Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(2):256-262
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis of 2-[(substituted benzylidene)imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophenes for *in vitro* anti-inflammatory and antimicrobial evaluation

Barnali Das*[1], Sajal Srivastava[2], S. C. Dinda[3] and Y. Rajendra Prasad[4]

[1]School of Pharmaceutical Education & Research, Berhampur University, Berhampur, Odisha, India
[2]Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow, India
[3]School of Pharmaceutical Education & Research, Berhampur University, Berhampur, Odisha, India
[4]A U College of Pharmaceutical Sciences, Andhra University, India

---

ABSTARACT

In an attempt to find a new class of antimicrobial agents having anti-inflammatory activity, a series of thiophene containing Schiff base moiety were prepared via the reaction of ketone, 4-N-methyl piperidone with the propylcyanoacetamide using ammonium acetate/glacial acetic acid as an acidic catalyst with the arrangement of continuous removal of water followed by reacting starting compounds with substituted aryl aldehydes. These compounds were screened for their antibacterial activity against Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus), Gram-negative bacteria (Escherichia coli and Klebsiella pneumonia) and antifungal activity against (Aspergillus niger and Candida albicans) by using cup plate method. The results clearly revealed the potential antimicrobial and anti-inflammatory activity of all these piperidinothiophenes when compared with the standard drug Ampicillin, Miconazole and Ibuprofen. Structures of the newly synthesized compounds were established by elemental analysis and spectral data.

Key words: Gewald Reaction, fusedthiophene, piperidine, schiffs base, antibacterial, antifungal, anti- inflammatory activity.

INTRODUCTION

Inflammation is a natural host-defensive process in the innate immunity response. Bacterial and viral infection triggers the activation of numerous immune cells such as macrophages, monocytes, and neutrophils undergoing various cellular responses such as phagocytic uptake, and the production of inflammatory mediators such as nitric oxide (NO), prostaglandin E_2 (PGE_2) and tumour necrosis factor (TNF) (Kinne et al., 2000)[1]. The effective blockade of these inflammatory responses is an important therapeutic target.

Thiophene and its derivatives are known for its wide array of activities[2-13]. Thiophene fused with five and six membered rings attracted medicinal chemists' interest in designing better drug molecule. From the literature survey it is revealed that only few papers have been published [Das et al 2007 & 2013, Romagnoli et al] on thiophene fused with piperidine ring; have prompted us to synthesize and exploit the pharmacological potential of new piperidinothiophene derivatives.

MATERIALS AND METHODS

All the melting points were determined in open capillaries, using Veego VMP- DS melting point apparatus, expressed in ^{o}C and are uncorrected. All the chemicals used in the synthesis were obtained from standard commercial sources. Reactions were monitored by TLC using silica gel-G (S. D. Fine Chem. Pvt. Ltd, Mumbai,

India) as the adsorbent and the different solvent systems were used. The separations of the compounds were checked on TLC under UV lamp and also by Iodine Chamber.

The ^{1}H NMR spectra of the compounds were recorded either on HRMS (in $CDCl_3$/ D_2O) Bruker Avance 400 MHz (FTNMR) or Avance 300 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. The infrared spectra were recorded on Perkin Elmer RX1 FTIR spectrophotometer. The mass spectra of the compounds were recorded on positive mode ESI- HRMS (m/ z) 100- 30000 Th model Agilent 6520 (Q- TOF) mass spectrophotometer. Bovine serum albumin (Merck Limited), Ibuprofen, Ampicillin, Miconazole nitrate and all other chemicals were of analytical grade.

Synthesis of 2-amino-3-(N-propyl carboxamido-6-N-methyl piperidino thiophene via formation of 2-Cyano-2-(4-N-methyl piperidine-1-ylidene) propyl carboxamide (bd3)

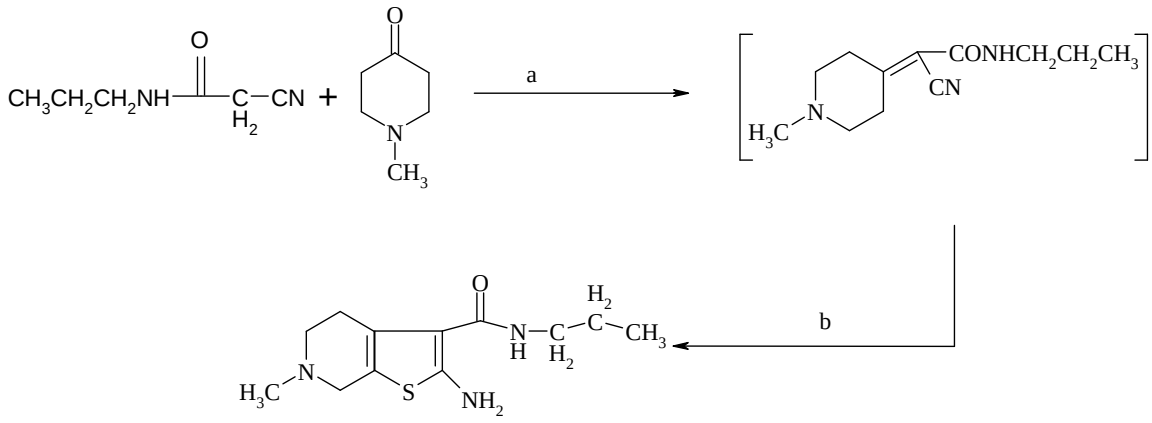
A mixture of propyl amine (0.50 mole) and ethylcyano acetate (0.50 mole) was taken in a conical flask and heated on oil bath at 160- 180^0 C for 6-7 hours. The reaction mixture was left at room temperature for overnight. The solid obtained was filtered, washed with water and dried, then recrystallized from acetone: water mixture (5:1).

A mixture of propylcyanoacetamide (5.04 g, 0.04 mole), 4-N-methylpiperidone (4.6 mL, 0.04 mole), ammonium acetate (2 g) and glacial acetic acid (2 mL) in benzene (100 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 5 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-(4-N-methyl piperidine-1-ylidene) propyl carboxamide in alcohol (30 mL) was added sulphur (1.28 g, 0.04 mole) in portions followed by the addition of, morpholine (6.0 mL) drop wise with stirring. The reaction mixture was stirred for 5 hours at 40-45 ºC and chilled over night. The solid obtained was filtered, washed with ethanol and crystallized from benzene.

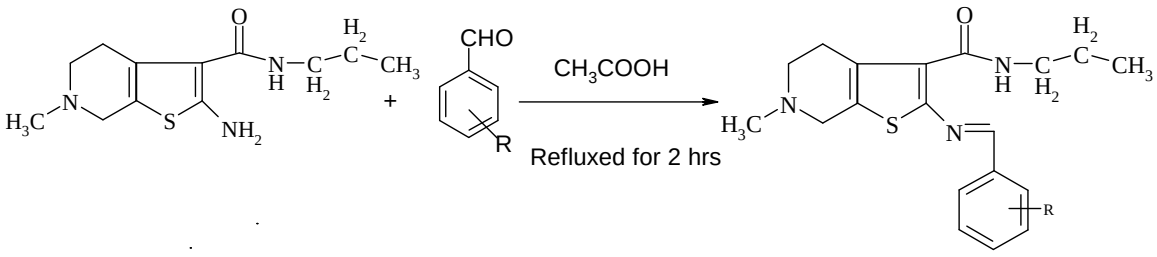
General method for the synthesis of 2-[(substituted benzylidene) imino]-3-N-propyl carboxamido-6-N-methyl piperidino thiophenes (Schiff bases) (bd3a to bd3l)

A mixture of the starting compound (**bd3**) (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 800 watt for 120 sec (2 min). The mixture was cooled to room temperature, the solid separated was filtered, washed with alcohol and crystallized with DMF: Water mixture.



a. reflux for 10 hours, C_6H_6, CH_3COOH, NH_4COOCH_3; b. Stirring for 60 min, S, Morpholine, Ethanol

Scheme 1



2-amino-3-N-propyl *(substituted aromatic aldehydes)* *bd3a- l carboxamido-6-N-methylpiperidino thiophene bd3*

Scheme 2

Compound bd3, analysed for $C_{12}H_{19}N_3OS$, m.p.138^{0}C, exhibited $[M+H]^+$ at m/z 254 in its positive ion mode electron spray ionization mass spectrum. The IR (cm^{-1}) spectrum, showed the characteristic absorption bands at 3385 (N-H), 2963 (C-H ali), 1664 (C=O), 1536 (C=C), 962 (C-N) and 682 (C-S).

The ^{1}HNMR spectrum (300 MHz, $CDCl_3$,) showed the characteristic signals of CO-NH and NH_2 at δ 8.65 and 4.43 as singlets respectively. The spectrum also showed three methylene groups of piperidine ring integrating for 6 protons at δ 3.82 (2H, s, CH_2), 2.7 (2H, t, CH_2) and 2.6 (2H, t, CH_2) respectively. The peaks at δ 2.98 integrated for two protons of side chain attached to amide nitrogen. The multiplet at δ 1.7 integrated for two protons of CH_2-CH_2-CH_3 of propyl chain. The peaks at 2.41 and 1.0 accounts for two methyl at piperidine nitrogen and propyl side chain. 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 bd3 was confirmed as 2-amino-3-N-propyl carboxamido-6-N-methylpiperidino thiophene.

1. *2-amino-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3),*** IR KBr (cm^{-1}) 3385 (N-H), 2963 (C-H ali), 1664 (C=O), 1536 (C=C), 962 (C-N), 682 (C-S). ^{1}HNMR (δ)- 8.65 (1H, s, CO-NH-CH_2), 4.43 (2H, s, NH_2), 3.82 (2H, s, CH_2 of piperidino ring), 2.98 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 1.65- 1.73 (2H, m, CH_2-CH_2-CH_3), 1.0 (3H, t, CH_2-CH_2-CH_3), M.P.(^{0}C)-134, m/z (M+1) 254. Anal. Calcd. for $C_{12}H_{19}N_3OS$: C, 56.89; H, 7.56; N, 16.58; S, 12.66. Found: C, 56.86; H, 7.57; N, 16.57; S, 12.65.

2. *2-(benzylidene imino-3-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3a)*** , IR KBr (cm^{-1}) 3440 (-NH-), 2925 (Ali. CH), 3115(Aro. CH), 1650 (C=O), 1584 (C=N), 1366 (Aro-C=C), 1052 (C-O), 815(C-N), 759(C-S). ^{1}HNMR (δ)- 8.65 (1H, s, CO-NH-CH_2), 8.3 (1H, s, N=CH), 7.6 (2H, d, CH of phenyl ring), 7.2 (3H, d, CH of phenyl ring), 3.82 (2H, s, CH_2 of piperidino ring), 2.98 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 1.65- 1.73 (2H, m, CH_2-CH_2-CH_3), 1.0 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-175, m/z (M+1) 342. Anal. Calcd. for $C_{19}H_{23}N_3OS$: C, 66.83; H, 6.67; N, 12.31; S, 9.39. Found: C, 66.80; H, 6.80; N, 12.32; S, 9.38.

3. *2-[(4'-chloro benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3b)***, IR KBr (cm^{-1}) 3400 (-NH-), 3115 (Aro. CH), 2935 (Ali.CH), 1653 (C=O), 1350 (C-N of N-CH_3),1266 (Aro-C=C), 1560 (C=N), 1177 (C-O) , 1071(Aro-Cl), 824 (C-N), 764 (C-S). ^{1}HNMR (δ)- 8.61 (1H, s, CO-NH-CH_2), 8.31 (1H, s, N=CH), 7.7 (2H, d, CH of phenyl ring), 7.5 (2H, d, CH of phenyl ring), 3.81 (2H, s, CH_2 of piperidino ring), 3.0 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.44 (3H, s, CH_3-N), 1.75- 1.70 (2H, m, CH_2-CH_2-CH_3), 1.1 (3H, t, CH_2-CH_2-CH_3), M.P.(^{0}C)-184, m/z (M+1) 376. Anal. Calcd. for $C_{19}H_{22}ClN_3OS$: C, 60.71; H, 5.90; N, 11.18; S, 8.53. Found: C, 60.70; H, 5.91; N, 11.71; S, 8.57.

4. *2-[(4'-fluoro benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3c)***, IR KBr (cm^{-1}) 3410 (-NH-), 3115 (Aro. CH), 2935 (Ali.CH), 1653 (C=O), 1560 (C=N), 1350 (C-N of N-CH_3), 1266 (Aro-C=C), 1234 cm^{-1} (C-F), 1177 (C-O), 764 (C-S), 824 (C-N). ^{1}HNMR (δ)- 8.6 (1H, s, CO-NH-CH_2), 8.31 (1H, s, N=CH), 7.7 (2H, d, CH of phenyl ring), 7.1 (2H, d, CH of phenyl ring), 3.81 (2H, s, CH_2 of piperidino ring), 3.0 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.44 (3H, s, CH_3-N), 1.75-1.70 (2H, m, CH_2-CH_2-CH_3), 1.1 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-188, m/z (M+1) 360. Anal. Calcd. for $C_{19}H_{22}FN_3OS$: C, 63.48; H, 6.17; N, 11.69; S, 8.92. Found: C, 63.46; H, 6.18; N, 11.67; 8.90.

5. *2-[(4'-methyl benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3d)***, IR KBr (cm^{-1}) 3363 (-NH-), 3187 (Aro. CH), 2935 (Ali.CH), 1641 (C=O), 1588 C=N), 1295 (Aro-C=C), 1127 (C-O), 828 (C-N), 779 (C-S). ^{1}HNMR (δ)- 8.65 (1H, s, CO-NH-CH_2), 8.3 (1H, s, N=CH), 7.6 (2H, d, CH of phenyl ring), 7.2 (2H, d, CH of phenyl ring), 3.82 (2H, s, CH_2 of piperidino ring), 2.98 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of

piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 2.35 (3H, s, CH_3 on phenyl ring), 1.65-1.73 (2H, m, CH_2-CH_2-CH_3), 1.0 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-205, m/z (M+1) 356. Anal. Calcd. for $C_{20}H_{25}N_3OS$: C, 67.57; H, 7.09; N, 11.82; S, 9.02. Found: C, 67.54; H, 7.10; N, 11.81; S, 9.04.

6. *2-[(4'-methoxy benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3e)***, IR KBr (cm^{-1}) 3224 (-NH-), 3116 (Aro-CH), 2946 (Ali-CH), 1633 (C=O), 1592 (C=N), 1313 (Aro C=C), 1230 (Aro-C-O of Aro-OCH_3), 1021 (C-O), 1076 (Aro C-O), 820 (C-N), 745(C-S). ^{1}HNMR (δ)- 8.64 (1H, s, CO-NH-CH_2), 8.3 (1H, s, N=CH), 7.6 (2H, d, CH of phenyl ring), 7.2 (2H, d, CH of phenyl ring), 3.82 (2H, s, CH_2 of piperidino ring), 3.72 (3H, s, OCH_3), 2.98 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 1.65- 1.73 (2H, m, CH_2-CH_2-CH_3), 1.0 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-198, m/z (M+1) 372. Anal. Calcd. for $C_{20}H_{25}N_3O_2S$: C, 64.66; H, 6.78; N, 11.31; S, 8.63. Found: C, 64.65; H, 6.79; N, 11.30; S, 8.62.

7. 2-[(4'-hydroxy benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene **(bd3f)**, IR KBr (cm^{-1}) 3478 (OH), 3210 (-NH-), 3112 (Aro. CH), 2935 (Ali.CH), 1653 (C=O), 1560 (C=N), 1518 (N=O of NO_2), 1266 (Aro-C=C), 1071 (C-O), 824 (C-N), 789 (C-S). ^{1}HNMR (δ)- 8.65 (1H, s, CO-NH-CH_2), 8.3 (1H, s, N=CH), 7.6 (2H, d, CH of phenyl ring), 7.2 (2H, d, CH of phenyl ring), 5.8 (1H, s, OH), 3.82 (2H, s, CH_2 of piperidino ring), 2.98 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 1.65-1.73 (2H, m, CH_2-CH_2-CH_3), 1.0(3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-188, m/z (M+1) 358. Anal. Calcd. for $C_{19}H_{23}N_3O_2S$: C, 63.84; H, 6.49; N, 11.75; S, 8.97. Found: C, 63.82; H, 6.48; N 11.74; S, 8.96.

8. 2-[(3'-methyl benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene **(bd3g)**, IR KBr (cm^{-1}) 3415 (-NH-), 3058 (Aro-CH), 2878 (Ali-CH), 1656 (C=O), 1532 (C=N), 1230 (Aro-C=C), 1015 (C-O), 826 (C-N), 771 (C-S. ^{1}HNMR (δ)- 8.63 (1H, s, CO-NH-CH_2), 8.3 (1H, s, N=CH), 7.5 (2H, d, CH of phenyl ring), 7.35 (1H, d, CH of phenyl ring), 7.2 (1H, s, CH of phenyl ring), 3.82 (2H, s, CH_2 of piperidino ring), 2.98 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 2.37 (3H, s, CH_3 on phenyl ring), 1.65- 1.73 (2H, m, CH_2-CH_2-CH_3), 1.0 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-192, m/z (M+1) 356. Anal. Calcd. for $C_{20}H_{25}N_3OS$: C, 67.57; H, 7.09; N, 11.82; S, 9.02. Found: C, 67.55; H, 7.08; N, 11.81; S, 9.01.

9. *2-[(3'-fluoro benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3h)***, IR KBr (cm^{-1}) 3423 (-NH-), 3058 (Aro. CH), 2878 (Ali. CH), 1656 (C=O), 1532 (C=N), 1238 (Aro-C-O of Aro-OCH_3), 1230 (Aro-C=C), 1214 cm^{-1} (C-F), 1015 (C-O), 826 (C-N), 771 (C-S). ^{1}HNMR (δ)- 8.63 (1H, s, CO-NH-CH_2), 8.32 (1H, s, N=CH), 7.64 (1H, s, CH of phenyl ring), 7.1- 7.43 (3H, m, CH of phenyl ring), 3.8 (2H, s, CH_2 of piperidino ring), 3.0 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.44 (3H, s, CH_3-N), 1.75- 1.70 (2H, m, CH_2-CH_2-CH_3), 1.1 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-190, m/z (M+1) 360. Anal. Calcd. for $C_{19}H_{22}FN_3OS$: C, 63.48; H, 6.17; N, 11.69; S, 8.92. Found: C, 63.47; H, 6.16; N, 11.70; S, 8.93.

10.*2-[(3'-chloro benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3i)***, IR KBr (cm^{-1}) 3280 (-NH-), 3125 (Aro-CH), 2936Ali-CH), 1640 (C=O), 1578 (C-N), 1300 Ar-C=C), 1158 (C-O), 1090 cm^{-1} (C-Cl), 820 (C-N), 766 (C-S). ^{1}HNMR (δ)- 8.6 (1H, s, CO-NH-CH_2), 8.3 (1H, s, N=CH), 7.7 (1H, s, CH of phenyl ring), 7.5- 7.2 (3H, m, 3X CH of phenyl ring), 3.81 (2H, s, CH_2 of piperidino ring), 3.0 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.44 (3H, s, CH_3-N), 1.75- 1.70 (2H, m, CH_2-CH_2-CH_3), 1.1 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-208, m/z (M+1) 376. Anal. Calcd. for $C_{19}H_{22}ClN_3OS$: C, 60.71; H, 5.90; N, 11.18; S, 8.53. Found: C, 60.71; H, 5.89; N, 11.19; S, 8.54.

11.*2-[(3',4'- dimethoxy benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3j)***, IR KBr (cm^{-1}) 3250 (-NH-), 3028 (Aro-CH), 2915 (Ali-CH), 1643 (C=O), 1593 (C= N), 1086 (C-N amine), 1369 (Aro C=C), 1242 (Aro-C-O of Aro-OCH_3), 1008 (C-O), 825 (C-N), 755(C-S). ^{1}HNMR (δ)- 8.64 (1H, s, CO-NH-CH_2), 8.3 (1H, s, N=CH), 7.1 (2H, d, CH of phenyl ring), 6.7 (1H, s, CH of phenyl ring), 3.82 (2H, s, CH_2 of piperidino ring), 3.70 (6H, s, OCH_3), 2.98 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 1.65- 1.73 (2H, m, CH_2-CH_2-CH_3), 1.0 (3H, t, CH_2-CH_2-CH_3). M.P. (^{0}C)-194, m/z (M+1) 402. Anal. Calcd. for $C_{21}H_{27}N_3O_3S$: C, 62.82; H, 6.78; N, 10.47; S, 7.99. Found: C, 62.82; H, 6.77; N, 10.48; S, 7.98.

12.*2-[(3',4',5'-trimethoxy benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3k)***, IR KBr (cm^{-1}) 3260 (-NH-), 3115 (Aro-CH), 2925 (Ali-CH), 1650 (C=O), 1583 (C=N), 1366(Aro-C=C), 1232 (Aro-C-O of Aro-OCH_3), 1052 (C-O), 825(C-N), 772 (C-S). ^{1}HNMR (δ)- 8.64 (1H, s, CO-NH-CH_2), 8.31 (1H, s, N=CH), 6.8 (2H, d, CH of phenyl ring), 3.82 (2H, s, CH_2 of piperidino ring), 3.70 (9H, s, OCH_3), 2.98 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 1.65- 1.73 (2H, m, CH_2-CH_2-CH_3), 1.0 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-215, m/z (M+1) 432. Anal. Calcd. for $C_{22}H_{29}N_3O_4S$: C, 61.23; H, 6.77; N, 9.47; S, 7.43. Found: C, 61.20; H, 6.79; N, 9.48; S, 7.44.

13.*2-[(4'-N,N-dimethyl benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3l)***, IR KBr (cm^{-1}) 3425 (-NH-), 3187 (Aro-CH), 2910 (Ali-CH), 1645 (C=O), 1568 (C=N), 1528 (N=O), 1300 (Ar-C=C), 1098 (C-O), 825 (C-N), 760 (C-S). ^{1}HNMR (δ)- 8.65 (1H, s, CO-NH-CH_2), 8.3 (1H, s, N=CH), 7.5 (2H, d, 2X CH of phenyl ring), 6.4 (2H, d, 2X CH of phenyl ring), 3.82 (2H, s, CH_2 of piperidino ring), 2.98 (2H, q, NH-CH_2-), 2.9 (6H, s, 2X N-CH_3), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.41 (3H, s, CH_3-N), 1.65-1.73 (2H, m, CH_2-CH_2-CH_3), 1.0(3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-196, m/z (M+1) 417. Anal. Calcd. for $C_{21}H_{28}N_4O_3S$: C, 60.55; H, 6.78; N, 13.45; S, 7.70. Found: C, 60.56; H, 6.67; N, 13.44; S, 7.71.

14.*2-[(2'-chloro benzylidine) imino]-3-N-propylcarboxamido-6-N-methyl piperidino thiophene* ***(bd3m)***, IR KBr (cm^{-1}) 3410 (-NH-), 3105 (Aro. CH), 2935 (Ali.CH), 1653 (C=O), 1560 (C=N), 1350 (C-N of N-CH_3), 1266 (Aro-C=C), 1177 (C-O), 1090 cm^{-1} (C-Cl), 824 (C-N), 764 (C-S). ^{1}HNMR (δ)- 8.61 (1H, s, CO-NH-CH_2), 8.31 (1H, s, N=CH), 7.6 (1H, d, CH of phenyl ring), 7.2 (1H, d, CH of phenyl ring), 7.0 (2H, t, CH of phenyl ring), 3.81 (2H, s, CH_2 of piperidino ring), 3.0 (2H, q, NH-CH_2-), 2.7 (2H, t, CH_2 of piperidino ring), 2.6 (2H, t, CH_2 of piperidino ring), 2.45 (3H, s, CH_3-N), 1.75- 1.70 (2H, m, CH_2-CH_2-CH_3), 1.1 (3H, t, CH_2-CH_2-CH_3). M.P.(^{0}C)-187, m/z (M+1) 377. Anal. Calcd. for $C_{19}H_{22}ClN_3OS$: C, 60.71; H, 5.90; N, 11.18; S, 8.53. Found: C, 60.72; H, 5.90; N, 11.18; S, 8.53. Found: C, 60.72; H, 5.91; N, 11.16; S, 8.52.

BIOLOGICAL EVALUATION

(a) Antibacterial Activity[20]: The antibacterial activity of synthesized thiophenes were conducted against two gram positive bacteria viz., *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacteria viz., *Escherichia coli and Klebsiella pneumoniae* by using cup plate method. Ampicillin was employed as reference standards to compare the results.

(b) Antifungal Activity[20]: All those compounds screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were *Aspergillus niger* and *Candida albicans*. Miconazole was employed as standard to compare the results.

(c**) Anti-Inflammatory Activity (Inhibition of bovine serum albumin denaturation[21] and anti- inflammatory activity)[23]:** Clinically established anti-inflammatory drugs have shown to inhibit heat coagulation of proteins. These anti-inflammatory drugs have exerted an inhibitory activity on immune haemolysis and also have suppressive effect on vascular reactivity. Denaturation as one of the causes of inflammation is well documented. Anti-inflammatory drugs interact in some way with proteins. To gauge the interaction between the drug and the proteins, the stability of proteins against heat coagulation can be measure (Inhibition of bovine serum albumin denaturation).

Biological activity of piperidinothiophenes (bd3a to bd3m):

Compound	R	Zone of inhibition (mm) *				Zone of inhibition (mm) *		Anti-inflammatory activity (% Bovine serum inhibition)
		S. aureus	*B. subtilis*	*E. coli*	*K. pneumonia*	*Aspergillus niger*	*Candida albicans*	
bd3a	H	09	06	03	05	12	10	32.2
bd3b	4'- chloro	17	13	11	16	25	22	38.4
bd3c	4'- fluoro	16	12	12	15	24	21	41.2
bd3d	4'- methyl	09	04	02	08	19	16	38.5
bd3e	4'- methoxy	10	04	03	08	18	14	41.2
bd3f	4'- hydroxy	10	05	04	09	17	18	51.1
bd3g	3'- methyl	08	04	03	08	14	10	37.4
bd3h	3'- fluoro	11	10	08	13	22	19	32.8
bd3i	3'- chloro	10	08	06	12	19	18	34.5
bd3j	3',4'- dimethoxy	07	03	04	09	12	08	57.2
bd3k	3',4',5'- trimethoxy	09	06	05	09	14	09	59.7
bd3l	4'-N-dimethyl	12	05	05	09	18	12	43.8
bd3m	2'- chloro	10	08	07	09	20	17	32.1
	Ampicillin	**19**	**16**	**14**	**18**	--	--	--
	Miconazole nitrate	--	--	--	--	**30**	**26**	--
	Ibuprofen	--	--	--	--	--	--	**68.55**

The test compounds were dissolved 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. 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 antibacterial activity of all the piperidinothiophenes synthesized have been evaluated by using cup plate method. The results of this activity are shown in table**.**

The results clearly revealed the potential antibacterial activity of all piperidinothiophenes, when compared with the standard drug ampicillin, but not at an identical dose level. Of all the compounds tested, compound **bd3b** having the chlorine substitution at the *para* position of the phenyl ring, showed maximum activity and this is followed by compounds **bd3c, bd3h** and **bd3m**, having fluoro group substitution at *para*, *meta* and chlorine substitution at *ortho* positions of the phenyl ring respectively. The rest of the compounds showed mild activity. The results demonstrated the necessity of halogen substituents on the aromatic ring, as they enhanced the activity.

Antifungal activity: The antifungal activity of the substituted piperidino 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 pertaining to the antifungal activity data of piperidinothiophenes revealed that all the compounds in this series have been found to be effective against both strains i.e. *A. niger* and *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, **bd3b** having chloro group substitution at *para* position of the phenyl ring showed the maximum activity followed by compounds **bd3c**, **bd3h** and **bd3m** have fluorine substitution at *para*, *meta* and chlorine at *ortho* positions of the phenyl ring respectively. The Schiff bases having two/ three halogens or having haloalkyl substituents can also be synthesized and screened for antifungal activity in order to get compounds with promising activity.

Anti- inflammatory activity: The anti-inflammatory activity of all the piperidinothiophenes synthesized have been evaluated by a method involving the inhibition of bovine serum albumin denaturation. The results of this activity were shown in table**.** The results clearly revealed the potential anti-inflammatory activity of all these piperidinothiophenes when compared with the standard drug ibuprofen. Of all the compounds tested, compound **bd3k** and **bd3j** having three methoxy group substitution at 3,4 and 5th position and two methoxy groups at 3rd and 5th position of the phenyl ring showed maximum activity and this is followed by compound **bd3f** having a hydroxy group at position 4th and hydroxy group at position 4 on the phenyl ring. The results demonstrated the necessity of electron donating substituents on aromatic ring, as they enhanced the activity.

Acknowledgement- Authors are thankful to Head, SAIF- CDRI, Lucknow for providing spectral data.

REFERENCES

[1] R W Kinne, R Brauer, B Stuhlmuller, E Palombo-Kinne,GR Burmester, *Arthritis Research*, **2000**, 2, 189–202.
[2] L Xiaoyun, B Wan, SG Franzblau, Q You, *Eur J Med Chem*, **2011**, 46, 3551- 63.
[3] K Balamurugan, S Perumal, ASK Reddy, P Yogeeswari, D Sriram, *Tetra Lett*, **2009**, 50, 6191–95
[4] JFD Chabert, B Marquez, L Neville, L Joucla, S Broussous, P Bouhours, E David, SP Rostaing, B Marquet, N Moreaub, M Lemaire, *Bioorg Med Chem*, **2007**, 15, 4482–97.
[5] JG Gonzalez, CE Stephens, T Wenzler, R Brun, FA Tanious, WD Wilson, T Barszcz, TA Werbovetz, DW Boykin, *Eur J Med Chem*, **2007**, 42, 552- 57.
[6] AM Isloor, B Kalluraya, KS Pai, *Eur J Med Chem*, **2010**, 45, 825–30.
[7] IMIFakhr, MAA Radwan, S El-Batran, OMEA El-Salam, SM El-Shenawy, *Eur J Med Chem*, **2009**, 44, 1718-25.
[8] DW Nelson, JM Frost, KR Tietje, AS Florjancic, K Ryther, WA Carroll, MJ Dart, AV Daza, BA Hooker, GK Grayson, F Yihong, TR Garrison, OF El-Kouhen, B Yao, M Pai, P Chandran, C. Zhu, C. Hsieh, M.D. Meyer, *Bioorg. Med. Chem. Let.*, **2012**, DOI, 10.1016/j.bmcl. 2012.01.121.
[9] CE Bennett, DA Burnett, WJ Greenlee, CE Knutson, P Korakas, C Li, D Tulshian, W Wu, R Bertorelli, S Fredduzzi, M Grilli, G Lozza, A Reggiani, A Veltri, *Bioorg Med Chem Lett*, **2012**, 22, 1575–78.
[10] VE Laing, DC Brookings, RJ Carbery, JG Simorte, MC Hutchings , BJ Langham, MA Lowe, RA Allen, JR Fetterman, J Turner, C Meier , J Kennedy, M Merriman, *Bioorg Med Chem Lett*, **2012**, 22, 472–75.
[11] AGE Amra, MH Sherif, MG Assy, MA Al-Omar, I Ragab, *Eur J Med Chem*, **2012**, 45, 5935- 42

[12] RDL Fuente, W Namkung, R Mills, ASVerkman, *Mol Pharm*, **2008**, 73, 758–68.
[23] AS Oganisyan, S Noravyan, A Dzhagatspanyan, G Akopyan G, *Pharm Chem J*, **2001**, 35, 127-9.
[14] S Srivastava, B Das, Y RajendraPrasad, *Int J Chem Sci*, **2012**, 10(4), 1999-2009.
[15] S Srivastava, B Das, R Shukla, Y Rajendra Prasad, *Asian J Pharm Life Sci*, **2012**, 2(2), 234-251.
[16] S Srivastava, B Das, Y Rajendra Prasad, *Der Pharm Let*, **2012**, 4(4), 1214-1220.
[17] S Srivastava, B Das, *Der Pharm Chem*, **2011**, 3(6), 103-111.
[18] S Srivastava, B Das, *Int J Chem Sci*, **2009**, 7(3), 1779-1783.
[19] S Srivastava, B Das, M Raghuprasad, S Mohan, *Asian J Chem*, **2007**, 19(4), 2813-2817.
[20] B Das, S Srivastava, J Sarvanan, S Mohan, *Asian J Chem*, **2007**, 19(5), 4118-20.
[21] B Das, S Srivastava, SC Dinda, Y Rajenrda Prasad, *Der Pharm Chem*, **2013**, 5(1), 161-166.
[22] R Romagnoli, PG Baraldi, CL Cara, E Hamel, G Basso, R Bortolozzi, G Viola, *Eur J Med Chem*, **2010**, 45, 5781-91.
[23] G Elias, MNA Rao, *Indian J Exp Biol*, **1988**, 26, 540-2.