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SYNTHESIS OF SOME NOVEL 2-AMINOTHIOPHENE DERIVATIVES AND EVALUATION FOR THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Chemistry of 2-aminothiophenes is arguably one of the most extensive and dynamic field of present-day research. Highly substituted thiophene derivatives are important heterocyclic compounds found in numerous biologically active and natural compounds.2-aminothiophenes forms a significant class of drugs which exhibits the excellent biological activities like antimicrobial, antifungal, anti-inflammatory, analgesic, antioxidant, antitumor activities. In continuation of our previous studies on synthesis of substituted 2-aminothiophenes and due to their significant biological properties, we now report the newly synthesized ethyl 2-amino-4-phenylthiophene-3-carboxylate derivatives are obtained by base protanated reaction between ketone, elemental sulphur with ethylcayanoacetate in the presence of diethyl amine. The newly synthesized targeted compounds were characterized by spectral analysis studies and screened for their antibacterial activity by using minimum inhibitory concentration (MIC) method by taking ampicilline and streptomycine as standard.

KEY WORDS

Substituted 2-aminothiophenes, Gewald synthesis, Antimicrobial activity, MIC method.

INTRODUCTION

A large number of medicinal compounds which have been discovered belong to a major class of heterocycles containing Nitrogen and Sulphur. The versatile synthetic applicability and biological activity of these heterocycles has helped the medicinal chemist to plan, organize and implement new approaches towards the discovery of novel drugs. Thiophenes and its derivatives are an important class of heterocyclic compound, specifically, 2-amino substituted thiophenes reported to possess a wide spectrum of biological properties such as antibacterial, antifungal, analgesic, anti-inflammatory, antioxidant and antitumor and local anaesthetic activity. For example, thiophene containing β-lactam antibiotics like Ticarcillin, Cefoxitin, Cephalothin and Cephalorodine have shown good antibacterial activity [1]. The significance of 2-aminothiophenes leads us to the present study where the data on synthesis and biological activities of various substituted 2-aminothiophenes are systemized and analysed by spectral analysis [2]. We have attempted the synthesis of some new compounds which contains thiophene nucleus in its structure [3]. We have synthesized ethyl 2-amino-4-phenylthiophene-3-carboxylate as the starting compound derivatized the starting compound to various substituted ethyl 2-amino-4-phenylthiophene-3-carboxylates (2_a-2_f).

MATERIALS AND METHODS

Chemistry

Reagents and Solvents were procured from commercial sources and were dried and purified according to literature survay. The melting points were determined with an electro thermal melting point apparatus. Infrared spectra (KBr disc) were perfumed on FTIR-8400 Shimadzu and the frequencies were expressed in cm-1. 1H NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard; the chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hertz (Hz). Signal multiplicities are represented by s(singlet), d(doublet), t (triplet), dd (double doublet), m(multiplet) and br s broad singlet). The reactions were followed and the purity of the compounds was checked by TLC on silica gel-precoated aluminium sheets.

General procedure for the synthesis of starting material ethyl 2-amino-4-phenylthiophene-3-carboxylate (1)

To a mixture of ethylcyanoacetate (0. 1 mol) and ketone (0. 1 mol) were dissolved in 150 ml of absolute ethanol. Elemental sulphur powder (0. 1 mol) and appropriate base (20 ml) were added. The mixture was heated at 55 to 65 °C during 2 hours and then was cooled in refrigerator for overnight. During the cooling the formed precipitate was separated out. The precipitate was collected and recrystalized with suitable solvent [4-7].

General procedure for the synthesis of starting material ethyl 2-amino-4-phenylthiophene-3-carboxylate derivatives

Dissolve 0.05M of 2-aminothiophene (1) and 0.05M of aldehydes (a-f),25ml of dry Dimethylformamide and HCl 0.2ml was taken in a round bottomed flask for condensation and allow the reaction mixture to reflux for 3hrs. Then the reaction mixture was stand overnight in the refrigerator and was poured into crushed ice.The precipitate thus obtained was filtered off and washed with water and recrystallized with suitable solvent[8,9].

Ethyl 2-(2-chlorophenylamino)-4-phenylthiophene-3-carboxylate(2_a)

Yield 71%, M.P 203^0 C,IR(KBr) :3312(N-H str), 1523 (C-N),1714(C=O),1446(C=C),2951(C-H),1H NMR(CDCl3) δ: 1.30{t,3H,H-ester},4.29{q,2H,-OCH_2},6.3{S,1H,H-thiophene},6.40-7.48{m,9H,H-Ar and Ph},4.0{S,1H,C-NH}. Anal. calcd: C,63.77; H,4.51; Cl,9.91; N,3.91; O,8.94;S,8.96.

Ethyl 2-(3-nitrophenylamino)-4-phenylthiophene-3-carboxylate(2_b)

Yield 73%, M.P 227^0 C, IR(KBr) : 3256(N-H str), 1462 (C-N), 1692(C=O), 1456(C=C),2392(C-H), 1H NMR(CDCl3) δ: 1.29{t,3H,H-ester},4.29{q,2H,-OCH_2},6.3{S,1H,H-thiophene},6.40-7.48{m,9H,H-Ar and Ph},4.1{S,1H,C-NH}. Anal. Calcd: C, 61.94; H, 4.38; N, 7.60; O, 17.37; S, 8.70.

Ethyl 2-(4-hydroxyphenylamino)-4-phenylthiophene-3-carboxylate(2_c)

Yield 74%, M.P 216^0 C, IR (KBr) :3326(N-H str), 1352(C-N), 1646(C=O), 1561(C=C), 3056(C-H), 3363(-OH),1H NMR (CDCl3) δ: 1.30 {t,3H,H-ester},5.0{S,1H,C-OH},4.29{q,2H,-OCH_2},6.29{S,1H,H-thiophene},6.40-7.48{m,9H,H-Ar and Ph},4.2{S,1H,C-NH}. Anal.calcd: C, 67.24; H, 5.05; N, 4.13; O, 14.14; S, 9.45.

2-(Furan-2-ylamino)-4-phenyl-thiphene-3-carboxylicacid ethyl ester(2_d)

Yield 71%, M.P 232^0 C, IR(KBr) :3398(N-H str), 1275(C-N), 1682(C=O), 1448(C=C), 2859(C-H), 1H NMR(CDCl3) δ: 1.30 {t,3H,H-ester},6.30-7.48{m,8H, H-Ar and Ph},5.0{S,1H,C-OH},4.30{q,2H,-OCH_2},6.30{S,1H,H-thiophene}, 4.0{S,1H,C-NH}.Anal. calcd C, 65.16; H, 4.82; N, 4.47; O, 15.32; S, 10.23.

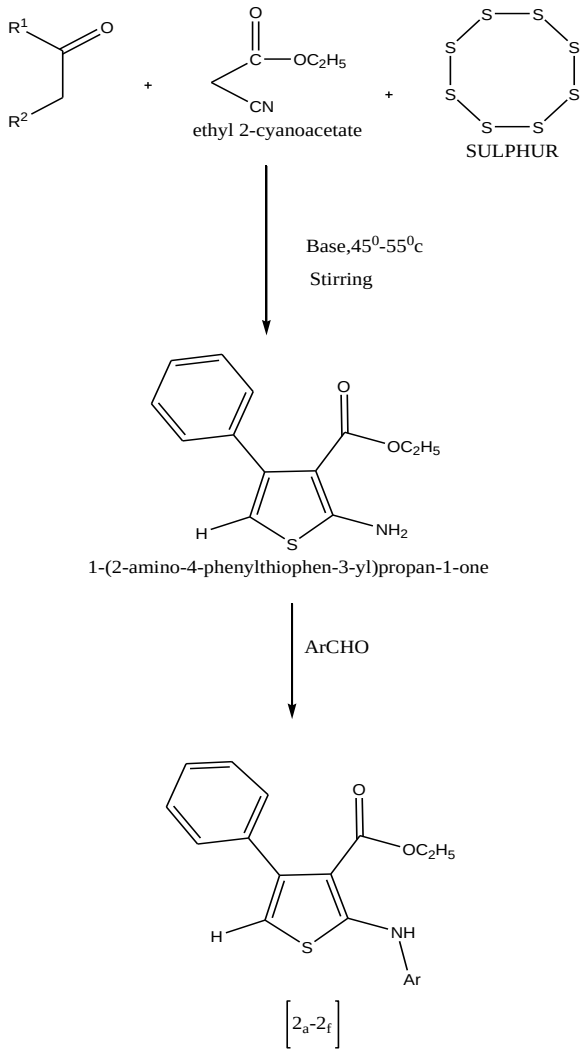
2-(4-Methoxy-phenylamino)-4-phenyl-thiophene-3-carboxylicacid ethyl ester (2_e)

Yield 70.5%, M.P 253^0 C, IR(KBr) :3258(N-H str), 1316(C-N), 1756(C=O), 1398(C=C), 2836(C-H), 1H NMR(CDCl3)δ:1.30{t,3H,H-ester},4.29{q,2H,-OCH_2},6.30-7.48{m,9H,H-Ar and Ph},3.73{t,3H,-OCH_2},4.0{S,1H,C-NH},6.30{S,1H,H-thiophene}.Anal.calcd: C, 67.97; H, 5.42; N, 3.96; O, 13.58; S, 9.07.

4-phenl-2-(thiophe-2-ylamino)-thiophene-3-carboxylicacid ethyl ester (2_f)

Yield 72%, M.P 256^0 C, IR(KBr) : 3359(N-H str),1217(C-N), 1712(C=O), 1515(C=C), 2859(C-H), 1H NMR(CDCl3) δ: 1.30{t,3H,H-ester},4.29{q,2H,-OCH_2},6.3{S,1H,H-thiophene},6.30-7.48{m,8H,H-Ar and Ph},4.0{S,1H,C-NH}. Anal calcd: C, 61.98; H, 4.59; N, 4.25; O, 9.71; S, 19.47.

SCHEME



BIOLOGICAL SCREENING

Antibacterial tests were performed at the Department of Microbiology, Swamiramanandatirtha Institute of pharmaceutical sciences, Nalgonda.

ANTIBACTERIAL SCREENING

The antibacterial studies of 2-aminothiophene derivatives were carried out against a battery of microorganisms. The antibacterial activity of the test compounds was assayed systematically against '4' different strains of bacteria i.e *E.coli, P.vulgaris,* and *B.subtilis* by S.aureus serial dilution method. Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth by Minimum inhibitory concentration method.

MINIMUM INHIBITORY CONCENTRATION

The newly synthesized compounds were screened for anti-bacterial activity studies at a concentration of 20µg/ml using Di methyl formamide as a control against gram^{+}ve Bacillus Substilis and gram^{-}ve E.Coli bacteria. The antibacterial activity of the test compounds were compared with Ampicillin and Streptomycin.

Serial dilutions of the test compounds and standard drugs were prepared in DMF.

Minimum inhibitory concentration is the lowest concentration of an compound that will inhibit the visible growth of a microorganism after 24hr incubation.

Minimum inhibitory concentration is important in diagnostic laboratories to confirm resistance to

microorganism to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

A Minimum inhibitory concentration is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against a organism.

DETERMINATION

- Preparation of antibiotic stock solution
- Preparation of antibiotic dilution range
- Preparation agar dilution plates
- Preparation of inoculum
- Inoculation
- Incubation
- Reading and interpreting results

PREPARATION OF NUTRIENT BROTH MEDIUM:

13.6grs of nutrient broth dissolved in 1000ml of distilled water. The prepared media was sterilized under autoclave at 120lb/1 hour.

PREPARATION OF TEST SOLUTIONS:

10mg of test sample was dissolved in 10ml of Dimethylformamide.

PREPARATION OF STANDARD SOLUTIONS:

10mg of standard drug was dissolved in 10ml of DMF.

PREPARATION OF STOCK SOLUTION:

From test and standard solutions 2ml of solutions were taken in different volumetric flasks and these are made up with DMF up to 20ml.this equivalent to 200 µg/ml.

PREPARATION OF SERIAL DILUTIONS:

DILUTION 1:

4ml of stock solution taken in a test tube and add 2ml of nutrient broth medium. This equivalent to 200µg/ml.

DILUTION 2:

From dilution 1 we have taken the 2ml sample and add 2ml of nutrient broth medium. This equivalent to 100µg/ml.

DILUTION 3:

From dilution 2 we have taken the 2ml sample and add 2ml of nutrient broth medium. This equivalent to 50µg/ml.

DILUTION 4:

From dilution 3 we have taken the 2ml of sample and add 2ml of nutrient broth medium. This equivalent to 25µg/ml.

DILUTION 5:

From dilution 4 we have taken the 2ml of sample and add 2ml of nutrient broth medium. This equivalent to 12.5µg/ml. In this 2ml was discarded.

These 5 dilutions were prepared individually for 3 micro-organisms. The organisms were inoculated in test tubes under laminar air flow in aseptic conditions. The test tubes were incubated at 37^0c for 24 hrs.

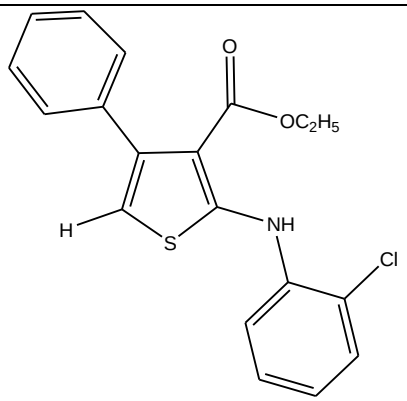
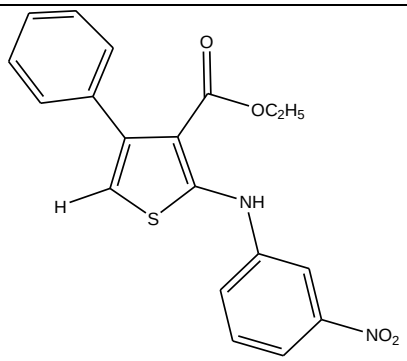
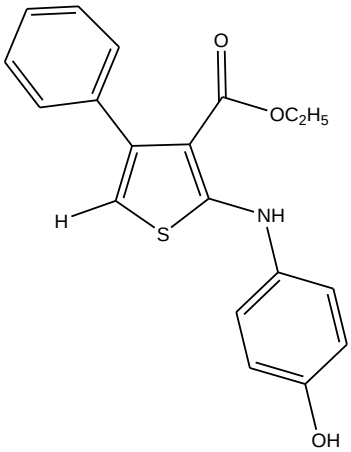
The MIC was the lowest concentration of the tested compound that yields no visible growth on the test tube.

RESULTS & DISCUSSION

All the compounds have been evaluated for their antibacterial activity against *Bacillus substilis, Escherichia coli, Proteus vulgarise (G-ve)* and S.aureus. The results of the evaluation have been viewed by taking Ampicillin and Streptomycin a broad spectrum antibiotic as the standard. Table 2 and 3 pertaining to the antibacterial data of 2-aminothiophene derivatives shows all the compounds of the series have been relatively more active against *B.subtilis* among all the compounds, compound **2_c** has been found to be greater inhibitory effect against the organisms employed , particularly against *B.subtilis, E.coli*, *P.vulgaris* and S.aureus with MIC.

Derivatives of 2-aminothiophenes

Table 1. Physico chemical properties

Compound structure	*Molecular formula*	*Molecular weight*	*Percentage yield*	*Melting point* ^{0}C	*$R_f$values*
	***$C_{19}H_{16}ClNO_2S$***	***357***	***71%***	***203***	***0.75***
	***$C_{19}H_{16}N_2O_4S$***	***368***	***73%***	***227***	***0.69***
	***$C_{19}H_{17}NO_3S$***	***339***	***74%***	***216***	***0.65***

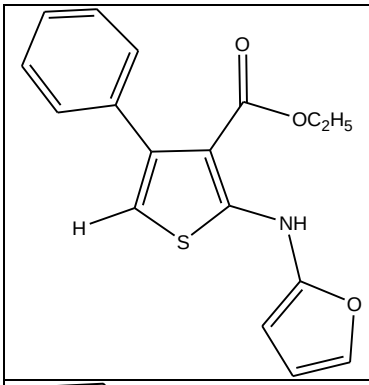
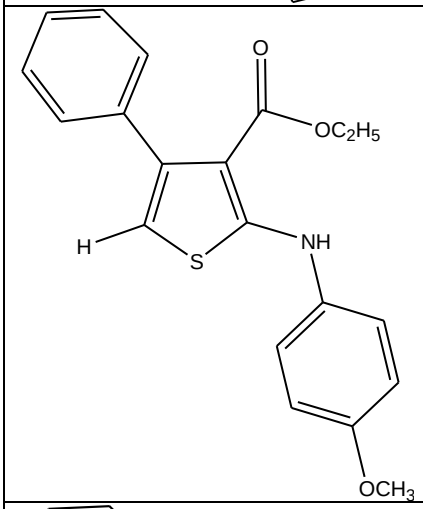
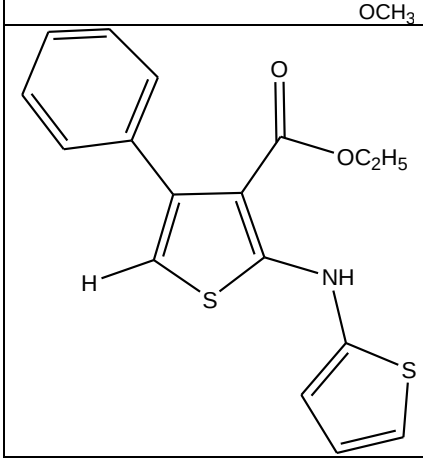
	***$C_{17}H_{15}NO_3S$***	***313***	***71%***	***232***	***0.71***
	***$C_{20}H_{19}NO_3S$***	***355***	***70.5%***	***253***	***0.69***
	***$C_{17}H_{15}NO_2S_2$***	***329***	***72%***	***256***	***0.67***

Table 2. Antimicrobial activity of standard drugs

S.NO	Standard drug	Proteusvulgaris	Bacillussubstilis	E.coli	S.aureus
1	Ampicillin	25µg	50µg	12.5µg	50µg
2	Streptomycin	12.5µg	50µg	12.5µg	25µg

Table 3. Antimicrobial activity of 2-aminothiophene derivatives

S.NO	Sample Name	Proteusvulgaris	Bacillussubtilis	E.Coli	S.aureus
1	2_a	12.5µg	12.5µg	12.5µg	25µg
2	2_b	100µg	50µg	12.5µg	25µg
3	2_c	25µg	50µg	12.5µg	50µg
4	2_d	25µg	50µ	25µg	12.5µg
5	2_e	12.5µg	25µg	50µg	25µg
6	2_f	25µg	50µg	25µg	50µg

CONCLUSION

In continuation of our research program on 2-aminothiophene, the present study focused on synthesizing 2-aminothiophene derivatives possessing different heterocyclic rings. The present study has given deep insight as the 2-aminothiophene bearing 4-hydroxy benzaldehyde shown significant anti-microbial activity. In the light of results of this study the further research will be carried out considering each heterocyclic ring individually with the 2-aminothiophene ring.

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