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Bioactivity Study of Thiophene and Pyrazole Containing Heterocycles

NITIN V. KALE[1], SUPRIYA P. SALVE[1], BHAUSAHEB K. KARALE[1], SADHANA D. MHASKE[2], SUSHAMA B. DARE[1], ANIL E. ATHARE[1] and SUSHAMA J. TAKATE[1]*

[1]Department of Chemistry, New Arts, Commerce and Science College, Ahmednagar, 414001, affiliated to SPPU, Pune, Maharashtra, India.
[2]Department of Chemistry, Dadapatil Rajale Arts, Science and Commerce College, Adinathnagar, 414505, Affiliated to SPPU, Pune, Maharashtra, India.
*Corresponding author E-mail: sjtakate26@gmail.com

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ABSTRACT

Chalcones **3a-f** were prepared by reacting thiophene containing pyrazolyl aldehyde **(2)** with different 2-hydroxy acetophenones **1a-f**. The compounds **3a-f** were transformed into different Pyrazolines **4a-f**. The formation of chromene derivatives **5a-f** occurred from the cyclization of **3a-f**, which were then transformed into pyrazole derivatives **6a-f**. Newly synthesized compounds have promising antibacterial activity against *S. typhii* and *S. aureus*, while weak activity against *B. subtilis* and *E. coli*. Compounds **5d** and **6d** had significant antifungal action towards *A. niger*, while most of the compounds were moderately active towards *T. viride*. Some of the synthesized compounds showed promising α-amylase inhibitory activity at 1 mg/mL concentration.

Keywords: 2-Hydroxyacetophenone, Pyrazole, Chromone, Antimicrobial activity.

INTRODUCTION

In the field of medicinal chemistry, most drugs have different heterocyclic scaffolds that show potential biological activities. Microbes are responsible for different human diseases. As these microorganisms develop resistance towards many of the present drug molecules, there is a need for continuous research on developing new potential medicinal agents. The presence of oxygen, sulphur and nitrogen containing heterocyclic nucleus imparts very effective pharmacological properties to therapeutic agents. In these scaffolds, the presence of Fluorine increases bioactivity of molecules several times[1,2].

Thiophene derivatives have varied therapeutic applications. Thiophene containing heterocyclic compounds have created interest among the researchers owing to their vast spectrum of biological functions including antimicrobial[3,4], antiparasitic[5,6], antiviral[7], anticancer[8,9], enzyme inhibitors[10], anti-inflammatory and analgesic[11] properties. Some of the commercially available drugs that contain thiophene as an integral component are Suprophen and Tiaprofenic acid

as an anti-inflammatory, Rolaxifene and OSI-930 as an anticancer, Methapyrilene as anti-histamine, Tienilic acid as an antihypertensive, Ticlopidine as antiplatelet, Olanzapine as antisychotic, Etizolam as anti-anxiety and Tigabine as anticonvulsant agents.

In recent years, pyrazole is the most studied heterocycle among the azole family due to its innumerable chemical, agrochemical and pharmacological[12,13] properties. Pyrazole containing compounds are reported for anticancer[14,15] antibacterial, antifungal[16–18], antiviral[19,20], antinflammatory[18,21], anti FAAH (Fatty Acid Amide Hydrolase)[22], anti-enzymatic(Anti-S IRT 1 and SIRT 2)[23], analgesic[24], 5α-Reductase inhibitor[25], antioxidant[26,27], and insecticidal[28]. The Pyrazole scaffold has fascinating medicinal potential and is found as a pharmacophoric group of the drug molecules available on the market such as anti-inflammatory agents Meprizole, Celecoxib and Lonazolac, Rimonabant acts as cannabinoid receptor and used to treat obesity, Difenamizole functions as an analgetic, Fomepizole inhibits alcohol dehydrogenase, Fezolamine acts as antidepressant, CDPPB functions as anti-psychotic and sildenafil inhibits phosphodiesterase (Figure 1).

Chalcones have attracted much attention from medicinal chemists, not only as synthon for biosynthetic perspectives but also as bioactive moiety[29–32]. Several heterocyclic rings can be obtained from chalcones through ring closure reactions. Chalcone shows diversified medicinal and biological activities such as antimalarial[33,34], anticancer[35,36], anti-inflammatory[37,38], anti-tubercular[39,40], Antioxidant[41,42], anti-alzheimer[43], antibacterial and antifungal[44–46].

Chromones belong to the flavonoid family and are widely used in folk medicine because of their interesting biological activities[47,48]. The large number of bioactive molecules have chromone moiety as an essential pharmacophore[49,50]. Many of the biologically active chromones show anti-bacterial and antifungal[51,52], anti-inflammatory[53,54], anti-oxidant[55,56], anticancer[57–59], anti-HIV[60], anti-obesity[61], antiviral[62], antidiabetic[48], anticonvulsant[63] and anti-tubercular activities[64]. In light of the significance of thiophene, pyrazole, chalcone and chromone in numerous areas, particularly in medicinal chemistry (biological activity), the present study focuses on synthesis of these scaffolds and their biological activities.

Inhibition of the digestive enzymes is one of the approaches to manage diabetis. These enzymes catalyse the hydrolysis of starch into smaller carbohydrates like glucose[65]. This is one of the important steps in maintaining blood sugar level. In diabetics, hypoglycaemia can be achieved by inhibiting the enzyme alpha-amylase and the inhibitor can prove a potential anti-diabetic agent.

Heterocycles like flavones, pyrazoles and thiophenes are reported for their α-amylase inhibitory activity[66-69].

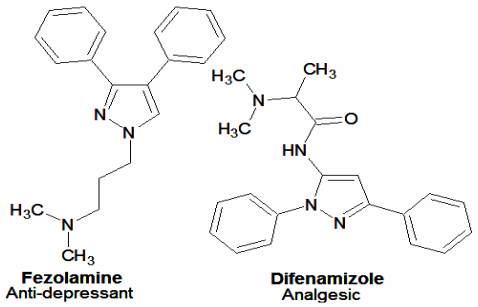


Fig. 1.

RESULT AND DISCUSSION

A well-known literature approach was used to synthesize various acetophenones **(1a-f)** and arylaldehyde **(2)** derivatives, as illustrated in Scheme-I and Scheme-II. The synthesis of chalcones, **3a-f** was carried out by Claisen-Schmidt condensation by using **1a-f** and **2**. Different spectral methods have verified the formation of **3a-f**. I.R. spectra of compound **3d** shows band at 3439, 1639 cm^{-1}. HRMS shows a molecular ion peak at 459.0135 support **3d** formation. ^{1}HNMR signal at 5.88 indicate olefinic proton and also the signal at δ 8.85 shows proton of pyrazole ring. In ethanol, the molecule **3a-f** interacts with hydrazine hydrate to produce bipyrazolyl phenols, **4a-f**. This formation of pyrazolines **4a-f** was confirmed by spectral technique. The I.R. Spectrum 3334 cm^{-1} and Molecular ion peak at 473.0410 in HRMS validated formation of **4d**. The most important confirmation of **4d** formation is in ^{1}HNMR spectra which shows two doublets at δ 3.17 and δ 3.71 confirm the presence of diastereotopic protons of methylene group of pyrazoline ring while the δ 5.09 triplet of methine in pyrazoline ring. These signals strongly support the formation of **4a-f**. Refluxing chalcone **3a-f** in DMSO with a catalytic quantity of iodine yielded 2-substituted chromone **5a-f**. The I.R. spectrum at 1653 cm^{-1}

and the molecular ion peak at 456.9967 verify **5d**. ^{1}HNMR spectra validate chromone formation as there is absence of downfield signal above δ 10.0 implies absence of O-H and also singlet at δ 6.80 is due to 3-position proton chromone. Chromones **5a-f** when refluxed in ethanol and hydrazine hydrate were transformed into pyrazoles **6a-f**. The I.R. Spectrum at 3404 cm^{-1} and In HRMS molecular ion peaks at 471.0243 supports **6d** formation. The most significant confirmation is the presence of a singlet at δ 12.70 in ^{1}HNMR spectra of O-H proton.

Amylase Inhibitory Activity

In a test tube 500 µL of test sample, 500 µL solution of α-amylase whose concentration is 0.5 mg/mL and phosphate buffer of 0.2 mM concentration were mixed and kept undisturbed 10 min at room temperature. Then the contents of the test tube were allowed to react 1% starch solution in phosphate buffer having pH 6.9. Then dinitrosalicylic acid was used to extinguish reaction. After incubation of 5 min test tubes were cooled and the contents were diluted with distilled water. The absorbance of resulting solutions was recorded at the wavelength 540nm. Then the results were compared with well-known inhibitor of the acarbose.

The results are recorded as % inhibition of enzyme activity.

%Inhibition = (Ac-As)/Ac x 100

Where AC - Absorbance for Control and AS - Absorbance for Sample

Table II: Amylase inhibitory Activity: (Concentration 1 mg/mL)

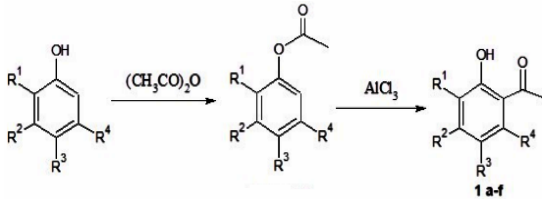
Compound	% Inhibition
6a	34
6b	31
6e	09
6f	10
Acarbose	45

Microbial analysis

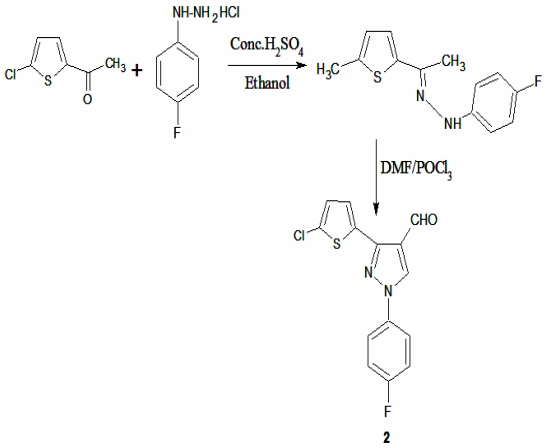
In vitro tests were performed on the compounds **2, 3a-f, 4a-f, 5a-f,** and **6a-j** against four bacterial and two fungal strains (Table 1). The agar well diffusion technique was utilized in this experiment. Ciprofloxacin and fluconazole were utilized as antibacterial and antifungal reference drugs respectively, while DMSO was used as a negative control. All compounds are inactive towards *Gram-positive* bacteria *Bacillus subtilis* and have modest action against *Escherichia coli* and *Staphylococcus aureus*. Compound **3c, 5a** and **5b** showed good activity against Salmonella typhii while all other compounds have a moderate level of action. Compounds **5d** and **6d** were shown to have promising action against *Aspergillus niger*, while others had modest activity against *Trichoderma viride*. The results were averaged over three experimental sets and reported as zone of inhibition in millimeters.

Table 1: Antimicrobial Activity (Zone of Inhibition at 1 mg/mL in mm)

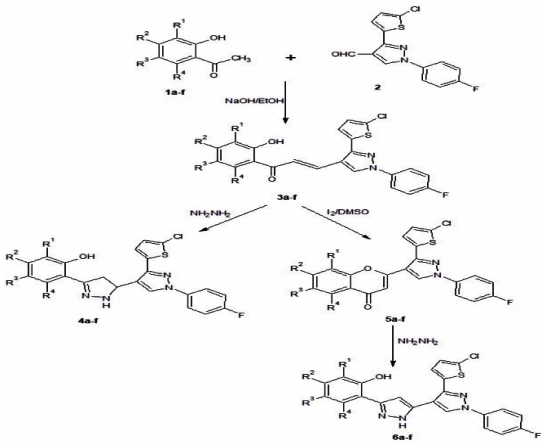
Compound	Antibacterial Activity				Antifungal Activity	
	Bacillus subtilis	*Staphylococcus aureus*	*Salmonella typhii*	*Escherichia coli*	*Aspergillus niger*	*Trichoderma viride*
2	-	11	13	+	-	-
3a	-	13	12	+	-	13
3b	-	15	13	+	-	14
3c	-	14	18	10	-	-
3d	-	10	11	10	-	-
3e	-	11	12	+	-	12
3f	-	12	12	10	-	-
4a	-	13	13	+	-	11
4b	-	12	15	+	-	14
4c	-	11	12	10	-	13
4d	-	12	13	+	-	-
4e	-	13	12	+	-	12
4f	-	12	13	+	-	-
5a	-	15	17	10	-	15
5b	-	13	18	+	-	21
5c	-	14	16	+	-	-
5d	-	13	16	+	21	20
5e	-	13	16	10	-	-
5f	-	14	15	10	-	-
6a	-	15	13	11	-	-
6b	-	12	12	11	-	-
6c	-	12	13	11	-	-
6d	-	13	13	11	22	21
6e	-	13	13	11	-	17
6f	-	11	-	-	-	-
Ciprofloxacin	28	23	40	26	-	-
Fluconazole	-	-	-	-	28	29



Scheme 1



Scheme 2



Scheme 3

EXPERIMENTAL

Open capillary technique was used for melting points which are uncorrected. IR Affinity-I Fourier transform infrared spectrophotometer (Shimadzu), Bruker Avance II 500 MHz spectrophotometer and Waters SYNAPT G2 HDMS were used to record the IR, ^{1}H NMR and Mass spectra. Samples for NMR were prepared in DMSO-d_6 and TMS was internal reference. Absorption frequencies in terms of chemical shift were expressed in δ ppm. Mass spectra were recorded on.

Table 2: Physical data for synthesized compound

Compound	R1	R2	R3	R4	m. p.(°C)	Yield (%)
3a	H	H	H	H	170	65
3b	H	CH_3	H	H	172-174	61
3c	H	CH_3	Cl	CH_3	220-222	63
3d	H	H	Cl	H	262-264	66
3e	Cl	H	Cl	H	280-282	68
3f	H	H	CH_3	H	262-264	58
4a	H	H	H	H	176-178	84
4b	H	CH_3	H	H	188-190	78
4c	H	CH_3	Cl	CH_3	242-244	86
4d	H	H	Cl	H	252-254	82
4e	Cl	H	Cl	H	294-296	84
4f	H	H	CH_3	H	264-266	80
5a	H	H	H	H	206-208	76
5b	H	CH_3	H	H	218-220	78
5c	H	CH_3	Cl	CH_3	222-224	80
5d	H	H	Cl	H	232-234	82
5e	Cl	H	Cl	H	256-258	78
5f	H	H	CH_3	H	248-252	80
6a	H	H	H	H	196-198	82
6b	H	CH_3	H	H	204-206	78
6c	H	CH_3	Cl	CH_3	208-210	72
6d	H	H	Cl	H	202-204	76
6e	Cl	H	Cl	H	218-220	82
6f	H	H	CH_3	H	212-214	74

(2E)-3-[3-(5-Chlorothiophen-2-yl)-1-(4-fluorophenyl)-1H-pyrazol-4-yl]-1-(2-hydroxyphenyl)prop-2-en-1-one, 3a

In 25 mL of ethanol and 12 mL of 30 percent NaOH solution, 2-hydroxyacetophenone 1a (0.015 mol) and 1,3-disubstituted-pyrazole-4-carbaldehyde 2 (0.015 mol) were dissolved and stirred at ambient temperature for 40-48 h with TLC monitoring. The contents were transferred to a beaker containing crushed ice. Then it was acidified using dil. acetic acid and the product was filtered and purified using alcohol to get **3a**. The compounds **3b-3f** were prepared using the same procedure.

3a

Yellow Solid, IR (KBr): 3124, 3066, 2926, 1685, 1639, 1514, 831, 748 cm^{-1}; HRMS: m/z 424.8751 (M+) ; ^{1}HNMR: δ 11.8 (1H, s), 8.89 (1H, s), 7.95 (2H, dd, *J*=9.12, 4.12Hz), 7.81 (1H, dd, *J*=8.88, 2.82Hz), 7.71 (1H, ddd, *J*=8.92, 8.96, 2.84Hz), 7.46 (2H, t, *J*=9.12Hz), 7.37 (1H, d), 7.28 (1H, ddd, *J*=8.96, 8.88, 2.90Hz), 7.21-7.24`(3H,`m),`5.92 (2H, q).

3b

Yellow Solid, IR(KBr): 3398, 3132, 3080, 1639, 1573, 1514, 833, 800 cm-1; HRMS: m/z 438.9017(M+); ^{1}HNMR: δ 10.2 (1H, s), 8.81 (1H, s),

7.87 (2H, dd, *J*=8.88, 3.88 Hz), 7.71 (1H, dd, *J*=8.64, 2.58Hz), 7.38 (2H, t, *J*=8.88Hz), 7.12-7.16 (2H, m), 7.09 (1H, dd, *J*=8.64, 2.56Hz), 5.84 (2H, q), 2.34 (3H, s).

3c

Yellow Solid, IR(KBr): 3352, 3124, 2924, 1672, 1639, 1585, 1514, 831, 752 cm^{-1}; HRMS: m/z 487.3733(M+); ^{1}HNMR: δ 12.4 (1H, s), 8.93 (1H, s), 7.99 (2H, dd, *J*=9.14, 4.14 Hz), 7.50 (2H, t, *J*=9.14Hz), 7.41 (1H, d), 7.24-7.28 (2H, m), i5.96i (2H,iq), i2.56i(3H,is), i2.48 (3H,is).

3d

Yellow Solid, IR(KBr): 3446, 3132, 3070, 1639, 1573, 1514, 833, 800 cm^{-1}; HRMS: m/z 459.0135 (M+); ^{1}HNMR: δ 11.3 (1H, s), 8.85 (1H, s), 7.91 (2H, dd, *J*= 9.4Hz), 7.79 (1H, d, *J*=2.7Hz), 7.69 (1H, dd, *J*=8.8, 2.72Hz), 7.42 (2H, t, *J*=9Hz), 7.33 (1H, d), 7.17-7.20 (2H, m), 5.88 (2H, AB-Quartet).

3e

Yellow Solid, IR(KBr): 3446, 3130, 3070, 1643, 1570, 1508, 835, 808 cm^{-1}; HRMS: m/z 473.7652(M+); ^{1}HNMR: δ 10.8 (1H, s), 8.79 (1H, s), 7.85 (2H, dd, *J*=8.86, 3.86 Hz), 7.71 (1H, d, *J*=2.56Hz), 7.61 (1H, d, *J*=2.58Hz), 7.36 (2H, t, *J*=8.86Hz), 7.27 (1H, d), 7.11 (1H, d), 5.82 (2H, q).

3f

Yellow Solid, IR(KBr): 3446, 3134, 1683, 1637, 1560, 1514, 831, 804 cm^{-1}; HRMS: m/z 438.9017(M+); ^{1}HNMR: δ 11.3 (1H, s), 8.91 (1H, s), 7.97 (2H, dd, *J*=9.06,4.06 Hz), 7.83 (1H, d, *J*=2.76Hz), 7.73 (1H, dd, *J*=8.86, 2.78Hz), 7.49 (2H, t, *J*=9.06Hz), 7.39 (1H, d), 7.22-7.26i(2H,im), i5.94i(2H,iq), i2.32i(3H,is).

2-[(3S)-3'-(5-Chlorothiophen-2-yl)-1'-(4-fluorophenyl)-3,4-dihydro-1'H,2H-3,4'-bipyrazol-5-yl]phenol, 4a

A mixture of 2 mL hydrazine hydrate, 15 mL ethanol and substituted pyrazolyl chalcone 3a (0.0015 mol) was taken in R.B. flask and refluxed for 4 hours. Then by adding 2 mL of glacial acetic acid, heated for further 4 hours. Once the reaction was finished the contents were taken into crushed ice. The resulting product had been filtered. On crystallization from ethanol, pure compound **4a** was obtained. The compound **4b-4f** were prepared using the same procedure.

4a

Yellow Solid, IR(KBr): 3309, 3084, 1593, 1514, 835, 802 cm^{-1}; HRMS: m/z 438.9050 (M+); ^{1}HNMR: δi12.26 (s, 1H), 8.64i(s,i1H), i7.90-8.01 (m,i3H), 7.37-7.50 (m, 4H), 7.32 (1H, ddd, *J*=8.84, 8.82, 2.68Hz), 7.22 (1H, d, *J*=8.92Hz), 7.14 (1H, ddd, *J*=8.86, 8.80, 2.94Hz), 6.97 (1H, dd, *J*=8.62, 2.94Hz), 5.13 (1H, t, *J*=10.12Hz), 3.75 (1H, dd, *J* =17.02, 10.12Hz), 3.21 (1H, dd, *J*=17.01Hz, 10.12Hz).

4b

Yellow Solid, IR(KBr): 3361, 3338, 3082, 1593, 1516, 829, 813 cm^{-1}; HRMS: m/z 452.9316 (M+); ^{1}HNMR: δ 11.64i(1H,is), 8.56i(1H,is), T7.84-7.93i(3H, m), 7.29-7.44 (4H, m), 7.13 (1H, d, *J*=8.68Hz) 7.04 (1H, dd, *J*=8.62, 2.52Hz), 6.90 (1H, d, *J*=2.52Hz), 5.05 (1H, t, 9.88Hz), 3.67 (1H, dd, *J*=17.04, 9.88Hz), 3.13 (1H, dd, *J*=17.05, 9.88Hz), 2.28 (3H, s).

4c

Yellow Solid, IR(KBr): 3292, 3078, 1514, 835, 802 cm^{-1}; iHRMS: m/z 501.4032 (M+) ; ^{1}HNMR: δ 10.74 (1H, s), 8.68 (1H, s), 7.94-8.05 (3H, m), 7.41-7.54 (3H, m), 7.27 (1H, d, *J*=8.94Hz), 7.02 (1H, s), 5.17 (1H, t, *J*=10.14Hz), 3.79i(1H,idd, *J*=16.94, i10.14Hz), 3.25 (1H, dd, *J*=16.94,i10.14Hz), 2.40i (3H,is), i2.30i(3H,is).

4d

Yellow Solid, IR(KBr): 3334, 3084, 1514, 835, 817 cm^{-1}; HRMS: m/z 473.0410(M+); ^{1}HNMR: δ 11.68 (1H, s), 8.6 (1H, s), 7.86-7.97 (3H, m), 7.33-7.46 (4H, m), 7.27 (1H, dd, *J*=8.72, 2.56Hz), 7.21 (1H, d, *J*=8.8Hz), 6.94 (1H, d, *J*=8.5Hz), 5.09 (1H, t, 10Hz), 3.71 (1H, dd, *J*=17.01, 10Hz), 3.17 (1H, dd, *J*=17.01, 10Hz).

4e

Yellow Solid, IR(KBr): 3327, 3080, 1514, 835 cm^{-1}; HRMS: m/z 507.7951 (M+); ^{1}HNMR: δ 12.08 (1H, s), 8.54 (1H, s), 7.80-7.91 (3H, m), 7.27-7.40 (4H, m), 7.21 (1H, d, *J*=2.42Hz), 7.13 (1H, d, *J*=8.66Hz), 5.03 (1H, t, 9.86Hz), 3.65 (1H, dd, *J*=15.97, 9.86Hz), 3.11 (1H, dd, *J*=15.96, 9.86Hz).

4f

Yellow Solid, IR(KBr): 3277, 3093, 1514, 821, 804 cm^{-1}; HRMS: m/z 452.9316 (M+); ^{1}HNMR: δ 10.14 (1H, s), 8.66 (1H, s), 7.92-8.04 (3H, m), 7.39-7.52 (4H, m), 7.33 (1H, dd, *J*=8.72, 2.56Hz), 7.25 (1H, d, *J*=8.8Hz), 7.00 (1H, d, *J*=8.5Hz), 5.15

(1H, t, *J*=10Hz), 3.77 (1H, dd, *J*=17.03, 10Hz), 3.23 (1H, dd, *J*=17.03, 10Hz), 2.30 (3H, s).

2-[3-(5-Chlorothiophen-2-yl)-1-(4-fluorophenyl)-1H-pyrazol-4-yl]-4H-chromen-4-one, 5a

Substituted pyrazolyl propenone **3a** (0.001 mol) was refluxed in 12 mL of DMSO containing 0.2 g Iodine at 135-145°C for 3-4 h and kept aside for 24 hours. Then the mixture was transferred to smashed ice and filtered solid was treated with 15% sodium thiosulphate for removal of unreacted Iodine. The compound **5a** was purified by recrystallizing it from ethanol. The compound **5b-5f** were prepared using the same procedure.

5a

Faint Solid, IR(KBr): 3124, 3059, 1653, 1516, 833 cm^{-1}; HRMS: m/z 422.8592(M+); ^{1}HNMR: δ 8.00-8.05 (4H, m), 7.65 (1H, dd, *J*=8.92, 2.88 Hz), 7.56 (1H, ddd, *J*=8.90, 8.84, 2.88Hz), 7.49-7.53i (4H,im), i7.29i(1H, d,i *J*=3.92Hz), 6.84 (1H,is).

5b

Yellowish Brown Solid, IR(KBr): 3122, 3059, 1654, 1516, 833, 800 cm^{-1}; HRMS: m/z 436.8858(M+); ^{1}HNMR: δ 7.92-7.97 (4H, m), 7.57 (1H, d, *J*=2.42Hz), 7.50 (1H, dd, *J*=8.56, 2.42Hz), 7.41-7.44 (3H, m), 7.21 (1H, d, *J*=3.68Hz), 6.76 (1H, s), 2.38 (3H, s).

5c

Yellowish Brown Solid, IR(KBr): 3113, 3057, 1645, 1514, 833, 792 cm^{-1}; HRMS: m/z 485.3575(M+); ^{1}HNMR: δ 8.04-8.09 (3H, m), 7.69 (1H, s), 7.53-7.57 (3H, m), 7.33 (1H, d, *J*=3.94Hz), 6.88 (1H, s), i2.60i(3H,is), i2.54i(3H, s).

5d

Faint Yellow Solid, IR(KBr): 3140, 3066, 1653, 1541, 1517, 835, 796 cm^{-1}; HRMS: m/z 456.9967 (M+); ^{1}HNMR: δ 7.96-8.01 (4H, m), 7.61 (1H, d, *J*=8.8 Hz), 7.45-7.49 (4H, m), 7.25 (1H, d, *J*=3.8Hz), 6.80 (1H, s).

5e

Yellow Solid, IR(KBr): 3124, 3074, 1654, 829, 779 cm^{-1}; HRMS: m/z 491.7494 (M+); ^{1}HNMR: δ 8.02-8.07 (4H, m), 7.51-7.55 (4H, m), 7.31 (1H, d, *J*=3.66Hz), 6.86 (1H, s).

5f

Yellow Solid, IR(KBr): 3140, 3066, 1653, 1541, 835, 796 cm^{-1}; HRMS: m/z 436.8858 (M+); ^{1}HNMR (DMSO-d_6): δ 7.90-7.96 (4H, m), 7.55 (1H, d, *J*=8.86Hz), 7.39-7.43 (4H, m), 7.19 (1H, d, *J*=3.86Hz), 6.74 (1H, s), 2.26 (3H, s).

2-[3'-(5-Chlorothiophen-2-yl)-1'-(4-fluorophenyl)-1'H,2H-3,4'-bipyrazol-5-yl]phenol, 6a

In ethanol 2-substituted-chromen-4-one, **5a** (0.015 mol) and hydrazine hydrate (0.005 mol) were dissolved and refluxed for 4 hours. TLC was used to monitor the reaction, and once it was finished, the reaction liquid stirred with cold water containing small amount of ice. After that, glacial acetic acid was used to neutralize the resulting mixture. Pure 6a was obtained by filtering the resultant product and recrystallizing it from ethanol. The compound 6b-6f were prepared using the same procedure.

6a

Yellow Solid, IR(KBr): 3336, 3128, 3068, 1514, 835, 800 cm^{-1}; HRMS: m/z 436.8891 (M+); ^{1}HNMR: δ 13.12 (1H, s), 9.04 (1H, s), 8.14 (1H, d), 7.92-8.03 (3H, m), 7.37-7.49 (5H, m), 7.34 (1H, ddd, *J*=8.66, 8.48, 2.24Hz), 7.26 (1H, d, *J*=9.20, 2.24Hz), 7.19 (1H, d).

6b

Yellow Solid, R(KBr): 3421, 3186, 1514, 835, 698 cm^{-1}; HRMS: m/z 450.9157 (M+); ^{1}HNMR: δ 10.95 (1H, s), 8.96 (1H, s), 8.06 (1H, d), 7.84-7.95 (3H, m), I7.47i(1H,Idd, *J*=8.33,i2.25Hz), i7.29-7.39i (4H,im), I7.18i(1H,id, iJ=2.25 Hz), I7.13I(1H,Id), I2.19I(3H,Is).

6c

Yellow Solid, IR(KBr): 3313, 3113, 1514, 833, 802 cm^{-1}; HRMS: m/z 499.3873 (M+); ^{1}HNMR: δ 11.37 (1H, s), 9.08 (1H, s), 8.18 (1H, d), 7.96-8.03 (2H, m), 7.41-7.50 (4H, m), 7.30 (1H, s), 7.24 (1H, d), 2.55 (3H, s), 2.41 (3H, s).

6d

Yellow Solid, IR(KBr): 3404, 3298, 3145, 1514, 833, 800 cm^{-1}; HRMS: m/z 471.0243 (M+); ^{1}HNMR: δ 12.70 (1H, s), 9.0 (1H, s), 8.10 (1H, d), 7.88-7.99 (3H, m), 7.33-7.45 (5H, m), 7.22 (1H, d, *J*=9.08 Hz), 7.17 (1H, d).

6e

Yellow Solid, IR(KBr): 3419, 3086, 1514, 835, 802 cm^{-1}; HRMS: m/z 505.7792(M+); ^{1}HNMR: δ 10.77 (1H, s), 9.06 (1H, s), 8.16 (1H, d), 7.94-8.06 (3H, m), 7.39-7.51 (5H, m), 7.22 (1H, d).

6f

Yellow Solid, IR(KBr): 3292, 3124, 1514, 835, 815 cm^{-1}; HRMS: m/z 450.9157 (M+); ^{1}HNMR: δ 12.15 (1H, s), 8.94 (1H, s), 8.04 (1H, d), 7.82-7.93 (3H, m), 7.27-7.39 (5H, m), 7.17 (1H, d, *J*=9.14 Hz), 7.09 (1H, d), 2.12 (3H, s).

CONCLUSION

Flourine and thiophene containing different pyrazolyl compounds were prepared in this present work and spectroscopic evidence strongly supports the suggested compounds. Compound **6a** and **6b** are promising alpha-amylase inhibitory activity in comparison with reference compound Acarbose. These compounds can be considered as lead compounds as anti-diabetic agents. Results of the antimicrobial study show that all the synthesized compounds can be modified structurally to improve their antimicrobial profile.

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Conflict of interest

There are no conflicts of interest declared by the authors.

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