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Synthesis and evaluation of some novel thiophenes as potential antibacterial and mycolytic Agents

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ABSTRACT

A series of tetrahydrobenzothiophene was synthesized with an objective to develop novel and potent amtimicrobial agent of synthetic origin. First, N- propylamine and Ethyl cyanoacetate were reacted to yield Propyl cyanoacetamide I. Then, compound (I) was reacted with cyclohexanone to obtain an intermediate, which was processed to 2-amino-3-N-(propylcarboxamido)- 4, 5, 6,7-tetra hydrobenzo (b) thiophene (II) by well known and versatile Gewald reaction. Reaction of compound II with different aromatic aldehyde yielded the title compound IIa-k. The synthesized compound were purified, characterized and evaluated for antimicrobial activity. Most of the compound exhibited moderate to significant activities.

Key Words : Tetrahydrobenzothiophene, Schiff bases, Gewald Reaction, Antimicrobial activity.

INTRODUCTION

Thiophenes have exhibited an array of biological activities ranging from antibacterial [2,4,6,8,10,11], antifungal [2,9,25], antioxidant[5], anti-inflammatory activity [1,5,10], antihyperlipidemic [6] and so on. Among the antimicrobial agents thiophene derivatives like Cephalothin, Cephalorodine and Cefoxitin are known to have a promising activity. Antifungal agents like Ticonazole and Sertaconazole also contain the thiophene heterocycle.

So far various new thiophenes 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[6,11,14,17,23]. Hence the synthesis of "2-amino-3-N-(propylcarboxamido) 4,5,6,7-tetra hydro benzo(b)thiophene"(**I**) is achieved. The different derivatives of the parent compound **I** was achieved by using different aryl aldehydes to obtain a series of Schiff Bases (**IIa- k)**, as mentioned below:

Where R= OH, Cl, CH_3, OCH_3, NO_2 etc

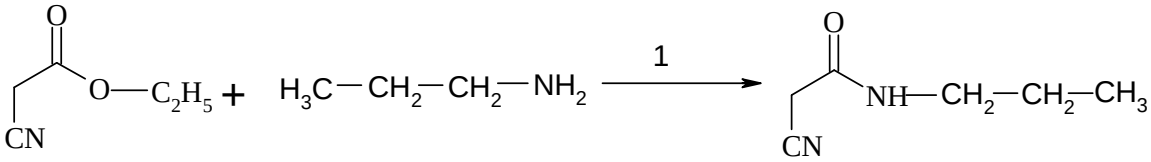
The new compounds were characterized by spectral data and screened for their in-vitro antimicrobial (antibacterial and antifungal) activity by agar diffusion method.

MATERIALS AND METHODS

The melting point of synthesized compounds was determined in open capillary tubes using melting point apparatus, expressed in ^{0}C and are uncorrected. Reactions were monitored by thin layer chromatography on pre-coated plates (SD fine Chem. Ltd) using different solvent systems. The purity of the compound was ascertained by TLC, using iodine vapours as visualizing agents. The structure of the compound were confirmed by I.R., NMR and Mass spectras.

SCHEME-I

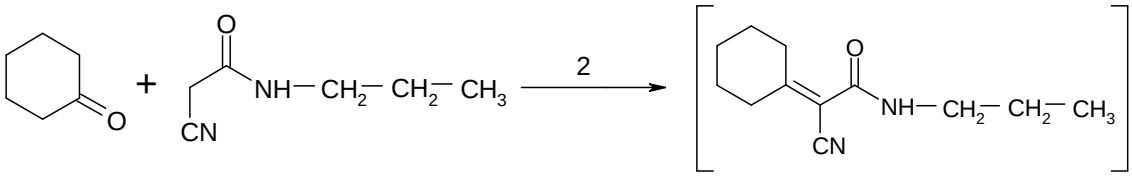
Step-I



I

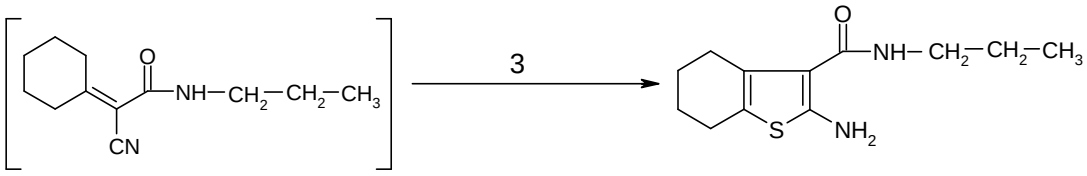
Ethyl cyano acetate *N- propylamine* *Propyl cyanoacetamide I*

Step-II



Cyclohexanone *propyl cyanoacetamide* *2-cyano-2-cyclohexylidene-N-propylacetamide*

Step-III



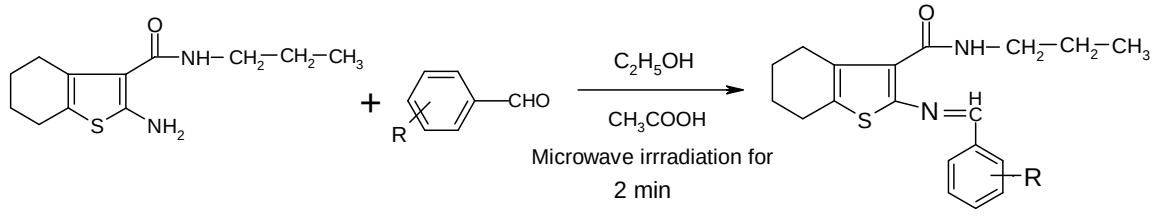
II

2-amino-3-N-(propylcarboxamido)-4,5,6,7- tetra hydro benzo(b) thiophene

1= microwave irradiation (2- 3 min at 750 watt); 2= CH_3COOH/ CH_3COONH_4 10 hrs. reflux ; 3= S, ethanol, DEA, at 40- 45 ^{0}C

SCHEME-II

General method for the syntheses of 2-[(substituted benzylidene) imino]-3-N-(propylcarboxamido) -4,5,6,7- tetra hydro benzo (b) thiophenes (Schiff bases) [IIa-k]:

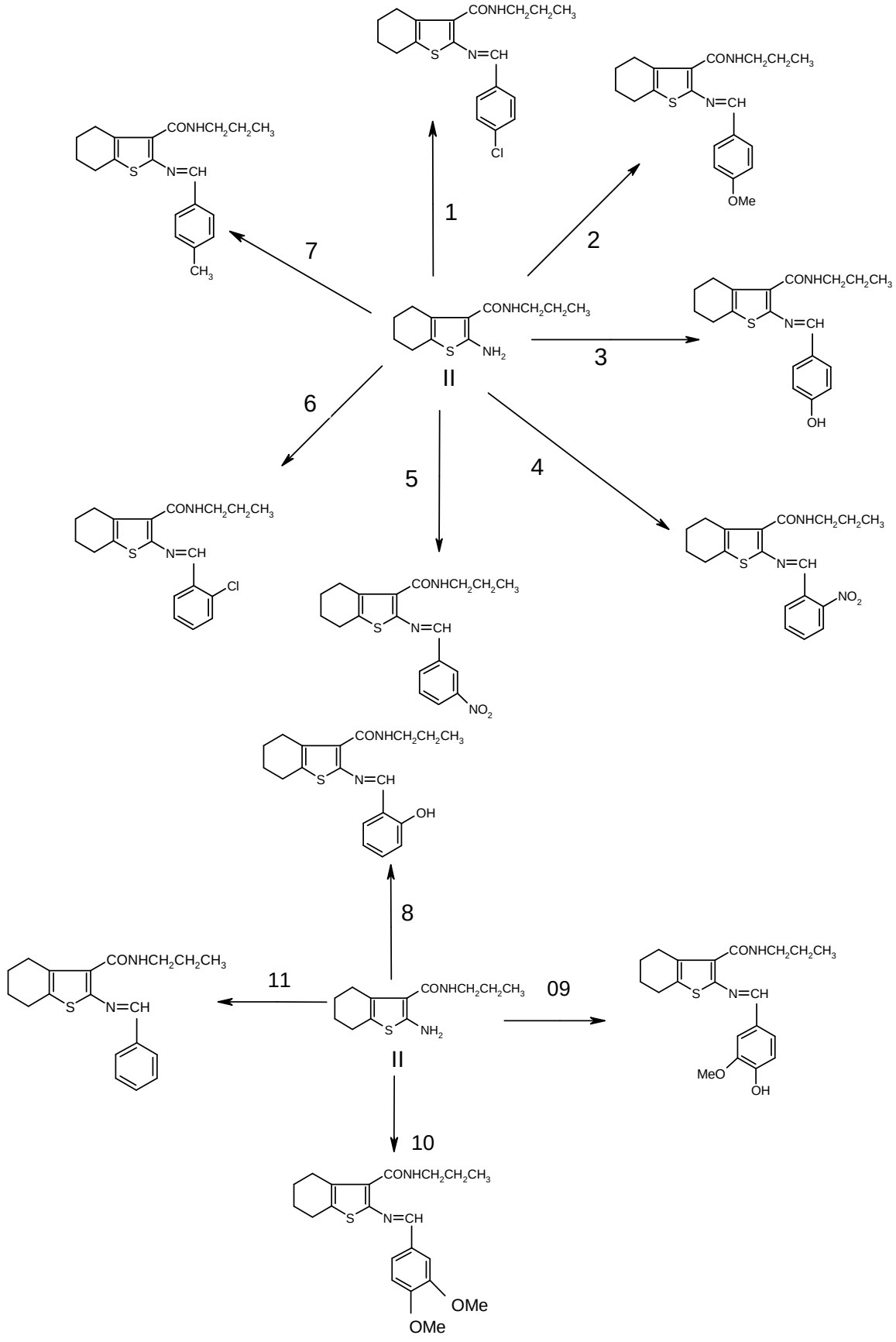


2-amino-3-(N-propylcarboxamido) -4,5,6.7-tetrahydro benzo(b) thiophene (II)

Substituted benzaldehydes (01-11)

IIa-k

Synthesis of 2-[(substituted benzylidene) imino]-3-N-(propylcarboxamido)-4,5,6,7-tetrahydrobenzo (b) thiophenes (Schiff bases) [IIa-k]



01 = 4-chloro benzaldehyde
02 = 4-methoxy benzaldehyde
03 = 4-hydroxy benzaldehyde
04 = 2-nitro benzaldehyde
05 = 3-nitro benzaldehyde
06 = 2-chloro benzaldehyde
07 = 4-methyl benzaldehyde
08 = 2-hydroxy benzaldehyde
09 = 4-hydroxy, 3-methoxy benzaldehyde
10 = 3,4-dimethoxy benzaldehyde
11 = benzaldehyde

Chemistry:

a) Synthesis of Propyl cyanoacetamide I :

A mixture of N-propylamine (0.5M) and ethyl cyano acetate (0.5M) was heated in microwave oven at 750 watt for 2-3min. The reaction mixture was left at room temperature for overnight. The solid obtained was filtered, washed with water and dried. Recrystallization was done by ethanol: water mixture (5:1).

b) Synthesis of 2-cyano-2-cyclohexylidene-N-propyl acetamide:

A mixture of propylcyanoacetamide (0.04M), cyclohexanone (0.04 M), ammonium acetate (1g) and glacial acetic acid (2ml) in benzene (80ml) was refluxed with an arrangement for continuous separation of water involving dean stark apparatus. After 10 hours the reaction mixture was cooled, diluted with 10 ml benzene and washed with sodium carbonate solution (10% w/v in water) and water successively and dried over anhydrous sodium sulphate. The solvent was removed under vacuum and the intermediate crude product obtained was immediately processed for next step.

c) Synthesis of 2-amino-3-N-(propylcarboxamido) 4,5,6,7- tetra hydro benzo(b) thiophene II:

To a mixture of 2-cyano-2-cyclohexylidene-N-propyl acetamide in alcohol (30 ml) was added sulphur (1.28 g; 0.04 M) in portions followed by the addition of, diethyl amine (6.0 ml) drop wise with stirring. The reaction mixture was stirred for 3 hours at 40-45 ºC and chilled over night. The solid obtained was filtered, washed with ethanol and crystallized from iso propyl alcohol: water mixture (9:1).

I.R. (in cm^{-1})- 3396,1591(-NH_2-); 3100(Ar CH); 2928(ali-CH); 1625 (C=O); 780(C-S); 1366(Ar-C=C); H^1 NMR- 8.0 (s, 1H, N-H), 6.0 (s, 2H,NH_2), 3.3 (q, 2H, -CH_2), 2.6 (d, 4H,2-CH_2), 1.8 (q,2H, -CH_2), 1.5 (m, 4H, 2-CH_2), 1.0 (t, 3H, -CH_3); MS (m/z, %) 239.4 [M^+, 100%].

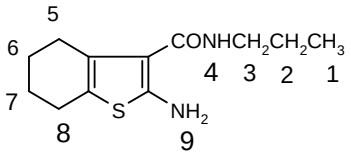
d) Synthesis of 2-[(substituted benzylidene) imino]-3-N-(propylcarboxamido)-4,5,6,7-tetra hydro benzo (b) thiophenes (Schiff bases):

A mixture of the starting compound (II) (0.005 M) and the required aryl aldehydes (Substituted benzaldehydes, 0.005 M) in ethanol and catalytic amount of glacial acetic acid (2 ml) was heated in microwave oven at 750 watt for 120 sec (2 min). The mixture was cooled to room temperature; the solid separated was filtered, washed with ethyl alcohol and crystallized with suitable solvent.

I.R. (in cm^{-1})- 3184 (Ar-CH); 2933 (Ali-CH); 1630 (C=O); 1545 C=N); 825 (C-N); 760 (C-S); 1528(N=O of NO_2);H^1 NMR- 1.0 (t, 3H,-CH_3, 1), 1.5-1.7 (m, 6H, 3-CH_2, 2,6&7), 2.6 (d, 2H, -CH_2, 5), 2.8 (d, 2H, -CH_2, 8), 3.3 (q, 2H, -CH_2, 3), 7.5 (t, 1H, -CH, 11), 7.6 (t, 1H, -CH, 12), 7.9 (d,2H, 2-CH, 10&13), 8.0 (s, 1H, -CONH, 4), 8.7 (s, 1H, N=CH, 9).

Spectral Analysis:

NMR spectrum of compound II:

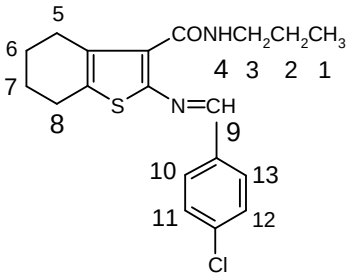


II

^{1}H NMR (CDCl3): δ values

8.0 (s, 1H, N-H, 4), 6.0 (s, 2H,NH_2, 9), 3.3 (q, 2H, -CH_2,3), 2.6 (d, 4H,2-CH_2, 5&8), 1.8 (q,2H, -CH_2, 2), 1.5 (m, 4H, 2-CH_2, 6&7), 1.0 (t, 3H, -CH_3, 1).

NMR spectrum of compound IIa:

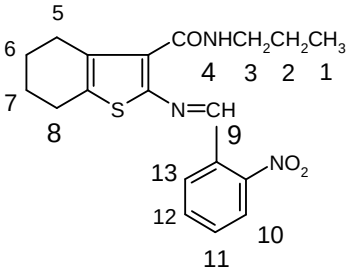


IIa

^{1}H NMR (CDCl3): δ values

1.0 (t, 3H, CH_3, 1), 1.6-1.8 (m, 6H, 3CH_2, 2,6&7), 2.6-2.9 (d, 4H, 2-CH_2, 5&8), 3.3 (q, 2H,-CH_2, 3), 8.3 (s, 1H, N-H, 4), 8.5 (s, 1H, N=CH, 9), 7.6 (d, 2H, 2-CH, 10&13),7.4 (d, 2H, 2-CH).

NMR spectrum of compound IId:



IId

^{1}H NMR (CDCl3): δ values

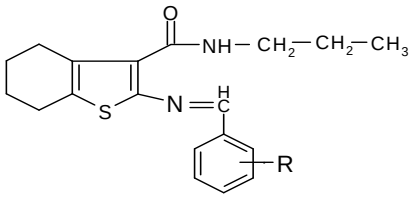
1.0 (t, 3H,-CH_3, 1), 1.5-1.7 (m, 6H, 3-CH_2, 2,6&7), 2.6 (d, 2H, -CH_2, 5), 2.8 (d, 2H, -CH_2, 8), 3.3 (q, 2H, -CH_2, 3), 7.5 (t, 1H, -CH, 11), 7.6 (t, 1H, -CH, 12), 7.9 (d,2H, 2-CH, 10&13), 8.0 (s, 1H, -CONH, 4), 8.7 (s, 1H, N=CH, 9).

Biological screening:

The antimicrobial activity [52,53,54] of the title compounds were evaluated by the agar diffusion method at concentration of 50 µg/0.1 ml using DMSO as a solvent. The zones of inhibition were measured in mm at the end of 24 h for bacteria and 48 h for fungi and are reported in related tables 2.

TABLE – 1 (PHYSICAL DATA)

2-[(substituted benzylidene) imino]-3-N-(propyl carboxamido)-4,5,6,7-tetra hydro benzo (b) thiophenes (Schiffs bases)[IIa-k)]:

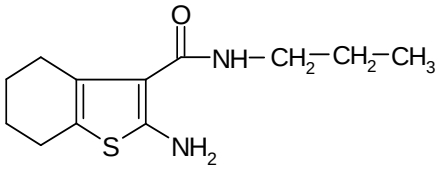


Comp. Code	R	Mol. Formula	M.W.	M.P.	% Yiel	R_f Val
IIa	4-chloro	$C_{19}H_{21}N_2SClO$	361	200	70.41	0.86
IIb	4-methoxy	$C_{20}H_{24}N_2OS$	340	137	54.11	0.92
IIc	4-hydroxy	$C_{19}H_{22}N_2O_2S$	342	220	50.29	0.78
IId	2-nitro	$C_{19}H_{21}N_3O_3S$	371	173	64.32	0.64
IIe	3-nitro	$C_{19}H_{21}N_3O_3S$	371	194	67.02	0.69
IIf	2-chloro	$C_{19}H_{21}N_2OSCl$	361	195	72.77	0.82
IIg	4-methyl	$C_{20}H_{24}N_2OS$	340	170	56.47	0.62
IIh	2-hydroxy	$C_{19}H_{22}N_2O_2S$	342	187	50.29	0.92
IIi	4-hydroxy 3-methoxy	$C_{20}H_{24}N_2O_3S$	372	149	67.04	0.65
IIj	3,4-dimethoxy	$C_{21}H_{26}N_2O_3S$	386	186	43.52	0.84
IIk	H	$C_{19}H_{22}N_2OS$	326	176	47.85	0.66

TLC solvent system: Benzene: Ethyl acetate (7:3); Recrystallization solvent: DMF : Water (9:1)

TABLE – 2 (SPECTRAL DATA)

2-Amino-3- N-(propyl carboxamido) -4,5,6,7- tetra hydro benzo (b)thiophene (II)



(II)

Compound Code	λ_{max} in nm	IR((KBr) cm^{-1}	^1H NMR
II	338	3396,1591(-NH_2-); 3100(Ar CH); 2928(Ali-CH); 1625 (C=O); 780(C-S); 1366(Ar-C=C).	8.0 (s, 1H, N-H, 4), 6.0 (s, 2H,NH_2, 9), 3.3 (q, 2H, -CH_2,3), 2.6 (d, 4H,2-CH_2, 5&8), 1.8 (q, 2H, -CH_2, 2), 1.5 (m, 4H, 2-CH_2, 6&7), 1.0 (t, 3H, -CH_3, 1).
IIa	435	3185 (Ar-CH); 2928(Ali-CH); 1630 (C=O); 1593 (C=N); 1085(Ar-Cl); 808(C-N); 746(C-S); 1541(Ar-C=C).	1.0 (t, 3H, CH_3, 1), 1.6-1.8 (m, 6H, 3CH_2, 2,6&7), 2.6-2.9 (d, 4H, 2-CH_2, 5&8), 3.3 (q, 2H,-CH_2, 3), 8.3 (s, 1H, N-H, 4), 8.5 (s, 1H, N=CH, 9), 7.6 (d, 2H, 2-CH, 10&13), 7.4 (d, 2H, 2-CH).
IIb	432	2935 (Ali-CH); 3123 (Ar-CH); 1629 (C=O); 1599 (C=N); 825 (C-N); 789 (C-S); 1250 (Ar-C-O of Ar-OCH_3)	--
IIc	408	2935 (Ali-CH); 3187 (Ar-CH); 1627 (C=O); 1535 (C=N); 833 (C-N); 779 (C-S); 3659(-OH)	---
IId	391	3184 (Ar-CH); 2933 (Ali-CH); 1630 (C=O); 1545 C=N); 825 (C-N); 760 (C-S);1528(N=O of NO_2)	1.0 (t, 3H,-CH_3, 1), 1.5-1.7 (m, 6H, 3-CH_2, 2,6&7),2.6 (d, 2H, -CH_2, 5), 2.8 (d, 2H, -CH_2, 8),3.3 (q, 2H, -CH_2, 3), 7.5 (t, 1H, -CH, 11), 7.6 (t, 1H, -CH, 12), 7.9 (d,2H, 2-CH, 10&13),
IIe	393	3112 (Ar-CH); 2935 (Ali-CH); 1653 (C=O); 1560 (C=N);835 (C-N); 789(C-S); 1518(N=O of NO_2)	--
IIf	400	3115 (Ar-CH); 2926 (Ali-CH); 1651 (C=O); 1593 (C=N); 815 (C-N); 759 (C-S); 1562 (Ar-C=C); 1071(Ar-Cl)	--
IIg	422	3184 (Ar-CH); 2935 (Ali-CH); 1645 (C=O); 1545 (C=N); 815 (C-N); 755 (C-S); 1517 (Ar-C=C).	--
IIh	442	2935 (Ali-CH); 3187 (Ar-CH); 1627 (C=O); 1535 (C=N); 833 (C-N); 779 (C-S); 3659(-OH)	--
IIi	412	3613 (O-H); 3058 (Ar-CH); 2935 (Ali-CH); 1656 (C=O); 1539 (C=N); 825 (C-N); 771 (C-S); 1512 (Ar-C=C); 1276 (Ar-C-O of Ar-OCH_3).	--
IIj	389	3123(Ar-CH); 2935 (Ali-CH); 1629 (C=O); 1599 (C-N); 1510 Ar-C=C); 1276 (Ar C-O of OCH_3); 825 (C-N); 789 (C-S).	--
IIk	41	2916 (Ali-CH); 1692 (C=O); 1589 (C= N); 828 (C-N); 789(C-S); 3176 (Ar-CH); 1511 (Ar C=C).	--

The antibacterial activity of newly synthesized derivatives has been evaluated against both gram-positive organisms *Staphylococcus aureus* and *Bacillus subtilis* and gram-negative organisms *Escherichia coli* and *Klebsiella pneumoniae.* The standard drug used was Ampicillin.

The antifungal screening was done by using *Aspergillus niger* and *Candida albicans.* The standard drug used was Miconazole nitrate.

From the screening results it was observed that, both electron donating and electron withdrawing groups on the aldehydic phenyl ring of the compounds influenced the activity. But aldehydic phenyl ring containing electron withdrawing group has shown promising result. Among all the compounds tested, IIf with 2-chloro substitution and IId with 2-nitro substitution at R was found to be most active against both Gram-positive and Gram-negative bacteria. The remaining compounds of both the series exhibited mild to moderate activities when compared to the standards. The antifungal screening results also suggest that the test compounds showed mild to moderate activity against *A.niger* only but no significant activity against *C.albicans* compared to the standard employed.

TABLE- 3 ANTIBACTERIAL ACTIVITY DATA FOR IIa –k

Comp. Code	R	Zone of Inhibition (mm)		Zone of Inhibition(mm)			
		Aspergillus *niger*	Candida *albicans*	S. *aureus*	B. *subtilis*	E. *coli*	K. *pneumoniae*
IIa	4-chloro	06	NA	08	10	07	08
IIb	4-methoxy	07	NA	06	07	NA	02
IIc	4-hydroxyl	05	03	04	03	NA	NA
IId	2- nitro	06	02	08	10	09	08
IIe	3-nitro	07	NA	05	06	05	07
IIf	2-chloro	10	05	09	09	07	10
IIg	4-methyl	03	NA	05	04	01	05
IIh	2-hydroxy	05	01	03	02	04	01
IIi	4-hydroxy,3-methoxy	03	NA	06	07	NA	NA
IIj	3,4-dimethoxy	06	NA	04	03	02	03
IIk	H	04	NA	06	05	NA	NA
Miconazole nitrate		18	15	--	--	--	--
Ampicillin		--	--	10	12	15	11

CONCLUSION

Gewald reaction is successfully utilized to synthesize the new 2-amino-3-N-(propyl carboxamido)-4,5,6,7-tetrahydro benzo(b)thiophene (II) from the corresponding (I) i.e. propyl cyanoacetamide (SCHEME-I).

A new series of compounds were synthesized from of 2-amino-3-N-(propyl carboxamido)-4,5,6,7-tetrahydro benzo(b)thiophene (II) by microwave irradiation method, with various substituted aromatic aldehydes in ethyl alcohol and glacial acetic acid as a catalyst to give various 2-[(substituted benzylidene) imino]-3-N-(propylcarboxamido)-4,5,6,7-tetra hydro benzo (b) thiophenes (Schiff bases) (II) to (IIa -k) **(**SCHEME-II).

The formation and purity of all the compounds were studied by melting point and TLC and are characterized by IR spectrum data of all the compounds, ^{1}H NMR, data of compounds (II, IIa and IId) and Mass spectrum data of compound II were analyzed.

All the compounds were screened for antimicrobial (antibacterial, antifungal) activity. Antibacterial activity against two Gram positive organisms *Staphylococcus aureus* and *Bacillus subtilis* and two Gram negative organisms *Escherichia coli* and *Klebsiella pneumoniae* using Ampicillin as the standard. The compounds were also screened for their antifungal activity against two strains of fungi *Aspergillus niger* and *Candida albicans* using Miconazole nitrate as

the standard. All the compounds as well as the standard were used at the concentration of 50 µg/0.1ml.

Evaluation of antibacterial and antifungal activities of all the titled compounds was performed by agar diffusion method. Many of the synthesized compounds showed mild to moderate antimicrobial activity and some were equipotent to the standards employed.

In conclusion, from the antibacterial activity results, it was observed that both electron donating and electron withdrawing groups on the aldehydic phenyl ring of the compounds influenced the activity. But aldehydic phenyl ring containing electron withdrawing group has shown promising result. Among all the compounds tested, IIf with 2-chloro substitution and IId with 2-nitro substitution at R was found to be most active against both Gram-positive and Gram-negative bacteria. The remaining compounds of both the series exhibited mild to moderate activities when compared to the standards. The antifungal screening results also suggest that the test compounds showed mild to moderate activity against *A.niger* only but no significant activity against *C.albicans* compared to the standard employed.

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