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Synthesis of New Pyrazolones and Fused Pyrazole Derivatives as Antimicrobial Agents

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SOME new pyrazolone and fused pyrazole derivatives start from enaminone-type precursor. This approach constitutes a novel and advantageous alternative for the synthesis of the target heterocycles. Thus, the reaction of pyrazolone 1 and DMFDMA afforded 4-(dimeth-ylamino)methylidenepyrazol-3-one (2). Enaminone 2 was utilized as a key intermediate for the synthesis of new pyrazolones 8, 9, 12, and 14, fused pyrazolones 4-7, and 10 and the spiro adducts 15a, b. The antimicrobial activity of some of the newly synthesized adducts were evaluated against six strains of Gram positive, Gram negative and fungi in comparison with Ampicillin and Nystatine.

Keywords: Pyrazolones, Pyrazoloisoxazole, Pyrazolopyrazole, Pyrazoloquinoline, Pyrazoloenaminone, Antimicrobial activity.

Pyrazole and its derivatives are potential building blocks in synthesis of new drugs[1-3]. They show broad spectrum biological activities such as anti-inflammatory[4-7], antitumor[8-10], and antimicrobial[11,12]. Most recently, pyrazolone derivatives showed high inhibition activity against Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis[13]. Pyrazoles also show potent inhibition against monoamine oxidase-B (MOA-B)[14], human telomerase[15], and dependent kinase (CDK)[16]. A new pyrazolone derivative known as edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) is being tested as a drug in clinical practice for brain ischemia[17,18]. It was also found to be effective against myocardial ischemia[19]. Pyrazolone derivatives such as antipyrine, aminopyrine and dipyrone are known as antipyretic and analgesic substances[20] and their pharmacological mechanism has been widely investigated. Some of the aryl pyrazole derivatives are useful in the treatment of a variety of disorders caused by Human Immunodeficiency Virus (HIV) and other genetic diseases caused by retroviruses such as Acquired Immune Deficiency Syndrome (AIDS)[21]. In the same context, substituted and fused pyrazoles are considered as the structural motif of many commercial drugs such as Zometapine[22], Celebrex[23], Sildenafil[24], and Rimonabant[25]. Pyrazoles also exhibit diverse properties[26] and useful as

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starting materials for the synthesis of other fused heterocycles[27,28]. In addition to their higher biological activities, pyrazoles are also used for the preparation of dyes[29] couplers for photographic materials[30] and as herbicides[31-33].

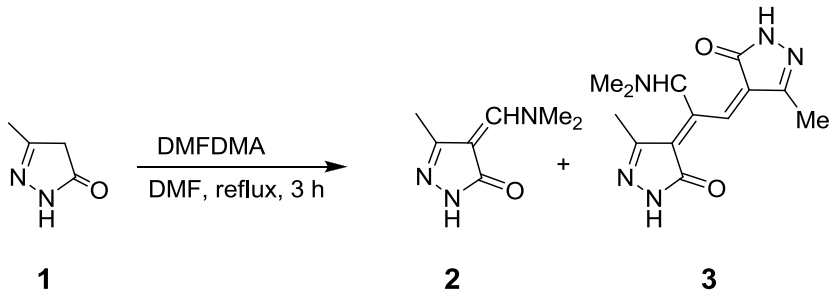
There is continuous medical need for new anti-infective therapies due to the global emergence of resistance to antimicrobial agents which is increasingly limiting the effectiveness of current drugs. This motivated us to synthesize some new pyrazole and fused pyrazole derivatives hoping that they show potential antimicrobial activity. Depending on all the previous information and in continuation to our previous work on synthesizing biologically active nitrogen-containing heterocycles[34,35], we report the use of 4-(dimethylamino) methylidene-pyrazol-3-one as a precursor for the synthesis of the new compounds reported herein.

Results and Discussion

Chemistry

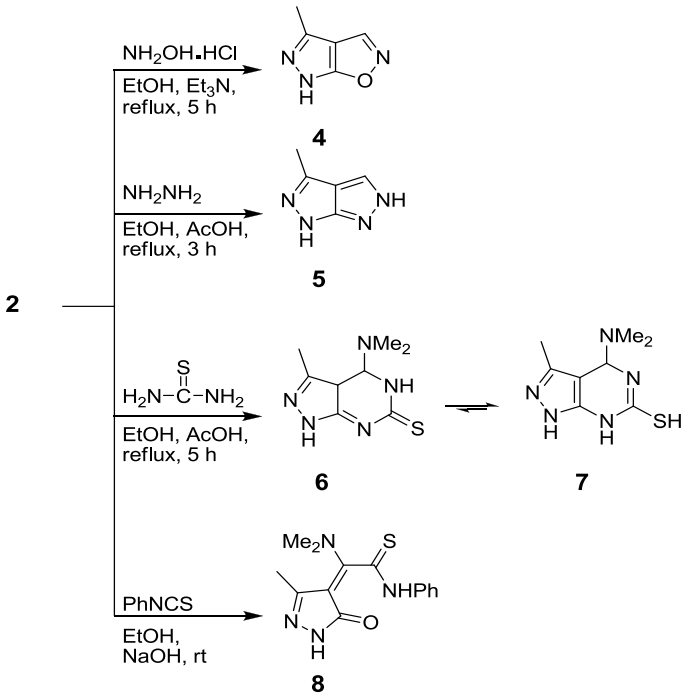
The reaction of active methylene-containing compound with dimethyl-formamide dimethyl acetal (DMFDMA) is the general and the most prevalent method to synthesize enaminones[36]. Thus, 5-methyl-2,4-dihydro-pyrazol-3-one (1)[37] was treated with DMFDMA in boiling DMF to afford a mixture of the pyrazoloenamine 2 in 80% yield in addition to the unexpected pyrazolodimethy-laminoethylidenepyrazol-3-one (3) in 20% yield in a molar ratio 4:1 (Scheme 1). A reasonable explanation of the formation of the adduct 3 could be attributed to the intermolecular interaction between two molecules of 2 followed by the loss of dimethylamine under the reaction conditions.

The structure of the two products 2 and 3 was elucidated on the basis of the analytical and spectral data. The mass spectrum of the products revealed molecular ion peaks at m/z = 153 and 261 (M^+), respectively. The ^{1}H-NMR of compound 3 showed a singlet at δ = 2.21 ppm corresponding to the NMe2 group, two singlets at δ = 2.73 and 2.89 ppm for the two CH_3 groups attached to the pyrazolone ring, singlet at δ = 6.04 ppm for the ethylidene proton and a broad D_2O-exchangeable signal at δ = 12.29 ppm for the two NH groups. The IR and ^{13}C-NMR spectrum were in agreement with both structures.



Scheme 1

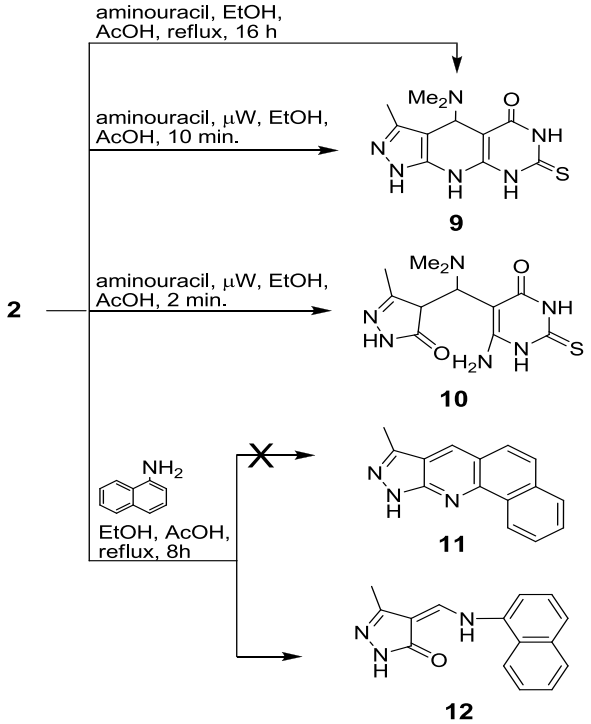
Next, we focused our attention on the synthesis of pyrazolone and fused pyrazole derivatives utilizing pyrazoloenamine 2 as a key precursor. As shown in Scheme 2, compound 2 was treated with hydroxylamine hydrochloride to yield the corresponding pyrazolo[4,3-d]isoxazole 4 in 75% yield. The formation of the isoxazole adducts upon the interaction between enaminone and hydroxylamine hydrochloride has been reported previously[38-40]. The structure of 4 was elucidated by the analytical and spectral data. It is noteworthy to mention here that the synthesis of 4-methyl-6H-pyrazolo[4,3-d]isoxazole (4) was claimed before by the reaction of 5-chloro-3-methyl-1H-pyrazole-4-carbaldehyde with hydroxylamine[41-43]. However, these reports are mystic since neither experimental procedure nor spectroscopic data of the reaction product was provided[44]. Interaction of 2 with hydrazine hydrate in ethanol containing acetic acid afforded pyrazolo[3,4-c]pyrazole 5 in 78% yield. The formation of pyrazole ring on treatment of enaminone with hydrazine hydrate has been investigated previously[38,40,45]. In addition, treatment of 2 with thiourea yielded the pyrazolopyrimidinethiole adducts 7. The 1H-NMR of the latter compound revealed a singlet at δ = 1.90 ppm for the SH proton beside the other characteristic signals of the structure. Compound 8 was formed via the interaction of 2 with phenylisothiocyanate in ethanol containing NaOH. The 1H-NMR spectrum supported the existence of the NMe2 as a singlet at δ = 2.33 ppm beside the other characteristic signals.



Scheme 2

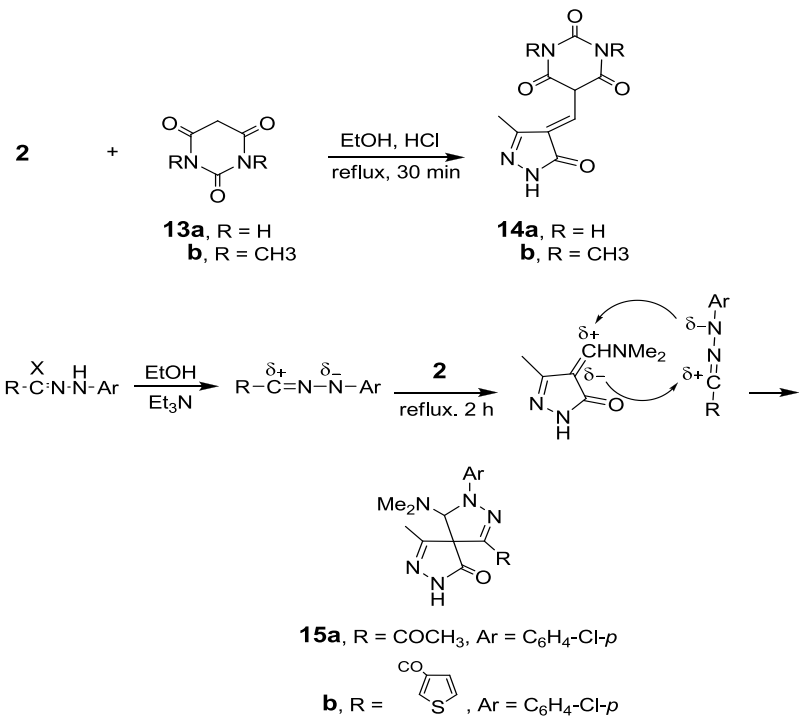
Scheme 3 outlines the reaction of 2 with aminothiouracil and α-naphthylamine. Pyrazoloenamine 2 was reacted with an equivalent amount of aminothiouracil in refluxing ethanol in presence of acetic acid to afford 9, however, the reaction time was long (16 hr) and the yield was poor (13%). To improve the yield and shorten the reaction time, the reaction was repeated by adopting MORE [Microwave-induced Organic Reactions Enhancement] technology invented by Bose[46,47]. Thus, the reaction of 2 with aminothiouracil could be completed in microwave oven using small amount of dimethylfor-mamide within 10 min. The yield of the product was also improved to 58%. The structure of 9 was confirmed by 1H-NMR which revealed the characteristic signals at δ = 2.09 and 2.51 ppm for CH3 and NMe2 groups, a singlet at δ = 5.04 ppm for the CH of the pyridine fused ring, in addition to the signals of 4 NH groups. Shorter reaction time (2 min) of pyrazoloenamine 2 and aminothiouracil under microwave irradiation afforded the open adduct 10.

We also attempted to synthesize compound 11 via the reaction between 2 and α-naphthylamine in refluxing ethanol in presence of acetic acid. Yet, this reaction did not yield the desired product, but instead transamination took place to afford adduct 12 in 69% yield. All the microanalytical and spectroscopic data were in accordance with the structure of compound 12 (Scheme 3).



Scheme 3

The previous results encouraged us to study the substitution reactions of compound 2 with heterocyclic C-nucleophiles such as barbituric acid as well as the cycloaddition reactions with hydrazonoyl halides. Compound 2 reacted with barbituric acid derivatives 13a, b in ethanol containing hydrochloric acid to form the corresponding substituted adducts 14a, b, respectively (Scheme 4). On the other hand, the reaction of compound 2 with hydrazonoyl halides was carried out in ethanol containing triethylamine. The latter converted the hydrazonoyl halides into nitrile imine dipolar form which underwent 3+2 cycloaddition reaction with compound 2 to furnish the corresponding spiro adducts 15a, b (Scheme 4). Enaminones are polyfunctional compounds possessing both electrophilic and nucleophilic properties. Typical electrophilic positions are C-3 (the dimethylaminomethylene center) and C-1 (the carbonyl carbon center) with the reactivity order C-3 > C-1 while a typical nucleophilic position is C-2[48-51]. The regioselectivity of the 3+2 cycloaddition reaction of compound 2 is in accordance with this hypothesis and excludes the formation of other products. The expected reaction sequence can be explained below (Scheme 4). Primarily, the microanalytical and the spectroscopic data confirmed the structures of the newly synthesized compounds 15a, b.



Scheme 4

Antimicrobial activity

The antimicrobial activity of the newly synthesized compounds 2, 3, 4, 7, 9, 12, and 14a,b against two strains of Gram positive bacteria (G^+) (*Bacillus Subtilis and Staphylococcus aureus*), two stains of Gram negative bacteria (G^-) (*Escherichia coli* and *Pseudomonas aeroginosa*) and two strains of fungi (Aspergillus *flavus and candida albicans*) was investigated in comparison with Ampicillin and Nystatine. The obtained results are presented in Table 1.

TABLE 1. Antimicrobial potentialities of the tested compounds expressed as size (mm/mg sample) of inhibition zone.

Microorganisms	Compounds									
	2	**3**	**4**	**7**	**9**	**12**	**14a**	**14b**	**Ampicillin**	**Nystatine**
Bacillus subtilis (G+)	12	12	13	13	13	15	12	15	18	-
Staphylococcus aureus (G+)	13	12	14	14	13	14	12	13	15	-
Escherichia coli (G-)	13	12	13	13	13	14	12	15	11	-
Pseudomonas aeroginosa (G-)	12	14	12	13	13	15	12	14	19	-
Aspergillus flavus (fungus)	14	14	0	13	0	0	0	0	-	12
Candida albicans (fungus)	11	11	13	12	12	13	12	13	-	12

In general, all tested compounds were capable of inhibiting the growth of all tested strains. Compounds 12 and 14b showed relatively high activity as antibacterial agents. The other compounds showed relatively moderate activity toward all tested strains.

Experimental

Synthetic methods, analytical and spectral data

Melting points were determined on an electro thermal apparatus (Buchi 535, Switzerland) in an open capillary tube and are uncorrected. IR spectra, expressed in (v, cm-1), were recorded in KBr pellets on a PA-9721 IR spectrophotometer. 1H-NMR & 13C-NMR spectra were obtained on a Varian EM-390 300 MHz spectrometer in DMSO-d6 as solvent, using TMS as an internal reference and chemical shifts (δ) were expressed in ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment. Elemental analyses were carried out by the Microanalytical Unit at the National Research Center, Giza, Egypt. Microbiological analyses were carried out by the Micro-analytical Center, Faculty of Science, Cairo University, Giza, Egypt.

Synthesis of compounds 2 and 3

General procedure

A mixture of pyrazolone 1 (4.9 g, 0.05 mol) and DMFDMA (6.6 ml, 0.05 mol) in DMF (30 ml) was heated under reflux for 3 hr. The reaction mixture was left to cool at room temperature. The precipitate formed was collected by filtration and crystallized from EtOH to afford 2 (80%). To the filtrate 10 ml of EtOH was added and the solution was left over night. The precipitate was collected by filtration and recrystallized from DMF/MeOH to afford 3 (20%).

4-[(Dimethylamino)methylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one (2)

Yellow crystals, mp 221-222°C (DMF). MS (m/z): 153 (M^+, 100%). IR (KBr), v, cm^{-1}: 3487 (NH), 2956, 2924, 2861 ($3CH_3$), 1671 (C=O). ^{1}H-NMR (300 MHz, DMSO-d6): 1.97 (s, 3H, CH3); 3.24 (s, 3H, N-CH_3); 3.75 (s, 3H, N-CH_3); 7.28 (s, 1H, CH); 10.37 (s, 1H, NH). Anal. Calcd. for $C_7H_{11}N_3O$: C, 54.89; H, 7.24; N, 27.43. Found: C, 54.77; H, 7.08; N, 27.29.

4-[2-(Dimethylamino)-2-(3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene) ethylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one (3)

Orange crystals, mp 347-348°C (EtOH). MS (m/z): 261 (M^+, 20%). IR (KBr), v, cm^{-1}: 3452 (NH); 2989, 2922 (CH_3); 1644 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.21 (s, 6H, $2CH_3$); 2.73 (s, 3H, CH3); 2.89 (s, 3H, CH_3); 7.36 (s, 1H, CH); 12.29 (br, 2H, 2NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 17.65 (two CH_3); 38.70 (two CH_3); 98.00 (pyrazolone); 130.12 (pyrazolone C4); 143.20 (ethylene CH); 154.80 (two pyrazolone C5); 167.20 (C-N); 168.10 (two C=O). Anal. Calcd. for $C_{12}H_{15}N_5O_2$: C, 55.16; H, 5.79; N, 26.80. Found: C, 54.91; H, 5.78; N, 26.42.

4-Methyl-6H-pyrazolo[4,3-d]isoxazole (4)

Hydroxylamine hydrochloride (0.01 mol) was added to a solution of pyrazoloenamine 2 (0.153 g, 0.01 mol) in EtOH (30 ml) containing few drops of Et_3N. The reaction mixture was heated under reflux for 5 hr, cooled and poured into ice/water. The yellow precipitate was collected by filtration and purified by crystallization from n-hexane.

Yellow crystals (75%), mp 194-195 °C (n-hexane). MS (*m/z*): 123 (M^+, 88%). IR (KBr), v, cm^{-1}: 3412 (NH); 2920 (CH_3). ^{1}H-NMR (300 MHz, DMSO-d_6, TMS): 2.25 (s, 3H, CH_3); 7.88 (s, 1H, CH); 10.48 (s, 1H, NH). Anal. Calcd. for $C_5H_5N_3O$: C, 48.78; H, 4.09; N, 34.13. Found: C, 48.68; H, 4.00; N, 34.15.

3-Methyl-1,5-dihydropyrazolo[3,4-c]pyrazole (5)

To a solution of compound 2 (0.153 g, 0.001 mol) in EtOH (30 ml), hydrazine hydrate (1 ml, 99%) and AcOH (1 ml) were added. The reaction mixture was heated under reflux for 3 hr. Compound 5 was filtered as yellow crystals.

Yellow crystals (78%), mp 309-310°C (EtOH). MS (*m/z*): 122 (M^+, 30%). IR (KBr), v, cm^{-1}: 3427 (NH); 2926 (CH_3). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.23 (s, 3H, CH_3); 7.37 (s, 1H, CH); 8.30 (s, 1H, NH); 12.26 (s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 19.44 (CH_3); 97.87; 148.02 (C4); 150.11 (C3); 159.25. Anal. Calcd. for $C_5H_6N_4$: C, 49.17; H, 4.95; N, 45.88. Found: C, 49.12; H, 4.80; N, 45.73.

4-(Dimethylamino)-3-methyl-4,7-dihydro-1H-pyrazolo[3,4-d]pyrimidine-6-thiol (7)

A mixture of pyrazoloenamine 2 (0.153 g, 0.001 mol) and thiourea (0.076 g, 0.001 mol) in EtOH (30 ml) containing 1 ml of AcOH was heated under reflux for 5 hr. The reaction mixture cooled and the solid product formed was filtered off and dried.

Yellow crystals, mp 251-252°C (EtOH). MS (m/z): 211 (M^+, 35%) 196 (M^+-CH_3, 35%). IR (KBr), v, cm^{-1}: 3440 (NH); 2961, 2919, 2851(CH_3). ^{1}H-NMR (300 MHz, DMSO-d_6): 1.90 (s, 1H, SH); 2.20 (s, 3H, CH_3); 2.21 (s, 6H, $2CH_3$); 7.35 (s, 1H, CH); 9.40 (s, 1H, NH); 12.26 (s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 12.65, 12.86, 13.00 ($3CH_3$); 66.37 (CH); 107.31 (pyrazole C4); 139.43 (pyrazole C3); 152.45 (pyrazole C5); 163.36 (C-SH). Anal. Calcd. for $C_8H_{13}N_5S$: C, 45.48; H, 6.20; N, 33.15. Found: C, 45.35; H, 6.01; N, 32.98.

(E)-2-(Dimethylamino)-2-(3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)-N-phenylethanethioamide (8)

To a solution of pyrazoloenamine 2 (0.153 g, 0.001 mol) in EtOH (30 ml) containing NaOH (0.04 g, 0.001 mol), PhNCS (0.01 mol) was added. The reaction mixture was stirred at room temperature overnight then poured into ice/H2O. The solid product formed was collected by filtration.

Yellow crystals, mp 158-159°C (benzene and n-hexane). MS (*m/z*): 288 (M^+, 20%). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.17 (s, 3H, CH_3); 2.33 (s, 6H, $2CH_3$); 6.97-7.71 (m, 5H, aromatic protons); 9.68 (br, s, 1H, NH); 12.29 (br, s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 17.78 (CH_3); 38.30 (2 CH_3, NMe2); 38.38 (pyrazole C4); 112.21 (pyrazole C3), [124.50, 125.13, 128.60, 139.42] (six aromatic carbons); 155.61 (C-N, linker); 168.00 (pyrazole C=O), 188.23 (C=S). Anal. Calcd. for $C_{14}H_{16}N_4OS$: C, 58.31; H, 5.59; N, 19.43; S, 11.12. Found : C, 58.01; H, 5.35; N, 19.21; S, 11.00.

4-(Dimethylamino)-3-methyl-7-thioxo-1,4,6,7,8,9-hexahydro-5H-pyrazolo [4',3':5,6] pyrido[2,3-d]pyrimidin-5-one (9)

Method A: Conventional heating

A mixture of pyrazoloenamine 2 (0.153 g, 0.001 mol) and thiouracil (0.143 g, 0.001 mol) in DMF (20 ml) containing AcOH (1 ml) was heated under reflux for 16 hr. The reaction mixture was left to cool and poured onto ice/H2O. The solid product formed was collected by filtration.

Method B: Microwave heating

An opened Erlenmeyer flask, containing a mixture of pyrazoloenamine 2 (0.153 g, 0.001 mol) and thiouracil (0.143 g, 0.001 mol) in 3 ml DMF was placed in domestic microwave oven and irradiated for 10 min. The reaction mixture was left to cool at room temperature. The resulting product was triturated with n-hexane. The formed precipitate was collected by filtration and crystallized from the suitable solvent.

Yellow solid (13% and 58% for conventional heating and microwave irradiation, respectively), mp >350°C (DMF). MS (*m/z*): 278 (M^+, 68%). IR (KBr), v, cm^{-1}: 3449 (NH); 2964, 2889(CH_3); 1619 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.09 (s, 3H, CH_3); 2.51 (s, 6H, 2 CH_3); 5.04 (s, 1H, CH); 7.21 (s, 1H, exchangeable NH); 8.17 (s, 1H, NH); 8.82 (b, H, NH); 11.45 (s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 17.40 (CH_3); 38.37 (2C, N(CH_3)2); 45.60, 88.60,

103.00, 142.70, 156.10, 162.70, 167.21 (C=O), 168.00 (C=S). Anal. Calcd. for $C_{11}H_{14}N_6OS$: C, 47.47; H, 5.07; N, 30.19; S, 11.52. Found: C, 47.25; H, 4.89; N, 30.02; S, 11.41.

6-Amino-5- [(dimethylamino) (3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl) methyl]-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (10)

An opened Erlenmeyer flask, containing a mixture of pyrazoloenamine 2 (0.153 g, 0.001 mol) and thiouracil (0.143 g, 0.001 mol) dissolved in DMF (2 ml), was placed in domestic microwave oven and irradiated for 2 min. The reaction mixture was left to cool at room temperature. The resulting product was triturated with n-hexane. The formed solid was collected by filtration.

Red crystals (63%), mp 288-290°C (EtOH). MS (*m/z*): 296 (M^+, 6%). IR (KBr), v, cm^{-1}: 3450 (NH); 2996, 2896 (CH_3); 1645, 1615 (2C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.22 (s, 3H, CH_3); 2.64 (s, 6H, $2CH_3$); 4.71 (d, 1H, pyrazole CH); 5.04 (d, 1H, methylidene CH); 6.69 (s, 1H, exchangeable NH); 7.37 (s, 1H, NH); 9.11 (br, 2H, NH_2); 11.30 (s, 1H, NH). Anal. Calcd. for $C_{11}H_{16}N_6O_2S$: C, 44.58; H, 5.44; N, 28.36; S, 10.82. Found: C, 44.45; H, 5.30; N, 28.18; S, 10.71.

5-Methyl-4-[(naphthalen-1-ylamino)methylidene]-2,4-dihydro-3H-pyrazol-3-one (12)

A mixture of pyrazoloenamine 2 (0.153 g, 0.001 mol) and α-naphthylamine (0.143 g, 0.001 mol) in absolute EtOH (30 ml) and AcOH (1 ml) was heated under reflux for 8 hr. The reaction mixture was left to cool. The solvent was evaporated under vacuum. The residue was triturated with n-hexane and the solid formed was collected by filtration.

Brown solid (69%), mp 282-283°C (EtOH). MS (*m/z*): 251 (M^+, 100%). IR (KBr), v, cm^{-1}: 3431 (NH); 2922 (CH_3); 1671 (C=O). 1H-NMR (300 MHz, DMSO-d_6): 2.21 (s, 3H, CH_3); 7.39 (s, 1H, CH); 7.59-8.09 (m, 7H, naphthalene); 8.78 (s, 1H, exchangeable NH); 11.19 (s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 12.71 (CH_3); 102.52 (pyrazole C3); 112.51, 118.72, 120.72, 123.00, 124.60, 125.66, 126.24, 128.32, 133.82, 134.37 (naphthalene carbons); 145.45 (methylidene carbon); 147.45 (pyrazole C5); 169.01 (C=O). Anal. Calcd. for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.55; H, 5.00; N, 16.35.

Synthesis of compounds 14a,b

General procedure

A mixture of pyrazoloenamine 2 (0.153 g, 0.001 mol) and barbituric acid (13a and 13b) (0.001 mol) in EtOH (25 ml) containing HCl (1 ml) was heated under reflux for 30 min. The reaction mixture was left to cool. The solid product formed was collected by filtration.

5-[(3-Methyl-5-oxo-1,5-dihydro -4H-pyrazol-4-ylidene) methyl] pyrimidine-2,4,6 (1H,3H,5H)-trione (14a)

Yellow crystals (90%), mp 354-355°C (EtOH). MS (*m/z*): 236 (M^+, 100%). IR (KBr), v, cm^{-1}: 3450 (NH); 2960 (M/Z %); 1744, 1683, 1638, 1618 (C=O).

^{1}H-NMR (300 MHz, DMSO-d_6): 2.19 (s, 3H, M/Z %); 3.46 (d, 1H, CH pyrimidine); 8.07 (d, 1H, CH); 11.26 (s, 2H, 2NH); 11.57 (s, 1H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6): 17.52 (CH_3); 48.22 (pyrimidine C5); 129.81 (pyrazole C4); 139.33 (methylene CH); 155.63 (pyrazole C3); 157.24 (pyrimidine C2); 168.00 (pyrazole C5); 170.70 (2C, pyrimidine C4 and C6). Anal. Calcd. for $C_9H_8N_4O_4$: C, 45.77; H, 3.41; N, 23.72. Found: C, 45.54; H, 3.22; N, 23.31.

1,3-Dimethyl-5- [(3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene) methyl] pyrimidine-2,4,6(1H,3H,5H)-trione (14b)

Yellow crystals (95%), mp 296-297°C (EtOH). MS (*m/z*): 264 (M^+, 25%). IR (KBr), v, cm^{-1}: 3430 (NH); 2961, 2920, 2853 (CH_3); 1779, 1728, 1665, 1633 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.21 (s, 3H, CH_3); 2.46 (s, 6H, CH_3); 3.47 (d, 1H, CH) 8.07 (d, 1H, CH) 11.55 (s, 1H, NH). Anal. Calcd. for $C_{11}H_{12}N_4O_4$: C, 50.00; H, 4.58; N, 21.20. Found: C, 49.88; H, 4.30; N, 21.01.

Synthesis of compounds 15a, b

General procedure

To a stirred solution of hydrazonoyl halide (0.01 mol) in EtOH containing Et3N (0.01 mol), pyrazoloenamine 2 (1.53 g, 0.01 mol) was added. The reaction mixture was stirred for 1 hr and then heated under reflux for 2 hr. The reaction mixture was left to cool and poured onto ice/H_2O. The solid product formed was collected by filtration and crystallized from EtOH.

6-Acetyl-8-(4-chlorophenyl)-9- (dimethylamino) -4- methyl-2,3,7,8-tetraazaspiro[4.4] nona-3,6-dien-1-one (15a)

Brown crystals (55%), mp 169-170°C (EtOH). MS (*m/z*): 347 (M^+). IR (KBr), v, cm^{-1}: 2978, 2928, 1890 (CH_3); 1664, 1627 (2C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 1.97 (s, 3H, CH_3); 2.20 (s, 3H, CH_3); 2.33 (s, 6H, $2CH_3$); 4.11 (s, 1H, CH); 7.37-7.73 (m, 5H, aromatic protons and NH). 13C-NMR (75 MHz, DMSO-d_6): 14.91 (CH_3), 19.33 (CH_3), 36.00 ($2CH_3$); 52.00 (spiro carbon); 70.52, 117.00 (pyrazole C5); 113.00, 122.22, 129.72, 141.61 (aromatic carbons); 155.00 (C6); 155.66 (C4); 177.00, 200.00 (2C=O). Anal. Calcd. for $C_{16}H_{18}ClN_5O_2$: C, 55.25; H, 5.22; Cl, 10.19; N, 20.14. Found: C, 55.05; H, 5.00; Cl, 9.91; N, 19.95.

8-(4-Chlorophenyl)-9-(dimethylamino)-4-methyl-6- (thiophen-3-ylcarbonyl) -2,3,7,8-tetraazaspiro[4.4]nona-3,6-dien-1-one (15b)

Yellow crystals (62 %), mp 180-181°C (EtOH). MS (*m/z*): 415 (M^+). IR (KBr), v, cm^{-1}: 3430 (NH); 2978, 2928 (CH_3); 1664, 1627 (2C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.28 (s, 3H, CH_3); 2.34 (s, 6H, $2CH_3$); 4.20 (s, 1H, CH); 7.34 (m, 2H, aromatic proton); 7.50 (m, 2H, aromatic protons); 8.07-8.15 (m, 3H, thiophene protons); 11.18 (s, 1H, NH). Anal. Calcd. for $C_{19}H_{18}ClN_5O_2S$: C, 54.87; H, 4.36; Cl, 8.52; N, 16.84; S, 7.71. Found: C, 54.55; H, 4.20; Cl, 8.32; N, 16.65; S, 7.50.

Bioassay

A filter paper sterilized disc saturated with measured quantity of the sample is placed on plate containing solid bacterial medium (nutrient agar broth) or fungal

medium (Dox's medium) which has been heavily seeded with spore suspension of the tested organism. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against test organism[52-54].

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تشييد بيرازولونات جديدة ومشتقات ملتحمة للبيرازول ذات نشاط بيولوجى عالى ضد الميكروبات

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تم فى هذا البحث تشييد بعض مركبات البيرازولونات و مشتقات جديدة للبيرازولات الملتحمة عن طريق استخدام أنواع من الإينامينونات .

ولقد أعطى تفاعل بيرازولون ١ مع ثنائى ميثيل فورماميد ثنائى ميثيل اسيتات ٤– ثنائى ميثيل أمينو ميثيليدين بيرازول-٣-اون ٢ واستخدمنا إينامينون ٢ كوسيط لتحضير بيرازولونات جديدة ٨، ٩، ١٢، و البيرازولونات الملتحمة ٤-٧، ١٠ .

وكذلك المركبات ١٥ أ، ب وقد تم تقييم النشاط البيولوجى لبعض المركبات المشيدة الجديدة ضد الميكروبات الموجبة والسالبة الجرام والفطريات مقارنة بالأمبيسلين و النيستاتين .