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CHARACTERIZATION AND BIOLOGICAL SCREENING OF SOME NEW SULFUR CONTAINING COMPOUNDS.

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ABSTRACT

*Two new classes of potential pharmacological thiophenes have been prepared. The obtained thiophenes were screened for in vitro anti- inflammatory. The new Schiffs bases of thiophene were prepared by mixing starting material with aromatic aldehyde in ethanol in microwave oven in presence of catalytic amount of glacial acetic acid. Among tested compounds **ss4h** and **ss4i** having hydroxy group at ortho and para positions of the phenyl ring showed maximum activity and this is followed by compounds **ss4q** and **ss4r** having a morpholinyl and piperazinyl substitution on the carbonyl carbon attached at the position 2 of the thiophene ring have shown significant activity when compared with standard drug.*

***Keywords**: Thiophenes , anti- inflammatory, Schiffs bases.*

INTRODUCTION

It is proved from the literature that, apart from possessing several biological activities[1-9], thiophenes are also useful intermediates[10-12] for the synthesis of several chemical and pharmacological classes[13-16] of therapeutic agents having heterocyclic structures in them. Also a number of thiophenes with novel substituents were earlier prepared in our laboratories[1-5]. These thiophenes were endowed with significant biological activities. Based on these observations, it was considered worthwhile to synthesize some new substituted thiophenes by Gewald reaction in the present study. To synthesize and characterize series of some novel 2-[(substituted benzylidene) imino]-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo(b) thiophenes.

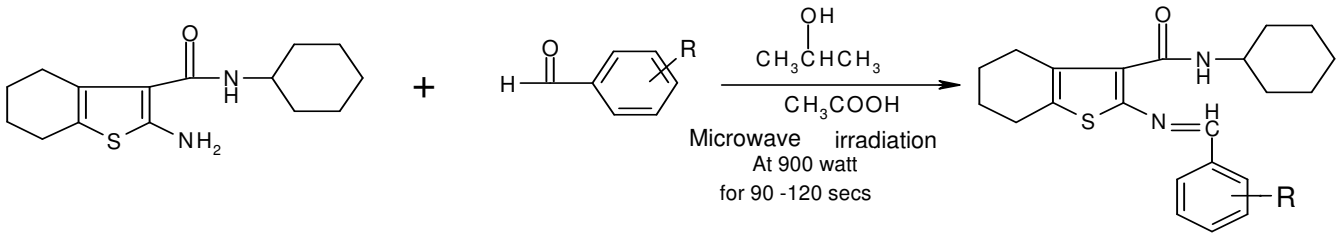
MATERIALS AND METHODS

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 vapors as visualizing agents. The structures of the compound were confirmed by I.R., NMR and Mass spectra.

EXPERIMENTAL:

(A) General method for the syntheses of 2-[(substituted benzylidene) imino]-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophenes (Schiff bases, ss4a to ss4l)

A mixture of the starting compound ss4 (0.005 mole) and the required aryl aldehydes (0.005 mole) in propan-2-ol (30 mL) and catalytic amount of glacial acetic acid (6 drops) was taken in a conical flask and was irradiated at 900W for 90 to 120 secs. The mixture was cooled to room temperature. The solid separated was filtered, washed with propan-2-ol and recrystallized from DMF: Water mixture (5:1).



2-amino-3- N-cyclohexyl -4,5,6,7- tetrahydrobenzo (b) Thiophene-3-caboxamide ss4

aromatic aldehydes(a-l)

ss4(a-l)

Scheme 1

(B) Synthesis of 2- benzamido-3- (N- cyclohexylamido)-4, 5, 6, 7- tetrahydrobenzo (b) thiophene (ss4m)

A mixture of the starting compound (ss4) i.e. 2-amino-3-(N-cyclohexylamido) -4, 5,6,7-tetrahydrobenzo(b)thiophene (0.015 mole) was thoroughly dissolved in 30 mL of dry pyridine. Benzoyl chloride (4 mL) was added drop wise in to the above solution with continuous stirring. Stirring was continued on a magnetic stirrer for about an hour. The reaction mixture was poured on to crushed ice. The solid obtained was filtered, washed with ice-cold water. Recrystallized from DMF/ Water mixture.

(C) Synthesis of 2-(p-acetamido benzamido)-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene: (ss4n)

A mixture of the starting compound (ss4) i.e. 2-amino-3-(N-cyclohexylamido) -4,5,6,7-tetrahydrobenzo(b)thiophene (1.39 g; 0.015 mole) was thoroughly dissolved in 30 mL of dry pyridine. The freshly prepared 4-amino benzoyl chloride was added drop wise in to the above solution with continuous stirring. The stirring was continued on a magnetic stirrer for about an hour. The reaction mixture was poured onto crushed ice. The solid obtained was filtered, washed with ice-cold water and recrystallized with DMF/Water mixture.

(D) Synthesis of 2-(p-chlorobenzamido)-3-(N-cyclohexylamido)- 4, 5, 6, 7-tetrahydrobenzo (b) thiophene (ss4o):

A mixture of starting compound (ss4) i.e. 2-amino-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo(b)thiophene. (1.39 g; 0.015 mole) was thoroughly dissolved in 30 mL of dry pyridine. The freshly prepared 4-chloro benzoyl chloride was added drop wise in to the above solution with continuous stirring. The stirring was continued on a magnetic stirrer for about an hour. The reaction mixture was poured onto crushed ice. The solid obtained was filtered, washed with ice-cold water and recrystallized from DMF/Water mixture.

(E) Synthesis of 2-(chloroacetamido)-3-(N-cyclohexylamido)-4,5,6,7-tetrahydro benzo (b) thiophene (ss4p):

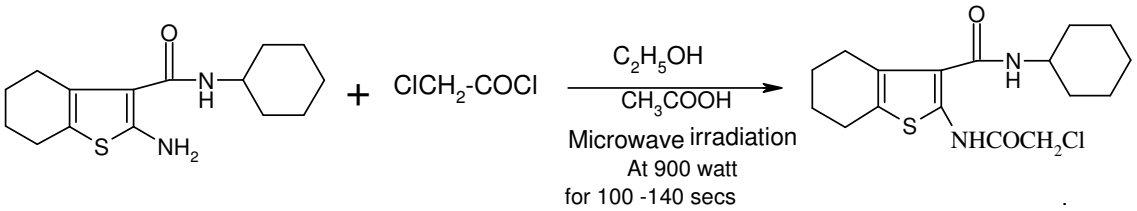
The compound 2-amino-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene (ss4) (2.78 g; 0.01 mole) in glacial acetic acid (30 mL) was added 1 mL of chloroacetyl chloride with constant stirring on room temperature. Then the reaction mixture was carried irradiated at 900 W for 100 to 140 secs. The reaction mixture was cooled to room temperature and poured the mixture in to ice cold water with stirring. The precipitate formed is filtered out, dried and recrystallized from Dioxan/ isopropyl alcohol (2:8) mixture.

(F) Synthesis of 2-(morpholino acetamido)-3-(N-cyclohexylamido)-4,5,6,7-tetra hydrobenzo (b) thiophene – (ss4q)

A mixture of the starting compound (ss4p) i.e. 2-(chloroacetamido)-3-(N-cyclohexyl amido)-4,5,6,7-tetrahydrobenzo(b)thiophene (1.77 g; 0.005 mole) and morpholine (1.30 g; 0.015 mole) and 30 mL of □ioxin was refluxed for 8 h. The excess solvent was removed under vacuum. The oily residue was cooled, triturated with cold water. Filtered and recrystallized from □ioxin and isopropyl alcohol (2:8) mixture.

(G) Synthesis of 2-(piperazino acetamido)-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene: (ss4r)

A mixture of the starting compound (ss4p) i.e. 2-(chloro acetamido)-3-(N-cyclohexylamido)- 4,5,6,7-tetrahydrobenzo(b) thiophene (1.77 g; 0.05 mole), piperazine (1.29 g; 0.015 mole) and 30 mL of Dioxan was refluxed for 6 h. The excess solvent was removed under vacuum. The oily residue was cooled, triturated with cold water. Filtered and recrystallized from dioxan and isopropyl alcohol (2:8) mixture.



Scheme 2

Compound ss4, analysed for $C_{15}H_{22}N_2OS$, m.p.130^{0}C, exhibited $[M+H]^+$ at m/z 279 in its positive ion mode electron spray ionization mass spectrum. The IR (cm^{-1}) spectrum showed the characteristic absorption bands at 3459 (NH_2), 2992 (C-H of ali.), 1652 (C=O) and 695 (C-S).

The ^{1}HNMR spectrum (300 MHz, $CDCl_3$) showed the characteristic signals of CO-NH and NH_2 at δ 8.3 and 4.2 as singlets respectively. The spectrum also showed nine methylene groups of cyclohexane ring integrating for 18 protons at δ 1.2-1.6 (10H, m, 5X CH_2), 1.9 (4H, m, 2X CH_2) and 3.5 (4H, t, 2X CH_2) respectively. The peak at δ 3.9 integrated for one proton, which was accounted for the -CO-NH-CH.

The results of elemental analysis were also in close agreement with those of the calculated values. Based on the above spectral data and elemental analysis the structure of the compound ss4 was confirmed as 2-amino-3-(N-cyclohexyl carboxamido)- 4,5,6,7- tetrahydrobenzo (b) thiophene.

CHEMISTRY:

1. *2-amino-3-(N-cyclohexyl carboxamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4):*** ESI m/z (M+1) 279; KBr (cm^{-1}) 3459 ($-NH_2$), 2992 (Ali-CH), 1652 (C=O), 695 (S-C); δ = 1.2-1.6 (10 H, m, -CH_2-CH_2-$CH_2$$CH_2$-$CH_2$- of cyclohexane); 1.9 (4H, m, -CH_2-CH_2-CH_2-CH_2 of tetrahydro benzene); 3.5 (4H, t, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.9 (1H, t, -CO-NH-CH of cyclohexane); 4.2 (2H, s, NH_2); 8.3 (1H, s, -CO-NH).

2. *2-[(3',4',5'-trimethoxy benzylidene)imino]-3-(N-cyclohexyl amido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4a)*** ESI m/z (M+1) 457; KBr (cm^{-1}) 3459 ($-NH_2$); 3293 (-NH-); 2992 (Ali-CH); 2927 (Ali-CH); 1652 (C=O); 815 (C-N); 695 (S-C); δ = 1.1-1.9 (14 H, m, -CH_2-CH_2-$CH_2$$CH_2$-$CH_2$- of cyclohexane and -$CH_2$-$CH_2$-$CH_2$-$CH_2$- of tetrahydrobenzene); 2.1(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.5 (1H, d, -CO-NH-CH of cyclohexane); 3.81 (3H, s, OCH_3), 3.89 (6H, m, 2X OCH_3), 7.45 (1H, m, Aro CH); 7.75 (1H, m, Aro CH); 8.3-(1H, s, -CO-NH); 8.5 (1H, d, -N=CH).

3. *2-[(3', 4'-dimethoxy benzylidene)imino]-3-(N-cyclohexyl amido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4b)*** ESI m/z (M+1) 427; KBr (cm^{-1}) 3449 ($-NH_2$); 29279 (Ali-CH); 1658 (C=O); 1549 (C=N); 760 (C-Cl); δ = 1.1-1.9 (14 H, m, -CH_2-CH_2-$CH_2$$CH_2$-$CH_2$- of cyclohexane and -$CH_2$-$CH_2$-$CH_2$-$CH_2$- of tetrahydrobenzene); 2.1(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.5 (1H, d, -CO-NH-CH of cyclohexane); 3.81 (6H, m, 2X OCH_3), 7.45 (1H, d, Aro CH); 7.75 (2H, d, Aro CH); 8.3-(1H, s, -CO-NH); 8.5 (1H, d, -N=CH).

4. *2-[(4'-methoxy benzylidene)imino]-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4c)*** KBr (cm^{-1}) 3413 (-NH); 3172 (Aro-CH); 2972 (Ali-CH); 1669 (C=O); 1546 (C=N); 826 (C-N); δ = 1.1-1.9 (14 H, m, -CH_2-CH_2-$CH_2$$CH_2$-$CH_2$- of cyclohexane and -$CH_2$-$CH_2$-$CH_2$-$CH_2$- of tetrahydrobenzene); 2.1(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.5 (1H, d, -CO-NH-CH of cyclohexane); 3.71 (3H, s, OCH_3), 7.45 (2H, d, Aro CH); 7.75 (2H, d, Aro CH); 8.3-(1H, s, -CO-NH); 8.5 (1H, d, -N=CH).

5. *2-[(2'-nitro benzylidene)imino]- 3-(N-cyclohexyl amido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4d)*** KBr (cm^{-1}) 3437 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1616 (C=O); 1678 (C=O); 689 (C-Cl); 1585, 1505, 1490,1401, (Ar C=C); δ = 1.2-1.9 (14 H, m, -CH_2-CH_2-$CH_2$$CH_2$-$CH_2$- of cyclohexane and -$CH_2$-$CH_2$-$CH_2$-$CH_2$- of tetrahydrobenzene); 2.2(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.6 (1H, d, -CO-NH-CH of cyclohexane); 7.46 (2H, d, Aro CH); 7.76 (2H, d, Aro CH); 8.2-(1H, s, -CO-NH); 8.4 (1H, d, -N=CH).

6. *2-[(3'-nitro benzylidene)imino]- 3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4e)*** KBr (cm^{-1}) 3337 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1697 (C=O); 1656 (C=O); 771 (C-Cl); δ = 1.3-2.0 (14 H, m, -CH_2-CH_2-$CH_2$$CH_2$-$CH_2$- of cyclohexane and -$CH_2$-$CH_2$-$CH_2$-$CH_2$- of tetrahydrobenzene); 2.2(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydro benzene); 3.6 (1H, d, -CO-NH-CH of cyclohexane); 7.5 (2H, d, Aro CH); 7.77 (2H, d, Aro CH); 8.4-(1H, s, -CO-NH); 8.4 (1H, d, -N=CH).

7. *2-[(2'-chloro benzylidene)imino]- 3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4f)*** KBr (cm^{-1}) 3249 (-NH); 3158 (-NH-); 2941 (Ali-CH); 1670 (C=O); 1250 (C-O); 830 (C-N); δ = 1.3-2.0 (14 H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.3(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydro benzene); 3.6 (1H, d, -CO-NH-CH of cyclohexane); 7.55 (2H, d, Aro CH); 7.76 (2H, d, Aro CH); 8.4-(1H, s, -CO-NH); 8.55 (1H, d, -N=CH).

8. *2-[(4'-chloro benzylidene)imino]- 3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4g)*** KBr (cm^{-1}) 3437 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1616 (C=O); 1678 (C=O); 689 (C-Cl); 1585, 1505, 1490,1401, (Ar C=C); δ = 1.1-1.9 (14 H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.1(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.5 (1H, d, -CO-NH-CH of cyclohexane); 7.45 (2H, d, Aro CH); 7.75 (2H, d, Aro CH); 8.3-(1H, s, -CO-NH); 8.5 (1H, d, -N=CH).

9. *2-[(2'-hydroxy benzylidene)imino]- 3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4h)*** KBr (cm^{-1}) 3337 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1697 (C=O); 1656 (C=O); 771 (C-Cl); δ = 1.3-2.1 (14 H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.3(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydro benzene); 3.7 (1H, d, -CO-NH-CH of cyclohexane); 5.3 (1H, s, OH) 7.55 (2H, d, Aro CH); 7.78 (2H, d, Aro CH); 8.3-(1H, s, -CO-NH); 8.6 (1H, d, -N=CH).

10. *2-[(4'-hydroxy benzylidene)imino]- 3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4i)*** KBr (cm^{-1}) 3249 (-NH); 3158 (-NH-); 2941 (Ali-CH); 1670 (C=O); 1250 (C-O); 830 (C-N); δ = 1.2-1.9 (14 H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.3(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydro benzene); 3.7 (1H, d, -CO-NH-CH of cyclohexane); 4.9 (1H, s, OH)7.6 (2H, d, Aro CH); 7.7 (2H, d, Aro CH); 8.1-(1H, s, -CO-NH); 8.3 (1H, d, -N=CH).

11. *2-[(4'-dimethyl amino benzylidene)imino]-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4j)*** KBr (cm^{-1}) 3437 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1616 (C=O); 1678 (C=O); 689 (C-Cl); 1585, 1505, 1490,1401, (Ar C=C); δ = 1.2-2.0 (14 H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.4(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.4 (1H, d, -CO-NH-CH of cyclohexane); 3.68 (6H, d, 2X CH_3)7.6 (2H, d, Aro CH); 7.78 (2H, d, Aro CH); 8.35-(1H, s, -CO-NH); 8.45 (1H, d, -N=CH).

12. *2-[(4'-methyl benzylidene)imino]- 3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b)thiophene* ***(ss4k)*** KBr (cm^{-1}) 3337 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1697 (C=O); 1656 (C=O); 771 (C-Cl); δ = 1.1-2.0 (14 H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.2(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.24 (3H, s, CH_3)3.6 (1H, d, -CO-NH-CH of cyclohexane); 7.6 (2H, d, Aro CH); 7.7 (2H, d, Aro CH); 8.4-(1H, s, -CO-NH); 8.6 (1H, d, -N=CH).

13. *2-[(3'-methoxy-4'-hydroxy benzylidene)imino]- 3-(N-cyclohexylamido)-4,5,6,7-tetrahydro benzo(b)thiophene* ***(ss4l)*** ESI m/z (M+1) 413; KBr (cm^{-1}) 3337 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1697 (C=O); 1656 (C=O); 771 (C-Cl); δ = 1.2-1.9 (14 H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.1(4H, d, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.6 (1H, d, -CO-NH-CH of cyclohexane);3.78 (3H, s, CH_3), 5.2 (1H, s, OH) 7.4 (2H, d, Aro CH); 7.75 (2H, d, Aro CH); 8.3-(1H, s, -CO-NH); 8.6 (1H, d, -N=CH).

14. *2-bezamido-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4m)*** KBr (cm^{-1}) 3249 (-NH); 3158 (-NH-); 2941 (Ali-CH); 1670 (C=O); 1250 (C-O); 830 (C-N); **δ =** 1.3- 1.8 (14H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetra hydrobenzene); 2.5 (4H, t, -CH_2-CH_2-CH_2-CH_2- of

tetrahydrobenzene); 3.5 (1H, t, -CO-NH-CH of cyclohexane); 7.3 (1H, m, Aro CH); 7.5 (2H, m, Aro CH); 8.1 (2H, m, Aro CH); 8.4 (2H, s, -CO-NH- and -NH-CO-C_6H_5).

15. *2-(4-amino benzamido)-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4n)*** KBr (cm^{-1}) 3437 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1616 (C=O); 1678 (C=O); 689 (C-Cl); 1585, 1505, 1490,1401, (Ar C=C); **δ =** 1.3- 1.8 (14H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.42 (3H, s, COCH_3), 2.5 (4H, t, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.5 (1H, t, -CO-NH-CH of cyclohexane); 7.3 (2H, d, Aro CH); 7.5 (2H, d, Aro CH); 8.4 (2H, s, -CO-NH- and -NH-CO-C_6H_5), 11.0 (1H, s, NH-$COCH_3$).

16. *2-(4-chloro benzamido)-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4o)*** KBr (cm^{-1}) 3337 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1697 (C=O); 1656 (C=O); 771 (C-Cl); **δ =** 1.3- 1.8 (14H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.5 (4H, t, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.5 (1H, t, -CO-NH-CH of cyclohexane); 7.3 (2H, d, Aro CH); 7.5 (2H, d, Aro CH); 8.4 (2H, s, -CO-NH- and -NH-CO-C_6H_5).

17. *2-(chloro acetamido)-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4p)*** KBr (cm^{-1}) 3449 (-NH_2); 2927 (Ali-CH); 1658 (C=O); 1549 (C=N); 760 (C-Cl); **δ =** 1.3- 1.8 (14H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.5 (4H, t, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 3.5 (1H, t, -CO-NH-CH of cyclohexane); 5.8 (2H, s, CH_2-Cl), 8.4 (2H, s, -CO-NH- and -NH-CO-C_6H_5).

18. *2-(morpholino acetamido)-3-(N-cyclohexylamido)-4, 5,6,7-tetrahydrobenzo (b) thiophene* ***(ss4q)*** ESI m/z (M+1) 406; KBr (cm^{-1}) 3413 (-NH); 3172 (Aro-CH); 2972 (Ali-CH); 1669 (C=O); 1546 (C=N); 826 (C-N); **δ =** 1.3- 1.8 (14H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.5 (4H, t, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.41 (4H, d, 2X CH_2), 3.4 (4H, d, 2X CH_2), 3.5 (1H, t, -CO-NH-CH of cyclohexane); 5.8 (2H, s, CH_2-Cl), 8.4 (2H, s, -CO-NH- and -NH-CO-C_6H_5).

19. *2-(piperazino acetamido)-3-(N-cyclohexylamido)-4,5,6,7-tetrahydrobenzo(b) thiophene* ***(ss4r).*** ESI (M+1) 405; KBr (cm^{-1}) 3337 (-NH); 3262 (-NH-); 2932 (Ali-CH); 1697 (C=O); 1656 (C=O); 771 (C-Cl); **δ =** 1.3-1.8 (14H, m, -CH_2-CH_2-CH_2CH_2-CH_2- of cyclohexane and -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.5 (4H, t, -CH_2-CH_2-CH_2-CH_2- of tetrahydrobenzene); 2.41 (4H, d, 2X CH_2), 3.4 (4H, d, 2X CH_2), 3.5 (1H, t, -CO-NH-CH of cyclohexane); 5.8 (2H, s, CH_2-Cl), 8.4 (2H, s, -CO-NH- and -NH-CO-C_6H_5), 8.1 (1H, s, NH).

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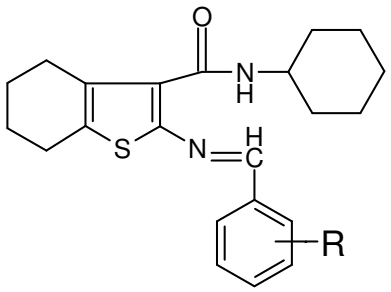
Table 1. Physical characterization data of thiophenes (ss4a- ss4l)



Compound	**R**	**Molecular Formula**	**Relative Molecular Mass (RMM)**	**Melting Point (^{o}C)**	**Yield %**
ss4	**-**	$C_{15}H_{22}N_2OS$	278	130	68.4
ss4a	3',4',5'-trimethoxy	$C_{25}H_{32}N_2O_4S$	456	170	76.13
ss4b	3',4'-dimethoxy	$C_{24}H_{30}N_2O_3S$	426	227	69.5
ss4c	4'-methoxy	$C_{23}H_{28}N_2O_2S$	396	210	58.37
ss4d	2'-nitro	$C_{22}H_{25}N_3O_3S$	411	238	60.45
ss4e	3'-nitro	$C_{22}H_{25}N_3O_3S$	411	218	62.56
ss4f	2'-chloro	$C_{22}H_{25}N_2OSCl$	400	209	69.25
ss4g	4'-chloro	$C_{22}H_{25}N_2OSCl$	400	179	56.35
ss4h	2'-hydroxy	$C_{22}H_{26}N_2O_2S$	382	253	69.84
ss4i	4'-hydroxy	$C_{22}H_{26}N_2O_2S$	382	232	75.07
ss4j	4'-dimethyl amino	$C_{24}H_{31}N_3OS$	409	110	52.66
ss4k	4'-methyl	$C_{23}H_{28}N_2OS$	380	180	61.5
ss4l	3'-methoxy-4'-Hydroxyl	$C_{23}H_{28}N_2O_3S$	412	222	67.46

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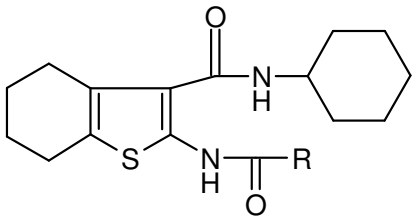
Table 2. Physical characterization data of thiophenes (ss4m- ss4r)



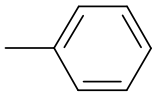
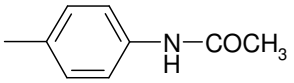
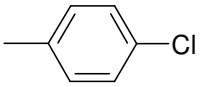
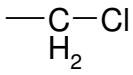
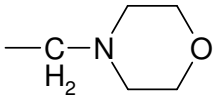
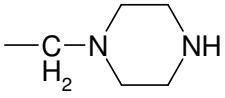
Compound Code	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (^{o}C)	Yield %
ss4m		$C_{22}H_{26}N_2O_2S$	382	205	52.98
ss4n		$C_{24}H_{29}N_3O_3S$	439	196	50.66
ss4o		$C_{22}H_{25}N_2O_2SCl$	416	230	48.2
ss4p		$C_{17}H_{23}N_2O_2SCl$	354	212	53.4
ss4q		$C_{21}H_{31}N_3O_3S$	405	185	55.3
ss4r		$C_{21}H_{32}N_4O_2S$	404	258	43.6

Table 3. Elemental Analysis data of new thiophenes (ss4, ss4a- r)

Compound	(% Calc.)			(% found)		
	C	H	N	C	H	N
ss4	64.74	7.96	10.06	64.72	7.95	10.03
ss4a	65.76	7.06	6.14	65.73	7.05	6.13
ss4b	67.58	7.09	6.57	67.56	7.08	6.56
ss4c	69.66	7.12	7.06	69.65	7.11	7.05
ss4d	64.21	6.12	10.21	64.20	6.11	10.20
ss4e	64.21	6.12	10.21	64.20	6.11	10.20
ss4f	65.90	6.28	6.99	65.89	6.27	6.98
ss4g	65.90	6.28	6.99	65.89	6.27	6.98
ss4h	69.08	6.85	7.32	69.06	6.84	7.32
ss4i	69.08	6.85	7.32	69.06	6.84	7.31
ss4j	70.38	7.63	10.26	70.37	7.62	10.25
ss4k	72.59	7.42	7.36	72.58	7.41	7.35
ss4l	66.96	6.84	6.79	66.94	6.82	6.78
ss4m	69.08	6.85	7.32	69.05	6.84	7.33
ss4n	65.58	6.65	9.56	65.54	6.63	9.54
ss4o	63.37	6.04	6.72	63.36	6.01	6.71
ss4p	57.53	6.53	7.89	57.50	6.51	7.88
ss4q	62.19	7.70	10.36	62.13	7.68	10.35
ss4r	62.34	7.97	13.85	62.31	7.95	13.82

BIOLOGICAL EVALUATION

Antibacterial Activity[15-17]- The test compounds were tested for their in vitro antibacterial activity by cup- plate method against strains of microbes, which are Escherichia *coli*, Bacillus *subtilis*, Staphylococcus *aureus* and Klebsiella *pneumoniae.*

Antifungal Acitvity[15-17]- The test compounds were tested for their in vitro antibacterial activity by cup- plate method against Aspergillus *niger* and Candida *albicans*. All the experiments were carried out in triplicate.

Anti- inflammatory Activity[18]- The test compounds were tested for in vitro anti- inflammatory activity by serum albumin denaturation method 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 $27^0 \square 1^0$C for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0 \square 1^0$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)

DISCUSSION AND CONCLUSION

Antibacterial Activity:

The results clearly revealed the potential antibacterial activity of all benzothiophenes, when compared with the standard drug ampicillin, but not at an identical dose level. Of all the compounds tested, compound **ss4d** having the nitro group at the *ortho* position of the phenyl ring, showed maximum activity and this is followed by compounds **ss4a** and **ss4f**, having chlorine substitution at *para* and *ortho* positions respectively.

The compounds **ss4m** and **ss4o**, having the unsubtituted phenyl ring and chlorine substitution at *para* position of the phenyl ring respectively also showed moderate degree of activity. The rest of the compounds showed mild to no activity. The results demonstrated the necessity of halogen substituents and nitro substituents on the aromatic ring, as they enhanced the activity.

Antifungal Acitvity:

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.

A close examination of the **Table-6** and **Table-7** pertaining to the antifungal activity data of benzothiophenes revealed that all the compounds in this series have been found to be effective against *A. niger* but not on *C. albicans*, when compared with the reference standard. The antifungal activity of compounds with halogen substitution was found to be more than those with electron releasing substituents. Of all the compounds tested, **ss4h** and **ss4g** having

hydroxy group and chlorine substitution at *ortho* and *para* positions of the phenyl ring showed the maximum activity followed by compounds **ss4i** and **ss4f** having hydroxy group and chlorine substitution at *ortho* and *para* positions of the phenyl ring respectively.

Anti-inflammatory Activity:

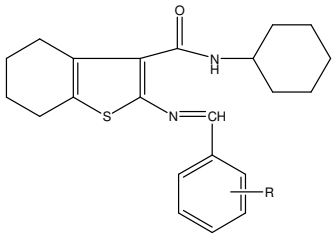
The anti-inflammatory activity of all the benzothiophenes synthesized has been evaluated by a method involving the inhibition of bovine serum albumin denaturation. The results of this activity were shown in **Table 8** and **Table 9.**

The results clearly revealed the potential anti-inflammatory activity of all these benzothiophenes when compared with the standard drug ibuprofen. Of all the compounds tested, compound **ss4h** and **ss4i** having hydroxy group at *ortho* and *para* positions of the phenyl ring showed maximum activity and this is followed by compounds **ss4q** and **ss4r** having a morpholin-yl and piperazin-yl substitution on the carbonyl carbon attached at the position 2 of the thiophene ring. The results demonstrated the necessity of electron donating substituents on aromatic ring, as they enhanced the activity.

ACKNOWLEDGEMENTS

Authors wish to thank the Central Drug Research Institute, Lucknow for providing spectral data and Amity University for providing facilities for research.

Table 4. Antibacterial activity of thiophenes (ss4a to ss4l)



Compound	R	Zone of inhibition (mm) *			
		S.aureus	*B.subtilis*	*E.coli*	*K. pneumoniae*
ss4 a	3',4',5'-trimethoxy	14	15	15	16
ss4 b	3',4'- dimethoxy	12	14	14	12
ss4 c	4'- methoxy	4	14	12	10
ss4 d	2'-nitro	20	20	22	19
ss4 e	3'-nitro	13	16	12	14
ss4 f	2'-chloro	21	19	18	20
ss4 g	4'-chloro	20	21	16	19
ss4 h	2'-hydroxy	13	12	16	15
ss4 i	4'- hydroxyl	17	16	16	14
ss4 j	4'- dimethyl amino	16	16	NA	NA
ss4 k	4'-methyl	12	14	NA	NA
ss4 l	3'-methoxy-4'-hydroxyl	14	13	12	12

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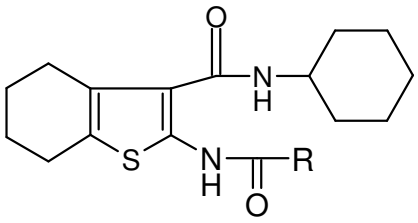
Table 5. Antibacterial activity of thiophenes (ss4m to ss4r)



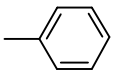
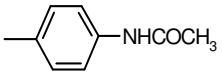
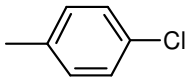
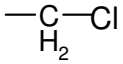
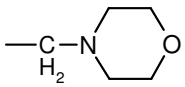
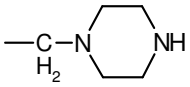
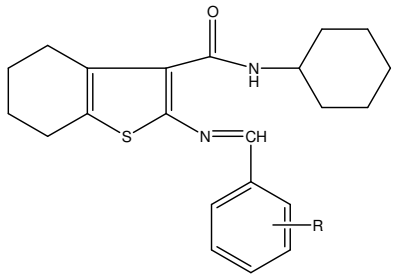
Compound	R	Zone of inhibition (mm) *			
		S.aureus	*B.subtilis*	*E.coli*	*K. pneumoniae*
ss4 m		14	14	18	17
ss4 n		10	12	11	10
ss4 o		20	14	19	16
ss4 p		12	16	10	12
ss4 q		11	14	18	11
ss4 r		10	13	16	13
Ampicillin	-	**23**	**19**	**25**	**20**

Table 6. Antifungal activity of thiophenes (ss4a to ss4k):



Compound	R	Zone of inhibition (mm) *	
		Aspergillus niger	*Candida albicans*
ss4 a	3',4',5'-trimethoxy	NA	NA
ss4 b	3',4'- dimethoxy	NA	NA
ss4 c	4'- methoxy	NA	NA
ss4 d	2'-nitro	13	12
ss4 e	3'-nitro	12	11
ss4 f	2'-chloro	13	11
ss4 g	4'-chloro	15	13
ss4 h	2'-hydroxy	16	12
ss4 i	4'- hydroxy	14	12
ss4 j	4'- dimethyl amino	NA	NA
ss4 k	4'-methyl	NA	NA
ss4 l	3'-methoxy-4'- hydroxy	NA	NA
Miconazole nitrate	-	**28**	**26**

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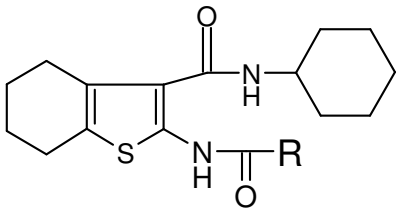
Table 7. Antifungal activity of thiophenes (ss4m to ss4r):



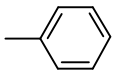
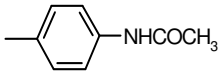
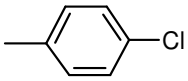
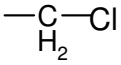
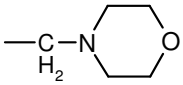
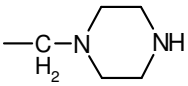
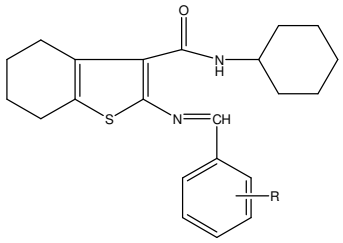
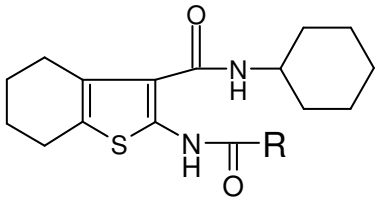
Compound	R	Zone of inhibition (mm) *	
		Aspergillus niger	*Candida albicans*
ss4m		NA	NA
ss4n		NA	NA
ss4o		9	13
ss4p		10	12
ss4q		NA	NA
ss4r		NA	NA
Miconazole nitrate	-	**28**	**26**

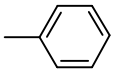
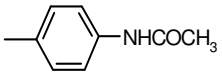
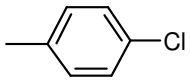
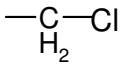
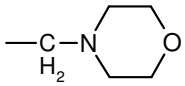
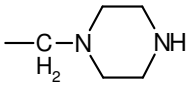
Table 8. *In vitro* Anti-inflammatory activity data of thiophenes (ss4a to ss4l):



Compound	R	Anti-inflammatory activity (% Bovine serum inhibition)
ss4 a	3',4',5'-trimethoxy	28.18
ss4 b	3',4'- dimethoxy	39.10
ss4 c	4'- methoxy	33.60
ss4 d	2'-nitro	23.72
ss4 e	3'-nitro	19.20
ss4 f	2'-chloro	42.56
ss4 g	4'-chloro	40.38
ss4 h	2'-hydroxy	58.28
ss4 i	4'- hydroxy	56.22
ss4 j	4'- dimethyl amino	33.80
ss4 k	4'-methyl	34.20
ss4 l	3'-methoxy-4'- hydroxy	50.48
Ibuprofen	--	**68.55**

Table 9. *In vitro* Anti-inflammatory activity data of thiophenes (ss4m to ss4r):



Compound	R	Anti-inflammatory activity (% Bovine serum inhibition)
ss4 m		58.40
ss4 n		60.43
ss4 o		62.20
ss4 p		54.80
ss4 q		64.38
ss4 r		62.60
Ibuprofen	-	**68.55**

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