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Antimicrobial and anti-inflammatory effect of some novel tetra substituted thiophenes

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ABSTRACT

A new series of 2-[(substituted benzylidene)imino]-3-(N-methylcarboxamido)-4,5-trimethylene thiophenes were synthesized by the reaction of aliphatic ketone with cyanoacetamide in strong basic medium followed by Schiff base formation. Then newly synthesized compounds were characterized by IR spectroscopy, ^{1}H NMR and mass spectral data. The newly synthesized compounds were screened for antifungal and antibacterial and anti- inflammatory activities. Among them ss6a, ss6f, ss6k and ss6l exhibited good antifungal and antibacterial activities and ss6d & ss6i showed promising anti-inflammatory activity.

Keywords: Thiophenes, Furfurylcyanoacetamide, Gewald Reaction, Schiff base, Antifungal, Antibacterial, Anti-Inflammatory.

INTRODUCTION

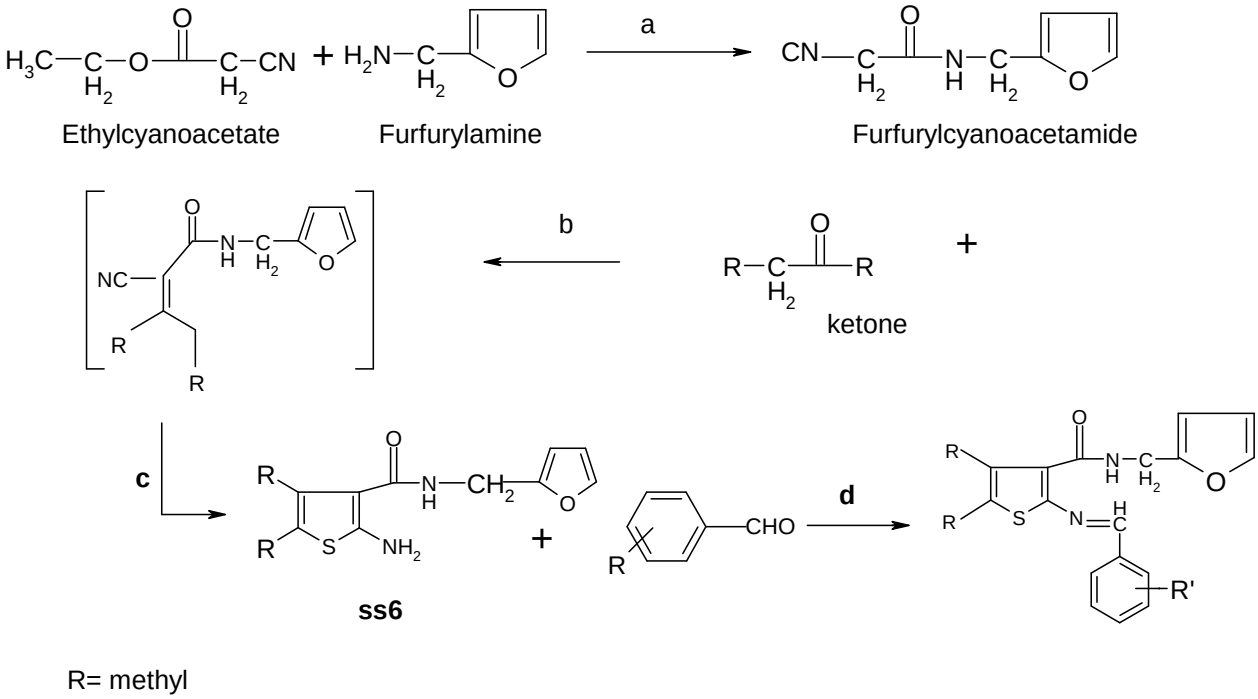
Thiophenes derivatives represent a class of important and well studied heterocycles. The interest in this kind of heterocycles has been spread from early dye chemistry to modern drug design[1-15], polymer science[16-19], biodiagnostics[20-24], macro molecule[25-28], metal ligands[29- 30] and many more[31- 49]. Generally all synthetic approaches to such kind of compounds either involve the functionalization at the position α and β to the sulphur atom of preconstructed thiophenes moiety or formation of thiophenes ring from open chain precursor. Gewald and co-worker developed the synthesis of 2- aminothiophene from the multicomponent condensation of ketones or aldehydes, cyanoacetate and elemental sulphur. Later on, there are several reviews and papers reported on variations and improvements on the originally published Gewald synthesis of polysubstituted thiophenes.

So far various new thiophenes have been synthesized and screened in our laboratories for antimicrobial[45, 47-49] and anti inflammatory activity[46]. The enthusiastic results prompted us to continue the investigation. So, an attempt was made to synthesize some new tetra substituted thiophenes from cyanoacetamide as antimicrobial agent adapting Gewald reaction. Hence the synthesis of "2-subtituted-3-(N-furfuryl amido)-4, 5-dimethyl thiophene is achieved. The different derivatives of the parent compound were achieved by using different aryl aldehydes to obtain a series of Schiff Bases, 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 activity by agar diffusion method and in vitro anti-inflammatory activity.

MATERIALS AND METHODS

The melting point of synthesized compounds was determined in open capillary tubes using melting point apparatus, expressed in ^{0} C and is 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 structures of the compound were confirmed by I.R., NMR and Mass spectra.



a=microwave irradiation, 750 watt, 180 sec; b= CH_3COOH/ CH_3COONH_4, C_6H_6, 10 hrs.; c= S, ethanol, DEA, 45- 50 ^{0}C; d= CH_3COOH, C-$_2H_5OH$, various aromatic aldehydes.

EXPERIMENTAL

Synthesis of furfurylcyanoacetamide (ss5)

A mixture of furfuryl amine (46.29 mL; 0.50 mole) and ethylcyano acetate (53.35 mL; 0.50 mole) was taken in a conical flask and heated in microwave oven at 750 watt for 180 sec. 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).

The same product was obtained by conventional heating on an oil bath at 160-170 ºC for 5-6 h. The yield was better with microwave assisted method.

Synthesis of 2-amino-3-(N-furfuryl amido)-4,5-dimethyl thiophene via formation of 2-Cyano-2-(isobutyl-1-ylidene) furfurylamide (ss6)

A mixture of furfurylcyanoacetamide (6.56 g; 0.04 mole), ethylmethylketone (3.58 mL; 0.04 mole), ammonium acetate (2 g) and glacial acetic acid (2 mL) in benzene (80 mL) was refluxed with an arrangement for continuous separation of water involving dean stark apparatus. After 10 h. 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.

To a mixture of 2-cyano-2-(isobutyl-1-ylidene)-furfurylamide in alcohol (30 mL) was added sulphur (1.28 g; 0.04 mole) in portions followed by the addition of, diethyl amine (6.0 mL) drop wise with stirring. The reaction mixture was stirred for 4 hours at 40-45 ºC and chilled over night. The solid obtained was filtered, washed with ethanol and crystallized from ethanol: water mixture (9:1).

2-amino-3-(N-furfuryl amido)-4,5-dimethyl thiophene ***(ss6)*** IR (KBr): 3283 cm $^{-1}$ (NH); 1684 cm $^{-1}$ (C=O); 2953cm^{-1} (Ali-CH); 1559 cm^{-1} (Ar-C=C), 1223cm^{-1}(thiophene); ^{1}H NMR ($CDCl_3$): δ 9.05 (1H, s, CO-NH-CH_2), 7.72 (1H, d, Ar-CH of furan), 6.38 (1H, d, Ar-CH of furan), 6.32 (1H, t, Ar-CH of furan), 4.2 (2H, s, NH_2), 4.65 (2H, d, NH-CH_2-), 2.41 (3H, s, -CH_3), 2.36 (3H,s, -CH_3); m/z [M+1] 250.

General method for the synthesis of 2-[(substituted benzylidene) imino]-3-(N-furfuryl amido)-4,5-dimethyl thiophenes (Schiff bases) (ss6a to ss6l)

A mixture of the starting composund (**ss6**) (0.005 mole) and the required aryl aldehydes (Substituted benzaldehydes, 0.005 mole) in ethanol (20 mL) 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.

1. *2-[(2'-chlorobenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6a)*** IR (KBr): 3460 (-NH-); 2925 (Ali. CH); 3115(Aro. CH); 1650 (C=O); 1584 (C=N); 1052 (C-O); 1071(Ar-Cl); 815(C-N); 759(C-S); 1366(Ar-C=C); ^{1}H NMR ($CDCl_3$): δ 9.05 (1H, s, CO-NH-CH_2), 7.78 (1H, s, N=CH), 7.37-7.42 (4H, m, Ar-CH of benzene ring), 7.72 (1H, d, Ar-CH of furan), 6.39 (1H, d, Ar-CH of furan), 6.32 (1H, t, Ar-CH of furan), 4.65 (2H, d, NH-CH_2-), 3.36 (3H, s, -CH_3), 2.41 (3H, s, -CH_3).

2. *2-[(4'-dimethylaminobenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophenes* ***(ss6b)*** IR (KBr): 3400 (-NH-); 3115 (Ar. CH); 2935 (Ali.CH); 1653 (C=O); 1560 (C=N); 1177 (C-O); 834 (C-N); 1350 (C-N of N-CH_3); 764 (C-S); 1266 (Ar-C=C); ^{1}H NMR ($CDCl_3$): δ 9.03 (1H, s, CO-NH-CH_2), 7.79 (1H, s, N=CH), 7.37-7.5 (4H, m, Ar-CH of benzene ring), 7.7 (1H, d, Ar-CH of furan), 6.48 (1H, d, Ar-CH of furan), 6.38 (1H, t, Ar-CH of furan), 4.55 (2H, d, NH-CH_2-), 3.7 (6H, s, NH(CH_3)$_2$)2.5 (3H, s, -CH_3), 2.4 (3H,s, -CH_3)

3. *2-[(3',4'-dimethoxybenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6c)*** IR (KBr): 3400 (-NH-); 3115 (Ar. CH); 2935 (Ali.CH); 1653 (C=O); 1560 (C=N); 1177 (C-O); 834 (C-N); 1350 (C-N of N-CH_3); 764 (C-S); 1266 (Ar-C=C); ^{1}H NMR ($CDCl_3$): δ 9.1 (1H, s, CO-NH-CH_2), 7.8 (1H, s, N=CH), 7.3-7.4 (4H, m, Ar-CH of benzene ring), 7.5 (1H, d, Ar-CH of furan), 6.4 (1H, d, Ar-CH of furan), 6.32 (1H, t, Ar-CH of furan),3.84 (6H, s, 2X OCH_3) 4.7 (2H, d, NH-CH_2-), 2.6 (3H, s, -CH_3), 2.5 (3H,s, -CH_3)

4. *2-[(4'-hydroxybenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6d)*** IR (KBr): 3659 (O-H); 3383 (-NH-); 2935 (Ali.CH); 3187 (Ar. CH); 1641 (C=O); 1588 C=N); 1295 (Ar-C=C); 1127 (C-O); 808 (C-N); 779 (C-S); ^{1}H NMR ($CDCl_3$): δ 9.1(1H, s, CO-NH-CH_2), 7.6 (1H, s, N=CH), 7.5-7.55 (4H, m, Ar-CH of benzene ring), 7.8 (1H, d, Ar-CH of furan), 6.4 (1H, d, Ar-CH of furan), 6.4 (1H, t, Ar-CH of furan), 5.11 (1H, s, OH) 4.7 (2H, d, NH-CH_2-), 2.6 (3H, s, -CH_3), 2.2 (3H,s, -CH_3).

5. *2-[(4'-methoxybenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6e)*** IR (KBr): 3244 (-NH-); 3116 (Ar-CH); 2946 (Ali-CH); 1633 (C=O); 1592 (C=N); 1021 (C-O); 1076 (Ar C-O); 830 (C-N); 745(C-S); 1313 (Ar C=C); ^{1}H NMR ($CDCl_3$): δ 9.3 (1H, s, CO-NH-CH_2), 8.2 (1H, s, N=CH), 7.4(2H, d, Ar-CH of benzene ring), 6.8 (2H, d, Ar-CH of benzene ring), 7.5 (1H, d, Ar-CH of furan), 6.61 (1H, d, Ar-CH of furan), 6.4 (1H, t, Ar-CH of furan), 4.63 (2H, d, NH-CH_2-), 3.86 (3H, s, OCH_3), 2.40 (3H, s, -CH_3), 2.32 (3H,s, -CH_3); m/z [M+1] 369.

6. *2-[(3'-nitrobenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6f)*** IR (KBr): 3400 (-NH-); 2935 (Ali.CH); 3112 (Ar. CH); 1653 (C=O); 1560 (C=N); 1071 (C-O); 834 (C-N); 789 (C-S); 1266 (Ar-C=C); 1518 (N=O of NO_2); ^{1}H NMR ($CDCl_3$): δ 9.02 (1H, s, CO-NH-CH_2), 7.8 (1H, s, N=CH), 7.42-7.52 (4H, m, Ar-CH of benzene ring), 7.7 (1H, d, Ar-CH of furan), 6.5 (1H, d, Ar-CH of furan), 6.4 (1H, t, Ar-CH of furan), 4.7 (2H, d, NH-CH_2-), 2.5 (3H, s, -CH_3), 2.4 (3H,s, -CH_3).

7. *2-[(3',4',5'-trimethoxybenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6g)*** IR (KBr): 3395 (-NH-); 2878 (Ali-CH); 3058 (Ar-CH); 1656 (C=O); 1532 (C=N); 1015 (C-O); 836 (C-N); 771 (C-S); 1230 (Ar-C=C); 1230 (Ar-C-O of Ar-OCH_3); ^{1}H NMR ($CDCl_3$): δ 9.0 (1H, s, CO-NH-CH_2), 7.71 (1H, s, N=CH), 7.4-7.5 (4H, m, Ar-CH of benzene ring), 7.7 (1H, d, Ar-CH of furan), 6.5 (1H, d, Ar-CH of furan), 6.4 (1H, t, Ar-CH of furan), 3.89 (6H, d, 2X OCH_3), 3.81 (3h, s, OCH_3), 4.6 (2H, d, NH-CH_2-), 2.5 (3H, s, -CH_3), 2.4 (3H,s, -CH_3).

8. *2-[(4'-hydroxy-3'-methoxybenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6h)*** IR (KBr): 3617 (O-H); 3423 (-NH-); 2878 (Ali. CH); 3058 (Ar. CH); 1656 (C=O); 1532 (C=N); 1015 (C-O); 836 (C-N); 771 (C-S); 1230 (Ar-C=C); 1230 (Ar-C-O of Ar-OCH_3); ^{1}H NMR ($CDCl_3$): δ 9.1 (1H, s, CO-NH-CH_2), 7.78 (1H, s, N=CH), 7.37-7.42 (4H, m, Ar-CH of benzene ring), 7.7 (1H, d, Ar-CH of furan), 6.4 (1H, d, Ar-CH of furan), 6.32 (1H, t, Ar-CH of furan), 4.91 (1H, s, OH), 4.65 (2H, d, NH-CH_2-), 3.78 (3H, s, OCH_3), 2.42 (3H, s, -CH_3), 2.3 (3H,s, -CH_3).

9. *2-[(2'-hydroxybenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6i)*** IR (KBr): 3630 (O-H); 3280 (-NH-); 2936Ali-CH); 3125 (Ar-CH); 1640 (C=O); 1578 (C-N); 1300 Ar-C=C); 1158 (C-O); 810 (C-N); 766 (C-S); ^{1}H NMR ($CDCl_3$): δ 9.0 (1H, s, CO-NH-CH_2), 7.8 (1H, s, N=CH), 7.4-7.48 (4H, m, Ar-CH of benzene ring), 7.8 (1H, d, Ar-CH of furan), 6.4 (1H, d, Ar-CH of furan), 6.4 (1H, t, Ar-CH of furan), 5.4 (1H, s, OH), 4.7 (2H, d, NH-CH_2-), 2.5 (3H, s, -CH_3), 2.4 (3H,s, -CH_3).

10. *2-[(4'-methylbenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6j)*** IR (KBr): 3240 (-NH-); 3028 (Ali-CH); 1643 (C=O); 1593 (C= N); 1086 (C-N amine); 815 (C-N); 755(C-S); 1008 (C-O); 2915 (Ar-CH);

1369 (Ar C=C); ^{1}H NMR ($CDCl_3$): δ 9.0 (1H, s, CO-NH-CH_2), 7.8 (1H, s, N=CH), 7.37-7.42 (4H, m, Ar-CH of benzene ring), 7.72 (1H, d, Ar-CH of furan), 6.38 (1H, d, Ar-CH of furan), 6.5 (1H, t, Ar-CH of furan), 4.25 (2H, d, NH-CH_2-), 2.2 (3H, s, -CH_3), 2.6 (3H,s, -CH_3), 2.32 (3h, s, CH_3).

11. *2-[(4'-chlorobenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6k)*** IR (KBr): 3240 (-NH-); 2925 (Ali-CH); 3115 (Ar-CH); 1650 (C=O); 1583 (C=N); 1052 (C-O); 1078(Ar-Cl); 815(C-N); 772 (C-S); 1366(Ar-C=C); ^{1}H NMR ($CDCl_3$): δ 9.1 (1H, s, CO-NH-CH_2), 7.78 (1H, s, N=CH), 7.32-7.42 (4H, m, Ar-CH of benzene ring), 7.7 (1H, d, Ar-CH of furan), 6.3 (1H, d, Ar-CH of furan), 6.4 (1H, t, Ar-CH of furan), 4.6 (2H, d, NH-CH_2-), 2.41 (3H, s, -CH_3), 2.36 (3H,s, -CH_3).

13. 2-*[(2'-nitrobenzylidene)imino]-3-(N-furfurylamido)-4,5-dimethyl thiophene* ***(ss6l)*** IR (KBr): 3415 (-NH-); 2910 (Ali-CH); 3187 (Ar-CH); 1645 (C=O); 1568 (C=N); 1098 (C-O); 825 (C-N); 760 (C-S); 1300Ar-C=C); 1528 (N=O); ^{1}H NMR ($CDCl_3$): δ 9.0 (1H, s, CO-NH-CH_2), 7.78 (1H, s, N=CH), 7.37-7.42 (4H, m, Ar-CH of benzene ring), 7.72 (1H, d, Ar-CH of furan), 6.38 (1H, d, Ar-CH of furan), 6.32 (1H, t, Ar-CH of furan), 4.65 (2H, d, NH-CH_2-), 2.48 (3H, s, -CH_3), 2.34 (3H,s, -CH_3).

BIOLOGICAL SCREENING-

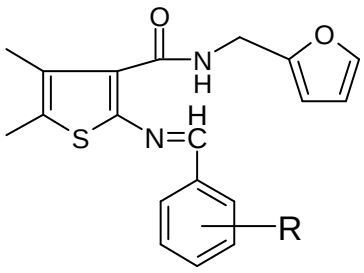
Antibacterial Activity- The test compounds were tested for their in vitro antibacterial activity by cup- plate method[53-55] against strains of microbes, which are Escherichia *coli*, Bacillus *subtilis*, Staphylococcus *aureus* and Klebsiella *pneumoniae.*

Antifungal Acitvity- The test compounds were tested for their in vitro antibacterial activity by cup- plate method[53-55] against Aspergillus *niger* and Candida *albicans*. All the experiments were carried out in triplicate.

Anti- inflammatory Activity- The test compounds were tested for in vitro anti- inflammatory activity by serum albumin denaturation method [46,56] by dissolving them in minimum amount of DMF and diluted with phosphate buffer (0.2 mole, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1mL) containing different concentrations of drug was mixed with 1 mL of 1 mmole albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$C for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0 \pm 1^0$C in a water bath for 10 min. After cooling the turbidity was measured at 660 nm. (Elico Spectrometer). Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken. The percentage of inhibition is calculated from the following formula.

% Inhibition =100 (1-Vt/Vc)

Physical characterization data of thiophenes



Code	R	Molecular Formula	Relative Molecular Mass	Melting Point	Percentage yield
ss6	-	$C_{12}H_{14}N_2O_2S$	250	138	27.19
ss6a	2'-chloro	$C_{19}H_{17}N_2O_2SCl$	372	165	72.41
ss6b	4'-dimethyl amino	$C_{21}H_{23}N_3O_2S$	381	124	28.66
ss6c	3', 4'-dimethoxy	$C_{21}H_{22}N_2O_4S$	398	138	31.00
ss6d	4'-hydroxy	$C_{19}H_{18}N_2O_3S$	354	235	20.25
ss6e	4'-methoxy	$C_{20}H_{20}N_2O_3S$	368	138	47.44
ss6f	3'-nitro	$C_{19}H_{17}N_3O_4S$	383	168	55.00
ss6g	3',4',5'-trimethoxy	$C_{22}H_{24}N_2O_5S$	428	172	61.33
ss6h	3'-methoxy-4'-hydroxy	$C_{20}H_{20}N_2O_4S$	384	180	30.00
ss6i	2'-hydroxy	$C_{19}H_{18}N_2O_3S$	354	218	66.03
ss6j	4'-methyl	$C_{20}H_{20}N_2O_2S$	352	174	64.63
ss6k	4'-chloro	$C_{19}H_{17}N_2O_2SCl$	372	165	25.5
ss6l	2'-nitro	$C_{19}H_{17}N_3O_4S$	383	185	55.00

Anitbacterial Activity					
Compound	**R**	**Zone of inhibition (mm) ***			
		S.aureus	***B.subtilis***	***E.coli***	***K. pneumonia***
ss6a	2'-chloro	08	04	01	NA
ss6b	4'-dimethyl amino	03	04	02	03
ss6c	3', 4'-dimethoxy	05	04	05	06
ss6d	4'-hydroxy	06	05	06	04
ss6e	4'-methoxy	06	06	05	02
ss6f	3'-nitro	08	09	05	01
ss6g	3',4',5'-trimethoxy	04	02	NA	NA
ss6h	3'-methoxy -4'-hydroxy	05	04	02	06
ss6i	2'-hydroxy	05	06	NA	NA
ss6j	4'-methyl	04	02	04	03
ss6k	4'-chloro	11	09	08	11
ss6l	2'-nitro	10	08	11	09
Ampicillin	-	**11**	**12**	**19**	**12**

Antifungal Activity			
Compound	**R**	**Zone of inhibition (mm) ***	
		Aspergillus niger	***Candida albicans***
ss6a	2'-chloro	09	07
ss6b	4'-dimethyl amino	02	NA
ss6c	3', 4'-dimethoxy	08	01
ss6d	4'-hydroxy	05	02
ss6e	4'-methoxy	06	03
ss6f	3'-nitro	10	04
ss6g	3',4',5'-trimethoxy	05	03
ss6h	3'-methoxy-4'-hydroxy	02	NA
ss6i	2'-hydroxy	05	NA
ss6j	4'-methyl	04	NA
ss6k	4'-chloro	09	06
ss6l	2'-nitro	05	02
Miconazole nitrate	-	**20**	**15**

Anti- Inflammatory Activity		
Compound	**R**	**Anti-inflammatory activity (% Bovine serum inhibition)**
ss6a	2'-chloro	34.68
ss6b	4'-dimethyl amino	25.84
ss6c	3', 4'-dimethoxy	22.40
ss6d	4'-hydroxy	56.32
ss6e	4'-methoxy	30.56
ss6f	3'-nitro	22.30
ss6g	3',4',5'-trimethoxy	30.24
ss6h	3'-methoxy-4'-hydroxy	51.30
ss6i	2'-hydroxy	56.43
ss6j	4'-methyl	24.56
ss6k	4'-chloro	42.10
ss6l	2'-nitro	34.23
Ibuprofen	--	**68.55**

RESULTS AND DISCUSSION

The antibacterial activity of Schiff base of thiophenes have been evaluated by using cup-plate method or agar diffusion method against *K. pneumoniae, E.coli, S. Aureus and B. subtilis*. The results clearly revealed the potential antibacterial activity of all thiophenes, when compared with the standard drug Ampicillin. Of all the compounds tested, compound **ss6k** and **ss6l** having the *para* chloro and *ortho* nitro group on benzene ring, showed maximum activity. The rest of the compounds showed moderate to mild activity and few compounds failed to produce activity against *E. coli* and *K. pneumoniae*.

The antifungal activity of the substituted thiophenes was evaluated against *A. niger* and *C. albicans*, employing miconazole nitrate as the standard drug using the cup-plate method. Of all the compounds tested, **ss6a** and **ss6f** having chloro group substitution at *ortho* and nitro group at *meta* position of the phenyl ring showed the maximum activity.

The anti-inflammatory activity of all the new thiophenes synthesized has been evaluated by using inhibition of bovine serum albumin denaturation method as compared with standard drug Ibuprofen. Of all the compounds tested, compound **ss6d** and **ss6i** having hydroxy group substitution at *para* and *ortho* position of the aromatic ring of thiophene showed maximum activity.

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