Int.J.Curr.Microbiol.App.Sci (2016) *5*(1): 364-375

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 1(2016) pp. 364-375**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.501.034

Synthesis and Evaluation of Ethyl 2-(2-Cyano-3-(Substituted Phenyl) Acrylamido)-4, 5, 6, 7-Tetrahydrobenzo[b] Thiophene-3-Carboxylates for Antioxidant and Antibacterial Activities

K. Madhavi* and K. V. Ramanamma

Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam (Women's University), Tirupati – 517 502 (A.P), India

**Corresponding author*

Keywords

Substituted 2-aminothiophenes, Substituted benzaldehydes, Knoevenagel condensation.

Article Info

Accepted:
16 December 2015
Available Online:
10 January 2016

A B S T R A C T

A series of ethyl 2-(2-cyano-3-(substituted phenyl)acrylamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylates were prepared by knoevenagel condensation of active methylene group of ethyl 2-(2-cyanoacetamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate with substituted benzaldehydes in toluene containing catalytic amounts of glacial acetic acid and piperidine. All the compounds were purified by recrystallization from suitable solvent and characterized by spectral and analytical methods. The antioxidant properties and antibacterial effects of the synthesized compounds were studied. Among the series, compounds with 4-hydroxy substituent on phenyl ring exhibited greater antioxidant activity at 100 µM concentration. The results of antibacterial activity revealed that 4-dimethyl amino derivative exhibited highest activity against *B. subtilis, E.coli* and *S. aureus*.

Introduction

The chemistry of substituted 2-aminothiophenes has received much attention upon their convenient availability through the most versatile synthetic method developed by Gewald (Gewald et al., 1966). The core structure is formed in the multicomponent reaction between a ketone or aldehyde, an activated nitrile and sulfur in the presence of suitable base.

Substituted 2-aminothiophene derivatives are important heterocycles found in numerous biologically active compounds. Among which Olanzapine, an antipsychotic drug and Tinoridine, a potent nonsteroidal anti-inflammatory drug with anti-peroxidative properties are available in the market. Some of the molecules containing 2-aminothiophene structure are under development (Alexander Domling, 2011). In particular AX20017, a tetrahydrobenzo[b] thiophene was identified as a promising

compound with antituberculosis properties (Székely et al., 2008). Further, in our laboratory cyanoacetylation of 2-amino-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate was carried out to give the respective N-substituted cyanoacetamide, 2-(2-cyanoacetamido)- 4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylate and it was evaluated as analgesic agent with antioxidant properties (Madhavi and Sudeepthi, 2012).

In the literature, synthesis of various non-natural acrylamides have been reported using N-substituted cyanoacetamides and evaluated for antitumor (Wei Zhou et al., 2009), antiparkinson's (Yasunori, et al., 1993), anti-inflammatory (Katsumi, et al., 1986), antioxidant activities (Madhavi Kuchana, 2014). Hence the present study has been planned to synthesize a series of non-natural acrylamides, ethyl 2-cyano-3-(substituted phenyl)acrylamido)-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carboxylate by knoevenagel condensation of 2-(2-cyanoacetamido)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate with various substituted aryl aldehydes and to evaluate all the compounds for *in vitro* antioxidant and antimicrobial activities.

Materials and Methods

All the chemicals were procured from Merck, Sigma Aldrich and SD fine of AR grade. Melting points were determined in open capillaries on a digital Stuart melting point apparatus and are uncorrected. UV spectra were recorded on Systronics UV-visible spectrometer. The IR spectra were recorded using KBr Pellets on a BRUKER Infrared spectrophotometer (cm^{-1}). ^1H NMR spectra were recorded on Avance-400 MHz spectrometer using TMS as internal standard (chemical shifts in δ ppm) using $CDCl_3$ as solvent. Mass spectra were recorded by Applied EI technique on Shimadzu QP 2011 PLUS GC-MS system. Purity of the compounds was checked by using the glass plates coated with Silica gel-G, and spots are detected by iodine vapour.

Synthesis of Ethyl 2-(2- cyanoacetamido)-4,5,6,7- tetrahydrobenzo [b] thiophene-3-carboxylate (Compound A)

1-Cyanoacetyl-3,5-Dimethylpyrazole (1.63 gm, 10 mM) was added to a solution of 1.0 equivalent amount of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate in toluene (10 ml). The mixture was refluxed for about 1 hr and the reaction was monitored by TLC. After completion of reaction the solution was cooled to room temperature. The crude product formed was filter and recrystallized with ethanol (Melting point is 111-113°C).

IR: 3256cm^{-1} (N-H), 2939cm^{-1}(C-H), 2259cm^{-1}(C≡N), 1649cm^{-1} (C=O), 1537cm^{-1}(C=C); ^1H NMR: δ 1.38-1.42 (t, 3H, -CH_3), δ 1.80-1.83 & δ 2.65-2.79 (m, 8H, CH_2), δ 3.36 (s, 2H, -CH_2-), δ4.45-4.50 (q, 2H, -CH_2-), δ 11.95 (s, N-H); Mass m/z 292(M^+).

General Method of Synthesis of Ethyl 2-(2-cyano-3- (substituted phenyl) acrylamido)- 4,5,6,7-tetrahydrobenzo[b] thiophene-3- carboxylates (Compounds B-M)

10 mM of compound A and 11mM of substituted benzaldehyde were added to 50 ml of toluene. To this mixture, 0.35 ml piperidine and 1.3 ml of acetic acid was added and refluxed for 5-6 hours. The completion of the reaction was monitored by performing TLC. Then the reaction mixture was cooled to room temperature, the precipitate was separated by filtration and recrystallized with suitable solvent.

Ethyl 2-(2- cyano-3-phenylacrylamido)-4,5,6,7- tetrahydrobenzo[b] thiophene-3-carboxylate(Compound B)

IR: 3418cm^{-1} (N-H), 2857cm^{-1}(Ar C-H), 2211cm^{-1}(C≡N), 1661cm^{-1}(C=O), 1528cm^{-1}(C=C); ^{1}H NMR: δ 1.38-1.42 (t, 3H, -CH_3), δ 1.80-1.82 & δ 2.67-2.82 (m, 8H, CH_2), δ 4.41-4.46 (q, 2H, -CH_2-), δ 7.50-7.58 & 7.99-8.01 (m, 5H, Ar), δ 8.40 (s, 1H, -CH=), δ 12.33(s, N-H); Mass m/z 380 (M^+).

Ethyl 2-(3- (4-chlorophenyl)-2-cyano-acrylamido) -4,5,6,7- tetrahydrobenzo[b] thiophene-3-carboxylate (Compound C)

IR : 3157cm^{-1} (N-H), 2983cm^{-1}(Ar C-H), 2216cm^{-1}(C≡N), 1665cm^{-1} (C=O), 1529cm^{-1}(C=C), 787cm^{-1}(C-Cl); ^{1}H NMR: δ 1.38-1.41 (t, 3H, -CH_3), δ 1.80-1.82 & δ 2.67-2.82 (m, 8H, CH_2), δ 4.40-4.46 (q, 2H, -CH_2-), δ 7.48-7.50 & 7.93-7.95 (m, 4H, Ar), δ 8.34 (s, 1H, -CH=), δ 12.35 (s, N-H); Mass m/z 414(M^+)

Ethyl 2-(2- cyano-3-p-tolylacrylamido)-4,5,6,7- tetrahydrobenzo[b] hiophene-3-carboxylate (Compound D)

IR : 3149cm^{-1} (N-H), 2928cm^{-1}(Ar C-H), 2211cm^{-1}(C≡N), 1663cm^{-1} (C=O), 1522cm^{-1}(C=C); ^{1}H NMR: δ 1.38-1.42 (t, 3H, -CH_3), δ 1.80-1.81 & δ 2.68-2.81 (m, 8H, CH_2), δ2.44 (s, 3H, -CH_3), δ 4.41-4.46 (q, 2H, -CH_2-), δ 7.27-7.33 & 7.90-7.92 (m, 4H, Ar), δ 8.36 (s, 1H, -CH=), δ 12.29 (s, N-H); Mass m/z 394 (M^+).

Ethyl 2-(2- cyano-3- (2-hydroxyphenyl) acrylamido)- 4,5,6,7-tetrahydrobenzo[b] thiophene-3- carboxylate (Compound E)

IR: 3380cm^{-1} (N-H), 3325cm^{-1} (O-H), 2979cm^{-1}(Ar C-H), 1930cm^{-1} (C≡N), 1675cm^{-1}(C=O), 1560cm^{-1}(C=C); ^{1}H NMR: δ 1.40-1.43 (t, 3H, -CH_3), δ 1.81-1.86 & δ 2.70-2.86 (m, 8H, CH_2), δ 4.40-4.45 (q, 2H, -CH_2-), δ 7.18-7.29 & 7.50-7.55 (m, 4H, Ar), δ 7.89 (s, 1H, -OH) δ 8.56 (s, 1H, -CH=), δ 14.06 (s, N-H); Mass m/z 396 (M^+).

Ethyl 2-(2-cyano-3-(4-hydroxyphenyl) acrylamido)- 4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate (Compound F)

IR: 3421cm^{-1} (N-H), 3241cm^{-1} (O-H),2944cm^{-1}(Ar C-H), 2212cm^{-1} (C≡N), 1664cm^{-1} (C=O), 1508cm^{-1}(C=C); ^{1}H NMR: δ 1.38-1.41 (t, 3H, -CH_3), δ 1.80-1.82 & δ 2.68-2.83 (m, 8H, CH_2), δ 4.40-4.46 (q, 2H, -CH_2-), δ 6.95-6.97 & 7.96-7.99 (m, 4H, Ar), δ 8.32 (s, 1H, -CH=), δ 12.27 (s, N-H); Mass m/z 396 (M^+).

Ethyl 2-(2- cyano-3-(4-hydroxy-3-methoxyphenyl) acrylamido) -4,5,6,7-tetra hydrobenzo[b] thiophene-3-carboxylate (Compound G)

IR: 3339cm^{-1} (N-H), 3171cm^{-1} (O-H), 2934cm^{-1}(Ar C-H), 2211cm^{-1} (C≡N), 1661cm^{-1}(C=O), 1511cm^{-1}(C=C), 1240 & 1028cm^{-1}(-C-O-C asymmetric & symmetric); ^{1}H NMR: δ 1.38-1.41 (t, 3H, -CH_3), δ 1.80-1.81 & δ 2.66-2.82 (m, 8H, CH_2), δ 3.89 (s,3H, -OCH_3), δ 4.39-4.45 (q, 2H, -CH_2-), δ 6.99-7.01 & 7.41-7.44 (m, 3H, Ar), δ 8.27 (s, 1H, -CH=), δ 12.24 (s, N-H), Mass m/z 426 (M^+).

Ethyl 2-(2- cyano-3- (4- hydroxy- 3,5-dimethoxyphenyl) acrylamido) -4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate (Compound H)

IR: 3449cm^{-1} (N-H), 2854cm^{-1}(Ar C-H), 2188cm^{-1} (C≡N), 1651cm^{-1}(C=O), 1534cm^{-1}(C=C), 1230 & 1033cm^{-1} (-C-O-C asymmetric & symmetric); ^{1}H NMR: δ 1.38-1.41 (t, 3H, -CH_3), δ 1.80-1.81 & δ 2.68-2.96 (m, 8H, CH_2), δ 3.90 (s, 6H, -OCH_3), δ 4.40-4.45 (q, 2H, -CH_2-), δ 7.26-7.36 (2s,

2H, Ar) δ 8.19 (s, 1H, -CH=), δ 12.13 (s, N-H); Mass m/z 456 (M^+).

Ethyl 2-(2- cyano-3- (4-methoxyphenyl) acrylamido)- 4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate (Compound I)

IR: 3171cm^{-1} (N-H), 2936cm^{-1}(Ar C-H), 2205cm^{-1}(C≡N), 1664cm^{-1}(C=O), 1523cm^{-1}(C=C), 1263 & 1021cm^{-1}(-C-O-C asymmetric & symmetric); ^{1}H NMR: δ 1.38-1.42 (t, 3H, -CH_3), δ 1.8-1.82 & δ 2.68-2.83 (m, 8H, CH_2), δ 3.90 (s, 3H, -OCH_3), δ 4.41-4.46 (q, 2H, -CH_2-), δ 7.0-7.02 & 8.0-8.02 (m, 4H, Ar), δ 8.32 (s, 1H, -CH=), δ 12.25 (s, N-H); Mass m/z 410 (M^+).

Ethyl 2- (2-cyano- 3-(3,5- dimethoxy-phenyl) acrylamido)- 4,5, 6,7-tetrahydro-benzo [b] thiophene- 3-carboxylate (Compound J)

IR: 3168cm^{-1} (N-H), 2932cm^{-1}(Ar C-H), 2204cm^{-1}(C≡N), 1666cm^{-1} (C=O), 1508cm^{-1}(C=C), 1254 & 1021cm^{-1} (-C-O-C asymmetric & symmetric); ^{1}H NMR: δ 1.41 -1.43 (t, 3H, -CH_3), δ 1.83-1.82 & δ 2.70-2.85 (m, 8H,CH_2), δ 3.90 & 4.0 (d, 6H, -OCH_3), δ 4.43-4.47 (q, 2H, -CH_2-), δ 7.52-7.54 & 7.80-7.81 (m, 3H, Ar), δ 8.33 (s, 1H, -CH=), δ 12.31 (s, N-H); Mass m/z 440 (M^+).

Ethyl 2-(2-cyano- 3-(3,4,5 -trimethoxy-phenyl) acrylamido) -4,5,6,7-tetrahydro-benzo[b] thiophene-3- carboxylate (Compound K)

IR: 3171cm^{-1} (N-H), 2927cm^{-1} (O-H), 2842cm^{-1}(Ar C-H), 2205cm^{-1} (C≡N), 1665cm^{-1}(C=O), 1531cm^{-1}(C=C), 1250 & 1030cm^{-1}(-C-O-C asymmetric & symmetric); ^{1}H NMR: δ 1.38-1.42 (t, 3H, -CH_3), δ 1.79-1.83 & δ 2.68-2.82 (m, 8H, CH_2), δ 3.96 (s, 9H, -OCH_3), δ 4.40-4.46 (q, 2H, -CH_2-), δ 7.27-7.30 (2s, 2H, Ar), δ 8.28 (s, 1H, -CH=), δ 12.31 (s, N-H); Mass m/z 470 (M^+).

Ethyl 2-(2- cyano-3- (4 -isopropylphenyl) acrylamido)- 4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate (Compound L)

IR: 3153cm^{-1} (N-H), 2935cm^{-1}(Ar C-H), 2208cm^{-1}(C≡N), 1664cm^{-1}(C=O), 1526cm^{-1}(C=C); ^{1}H NMR: δ 1.38-1.42 (t, 3H, -CH_3), δ 1.80-1.82 & δ 2.68-2.83 (m, 8H, CH_2), δ 1.28-1.30 (d, 6H, CH-(CH_3)$_2$), δ 2.94-3.04(se, 7H, CH), δ 2.44 (s, 3H, -CH_3), δ 4.41-4.46 (q, 2H, -CH_2-), δ 7.37-7.39 & 7.94-7.96 (m, 4H, Ar), δ 8.38 (s, 1H, -CH=), δ 12.30 (s, N-H); Mass m/z 422 (M^+).

Ethyl 2- (2-cyano-3- (4- (dimethylamino phenyl) acrylamido)-4,5,6,7-tetrahydro-benzo[b] thiophene- 3-carboxylate (Compound M)

IR: 3414cm^{-1} (N-H), 2932cm^{-1}(Ar C-H), 2197cm^{-1}(C≡N), 1660cm^{-1} (C=O), 1512cm^{-1}(C=C); ^{1}H NMR: δ 1.38-1.41 (t, 3H, -CH_3), δ 1.80-1.81 & δ 2.66-2.82 (m, 8H, CH_2), δ 3.11(s, 6H, -N(CH_3)$_2$), δ 4.40-4.45 (q, 2H, -CH_2-), δ 6.70-6.72 & 7.93-7.95 (m, 4H, Ar), δ 8.21 (s, 1H, -CH=), δ 12.10 (s, N-H); Mass m/z 423 (M^+).

Antioxidant Studies

Interaction with Stable Free Radical 1,1-Diphenyl Picrylhydrazyl (DPPH)

Solutions of test compounds and reference standard at 100 μM concentration were added to 100 μM DPPH in 95% ethanol. These solutions were kept at an ambient temperature for 20 minutes and absorbance was measured at 517 nm (Blios *et al*., 1958). Control experiment was carried out with solvent only. All the measurements were run in triplicate and the absorbance percentage inhibition of DPPH by the test compounds was calculated using the following formula,

Inhibition Percentage = [Control-Test]/Control × 100

Assay of Nitric Oxide (NO) Scavenging Activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by Griess reagent. Sodiumnitroprusside (10μM) in phosphate buffer pH 7.4; was incubated with 100 μM concentrations of test compounds dissolved in methanol and the solutions were incubated at 25°C for 150 minutes. Control experiment was conducted in an identical manner without test compound but with equal amount of solvent. The incubation solution (2 ml) was removed and diluted with 2 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent napthylethylene diamine was measured at 546 nm (Sreejayan and Rao MNA 1997). The results were obtained in triplicate and the percentage of scavenging activity was calculated as follows:

Percentage Scavenging = [Control-Test]/Control × 100

Iron Induced Lipid Peroxidation in Rat Brain Homogenate

Male albino rats weighing 180-200 g of either sex were used for study. Prior to decapitation and removal of brain, the animals were anesthetized with ether and perfused transcardially with ice-cold normal saline to prevent contamination of brain tissue with blood. Tissue was weighed and homogenate (10% w/v) was prepared in 0.15 M KCl and centrifuged at 800 g for 10 minutes. The supernatant was used immediately for the study. The incubation mixture contained in a final volume of 1 ml, brain homogenate (0.5 ml), KCl (0.15 M) and ethanol (10 μl) or test compound dissolved in ethanol. Peroxidation was initiated by adding, Fe^{3+} (100 μM) to give the final concentration stated. After incubating for 20 minutes at 37ºC, reactions were stopped by adding 2 ml of ice-cold 0.25 M HCl containing 15% trichloroacetic acid, 0.38% thiobarbituric acid and 0.05% BHT. Following heating at 80ºC for 15minutes samples were cooled and centrifuged at 1000g for 10 minutes. The absorbance of the supernatant was measured at 532 nm. Percentage inhibition of TBARS formed by test compounds was calculated by comparing with vehicle only experiments (Sreejayan and Rao MNA. 1994).

Superoxide Scavenging Activity

The Scavenging activity towards the superoxide radical ($O_2^{\cdot-}$) was measured in terms of inhibition of generation of $O_2^{\cdot-}$ by following alkaline DMSO method. 0.3g Potassium superoxide and 0.4ml of dry DMSO were allowed to stand in contact for 24hr and the solution was filtered immediately before use. Filtrate (200 μl) was added to 2.8ml of an aqueous solution containing NBT (56 μM), EDTA (10 μM) and potassium phosphate buffer (10 mM). Test compounds (1ml) at 100μM concentration were added, the absorbance was recorded at 560 nm against control (Shirwaikar et al., 2006).

Antibacterial Activity

The antibacterial activity of synthesized compounds at a concentration 1mg/ml was conducted against two gram positive bacteria viz., B. *subtilis* and S. *aureus* and gram negative bacteria viz., E.*coli* by using cup plate method. Streptomycin was employed as standard to compare the results.

The nutrient agar medium was sterilized by

autoclaving at 121ºC (15lb/sq inches) for 15 min. The petriplates, boiling tubes and flasks plugged with cotton were sterilized in hot-air oven at 160ºC, for an hour. Into each sterilized petriplate (10cm diameter), about 27ml of molten nutrient agar medium was poured and inoculated with the respective strain of bacteria (6ml of inoculum to 300ml of nutrient agar medium) was transferred aseptically. The plates were left at room temperature to allow the solidification. In each plate, four cups of 6mm diameter were made with sterile borer. Then 100µl of the test solution was added to the respective cups aseptically and labeled, accordingly. The plates were kept undistributed at 37ºC for at least 24hours to allow diffusion of the solution properly into nutrient agar medium. Then plates were examined for clear zones of inhibition.

All the experiments were carried out in quadruplets. Simultaneously, controls were maintained employing 100µl of dimethyl formamide to observe the solvent effects. The diameter of zone of inhibition (mm) was measured as an indicator for antibacterial activity of the compounds.

Results and Discussion

Chemistry

In the present research work, ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate and 1-cyanoacetyl-3,5-dimethylpyrazole were synthesized based on the reported methods. Cyanoacetylation of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate with 1-cyanoacetyl-3,5-dimethylpyrazole yielded ethyl 2-(2-cyano-acetamido)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate as an intermediate, which was purified and utilized in the synthesis of title compounds (Scheme-I).

Ethyl 2-(2-cyano-3-(substituted phenyl) acrylamido)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylates were synthesized by knoevenagel condensation of active methylene group of ethyl 2-(2-cyano-acetamido)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate with various substituted benzaldehydes in toluene containing catalytic amounts of piperidine and acetic acid. The reaction was completed within 5-6 hours, which gave the title compounds almost in pure form. These compounds were obtained in good yields ranging from 55-95%. All the compounds were recrystallized with ethanol and characterized by their physical data (table-1). The structures of these compounds were confirmed by IR, ^{1}H NMR and Mass spectra.

The IR spectrum of intermediate, ethyl 2-(2-cyanoacetamido)-4,5,6,7-tetra-hydrobenzo[b] thiophene-3-carboxylate, showed absorption bands at 3256 cm^{-1} and 1649 cm^{-1} indicative of N-H stretching and C=O stretching corresponds to amide group. An absorption band at 2259 cm^{-1} indicates C≡N stretching.

The IR spectra of final compounds showed an absorption band at 3449-3148 cm^{-1} indicative of N-H stretching. The absorption band in the region of 3009-2854 cm^{-1} indicates aromatic C-H stretching. The absorption band corresponding to C≡N stretching was appeared in the region of 2216-1928 cm^{-1} and the carbonyl group of amide appeared in the region of 1675-1651cm^{-1}. Compounds containing phenolic hydroxyl group showed an absorption band in the region of 3325-3171 cm^{-1} indicative of O-H stretching. Compounds with methoxy substitution exhibited absorption band in the region of 1263-1230 cm^{-1} and 1033-1221 cm^{-1} due to C-O-C asymmetric and symmetric stretching vibrations.

The ^{1}H NMR spectrum of intermediate, ethyl 2-(2-cyanoacetamido)-4,5,6,7-tetra-

hydrobenzo[b] thiophene-3- carboxylate showed triplet in the region of δ 1.38-1.42 due to methyl protons of ethyl ester group. Multiplets in the region of δ 1.80-1.83 and δ 2.65-2.79 due to -CH_2- protons of tetra-hydrobenzo[b]thiophene. The methylene protons of -CH_2 appeared as singlets in the region of δ 3.36. The spectra also exhibited quartets in the region of δ 4.45-4.50 due to -CH_2- protons of ethyl ester. Singlet in the region of δ 11.95 indicates of NH proton.

The NMR spectra of title compounds (B-M) showed triplet in the region of δ 1.30-1.49 due to methyl protons of ethyl ester. Multiplets in the region of δ 1.80-1.82 and δ 2.67-2.82 due to -CH_2- protons of tetrahydrobenzo[b] thiophene. The spectra also exhibited quartets in the region of δ 4.45-4.67 due to -CH_2- protons of ethyl ester. Multiplets in the region of δ 6.42-8.01 were assignable to aromatic protons. The spectra of the compounds showed singlets in the region of δ 8.18-8.40 due to benzylidene protons and also singlets in the region of δ 12.09-12.35 indicative of NH protons of amide group. Compounds containing methoxy group exhibited characteristic signals in the region of δ 3.89-4.0. The methyl protons of dimethylamino derivatives appeared as singlets in the region of δ 3.11. The disappearance of signal corresponding to active methylene protons of intermediate at δ 3.36 in all the NMR spectra of final compounds and appearance of benzylidene protons at δ 8.18-8.40 confirms the structures of title compounds.

The Mass spectra of the intermediate and the final compounds showed their characteristic molecular ion peak. Thus, the structures of the compounds were confirmed by IR, NMR and Mass spectral data.

Antioxidant Studies

All the compounds were tested for their antioxidant properties in four different methods viz., interaction with stable free radical DPPH, scavenging of Nitric Oxide free radical, inhibition of iron induced lipid peroxidation and ability to scavenge Super Oxide at 100 μM concentration. The activity data was presented in Table-2. Among all the evaluated compounds ethyl 2-(2-cyano-3-(4-hydroxy-3,5-dimethoxyphenyl) acrylamido)-4,5,6,7-tetra-hydrobenzo[b] thiophene-3-carboxylate (Compound H) showed grater activity comparable to that of standard employed. The highest activity of this compound may be attributed to sterically hindered phenolic moiety. The activity data revealed that ethyl 2-(2-cyano-3- (4-hydroxy- 3-methoxyphenyl) acrylamido)- 4,5,6,7- tetrahydrobenzo[b] thiophene-3- carboxylate (compound G) and ethyl 2-(2-cyano- 3-(4-hydroxyphenyl) acrylamido)- 4,5,6,7- tetrahydrobenzo[b] thiophene-3-carboxylate (Compound F) also exhibited good activity. The reason may be due to the presence of phenolic hydroxyl group at 4^{th} position on phenyl ring.

On change of hydroxyl group from 4^{th} position to 2^{nd} position on phenyl ring as in compound E (Ethyl 2-(2-cyano-3-(2-hydroxy-phenyl) acrylamido)-4,5,6,7-tetrahydrobenzo [b]thiophene-3-carboxylate) causes reduction in activity. Conversion of hydroxyl group into methoxy group also resulted in drastic reduction in their activity. Further, the activity data of the compounds in four different models was found to be correlative with each other. This observation clearly indicates that the phenolic moiety of compounds confer antioxidant and radical scavenging properties.

Antibacterial Activity

The antibacterial activity of synthesized compounds was conducted against two gram positive bacteria *B. subtilis, S. aureus* and gram negative bacteria *E.coli* by cup plate method. Streptomycin was employed as

standard to compare the results. The data was presented in Table-3.

From the antibacterial activity data, it was found that the synthesized compounds exhibited mild to good antibacterial activity against *B.subtilis*, *E. coli* and*S. aureus*, at a concentration of 100µg/ml. Ethyl 2-(2-cyano-3-(4-dimethylaminophenyl) acrylamido)-4,5 ,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate (Compound M) exhibited maximum zone of inhibition against *B.subtilis* (16 mm), *E. coli* (17mm) and *S.aureus* (14mm).

These results were almost nearer to the results obtained with the standard drug, Streptomycin. The standard drug (Streptomycin) gave 17mm zone of inhibition against *B.subtilis*, 19mm zone of inhibition against *E. coli* 15mm zone of inhibition against *S. aureus*. Replacement of 4-dimethylamino group on phenyl ring in compound M with its isosteric equivalent 4-isopropyl group, as in compound L (2-(2-cyano-3- (4-isopropylphenyl) acrylamido)-4,5,6,7- tetrahydrobenzo[b] thiophene-3-carboxylate) causes reduction in antibacterial activity.

Greater antibacterial activity of 4-dimethyl amino derivative may be due to the availability of lone pair of electrons on electronegative nitrogen atom.

From the activity data it is evident that compounds containing phenolic hydroxyl group at *para* position also exhibited good activity. Since, several title compounds possess good antibacterial activity, ethyl 2-(2-cyano-3-(substituted phenyl)acrylamide)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate could be used as a template for the future development of more potent therapeutic agents.

Scheme-I

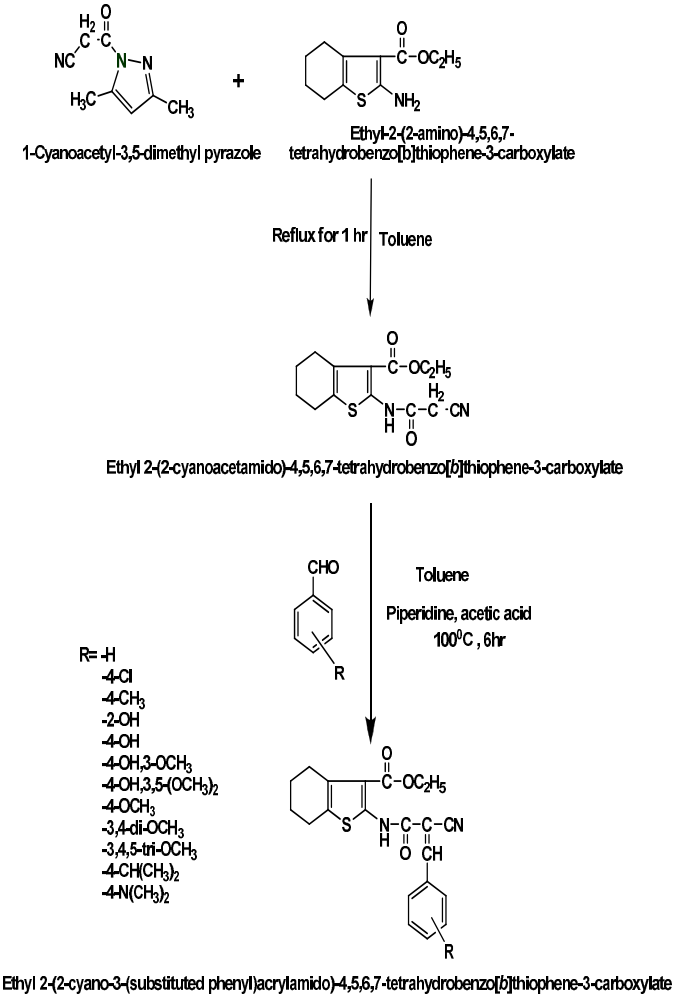


Table.1 Physical Data of Ethyl 2-(2-Cyano-3-(Substituted Phenyl)Acrylamido)-4,5,6,7-Tetrahydrobenzo[b]Thiophene-3-Carboxylates (B-M)

COMPOUND	R	M.F	M.W	M.P (°C)	YIELD (%)	R_f*
B	H	$C_{21}H_{20}N_2O_3S$	380	208-210	65	0.76
C	4-Cl	$C_{21}H_{19}N_2O_3SCl$	414	209-211	74	0.71
D	4-CH_3	$C_{22}H_{22}N_2O_3S$	395	218-220	90	0.85
E	2-OH	$C_{21}H_{20}N_2O_4S$	396	223-225	54	0.88
F	4-OH	$C_{21}H_{20}N_2O_4S$	396	272-274	94	0.88
G	4-OH,3-OCH_3	$C_{22}H_{23}N_2O_5S$	426	216-218	88	0.48
H	4-OH,3,5-$(OCH_3)_2$	$C_{23}H_{25}N_2O_6S$	456	210-212	79	0.69
I	4-OCH_3	$C_{22}H_{22}N_2O_4S$	411	247-249	72	0.88
J	3,4-di-OCH_3	$C_{23}H_{24}N_2O_5S$	441	218-220	69	0.76
K	3,4,5-tri-OCH_3	$C_{24}H_{27}N_2O_6S$	471	203-205	83	0.60
L	4- $CH(CH_3)_2$	$C_{24}H_{26}N_2O_3S$	422	212-214	76	0.90
M	4-$N(CH_3)_2$	$C_{23}H_{25}N_3O_3S$	423	256-258	81	0.56

R_f* solvent system – 10:1 ratio of chloroform and methanol

Table.2 Antioxidant Activity of Ethyl 2-(2-Cyano-3-(Substituted Phenyl)Acrylamido)-4,5,6,7-Tetrahydrobenzo[b]Thiophene-3-Carboxylates (B-M)

COMPOUND	R	% Inhibition of DPPH at 100µM	% Scavenging of Nitric Oxide at 100µM	% Inhibition of Lipid Peroxidation at 100µM	% Scavenging of Super Oxide at 100µM
B	H	11.0	56.7	32.3	35.2
C	4-Cl	33.7	52.9	16.4	51.7
D	4-CH_3	17.8	33.9	17.2	17.6
E	2-OH	34.1	46.1	48.6	60.1
F	4-OH	63.1	78.1	52.2	61.3
G	4-OH,3-OCH_3	66.7	70.2	62.1	67.4
H	4-OH,3,5-$(OCH_3)_2$	73.4	82.1	75.3	70.4
I	4-OCH_3	15.9	35.5	16.3	55.6
J	3,4-di-OCH_3	19.1	41.1	14.9	52.8
K	3,4,5-tri-OCH_3	24.0	38.6	26.3	51.0
L	4-$CH(CH_3)_2$	28.7	36.8	19.8	49.2
M	4-$N(CH_3)_2$	15.7	52.1	11.4	46.6
Standard	Ascorbic acid	77.08	89.6	-	78.8
Standard	α-Tocopherol	-	-	78.9	-

Table.3 Anti Bacterial Activity Data of Ethyl 2-(2-Cyano-3-(Substituted Phenyl)Acrylamido)-4,5,6,7-Tetrahydrobenzo[b]Thiophene-3-Carboxylates (B-M)

COMPOUND	R	Zone of inhibition (mm)		
		B.subtilis **(+ve)**	***E.coli*** **(-ve)**	***S.aureus*** **(+ve)**
B	H	07	09	10
C	4-Cl	10	12	11
D	4-CH_3	11	12	11
E	2-OH	09	11	10
F	4-OH	14	12	11
G	4-OH,3-OCH_3	16	13	10
H	4-OH,3,5-$(OCH_3)_2$	13	11	09
I	4-OCH_3	14	10	13
J	3,4-di-OCH_3	09	11	11
K	3,4,5-tri-OCH_3	10	09	13
L	4-$CH(CH_3)_2$	12	09	10
M	4-$N(CH_3)_2$	16	17	14
Standard	Streptomycin	17	19	15

Fig.1 Antioxidant Activities of Ethyl 2-(2-Cyano-3-(Substitutedphenyl)Acrylamido)-4,5,6,7-Tetrahydrobenzo[b]Thiophene-3-Carboxylates (B-M)

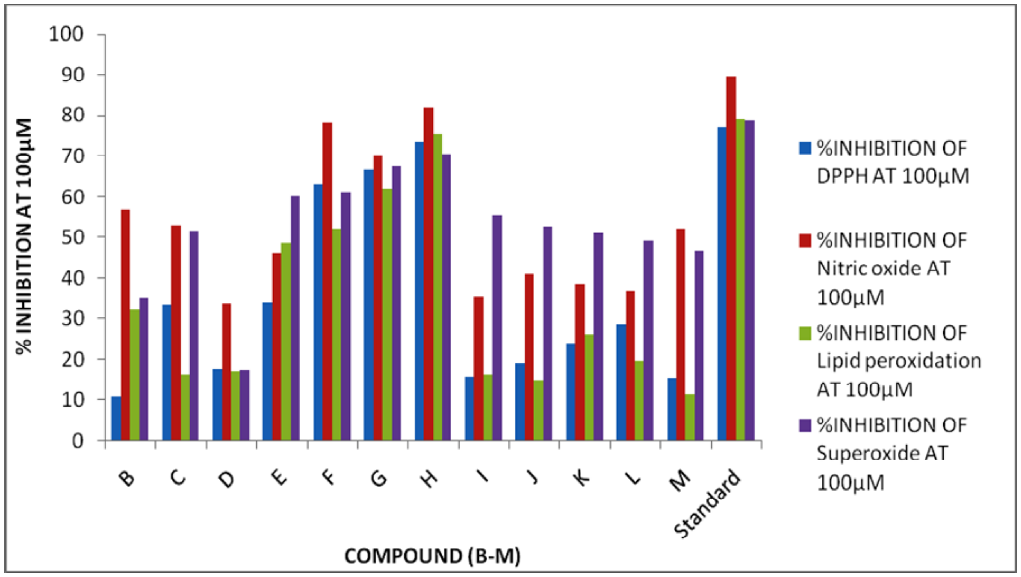
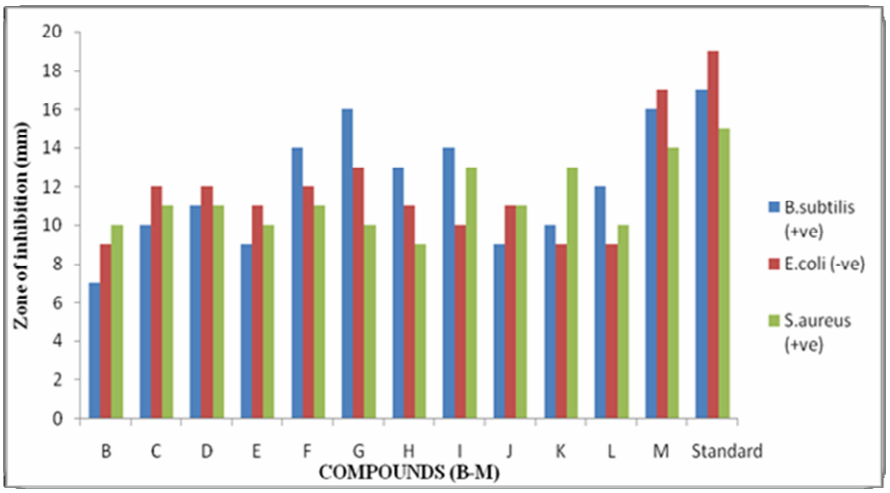


Fig.2 Antibacterial Activitiy of Ethyl 2-(2-Cyano-3-(Substitutedphenyl)Acrylamido)-4,5,6,7-Tetrahydrobenzo[b]Thiophene-3-Carboxylates (B-M)



In conclusion, the present work revealed that the compounds of ethyl 2-(2-cyano-3-(substituted phenyl) acrylamido)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxylate containing phenolic substitution exhibited greater activity. In particular, 4-hydroxy-3,5-dimethoxy derivative showed highest antioxidant activity in all four different models (Fig-1). This clearly indicates that sterically hindered phenolic moiety of the above compound conferred good antioxidant and radical scavenging properties. Ethyl 2-(2-cyano-3-(substitutedphenyl) acrylamido)-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboylate compounds were also screened for antibacterial activity. Among the series, the compound containing dimethylamino substitution (Compound M) showed maximum zone of inhibition against *B.subtilis* (15mm), *E. coli* (17mm) and *S.aureus* (14mm). These results were almost comparable to that of standard drug, Streptomycin (Fig-2). Since, these compounds possess good antioxidant and antibacterial activities, further investigations needed to be carried out to know their toxicity and therapeutic value.

References

1. Gewald K, Schinke E and Bottcher H. 1966. Heterocyclenaus CH-aciden Nitrilen VIII. 2-Amino-thiophene aus methylenaktiyen Nitrilen, Cabonylverbindungen und Schwefel. ChemBer. 99(1): 94-100.
2. Alexander DomlingYH. 2011. The Gewald multi component reaction. Molecular Diversity. 15(1): 3-33**.**
3. Székely R, Wáczek F, Szabadkai I, Németh G, Hegymegi-Barakonyi B, Eros D, Szokol B, Pató J, Hafenbradl D, Satchell J, Saint-Joanis B, Cole ST, Orfi L, Klebl BM andKéri G. 2008. A novel drug discovery concept for tuberculosis: inhibition of bacterial and host cell signaling.Immunol Lett 116(2): 107-115.
4. Madhavi K and Sudeepthi P. 2012. Synthesis of cyanoacetylated derivatives some heteroaryl amines as analgesic and antioxidant agents. International Journal of Pharmaceutical Sciences and Nanotechnology. 5(4): 1879-1884

5. Wei Zhou, Hai-bo Li, Chun-nian Xia, Xian-ming Zheng and Wei-xiao Hua. 2009. The synthesis and biological evaluation of some caffeic acid amide derivatives: E-2-Cyano-(3- substituted phenyl) acrylamides. Bioorg. Med. Chem. Letters**.**19: 1861–1865.
6. Yasunori K, Hisao T, Koichi S, Hiroto H, Hideo N and Toshiko O. 1993. Styrene derivatives. EP 0537742 A3.
7. Katsumi I, Kondo H, Fuse Y, Yamashita K, Hidaka T, Hosoe K, Takeo K, Yamashita T and Watanabe K. 1986. Synthesis and anti-inflammatory activity of 3,5-Di-tert-butyl-4-hydroxystyrenes. Chem. Pharm. Bull. 34 (4): 1619-1627.
8. Madhavi Kuchana. 2014. Synthesis and *in vitro* antioxidant activity of substituted α-cyano-N- (5- methyl-isoxazol- 3yl) cinnamides. World Journal of Pharmacy and Pharmaceutical Sciences. 3(6): 1800-1808.
9. Blois MS. 1958. Antioxidant determination by the use of stable free radical. Nature. 181: 119-120.
10. Sreejayan, and Rao MNA. 1997. Nitric oxide scavenging by curcuminoids. Pharm.Pharmcol. 49: 105-107.
11. Sreejayan and Rao MNA. 1994. Curcuminoids as potent inhibitors of lipid peroxidation. Pharm Pharmacol. 46(12): 1013-1016.
12. Shirwaikar A, Prabhu KS and Punitha ISR. 2006. *In vitro* antioxidant studies of *Sphaeranthus indicus* (Linn). Indian J Exp Biol. 44: 993-996.

How to cite this article:

K. Madhavi and K. V. Ramanamma. 2016. Synthesis and Evaluation of Ethyl 2-(2-Cyano-3-(Substituted Phenyl) Acrylamido)-4,5,6,7-Tetrahydrobenzo[b]Thiophene-3-Carboxylates for Antioxidant and Antibacterial Activities. *Int.J.Curr.Microbiol.App.Sci.* 5(1): 364-375
http://dx.doi.org/10.20546/ijcmas.2016.501.034