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Synthesis, characterization, POM analysis and antifungal activity of novel heterocyclic chalcone derivatives containing acylated pyrazole

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Abstract A series of heterocyclic chalcone derivatives (**4a–h**) were synthesized and characterized by IR, ^{1}H and ^{13}C NMR as well as MS. All the synthesized compounds were evaluated for their antifungal activity on *Candida albicans* and *Aspergillus niger*. The assay revealed that compounds **3d** and **4f** showed significant activity against both tested fungal strains. POM analyses showed that the compounds are highly lipophyllic but present a potential bioactivity. Moreover, they have no NH–O or N–HO intramoleculcular interaction which is a crucial parameter controlling solubility of compounds possessing these encouraging pharmaceutical properties. This series gives us an important lesson in drug design: We should take the balance of hydrosolubility/lipophilicity into consideration. POM analyses were in agreement with the idea of coexistence of two combined antifungal N,O and O,S-pharmacophore sites for series **4a–h**. On the other hand, two coexistents and identical N,Cl-pharmacophore sites have been identified for Fluconazole.

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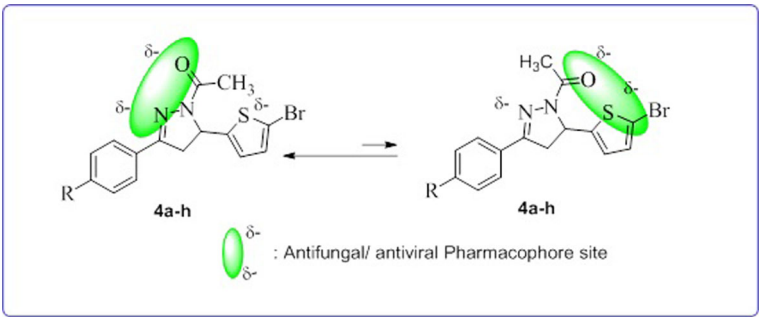
Graphical Abstract



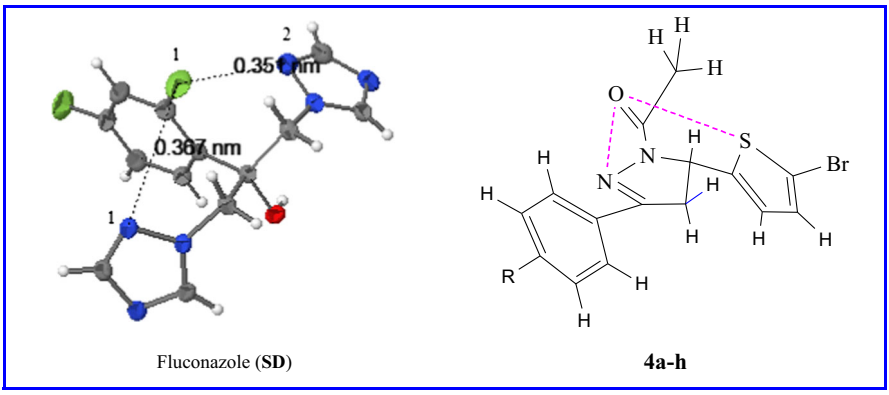
Keywords Chalcone-pyrazole derivatives · Antifungal · Petra/Osiris/Molinspiration (POM) analyses · Pharmacophore site Identification

Introduction

Fungal infections are serious types of health problems infecting humans around the world [1]. The emergence of antibiotics to fight fungi and other microbes has resulted in the presence of pathogens that are resistant to pharmaceutical agents. The presence of drugs such as Fluconazole, Methotrexate, Azathioprine, Cyclosporine, Voriconazole, Itraconazole, Micafungin, Posaconazole, Flucytosine, Anidulafungin, Caspofungin and others can help to fight against fungal infections. Nevertheless, these drugs gave side effects when used in combination with other drugs [2, 3].

Therefore, pyrazole derivatives are some of the compounds that can act against fungi as they exhibit a broad range of pharmacological activities such as antibacterial, anticancer, antifungal, anti-inflammatory, anti-oxidant and others [4, 5]. The presence of a F–N interaction between fluoro-1 and the two nitrogen (N-1 and N-2) atoms in the crystalline structure of Fluconazole [6–8] is an indication of the coexistence of two combined N,F-pharmacophore sites, and the removal or modification of this topology could obstruct or increase its various pharmacological activities (Fig. 1).

Based on its diversity of biological activities, Flcuconazole has attracted our attention for the synthesis of bis-armed pyrazole derivatives **4a–h** (Fig. 1) instead of mono-armed pyrazole derivatives as an antifungal drug. Previous studies carried out by Ben Hadda et al reported that various organic compounds showed improved antifungal activity against a wide range of fungal strains [9–12]. Encouraged by this, a series of heterocyclic bis-armed pyrazole derivatives were synthesized by focusing on the terminal aryl electron rich with different substituents of the phenyl ring. All the compounds were tested for their ability to act as antifungal agents in order to develop a novel therapeutic agent for the better treatment of fungal infections.



Fluconazole (**SD**) **4a-h**

Fig. 1 Structure of Flcuconazole (SD) and new chalcone derivatives containing acylated pyrazole **4a–h**

Experimental

Materials and methods

Melting points (uncorrected) were determined using a Barnstead Electrothermal 9100 melting point. ^{1}H and ^{13}C NMR spectra (400 and 300 MHz respectively) were recorded on a Bruker Avance II spectrometer using deuterated chloroform ($CDCl_3$) as solvent. Chemical shift values were given in δ (ppm) scales. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer. The ESMS were recorded on a MICROMASS Quattro-II LCMS system (Waters, Milford, MA, USA). Thin layer chromatography (TLC) alumina sheets precoated with silica gel 60 F254 (0.2 mm thickness) was used to monitor and detect the compounds, and the spots were visualized under a UV lamp at 254 nm. All chemicals and reagents were of analytical grade and were used without further purification.

General procedure for synthesis of chalcones (3a–h)

A mixture of desired substituted acetophenones (**2a–h**) (1 mol) with 5-bromo-2-thiophenecarboxaldehyde (**1**) (1 mol) was dissolved in methanol (25 mL). Aqueous potassium hydroxide (15 mL) was added dropwise to the mixture. The reaction mixture was stirred at room temperature overnight. The solid product was filtered, washed with water, dried to obtain the solid and then recrystallized using ethanol to produce the corresponding chalcones (**3a–h**). Completion of the reaction was monitored by TLC [13].

*(E)-3-(5-bromothiophen-2-yl)-1-phenylprop-2-en-1-one (**3a**)*

Pale yellow solid, yield: (2.01 g, 42.86 %). M.p: 72–74 °C; IR (cm^{-1}): 3087 (C–H–sp^2), 1658 (C=O), 1590, 1420 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (d, 1H, H-4, $J = 4.00$ Hz), 7.13 (d, 1H, H-3, $J = 4.00$ Hz), 7.25 (d, 1H, H-α, $J = 15.40$ Hz), 7.50–7.64 (m, 3H, H-3′ and H-5′ and H-4′), 7.84 (d, 1H, H-β,

$J = 15.40$ Hz), 7.99 for funding this Research group 8.04 (m, 2H, H-2′ and H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 116.42, 120.94, 128.41, 128.70, 131.36, 132.41, 132.96, 136.30, 137.91, 141.94, 189.61; MS (m/z): 293.1 [M+, 100 %], 295.1 [M+2].

(E)-3-(5-bromothiophen-2-yl)-1-(4-chlorophenyl)prop-2-en-1-one ***(3b)***

Pale yellow solid, yield: (4.99 g, 83.3 %). M.p: 129–130 °C; IR (cm^{-1}): 3069 (C–H–sp^2), 1655 (C=O), 1590, 1420 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (d, 1H, H-4, $J = 4.00$ Hz), 7.14 (d, 1H, H-3, $J = 4.00$ Hz), 7.20 (d, 1H, H-α, $J = 15.40$ Hz), 7.50 (d, 2H, H-3′ and H-5′, $J = 8.80$ Hz), 7.85 (d, 1H, H-β, $J = 15.40$ Hz), 7.96 (d, 2H, H-2′ and H-6′, $J = 8.40$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 116.77, 120.29, 129.01, 129.80, 131.43, 132.71, 136.21, 136.72, 139.39, 141.75, 188.19; MS (m/z): 327.0 [M+, 100 %], 329.0 [M+2].

(E)-3-(5-bromothiophen-2-yl)-1-(4-fluorophenyl)prop-2-en-1-one ***(3c)***

Pale yellow solid, yield: (5.78 g, 84.4 %). M.p: 100–102 °C; IR (cm^{-1}): 3108 (C–H–sp^2), 1655 (C=O), 1587, 1426 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (d, 1H, H-4, $J = 4.00$ Hz), 7.14 (d, 1H, H-3, $J = 4.00$), 7.22 (d, 1H, H-α, $J = 15.20$ Hz), 7.18–7.23 (m, 2H, H-3′ and H-5′), 7.85 (d, 1H, H-β, $J = 15.20$ Hz), 8.02–8.08 (m, 2H, H-2′ and H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ115.69, 115.98, 116.59, 120.38, 130.95, 131.07, 131.40, 132.59, 134.25, 136.48, 141.80, 187.84; MS (m/z): 311.0 [M+, 100 %], 313.0 [M+2].

(E)-1-(4-bromophenyl)-3-(5-bromothiophen-2-yl)prop-2-en-1-one ***(3d)***

Pale yellow solid, yield: (8.61 g, 89.0 %). M.p: 147–149 °C; IR (cm^{-1}): 3066 (C–H–sp^2), 1655 (C=O), 1587, 1420 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (d, 1H, H-4, $J = 3.80$ Hz), 7.14 (d, 1H, H-3, $J = 3.80$), 7.18 (d, 1H, H-α, $J = 15.20$ Hz), 7.67 (d, 2H, H-3′ and H-5′, $J = 8.80$ Hz), 7.82–7.90 (m, 3H, H-β and H-2′ and H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 116.82, 120.24, 128.10, 129.92, 131.45, 132.00, 132.77, 136.62, 136.80, 141.73, 188.40; MS (m/z): 372.9 [M^+, 100 %], 375.0 [M+2].

(E)-3-(5-bromothiophen-2-yl)-1-(4-iodophenyl)prop-2-en-1-one ***(3e)***

Pale yellow solid, yield: (6.21 g, 92.6 %). M.p: 146–148 °C; IR (cm^{-1}): 3090 (C–H–sp^2), 1655 (C=O), 1587, 1420 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (d, 1H, H-4, $J = 3.80$ Hz), 7.14 (d, 1H, H-3, $J = 3.80$ Hz), 7.18 (d, 1H, H-α, $J = 15.20$ Hz), 7.72 (d, 2H, H-3′ and H-5′, $J = 8.80$ Hz), 7.84 (d, 1H, H-β, $J = 15.20$ Hz), 7.89 (d, 2H, H-2′ and H-6′, $J = 8.80$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 100.84, 116.81, 120.22, 129.79, 131.44, 132.74, 136.78, 137.17, 137.99, 141.74, 188.71; MS (m/z): 419.0 [M^+, 100 %], 420.0 [M+1], 421.0 [M+2].

*(E)-3-(5-bromothiophen-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (**3f**)*

Pale yellow solid, yield: (6.11 g, 90.0 %). M.p: 142–143 °C; IR (cm^{-1}): 3075 (C–H–sp^2), 1655 (C=O), 1590, 1512, 1426 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.90 (s, 3H, CH_3), 7.01 (d, 2H, H-3′ and H-5′, $J = 8.80$ Hz), 7.07 (d, 1H, H-4, $J = 4.00$ Hz), 7.11 (d, 1H, H-3, $J = 4.00$ Hz), 7.27 (d, 1H, H-α, $J = 15.20$ Hz), 7.83 (d, 1H, H-β, $J = 15.20$ Hz), 8.03 (d, 2H, H-2′ and H-6′, $J = 9.20$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.53, 113.90, 115.95, 120.83, 130.74, 130.80, 131.28, 132.05, 135.51, 142.14, 163.54, 187.77; MS (m/z): 323.2 [M^+, 100 %], 325.0 [M+2].

*(E)-3-(5-bromothiophen-2-yl)-1-p-tolylprop-2-en-1-one (**3g**)*

Pale yellow solid, yield: (5.60 g, 82.8 %). M.p: 91–93 °C; IR (cm^{-1}): 3078(C–H–sp^2), 1652 (C=O), 1587, 1426 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (d, 1H, H-4, $J = 4.00$ Hz), 7.12 (d, 1H, H-3, $J = 4.00$ Hz), 7.25 (d, 1H, H-α, $J = 15.40$ Hz), 7.33 (d, 2H, H-3′ and H-5′, $J = 8.00$ Hz), 7.83 (d, 1H, H-β, $J = 15.40$ Hz), 7.93 (d, 2H, H-2′ and H-6′, $J = 8.40$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.73, 116.16, 120.99, 128.55, 129.40, 131.31, 132.21, 135.33, 135.88, 142.06, 143.87, 189.05; MS (m/z): 307.2 [M^+, 100 %], 309.2 [M+2].

*(E)-3-(5-bromothiophen-2-yl)-1-(4-nitrophenyl)prop-2-en-1-one (**3h**)*

Yellow solid, yield: (8.43 g, 77.9 %). M.p: 166–167 °C; IR (cm^{-1}): 3096 (C–H–sp^2), 1655 (C=O), 1577, 1515, 1423 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (d, 1H, H-4, $J = 3.80$ Hz), 7.19 (d, 1H, H-3, $J = 3.80$ Hz), 7.20 (d, 1H, H-α, $J = 15.40$ Hz), 7.88 (d, 1H, H-β, $J = 15.40$ Hz), 8.15 (d, 2H, H-3′ and H-5′, $J = 8.80$ Hz), 8.38 (d, 2H, H-2′ and H-6′, $J = 9.20$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 117.75, 119.91, 123.93, 129.32, 131.64, 133.51, 138.02, 141.35, 142.73, 150.11, 187.99; MS (m/z): 338.3 [M^+, 100 %], 340.1 [M+2].

***General procedure for synthesis of pyrazolines derivatives* (4a–h)**

A mixture of chalcones (**3a–h**) (1 mol) was refluxed with appropriate hydrazine hydrate (2.5 mol) in glacial acetic acid overnight. The reaction mixture was cooled and poured into crushed ice, then the obtained precipitate was filtered off, washed, and dried to give compounds (**4a–h**). The progress of the reaction was checked by TLC [14].

*1-(5-(5-bromothiophen-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4a**)*

Cream solid, yield: (0.63 g, 60.1 %). M.p: 112–115 °C; IR (cm^{-1}): 3072(C–H-sp^2), 2929 (C–H sp^3), 1643 (C=O), 1596 (C=N), 1440 (C=C aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H, H-1), 3.35 (dd, 1H, H-6a, $J = 4.40$ Hz, $J = 17.60$ Hz), 3.73 (dd, 1H, H-6b, $J = 17.60$ Hz, $J = 11.20$ Hz), 5.83 (dd, 1H, H-7, $J = 4.00$ Hz, $J = 11.60$ Hz), 6.80 (d, 1H, H-‘’4, $J = 4.00$ Hz), 6.89 (d, 1H,

H-‘‘3, $J = 4.00$ Hz), 7.45–7.79 (m, 5H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.96, 41.46, 55.28, 111.60, 125.04, 126.62, 128.82, 129.45, 130.58, 131.04, 145.57, 153.84, 169.05; MS (m/z): 349.1 [M^+, 100 %], 351.1 [M+2].

*1-(5-(5-bromothiophen-2-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4b**)*

Cream solid, yield: (0.83 g, 72.2 %). M.p: 120–123 °C; IR (cm^{-1}): 3092(–C–H–sp^2), 2916 (–C–H–sp^3), 1642 (C=O), 1590 (C=N), 1424 C=C aromatic ring). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H, H-1), 3.31 (dd, 1H, H-6a, $J = 4.40$ Hz, $J = 1$ 8.00 Hz), 3.70 (dd, 1H, H-6b, $J = 11.60$ Hz, $J = 17.60$ Hz), 5.83 (dd, 1H, H-7, $J = 4.00$ Hz, $J = 11.60$ Hz), 6.80 (d, 1H, H-‘‘4, $J = 3.60$ Hz), 6.90 (d, 1H, H-‘‘3, $J = 4.00$ Hz), 7.43 (d, 2H, H-‘3 and H-‘5, $J = 8.80$ Hz), 7.70 (d, 2H, H-‘2 and H-‘6, $J = 8.80$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.95, 41.38, 55.44, 111.69, 125.12, 127.84, 129.11, 129.49, 129.54, 136.57, 145.35, 152.69, 169.03; MS (m/z): 383.1 [M^+, 100 %], 385.1 [M+2].

*1-(5-(5-bromothiophen-2-yl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4c**)*

Cream solid, yield: (1.02 g, 92.6 %). M.p: 118–120 °C; IR (cm^{-1}): 3071(C–H–sp^2), 2971 (C–H sp^3), 1639 (C=O), 1605 (C=N), 1511, 1440 (C=C aromatic ring). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H, H-1), 3.33 (dd, 1H, H-6a, $J = 4.00$ Hz, $J = 17.60$ Hz), 3.70 (dd, 1H, H-6b, $J = 11.20$ Hz, $J = 17.60$ Hz), 5.84 (dd, 1H, H-7, $J = 4.00$ Hz, $J = 11.60$ Hz), 6.80 (d, 1H, H-‘‘4, $J = 3.60$ Hz), 6.89 (d, 1H, H-‘‘3, $J = 3.60$ Hz), 7.10–7.80 (m, 4H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.95, 41.53, 55.38, 111.65, 115.88, 116.18, 125.08, 128.57, 128.68, 129.48, 145.44, 152.77, 168.99; MS (m/z): 367.2 [M^+, 100 %], 369.2 [M+2].

*1-(3-(4-bromophenyl)-5-(5-bromothiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4d**)*

Cream solid, yield: (1.21 g, 94.2 %). M.p: 139–141 °C; IR (cm^{-1}): 3087(C–H–sp^2), 2917 (C–H sp^3), 1640 (C=O), 1587 (C=N), 1444 (C=C aromatic ring). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H, H-1), 3.30 (dd, 1H, H-6a, $J = 4.40$ Hz, $J = 17.60$ Hz), 3.70 (dd, 1H, H-6b, $J = 11.60$ Hz, $J = 17.60$ Hz), 5.84 (dd, 1H, H-7, $J = 4.00$ Hz, $J = 11.60$ Hz), 6.79 (d, 1H, H-‘‘4, $J = 3.60$ Hz), 6.89 (d, 1H, H-‘‘3, $J = 3.60$ Hz), 7.57–7.65 (m, 4H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.94, 41.33, 55.46, 111.70, 124.93, 125.13, 128.03, 129.49, 129.98, 132.06, 145.33, 152.78, 169.06; MS (m/z): 429.1 [M+1, 100 %], 431.0 [M+3].

*1-(5-(5-bromothiophen-2-yl)-3-(4-iodophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4e**)*

Cream solid, yield: (0.76 g, 80.0 %). M.p: 128–131 °C; IR (cm^{-1}): 3081 (C–H–sp^2), 2914 (C–H sp^3), 1640 (C=O), 1593 (C=N), 1447 (C=C aromatic ring). ^{1}H

NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H, H-1), 3.30 (dd, 1H, H-6a, $J = 4.00$ Hz, $J = 1$ 7.60 Hz), 3.70 (dd, 1H, H-6b, $J = 11.20$ Hz, $J = 17.60$ Hz), 5.80 (dd, 1H, H-7, $J = 4.40$ Hz, $J = 11.60$ Hz), 6.79 (d, 1H, H-''4, $J = 3.60$ Hz), 6.89 (d, 1H, H-''3, $J = 4.00$ Hz), 7.48 (d, 2H, H-'3 and H-'5, $J = 8.00$ Hz), 7.80 (d, 2H, H-'2 and H-'6, J = 8.40 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.97, 41.22, 55.43, 96.92, 111.69, 125.12, 128.07, 129.49, 130.52, 137.99, 145.33, 152.89, 169.01; MS (m/z): 475.0 [M^+, 100 %], 477.0 [M+2].

1-(5-(5-bromothiophen-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethanone ***(4f)***

Cream solid, yield: (0.93 g, 81.7 %). M.p: 108–110 °C; IR (cm^{-1}): 3089 (C–H–sp^2), 2919 (C–H sp^3), 1645 (C=O), 1590 (C=N), 1427 (C=C aromatic ring). ^1H NMR (400 MHz, $CDCl_3$): δ 2.39 (s, 3H, H-1), 3.30 (dd, 1H, H-6a, $J = 4.00$ Hz, $J = 17.60$ Hz), 3.68 (dd, 1H, H-6b, $J = 11.60$ Hz, $J = 17.60$ Hz), 3.87 (s, 3H, H-'7), 5.80 (dd, 1H, H-7, $J = 3.60$ Hz, $J = 11.20$ Hz), 6.79 (d, 1H, H-''4, $J = 4.00$ Hz), 6.87 (d, 1H, H-''3, $J = 4.00$ Hz), 6.96 (d, 2H, H-'3 and H-'5, $J = 8.80$ Hz), 7.70 (d, 2H, H-'2 and H-'6, $J = 8.80$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.95, 41.52, 55.14, 55.44, 111.49, 114.21, 123.64, 124.95, 128.24, 129.41, 145.73, 153.65, 161.49, 168.82; MS (m/z): 379.1[M^+, 100 %], 381.1[M+2].

1-(5-(5-bromothiophen-2-yl)-3-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(4g)***

Cream solid, yield: (0.71 g, 65.1 %). M.p: 119–120 °C; IR (cm^{-1}): 3077(C–H–sp^2), 2916 (C–H–sp^3), 1642 (C=O), 1590 (C=N), 1444 (C=C aromatic ring); ^1H NMR (400 MHz, $CDCl_3$): δ 2.39–2.46 (s, 6H, H-1 and H-'7), 3.33 (dd, 1H, H-6a, $J = 4.00$ Hz, $J = 17.60$ Hz), 3.70 (dd, 1H, H-6b, $J = 17.60$ Hz, $J = 11.40$ Hz), 5.81 (dd, ^1H, H-7, $J = 4.00$ Hz, $J = 11.40$ Hz), 6.80 (d, 1H, H-''4, $J = 4.00$ Hz), 6.89 (d, 1H, H-''3, $J = 3.60$ Hz), 7.27 (d, 2H, H-'3 and H-'5, $J = 8.00$ Hz), 7.66 (d, 2H, H-'2 and H-'6, $J = 8.00$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.55, 21.96, 41.49, 55.17, 111.54, 124.99, 126.58, 128.25, 129.42, 129.52, 140.98, 145.66, 153.97, 168.97; MS (m/z): 363.1 [M^+, 100 %], 365.1 [M+2].

1-(5-(5-bromothiophen-2-yl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(4h)***

Cream solid, yield: (0.86 g, 72.7 %). M.p: 132–134 °C; IR (cm^{-1}): 3080 (C–H–sp^2), 2919 (C–H–sp^3), 1642 (C=O), 1590 (C=N), 1441 (C=C aromatic ring); ^1H NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H, H-1), 3.38 (dd, 1H, H-6a, $J = 4.20$ Hz, $J = 17.60$ Hz), 3.77 (dd, 1H, H-6b, $J = 11.60$ Hz, $J = 17.60$ Hz), 5.89 (dd, 1H, H-7, $J = 4.20$ Hz, $J = 11.60$ Hz), 6.82 (d, 1H, H-''4, $J = 4.00$ Hz), 6.91 (d, 1H, H-''3, $J = 4.00$ Hz), 7.92 (d, 2H, H-'3 and H-'5, $J = 8.80$ Hz), 8.32 (d, 2H, H-'2 and H-'6, $J = 8.80$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.99, 41.25, 55.90, 111.95, 124.12, 125.38, 127.28 129.58, 137.03, 144.86, 148.60, 151.35, 169.24; MS (m/z): 394.1 [M^+, 100 %], 396.0 [M+2].

Antifungal screening

The synthesized compounds were screened for in vitro antifungal activity against two fungal strains, *Candida albicans* (MTCC 3958) and *Aspergillus niger* (MTCC 9933). Antifungal activity of the test compounds was expressed in terms of ZOI (zone of Inhibition) and MIC (minimum inhibitory concentration). Fluconazole was used as the reference drug for the study and DMSO was used as a negative control. Tests were performed in triplicate with the following procedure as described briefly. Autoclaved suitable growth media (Malt Yeast Agar for *Candida albicans* and Czapek Yeast Extract Agar-CYA for *Aspergillus niger*) were poured into autoclaved Petri plates. Further, the agar plates were swabbed with 100 µl inocula of each test organism under aseptic conditions. After the adsorption, wells of 6 mm diameter were made by a sterile metallic borer and the solutions of the working compounds (128 µg/20 µL) was poured into the wells. The plates were incubated at 28°C for 48 h and the ZOI was calculated. Similarly, the MIC values were calculated using the broth double-dilution method for each compound, taking 100 µl inoculum of each fungal culture, after incubation at 28°C for 48 h [15, 16].

POM analyses

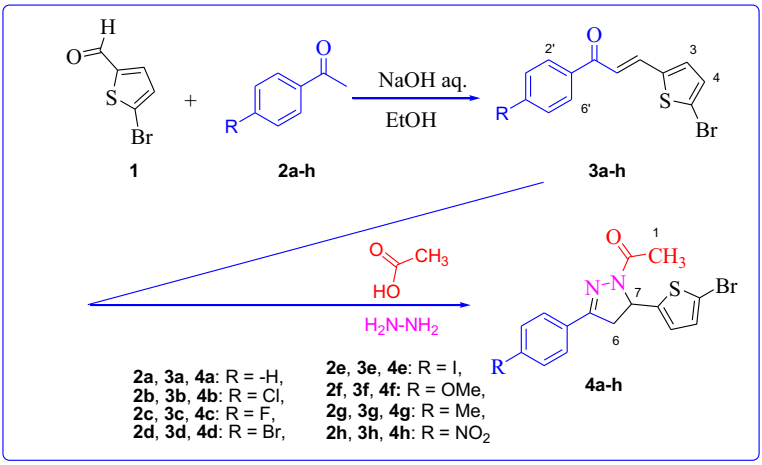
On the basis of POM analyses, we can conclude that the synthesized compounds are suitable for antiviral/antifungal activity as they possess ($X^{\delta-}$–$Y^{\delta-}$) pharmacophoric sites due to the presence of electronegative heteroatoms. It can also be hypothesized that the difference in charge between X and Y of the same dipolar pharmacophoric site should facilitate the inhibition of bacteria more than viruses and fungi [6–12, 17–22].

Results and discussion

Chemistry

The synthesis of chalcones (**3a–h**) was carried out by the condensation of substituted acetophenones (**2a–h**) with 5-bromo-2-thiophenecarboxaldehyde in the presence of sodium hydroxide and methanol. Pyrazoline derivatives (**4a–h**) were synthesized by the cyclization reaction of chalcones (**3a–h**) with hydrazine hydrate in the presence of glacial acetic acid (Scheme 1).

Chalcones (**3a–h**) were characterized by IR, ^{1}H NMR, ^{13}C NMR and MS. The IR spectrum of the chalcones (**3a–h**) showed a characteristic band for –C–H–sp^2 stretching of the aromatic ring at 3010–3080 cm^{-1}. The chalcones (**3a–h**) revealed bands at 1650–1675 cm^{-1} indicating the presence of a carbonyl group (–C=O). The low frequency for carbonyl is due to the conjugation of the olefinic group with those on the pi-electrons on the benzene ring in the enone moiety. The compounds (**3a–h**) also displayed absorption bands corresponding to the C=C aromatic ring at 1580–1600 and 1410–1430 cm^{-1}. The structure of compounds (**3a–h**) was confirmed by ^{1}H NMR spectra. Two vinylic protons of H-α and H-β appeared as



Scheme 1 Synthesis of chalcone (**3a–h**) and pyrazole derivatives (**4a–h**)

doublet at 7.20 ppm and 7.85 ppm, respectively. *Trans*-configuration of the chalcones (**3a–h**) was confirmed by the large coupling constant of olefinic protons (15.2–15.6 Hz). In the ^{13}C NMR spectra, the appearance signal in the low field region of δ 180–190 was assigned to the carbonyl group.

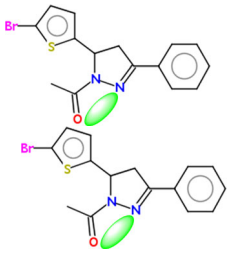
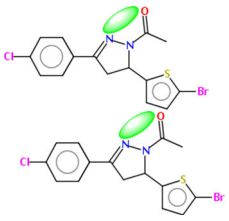
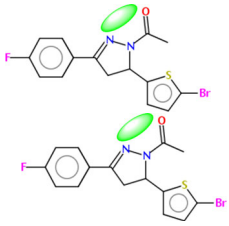
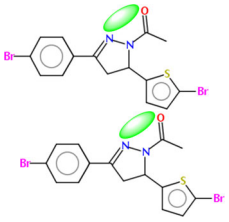
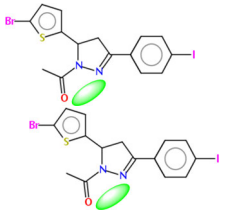
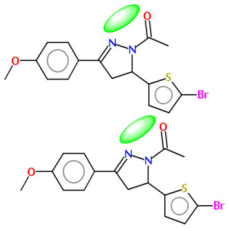
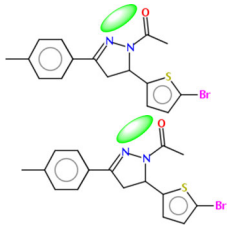
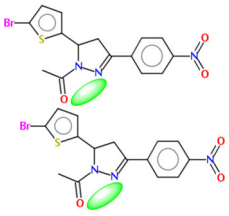
The IR spectrum of compounds (**4a–h**) exhibited the characteristic bands around 1570–1595 cm^{-1} and 1640–1660 cm^{-1} which indicated the presence of –C=N and carbonyl C=O groups, respectively. The IR of the compounds (**4a–h**) also revealed characteristic bands at 2910–2925 cm^{-1} due to C–H sp^3 stretching of the –$COCH_3$ group. The ^{1}H NMR spectra of compounds **4a–h** showed the pyrazoline ring CH_2 protons (H-6a and H-6b) resonated as a pair of doublet of doublets at δ 3.31 ppm (J = 4.30–4.45 Hz, J = 18.00–18.15 Hz) and δ 3.70 ppm (J = 11.40–11.60 Hz, J = 18.00–18.15 Hz), respectively. The Hx-7 also appeared as a doublet of doublets at δ 5.83 (J = 4.30–4.45 Hz, J = 11.40–11.60 Hz). The singlet signals integrating for three protons in the high field region, δ 2.3–2.5 ppm, was assigned to –CH_3 protons. The ^{13}C NMR supported the *N*-acetyl group C-1 and C-2 carbon atoms at δ 21.70–21.95 and δ 165–170, respectively.

The hydrophilicity character of each compound has been expressed in terms of its cLog*P* value since it has been established that the absorption or permeation is greatly affected by this quantity (the value of cLog*P*). Accordingly, when the value of cLog*P* is higher than 5, the absorption or permeation decreases (Table 1) [6–12].

Antifungal activity

Among the tested compounds, **3d**, **3f** and **4f** showed significant inhibition against *C.albicans* while compounds **3g**, **4g** and **4h** exhibited moderate inhibition against *C. albicans*. Compounds **3c**, **3d**, **3h**, **4a**, **4b**, **4d**, **4f** and **4g** showed significant inhibition against *A.niger*. The compounds **3a** and **3g** possessed moderate inhibition against *A. niger*. Both compounds **3d** and **4f** gave significant activity against both of the tested fungal strains. Detail of the result is shown in Table 2.

Table 1 Structure pyrazole derivatives **4a–h**; the hypothetic N,O-pharmacophore site (green color) is in *cis*/*cisoidal* conformation (Molinspiration Optimization)

Compound	Optimized structure	cLog*P*	Compound	Optimized structure	cLog*P*
4a		4.46	**4e**		5.55
4b		5.14	**4f**		4.52
4c		4.63	**4g**		4.91
4d		5.27	**4h**		4.42

Results from MIC values revealed that all the bis-armed pyrazole derivatives possess antifungal activity in the range of 32 to 130 μg/mL against the two selected fungi strains. Among all heterocyclic pyrazole derivative bearing an electron donor

Table 2 Antifungal activity [a] of compounds **3a–h**, **4a–h** and standard drug **SD** (Fluconazole)

Compound	R	MW (g/mole)	*Candida albicans*[a]		*Aspergillus niger*[a]	
			Z.I. (mm)	MIC (μg/mL)	Z.I. (mm)	MIC (μg/mL)
3a	H	293	–	–	10	>128
3b	Cl	327	–	–	–	–
3c	F	311	–	–	14	>64
3d	Br	372	12	>64	13	>128
3e	I	419	–	–	–	–
3f	OCH_3	323	13	>64	–	–
3g	CH_3	307	11	>128	10	>128
3h	NO_2	338	–	–	12	>64
4a	H	349	–	–	14	32
4b	Cl	384	–	–	14	64
4c	F	367	–	–	–	–
4d	Br	428	9	>128	15	>32
4e	I	475	–	–	–	–
4f	OCH_3	379	13	>64	13	>64
4g	CH_3	363	10	>128	12	>128
4h	NO_2	394	11	>128	–	–
SD[b]	–	306	18	16	20	8

[a] *Z.I.* Zone of Inhibition (mm)

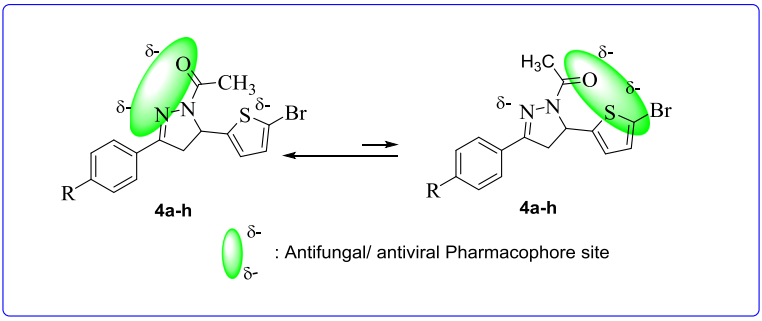
[b] *SD* Standard Drug (Fluconazole)

(–): Not Active

group, OMe (**4f**) exhibited the highest antifungal activity against both fungal strains (MIC = 64 μg/mL). This is in agreement with reported work by Ben Hadda et al., in which compounds with U geometry containing negatively charged groups emerged as the most potent antifungal and antiviral drugs [17–22]. However, the introduction of more electron-donor halogen groups such as iodine at the same position of the phenyl ring of heterocyclic pyrazole, especially compound (**4e**) caused a decrease in antifungal activity (no MIC) compared to the unsubstituted pyrazole derivative (**4a**) with (MIC = 32 μg/mL)

POM analyses

Petra/Osiris/Molinspiration analysis (POM) [17–22] is used to identify and indicate the type of pharmacophore site that affects biological activity with a change in the chemical substitution. POM is a well-known and simple bioinformatics but efficient method that has been used regularly to produce the two-dimensional models. It has the ability to predict the biological activities of the molecules and to represent the relationships between steric/electrostatic property as well as biological activity in the form of pharmacophore site which gives the key features on not only the ligand–receptor interaction but also on the topology of the receptor [17–22]. Potential antifungal N,O and O,S-pharmacophore sites is shown in Scheme 2.



Scheme 2 Potential antifungal N,O and O,S-pharmacophore sites

Table 3 shows the results of theoretical drug scores of tested compounds calculated with the aid of the Molinspiration program. Our findings concerning the screened molecules revealed that most of these compounds are moderate or not active (Table 3). They present low and negative drug scores and cannot be utilized as therapeutic agents if we take the five Lipinski rules. In fact, structures of the investigated biotargets inhibitors are supposed to present no violation and good bioavailability (cLogP < 5), but these compounds are the subject of a violation (VIOL $= 1$).

Thus, most of them are moderately or inactive. These analyses reveal that the compounds are highly lipophyllic but present a potential bioactivity. Moreover, they have no NH–O or N–HO intramoleculcular interactions which are a crucial parameter of controlling the solubility of compounds possessing these encouraging pharmaceutical properties. This series gives us an important lesson in drug design: We should take the balance of hydrosolubility/lipophicity into consideration (Table 3).

Our virtual POM screening results show clearly that Fluconazole (SD) is far away to be compared with the series **4a–h** because it presents better physico-chemical properties than our candidate drugs. The cLogP of SD is equal to -0.14 which constitutes a crucial parameter of bioavailability. This can be attributed to the existence of a hydroxyl group on the central carbon of SD (Fig. 1). In contrast to SD, the series **4a–h** has no hydrosolubilisator OH group (OHN $= 0$). So all eight of the new compounds have cLog*P*, without the acceptable criteria (cLog*P* $\ll 5$; not approximately equal to 5).

As the molecular weight of 8 of the 13 tested molecules **3**, **4** is 293–372 < 500 g/mole, it is possible to realize a more beneficial chemical modification (hydroxylation) in the goal to make more potentially active analogues with acceptable solubility, drug-likeness and drug score. The actual drug scores of most of the compounds are less encouraging (less positive values of DS), as shown in Table 3. Thus, the cLog*P* and drug score parameters should be taken into consideration and serve as a guide for further enzymatic screening investigations.

Table 3 Molinspiration calculations of compounds **3a–h** and **4a–h**

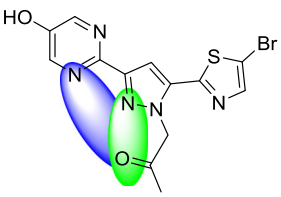
Compound	Molecular properties[a]				Bioactivity scores[b]					
	TPSA	*ONH*	VIOL	VOL	GPCR	*ICM*	KI	NRL	PI	EI
3a	17	0	0	210	−0.78	−0.42	−0.87	−0.91	−0.77	−0.32
3b	17	0	1	224	−0.68	−0.38	−0.79	−0.81	−0.73	−0.32
3c	17	0	0	215	−0.67	−0.39	−0.71	−0.75	−0.70	−0.29
3d	17	0	1	228	−0.78	−0.45	−0.81	−0.90	−0.78	−0.35
3e	17	0	1	234	−0.67	−0.37	−0.74	−0.74	−0.76	−0.35
3f	26	0	0	236	−0.65	−0.45	−0.71	−0.69	−0.65	−0.29
3g	17	0	0	227	−0.74	−0.48	−0.82	−0.83	−0.75	−0.35
3h	63	0	0	234	−0.71	−0.40	−0.76	−0.73	−0.68	−0.35
4a	33	0	0	258	−0.90	−1.26	−1.27	−1.19	−0.90	−0.51
4b	34	0	0	272	−0.83	−1.21	−1.20	−1.11	−0.87	−0.53
4c	33	0	0	263	−0.81	−1.22	−1.14	−1.06	−0.85	−0.51
4d	33	0	0	276	−0.91	−1.27	−1.22	−1.18	−0.91	−0.55
4e	33	0	0	282	−0.82	−1.21	−1.16	−1.06	−0.90	−0.55
4f	42	0	0	284	−0.81	−1.23	−1.14	−1.02	−0.81	−0.52
4g	33	0	0	275	−0.87	−1.28	−1.22	−1.12	−0.89	−0.55
4h	79	0	0	282	−0.89	−1.16	−1.20	−1.6	−0.86	−0.57
SD[c]	82	1	0	249	0.06	−0.04	−0.10	−023	−0.17	0.04

[a] *TPSA* Total of polar surface area, *ONH* OH–N or O–HN interraction, *VOL* volume

[b] *GPCR* GPCR ligand, *ICM* ion channel modulator, *KI* kinase inhibitor, *NRL* nuclear receptor ligamd, *PI* protease inhibitor, *EI* enzyme inhibitor

[c] *SD* standard drug (Fluconazole)

miLogP	1.59
TPSA	93.80
MW	380.23
nOHNH	1
nviolations	0
volume	264.56



GPCR ligand	-0.19
Ion channel modulator	-0.26
Kinase inhibitor	0.22
Nuclear receptor ligand	-0.22
Protease inhibitor	-0.38
Enzyme inhibitor	0.25

Fig. 2 Molecules in perspective

Conclusion

A series of heterocyclic chalcone derivatives containing acylated pyrazole and thiophene groups have been successfully synthesized in good yields. The potential bioactive compounds were evaluated for their antifungal activity. The results showed that compound **4f** exerts significant activity against *C. albicans* compared to compounds **4** and **4h** which exhibited moderate inhibition against *C. albicans*. Since this is a preliminary study and the number of fungal strains used here is limited, this

study should be extended to other strains. For example, compounds **4b**, **4g**, **4f**, and **4d**, showed significant activity against *A. niger*. Two compounds, **3d** and **4f**, showed significant activity against both the tested fungal strains. In future, this series of bis-armed pyrazoles need to be further modified (Fig. 2) and tested on a wide range of fungal and viral strains. All the tested compounds were filtered according to the POM platform and Lipinski rules and all had problems passing the filter. In addition, they showed varied physicochemical properties according to their ADME descriptors.

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