Synthesis, Characterization and Antimicrobial Activity of New Chalcones and Their 6-Aryl-4-(2,5-dichlorothiophen-3-yl)-6*H*-1,3-thiazin-2-amine Derivatives

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Abstract

Chalcones (**5a-h**) were prepared in high yields *via* the condensation of 3-acetyl-2,5-dichlorothiophene (**3**) with aryl aldehydes (**4a-h**). The reaction of the resulting chalcones with thiourea in the presence of a catalytic amount of hydrochloric acid provided the 1,3-thiazin-2-amine derivatives (**6a-g**) in moderate yields. All new compounds were characterized by extensive NMR analysis data, including 1D NMR experiments (^{1}H and ^{13}C) and 2D NMR experiments (COSY, HMBC, HSQC), as well as ESIMS and HRESIMS data. The newly synthesized compounds were evaluated for their biological activities, which demonstrate a very good to low bioactivities as antibacterial and antifungal, respectively.

Keywords

Thiazine; Thiophene; Thiourea; Condensation; Chalcone.

Introduction

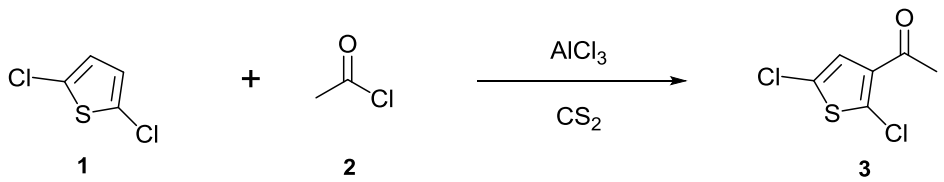
Chalcones exhibit a wide range of biological properties including antioxidant[1-4], anticancer[5-7], antiviral[8,9], anti-inflammatory[10-12], antimalarial[13,14], antileishmanial[15-19], antitubercular[20] and antimicrobial activities[21,22]. During the studies on the structure of clavicin, it was found that the biological activity of chalcones could be attributed to the presence of the *α,β*-unsaturated keto

functional group[23]. In addition to this, chalcones are useful building blocks for the preparation of synthetically significant heterocycles such as pyrazoles[24-28], isoxazoles[29], thiazine[30], oxazine[31], and many others related motifs[32,33].

1,3-Thiazine is a six membered heterocyclic ring which contains two hetero atoms (N and S) placed in the heterocyclic ring at the 1,3 positions. Many thiazine derivatives have been synthesized because of their wide range of biological activities[34,35] such as fungicide[36-39], anti-inflammatory[40,41], analgesic and antipyretic[42,43], antimicrobial and antiviral[44-48], and antitumor properties[49,50]. One of the most convenient approaches to synthesize chalcones is the condensation of aryl ketones with aromatic aldehydes mediated by a suitable base such as potassium or sodium hydroxide. Herein, we report the synthesis, characterization and antimicrobial activity of 1,3-thiazin-2-amine derivatives derived from chalcones bearing a thiophene nucleus (Schemes 2 and 3).

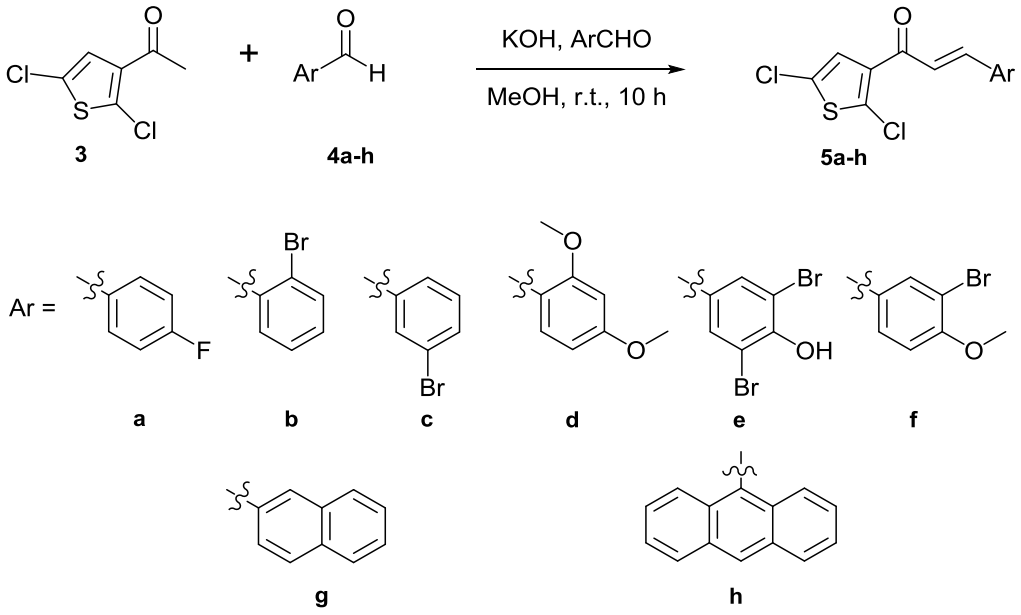
Results and Discussion

A large scale synthesis of 3-acetyl-2,5-dichlorothiophene **(3)** was achieved by Friedel-Crafts acylation using 2,5-dichlorothiophene **(1)**, acetyl chloride, anhydrous aluminium chloride as a Lewis acid catalyst and carbon disulfide as the solvent (Scheme 1).[51]



Scheme 1: Preparation of 3-acetyl-2,5-dichlorothiophene **(3)**.

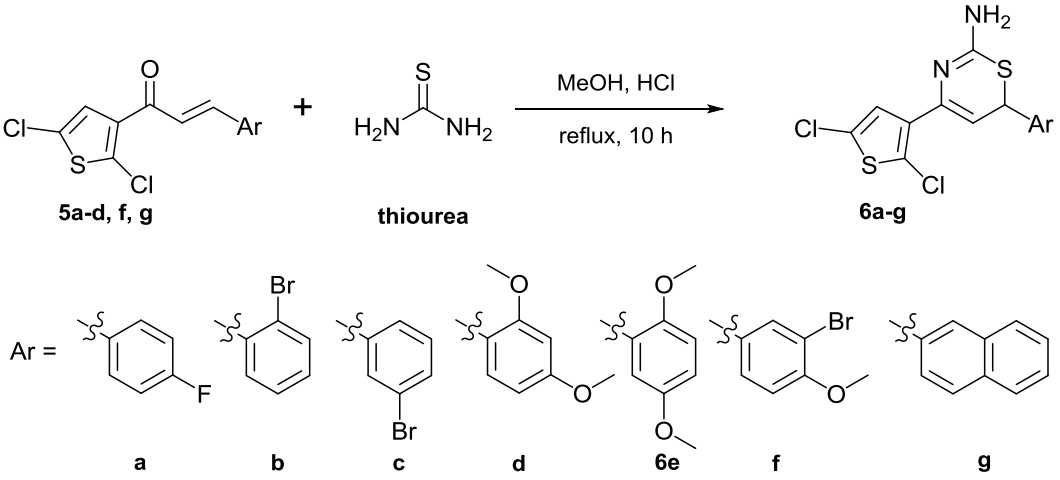
It was envisaged to prepare chalcones using Claisen-Schmidt condensation. For this purpose, the precursor, 3-acetyl-2,5-dichlorthiophene (**3**) was condensed with an array of aromatic aldehydes **(4a-h)** in the presence of potassium hydroxide. The reaction involves the formation of the enolate anion that is subsequently condensed with the aldehydes to produce the desired chalcones **(5a-h)** in excellent yields (Scheme 2). Chalcones **5a-c, g** have been reported previously [27,52-54], while all other chalcones are new.



Scheme 2: Base catalyzed *Claisen-Schmidt* synthesis of chalcones **(5a-h)**.

Condensation of the synthesized chalcones **(5a-d, f, g)** with thiourea in the presence of hydrochloric acid as catalyst afforded the 1,3-thiazine derivatives **(6a-d, f, g)** in moderate yields

(Scheme 3). This two-step approach to synthesize thiazine derivatives has also been successfully applied to one-pot multi-step transformation by achieving the synthesis of thiazine **(6e)** in a good yield.



Scheme 3: Synthesis of substituted 1,3-thiazines **(6a-g)**.

All new isolated chalcones **(5d-f, h)** and thiazines **(6a-g)** were characterized using different spectroscopic methods including IR, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, and mass spectrometry. A detailed analysis of 2D NMR data enabled an unambiguous determination of the exact chemical shifts of all protons and carbons for all compounds. Complete assignments for all protons and carbons are given in the experimental part. The ESIMS and HRESIMS data analyses revealed the correct molecular ion peaks for all compounds as suggested by their molecular formulas.

The ^{1}H NMR spectra of all new chalcones **(5d-f, h)** showed two doublet peaks in the olefinic region at 7.45-8.65 ppm due to the *α, β*-protons with a coupling constant value of ^{3}J = 15.59-16.87 Hz which supports the formation of the *E* isomers. In addition, the proton of the thienyl moiety in all chalcones appeared as singlet at δ 7.70–7.89 ppm. The ^{1}H NMR spectra of the newly prepared heterocycles are in good agreement with the proposed structures. As the ^{1}H NMR spectra of the final products showed only one exchangeable proton signal, and the ^{13}C NMR have no C=S signal, it is plausible to conclude the formation of thiazine compared to competing pyrimidine structure. The thiazine NH_2 exchangeable proton signals appeared as broad singlet at *δ* 6.92-7.14 ppm. The two mutually coupled doublet signals at *δ* 5.58-5.78 ppm and *δ* 5.10-5.87 ppm are assigned to H-5 and H-6 thiazine protons, with coupling constants in the range 6.24-7.52 Hz. The singlet appeared at *δ* 7.21-7.28 ppm is related to the thienyl proton (see supporting information for details).

The ^{13}C NMR spectra of the new chalcones **(5d-f, h)** showed the carbonyl carbon at 183.1-183.7 ppm. The ^{13}C NMR spectra of thiazines **(6a-g)** revealed absorption in the range 36.0-42.1 ppm corresponding to the C-6 carbon. The absorption appeared at 101.1-103.0 attributed to the C-5 carbon of the thiazine ring.

Biological Screening

All the newly synthesized compounds were tested for their bioactivities using *S. aureus* and pleasingly, chalcone **5d** exhibited significant antibacterial activities compared to standard

antibacterial agents. All remaining chalcones showed inferior activities against all the applied bacterial species. Notably, all the thiazine compounds **(6a-e)** exhibited significant antibacterial activity against *S. aureus* compared to the standard antibiotics, cefaclor and cefuroxime.

In contrast to antibacterial activities, both chalcones and thiazines possess low antifungal properties when tested against *C. albicans* and no activity was observed against *A. niger*. A summary of the biological activity is presented in Figures S 1 and S 2 (Supplemental Materials).

Experimental

Materials

Aromatic aldehydes and thiourea were purchased from Aldrich. 2,5-dichlorothiophene was purchased from Acros. 3-Acetyl-2,5-dichlorothiophene was prepared according to literature procedure[51]. Solvents were dried and distilled according to standard methods[55].

Instrumentation

^{1}H (500 MHz, 600 MHz) and ^{13}C (125 MHz, 150 MHz) NMR spectra were recorded in DMSO-d_6 which was used as an internal standard at 300 K on Bruker spectrometers (500 MHz, 600 MHz). Chemical shifts δ are given in parts per million (ppm) and were determined from the center of the respective coupling pattern (s: singlet, d: doublet, dd: doublet of doublet, t: triplet). ESI-HMRS measurements were performed on a LTQ-FT mass spectrometer (Thermo Fisher Scientific).

Thin layer chromatography (TLC) monitoring of the reaction was carried out using analytical TLC plates coated with silica gel (60 F254, Merck). Preparative thin layer chromatography (PTLC) was carried out at room temperature (r.t.) with the mobile phase being $CHCl_3$/pentane (30 : 70) mixture. The Supplemental Materials contains sample ^{1}H, ^{13}C NMR spectra and HRMS data for compounds 5 and 6 (Figures S 3 – S 45)

Synthesis

General procedure for the synthesis of Chalcones 5a-h

An equimolar mixture of the aryl aldehyde, KOH were dissolved in MeOH (50 mL). To this solution 3-acetyl-2,5-dichloro-thiophene, **3**, in MeOH (10 mL) was added dropwise. After the addition was completed, the reaction mixture was stirred at r.t. for 10 h. The precipitate formed was filtered off, washed with MeOH and dried without any further purification.

(*E*)-1-(2,5-dichlorothiophen-3-yl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (5d). Yield: 95%, – **^{1}H NMR** (DMSO-d_6, 500 MHz): δ = 7.92 (d, J = 16.87 Hz, 1H, H-3), 7.85 (d, J = 8.62 Hz, 1H, H-6''), 7.70 (s, 1H, H-4'), 7.45 (d, J = 16.87 Hz, 1H, H-2), 6.67 (d, J = 2.20 Hz, 1H, H-3''), 6.66 (dd, J = 7.33, 2.20 Hz, 1H, H-5''), 3.91 (s, 3H, 2''-OCH_3), 3.86 (s, 3H, 4''-OCH_3). – **^{13}C NMR** (DMSO-d_6, 125 MHz) δ = 183.7 (C-1, C=O), 164.0 (C_q-4''),160.7 (C_q-2''), 140.4 (CH-3), 138.0 (C_q-2'), 131.1 (CH-6''), 130.5 (C_q-5'), 128.4 (CH-4'), 126.0 (C_q-3'), 121.6 (CH-2), 116.0 (C_q-1''), 107.0 (CH-5''), 98.8 (CH-3''), 56.4 (2''-OCH_3), 56.1 (4''-OCH_3). – **(+)-HRESIMS** *m/z* 364.9773 $[M+Na]^+$, 366.9745 $[M+Na+2]^+$, 368.9717 $[M+Na+4]^+$, (calcd for $C_{15}H_{12}Cl_2O_3SNa$, 364.9773).

(*E*)-3-(3,5-dibromo-4-hydroxyphenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-en-1-one (5e). Yield: 80%, – **^{1}H NMR** (DMSO-d_6, 500 MHz): δ = 10.60 (bs, 1H, OH), 8.14 (s, 2H, 2'', 6''), 7.89 (s, 1H, H-4'), 7.59 (d, *J* = 15.59 Hz, 1H, H-3), 7.52 (d, *J* = 15.59 Hz, 1H, H-2). – **^{13}C NMR** (DMSO-d_6, 125 MHz) δ = 183.1 (C-1, C=O), 153.6 (Cq-4''),142.9 (CH-3), 137.1 (C_q-2'), 133.4 (CH-2'',6''), 131.8 (C_q-5'), 129.3 (C_q-1''),128.5 (CH-4'), 125.9 (C_q-3'), 123.9 (CH-2), 112.7 (C_q-3'',5''). – **(-)-ESIMS** *m/z* 453 ([M - H]$^-$, 38), 455 ([M-H+2]$^-$, 100), 457 ([M-H+4]$^-$, 92), 459 ([M-H+6]$^-$, 36), 461([M-H+8]$^-$, 5). −**(-)-HRESIMS** *m/z* 454.7739 [M-H+2]$^-$, 456.7715[M-H+4]$^-$,458.7687 [M-H+6]$^-$ (calcd for $C_{13}H_5Br_2Cl_2O_2S$, 452.7760).

(*E*)-3-(3-bromo-4-methoxyphenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-en-1-one (5f). Yield: 94%, – **^{1}H NMR** (DMSO-d_6, 500 MHz): δ = 8.20 (d, *J* = 2.20 Hz, 1H, H-2''), 7.83 (s, 1H, H-4'), 7.82 (dd, *J* = 8.62, 2.20 Hz, 1H, H-6''), 7.62 (d, *J* = 15.77 Hz, 1H, H-3), 7.48 (d, *J* = 15.77 Hz, 1H, H-2), 7.18 (d, *J* = 8.62 Hz, 1H, H-5''), 3.91 (s, 3H, 4''-OCH_3). **^{13}C NMR** (DMSO-d_6, 125 MHz) δ = 183.3 (C-1, C=O), 157.9 (C_q-4''),144.0 (CH-3), 137.4 (C_q-2'), 133.4 (CH-2''), 131.5 (CH-6''), 131.4 (C_q-5'), 128.9 (C_q-1''), 128.5 (CH-4'), 125.9 (C_q-3'), 123.3 (CH-2), 113.2 (CH-5''), 111.9 (C_q-3''), 57.1 (4''-OCH_3). – **(+)-ESIMS** *m/z* 413 ([M+Na]$^+$, 45), 415 ([M+Na+2]$^+$, 76), 417 ([M+Na+4]$^+$, 36). − **(+)-HRESIMS** *m/z* 412.8769 [M+Na]$^+$, 414.8745 [M+Na+2]$^+$, 416.8719 [M+Na+4]$^+$, (calcd for $C_{14}H_9BrCl_2O_2SNa$, 412.8776).

(*E*)-3-(anthracen-9-yl)-1-(2,5-dichlorothiophen-3-yl)prop-2-en-1-one (5h). Yield: 83%, – **^{1}H NMR** (DMSO-d_6, 500 MHz): δ = 8.73 (s, 1H, H-8''), 8.65 (d, *J* = 16.02 Hz, 1H, H-3), 8.30 (dd, *J* = 8.54,1.22 Hz , 2H, H-3''), 8.18 (dd, *J* = 8.24, 1.53 Hz, 2H, H-6''), 7.77 (s, 1H, H-4'),7.63 (m, 2H, H-4''), 7.46 (d, *J* = 16.02 Hz, 1H, H-2), 7.61 (m, 2H, H-5''). – **^{13}C NMR** (DMSO-d_6, 125

MHz) δ=183.1 (C-1, C=O), 141.8 (CH-3), 137.3 (C_q-2'), 133.3 (CH-2), 131.8 (C_q-5'), 131.3 (C_q-1''), 129.6 (C_q-7''), 129.5 (C_q-2''), 129.4 (CH-6''), 129.2 (CH-8''), 128.6 (CH-4'), 127.4 (CH-4''), 126.3 (C_q-3'), 126.2 (CH-5''), 125.5 (CH-3''). – **(+)-ESIMS** *m/z* 405 ($[M+Na]^+$, 100), 407 ($[M+Na+2]^+$, 73), 409 ($[M+Na+4]^+$, 17). – **(+)-HRESIMS** *m/z* 404.9877 $[M+Na]^+$, 406.9848 $[M+Na+2]^+$, (calcd for $C_{21}H_{12}Cl_2OSNa$, 404.9878).

General procedure for the synthesis of thiazines 6a-g

A mixture of 1,3-diaryl-2-propen-1-ones (chalcones) (**5a-g**) (0.01 mol), thiourea (0.03 mol) and concentrated hydrochloric acid (0.01 mol) in methanol (50 mL) was refluxed for about 10 hr, the reaction mixture was cooled and then poured into ice water (150 mL), and then neutralized with dilute potassium hydroxide solution. The obtained solid was filtered off, air-dried, and purified using preparative thin layer chromatography (PTLC).

4-(2,5-dichlorothiophen-3-yl)-6-(4-fluorophenyl)-6*H*-1,3-thiazin-2-amine (**6a**). Yield: 48%, – **^{1}H NMR** (DMSO-d_6, 600 MHz): δ = 7.38 (dd, *J* = 8.62, 5.50 Hz, 2H, H-2'',6''), 7.24 (s, 1H, H-4'), 7.18 (t, *J* = 8.80 Hz, 2H, H-3'',5'')' 7.09 (bs, 2H, 2-NH_2), 5.71 (d, *J* = 6.24 Hz, 1H, H-5), 5.11 (d, *J* = 6.24, 3.0 Hz, 1H, H-6). – **^{13}C NMR** (DMSO-d_6, 150 MHz) δ = 162.8, 161.3 (d, *J* = 244.8 Hz, C_q-4''), 154.0 (C_q-2), 142.1 (C_q-4), 138.8 (C_q-1''), 138.3 (C_q-3'), 129.9, 129.8 (d, 8.67 Hz, CH-2'',6''), 128.6 (CH-4'), 124.6 (C_q-5'), 120.9 (C_q-2'), 116.0, 115.9 (d, *J* = 21.35 Hz, CH-3'',5''), 103.01 (CH-5), 41.4 (CH-6). **–(+)-HRESIMS** *m/z* 380.9461 $[M+Na]^+$, 382.9433 $[M+Na+2]^+$, 384.2077 $[M+Na+4]^+$, (calcd for $C_{14}H_9Cl_2FN_2S_2Na$, 380.9460).

6-(2-bromophenyl)-4-(2,5-dichlorothiophen-3-yl)-6*H*-1,3-thiazin-2-amine (6b). Yield: 57%, – **^{1}H NMR** (DMSO-d_6, 600 MHz): δ = 7.66 (dd, *J* = 7.89, 1.10 Hz, 1H, H-3''), 7.39 (td, *J* = 7,70, 1.10 Hz, 1H, H-4''), 7.33 (dd, *J* = 7.89, 1.83 Hz, 1H, H-6''), 7.25 (s, 1H, H-4'), 7.24 (td, *J* = 7.70, 1.65 Hz, 1H, H-5''), 7.14 (bs, 2H, 2-NH_2) 5.69 (d, *J* = 7.15 Hz, 1H, H-5), 5.21 (d, *J* = 7.15 Hz, 1H, H-6). – **^{13}C NMR** (DMSO-d_6, 150 MHz) δ = 153.9 (C_q-2), 142.7 (C_q-4), 140.1 (C_q-1''), 138.4 (C_q-3'), 133.6 (CH-3''), 130.1 (CH-5''), 129.5 (CH-6''), 128.7 (CH-4''), 128.6 (CH-4'), 124.6 (C_q-5'), 123.3 (C_q-2''), 121.2 (C_q-2'), 101.2 (CH-5), 40.3 (CH-6). – **(+)-ESIMS** *m/z* 419 ($[M+H]^+$, 62), 421 ($[M+H+2]^+$, 100), 423 ($[M+H+4]^+$, 52), 425 ($[M+H+6]^+$, 12) . **(+)-HRESIMS** *m/z* 418.8839 $[M+H]^+$, 420.8815 $[M+H+2]^+$, 422.8790 $[M+H+4]^+$, 424.8762 $[M+H+6]^+$, (calcd for $C_{14}H_{10}BrCl_2N_2S_2$, 418.8840).

6-(3-bromophenyl)-4-(2,5-dichlorothiophen-3-yl)-6*H*-1,3-thiazin-2-amine(6c). Yield: 51%, – **^{1}H NMR** (DMSO-d_6, 600 MHz): δ = 7.53 (d, *J* = 3.66, 1H, H-2''), 7.48 (dd, *J* = 7.52, 3.30 Hz, C-4''), 7.33 (m, 2H, H-5'',6''), 7.23 (s, 1H, H-4'), 7.11 (bs, 2H, 2-NH_2), 5.71 (d, *J* = 7.52 Hz, 1H, H-5), 5.10 (d, *J* = 7.52 Hz, 1H, H-6). – **^{13}C NMR** (DMSO-d_6, 150 MHz) δ = 153.4 (C_q-2),145.4 (C_q-1''), 142.1 (C_q-4), 138.5 (C_q-3'), 131.4 (CH-5''), 130.4 (CH-2''), 130.5 (CH-4''), 128.6 (CH-4'), 126.8 (CH-6''), 124.6 (C_q-5'), 122.2 (C_q-3''), 121.2 (C_q-2'), 102.2 (CH-5), 41.4 (CH-6). – **(+)-ESIMS** *m/z* 419 ($[M+H]^+$, 62), 421 ($[M+H+2]^+$, 100), 423 ($[M+H+4]^+$, 52), 425 ($[M+H+6]^+$, 12) . – **(+)-HRESIMS** *m/z* 418.8839 $[M+H]^+$, 420.8815 $[M+H+2]^+$, 422.8790 $[M+H+4]^+$, 424.8762 $[M+H+6]^+$, (calcd for $C_{14}H_{10}BrCl_2N_2S_2$, 418.8840).

4-(2,5-dichlorothiophen-3-yl)-6-(2,4-dimethoxyphenyl)-6*H*-1,3-thiazin-2-amine (6d). Yield: 68%, – **^{1}H NMR** (DMSO-d_6, 600 MHz): δ = 7.22 (s,1H, H-4'), 7.08 (d, J = 8.80 Hz, 1H, H-6"), 6.94 (bs, 2H, 2-NH_2), 6.58 (d, J = 2.57 Hz, 1H, H-3"), 6.50 (dd, J = 8.80, 2.20 Hz, 1H, H-5"), 5.58 (d, J = 7.15Hz, 1H, H-5), 5.11 (d, J = 7.15 Hz, 1H, H-6), 3.83 (s, 2"-OCH_3), 3.75 (s, 4"-OCH_3). – **^{13}C NMR** (DMSO-d_6, 150 MHz) δ =160.7 (C_q-4"), 157.3 (C_q-2"), 154.7 (C_q-2), 141.6 (C_q-4), 138.4 (C_q-3'), 129.0 (CH-6"), 128.6 (CH-4'), 124.5 (C_q-5'), 121.3 (C_q-1"), 120.6 (C_q-2'), 105.2 (CH-5"), 102.3 (CH-5), 99.1 (CH-3"), 56.2 (2"-OCH_3), 55.7 (4"-OCH_3-), 35.8 (CH-6). – **(+)-ESIMS** *m/z* 401 ($[M+H]^+$, 100), 403 ($[M+H+2]^+$, 71), 405 ($[M+H+4]^+$, 16). – **(-)-HRESIMS** *m/z* 398.9802 $[M-H]^-$, 400.9771 $[M-H+2]^-$, 402.8047 $[M-H+4]^-$, (calcd for $C_{16}H_{13}Cl_2N_2O_2S_2$, 398.9801).

4-(2,5-dichlorothiophen-3-yl)-6-(2,5-dimethoxyphenyl)-6*H*-1,3-thiazin-2-amine (6e). Yield: 71%, – **^{1}H NMR** (DMSO-d_6, 600 MHz): δ = 7.21(s, 1H, H-4'), 6.99 (bs, 2H, 2-NH_2), 6.96 (d, J = 8.99 Hz, 1H, H-3"), 6.85 (dd, J = 8.99, 3.12 Hz, 1H, H-4"), 6.77 (d, J = 3.12 Hz, 1H, H-6"), 3.80 (s, 3H, 2"-OCH_3), 3.67 (s, 3H, 5"-OCH_3), 5.59 (d, J = 6.79 Hz, 1H, H-5), 5.15 (d, J= 6.79 Hz, H-6). – **^{13}C NMR** (DMSO-d_6, 150 MHz) δ = 154.4 (C_q-2), 153.5 (C_q-5"), 150.3 (C_q-2"), 142.0 (C_q-4), 138.7 (C_q-3'), 130.3 (C_q-1"), 128.5 (CH-4'), 124.6 (C_q-5'), 120.9 (C_q-2'), 114.8 (CH-6"), 113.1 (CH-4"), 112.5 (CH-3"), 102.0 (CH-5), 56.5 (2"-OCH_3), 55.7 (5"-OCH_3), 36.0 (CH-6). – **(-)-ESIMS** *m/z* 399 ($[M-H]^-$, 100), 401 ($[M-H+2]^-$, 69), 403 ($[M-H+4]^-$, 16). – **(-)-HRESIMS** *m/z* 398.9803 $[M-H]^-$, 400.9773 $[M-H+2]^-$, 402.9743 $[M-H+4]^-$, (calcd for $C_{16}H_{13}Cl_2N_2O_2S_2$, 398.9801).

6-(3-bromo-4-methoxyphenyl)-4-(2,5-dichlorothiophen-3-yl)-6*H*-1,3-thiazin-2-amine (6f). Yield: 42%, – **^{1}H NMR** (DMSO-d_6, 600 MHz): δ = 7.52 (d, J = 2.13 Hz, 1H, H-2''), 7.30 (dd, J = 8.59, 2.13 Hz, 1H, H-6''), 7.21 (s,1H, H-4'), 7.08 (d, ^{3}J = 8.59 Hz, 1H, H-5''), 7.01 (bs, 2H, 2-NH_2), 5.67 (d, J = 6.65 Hz, 1H, H-5), 5.03 (d, J = 6.64 Hz, 1H, H-6), 3.83 (s, -4''-OCH_3). – **^{13}C NMR** (DMSO-d_6, 150 MHz) δ = 154.7 (C_q-4''), 153.2 (C_q-2), 141.2 (C_q-4), 135.7 (C_q-1''), 131.6 (CH-2''), 128.0 (CH-4'), 127.8 (CH-6''), 124.0 (C_q-5'), 120.3 (C_q-2'), 112.7 (CH-5''), 110.4 (C_q-3''), 102.2 (CH-5), 56.2 (4''-OCH_3), 42.1 (CH-6). – **(-)-HRESIMS** *m/z* 446.8804 $[M-H]^-$, 448.8780 $[M-H+2]^-$, 450.8745 $[M-H+4]^-$, 452.8949 $[M-H+6]^-$ (calcd for $C_{15}H_{10}BrCl_2N_2OS_2Na$, 446.8800). – **(+)-HRESIMS** *m/z* 470.8765 $[M+Na]^+$, 472.8740 $[M+Na+2]^+$, 474.8712 $[M+Na+4]^+$, 476.3734 $[M+Na+6]^+$(calcd for $C_{15}H_{11}BrCl_2N_2OS_2Na$, 470.8765).

4-(2,5-dichlorothiophen-3-yl)-6-(naphthalen-3-yl)-6*H*-1,3-thiazin-2-amine (6g). Yield: 65%, – **^{1}H NMR** (DMSO-d_6, 600 MHz): δ = 8.26 (d, J = 8.37 Hz, 1H, 4''), 7.97 (dd, J = 7.92, 1.50 Hz, 1H, H-7''), 7.86 (dd, J = 6.27, 3.15 Hz, 1H, H-9''), 7.60 (td, 8.30, 1.30 Hz, 1H, H-5''), 7.55 (td, 7.87, 1.30 Hz, 1H, H-6''), 7.48 (m, 2H, H-2'', 10''), 7.28 (s, 1H, H-4'), 6.92 (bs, 2H, 2-NH_2), 5.78 (d, J = 6.40 Hz, 1H, H-5), 5.87 (d, J = 6.40 Hz, 1H, H-6). – **^{13}C NMR** (DMSO-d_6, 150 MHz) δ = 153.8 (C_q-2), 142.3 (C_q-4), 138.3 (C_q-3'), 135.9 (C_q-1''), 133.8 (C_q-8''), 129.8 (C_q-3''), 128.8 (CH-7''), 128.1 (CH-9''), 128.1 (CH-4'), 126.2 (CH-5''), 125.7 (CH-6''), 125.4 (CH-10''), 125.3 (CH-2''), 123.9 (C_q-5'), 123.5 (CH-4''), 120.2 (C_q-2'), 102.2 (CH-5), 38.2 (CH-6). – **(+)-ESIMS** *m/z* 389 ($[M-H]^-$, 100), 391 ($[M-H+2]^-$, 66), 393 ($[M-H+4]^-$, 17). – **(+)-HRESIMS** *m/z* 390.9890 $[M+H]^+$, 392.9861 $[M+H+2]^+$, 394.9833 $[M+H+4]^+$, (calcd for $C_{18}H_{13}Cl_2N_2S_2$, 390.9892).

Biological screening test

The *in vitro* antibacterial activity of the newly synthesized compounds (chalcones **5d-f, h** and thiazines **6a-g**) was carried out against 24h old cultures of *E. coli*, *S. aureus* and *B. subtilis* bacterial species, whereas the antifungal activity was carried out against 48h old culture of *C. albicans*, and *A. niger* spore suspension using well diffusion method at concentrations of 100 µg/mL in DMSO[56]. Nutrient agar and potato dextrose agars were used to culture the bacteria and fungi, respectively. The plates were inoculated by the bacteria or fungi and incubated for 24h at 37 ºC for bacteria and for 72h at 27 ºC for fungi. Simultaneously, cefaclor **(S1)** and cefuroxime **(S2)** at concentrations 100 µg/mL was used as standard against bacteria while fluconazole at the same concentration was used as standard antifungal agents.

Conclusion

Several novel structures of chalcones and thiazine heterocycles have been successfully synthesized. All compounds were characterized using different spectroscopic methods. The antimicrobial test of all new compounds was carried out against bacteria and fungi, which shows significantly better antibacterial activities compared to standard antibiotics.

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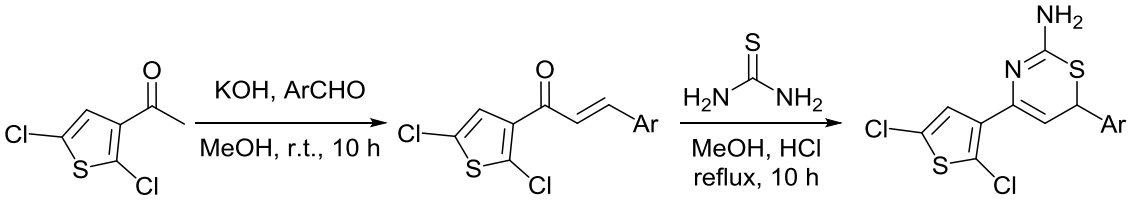

3 **5a-h** **6a-g**