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Green synthesis, antibacterial activity and computational study of pyrazoline and pyrimidine derivatives from 3-(3,4-dimethoxy-phenyl-1-(2,5-dimethyl-thiophen-3-yl)-propenone

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ABSTRACT

Various pyrazoline and pyrimidine derivatives were synthesized by the reaction of thiosemicarbazide / phenyl hydrazine / hydrazine hydtate / thiourea / urea with 3-(3,4-dimethoxy-phenyl-1-(2,5-dimethyl-thiophen-3-yl)-propenone under microwave irradiation, which itself was derived from the reaction of 3-acetyl-2,5-dimethylthiophene with 3,4-dimethoxy benzaldehyde. The corresponding pyrazoline and pyrimidine derivatives were obtained in good to excellent yields. All of the new compounds obtained were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analyses. The anti-bacterial activity of these compounds were tested *in-vitro* by the disk diffusion assay against two Gram-positive and two Gram-negative bacteria. The results showed that one of the pyrazoline derivatives is better at inhibiting the growth as compared to chloramphenicol against both types of the bacteria (Gram-positive and Gram-negative). Furthermore, all the molecules were subjected to computational calculation using the density functional theory with B3LYP method to corroborate their antibacterial activities.

1. Introduction

Chalcones, the α,β-unsaturated carbonyls, plays a vital role in many biological processes as well as an important intermediate for medicinal applications and is an area of continued attention for chemists and biologists [1-6]. In addition, these molecules exhibit wide range of pharmacological properties, like antibacterial [7-10], antimalarial [11], anticancer [12-14], antiviral, anti-inflammatory [15,16] and antituberculosis activities [17]. The application profile of chalcones is not limited to pharmaceutics but also useful in materials science fields such as non-linear optics (NLO) [18,19], optical limiting [20], as these molecules have interesting photodynamic and spectral properties [21,22]. Furthermore, chalcones are excellent precursors for the synthesis of variety of heterocyclic systems like pyrazolines, pyridines, pyrimidines, diazepines. Among these nitrogen heterocycles, pyrazolines and pyrimidine derivatives are of prime importance [23]. The pyrazoline ring system has been shown to possess number of biological activities including antibacterial [24], antitubercular [25], antidepressant [26], anti-inflammatory [27], anticancer [28], and anticonvulsant activities [29]. On second line, the pyrimidine derivatives are ubiquitous in nature as nucleic acids and vitamin B1 and also have remarkable pharmaceutical importance because of their biological activity as anti HIV [30], antitubercular [31], antifungal [32], antimalarial [33] and antitumor agents [34]. This prompted us to synthesize heterocyclic chalcone, 3-(3,4-dimethoxy-phenyl-1-(2,5-dimethylthiophen-3-yl)-propenone, and then cyclise with hydrazine and urea derivatives to generate thiphen-3-yl appended pyrazoline and pyrimidines derivatives (Scheme 1).

2. Experimental

All the chemicals and solvents used for this work were obtained from Merck (Germany) and Aldrich chemical company (USA). Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart- SMP10 melting point apparatus and are uncorrected. IR absorption spectra were recorded on Shimadzu FTIR-8400s using KBr pellets in the range of 4000-400 cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on Bruker-AVANCE-III 600 MHz spectrophotometer and TMS (tetramethylsilane) as an internal standard.

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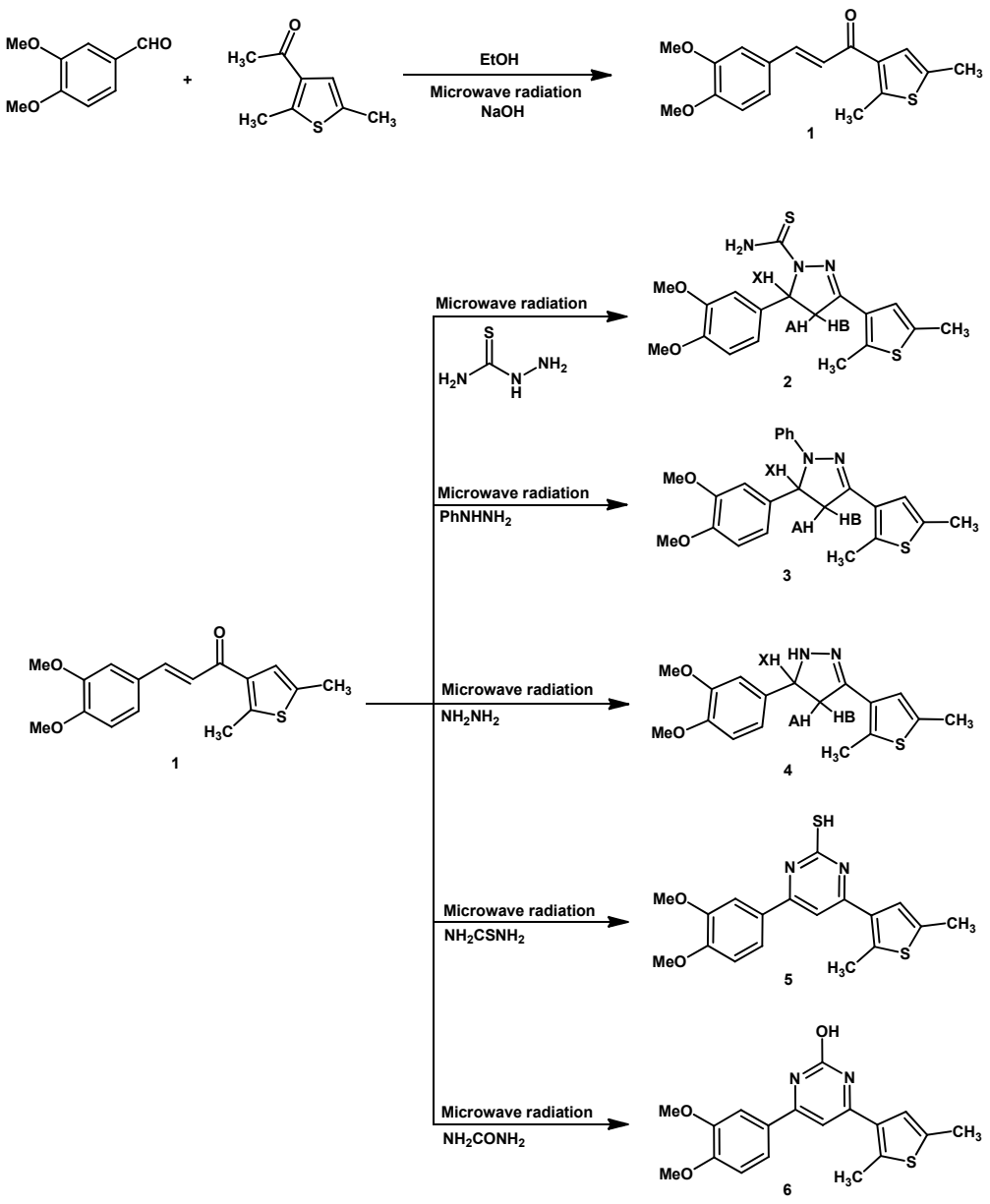

Scheme 1

The ^{1}H and ^{13}C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS. The splitting patterns are designated as follows; s, singlet; d, doublet; m, multiplet. Mass spectra were recorded on GC-MS spectrometer. Elemental analyses (C, H, N) were done on a CHN rapid analyzer. All the new compounds gave C, H and N analysis within 0.03% of the theoretical values. Purity of the compounds were checked by thin layer chromatography (TLC) on Merck silica gel 60 F_{254} precoated sheets in chloroform/methanol mixture and spots were developed using iodine vapours/ultraviolet light as visualizing agent.

2.1. 3-(3,4-Dimethoxyphenyl)-1-(2,5-dimethylthiophen-3-yl)-prop-2-en-1-one (1)

A solution of 3-acetyl-2,5-dimethythiophene (0.005 mol) and 3,4-dimethoxy-benzaldehyde (0.5 g, 0.0033 mol) in dry ethanol (20 mL) was added a catalytic amount of sodium hydroxide (1 pellet). The reaction mixture was heated inside a microwave oven for 43 sec. (at 210 Watts, i.e. 30% microwave power). When the reaction was completed, reaction mixture was allowed to cool in an ice bath. The product thus formed was filtered, washed with ethanol followed by water, then dried and recrystallized by distilled ethanol and chloroform (Scheme 1). Color: Yellow. Yield: 82%. M.p.: 115 °C. FT-IR (KBr, ν, cm^{-1}): 3045 (C-H), 2909 (C-H), 1647 (C=O), 1583 (C=C). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.66 (d, 1H, C=CH, J = 15.6 Hz), 7.27 (s, 1H, Ar-CH), 7.20 (d, 1H, Ar-CH, J = 8.4 Hz), 7.14 (d, 1H, CH=C, J = 15.6 Hz), 6.89 (d, 1H, Ar-CH, J = 8.4 Hz), 6.89 (s, 1H, $CH_{thiophen}$), 3.97 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 2.69 (s, 3H, CH_3), 2.44 (s, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 151.12, 149.09, 146.79, 143.76, 136.78, 135.12, 127.88, 125.91, 122.95, 122.90, 110.97, 109.82, 77.28, 55.96, 55.91, 15.82, 15.06. EI-MS (m/z, (%)): 304 (74) $[M+1]^{+\cdot}$. Anal. calcd. for $C_{17}H_{18}O_3S$: C, 67.52, H, 6.00, S, 10.60. Found: C, 67.46, H, 5.96, S, 9.97%.

2.2. 5-(3,4-Dimethoxy-phenyl)-3-(2,5-dimethyl-thiophen-3-yl),-4,5-dihydro-pyrazole-1-carbothioic acid amide (2)

A mixture of chalcone (0.0025 mol) (**3**), thiosemicarbazide (0.0025 mol) was dissolved in acetone (5 mL) and ethanol (5 mL), then K_2CO_3 (0.5 g) was added and stirred vigorously. After 6 min, the solvent was removed under vacuum and the dry powder was irradiated in a microwave oven for the 6 min at 110 W. After completion of reaction as followed by TLC examination, chilled water was added to the reaction mixture. The solid product was obtained, which was filtered, dried and crystallized from mixture of acetone:ethanol (6:4, *v:v*) (Scheme 1). Color: Brown. Yield: 78.2%. M.p.: 175 °C. FT-IR (KBr, *v*, cm^{-1}): 3426 (NH_2), 3245 (C-H), 2914 (C-H), 1582 (C=C), 1503 (C=N), 1249 (C=S), 1137 (C-N). ^{1}H NMR (600 MHz, DMSO-*d*6, δ, ppm): 7.20 (s, 2H, NH_2), 6.81 (dd, 2H, Ar-CH, *J* = 8.4 Hz), 6.76 (dd, 1H, Ar-CH, *J* = 8.4 Hz), 6.74 (s, 1H, $CH_{thiophen}$), 5.91 (dd, 1H, HX, J_{AX} = 3.6, J_{BX} = 3.2 Hz), 3.86 (dd, 1H, HA, J_{AX} = 11.4, J_{AB} = 10.2 Hz), 3.16 (dd, 1H, HB, J_{BA} = 3.6, J_{BX} = 3.6 Hz), 2.62 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 2.40 (s, 3H, OCH_3), 2.42 (s, 3H, OCH_3). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 176.17, 153.39, 149.12, 148.32, 140.37, 136.43, 134.41, 127.39, 125.56, 117.33, 111.31, 108.93, 62.39, 55.98, 45.48, 16.15, 14.99. MS (EI, *m/z* (%)): 377 (52) $[M+1]^{+\cdot}$. Anal. calcd. for $C_{18}H_{21}N_3O_2S_2$: C, 57.57, H, 5.64, N, 11.19. Found: 57.52, H, 5.58, N. 11.14%.

2.3. 5-(3,4-dimethoxyphenyl)-3-(2,5-dimethylthiophen-3-yl)-1-phenyl-4,5-dihydro-1H-pyrazole (3)

A mixture of chalcone (0.004 mol) (**1**) and phenyl-hydrazine (0.800 mL, 0.004 mol) was dissolved in ethanol (5 mL) then K_2CO_3 (0.003 mol) was added stirred vigorously. After 6 min, the solvent was removed under vacuum and the dry powder was irradiated in a microwave oven for the 6 min at 110 W. After completion of reaction as followed by TLC examination, chilled water was added to the reaction mixture. The solid product was obtained, which was filtered, dried and crystallized from ethanol (Scheme 1). Color: Dark Brown. Yield: 68.46%. FT-IR (KBr, *v*, cm^{-1}): 3292 (C-H), 2919 (C-H), 1670 (C=C), 1596 (C=N), 1135 (C-N). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.53 (s, 1H, Ar-$CH_{thiophen}$), 7.38-7.01 (m, 8H, Ar-CH), 5.08 (dd, 1H, HX, J_{AX} = 7.8, J_{BX} = 7.8 Hz), 3.94 (dd, 1H, HA, J_{AB} = 13.0, J_{AX} = 12.0 Hz), 3.12 (dd, 1H, HB, J_{BA} = 7.8, J_{BX} = 7.8 Hz), 2.54 (s, 3H, -OCH_3), 2.43 (s, 3H, -OCH_3), 2.25 (s, 3H, -CH_3), 2.14 (s, 3H, -CH_3). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 149.85, 148.24, 145.55, 144.81, 135.47, 131.39, 129.24, 128.83, 127.11, 125.31, 120.09, 118.04, 113.27, 108.59, 120.09, 118.04, 113.27, 108.59, 63.95, 55.89, 55.82, 15.88, 15.38, 15.05, 13.89. MS (EI, *m/z* (%)): 394(76) $[M+1]^{+\cdot}$. Anal. calcd. for $C_{23}H_{24}N_2O_2S$: C, 70.38, H, 6.16, N, 7.14. Found: C, 70.32, H, 6.12, N, 7.11%.

2.4. 5-(3,4-Dimethoxy-phenyl)-3-(2,5-dimethyl-thiophen-3-yl)-4,5-dihydro-1H-pyrazole (4)

A mixture of chalcone (0.004 mol) (**1**) and hydrazine hydrate (0.800 mL, 0.004 mol) was dissolved in ethanol (5 mL) then K_2CO_3 (0.003 mol) was added stirred vigorously. After 5 min, the solvent was removed under vacuum and the dry powder was irradiated in a microwave oven for the 6 min at 110 W. After completion of reaction as followed by TLC examination, chilled water was added to the reaction mixture. The solid product was obtained, which was filtered, dried and crystallized from ethanol (Scheme 1). Color: Dark Brown. Yield: 72.5%. M.p.: Semi-solid. FT-IR (KBr, *v*, cm^{-1}): 3246 (NH), 2928 (C-H), 1634 (C=C), 1562 (C=N), 1138(C-N). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 8.26 (s, NH), 7.31 (s, 1H, Ar-CH), 7.27 (d, 1H, Ar-CH, *J* = 8.4 Hz), 6.90 (d, 1H, Ar-CH, *J* = 8.4 Hz), 6.55 (s, 1H, $CH_{thiophen}$), 3.95 (dd, 1H, HX, J_{XA} = 10.0, J_{XB} = 12.0 Hz), 3.88 (dd, 1H, HA, J_{AX} = 13.0, J_{AB} = 11.5 Hz), 2.70 (dd, 1H, HB, J_{BA} = 5.4, J_{BX} = 6.8 Hz), 2.65 (s, 3H, -OCH_3), 2.64 (s, 3H, -OCH_3), 2.24 (s, 3H, CH_3), 2.18 (s, 3H, -CH_3). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 149.82, 134.54, 130.35, 127.29, 126.90, 126.13, 125.85, 112.27, 111.97, 112.21, 112.97, 112.11, 42.98, 40.44, 15.17, 15.11, 14.64. MS (EI, *m/z* (%)): 318(64) $[M+1]^{+\cdot}$. Anal. calcd. for $C_{17}H_{20}N_2O_2S$: C, 64.53, H, 6.37, N, 8.85. Found: 64.48, H, 6.32, N. 8.81%.

2.5. 4-(3,4-Dimethoxyphenyl)-6-(2,5-dimethylthiophen-3-yl)-pyrimidine-2-thiol (5)

A mixture of chalcone (1 mmol) (**3**), thiourea (1.5 mmol) and KOH (0.002 mole) were dissolved in 10 mL ethanol. The contents were thoroughly mixed. The reaction mixture was subjected to microwave irradiation in a commercially available IFB domestic microwave oven having a maximum power output of 110 W operating at 2450 Hz intermittently at 5 min, a completion of reaction as monitored by TLC. It was then cooled and poured in cold water acidified with dil. HCl. Filtered, washed and dried. The product was recrystallized from ethanol to get product (Scheme 1). Color: Reddish Brown. Yield: 72.52%. M.p.: 97 °C. FT-IR (KBr, *v*, cm^{-1}): 3192 (C-H), 2915(C-H), 1648 (C=C), 1578 (C=N), 1190 (C-N), 683 (C-SH). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 7.63 (s, 1H, $CH_{pyr.}$) 7.27 (d, 1H, Ar-CH, *J* = 6.6 Hz), 7.20 (d, 1H, Ar-CH, *J* = 6.6 Hz), 7.14 (s, 1H, Ar-CH), 7.07 (s, 1H, $CH_{thiophen}$), 3.95 (s, 1H, S-H), 2.45 (s, 3H, -OCH_3), 2.41 (s, 3H, -OCH_3), 2.34 (s, 3H, CH_3), 2.37 (s, 3H, -CH_3). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 186.67, 174.87, 151.17, 149.53, 149.14, 143.76, 136.82, 135.82, 129.94, 127.93, 122.94, 111.18, 109.77, 77.23, 55.99, 15.11, 14.64. 15.82, 15.07, 14.12. MS (EI, *m/z* (%)): 360 (82) $[M+1]^{+\cdot}$. Anal. calcd. for $C_{18}H_{18}N_2O_2S_2$: C, 60.31, H, 5.06, N, 7.81. Found: C, 60.27, H, 4.98, N, 7.78%.

2.6. 4-(3,4-dimethoxyphenyl)-6-(2,5-dimethylthiophen-3-yl)pyrimidin-2-ol (6)

A mixture of chalcone (1 mmol) (**3**), urea (1.5 mmol) and KOH (0.002 mole) were dissolved in 10 mL ethanol. The contents were thoroughly mixed. The reaction mixture was subjected to microwave irradiation in a commercially available IFB domestic microwave oven having a maximum power output of 110 W operating at 2450 Hz intermittently at 5 min, a completion of reaction as monitored by TLC. It was then cooled and poured in cold water acidified with dil. HCl. Filtered, washed and dried. The product was recrystallized from ethanol to get product (Scheme 1). Color: Brown. Yield: 82.90 %. M.p.: 107 °C. IR (KBr, *v*, cm^{-1}): 3416 (OH), 2935(C-H), 1634 (C=C), 1529 (C=N), 1118 (C-N). ^{1}H NMR (600 MHz, DMSO-d_6, δ, ppm): 9.86 (s, 1H, OH), 7.66 (d, 1H, Ar-CH, *J* = 7.8 Hz), 7.21 (d, 1H, Ar-CH *J* = 7.4 Hz), 7.07 (s, 1H, Ar-CH), 7.14 (s, 1H, $CH_{thiophen}$), 6.90 (s, 1H, $CH_{pyr.}$), 2.67 (s, 3H, OCH_3), 2.68 (s, 3H, OCH_3), 2.45 (s, 3H, CH_3), 2.45 (s, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 186.67, 151.18, 149.16, 146.76, 143.75, 136.83, 135.29, 127.94, 125.92, 123.00, 122.93, 111.09, 109.92, 55.99, 55.95, 15.81, 15.07. MS (EI, *m/z* (%)): 344(76) $[M+1]^{+\cdot}$. Anal. calcd. for $C_{18}H_{18}N_2O_3S$: C, 63.14, H, 5.30, N, 8.18. Found: C, 63.11, H, 5.27, N, 8.15%.

2.7. Organism culture and in vitro screening

Antibacterial activity was assayed by the disk diffusion method with minor modifications [35]. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimurium*, and *Escherichia coli* were subcultured in BHI medium and incubated for 18 h at 37 °C, and then the bacterial cells were suspended, according to the McFarland protocol in saline solution to produce a suspension of about 10^5 CFU/mL. Ten µL of this suspension was mixed with sterile antibiotic agar (10 mL) at 40 °C and poured onto an agar plate in a laminar flow cabinet. Five paper disks (6.0 mm diameter) were fixed onto nutrient agar plate.

Table 1. Antibacterial activity of chalcone and their cyclized products, positive control chloramphenicol and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

Compounds	Corresponding effect on microorganisms *			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
1	9.8±0.3	10.5±0.4	10.8±0.2	11.2±0.5
2	17.6±0.5	18.6±0.4	17.6±0.4	20.6±0.5
3	17.3±0.5	17.8±0.5	17.4±0.4	20.2±0.8
4	16.6±0.4	16.2±0.5	16.8±0.5	17.8±0.5
5	14.4±0.4	14.8±0.5	15.6±0.4	16.2±0.5
6	15.2±0.4	15.2±0.4	14.8±0.4	16.4±0.4
Chloramphenicol	17.0±0.5	18.2±0.4	17.2±0.8	20.0±0.2
DMSO	-	-	-	-

* "-": No activity.

Table 2. Minimum inhibition concentration (MIC) of chalcones (**1**-**6**) products, positive control: chloramphenicol.

Bacterial Strain	MIC (µg/mL) Compound						Positive control
	1	2	3	4	5	6	
S. aureus	512	32	16	128	64	128	32
S. pyogenes	512	32	32	128	128	128	32
S. typhimurium	128	16	16	64	64	64	32
E. coli	128	16	32	64	64	64	32

Ten mg of each test compound was dissolved in DMSO (100 µL) to prepare stock solution and from stock solution different concentration of 10 (1 µL stock solution + 9 µL solvent), 20 (1 µL stock solution + 4 µL solvent), 25 (1 µL stock solution + 3 µL solvent), 50 (1 µL stock solution + 1 µL solvent), and 100 µg/µL of each test compound were prepared. These compounds of different concentration were poured over disk plate on to it. Chloramphenicol (30 µg) was used as standard drug (positive control). A DMSO-wetted disk was used as negative control. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. Table 1 and 2 reports the inhibition zones (mm) of each compound and the controls this experiment was repeated two times for each compound and found same results.

2.8. Computational method

The computational calculations of compounds **1** to **6** were performed using Spartan'04 Windows graphical software [36], with density functional theory (DFT) with RB3LYP method. This method previously has been used effectively for the calculation of the small molecules [37].

However, all the molecules were modeled and sent to energy minimization first, and then their lower energy conformational structure was calculated using Semi-Empirical PM3 calculations. All the calculated molecules were submitted for the higher level of calculations i.e. Hartree-Fock (HF)/3-21G* basis set, and then to HF/6-31G* basis set. The resulting wave function, Hessian matrix, and geometry of the molecules were used and submitted for the final level of calculation that is DFT/B3LYP with 6-31G* basis set. The fundamental frequencies of finally obtained structures were also calculated and assigned as minima (all real frequencies).

3. Results and discussion

3.1. Chemistry

In the present work, pyrazolines (**2**-**4**) and pyrimidines (**5**-**6**) were prepared by the reaction of 3-(3,4-dimethoxy-phenyl-1-(2,5-dimethyl-thiophen-3-yl)-propenone (**1**) with thiosemi-carbazide / phenyl hydrazine / hydrazine/ thiourea / urea [38,39] by the microwave irradiation. The synthetic route of compounds is outlined in Scheme 1. The chemical structures of the synthesized compounds were established by spectroscopic (FT-IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analyses.

Assignments of selected characteristic IR band positions provide significant indication for the formation of the Schiff base derivative. The IR spectrum of compound **1** shows the characteristic band at 1647 cm^{-1} which indicates the presence of C=O group. The IR spectrum of compound **2** shows the characteristic bands at 1596 cm^{-1} and 1135 cm^{-1} which indicate the presence of C=N and C-N group. The IR spectrum of compound **3** shows the characteristic bands at 1503 cm^{-1} and 1249 cm^{-1} which indicate the presence of C=N and C=S group. The IR spectrum of compound **4** shows the characteristic bands at 1562 cm^{-1} and 1138 cm^{-1} which indicate the presence of C=N and C-N group. The IR spectrum of compound **5** shows the characteristic bands at 1578 cm^{-1} and 683 cm^{-1} which indicate the presence of C=N and –S-H group. The IR spectrum of compound **6** shows the characteristic bands at 1529 cm^{-1} and 3416 cm^{-1} which indicate the presence of C=N and OH group. ^{1}H NMR spectrum of compound **1** shows two doublets at 7.66 ppm (J = 15.6 Hz) and 7.14 ppm (J = 15.6 Hz), indicating that the ethylene moiety in the enone linkage is in the *trans*-conformation in the chalcone. ^{1}H NMR spectra of compounds **2**, **3** and **4** show doublet of doublet (*dd*) of –CH_2 at 2.70-5.91 ppm confirmed the cyclisation in pyrazoline moiety in 2, 3 and 5. ^{1}H NMR spectrum of compound **5** shows a sharp singlet at δ 3.95 ppm due to S-H proton; and also have a sharp singlet of C-H at δ 7.63 ppm, confirmed the cyclisation to form pyrimidine. Spectrum of compound **6** shows a sharp singlet at 9.86 due to OH proton. It also shows a sharp singlet of C-H at δ 6.90 ppm, confirmed the cyclisation to form pyrimidine. ^{13}C NMR ($CDCl_3$) spectrum of chalcone and their cyclised products were recorded in $CDCl_3$. Spectral signals are in good agreement with the probable structural details of ^{13}CNMR spectra of all the compounds, see the experimental section. Finally, characteristic peaks were also observed in the mass spectra of chalcone and its cyclized products by the molecular ion peak. The mass spectrum of compound **5** shows a molecular ion peak ($M^{+\cdot}$) m/z 360.

3.2. Antimicrobial activity

The *in vitro* antibacterial activity of chalcone and their cyclized products (**1**-**6**) assayed by the disk diffusion method using cultures of *S. aureus, S. pyogenes, S. typhimurium*, and *E. coli*. Chloramphenicol (30 mg) was used as the standard drug, whereas a DMSO-wetted disk was used as negative control [40].

The results showed that compound **2** and **3** is better at inhibiting the growth of both types of the bacteria (Gram-positive and Gram-negative) as compared to chloramphenicol. Results are summarized in Table 1 and 2.

Table 3. All the descriptors calculated using DFT with B3LYP method.

Structure	Total energy (au)	LUMO (eV)	Molecular weight	Volume (Å^3)	Density
1	-1282.44604	-1.98	302.394	314.62	0.961
2	-1809.57252	-2.00	375.517	366.45	0.958
3	-1548.95936	-1.99	392.523	409.01	0.960
4	-1317.91369	-0.58	316.425	324.30	0.976
5	-1753.04767	-1.53	358.486	350.36	1.023
6	-1430.09129	-1.49	342.419	339.97	1.007

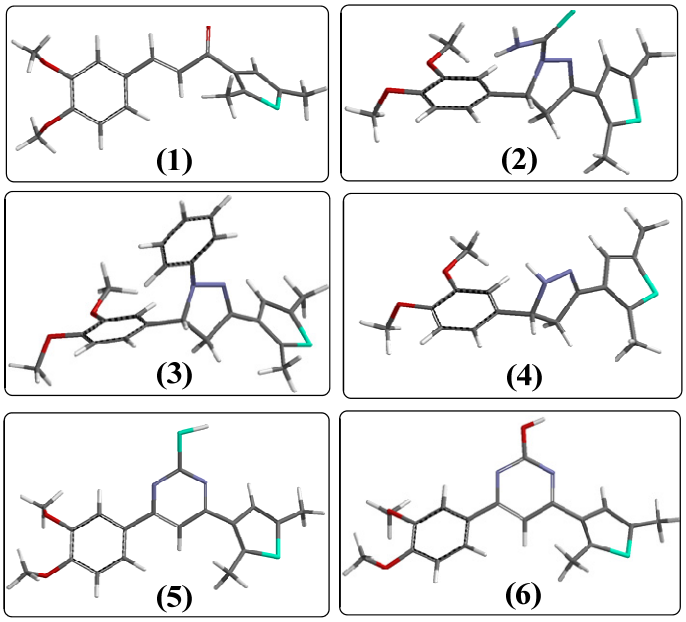

Figure 1. Three dimensional optimized structures **1** to **6** calculated with DFT/B3LYP method.

3.3. Computation

All the compounds **1** to **6** were calculated using DFT with RB3LYP method. Their calculated three dimensional structures are shown in Figure 1. Some selected descriptors such as total energy, molecular weight, LUMO (Lowest Unoccupied Molecular Orbital), volume and density, are presented in Table 3.

Antibacterial activity of a molecule is a function of its LUMO and density.

LUMO is an electronic parameter, which measures the electrophilicity of the molecules. When a molecule acts as a Lewis acid, incoming electrons are received in its LUMO. Molecules with low-lying LUMO are more capable to accept electrons than those with higher energy LUMO, and thus will show higher activity.

Density is a 3D-descriptor, it reflects the type of atoms and how tightly they are packed in a molecule. However, the density is negatively correlated with the activity. This means molecule with lower density, will increase the activity. However, this does not hold true for the compound **1**, which has shown the lowest antibacterial activity experimentally. It was observed that the molecules **2** and **3** have lower LUMO energies, and lower density values (Table 3). Thus, one can conclude the higher antibacterial activity of compounds **2** and **3**. These theoretical results are in good agreement in our experimental results.

4. Conclusion

A chalcone was prepared by the reaction of 3,4-dimethoxy benzaldehyde with 3-acetyl-2,5-dimethylthiophene, treatment of chalcone with thiosemicarbazide/phenyl hydrazine/ hydrazine hydrate/thiourea/urea resulted in the corresponding pyrazolines, pyrimidines with good yields. The antibacterial activity of this compound was examined using culture of bacteria and the results showed that the pyrazoline and pyrimidine have the promising antibacterial activity. Among the entire six compounds, pyrazolines derivative (**2** and **3**) showed better antibacterial activity as compared with the reference drug chloramphenicol. It was also observed theoretically that the compounds **2** and **3** with lower LUMO values and lower density values concluded their higher activity. These theoretical results were found in good support to the experimental results.

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