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Conventional and microwave-assisted synthesis, anticancer and antimicrobial evaluation of some new pyrazolone, pyrazole and pyrimidine derivatives

Kurls E. Anwer [1,*] and Galal H. Sayed[1]

[1] Heterocyclic Synthesis Lab., Department of Chemistry, Faculty of Science, Ain Shams University, Abbassia, 11566, Cairo, Egypt

*E-mail: kurlsekram@sci.asu.edu.eg

Abstract:

Under conventional and microwave methods, reactions of ethyl 2-((4-nitrophenyl)diazenyl)-3-oxobutanoate **2** with nicotinohydrazide and thiosemicarbazide furnished the pyrazolone **5** and pyrimidine **7** derivatives, respectively. Treatment of compound **7** with acetic anhydride, phenyl isothiocyanate, ethyl bromoacetate, and phenacyl bromide produced the corresponding pyrimidine derivatives **8–11**. Similarly, the reaction of 2-((4-nitrophenyl)diazenyl)malononitrile **3** with nicotinohydrazide and thiosemicarbazide gave the corresponding pyrazole **12** and pyrimidine **14** derivatives, respectively. Also, the addition reaction of ethyl 2-cyano-2-((4-nitrophenyl)diazenyl)acetate **4** with 2-aminobenzohydrazide and nicotinohydrazide give the corresponding pyrazolone derivatives **15** and **16**, respectively. These entire novel scaffolds have been proved using elemental analysis, spectral data including IR, ^{1}H-NMR in addition to mass spectra. These new scaffolds were screened for in vitro anticancer and antimicrobial activities. Most analogs revealed excellent to good results.

Keywords:

Pyrazole, pyrazolone, pyrimidine, microwave, anticancer and antimicrobial.

1. Introduction:

A large number of pyrimidine derivatives are reported to exhibit antimicrobial [1], antifolates [2], anticancer [3], antiviral [4], antagonist [5], antitumor [6], anti-inflammatory [7], analgesic [8], antiproliferative [9], antihistaminic [10], antiparasitic [11] and antimicrotubule [12] activities. On the other hand, pyrazole compounds are widely used in the petrochemical industry [13, 14], catalytic [15] and polymer [16] manufacturing. Pyrimidine and pyrazole derivatives have partially harmful effects on the environment and humans and there are requirements for converting them into safe and useful products [17, 18].

During the last decades, derivatives of pyrazole showed wide-range of biological and pharmacological activities such as anti-inflammatory, antagonist, analgesic, anthelmintic, anticancer, herbicidal, acaricidal, antiviral, antimitotic, antioxidant, insecticidal, and antimicrobial activities [19-29]. Furthermore, synthetic heterocyclic compounds containing nitrogen atoms, have proven to have significant and diverse therapeutic potential activity. Preparation of heterocyclic compounds by using microwave technique was previously reported, where microwave irradiation is considered one of the green chemistry techniques due to improving the yield, friendly environment, and reducing the reaction times [30-32]. Based on what has been paved, and according to our continuation efforts in synthesized novel heterocyclic derivatives are stemmed from their previous favorable applications in biology and industry [33-48].

Ethyl 2-((4-nitrophenyl)diazenyl)-3-oxobutanoate **2**, 2-((4-nitrophenyl)diazenyl)malononitrile **3** and ethyl 2-cyano-2-((4-nitrophenyl)diazenyl)acetate **4** were prepared according to the previously reported methods [49-51] and then used as key starting materials to synthesize the target compounds via one-pot multicomponent reaction using microwave technique, also investigate its behavior and reactivity toward some nucleophilic and electrophilic reagents. On the other hand, the reactions were pressed using the thermal method according to the literature method [52, 53].

Comparison between the percentage yields and consumed times, which resulted from the two techniques were performed. The yield economy "YE", atomic economy "AE", reaction mass efficiency "RME" and optimum efficiency "OE" were used for comparison between the consumed times and % of the yields resulting from the two techniques. The various synthesized compounds were illustrated by using different spectroscopic and analytical tools. The new compounds were screened in vitro anticancer against two cancer cell lines, namely, *hepatocellular carcinoma* (HePG-2), and *colorectal carcinoma* (HCT-116). The newly synthesized compounds were tested as an antibacterial against two gram-positive (*S. aureus, B. subtilis*) and two gram-negative bacteria, (*E. coli, P. aeuroginosa*). The new compounds were also tested against two fungi (*C. albicans, A. flavus*).

2. Experimental:

2.1. Synthesis:

All solvents, reagents, and chemicals were bought from Sigma Aldrich. The used solvents were purified according to the standard methods. TLC was carried out on the plates of silica gel (Merck Kiesel gel $60F_{254}$, BDH) to monitor the progress of all synthesized compounds homogeneity and reactions. Microwave reactions were carried out with microwave reactor Anton Paar (mono wave 300) using 10 mL borosilicate glass vials. All melting points were measured on a digital Stuart electric melting point apparatus "SMP3" and were uncorrected. Infrared spectra measurements (cm^{-1}) were determined using KBr disks on a PerkinElmer 293 spectrophotometer. The ^{1}H-NMR spectra were measured on a Varian Mercury 300 MHz spectrometer. All synthesized compounds were dissolved in DMSO–d_6 as a solvent using tetramethyl silane as an internal standard. A GC-2010 Shimadzu Gas chromatography mass spectrometer (EI, 70 eV) was used for Mass spectrometry measurements. A Perkin-Elmer CHN-2400 analyzer was used for elemental microanalyses (CHN), the data were found to be in good agreement within ±0.4% of the theoretical values. The biological evaluations were carried out at the Department of Pharmacology, Faculty of Pharmacy, Mansoura University, Egypt.

2.1.1. 5-Methyl-2-nicotinoyl-4-((4-nitrophenyl)diazenyl) -2,4-dihydro-3H-pyrazol-3-one (5)

Conventional method. A mixture of compound **2** (2.79 g, 0.01 mol.), nicotinohydrazide (1.37 g, 0.01 mol.) in butanol (20 mL) was refluxed for 8h. The solid separated during reflux was filtered off and crystallized from ethanol to give yellow crystals **5**.

Microwave method. An equimolar amount of compound **2** (2.79 g, 0.01 mol.), nicotinohydrazide (1.37 g, 0.01 mol.) in butanol (2 mL) was irradiated for 3 min. m.p. 267-268 ^{O}C. IR (KBr) ν cm^{-1}: 1674, 1660 (C=O), 1610 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 2.25 (s, 3H, CH_3), 6.63 (s, 1H, N-CH) and 9.58-7.42 (m, 8H, Ar-H). MS: *m/z* 352 [M^+] (23.90%). Anal. Calcd. for $C_{16}H_{12}N_6O_4$ (352): C, 54.55; H, 3.43; N, 23.85. Found: C, 54.62; H, 3.51; N, 23.63.

2.1.2. 3-Amino-6-methyl-5-((4-nitrophenyl)diazinyl)-2 thioxo-2,5-dihydropyrimidin-4(3H)-one (7)

Conventional method. A mixture of compound **2** (2.79 g, 0.01 mol.), thiosemicarbazide (0.91g, 0.01 mol.) in absolute ethanol (20 mL) was refluxed for 6 h. The solid separated during reflux was filtered off and crystallized from ethanol to afford orange crystals **7**.

Microwave method. An equimolar amount of compound **2** (2.79 g, 0.01 mol.), thiosemicarbazide (0.91g, 0.01 mol.) in DMF (2 mL) was irradiated for 2 min. m.p. 222-224 ^{O}C. IR (KBr) ν cm^{-1}: 3385, 3289 (NH_2), 1693 (C=O), 1596 (C=N), 1482 (C=S). ^{1}H-NMR (DMSO-d_6) δ: 2.25 (s, 3H, CH_3), 7.56-7.25 (5m, 5H, Ar-H and N-CH), 9.84 (s, 2H, NH_2, D_2O exchangeable). MS: *m/z* 306 [M^+] (25.94%). Anal. Calcd. for $C_{11}H_{10}N_6O_3S$ (306): C, 43.13; H, 3.29; N, 27.44; S, 10.47. Found: C, 42.95; H, 3.14; N, 27.55; S, 10.64.

2.1.3. N-(4-Methyl-5-((4-nitrophenyl)diazenyl)-6-oxo-2-thioxo-5,6-dihydropyrimidin-1(2H)-yl)acetamide (8)

Conventional method. A solution of compound **7** (3.06 g, 0.01 mol.) in mixture of acetic anhydride (20 mL) and acetic acid (10 mL) was refluxed for 2h. The reaction mixture after cooling, was poured onto ice water (250 mL), the solid that separated out was filtered off, washed with water and recrystallized from ethanol to produce brown crystals **8**.

Microwave method. An equimolar amount of compound **7** (3.06 g, 0.01 mol.) sin mixture of acetic anhydride (3 mL) and acetic acid (1.5 mL) was irradiated for 2 min. m.p. 248-250 ^{O}C. IR (KBr) ν cm^{-1}: 3278 (NH), 1687 (C=O), 1597 (C=N), 1481 (C=S). ^{1}H-NMR (DMSO-d_6) δ: 2.26 (s, 3H, $COCH_3$), 2.46 (s, 3H, CH_3), 6.48 (s, 1H, NH, D_2O exchangeable) and 7.30 (s, 1H, N-CH) and 8.56-8.16 (m, 4H, Ar-H). MS: *m/z* 348 [M^+] (27.21%). Anal. Calcd. for $C_{13}H_{12}N_6O_4S$ (348): C, 44.83; H, 3.47; N, 24.13; S, 9.21. Found: C, 44.69; H, 3.31; N, 24.30; S, 9.32.

2.1.4. 1-(4-Methyl-5-((4-nitrophenyl)diazenyl)-6-oxo-2-thioxo-5,6-dihydropyrimidin-1(2H)-yl)-3-phenyl thiourea (9)

Conventional method. A mixture of compound **7** (3.06 g, 0.01 mol.), phenyl isothiocyanate (1.35 mL, 0.01 mol.) in absolute ethanol (20 mL) was refluxed for 8h. After cooling, the solid formed was filtered off and recrystallized from ethanol to obtain brown crystals **9**.

Microwave method. An equimolar amount of compound **7** (3.06 g, 0.01 mol.), phenyl isothiocyanate (1.35 mL, 0.01 mol.) in DMF (2 mL) was irradiated for 1 min. m.p. 248-250 ^{O}C. IR (KBr) ν cm^{-1}: 3288, 3204 (NH_2), 1693 (C=O), 1596 (C=N), 1482 (C=S). ^{1}H-NMR (DMSO-d_6) δ: 2.53 (s, 3H, CH_3), 6.50 (s, 2H, 2NH, D_2O exchangeable),7.32 (s, 1H, N-CH) and 8.55-8.18 (m, 9H, Ar-H). MS: *m/z* 441 [M^+] (30.15%). Anal. Calcd. for $C_{18}H_{15}N_7O_3S_2$ (441): C, 48.97; H, 3.42; N, 22.21; S, 14.53. Found: C, 49.13; H, 3.36; N, 22.40; S, 14.63.

2.1.5. Ethyl-(4-methyl-5-((4-nitrophenyl)diazenyl)-6-oxo-2-thioxo-5,6-dihydropyrimidin-1(2H)-yl)glycinate (10)

Conventional method. A mixture of compound **7** (3.06 g, 0.01 mol.), anhydrous potassium carbonate (1.38 g, 0.01 mol.), ethyl bromoacetate (1.67ml, 0.01 mol.) in dry acetone (50 mL) was refluxed for 24 h. After filtration while hot and evaporation of the solvent the solid formed was recrystallized from ethanol to give black crystals **10**.

Microwave method. An equimolar amount of compound **7** (3.06 g, 0.01 mol.), anhydrous potassium carbonate (1.38 g, 0.01 mol.), ethyl bromoacetate (1.67ml, 0.01 mol.) in DMF (2 mL) was irradiated for 3 min. m.p. > 300 ^{O}C. IR (KBr) ν cm^{-1}: 3181 (NH), 1705, 1651 (C=O), 1585 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 1.25 (t, 3H, $CH_2\underline{CH_3}$), 2.53 (s, 3H, CH_3), 3.92 (s, 2H, $NH\underline{CH_2}$), 4.18 (q, 2H, $\underline{CH_2}CH_3$), 7.30 (s, 1H, N-CH),

8.56-8.16 (m, 4H, Ar-H) and 10.12 (s, 1H, NH, D_2O exchangeable). MS: *m/z* 392 [M^+] (15.26%). Anal. Calcd. for $C_{15}H_{16}N_6O_5S$ (392): C, 45.91; H, 4.11; N, 21.42; S, 8.17. Found: C, 45.78; H, 3.96; N, 21.30; S, 8.35.

2.1.6.6-Methyl-5-((4-nitrophenyl)diazenyl)-3-((2-oxo-2-phenylethyl)amino)-2-thioxo-2,5-dihydropyrimidin-4(3H)-one (11)

Conventional method. A mixture of compound **7** (3.06 g, 0.01 mol.), phenacyl bromide (1.99 g, 0.01 mol.) in ethanol (50 mL) was refluxed for 6 h. After cooling, the solid formed was filtered off and recrystallized from methanol to afford green crystals **11**.

Microwave method. An equimolar amount of compound **7** (3.06 g, 0.01 mol.), phenacyl bromide (1.99 g, 0.01 mol.) in DMF (2 mL) was irradiated for 3 min. m.p. 256-258 ^{O}C. IR (KBr) ν cm^{-1}: 3289 (NH), 1692, 1671 (C=O), 1610 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 2.51(s, 3H, CH_3), 4.34 (s, 2H, NH$\underline{CH_2}$), 7.31 (s, 1H, N-CH), 8.54-8.18 (m, 9H, Ar-H) and 9.94 (s, 1H, NH, D_2O exchangeable). MS: *m/z* 424 [M^+] (35.24%). Anal. Calcd. for $C_{19}H_{16}N_6O_4S$ (424): C, 53.77; H, 3.80; N, 19.80; S, 7.56. Found: C, 53.59; H, 3.71; N, 19.72; S, 7.71.

2.1.7.(3,5-Diamino-4-((4-nitrophenyl)diazenyl)-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (12)

Conventional method. A mixture of compound **3** (2.15 g, 0.01 mol.), nicotinohydrazide (1.37 g, 0.01 mol.) in butanol (20 mL) was refluxed for 10h. The solid separated during reflux was filtered off and crystallized from ethanol to produce orange crystals **12**.

Microwave method. An equimolar amount of compound **3** (2.15 g, 0.01 mol.), nicotinohydrazide (1.37 g, 0.01 mol.) in butanol (2 mL) was irradiated for 2 min. m.p. 244-246 ^{O}C. IR (KBr) ν cm^{-1}: 3336, 3298, 3261 (NH_2), 1695 (C=O), 1625 (C=N). ^{1}H-NMR (DMSO-d_6) δ: 7.60 (s, 4H, $2NH_2$, D_2O exchangeable) and 8.23-9.12 (m, 8H, Ar-H). MS: *m/z* 352 [M^+] (19.15%). Anal. Calcd. for $C_{15}H_{12}N_8O_3$ (352): C, 51.14; H, 3.43; N, 21.81. Found: C, 52.10; H, 3.50; N, 31.90.

2.1.8. 1,4,6-Triamino-5-((4-nitrophenyl)diazenyl) pyrimidine-2(1H)-thione (14)

Conventional method. A mixture of compound **3** (2.15 g, 0.01 mol.), thiosemicarbazide (0.91g, 0.01 mol.) in butanol (20 mL) was refluxed for 10h. The solid separated during reflux was filtered off and crystallized from ethanol to obtain brown crystals **14**.

Microwave method. An equimolar amount of compound **3**(2.15 g, 0.01 mol.), thiosemicarbazide (0.91g, 0.01 mol.) in butanol (2 mL) was irradiated for 2 min. m.p. 196-198 ^{O}C. IR (KBr) ν cm^{-1}: 3311, 3275 (NH_2), 1610 (C=N), 1452 (C=S). ^{1}H-NMR (DMSO-d_6) δ: 7.83-8.76 (m, 4H, Ar-H) and 10.03 (s, 6H, $3NH_2$, D_2O exchangeable). MS: *m/z* 306 [M^+] (23.21%). Anal. Calcd. for $C_{10}H_{10}N_8O_2S$ (306): C, 39.21; H, 3.29; N, 36.58; S,10.47. Found: C, 39.39; H, 3.18; N, 36.55; 10.22.

2.1.9. General procedure for synthesis of compounds (15 and 16)

Conventional method. 2-Aminobenzohydrazide (1.51 g, 0.01 mol.) and/or nicotinohydrazide (1.37 g, 0.01 mol.) was added to a solution of compound **4** (2.62 g, 0.01 mol.) in butanol (50 mL) and the resulting mixture was refluxed for 8h. The solid that separated during reflux was filtered off while hot and recrystallized from ethanol to give compounds **15** and **16**.

Microwave method. 2-Aminobenzohydrazide (1.51 g, 0.01 mol.) and/or nicotinohydrazide (1.37 g, 0.01 mol.) was added to a solution of compound **4** (2.62 g, 0.01 mol.) in DMF (2 mL) and the resulting mixture irradiated for 2min.

2.1.9.1. 5-Amino-1-(3-aminobenzoyl)-4-((4-nitrophenyl)diazenyl)-1,2-dihydro-3H-pyrazol-3-one (15)

m.p. > 300 ^{O}C. IR (KBr) ν cm^{-1}: 3402, 3365, 3312, 3286 (NH_2), 3190 (NH), 1680, 1652 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 7.39-8.64 (m, 8H, Ar-H), 9.92 (s, 4H, $2NH_2$, D_2O exchangeable) and 11.63 (s, 1H, NH, D_2O exchangeable). MS: *m/z* 367 [M^+] (18.21%). Anal. Calcd. for $C_{16}H_{13}N_7O_4$ (367): C, 52.32; H, 3.57; N, 26.69. Found: C, 52.47; H, 3.44; N, 26.51.

2.1.9.2. 5-Amino-1-nicotinoyl-4-((4-nitrophenyl)diazenyl)-1,2-dihydro-3H-pyrazol-3-one (16)

m.p. 292-294 ^{O}C. IR (KBr) ν cm^{-1}: 3391, 3352, 3342, 3289 (NH_2), 3213 (NH), 1678, 1650 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 7.37-8.78 (m, 8H, Ar-H), 9.87 (s, 2H, NH_2, D_2O exchangeable) and 11.43 (s, 1H, NH, D_2O exchangeable). MS: *m/z* 353 [M^+] (29.07%). Anal. Calcd. for $C_{15}H_{11}N_7O_4$ (353): C, 51.00; H, 3.14; N, 27.75. Found: C, 50.92; H, 3.19; N, 27.70.

2.2. Comparison between microwave and conventional methods.

The comparison in terms of yields and times between the prepared compounds by using microwave and conventional techniques were reported. However, we used the yield economy (YE) as a term to determine the microwave and conventional synthetic different efficiencies of the same reaction. Calculation of YE was occurred through: $E = \frac{yield\%}{Reaction\ time\ "min"}$. In this report, the YE was used to provide the yields obtained conclusively enhanced under the microwave and conventional conditions. The equation of RME is: $\mathrm{RME} = \frac{Wt\ of\ isolated\ product}{Wt\ of\ reactants}$. While, OE was used for the direct comparison between the three reaction types and can be calculated through $OE = \frac{RME}{AE}\ x\ 100$. So we can consider the yield economy (YE) as a metric to enhancing the conversion efficiencies of these three different synthetic methods of the same reaction. The reaction theoretical maximum efficiency were represented by using AE, while, RME gives the observed mass efficiency. The conventional and microwave reactions atomic economy (AE) have the same values due to using two different reaction conditions to obtain the same desired compounds, as shown in (Table 1).

Table 1. comparison in terms of physical data between the desired compounds (5-16) under microwave, and conventional techniques

Cpd. no.	Time "min"		Yield %		YE		OE		RME		AE
	M.W.	Th.	M.W.	Th.	M.W.	Th.	M.W.	Th.	M.W.	Th.	
5	3	480	91	50	30.33	0.1042	65.37	35.92	77.25	42.45	84.62
7	2	360	90	50	45	0.1389	66.20	36.78	80.04	44.47	82.70
8	2	120	93	65	46.50	0.5417	71.44	49.93	83.76	58.54	85.29
9	1	480	90	75	90	0.1563	81.50	67.92	81.50	67.92	100
10	3	1440	92	60	30.67	0.4167	53.91	35.16	84.02	54.80	64.16
11	3	360	93	75	31	0.2083	71.56	57.71	85.23	68.74	83.96
12	2	600	92	75	37.5	0.0833	76.02	61.97	76.02	61.97	100
14	2	600	91	65	45.5	0.1083	73.28	52.34	73.28	52.34	100
15	2	480	90	60	45	0.1250	67.82	45.22	76.32	50.89	88.86
16	2	480	90	62	45	0.1292	67.17	46.27	75.92	52.30	88.47

2.3. In vitro anticancer evaluation:

Materials and methods

Cell line

Hepatocellular carcinoma (HePG-2), and colorectal carcinoma (HCT-116). The cell lines were obtained from ATCC via a Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Chemical reagents

The reagents RPMI-1640 medium, MTT and DMSO (sigma co., St. Louis, USA), and Fetal Bovine serum (GIBCO, UK). 5-Fluorouracil was used as a standard anticancer drug for comparison.

MTT assay (1)

The different cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100µg/mL streptomycin at 37^{O}C in a 5% CO_2 incubator. The cells were seeded in a 96-well plate at a density of 1.0x10^{4} cells/well at 37^{O}C for 48 h under 5% CO_2. After incubation, the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in the volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

Table 2. Anticancer activity as mean zones of inhibition (mm) against some microorganisms:

Comp.	In vitro Cytotoxicity IC50 (µM)•	
	HePG2	HCT-116
5-Fu	7.86±0.5	5.35±0.3
5	8.4± 5.3	12.8 ± 3.9
7	16.5 ± 2.8	14.2 ± 2.4
8	65.7 ± 2.7	32.6 ± 2.1
9	9.4 ± 7.8	6.8 ± 4.8
10	24.8 ± 2.1	32.4 ± 2.3
11	10.1 ± 2.1	15.2 ± 2.3
12	88.4 ± 4.2	68.0 ± 2.7
14	11.1± 5.1	19.4 ± 3.9
15	16.5 ± 2.7	17.4 ± 2.4
16	9.1 ± 2.8	8.8 ± 1.9

• IC50 (µM): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic)
•• 5-Fu: 5-Fluorouracil

The results in Table **2** revealed that compounds **5**, **9**, and **16** showed a very strong cytotoxic activity against HePG2, while compounds **9** and **16** showed a very strong cytotoxic activity and compounds **5**, **7**, **11**, **14** and **15** showed a strong cytotoxic activity against HCT-116.

2.4. Antimicrobial activities:

The antimicrobial activities of the synthesized compounds were tested against a panel of two gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis), and two gram-negative bacteria (Escherichia coli, Pseudomonas aeuroginosa). The anti-fungal activity of the compounds was tested against two fungi (Candida albicans, Aspergillus flavus). Each of the compounds was dissolved in DMSO and a solution of the concentration 1 mg /ml were prepared separately paper discs of Whatman filter paper were prepared with standard size (5cm) and were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were placed aseptically in the petri dishes containing nutrient agar media (agar 20g + beef extract 3g + peptone 5g) seeded with *S. aureus, B. subtilis, E. coli, P. aeuroginosa, C. albicans and A. flavus*. The petri dishes were incubated at 36 ^{O}C and the inhibition zones were recorded after 24h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic Ampicillin and antifungal Colitrimazole was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the following formula:

$$\%\ \boldsymbol{Activity\ Index} = \frac{\boldsymbol{Zone\ of\ inhibition\ by\ test\ compound\ (diametre)}}{\boldsymbol{Zone\ of\ inhibition\ by\ standard\ (diametre)}} \times 100$$

At the end of the incubation period, in terms of % Activity index was recorded (Table 3) as the lowest concentration of the substance that had no visible turbidity. Control experiments with DMSO and uninoculated media were run parallel to the test compounds under the same conditions.

The results demonstrate that tested fungi were more sensitive to all compounds compared with bacteria. The most active compounds against fungi were **5, 9, 14, 15** and **16**, while the most active compounds were **5, 15** and **16** for Gram-negative and **5, 9, 15** and **16** for Gram-positive bacteria. In addition, Gram-negative bacteria were more sensitive to the compounds compared with Gram-positive ones (Figures 1 and 2).

Table 3. Show the antimicrobial activities in terms of % Activity index for the desired derivatives.

Cpds. No.	E. Coli		P. Aeuroginosa		S. Aureus		B. Subtilis		C. Albicans		A. Flavus	
	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index
5	20	80	22	95.7	19	79.2	23	100	21	77. 8	24	96
7	11	44	12	52.2	16	66.77	14	60.9	15	556	22	88
8	NA	---	NA	---	NA	---	3	13.0	NA	---	4	16
9	16	64	22	95.7	12	50	19	82.6	25	92.6	24	96
10	7	28	9	39.1	9	37.5	6	261	3	11.1	10	40
11	13	52	12	52.2	11	45.8	14	60.9	6	22.2	13	52
12	NA	---	5	21.7	6	25	3	13.0	NA	---	NA	---
14	15	60	18	78.3	13	54.2	20	86.9	15	55.6	22	88
15	14	56	16	69.6	18	75	16	696	18	66.7	23	92
16	16	64	22	95.7	14	58.3	19	82.6	12	44.4	20	80
Ampicillin	**25**	**100**	**23**	**100**	**24**	**100**	**23**	**100**	**NA**	**----**	**NA**	**----**
Colitrimazole	**NA**	**----**	**NA**	**----**	**NA**	**----**	**NA**	**----**	**27**	**100**	**25**	**100**

NA → No Activity.

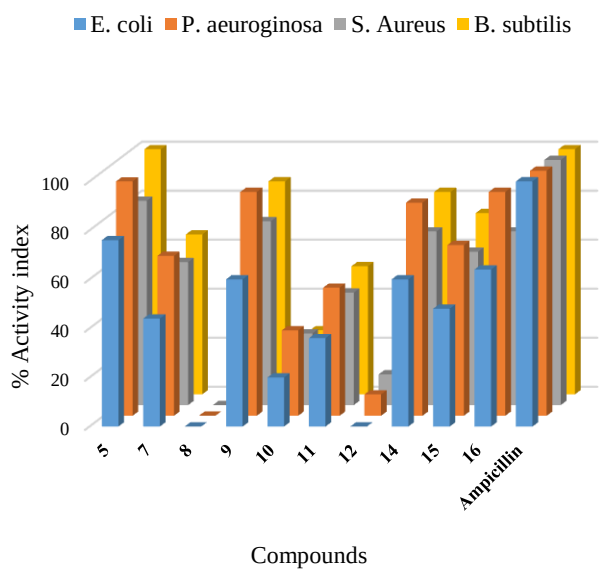


Figure 1. % Activity index of most potent compounds against bacteria.

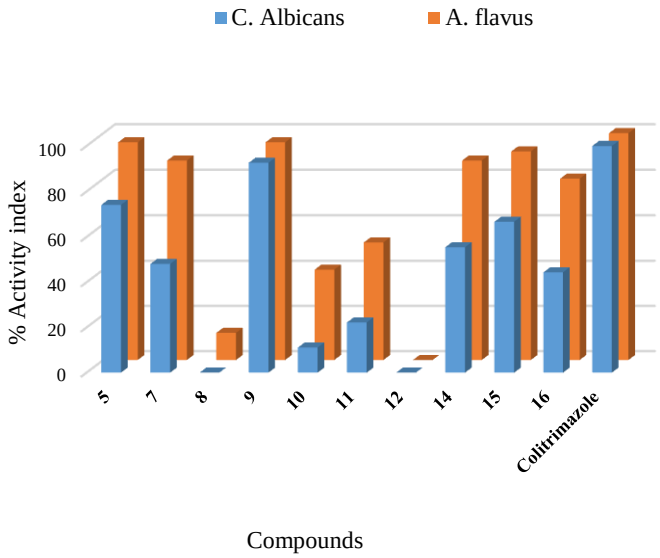
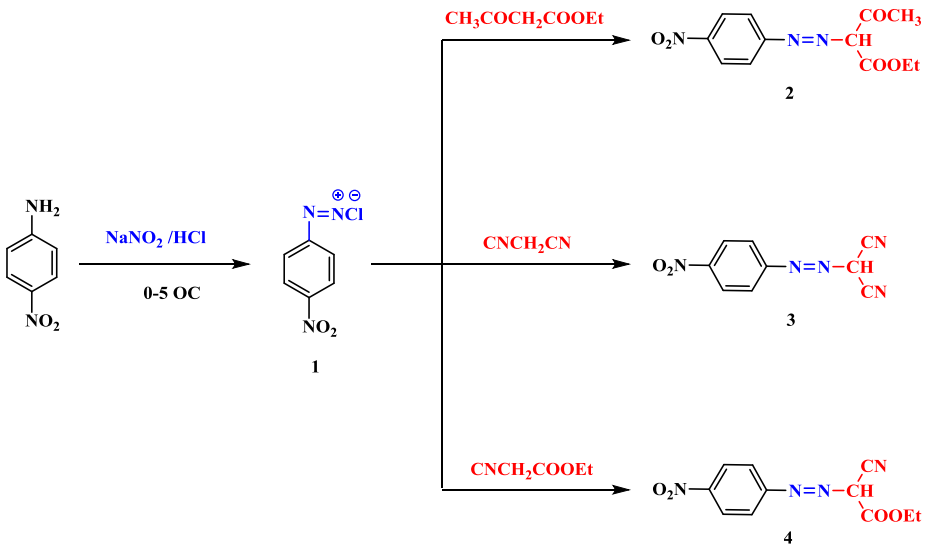


Figure 2. % Activity index of most potent compounds against fungal.

3. Results and discussion:

The new pyrazole and pyrimidine derivatives were synthesized following the reactions depicted in Schemes 1-4. The starting materials ethyl 2-((4-nitrophenyl)diazenyl)-3-oxobutanoate **2**, 2-((4-nitrophenyl)diazenyl)malononitrile **3** and ethyl 2-cyano-2-((4-nitrophenyl)diazenyl)acetate, respectively. The primary amino group of p-nitroaniline is capable of forming the corresponding diazonium salt **1** upon treatment with nitrous acid at 0-5^{O}C and the subsequent azo coupling with ethyl acetoacetate, malononitrile and/or ethyl cyanoacetate as a carbon nitrile afforded the starting material compound **2, 3** and **4**, respectively. (Scheme 1).



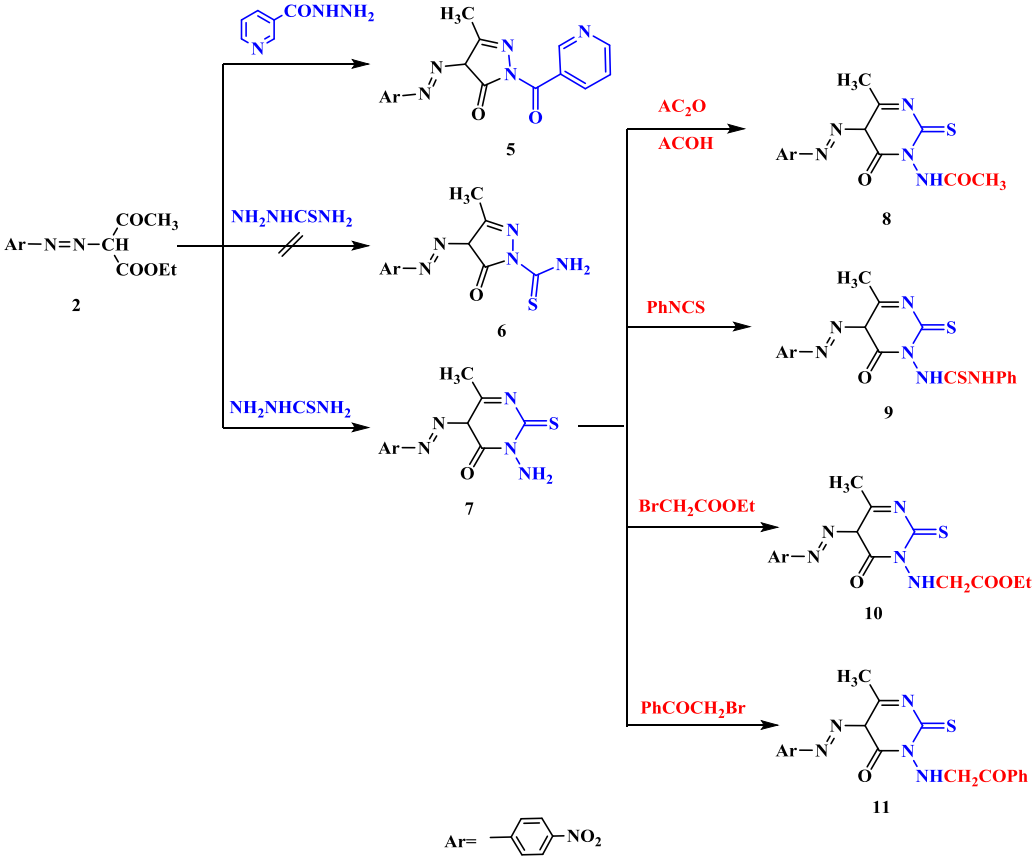
Scheme 1. Synthesis of diazenyl derivatives 2-4.

The reaction of compound **2** with nicotinohydrazide in butanol afforded pyrazolone derivative **5**, through the nucleophilic attack of the NH_2 group on the positively polarized carbonyl of the acetyl group then elimination of one molecule of water, followed by ring cyclization through the nucleophilic attack of NH on the polarized carbonyl group of the ester group, then elimination of one molecule of ethanol. The structure of compound **5** was confirmed from its IR spectrum displayed bands of 2C=O at ν 1674 and 1660 cm^{-1}. Its mass spectrum showed a molecular ion peak at m/z=352 (23.90%) corresponds to the molecular formula $C_{16}H_{12}N_6O_4$.

According to the previous method [54], we tried to synthesize the pyrazole derivative **6** via the reaction of diazenyl derivative **2** with

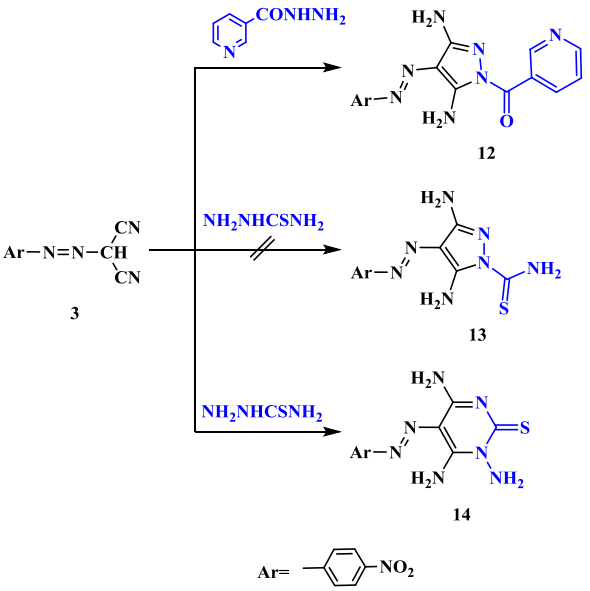
thiosemicarbazide. However, the pyrimidine derivative **7** was found to be the isolated product. The structure of compound **7** was confirmed from its IR spectrum which displayed bands of NH_2 and C=O at ν 3385, 3289, and 1693 cm^{-1}, respectively. Its mass spectrum showed a molecular ion peak at m/z=306 (25.94%) which corresponds to the molecular formula $C_{11}H_{10}N_6O_3S$.

However, compound **7** was used as a key intermediate for the preparation of new interesting heterocyclic compounds via its reactions with different electrophiles such as acetic anhydride, phenyl isothiocyanate, ethyl bromoacetate and phenacyl bromide to afford the corresponding dihydropyrimidine derivatives **8–11**. Compound **8** was formed through a nucleophilic attack of NH_2 on C=O of AC_2O followed by the elimination of one molecule of acetic acid. The structure of compound **8** was confirmed from its IR spectrum which displayed bands at 3278 (NH), 1687 (C=O), 1597 (C=N), 1481 cm^{-1} (C=S), respectively. Its mass spectrum showed a molecular ion peak at m/z=348 (27.21%) which corresponds to the molecular formula $C_{13}H_{13}N_6O_4S$. Also, the structure of compound **9** was confirmed from its mass spectrum which showed a molecular ion peak at m/z=441 (30.15%) corresponding to the molecular formula $C_{18}H_{15}N_7O_3S_2$. On the other hand, compound **10** was formed through the elimination of one molecule of HBr, the structure of compound **10** was confirmed from its ^{1}H-NMR which showed peaks of CH_2 protons at δ 3.92 ppm. Similarly, compound **11** formed via the elimination of one molecule of HBr. The structure of compound **10** was confirmed from its ^{1}H-NMR which showed the peak of the CH_2 protons at δ 4.34 ppm. (Scheme 2).



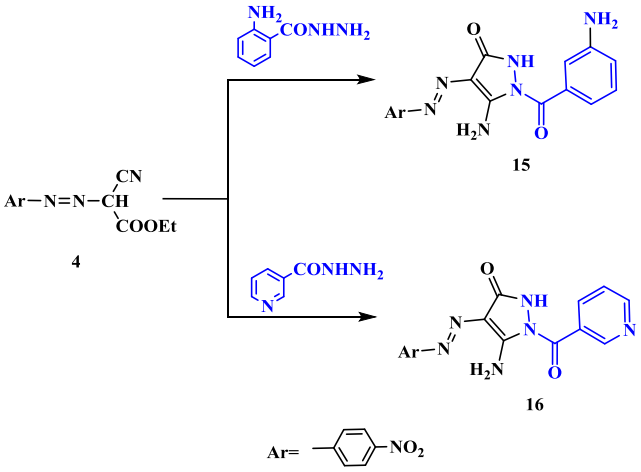
Scheme 2. Synthesis of pyrazolone and pyrimidine derivatives 5, 7-11.

While the reaction of compound **3** with nicotinohydrazide in butanol afforded pyrazole derivative **12**. The structure of compound **12** was confirmed from its IR spectrum which displayed bands of NH_2 at ν 3336, 3298, 3261 cm^{-1} and C=O at ν 1695 cm^{-1}. Its mass spectrum showed a molecular ion peak at m/z=352 (19.15%) which corresponds to the molecular formula $C_{15}H_{12}N_8O_3$. Furthermore, we tried to synthesize the pyrazole derivative **13** via the reaction of diazenyl derivative **3** with thiosemicarbazide. However, the pyrimidine derivative **14** was found to be the isolated derivative. The structure of the obtained product was confirmed from its spectroscopic data and elemental analysis (Scheme 3).



Scheme 3. Synthesis of pyrazole and pyrimidine derivatives 12 and 14.

Similarly, compound **4** when allowed to react with 2-aminobenzohydrazide and/or nicotinohydrazide gave the corresponding pyrazolone derivatives **15** and **16**, respectively. The structure of compound **15** was illustrated from its IR spectrum which displayed bands at ν 3402, 3365, 3312, 3286 (NH_2), 3190 (NH), 1680, 1652 (C=O). Its ^{1}H-NMR spectrum showed peaks at δ 9.92 and 11.63 ppm corresponding to $2NH_2$ and NH. Its mass spectrum showed a molecular ion peak at m/z=367 (18.21%) which corresponding to molecular formula $C_{16}H_{13}N_7O_4$. Also, the structure of compound **16** was illustrated from its IR spectrum which displayed bands at ν 3391, 3352, 3342, 3289 (NH_2), 3213 (NH), 1678, 1650 (C=O). Its ^{1}H-NMR spectrum showed peaks at δ 9.87 and 11.43 ppm corresponding to NH_2 and NH. Its mass spectrum showed a molecular ion peak at m/z=353 (29.07%) which corresponding to molecular formula $C_{15}H_{11}N_7O_4$.



Scheme 4. Synthesis of pyrazolone derivatives 15 and 16.

4. Conclusion:

Ethyl 2-((4-nitrophenyl)diazenyl)-3-oxobutanoate 2, 2-((4-nitrophenyl)diazenyl)malononitrile 3 and ethyl 2-cyano-2-((4-nitrophenyl)diazenyl)acetate 4 were used as starting materials for preparation of some novel pyrazolone, pyrazole and pyrimidine derivatives. The new compound structures were established from spectroscopic data. All the newly synthesized compounds were prepared by conventional method and under microwave irradiation. The newly synthesized compounds were tested against two cancer cells, Gram-positive and Gram-negative bacteria, and fungi.

References:

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Human and Animal rights

No animals/humans were used for studies that are the base of this research.

Consent for publication

Not applicable

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