Synthesis and Antimicrobial activities of Novel Aryloxymethylideneamino derivatives of 4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile

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Abstract

Series of 2-(Aryloxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile derivatives were synthesized from 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile. The new compounds showed good antimicrobial activities against S. Pyrgens, E.aerogens and C. Tropicallis , A. Flavus microbes.

Keywords: Dimethylformamidine, 2-(Aryloxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carbonitrile.

INTRODUCTION

Many natural product has benzothiophene molecules are important because they shows anticancer [1,2], anti-inflammatory[3] antimicrobial [4,12], antianxiety [5] activities. Substituted benzothiophene derivatives are potent and selective inhibitor of human leukocyte elastase [6], kinesin spindle protein (KSP) [7], tubulin [8] and tyrosine kinase of the fibroblast growth factor receptors (FGRF) [9] as well as adenosine A1 receptor allosteric enhancers [10]. K. Madhavi prepared 4, 5, 6, 7-Tetrahydrobenzo[b] Thiophene-3-Carboxylates and evaluated antioxidant and antibacterial activities [11, 13] and 2-amino-3-N-(propylcarboxamido)- 4, 5, 6,7-tetra hydrobenzo (b) thiophene showed mycolytic activities [12] which resulted in marketing as antifungal agents sertaconazol. Cytotoxic and anti-leshmanial activity [13] N-ethoxymethino derivatives, *N*-phenylaminomethino derivatives, hydrazine derivatives, pyrazole derivatives, and *N*-methinonitrilo derivatives of 4,5,6,7-tetrahydrobenzo[*b*]thiophene were synthesized. Thieno (3,2-*d*)- (1,2,3)-triazine derivatives and *N*-(3-cyano-5,6-dihydro-4*H*-cyclopenta (*b*) thiophene derivatives were prepared and exhibited anticancer activity [15]. [23-26] However aryloxy derivatives of tetrahydrobenzo[b]thiophene-3-carbonitrile arenot reported . Hence we thought synthesis of a series of 2-(Aryloxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile **7.** There using N'-(3-cyano-4,5,6,7-tetrahydrobenzo [b]thiophen-2-yl) -N,N-dimethylformamidine **5** was reacted with substituted phenols. Yieled new compounds 2-(Aryloxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile derivatives **(7a-e)** .The synthesisized compound were evaluated for biological activity and showed moderate to good antimicrobial activity compared with standard drugs Gentamicin and Fluconazole.

EXPERIMENTAL SECTION

General procedures

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (3)

The mixture of cyclohexanone (0.01 mol) and malononitrile (0.01 mol, 0.66 g) was stirred in ethanol at 50 °C for 30 min. After 30 min elemental sulfur (0.02 mol) was added portion wise followed by dropwise addition of morpholine (0.01 ml). The reaction mixture was stirred further 7-8 h in an oil bath at 70-80 ^{o}c (TLC check, toluene: acetone, 9:1, v/v). After cooling, the reaction mixture was poured in ice-cold water (15 ml), stirred at room temperature for further 30 minutes. The aqueous layer was extracted with chloroform (3 x 100 ml) and organic layer were dried over sodium sulphate. The organic layer was concentrated under vacuum afforded solid was further recrystallized from chloroform: n-hexane (80:20) afforded compound **3** in 70-75% yields. This compound was characterized by IR, NMR data and compared with the literature M.P. [15, 16].

(E)-N'-(3-cyano-4,5,6,7-tetrahydrobenzo [b]thiophen-2-yl)-N,N-dimethyl-formamidine (5)

Compound **3** (2.56 g, 0.01mol) and DMF-DMA (1.32 mL, 0.01mol) in dry p-xylene (15 mL) was refluxed for 5 hr (TLC checked, chloroform: methanol 9:1v/v).

The excess of solvent was removed under reduced pressure. The solid obtained was stirred in 20 mL hexane for 4 hr and was filtered washed with cold methanol. It was recrystallized from toluene as pale yellowish brown solid.

Light brown solid, Yield 80%, 4g, m.p. 130 °C; IR (Platinum ATR) 2288 (CN), 2935 (CH), 1613 (C=N), 1620 (C=C) 2825(CH_3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.82 (t, J = 4 Hz, 2H, CH_2), 2.56 (t, J = 4 Hz, 2H, CH_2), 7.66 (s , 1H , NHCH), 3.07 (s , 3H ,CH_3), $C_{12}H_{15}N_3S$ (233.10) :Calcd C, 61.77; H, 6.48; N, 18.01; S, 13.74 Found C, 61.81; H, 6.45; N, 18.04; S, 13.70

(E)-2-(Aryloxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile derivatives (7a-e)

Compound **5** (0.1 gm 0.43 mmol) and Phenol **6a-e** (0.43 mmol) in ethanol and 2-3 drops glacial acetic acid refluxed for 8 hr (TLC Checked, Hexane: Ethyl acetate ,8:2 v/v). The solid obtained was filtered and recrystallized from ethanol afforded compound **7a-e**.

(E)-2-(p-chlorophenoxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (7a).

White solid, Yield : 80%, 0.91mg, m.p. 132°C; IR (Platinum ATR) cm^{-1}: 2931 (NH), 1627 (C=N), 2198 (CN), 1573 (C=C) ,864 (Cl); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-7.70 (t, J=4 Hz 2H, CH), 2.56-1.82 (t, J = 4 Hz, 4H, CH_2), 7.50 (s, 1H, CH). $C_{16}H_{13}ClN_2OS$ (316.81): Calcd C, 60.66; H, 4.14; N, 8.84 Found C, 60.56; H, 4.20; N, 8.88.

(E)-2-(p-bromophenoxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (7b).

White solid, Yield 68%, 0.82 mg, m.p. 140 °C; IR (Platinum ATR) cm^{-1}: 2931 (NH), 1620 (C=N), 2198 (CN), 1599(C=C) , 702 (Br) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40-7.62 (t, J=4 Hz 2H, CH), 2.56-1.82 (t, J = 4 Hz, 4H, CH_2), 7.50 (s, 1H, CH). $C_{16}H_{13}BrN_2OS$ (361.26): Calcd C, 53.20; H, 3.63; N, 7.75 Found C, 53.23; H, 3.65; N, 7.70.

(E)-2-(m-hydroxyphenoxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (7c)

White solid, 62%, 0.84 mg, m.p. 99°C; IR (Platinum ATR) 2198 (CN), 2935 (CH), 1766 (C=N), 1620 (C=C); 3200 (OH) and ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.85-2.59 (t, J = 4 Hz, 2H, CH_2), 7.67(s,1H,CH),8.01 (s, 1H, OH). 6.38(d,J=4Hz 1H, CH),7.02 (t,1H,CH),7.34(s,1H,CH) $C_{16}H_{14}N_2O_2S$ (298.36): Calcd. C, 64.41; H, 4.73; N, 9.39. Found C, 64.36; H, 4.78; N, 9.44.

(E)-2-(p-methoxyphenoxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (7d).

White solid, Yield: 61%, 0.782 mg, m.p. 102°C; IR (Platinum ATR) cm^{-1}: 1573(C=N), 2198(CN), 1620 (C=C) cm^{-1}1766 (C=N); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.60-6.80 (t, J=1.5 Hz 2H, CH), 3.41 (s, 3H, CH_3), 2.56-1.82 (t, J = 4 Hz, 4H, CH_2), 7.50 (s, 1H, CH). $C_{17}H_{16}N_2O_2S$ (312.39): Calcd; C, 65.36; H, 5.16; N, 8.97. Found C, 65.26; H, 5.28; N, 8.95.

(E)-2-(2-napthoxymethylideneamino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (7e).

White solid, Yield 70%, 0.98 mg, m.p. 100 °C; IR (Platinum ATR) cm^{-1}: 1620 (C=N), 2175 (CN), 1470 (CH) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.98 (d, 2H, ArCH), 7.62-7.63 (d, J=4 Hz 1H,Ar CH), 7.53(m, 1H,ArCH), 7.30 (m, 1H,ArCH) ,7.21 (m, 1H, ArCH)2.56-1.82 (t, J = 4 Hz, 4H, CH_2), 7.50 (d, 1H, CH). $C_{20}H_{16}N_2OS$ (332.42): Calcd C, 72.26; H, 4.85; N, 8.43; Found C, 72.30; H, 4.71; N, 8.53;

MATERIALS AND METHODS

The physical constant ie melting point of all new compounds was reported with the help of Gallencamp melting point equipment (Model no MFB-595) using open capillary tubes. All the recorded melting points are uncorrected. Bruker FTIR-TENSOR-II was used to record IR spectra of the compounds. ^{1}HNMR spectra of the compounds were recorded on Bruker Avance II NMR instrument at 500 mhz frequency. The $cdcl_3$ or DMSO was used to record the NMR using TMS as internal standard. Chemical shifts are given in δ ppm and splitting of NMR samples are given as singlet(s), broad singlet (bs), doublet (d), triplet (t), multiplets (m).The reactions were monitored on thin layer chromatography (TLC 0.2 mm silica gel 60 F_{254} Merck plates) plates using UV light 254 and 366 nm. All commercial grade chemicals were purchased from S.D. Fine chemicals, Sigma Aldrich, Merck, Loba chemie and used without further purification while solvents were purified by standard literature procedures.

Antimicrobial assay:

The antimicrobial assay of compound **7a-e** was carried out using agar well plate method. The antibacterial and antifungal assays were performed in Muller-Hinton agar and Crazek Dox agar. The standard strains used for the antimicrobial assay was procured from Microbial Culture Collection Centre, Pune, India. Antimicrobial evaluation was performed using the bacteria reseeded in Muller-Hinton broth for 24 hr at 37 ^{o}C and fungi reseeded in Crazek Dox agar for 48 hr at 25 ^{o}C. The antibacterial activity of tested samples were studied in triplicate against Gram positive bacteria *S.pyrgens* (ATCC 29737) and Gram negative bacteria *E.aerogens* (ATCC 25922). The same samples were tested for antifungal activity in triplicate against *C. tropicallis* (MTCC 277) and *A. flavus* (MCIM 545).

Table 2: Antimicrobial screening of compounds (7a-h): *Inhibition Zone Diameter (mm)*

Compound	Ar	*E.aerogens*	*S. pyrgens*	*A.flavus*	*C.tropicallis*
7a	4-Chlorophenol	18 ± 0.8	17 ± 0.4	19 ± 0.3	18 ± 0.5
7b	4-Bromophenol	16 ± 0.8	16 ± 0.8	18 ± 0.5	18 ± 0.9
7c	Resorcinol	14 ± 0.9	15 ± 1.1	16 ± 1.2	15 ± 0.8
7d	3-methoxyphenol	17 ± 0.8	18 ± 0.3	17 ± 0.7	18 ± 0.4
7e	2 -Naphthol	12 ± 0.6	13 ± 0.5	14 ± 1.1	15 ± 1.3
	DMSO	11 ± 0.7	12 ± 0.9	12 ± 0.6	13 ± 0.3
	Gentamicin	22 ± 0.4	23 ± 0.7	-	-
	Fluconazole	-	-	23 ± 0.8	24± 0.5

Gentamicin (10 µg/ mL) and Fluconazole (20 µg/ mL) Inhibition Zone = 9-14 mm: slight activity, 15-19 mm: moderate activity, 20 -24 mm : high activity, >25 mm: excellent activity

The compounds were dissolved in DMSO at desired concentrations of 40, 20, 10 µg/ mL. DMSO was loaded as negative control. Gentamicin (10 µg/ mL) and Fluconazole (20 µg/ mL) were used as standards for evaluating the antibacterial and antifungal activity. The zone of inhibition (mm) was determined from the diameter of the zone of inhibition using caliper as per National Committee for Chemical Laboratory Standards (NCCLS, M7-A5, January 2000). The antimicrobial activity of 4-Chlorophenol was more than 4-Bromopheno and 3-methoxyphenol.

The compound **7a** exhibited excellent antibacterial activities against Gram positive and Gram negative bacteria viz. *S.pyrgens* , *E.aerogenns* with MIC *10 µg/ mL* when compared with standard antibacterial drug Gentamicin (*10 µg/ mL*). Similarly, compound **7a** also showed excellent antifungal activities against *A.flavus* (MCIM 545) and *C.tropicallis* with MIC *10 µg/ mL* when compared with standard antifungal drug Fluconazole (*20 µg/ mL*). The compound **7c** and **7d** showed moderate antibacterial activity against *E.aerogens* with MIC *20 µg/ mL* when compared with standard antibacterial drug Gentamicin (*10 µg/ mL*). The compounds **7c, 7d** and **7e** showed excellent antifungal activities against *A.flavus.* Similarly, compounds **7b**, **7c**, **7d** showed equivalent antifungal activities against *C.tropicallis* with MIC *20 µg/ mL* when compared with standard antifungal drug Fluconazole (*20 µg/ mL*). The results of antimicrobial activity are shown in Table 2 and 3.

Table 3: Antimicrobial screening of compounds (7a-h): MIC in µg / mL values

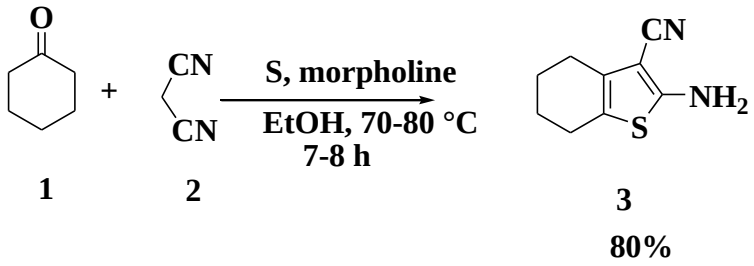
Compound	Ar	*E.aerogens*	*S. pyrgens*	*A.flavus*	*C.tropicallis*
7a	4-Chlorophenol	80	40	80	40
7b	4-Bromophenol	40	40	20	20
7c	Resorcinol	20	20	20	20
7d	3-methoxyphenol	20	20	20	20
7e	2 -Naphthol	40	20	20	40

Gentamicin	10	10	-	-
Fluconazole	-	-	20	20

Gentamicin (10 µg/ mL) and Fluconazole (20 µg/ mL) Inhibition Zone = 9-14 mm: slight activity, 15-19 mm: moderate activity, 20 -24 mm : high activity, >25 mm: excellent activity NT: Not Tested

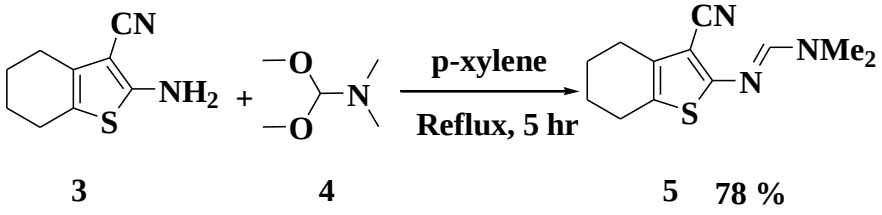
RESULTS AND DISCUSSIONS

Gewald thiophene method is used for synthesis of 2-amino-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carbonitrile **3** by treating cyclohexanone , malononitrile and sulfur in presence of morpholine as catalyst [27-29]. This compound was characterized by IR, ^1H NMR data and compared with the literature M.P. (Scheme 1)

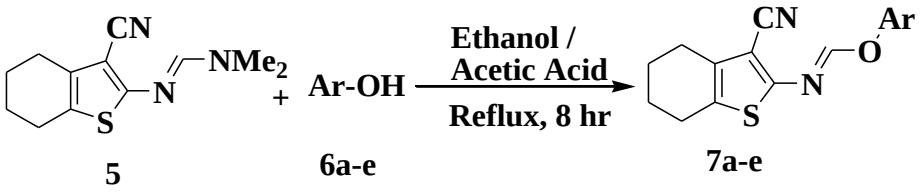


Scheme 1

The key intermediate 4,5,6,7-tetrahydrobenzo [b]thiophen-2-yl)-*N*,*N*-dimethylformamidine **5** was obtained our earlier procedure, by the reaction of 2-amino-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carbonitrile **3** with DMF/DMA (Scheme 2). Confermation of compound **3** by IR, ^1H NMR data. For e.g. IR spectrum showed stretching frequencies at 1613, 2288 cm^{-1} assigned for C=N and CN. The ^{1}H NMR spectrum of **5** showed triplets at δ 1.82 and 2.52 for 2×CH_2-CH_2 , singlet at 7.66,for =CH singlet at δ 3.07 assigned CH_3 group. In our previous work compound 5 was treated with various amine yielded various biologically active formamidine derivatives **[30]** from this idea we thought to replace aromatic amine with phenols for synthesized new biological active heterocyclic compound. The compound **5** was reacted with substituted phenols in presence of catalytic amount of glacial acetic acid in ethanol afforded *N*'-(3-cyano-4,5,6,7-tetrahydrobenzo[b] thiophen-2-yl)-2-((E) formamido) acetic acid **7a-e.** with 60-70% yields. (Scheme 3)



Scheme 2



Scheme 3

Compd.	Ar	% Yield
7a	4-Chlorophenol	80
7b	4-Bromophenol	68
7c	Resorcinol	62
7d	3-methoxyphenol	61
7e	2 -Naphthol	70

The compound **7a** was characterized as the IR spectrum of **7a** showed stretching frequencies at 1325 (COAr), 1627 (C=N), 2198 (CN), 1573 (C=C) ,864 (Cl); The ^{1}H NMR spectra of this compound showed triplet at δ 7.28-7.70 for aromatic protons , 2.56 triplet for CH_2-CH_2,and 7.50 for (CH).

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