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Synthesis and anticancer / antibacterial activity of compounds containing thiophene ring linked to a chalcone derivatives

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Abstract: In the context a novel series of new anticancer, antibacterial targets and in aspect of potential several thiophene linked to chalcone derivatives described in the present study. In present work **9** with remarkable improvement in lipophilicity as compared to the parent thiophenes was designed, synthesized, and characterized by ^{1}H NMR, ^{13}C NMR, IR and Mass spectral analysis. We have synthesized some 5-aryl-thieno[3,2-b]thiophene-chalcone derivatives (**9a-9j**) from 5-bromo-thieno[3,2-b]thiophene-2-carbaldehyde **7**. Among the new derivatives tested **9a, 9b, 9f,** and **9i** exhibited most potent activity against *S.aureus* NCCS2079*, E.coli* MTCC739 and *P.aeruginosa* NCCS2200 bacterial strains which is more promising than Ampicillin. The compounds **9a, 9g, 9d,** and **9j** were showed good antifungal activity against fungal strains *C. albicans, A. flavus* and *A. fumigatus*. Completely a good number of these analogues exhibited superior cytotoxicity than compared to the reference compound. In the series, **9g**, **9h, 9d** best antitumor activity was display with highest % of inhibition in A549 cell line compared reference Doxorubicin. Compound **9e, 9b** indicating the potential cytotoxic properties of the new compounds against SKNSH cancer cell line.

Keywords: Claisen-schmidt condensation, suzuki-coupling, vilsmeier-haack reaction, antibacterial activity, cytotoxicity.

Introduction

Thiophenes, a significant class of heterocycles and have found to great interest in a variety of fields from medicinal chemistry to material science. They are also frequently found in various pharmaceuticals and drug candidates [1], semiconductors [2], liquid crystals [3] and other molecular functional materials [4]. Thieno[3,2-b]thiophenes skeletons

are important in pharmaceutical research because of their versatile biological activities such as antitumor, antiviral, antibacterial, anticancer, antioxidant and α-glucuronidase and α-glucosidase inhibition, anti glaucoma activity, and inhibitors of platelet aggregation properties [5]. The Vilsmeier-Hacck reagent is an excellent, eco friendly and mild reagent for the formylation of reactive aromatic and hetero aromatic molecules. The application of the vilsmeier-haack reagent [$POCl_3$/DMF] for the formylation of variety of aryl and hetero aryl compounds [6]. Additionally, its enaminones derivatives are versatile synthons and have a lot of synthetic applications such as N-heterocycles, wide variety of naturally occurring alkaloids and pharmaceutical drugs. The efficiency of palladium-catalyzed Suzuki cross-coupling for the reaction of aryl boronic acids was well documented in the literature [7].

From literature review, the chemistry of bimolecular coupling of 5-aryllation with 5-bromo thieno[3,2-b]-thiophene-2-carbaldehyde derivatives via Pd-catalyzed has not been reported. It consists of directly arylating hetro aromatics via a C-H bond activation of the heteroarenes chalcones [8] are flavonoid and isoflavonoid precursors which are abundant in edible plants and are considered as important intermediate in the flavonoid biosynthesis. Chalcones are flavonoid and isoflavonoid precursors [9] which are abundant in edible plants and are considered as important intermediate in the flavonoid biosynthesis. Chalcones of heterocycles from nature or synthetic origin exhibit wide and diverse pharmacological activities like antioxidant [10], antibacterial [11], anti-leishmanial [12,13] anticancer [14,15,16] antiangiogenic [17] anti-infective, anti-inflammatory activities [18] and are widely used in traditional medicine practices [19]. Crotaorixin, Hydroxyderricin, Crotmadine, Chromenochalcone, Xanthohumol B (I-V in **Fig. 1**) and furan, salicyclic acid based chalcone derivatives (VI-VIII in **Fig. 1**) are the examples of naturally occurring and synthetic thiophene-chalcone derivatives with numerous biological activities [20] (**Fig. 1**).

Herein, we report synthetic details of the preparation of the 5-arylthieno[3,2-b]thiophene linked to chalcone derivatives and the analysis of the structure–activity relationships in terms of their anticancer, antibacterial activities.

EXPERIMENTAL

Chemistry

Materials and Methods

All the used reagents and solvents were obtained from commercial sources and were of analytical grade, melting points were determined by open capillary method. IR spectra were recorded on a Perkin Elmer spectrometer in KBr pellets. ^{1}H NMR (DMSO-d_6 300, 400MHz) and ^{13}C NMR (DMSO-d_6, 75, 125 MHz) were recorded on spectrometer TMS as internal standard (chemical shifts and ppm). Mass spectra were recorded on a VG micro mass 70-70 Hz instrument. All the reactions were monitored by TLC on pre coated silica gel plates (60F 254; Merck). Column chromatography was performed on 100-200 mesh silica gel using 10-20 fold excess (by weight) of the crude product. The organic extracts were dried over anhydrous Na_2SO_4.

General procedure for the synthesis of 3-bromothiophene-2-carbaldehyde (2)

To a solution mixture of DMF (6.7 g, 1.2 mol) and $POCl_3$ (1.4 g, 1.2 mol) stirred at 0 °C for 1 hr, compound **1** (2 g, 1.0 mol) was added, and heated to 90 °C for 40 hr. After checked TLC, reaction was completed and then quenched with ice – water, extracted by Diethyl ether 3 x 75 mL, Total organic layers were dried over anhydrous

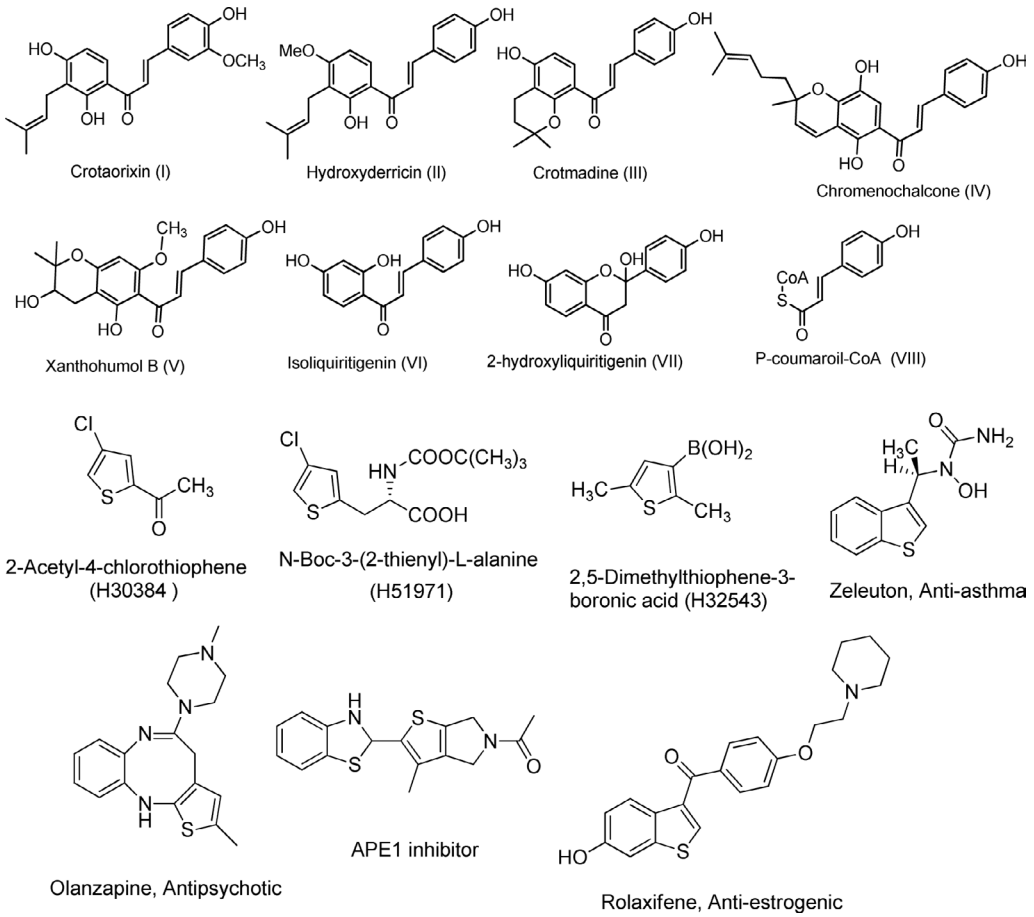
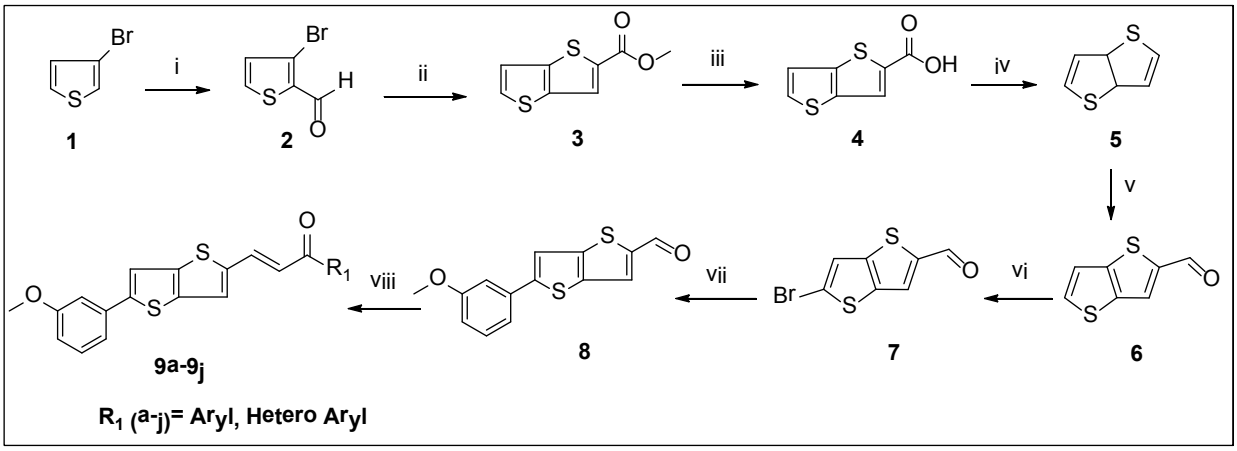


Figure 1: Naturally occurring (**I-V**) and synthetic aryl/heteroaryl chalcones (**VI-VIII**) and different biological importance of thiophene ring contains heterocycles



Scheme 1: Synthesis of chalcone-thiophene derivatives (**9a-9j**) Reagent and Conditions: i) $POCl_3$/ DMF, 1 hr, 65 % ii) Methyl thioglycolate, DMF/ K_2CO_3, rt, 3 hr, 83 % iii) LiOH/ THF/H_2O, 65 °C, 2 hr, 90 % iv) neat reaction/reflux, 2 hr, 230 °C, 60 % v) $POCl_3$/DMF, 90 ° C, 5 hr, 97 % vi) NBS/DMF, 25 °C, 2 hr, 80 % vii) DME/Pd $(Ph_3P)_4$/ K_2CO_3, reflux, 5 hr, 60 % viii) Substituted aromatic acetophenones, NaOH in methanol (2 molar), rt, 5-8 hr, 91-94 %.

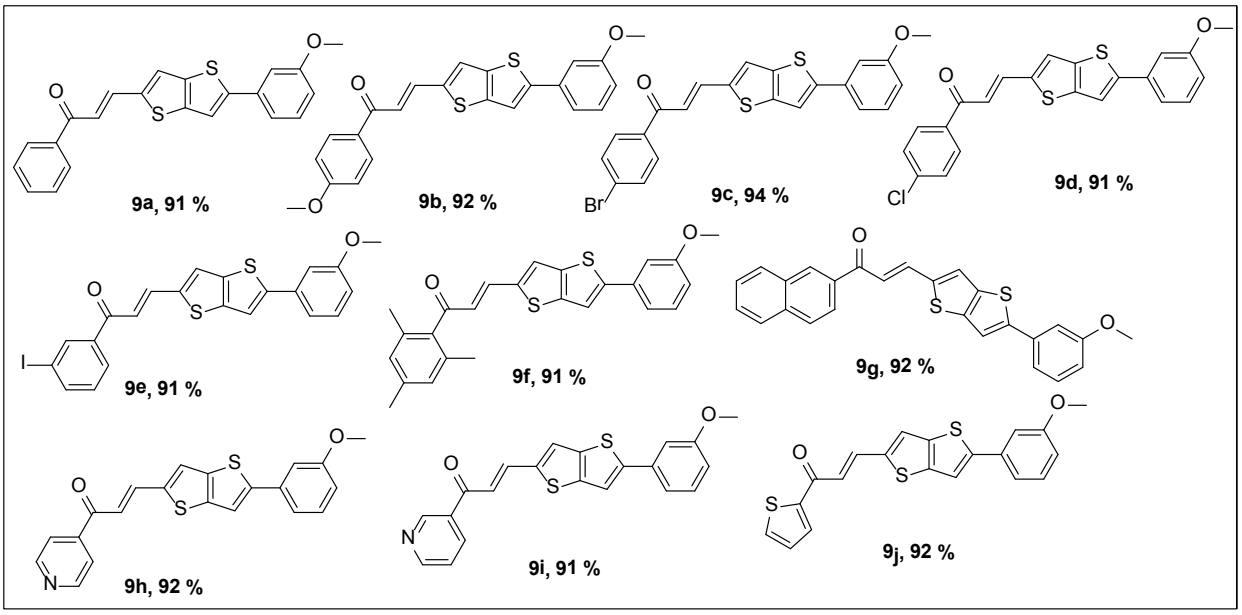


Figure 2: Chemical structures and yields of target compounds (**9a-9j**)

sodium sulphate Na_2SO_4 and distilled reduced pressure to get the desired compound (**2**) in 65 % yield.

Synthesis of thieno[2,3-b]thiophene-2-carboxylic acid methyl ester (3)

3-bromo-thiophene-2-carbaldehyde-2 (**2**) (2.5 g, 1.3 mol) was added to a stirred mixture of methyl thioglycolate (1.8 g, 1.3 mol), potassium carbonate (5.4 g, 3.9 mol) and DMF (25 mL) at ambient temperature and the resulting mixture was stirred about 3 hr. The reacting solvent DMF was removed under reduced pressure. Then the residual was poured into ice water (500 mL) and the extracted with diethyl ether. The combined organic layers were dried over anhydrous Na_2SO_4 and the organic layer was concentrated to get the residue and isolated by hexane afford **3** as white powder. White solid, yield 83 %, mp 93-95 °C, IR (KBR, cm^{-1}) υ_{max}; 1656 (C=O) 1545, 1535 (C=C), 1310 (C-S-C), 1241 (C-O-C); ^{1}H NMR ($CDCl_3$, δ ppm): 8.09 (s, 1H, =CH), 7.64 (d, 1H, *J* = 5.6, =CH), 7.31 (d, 1H, *J* = 5.2, =CH), 3.80 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, δ ppm) 160.6, 134.4, 133.5, 128.0, 125.5, 120.2, 51.5; LC-MS: 199 $(M+1)^+$.

Synthesis of thieno[3,2-b]thiophene-2-carboxlic acid (4)

A mixture of lithium hydroxide monohydride (1.5 g, 2.0 mol) was dissolved in water (15 mL), and direct added tetrahydrofuran (15 mL), finally added Compound **3** (0.8 g, 1.0 mol), the reaction mass was heated to 65 °C for 2 hr. Reaction is completed and then THF solvent was distilled out under reduced pressure and the reaction mass was cooled to 0 °C and then added water, pH is 2 with 6 N HCl to get the solid, solid was filtered and dried (1.8 g, 90 %). White solid, mp 218-223 °C, IR (KBR, cm^{-1}) υ_{max}; 1654 (C=O), 1565, 1534 (ring-C=C), 1260 (C-S-C); ^{1}H NMR ($CDCl_3$, δ ppm): 8.09 (s, 1H, =CH), 7.74 (d, 1H, *J* = 5.6, =CH), 7.31 (d, 1H, *J* = 5.2, =CH). ^{13}C NMR ($CDCl_3$, δ ppm): 162.6, 134.7, 133.5, 127.9, 125.6, 122.2, 120.2. LC-MS: 184 $(M+1)^+$.

Synthesis of thieno[3,2-b]thiophene (5)

A stirred solution of Acid Compound (**4**) (1.5 g,

0.5 mol) was heated at 230 °C in a sand bath for 2 hr, when TLC analysis confirmed the absence of starting material and reaction mass was cooled to ambient temperature and isolated by hexane. White solid, yield 60 %, mp: 53-55 °C, IR (KBR, cm^{-1}) υ_{max}; 1576, 1540 (C=C) 1280 (C-S-C), ^{1}H NMR ($CDCl_3$, δ ppm): 7.80 (s, 1H, =CH), 7.31 (s, 1H, =CH); ^{13}C NMR ($CDCl_3$, δ ppm) 143.2, 143.1, 125.6, 125.5, 120.2, 120.1; LC-MS: 140 $(M+1)^+$.

Synthesis of thieno[3,2-b]thiophene-2-carbaldehyde (6)

To a stirred solution of DMF (2.1 g, 1.2 mol) and $POCl_3$ (0.2 g, 1.2 mol) stirred at 0 °C for 3 hr. Compound (**5**) (2.5 g) was added, and heated to 90 °C for 5 hr. After check the TLC reaction was completed and then quenched with ice-water, extracted by diethyl ether 3 x 30 mL, total organic layers were dried over sodium sulphate and distilled reduced pressure to get the desired compound (**6**). White solid, yield 97 %, mp: 83-85 °C. IR (KBR, cm^{-1}) υ_{max}; 1638 (C=O), 1575, 1555 (C=C), 1265 (C-S-C), ^{1}H NMR ($CDCl_3$, δ ppm): 9.90 (s, 1H, CHO), 7.84 (s, 1H, =CH), 7.32 (s, 1H, =CH) ^{13}C NMR ($CDCl_3$, δ ppm) 182.5, 143.5, 136.7, 130.0, 125.6, 122.9, 120.2,; LC-MS: 168 $(M+1)^+$.

Synthesis of 5-bromo-thieno[3,2-b]thiophene -2-carbaldehyde (7)

To a stirred solution of thieno[3,2-b]thiophene-2-carbaldehyde (**6)** (3 g, 0.000178 mol) in DMF stirred at 0 °C for 3 hr, N-bromosuccinimide (3.1 g, 0.178 mmol) was added, the reaction mass was allowed to 25 °C for 2 hr. After checked the TLC reaction was completed, and then quenched with ice water, extracted by diethyl ether 3 x 300 mL, Total organic layers were dried over sodium sulphate and distilled reduced pressure to get the desired compound **7**. White solid, yield 80 %, mp: 85 °C, IR (KBR, cm^{-1}) υ_{max}; 1648 (C=O), 1585, 1560 (C=C), 1270 (C-S-C). ^{1}H NMR ($CDCl_3$, δ ppm): 9.93 (s, 1H, CHO), 7.84 (s, 1H, =CH), 7.35 (s, 1H, =CH); ^{13}C NMR ($CDCl_3$, δ ppm) 182.5, 143.5, 136.7, 130.4, 122.6, 122.5, 112.1; LC-MS: 246, 248 $(M + 2)^+$.

Synthesis of 5-(3-methoxyphenyl) thieno[3,2-b]thiophene-2-carbaldehyde (8)

Compound (**7**) (10 g, 0.040 mmol) dissolved in 1,2–dimethoxyethane DME (80 ml) was treated with $(Ph_3P)_4Pd$ (0.42 g, 0.0004 mmol) with 3-methoxy phenyl boronic acid (5.6 g, 0.040 mmol) and an aq. Solution of 2 molar potassium carbonate and the reaction was refluxed for 5 hr. The resulting reaction mass was cooled to ambient temperature. The solvent was partially removed under reduced pressure and the resulting mixture was extracted with dichloroethane. The organic layers were dried over sodium sulphate and evaporated under reduced pressure. The crude was purified by column chromatography using 10 % Ethyl acetate-Hexane to get the desired product **8**. Light yellow solid, yield 60 %, mp: 85 °C, IR (KBR, cm^{-1}) υ_{max}; 1678 (C=O), 1575, 1555 (C=C) 1310 (C-S-C), 1257 (C-O-C). ^{1}H NMR ($CDCl_3$, δ ppm): 9.91 (s, 1H, CHO), 7.97 (s, 1H, =CH), 7.24 (d, 1H, ArH), 7.23 (m, 1H, ArH), 7.17 (d, 1H, ArH), 6.98 (m, 2H, ArH), 3.87 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, δ ppm) 182.2, 161.2,143.5, 138.3, 138.2, 136.7, 130.3, 122.8, 121.9, 119.8, 119.6, 119.5, 110.5, 55.9; LC-MS: 275 $(M+1)^+$.

General procedure for the preparation of thiophene-chalcone derivatives (9a-9j)

Ethanones (**a-j**) (0.76 g, 1.459 mmol) dissolved in alkaline methanolic solution (2.9 g, 2 mol) stirred for 10 min and then added 5-(3-methoxyphenyl)thieno[3,2-b]thiophene-2-carbaldehyde (**8**) (1 g, 1.459 mmol) the resulting mixture was stirred at ambient temperature for 5-8 hr. The reaction mixture was diluted with

water (10 mL) and filtered to get the solid and air dried to give 91-94 % yield (**9a-9j**).

(E)-3-(2-(3-methoxyphenyl)thieno[3,2-b] thiophene-5yl)-1-phenylprop-2-en-one **(9a)**
Yellow solid, mp: 128-130 °C, IR (KBR, cm^{-1}) υ_{max}; 1668 (C=O), 1597 (C=C), 1261 (C-S-C), 1167 (C-S-C); ^{1}H NMR ($CDCl_3$, δ ppm): 7.38 (s, 1H, =CH), 7.27 (m, 3H, ArH), 7.25 (s, 1H, =CH), 7.23 (d, 1H, ArH), 7.18 (s, 1H, ArH), 7.07 (m, 3H, ArH), 6.86 (d, *J* =7.6, 2H, ArH), 6.75 (d, *J* =7.5, 1H, ArH), 3.80 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, δ ppm) 184.41, 151.72, 139.42, 134.00, 133.12, 130.72, 129.06, 125.45, 120.24, 128.21, 126.73, 21.44. LC-MS: 377 $(M+1)^+$.

(E)-1-(4-methoxyphenyl)-3-(2-(3-methoxyphenyl)thieno[3,2-b]thiophene-5-yl) prop-2-en-1-one **(9b)**
Yellow solid, mp: 147-149 °C, IR (KBR, cm^{-1}) υ_{max}; 1648 (C=O), 1565, 1480 (C=C), 1292 (C-S-C), 1250 (C-O-C). ^{1}H NMR ($CDCl_3$, δ ppm): 8.32 (d, 1H, =CH), 7.99 (s, 1H, =CH), 7.48 (d, *J* = 7.6, 1H, ArH), 7.33 (s, 1H, ArH), 7.27 (d, *J* = 7.0, 1H, ArH), 7.17 (s, 1H, ArH), 7.01 (d, *J* = 6.5, 2H, ArH), 6.90 (d, *J* = 7.5, 1H, ArH), 6.88 (d, *J* = 7.0, 1H, ArH), 3.91 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$, δ ppm) 188.46, 159.51, 159.43, 146.47, 138.88, 136.10, 135.84, 134.25, 130.59, 129.92, 129.88, 129.32, 121.53, 121.13, 120.82, 119.08, 114.63, 113.75, 112.77, 55.18, 39.53; LC-MS: 407 $(M+1)^+$.

(E)-1-(4-bromophenyl)-3-(2-(3-methoxyphenyl)thieno[3,2-b]thiophene-5-yl) prop-2-en-1-one **(9c)**
Yellow solid, mp: 123 °C, IR (KBR, cm^{-1}) υ_{max}; 1685 (C=O), 1564, 1557 (C=C), 1261 (C-S-C), 1134 (C-O-C), 845 (C-Br): ^{1}H NMR ($CDCl_3$, δ ppm): 8.05 (d, 2H, =CH), 7.78 (d, 2H, ArH), 7.61 (m, 2H, ArH), 7.55 (d, *J* = 7.6, 1H, ArH), 7.51 (m, 1H, ArH), 7.44 (d, *J* = 7.6, 1H), 7.33 (m, 3H, ArH), 3.82 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, δ ppm) 187.47, 159.42, 146.70, 138.00, 136.08, 136.03, 136.05, 134.21, 130.59, 130.22, 129.88, 129.50, 128.84, 121.55, 120.58, 114.66, 113.72, 55.17, 39.9; LC-MS: 455 $(M+1)^+$.

(E)-1-(4-chlorophenyl)-3-(2-(3-methoxyphenyl)thieno[3,2-b]thiophene-5-yl) prop-2-en-1-one **(9d)**
Yellow solid, mp: 178-180 °C, IR (KBR, cm^{-1}) υ_{max}; 1662 (C=O), 1557, 1482 (C=C), 1261 (C-S-C), 1194 (C-O-C), 867 (C-Cl). ^{1}H NMR ($CDCl_3$, δ ppm): 8.86 (s, 1H, =CH), 8.03 (m, 3H, ArH), 7.51 (m, 4H, ArH), 7.17 (s, 1H, =CH), 6.92 (d, 1H, ArH), 3.82 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, δ ppm) 196.00, 187.94, 159.44, 159.06, 153.21, 149.41, 141.42, 138.14, 136.17, 136.05, 132.68, 130.46, 129.83, 129.65, 129.25, 127.21, 121.56, 120.77, 114.12, 113.65, 55.19, 45.84. LC-MS: 411 $(M+1)^+$

(E)-1-(3-iodophenyl)-3-(2-(3-methoxyphenyl) thieno[3,2-b]thiophene-5-yl)prop-2-en-1-one **(9e)**
Yellow solid, mp: 163-165 °C, IR (KBR, cm^{-1}) υ_{max}; 1663 (C=O), 1560, 1462 (C=C), 1251 (C-S-C), 1135 (C-O-C), 824 (C-I). ^{1}H NMR ($CDCl_3$, δ ppm): 8.01 (s, IH, =CH), 7.98 (m, 3H, ArH), 7.85 (m, 6H, ArH), 7.53 (s, IH, =CH), 7.31 (s, IH, ArH), 3.73 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, δ ppm) 189.7, 161.2, 138.3, 137.9, 134.8, 134.6, 134.2, 130.3, 129.9, 129.9, 129.3, 129.3, 127.4, 126.4, 121.9, 119.8, 119.7, 114.3, 111.3, 55.9, LC-MS: m/z; 502 $(M+1)^+$

(E)-1-mesityl-3-(2-(3-methoxyphenyl) thieno[3,2-b]thiophene-5-yl)prop-2-en-1-one **(9f)**
Yellow solid, mp: 164-165 °C, IR (KBR, cm^{-1}) υ_{max}; 1662 (C=O), 1545, 1462 (C=C), 1241 (C-S-C), 1142 (C-O-C). ^{1}H NMR ($CDCl_3$, δ ppm): 8.12 (s, 1H, =CH), 8.01 (s, 1H, =CH), 7.9 (d, 2H, ArH), 7.8 (d, *J* = 7.6, 1H, ArH), 7.52 (d, 3H, ArH), 7.41 (d, 4H, ArH), 7.3 (d, *J* = 7.6, 1H, ArH), 7.01 (s, 1H, ArH), 3.80 (s, 3H, CH_3), 2.22 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, δ ppm) 189.7, 161.2, 138.3, 137.9, 137.8, 134.8, 134.6, 134.2, 130.3, 129.9, 129.9 129.3, 127.4, 126.4, 121.9,

119.8, 119.7, 114.3, 111.3, 55.9. LC-MS: 419 $(M+1)^+$.

(E)-3-(2-(3-methoxyphenyl)thieno[3,2-b] thiophene-5-yl)-1-(naphthalene-3-yl)prop-2-en-1-one **(9g)**

Yellow solid, mp: 143-146 °C, IR (KBR, cm^{-1}) υ_{max}; 1682 (C=O), 1567, 1482 (C=C), 1261 (C-S-C), 975 (trans CH=CH). ^1H NMR ($CDCl_3$, δ ppm): 8.54 (d, 1H, =CH), 8.04 (m, 5H, ArH), δ 7.52 (s, 5H, ArH), 7.85 (d, *J* = 7.6, 1H, ArH), 7.68 (d, *J* = 7.6, 1H, ArH), 7.46 (s, 1H, =CH), 7.34 (d, 1H, ArH), 3.8 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, δ ppm) 189.7, 161.2, 138.3, 137.9, 137.8, 134.8, 134.6, 134.2, 130.3, 129.9, 129.9, 129.3, 129.3,127.4, 126.4, 121.9, 119.8, 119.7, 114.3, 111.3, 55.9. LC-MS: 427 $(M+1)^+$.

(E)-3-(2-(3-methoxyphenyl)thieno[3,2-b] thiophen-5-yl)-1-(pyridine-4-yl)prop-2-en-1-one **(9h)**

Yellow solid, mp: 175-177 °C, IR (KBR, cm^{-1}) υ_{max}; 1689 (C=O), 1567, 1491 (C=C), 1414 (C=N), 1261 (C-S-C), 984 (trans CH=CH). ^1H NMR ($CDCl_3$, δ ppm): 9.23 (s, 1H, PyH), 8.80 (d, 1H, *J* = 7.5, PyH), 8.40 (d, 1H, *J* = 7.5, PyH), 7.90 (2H, m, ArH), 7.43-7.09 (m, 3H, ArH), 7.42 (d, 1H, *J* = 7.6, ArH), 7.30 (d, 1H, *J* = 7.6, ArH), 3.8 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, δ ppm) 189.7, 161.2, 138.3, 137.9, 137.8, 134.8, 134.6, 134.2, 130.3, 129.9, 129.9, 129.3, 129.3, 127.4, 126.4, 121.9, 119.8, 119.7, 114.3, 111.3, 55.9. LC-MS (m/z): 378 $(M+1)^+$.

(E)-3-(2-(3-methoxyphenyl)thieno[3,2-b] thiophen-5-yl)-1-(pyridine-3-yl)prop-2-en-1-one **(9i)**

Yellow solid, mp: 176-179 °C, IR (KBR, cm^{-1}) υ_{max}; 1642 (C=O), 1546, 1462 (C=C), 1396 (C=N), 1261 (C-S-C). ^1H NMR ($CDCl_3$, δ ppm): 8.03 (m, 3H, ArH), 7.51 (m, 4H, ArH), 7.36 (s, 1H, =CH), 7.31 (m, 1H, ArH), 7.23 (d, *J* = 7.6, 1H, ArH), 7.49 (d, *J* = 7.6, 1H, ArH), 6.90 (d, 1H, ArH), 3.82 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, δ ppm) 189.7, 161.2, 138.3, 137.9, 137.8, 134.8, 134.6, 134.2, 130.3, 129.9, 129.9, 129.9, 129.3, 129.3, 127.4, 126.4, 121.9 119.8, 114.3, 111.3, 55.9; LC-MS (*m/z*) 378 $(M+1)^+$.

(E)-3-(2-(3-methoxyphenyl)thieno[3,2-b] thiophen-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one **(9j)**

Yellow solid mp: 187-189 °C, IR (KBR, cm^{-1}) υ_{max}; 1690 (C=O), 1594, 1515 (C=C), 1262 (C-S-C), 1234 (C-S-C), 1167 (C-O-C). ^1H NMR ($CDCl_3$, δ ppm): 8.03 (s, 1H, =CH), 7.98 (s, 1H, =CH), 7.85 (d, 2H, *J* = 7.5, =CH), 7.67 (d, 2H, *J* = 7.6, =CH), 7.49 (d, *J* = 7.6, 1H, ArH), 7.46 (d, *J* = 7.6, 1H, ArH), 7.192 (m, 3H, ArH), 3.82 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, δ ppm) 189.7, 161.2, 138.3, 137.9, 137.8, 134.8, 134.6, 134.2, 130.3, 129.9, 129.9, 129.3, 129.3, 127.4, 126.4, 121.9, 119.8, 119.7, 114.3, 111.3, 55.9 LC-MS (*m/z*): 383 $(M+1)^+$.

PHARMACOLOGY

Anticancer activity

Invitro anticancer activity of the test compounds was tested using MTT colorimetric assay as per ATCC protocol [21, 22]. Cell lines used in the present includes SKNSH derived from Human lung adeno carcinoma epithelial cell line (ATCC No. HTB-11), A549 derived from human colorectal adenocarcinoma cells (ATCC No. CCL-185) which were procured from American Type Culture Collection, Manassas, VA, USA. The toxicity profile of the test compounds on human normal cells HEK293T are summarised in **Fig 3.** Doxorubicin was used as the standard drug in the assay.

Procedure

Day one: One full confluent T-25 flask was trypsinized and 5 mL of complete media was added to trypsinized cells and centrifuged in a sterile 15 mL falcon tube at 500 rpm in the swinging bucket rotor (-400 x g) for 5

min. Media was removed and the cells were resuspended to 1.0 ml with complete media and cells were counted. The cells were diluted to 75,000 cells per ml incomplete media. 100 µL of cells (7500 total cells) were added in each well and incubated overnight in a humid incubator with 5% CO_2 at 37 °C so that the cells adhere to the surface. Different concentrations of compounds were prepared by dissolving in DMSO.

Day two: Different concentrations of compounds were added to the adherent cells in triplicates (l µL per each well) and incubated for 48 hr with DMSO alone as control.

Day Three: MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (sigma catalog no. M2128) was dissolved in PBS at 5 mg/mL and filter sterilized and stored at 4 °C. 10 µL of MTT solution was added to each well and incubated for 2 hr at 37 °C in the incubator. Then the media was aspirated and plates were dried and 100 µL of DMSO (solvent) was added to each well. Plate was covered with tinfoil and agitated on orbital shaker for 15 min.

Antibacterial activity

Glass wares and apparatus

Glass petridish, Glass tubes, Beakers, Erlenmeyer flasks, Bacterial loop and measuring cylinder. All the glass wares were of Borosilicate grade. Digital electronics balance (Shankar Scientific supplies, India), Yorco Horizontal Laminar air flow bench (Yorco sales Pvt. Ltd, New Delhi, India), Ausco incubator, Zone reader (Cintex industrial Corporation, India), hot air oven, autoclave and UV/Visible spectrophotometer (Shimadzu corporation, Japan). Nutrient Broth, Nutrient agar and 5 mm diameter antibiotic assay were obtained from Hi-Media Laboratories Limited, India. Barium chloride dehydrate GR, concentrated sulphuric acid GR, Dimethyl sulphoxide GR, Sodium chloride AR and Potassium dichromate were obtained from Ranbaxy Laboratories Ltd, Chemical Division, India. The standard bacterial and fungal strains were procured from National Centre from Cell Science (NCCS), Pune, India.

The antibacterial activity of synthesized compounds was studied by the disc diffusion method [23, 24] against the following pathogenic organisms. The gram-positive bacteria screened were *Staphylococcus aureus* NCCS 2079 (SA), Gram-negative bacteria screened were Escherichia *coli* NCCS 2065 (EC) and *Pseudomonas aeruginosa* NCCS 2200 (PA). The synthesized compounds were used at the concentration of 250 µg/mL and 500 µg/mL using DMSO as a solvent. The amoxicillin 10 µg/disc and Streptomycin 30 µg/disc were used as a standard (Himedia laboratories limited, Mumbai).

Disc Diffusion Method

A suspension of *Staphylococcus aureus* (SA) was added to sterile nutrient agar at 45 ºC. The mixture was transferred to sterile petridishes to give a depth of 3 to 4 mm and allowed to solidify. Precautions were observed to reduce uniform layer of medium on the plate. Sterile discs 5mm in diameter (made from Whatman Filter paper) were immersed in the solutions of synthesized compounds (250 µg/mL) and maintain an untreated control sample for comparison. Leave the plates to stand for 1 hr at room temperature as a period of preincubation diffusion to minimize the effects of variations in different time. Then the plates were incubated at 37 ºC for 24 hr and observed for antibacterial activity. The diameter of the zone of inhibition was measured for each plate in which the zone of inhibition was observed. The average zone of inhibition was calculated and compared with that of standard. A similar procedure was adopted for studying the antibacterial activity

against the other organisms.

Antifungal activity

The antifungal activity of synthesized compounds were studied by disc diffusion method against the organisms of *Aspergillus nigeri* NCCS 1196 (AN), *Candida albicans* NCCS 3471 (CA) and *A. fumigates* (AF). Compounds were treated at the concentrations of 250 µg/mL using DMSO as a solvent and Flucanazole 50 µg/mL used as a standard drug against these organisms.

Disc diffusion method

A suspension of *Aspergills nigeri* NCCS 1196 (AN), *Candida albicans* NCCS 3471 (CA) and *A. fumigatus* was added to a sterile sabouraud dextrose agar at 45 ºC. The mixture was transferred to sterile petridishes and allowed to solidify. Sterile discs 5 mm in diameter (made from whatmann Filter paper) immersed in the solutions of synthesized compounds and control were placed on the surface of agar medium with forceps and pressed gently to ensure even contact. Leave the plates to stand for 1 hr at rt as a period of preincubation diffusion to minimize the effects of variation at 37 ºC for 13 hr and observed for antibacterial activity. The diameters of the zone of inhibition were measured for the plates in which the zone of inhibition was observed. The average zone of inhibition was calculated with that of standard.

RESULTS AND DISCUSSIONS

Chemistry

In present research used hetero cyclic compounds such as fused five-membered heterocyclic compounds thiophene and thieno[3,2-b]thiophene, we have synthesized some 5-arylthieno[3,2-b] thiophene chalcone derivatives and the target compounds were screened for their antibacterial activities strains using disk diffusion method. Synthesis of aryl/ hetero aryl thiophenes involve the reaction compound **2** was Vilsmeier Haack reaction by using dry N,N-dimethylformamide (DMF) and phosphorus oxychloride ($POCl_3$) refluxed for 30 hr afforded **2** in 65 % yield as a colorless liquid. Cyclisation of ester **3** is 80 % yield. Hydrolysis of this ester was carried out by using 10 % aqueous solution in THF and water which gave acid **4** in 95 % yields as a white solid. The resulting acid was heated to 230 °C, Total CO_2 gas is going, reaction is completed observed, after removal of heating and filtered with hexane to obtained white solid **5** was treated with formylation to afforded mono aldehyde **6** in 83 % as a white solid. Direct go to next stage of brominating with N-bromosuccinamide and DMF to obtained white solid **7** followed by Suzuki coupling with palladium catalysis, DME, $NaHCO_3$ to afford **8**. The Claisen – Schmidt condensation reaction of chalcone compounds was treated with ethanones ethanones [**a-j**] were dissolved in alkaline methanolic solution and stirred for 10 mints and then added 5-(3-methoxyphenyl)thieno[3,2-b]thiophene-2-carbaldehyde (**8**) the resulting mixture was stirred at ambient temperature for 5-8 hr. The reaction mixture was diluted with water (10 mL) and filtered to get the solid and air dried to give **(9a-9j)** in good yields (91-94 %) (**Scheme 1**, **Fig. 2**).

The E-configuration was confirmed by the spectral analysis. In ^{1}H NMR spectrum, two doublet signals appeared at around δ 6.45 and 7.23 coupling to each other with J values of 7.5-7.6 Hz, which confirmed the E-geometry of olefin in chalcone compounds. To represent the series (E)-3-(2-(3-methoxyphenyl)thieno[3,2-b] thiophene-5yl)-1-phenylprop-2-enone (**9a**) is formed as pale yellow with 91-94 % yield and mp: 128-130 °C. The structure of IR signals υ cm^{-1} 1668 cm^{-1} for CO, 1597, 1565 cm^{-1} aromatic C=C vibrations, 1261 cm^{-1} C-S-C stretching,

1120 cm^{-1} C-O-C stretching; ^{1}H-NMR δ ppm at 7.38, 7.25 singlet for 1H's belongs to thiophene ring, δ 7.27 multiplet for aromatic hydrogens, δ 6.86, 6.75 doublet for aromatic hydrogens and δ at 3.80 singlet for OCH_3; ^{1}H NMR ($CDCl_3$, δ ppm) 189.7, 161.2, 138.3, 137.9, 137.8, 134.8, 134.6, 134.2, 130.0, 129.9, 129.8, 129.3, 129.2, 127.4, 126.4, 121.9, 119.8, 119.7, 114.3, 111.3, 55.9. LC-MS: 377 $(M+1)^+$ fragmentation pattern was in accordance with the assigned structure (Pl. see the experimental section for details).

BIOLOGY

Cytotoxicity

The results were represented as percentage of cytotoxicity/viability. From the percentage of cytotoxicity the IC_{50} values were calculated and presented in the table. Treatment with the compounds reduced the viability of human cancer cell lines in a concentration-dependent manner, with IC_{50} values in the low micromolar range. IC_{50} values of different compounds on different cell lines in µM. 4 different concentrations of target compounds **9a-9j** were tested on two cancer cell lines. IC_{50} values were given in micromolar concentrations (µM) present in **Table 1.**

Table-1: Anti-cancer activity of title compounds target **9a-9j** (Target **1-10**) [% of inhibition at 25 µM]

Entry	Compounds	A549	SKNSH
9a	Target **1**	8.81	32.07
9b	Target **2**	9.91	16.12
9c	Target **3**	18.21	13.6
9d	Target **4**	20.96	>100
9e	Target **5**	18.21	52.4
9f	Target **6**	18.79	14.3
9g	Target **7**	51.00	13.2
9h	Target **8**	47.17	>100
9i	Target **9**	3.67	10.02
9j	Target **10**	4.68	5.23
Reference		[a]	[b]

[a]Doxorubicin IC_{50} is 0.3 µM; [b]Doxorubicin IC_{50} is 1 µM

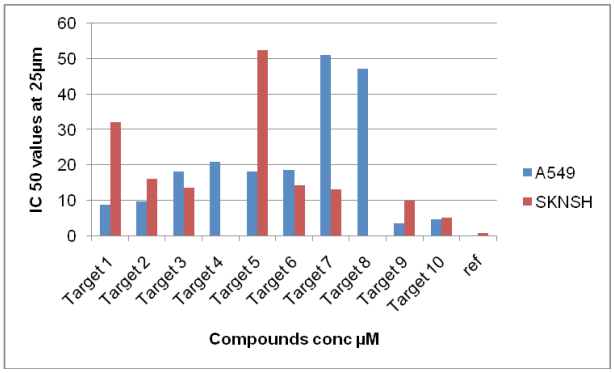


Figure 3: Cytotoxicity screening of title compounds

The title compounds (**Fig. 3**) were screened for anticancer activity employing A549, SKNSH cell lines by MTT colorimetric assay and the results are summarized in **Table 1**. The test compounds with > 50 % inhibition on cancer cell lines are assumed to have good activity in treating colon and breast cancers. From the results it is obvious that reductive target thiophene chalcone derivatives **9a–9j** displayed better biological response than their parent nuclei. Comparison of screening results of the title compounds with the reference compound is DXN.

SAR studies

The fact that all the derivatives showed enhanced activity except compounds **9g, 9h, 9d** against colon cancer cell lines, and compounds **9e, 9a** and **9b** against breast cancer cell lines. Among series the activity order is meta substituted methoxy benzyl moiety at eastern, naphthalene at western **9g** (51.00 % of inhibition) greater than derivative meta substituted methoxy benzyl moiety at eastern, pyridine at western **9h** (47.17 % of inhibition) which in turn greater than para substituted chloro benzyl moiety **9d** (20.96 % of inhibition) against A549 cancer cell line and for SKNSH cancer cell line the order is **9e** (52.40 % of inhibition) greater than **9a** (32.07 % of inhibition) which in turn greater than **9b** (16.12

% of inhibition) revealing that these compounds are selective towards a particular cell line.

Antibacterial activity

Quite a good number of the final candidates have shown superior antibacterial activity when compared to the ampicillin. **9** were tested *invitro* for their antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (MTCC96), and Gram-negative bacteria *Escherichia coli* (MTCC739), *Pseudomonas aeruginosa* (MTCC2453) and antifungal activity against *Aspergillus nigeri* NCCS 1196 (AN), *Candida albicans* NCCS 3471 (CA) and *A. fumigates* (AF). (**Table 2, Fig. 4).** Dimethyl sulphoxide (DMSO) was used as solvent control. All compounds were screened at 1 mg/mL concentration. Compounds **9d** and **9e** showed good activity against Gram-negative bacteria only i.e., *E. coli* and *P. aeruginosa* respectively. Compounds **9a, 9b, 9i,** and **9j** exhibited moderate to low antibacterial activity against both Gram-positive and gram-negative bacteria respectively. The order of activity was **9a > 9b > 9f > 9c > 9e > 9d.** These analogous were also tested for antifungal activity and compounds **9a, 9d, 9i,** and **9j** exhibited excellent activity against three fugal strains *Aspergillus nigeri* NCCS 1196, *Candida albicans* NCCS 3471 and *A. fumigates* compared with reference compound Ampicillin, Flucanazole.

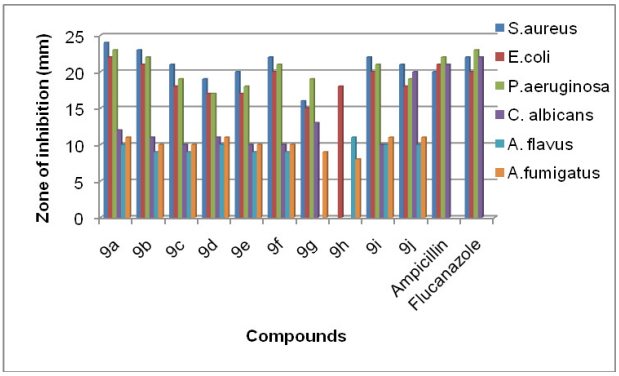


Figure 4: Antibacterial evaluation of final compounds

Table 2: Antimicrobial, antifungal activity of target compounds

Compounds	Zone of inhibition in mm					
	Antibacterial activity			Antifungal activity		
	S.aureus	*E.coli*	*P.aeruginosa*	*C. albicans*	*A. flavus*	*A. fumigatus*
9a	24	22	23	12	10	11
9b	23	21	22	11	9	10
9c	21	18	19	10	9	10
9d	19	17	17	11	10	11
9e	20	17	18	10	9	10
9f	22	20	21	10	9	10
9g	16	15	19	13	ND	9
9h	ND	18	ND	ND	11	8
9i	22	20	21	10	10	11
9j	21	18	19	12	10	11
Ampicillin	20	21	22	ND	ND	ND
Flucanazole	22	20	23	ND	ND	ND

ND: No zone of inhibition

Conclusion

In summary, we have developed a new efficient approach to the synthesis of 5-arylthieno[3,2-b] thiophene-chalcones has been reported, 5-arythieno[3,2-b]thiophene-2-carboxldehyde as key intermediate for the final step have been prepared by suzuki cross coupling by employing tetrakis(triphenylphosphine) palladium (0). The current methodology allows for the incorporation of many substitution patterns not available from the few previously reported approaches of this class. The final compounds **9a, 9b, 9i**, and **9f** have shown superior antibacterial activity against *S. aureus* strain (24, 23, 22 mm values respectively) than the reference antibiotic drug Ampicillin (20 mm). Further, cytotoxicity the targets **9e, 9g,** and **9h** have excellent activity against both the A549, SKNSH cancer cell lines (52.4, 51.00, 47.17 μM values respectively). Thus, suggesting that compounds from the present series can serve as important gateway for the design and development of new antibacterial, anticancer agents make them valid lead compounds for further optimization.

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