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One-Pot Synthesis of N-Benzyl Substituted 2-Aminothiophene-3-Carboxilic Acid Scaffold and their Antibacterial Activity

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Abstract: ***2-aminothiophene is an important pharmacophore in medicinal chemistry which exhibits a wide spectrum of biological activities. An efficient one pot synthesis has been established for the synthesis of novel series of N-benzyl substituted 2-aminothiophene-3-carboxylic acid derivatives. The synthesized compounds were characterized by nuclear magnetic resonance (^{1}H and ^{13}C NMR) and Mass spectrometry. The anti-microbial activity of these compounds were tested on Gram-negative bacteria and Gram-positive bacteria. Among all the synthesized compound 2b, 2c, 2f were found to have good antibacterial activity. Conjugation of the compound and carboxylic acid group has enhanced the activity of all the compounds.***

Keywords : 2-amino-3-carbomethoxythiophene, N-benzyl substituted 2-aminothiophene-3-carboxylic acid, microbiological activity.

I. INTRODUCTION

Bacterial infections are one of the main reasons of mortality all over the globe. Bacteria developed resistance towards the established drugs and antibiotics; this is one of the most lethal threats to the people's health issues throughout the world. Antibiotic resistant bacteria can cause illnesses even though new antibiotics were discovered. The over usage of antibiotics by humans is the main reason for the development of resistant bacteria which leads to dangerous infections and serious disability. In some cases, it leads to death. Researchers and scientists are working in this area to develop new drugs that can overcome the bacterial resistant. Hence, it is essential to develop new antimicrobial drugs which are capable of curing drug resistant bacterial strains.

Enormous anti-bacterial drugs containing heterocyclic moiety have been discovered, among which 2-aminothiophene and its analogs are an important class of compounds, which are having different pharmacological and biological attributes in medicinal chemistry[1].

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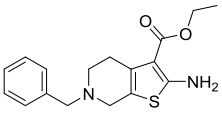
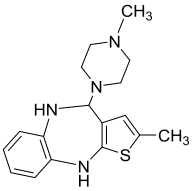
Currently, lot of investigation under progress throughout the world on their biological properties and development of new drugs containing aminothiophene analogs because of their stability, availability, and structural simplicity, 2-aminothiophenes occupy very important position in drug discovery.

Many drugs have been developed using these molecules; like Tinoridine and Olanzepine[2-4].



Tinoridine



Olanzepine

An exhaustive research has been going on 2-aminothiophenes which exposes a wide range of biological activities *viz.*, antimicrobial[5-6], anti-inflammatory[7], anxiolytic[8], antileishmanial[9], anti-diabetes[10], antifungal[11], antioxidant[12], antiplatelet activities[13]and anticancer activity[14]. For example thiophene containing ethyl 2-amino-4-phenylthiophene-3-carboxylates have been synthesized and evaluated their antimicrobial activity[15].. Another attractive feature of 2-aminothiophenes and its derivatives are their outstanding antimicrobial activity[16]. Extensive work has been done on the microbial activity and synthesis of thiophene derivatives[17] novel thieno pyrimidines and triazolo thienopyrimidines were prepared and evaluated for the antimicrobial activity[18]. Tetra substituted 2-acetylthiophene derivative were synthesized and evaluated for the antimicrobial activity[19]. There are several methods of synthesis of 2-aminothiophenes[20]. 2-aminothophenes with ester derivatives are synthesized using microwave assistance[21]. A three component one pot condensation was also reported for 2-aminothophene derivatives[22].

Carboxylic acids are extensively used as antibacterial agents in medicinal chemistry; a very less work has been done on the synthesis of amino thiophene carboxylic acid derivatives[23] and explored their biological activity. and these enormous biological properties of 2-aminothiophene has attracted us to attempt the synthesis of carboxylic acid derivatives of thiophene and tested their activity against gram -ve and gram +ve bacteria which showed enhanced antibacterial activity.

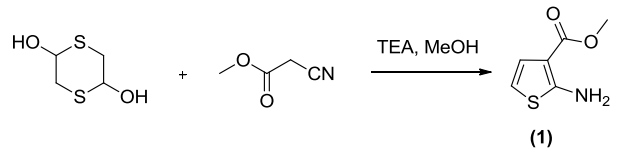
II. MATERIALS AND METHODS

The chemicals used for this research brought from Sigma Aldrich. TLC plates (60F-254) were used to monitor the reaction progress under short and long UV light. ^{1}H&^{13}C NMR spectra were recorded using 400 MHz on Bruker Avance spectrometer with tetramethylsilane (TMS) as an standard. Splitting patterns were presented as singlet (s), doublet (d), triplet (t), quartet (q) and broad (br) signals were also indicated. The values of coupling constants (J) in Hertz (Hz) and chemical shifts were given in ppm. Mass spectra were recorded using water LC–MS instrument using electro-spray ionization method (ESI).

III. EXPERIMENTAL

Synthesis of 2-amino-3-carbomethoxythiophene (1):

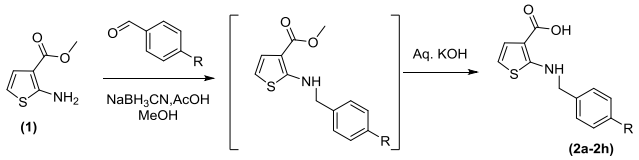
1,4-dithiane-2,5-diol (10 g, 65.7 mmol) was dissolved in methanol (70 mL) and methyl cyanoacetate (8.78 g, 88.69 mmol) was added at below 0 ^{0}C, to this reaction mixture triethylamine (3.32 g, 32.86 mmol) was slowly added over 1 h at 0 °C with constant stirring. The resulting reaction mixture was heated to 40 ^{0}C and maintained at the same temperature for 3h. The obtained solids were filtered at hot condition; the filtrate was slowly added into ice water (30 mL). The crude compound was filtered using Buchner funnel, which was purified in ethyl acetate and n-heptane mixture to give (1) as a crystalline solid. Synthetic scheme depicted in **scheme-1**



Scheme-1: Synthesis of 2-amino-3-carbomethoxythiophene

Synthesis of carboxylic acid derivatives2a-2h

To the solution of 2-amino-3-carbomethoxythiophene (1g, 6.36 mmol), substituted benzaldehydes (1.2 eq) in methanol (10 mL), added triactoxyborohydride (2.02 g, 9.540 mmol) and acetic acid (0.45 g, 7.63 mmol) and stirred for 18 h at 20-30 ^{0}C, to this reaction mixture added aqueous solution of 4 M KOH (10 mL). The reaction mixture was heated under reflux for 6 h. Partially evaporated the reaction mass under vacuum to remove methanol and the aqueous layer was diluted with process water (5 mL), washed with dichloromethane (10 mL), and adjusted pH-1.5 using 20% aqueous HCl. The compound was extracted using ethyl acetate , followed by 10% aq sodium chloride wash, dried over anhydrous sodium sulphate and distilled organic layer under vacuum resulted crude compound, which was further purified in isopropyl alcohol to give pure N-benzyl substituted 2-aminothiophene-3-carboxylic acid (2a-2h) in good yields summarized in **Table-1**. Synthetic scheme depicted in **scheme-2**



Scheme-2: Synthetic scheme for the preparation of 2a-2h

Spectral data:

2-amino-3-carbomethoxythiophene (1)

^{1}H NMR (400 MHz, DMSO-d_6) δ7.22 (s, br, 2H, NH_2),6.81 (d, J = 5.84, 1H, ThH), 6.26 (d, J = 5.84, 1H, ThH), 3.69 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 164.0, 124.9, 106.5, 103.7, 50.5;ESI-MS $[M+H]^+$ 158.20.

2-(benzylamino)thiophene-3-carboxylic acid (2a)

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, br, 1H, COOH), 7.57-7.41 (m, 6H, 5ArH, 1ThH), 6.74 (d,1H, J = 16.04 Hz, ThH), 4.81 (s,1H, NH), 4.09 (d, 2H, J = 5.84); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.05, 162.00, 139.39, 130.91, 128.79, 127.56, 127.44, 123.61, 106.63, 47.34;ESI-MS $[M+H]^-$232.10

2-((4-methylbenzyl)amino)thiophene-3-carboxylic acid (2b)

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, br, 1H, COOH),7.55-7.41 (m, 6H, 5ArH, 1ThH), 6.74 (d, 1H, J = 16.04 Hz, ThH), 4.83 (d, 1H, NH), 4.08 (d, 2H, J=5.83, CH_2), 3.07 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.05, 162.00, 139.29, 136.91, 130.91, 128.80, 127.15, 123.61, 106.63, 47.34, 20.40;ESI-MS $[M+H]^-$245.98

2-((4-methoxybenzyl)amino)thiophene-3-carboxylic acid (2c)

^{1}H NMR (400 MHz, DMSO-d_6) δ9.44 (s, br, 1H, COOH),7.66-7.54 (m, 5H, 4ArH, 1ThH), 6.56 (d, 1H, J = 16 Hz, ThH), 5.08 (d, 1H, J = 5.84 Hz, NH), 4.08 (d, 2H, J = 5.84, CH_2), 3.66 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.05, 162.00, 159.12, 136.70, 130.91, 128.71, 123.61, 114.13, 106.63, 55.16, 47.34; ESI-MS $[M+H]^-$262.30

2-((4-nitrobenzyl)amino)thiophene-3-carboxylic acid (2d)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, br, 1H, COOH), 7.66-7.54 (m, 5H, 4HArH, 1HThH), 6.56 (d, 1H, J = 16, ThH), 4.82 (d, 1H, J = 0.48 Hz, NH), 4.08 (d, 2H, J = 5.84, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.05, 162.00, 147.66, 145.05, 130.91, 127.72, 124.29, 123.61, 106.63, 47.34;ESI-MS $[M+H]^-$277.98

2-((4-cyanobenzyl)amino)thiophene-3-carboxylic acid (2e)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, br, 1H, COOH), 8.06-7.82 (m, 5H, 4HArH, 1HThH), 6.25 (d, 1H, J = 0.4 Hz, ThH), 4.08 (d, 1H, J = 5.84 Hz, NH), 3.77 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.05, 162.00, 147.66, 145.05, 130.91, 127.72, 124.29, 123.61, 106.63, 47.34, ESI-MS $[M+H]^-$257.30

2-((4-ethylbenzyl)amino)thiophene-3-carboxylic acid (2f)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.1 (s, br, 1H, COOH),7.86-7.45 (m, 5H, 4ArH, 1ThH), 6.06 (d, 1H, J = 16 Hz, ThH), 4.82 (d, 1H, J = 5.84 Hz, NH), 4.22 (d, 2H, J = 5.84, CH_2), 2.65 (q, 2H, J = 7.5 Hz, CH_2), 1.20 (t, 3H, J = 7.5, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.05, 162.00, 139.21, 137.82, 130.91, 128.64, 128.29, 123.61, 106.63, 47.34, 28.48, 15.53; ESI-MS $[M+H]^-$260.03

2-((4-(dimethylamino)benzyl)amino)thiophene-3-carboxylic acid (2g)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.6 (s, br, 1H, COOH),7.77-7.42 (m, 5H, 4ArH, 1ThH), 6.08 (d, 1H, J = 16 Hz, ThH), 4.74 (d, 1H, J = 5.84 Hz, NH), 4.11 (d, 2H, J = 7.54, CH_2), 2.51 (s, 6H, $N(CH_3)_2$);^{13}C NMR (100 MHz, $CDCl_3$) δ 164.05, 162.00, 151.51, 135.27, 130.91, 127.81, 123.61, 116.41, 106.63, 47.34, 39.43;ESI-MS $[M+H]^-$275.10

2-((4-bromobenzyl)amino)thiophene-3-carboxylic acid (2h)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, br, 1H, COOH), 7.98-7.66 (m, 5H, 4HArH, 1HThH), 6.33 (d, 1H, J = 0.4 Hz, ThH), 4.23 (d, 1H, J = 5.64 Hz, NH), 3.77 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.05, 162.00, 140.00, 131.96, 130.91, 129.00, 123.61, 121.94, 106.63, 47.34; ESI-MS $[M+H]^+$ 312.96 (100%), 310.96 (98.1%)

Protocol for Antimicrobial activity:

All the compounds were tested for their antibacterial activities against six bacterial cell lines (*Staphylococcusaureus, Bacillus cereus, Bacillus subtilis E. coli, Enterococcus faecalis, and Klebsiella pneumonia)*. The protocol for the tests were adopted from Karteek et. al., 2018 [24]**.** Tetracycline (30 µg/ml each) was used as a reference drug for this study**.**

IV. RESULT AND DISCUSSION

Chemistry

Synthetic scheme for the preparation of 2a-2h.

Preparation of 2-aminothiophene carboxylic acid derivatives was not explored by many researchers. Thus we have planned for the synthesis of new N-benzyl substituted 2-aminothiophene-3-carboxylic acid derivatives in a single step and succeed in this direction. The synthesis of 2-amino thiophene carboxylate has been reported earlier. The carboxylic acid derivatives were prepared via. Reductive amination by reacting the starting material (1) with different substituted benzaldehydes, and reduced to corresponding amines using triacetoxyborohydride in acetic acid. The reaction mixture was subjected to hydrolysis to carboxylic acid using KOH to afford **2a-2h** in good yields as shown in **Scheme-2.** All the synthesized compounds are characterized by ^{1}H and ^{13}C NMR and mass spectrometry which confirms that the peak at 10.6 belongs to carboxylic acid group and a peak at 4.22 for NH protons.

Table-1: Yields of synthesized compounds (2a-2h)

Compound	R	Color	Yield (%)	Molecular formula (M.W)
2a	H	Light brown	68	$C_{12}H_{11}NO_2S$ (233.29)
2b	CH_3	Light brown	83	$C_{13}H_{13}NO_2S$ (247.31)
2c	OCH_3	Light brown	72	$C_{13}H_{13}NO_3S$ (263.31)
2d	NO_2	Light yellow	59	$C_{12}H_{10}N_2O_4S$ (278.28)
2e	CN	Light yellow	49	$C_{13}H_{10}N_2O_2S$ (258.3)
2f	CH_2CH_3	Light brown	69	$C_{14}H_{15}NO_2S$ (261.34)
2g	$N(CH_3)_2$	Light brown	63	$C_{14}H_{16}N_2O_2S$ (276.35)
2h	Br	Off white	87	$C_{12}H_{10}BrNO_2S$ (312.18)

Antimicrobial activities:

All the synthesized compounds were tested for their *in vitro* antibacterial activity against six bacterial cell lines by well-plate diffusion method. The antibacterial results were summarized in **Table-2**. The values were given in zone of inhibition and were measured in millimetres (mm). All the synthesized compounds were active against the selected organisms. Specifically, compound 2b is showing good activity against all the bacterial cell lines. Whereas, compounds 2c and 2f are appreciable active against *Staphylococcusaureus*, *Bacillus subtilis*, and *Bacillus cereus* and 2g showed good activity against *E. coli* and *Enterococcus faecalis*. The activity of all the synthesized compounds may be due to the presence of carboxylic acid group attached to benzyl thophene. These structures are highly conjugated and the presence of electron donating groups at para position to thiophene enhances the conjugation and thus the molecule is stable which can cause the mortality to the bacterial cell walls.

Table-2: Antimicrobial activity of 2a-2h (Inhibition zone in mm)

S.No	Compound	*Staphylococcusaureus*	*Bacillus subtilis*	*Bacillus cereus*	*Escherichia coli*	*Enterococcus faecalis*	*Klebsiella pneumonia*
1	2a	18	17	18	18	14	19
2	2b	**22**	**23**	**23**	**20**	**21**	**20**
3	2c	**23**	**20**	**23**	18	19	17
4	2d	10	9	14	7	8	11
5	2e	9	8	13	10	11	11
6	2f	**23**	**21**	**23**	18	16	17
7	2g	18	18	20	**19**	**21**	18
8	2h	15	15	16	10	9	10
9	Tetracycline (30 mg/ml)	25	24	24	22	25	26

V. CONCLUSION

In this paper, we have reported an efficient one pot three component synthesis of novel N-benzyl substituted 2-aminothiophene-3-carboxylic acids in excellent yield and their structures were characterized by ^{1}H and ^{13}C NMR spectroscopy. These compounds were screened in vitro for their antibacterial activities. Results showed that upon replacing the substituent at Para position of benzene ring showed excellent effect on anti-microbial activity significantly. The electron donating groups like methyl, methoxy and ethyl in compounds 2b, 2c, 2f respectively showed excellent antimicrobial activity

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