 0.72.

Physical results

Molecular formula: $C_{11}H_{15}O_2NS$
Molecular weight: 225
Melting point: 92°C
Percentage yield: 72%
R_f value: 0.72.

Synthesis of 2-amino-4, 5, 6, 7-tetrahydrobenzo[b] thiophene-3-carbonitrile

Reaction



Procedure

A mixture of cyclohexanone (0.1 mole), sulfur (0.1 mole), malononitrile (0.1 mole), and ethanol (20 ml) were taken in the RBF and subjected for stirring, to this well stirred mixture diethylamine (0.125 mole) was added drop wise until sulfur went into the solution and continue the stirring for 3 h and kept in refrigerator for 24 h. The obtained precipitate was filtered and dried recrystallized using ethanol.

TLC studies of the compounds

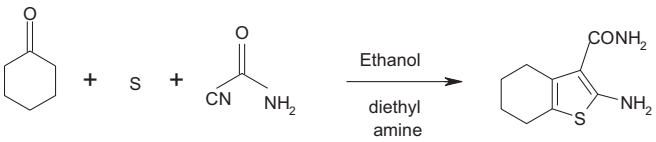
Solvent system: Petroleum ether: Ethyl acetate.
R_f values: 0.30.

Physical results

Molecular formula: $C_9H_{10}N_2S$.
Molecular weight: 178.
Melting point: 160°C.
Percentage yield: 60%.
R_f value: 0.30.

Synthesis of 2-amino-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate

Reaction



Procedure

A mixture of cyclohexanone (0.1 mole), sulfur (0.1 mole), cyanoacetamide (0.1 mole), and ethanol (20 ml) were taken in the RBF and subjected for stirring, to this well stirred mixture diethylamine (0.125 mole) was added drop wise until sulfur went into the solution and continue the stirring for 3 h and kept in refrigerator for 24 h. The obtained precipitate was filtered and dried recrystallized using ethanol.

TLC studies of the compounds

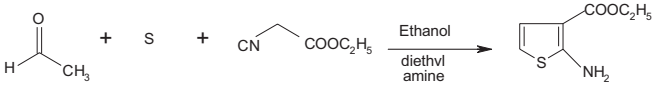
Solvent system: Petroleum ether: Ethyl acetate.
R_f values: 0.45.

Physical results

Molecular formula: $C_9H_{12}N_2SO$.
Molecular weight: 196.
Melting point: 110°C.
Percentage yield: 55%.
R_f value: 0.45.

Synthesis of ethyl 2-aminothiophene-3-carboxylate

Reaction



Procedure

A mixture of acetaldehyde (0.1 mole), sulfur (0.1 mole), ethylcyanoacetate (0.1 mole), and ethanol (20 ml) were taken in the RBF and subjected for stirring, to this well stirred mixture diethylamine (0.125 mole) was added drop wise until sulfur went into the solution and continue the stirring for 3 h and kept in refrigerator for 24 h. The obtained precipitate was filtered and dried recrystallized using ethanol.

TLC studies of the compounds

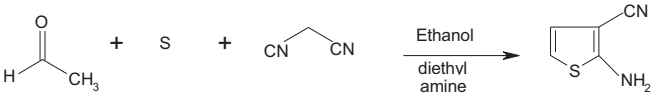
Solvent system: Petroleum ether: Ethyl acetate.
R_f values: 0.85.

Physical results

Molecular formula: $C_7H_9O_2NS$.
Molecular weight: 171.
Melting point: 125°C.
Percentage yield: 80%.
R_f value: 0.85.

Synthesis of 2-aminothiophene-3-carbonitrile

Reaction



Procedure

A mixture of acetaldehyde (0.1 mole), sulfur (0.1 mole), malononitrile (0.1 mole), and ethanol (20 ml) were taken in the RBF and subjected for stirring, to this well stirred mixture diethylamine (0.125 mole) was added drop wise until sulfur went into the solution and continue the stirring for 3 h and kept in refrigerator for 24 h. The obtained precipitate was filtered and dried recrystallized using ethanol.

TLC studies of the compounds

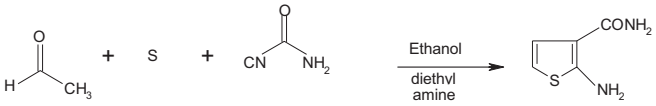
Solvent system: Petroleum ether: Ethyl acetate.
R_f values: 0.72

Physical results

Molecular formula: $C_5H_4N_2S$
Molecular weight: 124.
Melting point: 210°C.

Percentage yield: 75%.
R_f value: 0.72.

Synthesis of 2–aminothiphen-3-carboxamide
Reaction



Procedure

A mixture of acetaldehyde (0.1 mole), sulfur (0.1 mole), cyanoacetamide (0.1 mole), and ethanol (20 ml) were taken in the RBF and subjected for stirring, to this well stirred mixture diethylamine (0.125 mole) was added drop wise until sulfur went into the solution and continue the stirring for 3 h and kept in refrigerator for 24 h. The obtained precipitate was filtered and dried recrystallized using ethanol.

TLC studies of the compounds

Solvent system: Petroleum ether: Ethyl acetate.
R_f values: 0.6.

Physical results

Molecular formula: $C_5H_6N_2SO$.
Molecular weight: 142.
Melting point: 130°C.
Percentage yield: 72%.
R_f value: 0.6.

Antimicrobial activity

Introduction to antimicrobial activity
Various substituted 2-aminothiophene derivatives were reported to possess antimicrobial activity, they were prepared during the course of the present work was tested for antimicrobial activity. The antimicrobial activity was tested by biological assays.

Biological assays

In biological assays the compound being quantitative either depresses or stimulates growth of test microorganisms. Biological assays usually are more difficult to perform, provide greater error, and less reproducible than chemical or clinical assays. The test organisms for biological assays may be strains normally encountered in nature or they may be strains that have been artificially matured for their use in a specific assay.
Biological assays fall into four general categories:

1. Diffusion assays
2. Turbidimetric assays
3. Metabolic response assays
4. Enzymatic assays

In the present experiment diffusion assay was performed for antimicrobial activity of newly synthesized substituted 2-aminothiophene derivatives.

Diffusion assays

Diffusion assays are carried out on a solid medium usually an agar medium, which is suitable for the growth of the test organism. The compound to be assayed is allowed to diffuse through the medium in a radial fashion from a cup. Hence, the adjacent growth of the test organism is either depressed, as with an antibacterial which depresses the growth, or stimulated as with a growth factor. The diameter of this area reflects the concentration of the compound being assayed and it is compared with similar zones produced various known concentrations of standard or reference compound. There are two types of diffusion assays, although somewhat similar, each has its own particular advantages. Those methods are paper disc method and cup plate method.
In paper disk method paper disks are applied with 0.1 ml of testing substance. A standardized amount of agar medium, perhaps 10 ml, is placed in Petri plates and allowed to solidify. As soon as this base layer is solidified, a standardized amount of the same or a different agar medium inoculated with a test organism is added above the base layer and allowed to solidify to form the seeded agar layer. Then, paper discs are placed on solidified agar medium. The number of discs used for plate depends on expected sizes of the zones, since the zones should not overlap.

In cup plate method, instead of using discs, cups or cylinders are made on the solidified and seeded agar medium. These cylinders are filled with the appropriate dilutions of the solutions to be assayed or with solutions containing known concentrations of the reference compound and the plates are incubated for a specific period of time kept at constant temperature. The diameters of the zones are measured in millimeters and the concentrations in the solutions under assays are determined by comparison with standard.

Experimental work

In the present experiment, the antimicrobial activity was tested by Cup plate method [Figure 1]. The antimicrobial activity of substituted 2-aminothiophene derivatives was tested and compared with the standard Streptomycin. The concentration of different test solutions is 2 mg/ml compared with standard solution at a concentration of 5 mg/ml. Acetone and chloroform were used as a solvent.

Test organisms

Escherichia coli, *Staphylococcus aureus*, and *Bacillus cereus.*

Composition of nutrient medium

Mueller-Hinton agar medium

Per liter

Beef extract powder 2.0 g.
Acid digest of casein 17.5 g.
Starch 1.5 g.
Agar 17.0 g.
Final pH 7.3 ± 0.1 at 25°C

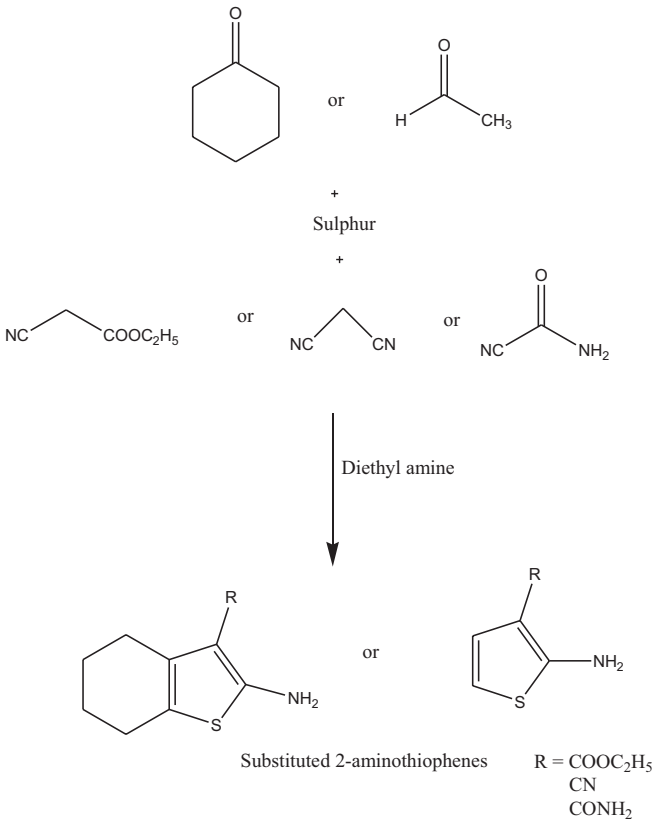
Preparation of medium

1. 38 g of the medium was suspended in one liter of purified water
2. Heated with frequent agitation and boiled for 1 min to completely dissolve the medium
3. The medium was finally adjusted to required pH
4. Autoclaved at 121°C for 15 min. Cooled at room temperature.

Procedure

The above medium was inoculated at 1% level with 18 h old cultures of the above mentioned test organisms and was transferred into sterile petri dishes[13-18]. The medium in the plates was allowed to set at room temperature for about 10 min and they were set to solidify in a refrigerator for 30 min. After that cylinders were made in the medium[19-23]. The test solutions which were prepared in acetone and chloroform along with the standard solution of Streptomycin were placed in their respective cylinders. The plates thus prepared were left to stand in a refrigerator for about 1 h to allow the test solution for diffusion. Then, incubation of the above plates was done for 24 h at 37°C. The plates were examined for zones of inhibition and the inhibition zone diameters were measured[24-34].

Scheme



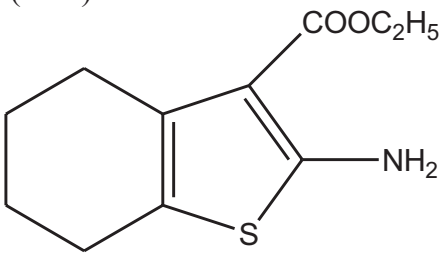
RESULTS-PHYSICAL PROPERTIES [TABLES 1-6]

Melting points were determined by open-ended capillary tube and are uncorrected. Purity of the

compounds was identified by the TLC using silica gel-G as stationary phase.

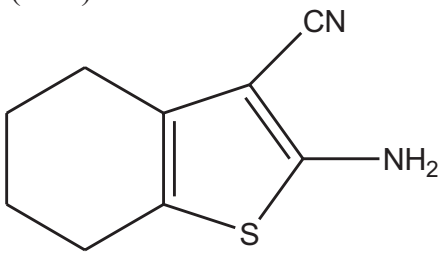
Spectral data ethyl 2-amino-4,5,6,7 tetrahydrobenzo[*b*]thiophene 3-carboxylate: (1a)

IR [Cm^{-1}, KBr]:
3405.52 (NH),
1646.72 (C=O of ester group),
780.88 (C-S),
1594.72 (C=C).



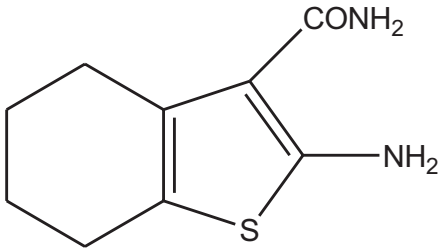
Spectral data of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile: (1b)

IR [Cm^{-1}, KBr]:
3445.08 (NH),
762.52 (C-S),
1619.76 (CN),
1519.63 (C=C)



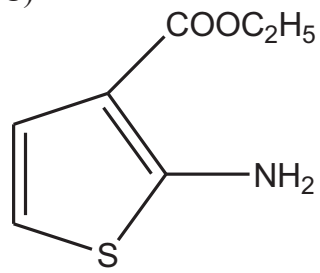
Spectral data of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate: (1c)

IR [Cm^{-1}, KBr]:
3406.43 (NH), 2927.23 (Amide NH),
621.85 (C-S),
1635.40 (C=O of Amide group),



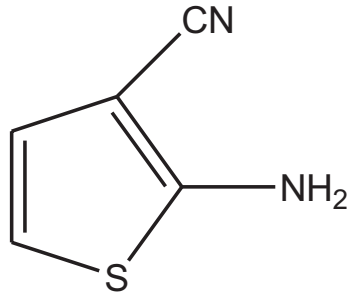
Spectral data of ethyl 2-aminothiophene-3-carboxylate: (2a)

IR [Cm^{-1}, KBr]:
3515.52 (NH),
1636.72 (C=O of ester group),
785.33 (C-S),
1574.72 (C=C)



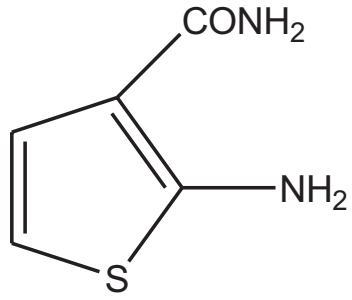
Spectral data of2-aminothiophene-3-carbonitrile: (2b)_

IR [Cm^{-1}, KBr]:
3316.59 (NH),
618.90 (C-S),
1620.46 (CN),
1567.01 (C=C)



Spectral data of2–aminothiphen-3-carboxamide: (2c)

IR [Cm^{-1}, KBr]:
3460.74 (NH), 3409.86 (Amide NH),
780.52 (C-S),
1680.60 (C=O of Amide group),



ANTIMICROBIAL ACTIVITY RESULTS

Name of organism	Average zone of inhibition (cm)						
	Standard (100 µg/ml)	1a (100 µg/ml)	1b (100 µg/ml)	1c (100 µg/ml)	2a (100 µg/ml)	2b (100 µg/ml)	2c (100 µg/ml)
Escherichia coli	10.8	10.6	10.5	10.8	9.4	10.2	9.5
Bacillus cereus	10.5	20	10.6	20.1	10.5	10.4	10.3
Staphylococcus aureus	10.4	10.2	10.7	20	10.3	10.6	10.4

Table 1: Physical data of Compound-1a

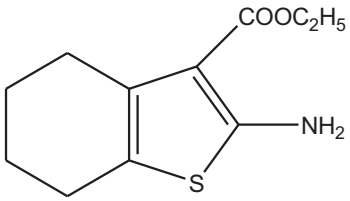
Compound number	Compound structure	Molecular formula	Molecular weight	Recrystallized solvent	Melting point (°C)	Yield (%)	R_f value
1		$C_{11}H_{15}O_2NS$	225	Ethanol	92	72	0.70

Table 2: Physical data of Compound-1b

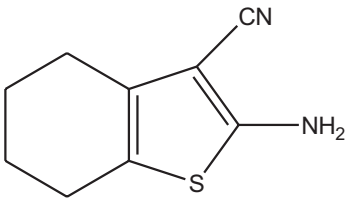
Compound number	Compound structure	Molecular formula	Molecular weight	Recrystallized solvent	Melting point (°C)	Yield (%)	R_f value
2		$C_9H_{10}N_2S$	178	Ethanol	160	60	0.30

Table 3: Physical data of Compound-1c

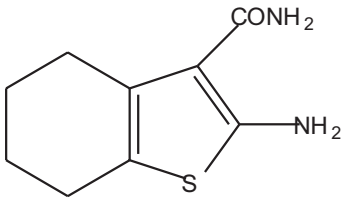
Compound number	Compound structure	Molecular formula	Molecular weight	Recrystallized solvent	Melting point (°C)	Yield (%)	R_f value
3		$C_9H_{12}N_2SO$	196	Ethanol	110	55	0.45

Table 4: Physical data of Compound-2a

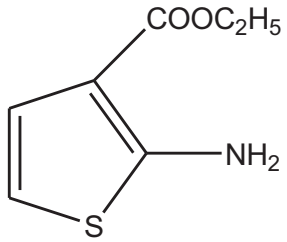
Compound number	Compound structure	Molecular formula	Molecular weight	Recrystallized solvent	Melting point (°C)	Yield (%)	R_f value
4		$C_7H_9O_2NS$	171	Ethanol	125	80	0.85

Table 5: Physical data of Compound-2c

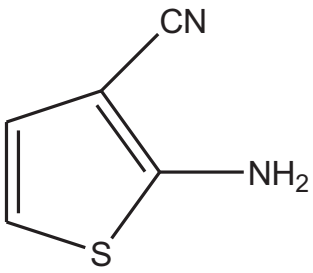
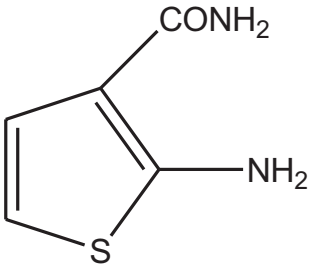
Compound number	Compound structure	Molecular formula	Molecular weight	Recrystallized solvent	Melting point (°C)	Yield (%)	R_f value
5		$C_5H_4N_2S$	124	Ethanol	210	75	0.72

Table 6: Physical data of Compound-2d

Compound number	Compound structure	Molecular formula	Molecular weight	Recrystallized solvent	Melting point (°C)	Yield (%)	R_f value
6		$C_5H_6N_2SO$	142	Ethanol	130	72	0.6

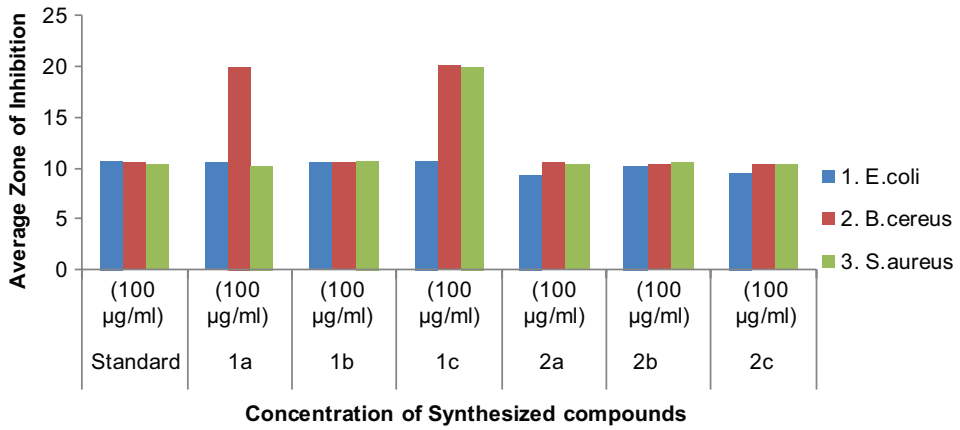


Figure 1: Comparative antimicrobial activity of synthesized compounds

SUMMARY AND CONCLUSION

- Various substituted 2-aminothiophenes were synthesized from cyclohexanone/acetaldehyde, sulfur treated with active methylene group containing compounds in the presence of diethyl amine and ethanol to give corresponding titled compounds (1a–1c) and (2a–2c) in good yields
- Synthesized compounds were characterized by physical data

Molecular formula:

- Molecular weight
- Melting point.

R_f value.

- Further titled compounds were characterized by spectral data (IR spectra)
- Synthesized compounds (1a–1c) and (2a–2c) tested for antimicrobial activity. All the synthesized compounds possess

good antimicrobial activity against *E. coli*, *B. cereus*, and *S. aureus* with respect to standard

- 1a compound possess good antimicrobial activity against *B. cereus* whereas 1c compound shows very good antimicrobial activity against *B. cereus* and *S. aureus*.

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