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Microwave and Conventional Synthesis of Some New Mercapto Pyrazolaldehyde Bonded to Indolyldihydropyrimidine Thione Derivatives as In Vivo Anti-inflammatory, and Analgesic, and In Vitro Antimicrobial Agents

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Abstract

The five-membered heterocyclic group of pyrazoles/pyrazolines plays an important role in drug discovery besides to their wide range of the biological, agrochemical and pharmacological properties. The synthesis of the pyrazole derivative **2** was achieved *via* the reaction between oxoketene *gem*-dithiol **1** and phenylhydrazine. Subsequently, the key synthon mercaptopyrazole derivative **2** was qualified to react with some reagents e.g. DMF/$POCl_3$ and/or sulfanilic acid to afford pyrazolecarbaldehyde **3** and/or pyrazolyl aminobenzenesulfonic acid derivative **4**. The Schiff bases **5a-c** and/or the cyclic adducts **7**, **8a**,**b** and/or **9** resulted from the condensation of the formylated adduct **3** with the appropriate substituted amines such as *p*-nitroaniline, *p*-chloroaniline, sulfanilic acid, guanidine sulfate, thiourea and/or acetophenone. The celecoxib analog **6** was obtained by the condensation of the terminal sulfonic acid group in the adduct **4** with 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one. The previously prepared compounds **2**, **4-9** were obtained in both conventional and/or microwave conditions and they were screened for their *in vivo* and *in vitro* activities. The structures of the newly prepared compounds have been confirmed by means of elemental analyses, FT-IR, MS, ^{1}H-NMR and/or ^{13}C-NMR.

Keywords Pyrazoles; oxoketene *gem*-dithiol; celecoxib analog; Vilsmeier-Haack formylation; Schiff bases; carrageenan paw edema.

1. Introduction

Pyrazole [1] is a one of the major tools to be investigated in the drug design and discovery. Many studies have been reported by researchers that have claimed the significant biological potential of these derivatives.[2] According to the literatures the pyrazole derivatives are supposed to have diverse pharmacological activities ranging from anti-fungal, anti-cancer, anti-tubercular, antimicrobial, anti-inflammatory, angiotensin-converting enzyme inhibitor, analgesic, anticonvulsant, antianxiety, anthelmintic, antipsychotic, antioxidant, antiobesity and herbicidal.[3]

Pyrazole, a five-membered heterocycle containing two nitrogen atoms, is extensively found as a core framework in a huge library of heterocyclic compounds that envelops promising agro-chemical, fluorescent and biological potencies.[4] In addition, pyrazoles have diversity of uses such as angiotensin converting enzyme (ACE) inhibitory, neuroprotective, cholecystokinin-1 receptor antagonist, and estrogen receptor (ER) ligand activity.[5-10] Herein we attributed to synthesize several potential pyrazole derivatives in the hope of rising in the significance and low side effects of a novel pyrazoles, disclosing innovative routes for synthesizing pyrazoles, examining different potencies of pyrazoles, and seeking for potential applications of pyrazoles

2. Experimental

All solvents and chemicals were commercially available from Sigma-Aldrich (USA). All melting points were determined on the Kofler melting point apparatus and were uncorrected. The progress of the reactions was followed up by the TLC technique. Infrared spectra (IR) were recorded on a Shimadzu IR 8101 infrared spectrophotometer and absorption was expressed in wave number (cm-1) using KBr disc. 1H-NMR and 13C-NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer and JEOL-ECA500II (100 MHz), at Mansoura University, Faculty of Pharmacy and Sohag University, using TMS as an internal standard; chemical shifts are expressed as δ and DMSO-d_6 as a solvent. Mass spectra were recorded on Shimadzu Qp-2010 Plus spectrometer at 70 eV (EI). Mass spectra were carried out at the "Micro Analytical Center" of Cairo University. *In-vivo* anti-inflammatory activity for the

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newly prepared compounds was performed based on the inhibition of the hind paw edema in rats. Experimental procedures were in accordance with the guidelines of the Animal Ethics Committee of the Faculty of Science, South Valley University, Qena, Egypt. Microwave reactions were performed with a Millstone Organic Synthesis Unit with touch control terminal (Micro SYNTH, Giza, Egypt) with a continuous focused microwave power delivery system in a pressure glass vessel (10 ml) sealed with a septum under magnetic stirring. The temperature of the reaction mixture was monitored using a calibrated infrared temperature control under the reaction vessel, and control of the pressure was performed with a pressure sensor connected to the septum of the vessel. Note that, for each reaction the ratios between the reactants in the convention and the microwave methods were the same, but in the case of using microwave irradiation the amounts of the reactants and the volumes of the solvents were modified to be suitable for the vessel used, keeping with the same ratios between the reactants.

Synthesis of 1-(4-(1*H*-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3,3-dimercaptoprop-2-en-1-one (1)[11]

Synthesis of 4-(1*H*-indol-3-yl)-5-(5-mercapto-1-phenyl-1*H*-pyrazol-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1*H*)-thione (2)

A few drops of acetic acid was added to a solution of oxoketene *gem*-dithiol **1** (0.361 gm, 0.001 mol) and phenylhydrazine (0.098 ml, 0.001 mol) in ethanol (20 ml). The reaction mixture was heated under reflux for 5 h and/or microwave irradiation for 6 min. The yields; 69.5%, 92.1%, respectively, M. P. > 300 ºC, colour: brown, recrystallization from: ethanol/benzene. Mass spectrum (*m/e*) (I, %) 417 ($M^{+\cdot}$, 10), (313, 100). IR (KBr): *v* (cm^{-1}): 3228 (NH's), 2364 (SH) and 1455 (C=S). ^{1}H-NMR (DMSO-d_6): δ (ppm.): 2.74 (s, 3H, CH_3), 3.61 (s, 1H, C(sp^3-H)-Pyrimid.), 6.56-7.69 (m, 11H, aromatic protons, (*J* = 2.6)), 8.13 (s, 1H, NH-Pyrimid.), 10.68 (s, 1H, NH-Indol.), 11.29 (s, 1H, NH-Pyrimid.) and 13.25 (s, 1H, SH). ^{13}C-NMR: 12.9, 62.8,100.1,110.1,112.2,115.8,117.9,117.9,118.4,120.6,121.3,125.1,126.0,129.5,132.0,136.1,140.3,141.1,141.6, 172.1. Elemental analysis for $C_{22}H_{19}N_5S_2$ (417.55). Calcd: C, 63.28; H, 4.59; N, 16.77; S, 15.36 Found: C, 63.47; H, 4.24; N, 17.03; S, 15.26.

Synthesis of 3-(4-(1*H*-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-5-mercapto-1-phenyl-1*H*-pyrazole-4-carbaldehyde (3)

A solution of the compound **2** (0.417 gm, 0.001 mol) in DMF (30 ml) was stirred in ice path at 0-5 ºC for 3 h in the presence of $POCl_3$ (10 ml); then it was triturated with a cold solution of K_2CO_3. The mixture was left aside to settle, then it was filtered off; the product was collected and left to dry. The yield; 71.4%, M. P. > 300 ºC, colour: dark brown, recrystallization from: ethanol. Mass spectrum: (*m/e*) (I, %) 445 ($M^{+\cdot}$, 8), (117, 100). IR (KBr): *v* (cm^{-1}): 3451 (NH's), 2547 (SH), 1663 (C=O), and 1453 (C=S). ^{1}H-NMR (DMSO-d_6): δ (ppm.): 2.74 (s, 3H, CH_3), 3.31 (s, 1H, C(sp^3-H)-Pyrimid.), 6.41-7.96 (m, 10H, aromatic-H, (*J*= 3.3)), 8.13 (s, 1H, NH-Pyrimid.), 9.25 (s, 1H, CHO), 10.71 (s, 1H, NH-Indol.) and 11.07 (s, 1H, NH-Pyrimid.) and 13.31 (s, 1H, SH). ^{13}C-NMR: 21.7,63.8,73.0, 111.1,111.7, 112.1,114.1,116.3,117.9,119.0,120.9,123.1,125.9, 127.4,131.5,136.7,137.1,141.1,142.1,162.3,179.0. Elemental analysis for $C_{23}H_{19}N_5S_2O$ (445.56). Calcd: C, 62.00; H, 4.30; N, 15.72; S, 14.39 Found: C, 61.89; H, 4.55; N, 15.90; S, 14.64.

Synthesis of 4-((3-(4-(1*H*-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1-phenyl-1*H*-pyrazol-5-yl)amino)benzenesulfonic acid (4)

A mixture of the compound 2 (0.417 gm, 0.001 mol) and sulfanilic acid (0.173 gm, 0.001 mol) in ethanol (30 ml) in the presence of a few drops of piperidine was heated under reflux for 6 h and/or under microwave for 5 min. The so formed product was collected by filtration and left to dry. The yields; 59.6%, 83.0% respectively, M. P. > 300 oC, colour: dark brown, recrystallization from: ethanol/benzene. Mass spectrum (m/e) (I, %) 556 (M+., 4), (57, 100). IR (KBr) v (cm-1): 3397 (OH), 3205 (NH's) and 1499 (C=S). 1H-NMR (DMSO-d6): δ (ppm.): 1.57 (s, 1H, OH), 2.74 (s, 3H, CH3), 3.30 (s, 1H, C(sp3-H)-Pyrimid.), 6.24-7.96 (m, 15H aromatic protons, (J= 3.6)), 8.20 (s, 1H, NH-Pyrimid.), 8.21 (s, 1H, NH, Pyraz.-NH-Ph.), 10.74 (s, 1H, NH-Indole) and 10.98 (s, 1H, NH-Pyrimid.). 13C-NMR: 22.1, 65.9, 109.2,111.0,113.5,115.4,116.6,117.2,118.5,126.7,127.7,128.1,129.3,132.5,133.1,134.6,135.0,139.5,140.1,142.2,143.6,144.1, 145.6 ,179.8. Elemental analysis for C28H24N6S2O3 (556.66). Calcd: C, 60.42; H, 4.35; N, 15.10; S, 11.52 Found: C, 59.89; H, 4.70; N, 15.09; S, 11.99.

General procedures for synthesizing of the compounds (5a-c)

An equimolar ratios of the compound 3 (0.445 gm, 0.001 mol) and 4-nitroaniline (0.138 gm, 0.001 mol); 4-chloroaniline (0.127 gm, 0.001 mol) or sulfanilic acid (0.173 gm, 0.001 mol) in ethanol (20 ml) in the presence of a catalytic amount of piperidine were heated under refluxed for 5 h or under microwave for 5-6 minutes. The progress of the reactions was followed up by TLC. Finally, the reaction mixtures were left overnight at room temperature to settle and the so formed precipitates were collected by filtration and left to dry. The obtained products were recrystallized from the appropriate solvents.

Synthesis of (E)-4-(1H-indol-3-yl)-5-(5-mercapto-4-(((4-nitrophenyl)imino)methyl)-1-phenyl-1H-

pyrazol-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (5a)

Obtained from the compound 3 and 4-nitroaniline. The yields; 61.8%, 90.3% respectively, M. P. > 300 oC, colour: brown, recrystallization from: ethanol. Mass spectrum (m/e) (I, %) 565 (M+., 2), (88, 100). IR (KBr) v (cm-1): 3404 (NH's), 2342 (SH), 1616 (C=N) and 1452 (C=S). 1H-NMR (DMSO-d6): δ (ppm.): 2.74 (s, 3H, CH3), 3.37 (s, 1H, C(sp3-H)-Pyrimid.), 6.53-7.96 (m, 15H, aromatic protons, (J= 2.4) + CH=N), 8.65 (s, 1H, NH-Pyrimid.), 10.97 (s, 1H, NH-Indole), 11.01 (s, 1H, NH-Pyrimid.) and 13.12 (s, 1H, SH). 13C-NMR: 21.7,63.4,99.5,111.1,111.4, 112.1, 114.0,116.3,117.8,119.0,120.9,123.2,125.2,126.2,127 .4,131.3, 134.3,136.4,137.5,140.1, 142.3,143.4, 150.0, 162.2, 178.1. Elemental analysis for C29H23N7O2S2 (565.67). Calcd: C, 61.58; H, 4.10; N, 17.33; S, 11.34. Found: C, 61.43; H, 4.21; N, 17.54; S, 11.11.

Synthesis of (E)-5-(4-(((4-chlorophenyl)imino)-methyl)-5-mercapto-1-phenyl-1H-pyrazol-3-yl)-4-(1H-indol-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1H)-thione (5b)

Obtained from the compound 3 and 4-chloroaniline. The yields; 68.7%, 88.5% 90.3%, M. P. > 300 oC, colour: brown, recrystallization from: ethanol. Mass spectrum (m/e) (I, %) 555 (M+., 5), (58 , 100). IR (KBr) v (cm-1): 3414 (NH's), 2371 (SH), 1617 (C=N) and 1452 (C=S). 1H-NMR (DMSO-d6): δ (ppm.): 2.74 (s, 3H, CH3), 3.30 (s, 1H, C(sp3-H)-Pyrimid.), 6.52-7.95 (m, 15H, aromatic protons, (J= 2.3) + CH=N), 10.77 (s, 1H, NH-Pyrimid.), 10.93 (s, 1H, NH-indole), 11.05 (s, 1H, NH-Pyrimid.) and 13.21 (s, 1H, SH). 13C-NMR: 21.7,63.7,111.1,111.7, 112.1,114.1,116.3,117.8,119.8,120.6,123.1,125.4,126 .3,127.1,129.2, 131.4,134.0,136.7,137.4, 140.2,142.3, 143.8,151.1,161.0,178.1. Elemental analysis for C29H23ClN6S2 (555.11). Calcd: C, 62.75; H, 4.18; Cl, 6.39; N, 15.14; S, 11.55. Found: C, 62.73; H, 4.20; Cl, 6.40; N, 15.13; S, 11.55.

Synthesis of (E)-4-(((3-(4-(1H-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-5-mercapto-1-phenyl-1H-pyrazol-4-yl)methylene)amino)benzenesulfonic acid (5c)

Obtained from the compound 3 and sulfanilic acid. The yields; 71.6%, 88.5% respectively, M. P. > 300 oC, colour: brown, recrystallization from: ethanol. Mass spectrum (m/e) (I, %) 600 (M+., 11), (117 , 100). IR (KBr) v (cm-1): 3301 (OH), 2347 (SH), 3225 (NH's), 1617 (C=N) and 1452 (C=S). 1H-NMR (DMSO-d6): δ (ppm.): 1.59 (s, 1H, OH), 2.74 (s, 3H, CH3), 3.81 (s, 1H, C(sp3-H)-Pyrimid.), 6.52-7.96 (m, 15H, aromatic protons, (J= 3.4) + CH=N), 8.95 (s, 1H, NH-Pyrimid.), 10.72 (s, 1H, NH-Pyraz.), 10.81 (s, 1H, NH-Indole) and 13.11 (s, 1H, SO2OH). 13C-NMR: 21.7,63.8,111.0,111.4,112.1, 116.0, 117.8, 119.7,120.9,121.6, 123.5,126.1,126.2, 127.4, 129.3, 131.5,136.5,140.2,141.3,142.7,144.0,148.5,151.1,162 .5,178.1. Elemental analysis for C29H24N6S3O3 (600.73). Calcd: C, 57.98; H, 4.03; N, 13.99; S, 16.01. Found: C, 58.05; H, 4.12; N, 13.87; S, 16.33

Synthesis of 4-((3-(4-(1*H*-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1-phenyl-1*H*-pyrazol-5-yl)amino)-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)benzenesulfonamide (6)

A mixture of the compound **4** (0.556 gm, 0.001 mol) and 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (0.203 gm, 0.001 mol) in ethanol (20 ml) in the presence of a few drops of piperidine was heated under reflux for 6 h and/or microwave for 5 min. The reaction progress was monitored by TLC. The reaction mixture was left overnight at room temperature; the so formed precipitate was collected by filtration and left to dry. The yields; 73.7%, 90.5% respectively, M. P. > 300 ^{o}C, colour: dark brown, recrystallization from: ethanol. Mass spectrum (*m/e*) (I, %) 741 ($M^{+\cdot}$, 1), (87, 100). IR (KBr) *v* (cm^{-1}): 3404, 3249 (NH's), 1656 (C=O) and1455 (C=S). ^{1}H-NMR (DMSO-d_6): δ (ppm.): 2.65 (s, 6H, $2CH_3$), 2.74 (s, 3H, CH_3), 3.37 (s, 1H, C(sp^3-H)-Pyrimid.), 6.56-7.50 (m, 21H, aromatic protons, (*J*= 2.0) + NH), 8.52 (s, 1H, NH-Pyrimid.), 10.70 (s, 1H, Pyraz.-NH-Ph), 10.93 (s, 1H, NH-Indole) and 11.51 (s, 1H, NH-Pyrimid.). ^{13}C-NMR: 15.0,21.9,22.2,110.2,111.3,112.3,113.0, 114.4,115.5,116.8,117.8,118.4, 120.3,122.4,123.9, 125.2,128.4,129.2,130.3,132.4,132.6,134.8,135.5, 136.9,137.4,138.7,139.0,142.3,143.3,144.1,148.3,167 .7, 179.9. Elemental analysis for $C_{39}H_{35}N_9S_2O_3$ (741.89). Calcd: C, 63.14; H, 4.76; N, 16.99; S, 8.64 Found: C, 63.03; H, 4.68; N, 16.55; S, 9.54.

Synthesis of 5-(1,6-diphenyl-1,6-dihydropyrazolo [3,4-*c*]pyrazol-3-yl)-4-(1*H*-indol-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1*H*)-thione (7)

A solution of the compound **3** (0.445 gm, 0.001 mol) and phenylhydrazine (0.098 ml, 0.001 mol) in ethanol (20 ml) in the presence of a few drops of piperidine was heated under reflux for 4 h and/or microwave for 5 min., then it was left overnight at room temperature to settle. The so formed precipitate was collected by filtration and left to dry. The yields; 81.3%, 92.7% respectively, M. P. > 300 ^{o}C, colour: brown, recrystallization from: ethanol. Mass spectrum (*m/e*) (I, %) 501 ($M^{+\cdot}$, 4), (117 , 100). IR (KBr) *v* (cm^{-1}): 3410 (NH's), 1616 (C=N) and 1452 (C=S). ^{1}H-NMR (DMSO-d_6): δ (ppm.): 2.74 (s, 3H, CH_3), 3.36 (s, 1H, C(sp^3-H)-Pyrimid.), 7.06-7.45 (s, 16H, aromatic protons, (*J*= 1.5)), 10.78, (s, 1H, NH-Pyrimid,) 10.98 (s, 1H, NH-Indole) and 11.09 (s, 1H, NH-Pyramid.). ^{13}C-NMR: 21.7,63.6,73.1,111.1, 111.4,112.1,114.1,116.3,117.8,119.9,120.9,121.6,123 .0,125.2,127.4,129.2,131.3,133.4,136.5,137.6,141.7, 142.3,149.1,149.7,179.2. Elemental analysis for $C_{29}H_{23}N_7S$ (501.17). Calcd: C, 69.44; H, 4.62; N,

19.55; S, 6.39. Found: C, 70.13; H, 4.74; N, 18.29; S, 6.84.

Synthesis of 5-(6-amino-1-phenyl-1*H*-pyrazolo [3,4-*d*]pyrimidin-3-yl)-4-(1*H*-indol-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1*H*)-thione (8a)

A solution of the compound **3** (0.445 gm, 0.001 mol) in ethanol (20 ml), guanidine sulfate (0.157 gm, 0.001 mol) and a few drops of piperidine was heated under reflux for 5 h and/or microwave for 5 min. The so formed precipitate was collected by filtration and left to dry. The yields; 86.4%, 92.3% respectively, M. P. 300-303 ºC, colour: brown, recrystallization from: ethanol. Mass spectrum (*m/e*) (I, %) 452 ($M^{+\cdot}$, 2), (117 , 100). IR (KBr) *v* (cm^{-1}): 3401 (NH_2), 3215 (NH), 1616 (C=N) and1453 (C=S). ^{1}H-NMR (DMSO-d_6): δ (ppm.): 2.74 (s, 3H, CH_3), 3.46 (s, 1H, C(sp^3-H)-Pyrimid.), 5.70 (s, 2H, NH_2), 6.65-7.96 (s, 11H, aromatic protons, (*J*= 2.4)), 9.00 (s, 1H, NH-Pyrimid.), 9.85 (s, 1H, NH-Indol), 10.46 (s, 1H, NH-Pyrimid.). ^{13}C-NMR: 21.7,63.7,100.5,104.9,111.8, 112.1,114.8, 116.7,117.8,119.6,120.8, 123.2,125.4, 127.3,132.3, 136.7,137.3,140.3,141.0,142.8,161.0,179.1.Elemental analysis for $C_{24}H_{20}N_8S$ (452.54). Calcd: C, 63.70; H, 4.45; N, 24.76; S, 7.08. Found: C, 63.22; H, 5.36; N, 24.15; S, 7.27.

Synthesis of 4-(1*H*-indol-3-yl)-5-(6-mercapto-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3-yl)-6-methyl-3,4-dihydropyrimidine-2(1*H*)-thione (8b)

A mixture of the **3** (0.445 gm, 0.001 mol) in ethanol (20 ml), thiourea (0.076 gm, 0.001 mol) and a catalytic amount of piperidine was heated under reflux for 5 h and/or microwave for 6 min. The progress of the reaction was followed up by TLC, finally the so formed product was collected by filtration and left to dry. The yields; 79.9%, 91.2% respectively, M. P. > 300 ºC, colour: brown, recrystallization from: ethanol. Mass spectrum (*m/e*) (I, %) 469 ($M^{+\cdot}$, 4), (117, 100). IR (KBr) *v* (cm^{-1}): 3402 (NH's), 2365 (SH) and 1452 (C=S). ^{1}H-NMR (DMSO-d_6): δ (ppm.): 2.74 (s, 3H, CH_3), 3.61 (s, 1H, C(sp^3-H)-Pyrimid.), 6.56-7.99 (s, 11H, aromatic protons), (*J*= 3.8)), 0.78 (s,1H, NH-Pyrimid.) 10.79 (s, 1H, NH-Iindol.), 11.03 (s, 1H, NH-Pyrimid.) and 13.00 (s, 1H, SH).

^{13}C-NMR: 21.7,63.7,104.9,111.1, 112.4,114.1,116.8, 117.1,117.8,119.9,120.7,123.0,125.1,127.4,132.3,136 .7,137.3,140.4,141.2, 142.3,163.3,179.0. Elemental analysis for $C_{24}H_{19}N_7S_2$ (469.59). Calcd: C, 61.39; H, 4.08; N, 20.88; S, 13.65. Found: C, 61.85; H, 4.91; N, 19.92; S, 13.32.

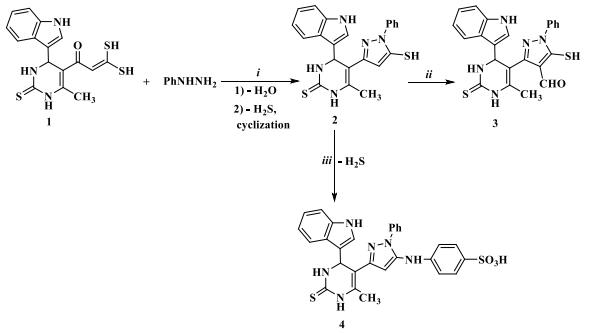
Synthesis of (3-(4-(1*H*-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-1-phenyl-4,5-dihydro-1*H*-thieno[2,3-*c*]pyrazol-5-yl)(phenyl)methanone (9)

A mixture of the compound **3** (0.445 gm, 0.001 mol) and potassium hydroxide (2N) was stirred in ethanol (20 ml) at room temperature for 1 h then; acetophenone (0.116 ml, 0.001 mol) was added. The previous mixture was heated under reflux for 6 h and/or microwave for 5 min. The reaction progress was followed up and monitored by TLC, after completion it was left overnight at room temperature to settle. The solution was concentrated, cooled and the so formed precipitate was collected by filtration and left to dry. The yields; 87.4%, 92.6% respectively, M. P. 260-262 ºC, colour: black, recrystallization from: ethanol. Mass spectrum (*m/e*) (I, %) 547 ($M^{+\cdot}$, 2), (105 , 100). IR (KBr) *v* (cm^{-1}): 3412 (NH's), 1661 (C=O) and 1455 (C=S). ^{1}H-NMR (DMSO-d_6): δ (ppm.): 2.74 (s, 3H, CH_3), 2.92 (d, 2H, CH_2), 3.32 (s, 2H, C(sp^3-H)-Pyrimid. + CHCO), 6.69-7.99 for (m, 16H, aromatic protons (*J*= 3.8) + NH), 10.29 (s, 1H, NH-Indole), 10.70 (s, 1H, NH-Pyrimid.). ^{13}C-NMR: 21.7,51.7,63.9,65.0, 111.1, 111.4,112.1,115.6,118.4, 119.8,120.9,121.6,123.0, 126.1,127.4,128.6,129.8, 130.2,133.1,134.1,136.5, 139.0,140.3,141.8, 148.2, 178.6, 189.4. Elemental analysis for $C_{31}H_{25}N_5S_2O$ (547.70). Calcd: C, 67.98; H, 4.60; N, 12.79; S, 11.71. Found: C, 67.15; H, 4.99; N, 13.48; S, 11.19

3. Results and discussion

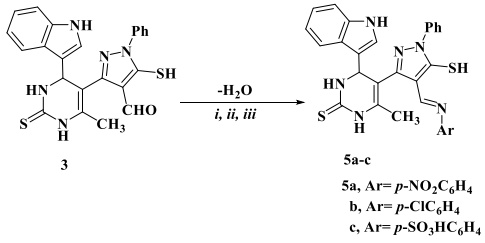
The substrate oxoketene *gem*-dithiol [11] **1** reacted with phenylhydrazine to afford the pyrazole derivative **2** in either conventional and/or microwave irradiation conditions. Vilsmeier-Haack formylation [12] ($POCl_3$/DMF reagent) of the compound **2** produced the formylated adduct **3**. On the other hand, subsequent, treatment of the pyrazole derivative **2** with sulfanilic acid in different reaction conditions e.g. reflux and/or MW; a sulfahydryl elimination reaction took place to afford the pyrazolyl derivative **4**. Optimization of the reactions conditions and yields for the compounds **2** and/or **4** was illustrated in the table 1.

The structures of the obtained compounds **2**-**4** were derived and confirmed depending on their analyses. Mass spectrum of the adduct **2** showed a molecular ion peak at *m/z*= 417, while IR spectrum revealed the disappearance of the carbonyl group which was present in the starting compound **1**, Scheme 1. ^{13}C-NMR for the product **3** postulated a signal at 162.3 characteristic for the C=O group. The molecular ion peak of the adduct **4** appeared at *m/z*= 556, while ^{13}C-NMR showed signals at 22.1-179.8 characteristic for aliphatic, aromatic and thiocarbonyl carbons.



Scheme 1 Synthesis of 2-4
Reagents and conditions
***i*, EtOH/AcOH/reflux 5 h or EtOH/AcOH/MW 6 min.**
***ii*, DMF/aq. K_2CO_3, $POCl_3$, stirr. 3 h.**
***iii*, Sulfanilic acid, EtOH/Pip./reflux 6 h or sulfanilic acid, EtOH/Pip./MW 5 min.**

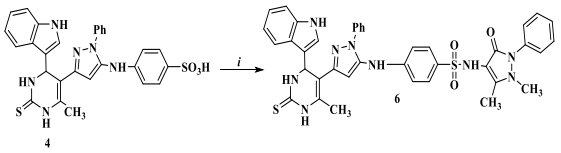
Owing to the reported on the biological activities of Schiff bases bearing pyrazole moieties as antitumor and anticancer agents [13,14], herein the authors could synthesize a new generation from Schiff bases *via* the reaction of pyrazolecarbaldehyde **3** with some aromatic amines e.g. 4-nitroaniline, 4-chloroaniline and/or sulphanilic acid to gather the target Schiff bases **5a-c**, Scheme 2. The reactions were carried out either in the conventional or the MW conditions as shown in the table 1. Remarkably, under the two different conditions the same products were obtained and this was confirmed depending on the analyses and the spectral data. IR spectra for the compounds **5a-c** confirmed the disappearance of the bands belong to the C=O group which was present in the starting compound **3**. Also, ^{13}C-NMR spectra for the adducts **5a-c** were on accordance with the previous observation obtained in IR analyses; where the signal characteristic for the C=O group disappeared.



5a, Ar= *p*-$NO_2C_6H_4$
b, Ar= *p*-ClC_6H_4
c, Ar= *p*-$SO_3HC_6H_4$

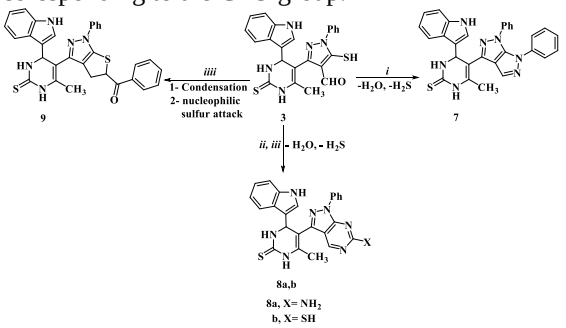
Scheme 2 Synthesis of 5a-c
Reagents and conditions:
5a, *i*, *p*-Nitroaniline, EtOH/Pip./reflux 5 h or *p*-nitroaniline, EtOH/Pip./MW, 5 min.
5b, *ii*, *p*-Chloroaniline, EtOH/Pip./reflux 5 h or *p*-chloroaniline, EtOH/Pip./MW, 6 min.
5c, *iii*, Sulfanilic acid, EtOH/Pip./reflux 5 h or sulfanilic acid, EtOH/Pip./MW, 6 min.

It was profitable and wealthy idea to exploit the substance **4** for preparing the celecoxib [15] analog **6**; upon the reaction of benzenesulfonic acid derivative **4** with 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one; benzenesulfonamide derivative **6** was obtained, Scheme 3.



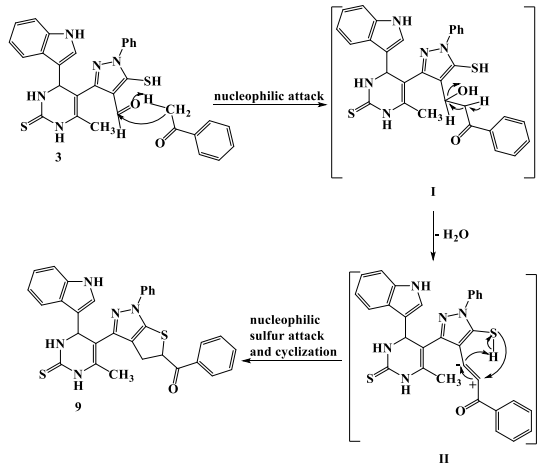
Scheme 3 Synthesis of 6
Reagents and conditions:
***i*, 4-Amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one, EtOH/Pip/reflux 6 h or 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one, EtOH/Pip/MW 5 min.**

Condensation of the pyrazolecarbaldehyde derivative **3** with phenylhydrazine, guanidine sulfate and/or thiourea and susequent cyclization *via* sulfahydryl elimination afforded the adducts **7** and/or **8a**,**b** in different reaction conditions, table 1, Scheme 4. The prepared compounds **7** and/or **8a**,**b** were subjected to different analyses to ensure their suggested chemical structures. Viewed from the structural features of 3-(4-(1*H*-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-5-mercapto-1-phenyl-1*H*-pyrazole-4-carbaldehyde **3**; the push-pull electronic effect of the *β*-thiol group and the α-carbonyl group makes the carbon-carbon double bond highly polarized. Owning to the foregoing information about the bioreactivity of the pyrazole derivatives [2], Knoevenagel condensation reaction of the adduct **3** with acetophenone was applied in the prescence of methanol and potassium hydroxide under reflux and/or microwave irradition to afford (3-(4-(1*H*-indol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl)-1-phenyl-4,5dihydro-1*H*-thieno[2,3-*c*]pyraz-ol-5-yl)(phenyl)methanone **9**, Scheme 4. Optimization of the conditions and yields for the compound **9** was illustrated in the table 1**.** Mass spectrum for the adduct **9** showed a molecular ion peak at *m/z*= 547, while IR spectrum showed a band at 1661 cm^{-1} belongs to the C=O group. Also, ^{13}C-NMR, revealed a value at 178.6 coresponding to the C=O group.



Scheme 4 Synthesis of 7-9
Reagents and conditions:
***i*, Phenylhydrazine, EtOH/Pip./reflux 4 h or phenylhydrazine, EtOH/Pip./MW, 5 min.**
8a, *ii*, Guanidine sulfate, EtOH/Pip./reflux 5 h or guanidine sulfate, EtOH/Pip./MW, 5 min.
8b, *iii*, Thiourea, EtOH/Pip./reflux 5 h or thiourea, EtOH/Pip./MW, 6 min.
***iiii*, Acetophenone, MeOH/KOH/reflux 6 h, or acetophenone, MeOH/KOH//MW, 5 min.**

Scheme 5 illustrates the proposed mechanism for the formation of the compound 9 as shown bellow.



Scheme 5 The suggested mechanism for the formation of 9

[a]In the present work we have studied the synthesis of different compounds by using different reactions conditions (i.e. conventional heating and/or microwave irradiation) as shown in the table 1. In all conditions the reactants were taken in the same ratios (except in the case of MW; the amounts of the reactants were modified to be suitable for the vessel used in this method) keeping with the same ratios between the reactants in all cases. The reactions progress were followed up and monitored by the thin layer chromatography. As shown in the above table 1, it was noticed that the reactions proceeded smoothly in different conditions and gave the same products and this was confirmed by different analyses and spectral data. From the above table 1, the ratios of the yields in the case of microwave irradiation, were the highest in comparison with that obtained in the conventional heating.

Table 1[a]: Optimization of the reactions conditions and the yields for the compounds 2,4-9

Entry	Solvent and catalysts	Reaction time	Reaction conditions	Yield %
2	EtOH/AcOH	5 h	Reflux	69.5
		6 min.	MW	92.1
4	EtOH/Pip.	6 h	Reflux	59.6
		5 min.	MW	83.0
5a	EtOH/Pip.	5 h	Reflux	61.8
		5 min.	MW	90.3
5b	EtOH/Pip.	6 h	Reflux	68.7
		5 min.	MW	88.5
5c	EtOH/Pip.	5 h	Reflux	86.4
		6 min.	MW	92.3
6	EtOH/Pip.	6 h	Reflux	75.3
		5 min.	MW	88.7
7	EtOH/Pip.	5 h	Reflux	71.6
		5 min.	MW	83.6
8a	EtOH/Pip.	4 h	Reflux	81.3
		5 min.	MW	91.7
8b	EtOH/Pip.	5 h	Reflux	79.9
		6 min.	MW	91.2
9	MeOH/KOH	6 h	Reflux	87.4
		5 min.	MW	92.6

4. *In-vivo* anti-inflammatory study
(Carrageenan paw edema inflammation model)

The *In-vivo* anti-inflammatory activity of the synthesized compounds were tested for their anti-inflammatory activities according to carrageenan induced paw edema method in comparison to celecoxib as a reference drug was performed based on the inhibition of the hind paw edema in rats.[16] Experimental procedures were in accordance with the guidelines of the Animal Ethics Committee of Faculty of Science, South Valley University, Qena, Egypt. Male albino thirty three rats weighing about (120-150 g) were used. All the rats were kept under standard laboratory conditions at temperature of 25°C and relative humidity of 55%. The rats were housed in polypropylene cages (three rats per cage) with free access to a standard laboratory diet (lipton feed) and water ad libitum. The thickness of the hind rat paws was measured before and thirty minutes before the application of each tested compound and after carrageenan injection to detect the carrageenan induced inflammation by means of a Vernier Caliper (SMEC, China). The tested is based on the pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 ml of 1% w/v solution in normal saline) into the right hind paw of the rats. Each test compound at a dose of 28 µmole/kg (dissolved in 1% Na-CMC in normal saline) was injected intraperitoneal to different groups of rats. The animals of control group received a vehicle (1% Na-CMC in normal saline), while reference groups received anti-inflammatory commercial drug (Nonsteroidal anti-inflammatory celecoxib: Celebrex®) intraperitoneal to different groups of rats. We used celecoxib as a reference compound, because of the synthesized compounds were based on celecoxib.[17] The difference between thicknesses of the two paws was taken as a measure of oedema. The measurements were carried out at 1, 2, 3, 4 and 5 hrs. After injection of tested compounds, reference drug. The increase in paw thickness was measured before carrageenan injection and immediately after carrageenan injection (zero time) and then every hour for up to 5 hrs using a Vernier Caliper. The percentages of oedema inhibition were calculated according to the following equation.

Post-hoc examination by Dunnett's multiple comparison testing was used for calculation.

$$\% \text{ Edema inhibition} = \frac{(V_R - V_L)\text{ control} - (V_R - V_L)\text{ treated}}{(V_R - V_L)\text{ control}} \times 100$$

Where, VR: Average right paw thickness, VL: Average left paw thickness.

Statistical Analysis

IBM SPSS statistics (Statistical Package for Social Sciences) software version 25.0, IBM Corp., Chicago, USA was used to code, tabulate and statistically analyze the collected data. In descriptive statistics, the mean ± SD (standard deviation) was also

done. To statistically measure difference among the thickness measurement at the time intervals within each compound, we used Repeated Measures test with comment on "tests within subjects effect", Green house-Geisser. To statistically measure difference among the thickness measurements at the time intervals; between the celecoxib and each individual of the tested compounds, we used the repeated measures, two ways ANOVA
test, the Post-hoc examination by Dunnett's multiple comparison testing was used. The p-value was used. Significance levels were considered as following: at ρ value ≥ 0.050 is non-significant, ρ value < 0.050 is significant and ρ value < 0.010 is highly significant.

I- Results of *In-vivo* anti-inflammatory activity of the tested compounds in to male rat's agents:

Analysis after five hours:

Four rats were included in each compound group and the thickness of the rats' paws was measured.

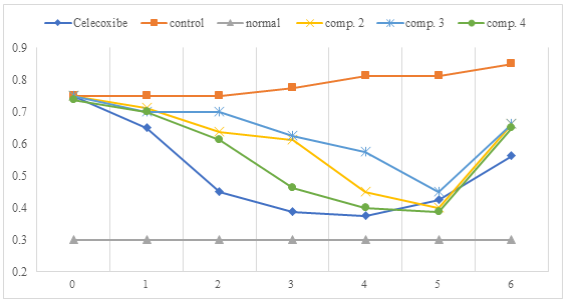


Figure 1: Measurements of the thickness of the rats' paws in the experiments compounds 2- 4.

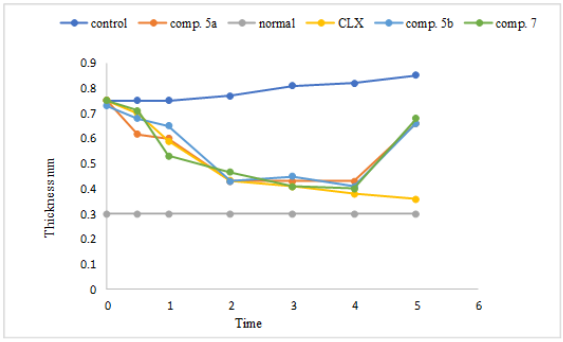


Figure 2: Measurements of the thickness of the rats' paws in the experiments compounds 5a,b and 7.

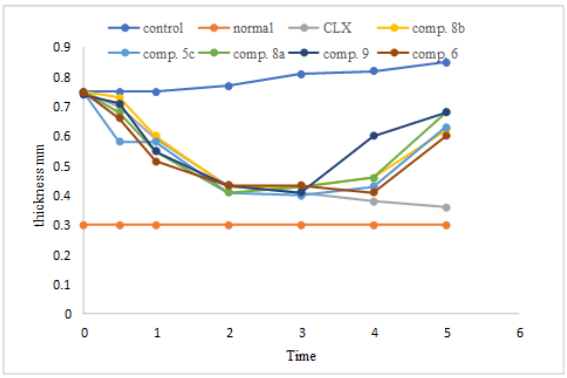


Figure 3: Measurements of the thickness of the rats' paws in the experiments compounds 5c, 6, 8a,b and 9

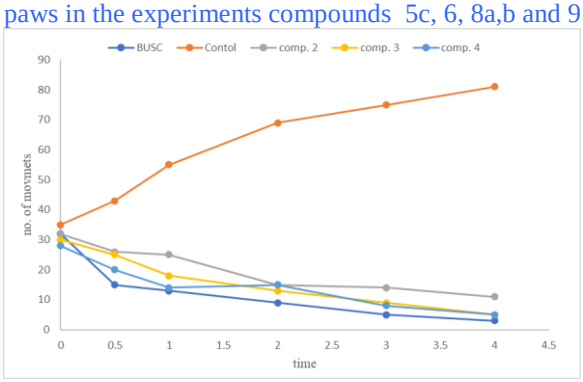


Figure 4: Number of movements in the experiments compounds 2-4.

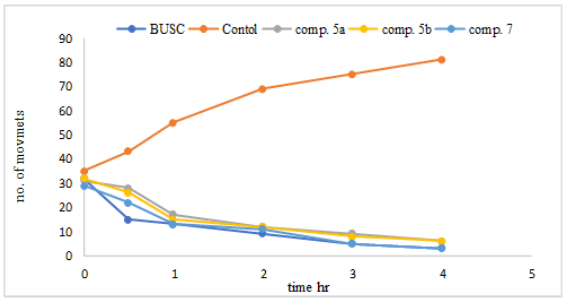


Figure 5: Number of movements in the experiments compounds 5a,b and 7.

Table 2: The measurements of the thickness of rats' paws in the experiment compounds.

compound		At baseline	At 30 minutes	At 1 hour	At 2 hours	At 3 hours	At 4 hours	At 5 hours	P-value[1]	P-Value[2]
Celecoxibe	Mean	0.75	0.7	0.58333333	0.4333333	0.41666	0.38333333	0.36666667	<0.0001	Ns
	SD	0	0.05	0.07637626	0.057735	0.02887	0.02886751	0.02886751		
2	Mean	0.75	0.7125	0.6375	0.6125	0.45	0.4	0.6625	<0.0001	0.002
	SD	0	0.025	0.04787	0.04787	0.04082	0	0.025		
3	Mean	0.75	0.7125	0.7	0.625	0.575	0.45	0.6625	0.052	

	SD	0	0.025	0.04082	0.06455	0.06455	0.05774	0.04787		<0.0001
4	Mean	0.7375	0.7	0.6125	0.4625	0.4	0.3875	0.65	0.003	0.045
	SD	0.025	0.04082	0.04787	0.06292	0	0.025	0.04082		
5a	Mean	0.75	0.61666667	0.61666667	0.43333333	0.43333333	0.43333333	0.66666667	0.0162	0.0002
	SD	0	0.1040833	0.07637626	0.05773503	0.02886751	0.02886751	0.02886751		
5b	Mean	0.73333333	0.68333333	0.65	0.43333333	0.45	0.41666667	0.66666667	<0.0001	0.045
	SD	0.02886751	0.05773503	0.05	0.05773503	0	0.02886751	0.02886751		
5c	Mean	0.75	0.58333333	0.58333333	0.41666667	0.4	0.43333333	0.63333333	0.004	0.004
	SD	0	0.07637626	0.02886751	0.02886751	0.05	0.02886751	0.02886751		
6	Mean	0.75	0.66666667	0.51666667	0.43333333	0.43333333	0.41666667	0.65	0.01755	0.0002
	SD	0	0.02886751	0.12583057	0.05773503	0.02886751	0.02886751	0.05		
7	Mean	0.75	0.71666667	0.53333333	0.46666667	0.41666667	0.4	0.68333333	<0.0001	0.0002
	SD	0	0.02886751	0.05773503	0.02886751	0.02886751	0	0.02886751		
8a	Mean	0.74333333	0.68333333	0.55	0.41666667	0.43333333	0.46666667	0.68333333	<0.0001	0.125
	SD	0.01154701	0.05773503	0.08660254	0.02886751	0.02886751	0.05773503	0.02886751		
8b	Mean	0.75	0.73333333	0.6	0.43333333	0.43333333	0.46666667	0.62	0.001	<0.0001
	SD	0	0.02886751	0.08660254	0.02886751	0.02886751	0.02886751	0.05196152		
9	Mean	0.74333333	0.71666667	0.55	0.43333333	0.41666667	0.6	0.68333333	<0.0002	Ns
	SD	0.01154701	0.02886751	0.08660254	0.02886751	0.02886751	0.08660254	0.02886751		
Control	Mean	0.75	0.75	0.75	0.775	0.8125	0.8125	0.85	0.074	<0.0001
	SD	0	0	0.04082	0.02887	0.04787	0.04787	0.04082		
Normal		0.3	0.3	0.3	0.3	0.3	0.3	0.3	Ns	<0.0001

P-Value[1] The difference among the thickness measurements at the time intervals within the compound.
P-Value[2] The difference between the celecoxib and each individual's thickness measurements at the time intervals.
Repeated measures test was used and the Post-hoc examination by Dunnett's multiple comparison testing.

Table 3: The mean of the number of the movements in the rats in the experiment's compounds.

Time	**2**	**3**	**4**	**5a**	**5b**	**5c**	**6**	**7**	**8a**	**8b**	**9**	**BUSCOPAN**	**control**
30 min.	32	30	28	31	32	31	25	29	33	30	33	32	35
1hr.	26	25	20	28	26	25	21	22	19	23	24	15	43
2hr.	25	18	14	17	15	19	19	13	15	15	20	13	55
3hr.	15	13	15	12	12	12	18	11	13	9	15	9	69
4hr.	14	9	8	9	8	9	16	5	7	5	13	5	75
5hr.	11	5	5	6	6	4	12	3	4	3	5	3	81

The analgesic activities of the compounds **2- 9** were tested compared to BUSCOPAN.

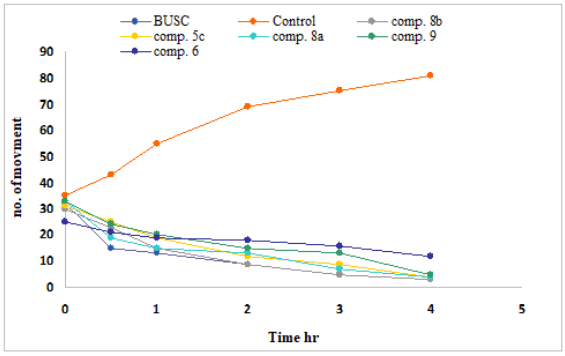


Figure 6: Number of movements in the experiments compounds 5c, 6, 8a,b, and 9.

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method.[18] Briefly, 100 µl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 108 cells/ ml for bacteria or 105 cells/ml for fungi.[19] 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method.[20] Plates inoculated with filamentous fungi as Aspergillus flavus at 25 oC for 48 hrs.; Gram (+) bacteria as Staphylococcus aureus, Bacillus subtilis; Gram (-) bacteria as Escherichia coli, Pseudomonas aeuroginosa they were incubated at 35-37 oC for 24-48 hours and yeast as Candida albicans incubated at 30 oC for 24-48 hours and, then the diameters of the inhibition zones were measured in millimeters.[21] Standard discs of Ampicillin (Antibacterial agent), Amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (distilled water, chloro-form, DMSO) were used as a negative control. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated 10µ of tested concentration of the stock solutions.

When a filter paper disc impregnated with a tested chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "**Zone of inhibition**" or" **Clear zone**". For the disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards.[22]

Agar-based methods such as Etest and disk diffusion can be good alternatives because they are simpler and faster than broth based methods.[23,24]

Table 4[a]: The effect of the compounds 2-9 against bacteria and fungi.

Sample		Inhibition zone diameter (mm/mg Sample)			
		Bacterial species		Fungi	
		***Bacillus subtilis* (G^+)**	***Escherichia Coli* (G^-)**	***Aspergillus flavus* (Fungus)**	***Candida albicans* (Fungus)**
Control: DMSO		0.0	0.0	0.0	0.0
Standard	**Ampicillin: Antibacterial agent**	23	21	----	---
	Amphotericin B: Antifungal agent	----	----	22	25
2		13	12	11	15
3		10	9	10	13
4		6	4	----	3
5a		13	14	10	14
5b		12	10	9	11
5c		12	13	11	12
6		----	----	----	----
7		15	11	9	11
8a		13	12	9	10
8b		12	11	5	6
9		8	9	----	----

[a] As shown from the above table 4, the newly prepared compounds **2-9** were *in vitro* screened for their biological activities against bacteria (Gram positive, Bacillus subtilis - Gram negative, Escherichia Coli) and fungi (Aspergillus flavus - Candida albicans) in comparison with those of Ampicillin: antibacterial and Amphotericin B: antifungal. Remarkably, the synthesized compounds **2-9** achieved a moderate antimicrobial and antifungal activity; the inhibition zones were expressed in numbers. The effect of the newly prepared compounds **2-9** on the above mentioned microorganisms was also illustrated in the figure 7 as shown below.

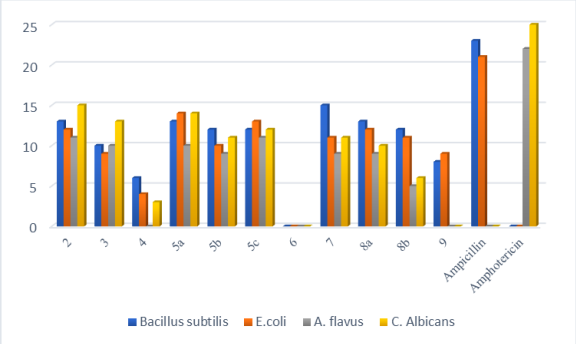


Figure 7: The effect of the compounds 2-9 against bacteria and fungi.

6- Conclusion

In brief we have synthesized some pyrazole derivatives incorporated to indolyldihydro-pyrimidine moiety in their constructions. The newly obtained compounds were characterized by different means of analyses. In addition, the compounds were in vivo and in vitro screened for their anti-inflammatory and microbiological activities and they gave satisfactory results.

7- Conflicts of interest

The authors would like to declare that, they have no conflicts of interest in publication of this paper in Egyptian Journal of Chemistry

8. Acknowledgments

The authors would like to thank the members of Micro analytical center and Microbiology lab. at Cairo University

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