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Synthesis, characterization and antimicrobial activity of N'-benzylidene-5-bromothiophene-2-carbohydrazide derivatives

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ABSTRACT

Some new N'-benzylidene-5-bromothiophene-2-carbohydrazide derivatives possessing thiophene nucleus were synthesized and characterized by IR, NMR and mass spectral analysis. All synthesized compounds were screened for antimicrobial activity using cup plate method. All the compounds showed moderate to good antimicrobial activity and anti fungal activity.

***Keywords*:** 2-carbohydrazide; Schiff base; antimicrobial activity

1. INTRODUCTION

Schiff bases are the important class of compounds owing to their wide range of biological activities and industrial applications. These compounds are now used to formulate anticancer[1], anti HIV[2], antitubercular[3], antiviral[4], antimalarial[5] drugs. Some complexes of Schiff base sows antibacterial, and antiviral activity[6-7]. Schiff base of some transition metal also sows Analgesic and anti-inflammatory activities[8]. Many potent antibacterial and antifungal agents have also been prepared. A large number of antibacterial agents are available to manage pathogenic microorganisms in nature. These treatments however could not completely destroy such organisms, probably due to the widespread irrational, unscientific and apathetic use of such agents. The survived microorganisms have matched the ingenuity in developing their own defenses. As a result such drugs gradually lose their effectiveness in action. Repetition and overdose of such drugs often cause severe environmental pollution. In order to get rid of this situation, it has become a common practice to find out safer, more effective and inexpensive new chemical compounds as antibacterial agents.

2. EXPERIMENTAL

All chemicals and solvents were purchased from Spectrochem Pvt Ltd., Mumbai of AR grade and were used without further purification. Melting points were taken in open capillary

method and are uncorrected. IR spectra were recorded on FTIR-8400 spectrophotometer (Shimadzu, Kyoto, Japan), using DRS probe KBr pallet. ^{1}H-NMR spectra of the synthesized compounds were recorded on a Bruker-Avance-II (400 MHz) DMSO-*d*6 solvent.

Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu, Kyoto, Japan). Physical constants of the synthesized compounds **5a-5j** are shown in Table 1.

2. 1. Synthesis of Methyl 5-bromothiophene carboxylate (int-1)

To a cooled solution of 5-bromothiophene2-carboxylic acid (10 gm, 0.048 mmol) and methanol (100 ml, 5v), conc H_2SO_4 (10 ml, 1v) was added drop wise at 15 °C. After addition, reflux reaction mixture at 65 °C for 6 hr. After completion of reaction, the mixture was poured in ice cold water. The resulting reaction mixture was further stirred at 5 °C for 5hr. The obtained solid was filtered, wash with water and dried it in oven.

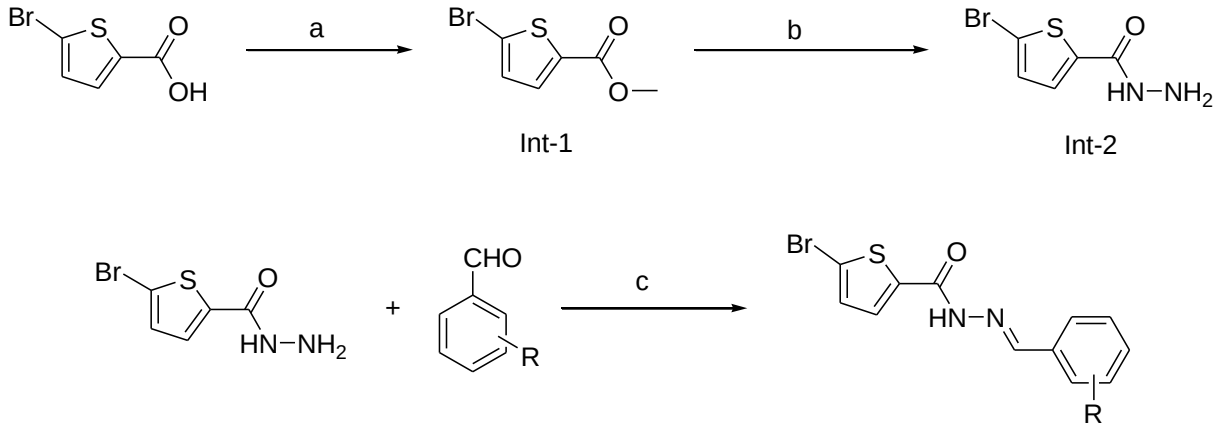
2. 2. Synthesis of 5-bromothiophene 2-carbohydrazide (int-2)

To a cooled solution of methyl 5-bromothiophene-2-carboxylate (10 gm, 0.045 mmol) and methanol (100 ml, 5v), hydrazine hydrate (10 ml, 1v)was added drop wise at 15 °C. After addition, reaction mixture was reflux at 65 °C for 6 hr. After completion of reaction, the mixture was poured in ice cold water. The resulting reaction mixture was further stirred at 5 °C for 5hr. The obtained solid was filtered, wash with water and dried it in oven.

2. 3. General synthesis of schiff base of 5-bromothiophene-2-carbohydrazide (HM-1a-j)

To a solution of 5-bromothiophene-2-carbohydrazide (2 gm, 0.0090 mmol) and methnol (10 ml, 5v), benzaldehyde was added at rt. After addition, reaction mixture was heated at reflux temperature for 30 min. after completion of reaction mixture was cooled. The obtained solid was filtered, wash with methanol and dried it in oven.

3. REACTION SCHEME



Scheme 1. (a) Methanol, sulfuric acid, 65 °C (b) Hydrazine Hydrate, 60 °C (c) Methanol, Reflux.

Entry	R	Time	Yield %	Mp ^{o}C
HM-1a	4-OCH_3	30 min	80	140-142
HM-1b	4-OH	50 min	82	135-138
HM-1c	3,4 di-OCH_3	40 min	88	156-158
HM-1d	2,5 di-OCH_3	50 min	90	168-170
HM-1e	3-OCH_3	1 hr	80	121-124
HM-1f	4-F	1.2 hr	68	178-180
HM-1g	4-Cl	1 hr	72	192-194
HM-1h	4-Br	1 hr	85	176-178
HM-1i	3-Cl	1.3 hr	82	210-212
HM-1j	2-Cl	1.5 hr	87	156-158
HM-1k	2-NO_2	1.3 hr	78	217-219
HM-1l	4-CN	1.4 hr	81	189-191
HM-1m	3-Br	1.2 hr	80	188-190
HM-1n	-H	1 hr	90	178-180
HM-1o	4-Me	1 hr	92	191-193
HM-1p	3-OH	1.2 hr	97	210-213
HM-1q	4-NO_2	1.3 hr	95	222-224
HM-1r	4-OH, 3-OMe	1 hr	75	186-188
HM-1s	2-F	1.5 hr	88	182-184
HM-1t	-C_3H_7	1.3 hr	72	162-164
HM-1u	-C_2H_5	1.2 hr	89	172-1744
HM-1v	4-(N,N-dimethyl) amino	1.7 hr	90	190-192

4. SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS

(z)-5-bromo-N'-(4-methoxybenzylidene) thiophene-2-carbohydrazide (HM-1a):
White solid; Rf 0.45 (4:6 hexane-EtOAc); mp 140-142 °C; IR (KBr): 3032, 2941, 2833, 1654, 1606, 1518, 1222, 1354, 1305, 1257, 1166, 1035, 949, 802, 713, 603, 538, 422 cm^{-1} ;

^{1}H NMR: δ 3.812 (1H, s, -OCH_3); 6.924-6.954 (2H, d, Ar-H); 7.088-7.099 (1H, d, Th-H) 7.657-7.679 (2H, d, Ar-H); 7.827 (1H, s, -N=CH); 7.888-7.898 (1H, d, Th-H); 10.115 (1H, s, -NH); MS (m/z): 339 (M^+); Anal. Calcd for: $C_{13}H_{11}BrN_2O_2S$: C, 46.03; H,3 .27; N, 8.26

(z)-5-bromo-N'-(3,4-methoxybenzylidene)thiophene-2-carbohydrazide (HM-1c):
White solid; Rf 0.42 (4:6 hexane-EtOAc); mp 156-158 °C; IR (KBr): 2949, 1645, 1595, 1496, 1413, 1354, 1222, 1103, 1041, 963, 813, 729 cm^{-1} ;^{1}H NMR: δ 3.82 (3H, s, -OCH_3), 3.88 (3H, s, -OCH_3), 7.029-7.081 (1H, d, Th-H), 7.21-7.24 (1H, d, Ar-H), 7.32-7.36 (1H, d, Ar-H), 7.51 (1H, s, Ar-H), 7.81 (1H, s, -N=CH), 8.029 (1H, s, Th-H), 11.957 (1H, s, -NH); MS (m/z): 367(M^+); Anal. Calcd for $C_{14}H_{13}BrN_2O_3S$; C, 45.54; H, 3.55; N, 7.59

(z)-5-bromo-N'-(2,5-methoxybenzylidene)thiophene-2-carbohydrazide (HM-1d):
White solid; Rf 0.44 (4:6 hexane-EtOAc); mp 168-170 °C; IR (KBr): 3161, 3045, 2947, 2833, 1654, 1585, 1516, 1465, 1386, 1354, 1269, 1180, 1043, 972, 941, 852, 785, 719, 682,580 cm^{-1}; ^{1}H NMR: δ 3.81 (6H, s, -OCH_3), 7.032 (1H, d, Th-H), 7.047 (1H, S, Ar-H), 7.34-7.36 (1H, d, Ar-H), 7.52-7.53 (1H, d, Ar-H), 7.81-7.82 (1H, d, Th-H), 8.41 (1H, s,-N=CH), 11.95 (1H, s, -NH); MS (M/Z); 367(M^+); Anal. Calcd for $C_{14}H_{13}BrN_2O_3S$; C, 45.54; H, 3.59; N, 7.29

(z)-5-bromo-N'-(3-methoxybenzylidene)thiophene-2-carbohydrazide (HM-1e):
White solid; Rf 0.45 (4:6 hexane-EtOAc); mp 121-124 °C; IR (KBr): 3161, 3043, 2947, 2835, 1654, 1585, 1516, 1465, 1386, 1354, 1269, 1180, 1043, 972, 941, 852, 785, 719, 682 cm^{-1}; ^{1}H NMR: δ 3.81 (3H, s, -OCH_3), 7.032 (1H, d, Th-H),7.81-7.82 (1H, d, Th-H), 8.41 (1H, s, -N=CH), 11.95 (1H, s, -NH); 7.05 (1H, d, Ar-H), 7.32 (1H, d, Ar-H), 7.39 (1H, d, Ar-H), 7.59 (1H, s, Ar-H); MS (M/Z); 339 (M^+); Anal. Calcd for $C_{13}H_{11}BrN_2O_2S$; C, 47.05; H, 3.27; N, 8.46.

(z)-5-bromo-N'-(4-fluorobenzylidene) thiophene-2-carbohydrazide (HM-1f):
White solid; Rf 0.45 (4:6 hexane-EtOAc); mp 178-180 °C; IR (KBr): 3160, 3040, 2945, 2830, 1650, 1580, 1560, 1459, 1380, 1350, 1260, 1185, 1040, 970, 940, 845, 680, 540 cm^{-1}; ^{1}H NMR: δ 7.032 (1H, d, Th-H), 7.81-7.82 (1H, d, Th-H), 8.410 (1H, s, -N=CH), 11.950 (1H, s, -NH); 7.340 (2H, d, Ar-H), 7.810 (2H, d, Ar-H); MS (M/Z); 325 (M^+); Anal. Calcd for $C_{12}H_8BrFN_2OS$; C, 43.06; H, 2.55; N, 8.50

(z)-5-bromo-N'-(4-chlorobenzylidene)thiophene-2-carbohydrazide (HM-1g):
White solid; Rf 0.48 (4:6 hexane-EtOAc); mp 192-194 °C; IR (KBr): 3155, 3045, 2950, 2836, 1658, 1590, 1520, 1470, 1389, 1358, 1270, 1190, 1045, 975, 945, 855, 725, 620, 590 cm^{-1}; ^{1}H NMR: δ 7.035 (1H, d, Th-H), 7.82-7.83 (1H, d, Th-H), 8.416 (1H, s, -N=CH), 11.954 (1H, s, -NH); 7.348 (2H, d, Ar-H), 7.816 (2H, d, Ar-H); MS (M/Z); 341 (M^+); Anal. Calcd for $C_{12}H_8BrClN_2OS$; C, 40.96; H, 2.65; N, 8.58

(z)-5-bromo-N'-(4-bromobenzylidene)thiophene-2-carbohydrazide (HM-1h):
White solid; Rf 0.46 (4:6 hexane-EtOAc); mp 176-178 °C; IR (KBr): 3156, 3050, 2958, 2830, 1652, 1596, 1527, 1474, 1380, 1370, 1280, 1105, 1060, 968, 941, 849,715, 625, 596 cm^{-1}; MS (M/Z); 388 (M^+); Anal. Calcd for $C_{12}H_8Br_2N_2OS$; C, 40.92; H, 2.55; N, 8.48

5. BIOLOGICAL ACTIVITY

Antimicrobial Sensitivity Testing

Well Diffusion/Agar Cup Method

In vitro affectivity of antimicrobial agents can be demonstrated by observing their capacity to inhibit bacterial growth on suitable media. The production of a zone depends on two factors namely bacterial growth and concentration of antimicrobial agent. The hole/well punch method was first used by Bennett. This diffusion method has proved more effective than many other methods. According to Lt. General Raghunath the well technique is 5-6 times more sensitive than using disk method.

Principle

When antimicrobial substance is added in agar cup (made in a medium previously inoculated with test organism) the redial diffusion of an antimicrobial agent through the agar, produces a concentration gradient. The test organism is inhibited at the minimum inhibitory concentration (MIC), giving rise to a clear zone of inhibition.

❖ **Antimicrobial Sensitivity Assay**

Sr. No.	**Code no.**	**MIC (µg/mL)**						
		antibacterial activity				antifungal activity		
		E.coli	*P.aeruginosa*	*S.aureus*	*S.pyogenus*	*C.albicans*	*A.niger*	*A.clavatus*
1	HM-1a	250	500	100	500	500	1000	1000
2	HM-1b	250	500	200	500	200	500	500
3	HM-1c	500	200	500	500	500	1000	1000
4	HM-1d	500	250	250	250	200	500	>1000
5	HM-1e	250	250	500	125	>1000	500	>1000
6	HM-1f	500	125	100	125	>1000	>1000	>1000
7	HM-1g	500	250	500	500	500	500	>1000
8	HM-1h	500	500	500	500	500	>1000	500
9	HM-1i	250	500	500	500	>1000	>1000	>1000
10	HM-1j	125	250	250	500	200	500	>1000
11	HM-1k	250	500	200	125	1000	>1000	>1000
12	HM-1l	62.5	125	100	62.5	100	200	200
13	HM-1m	125	250	200	125	200	500	500
14	HM-1n	500	1000	200	500	1000	500	500

15	HM-1o	62.5	100	500	100	1000	>1000	>1000
16	HM-1p	125	100	125	200	1000	>1000	>1000
Gentamycin		0.05	1	0.25	0.5	-	-	-
Ampicilin		100	100	250	100	-	-	-
Chloramphenicol		50	50	50	50	-	-	-
Ciprofloxacin		25	25	50	50	-	-	-
Norfloxacin		10	10	10	10	-	-	-
Nystatin		-	-	-	-	100	100	100
Greseofulvin		-	-	-	-	500	100	100

6. CONCLUSIONS

We have established facile and convenient method for the synthesis of Schiff base of 5-bromothiophene 2-carbohydrazide under a conventional reagent. All synthesized compounds were obtained in good to moderate yield. All synthesized compounds were characterized by IR, NMR and Mass spectrometry. All the synthesized compounds have been investigated for their antibacterial activities. The investigation of antibacterial and antifungal screening data revealed that, the compounds HM-1l, HM-11p and HM-1o shows very good activity against bacterial stain, HM-1o, HM-1a, HM-1b and HM-1d shows comparatively good activity against fungal stain.

All these compounds were found to possess cytotoxic effect. Therefore, these compounds may be used as new antibacterial drugs after performing further research works with advanced technology.

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