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UTILITY OF ENAMINONITRILE IN THE SYNTHESIS OF NOVEL HETEROCYCLES BEARING PYRAZOLE MOIETY AND THEIR ANTIMICROBIAL AND CYTOTOXIC ACTIVITY ASSESSMENT

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ABSTRACT:

Enaminonitrile derivatives 1a,b were used as a versatile material for the synthesis of different heterocyclic compounds such as pyrazoloacetamide, pyrazolopyrimidinone, pyrazolobenzamide, pyrazolopyrimidines, pyrazolothiazinethiones, pyrazolopyrimidinedithiones, pyrazolocynoacetamide, pyrazolopyrazoles, tetrazolopyrazoles, pyrazolotetrazolopyrimidines, and pyrazolotetrazolodiazepine derivatives, through the reaction with different electrophiles and nucleophiles. The newly synthesized compound were tested against gram positive and gram negative bacteria, fungi and two human tumor cell lines.

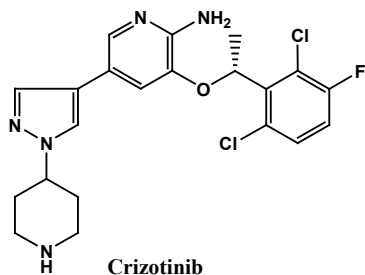
Keywords: pyrazolopyrimidine, pyrazolothiazinethiones, pyrazolotetrazolopyrimidines, pyrazolotetrazolodiazepine, antimicrobial, cytotoxic activity.

INTRODUCTION:

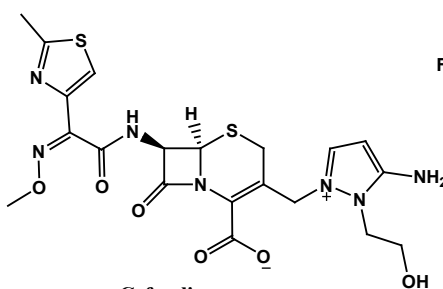
In the last several decades, pyrazole derivatives have received considerable attention due to their wide-range of biological activity. Pyrazoles are reported to exhibit antiviral [1], antagonist [2], antimicrobial [3], anti-bacterial [4], anticancer [5], anti-inflammatory [5], analgesic [6], anti-prostate cancer [7], herbicidal [8], acaricidal and insecticid-

al [9] activities and also as anti-Tobacco Mosaic Virus [10].

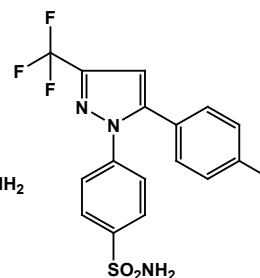
Among pyrazoles, Crizotinib is a drug used as anti-cancer [11], Cefoselis is a drug used as antibacterial [11] and Celebrex (celecoxib) is a drug used for rheumatoid arthritis and osteoarthritis [12].



Crizotinib



Cefoselis



Celebrex

For the above mentioned biological activity and in continuation of our efforts in the synthesis of biologically active heterocycles [13-17], we prepared an enaminonitriles containing pyrazole moiety, which were subjected to electrophilic and nucleophilic reactions in order to prepare novel heterocyclic compounds and study their antimicrobial and anticancer activities. The

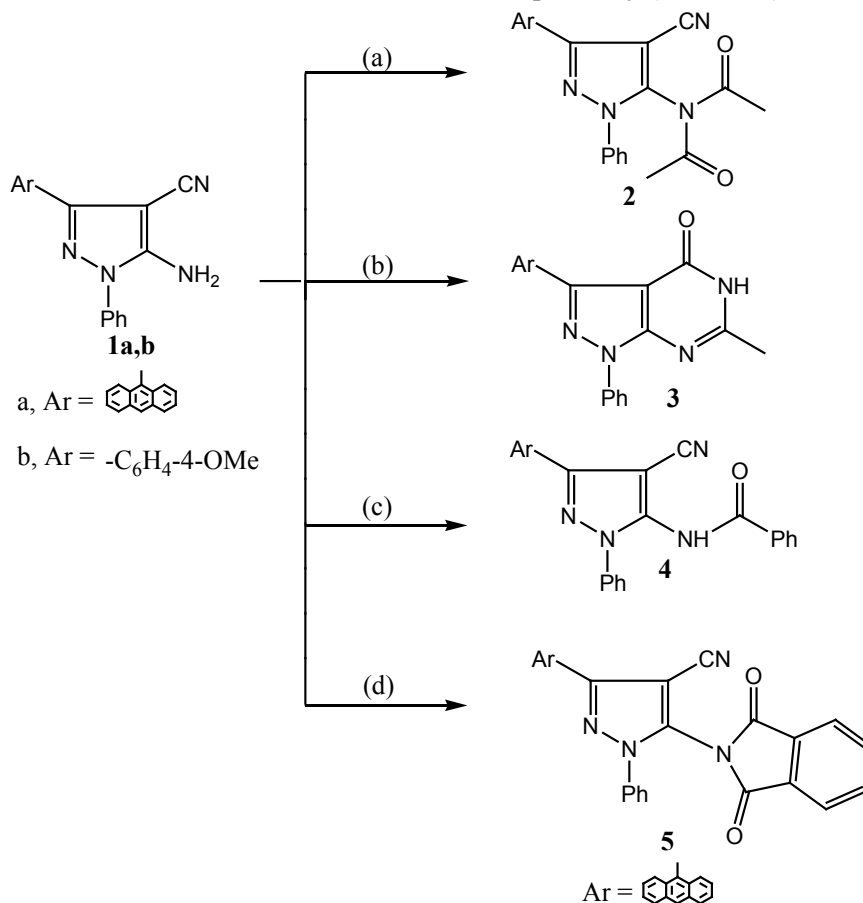
newly synthesized compounds were evaluated against gram positive *Staphylococcus aureus*, *Bacillus subtilis* and gram negative *Escherichia coli*, *Pseudomonas aeruginosa*. The anti-fungal activities of the compounds were tested against two fungi *Candida albicans*, *Aspergillus flavus*. Also, some of the compounds were tested against two human tumor cell lines namely; mammary

gland breast cancer (MCF-7) and human skin cancer (HFB4) some of the tested compounds showed high antimicrobial and cytotoxic activities.

RESULTS AND DISCUSSION

The starting enaminonitriles 1a,b were prepared malononitrile, phenyl hydrazine and

aromatic aldehydes in one pot reaction [18]. Compound 1a was treated with acetic anhydride, $\text{Ac}_2\text{O}/\text{AcOH}$ mixture, benzoyl chloride in the presence of triethyl amine and/or phthalic anhydride producing N,N-diacetyl pyrazole, pyrazolopyrimidine, N-benzoyl pyrazole and 1,3-dioxoisindolinyl pyrazole derivatives 2-5, respectively (Scheme 1).



Reagents and conditions: a) Ac_2O , reflux 3 h, b) Ac_2O , AcOH , reflux 7 h, c) PhCOCl , TEA, reflux 8 h, d) Phthalic anhydride, AcOH , reflux 4 h.

Scheme 1

Formation of compound 2 takes place through the reaction of the amino group with two moles of acetic anhydride. The $^1\text{H-NMR}$ of 2 indicated the presence of a two acetyl groups where it showed δ at 2.18 (s, 6H, 2COCH_3). While compound 3 is believed to take place through the hydrolysis of the cyano group to amide group and acetylation of the amino group followed by ring closure through elimination of water molecule to produce the pyrimidinone ring. The structure of the product gets support from its IR spectrum which devoid of cyano group absorption band.

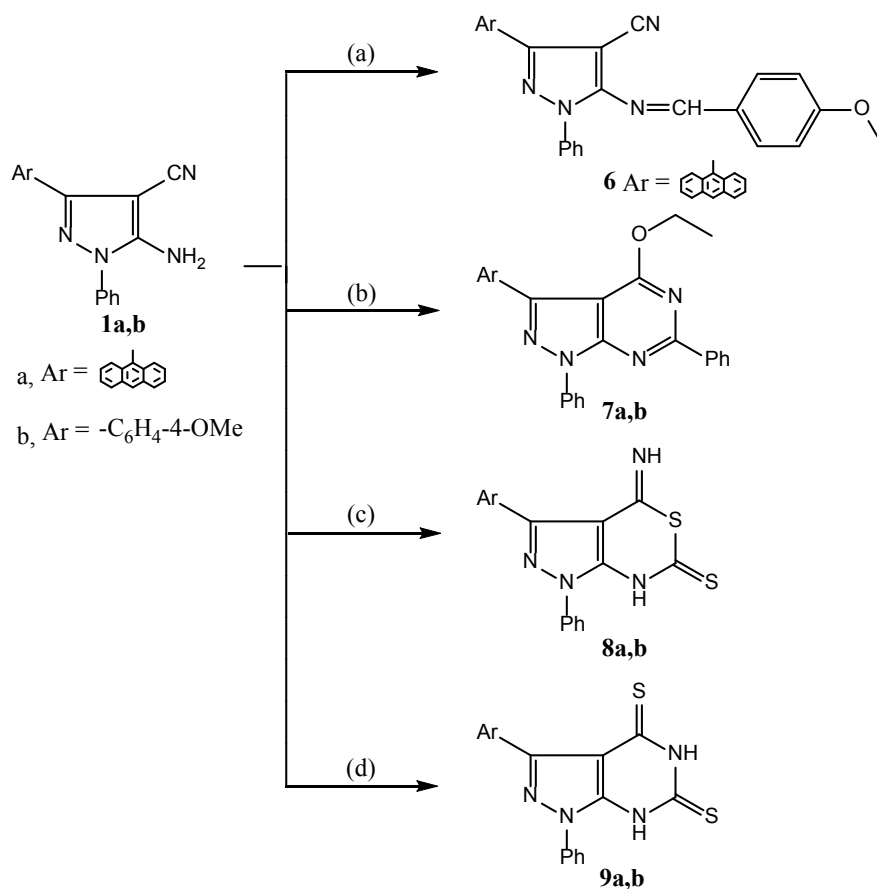
The structure of compound 4 was elucidated

from its IR which showed peaks at 3287 (NH), 2228 (CN), and 1654 ($\text{C}=\text{O}$), and the $^1\text{H-NMR}$ which displayed signals at δ : 7.49-8.88 (m, 19 H, Ar-H), 9.65 (s, 1H, NH, D_2O exchangeable). Also, the IR spectrum of compound 5 showed a peak at 1698 ($\text{C}=\text{O}$) and its MS: m/z 490 [M^+] (2.43%), which is in accordance with the structure.

On the other hand, reaction of 1a with 4-methoxybenzaldehyde, in the presence of catalytic amount of acetic acid, afforded the Schiff base 6, while the reaction of 1a,b with benzaldehyde in the presence of sodium hydroxide [19] pro-

duced the pyrazolopyrimidine derivatives 7a,b. Also, refluxing 1a,b with carbon disulfide gave the pyrazolothiazine derivatives 8a,b, but when

the same reaction was carried out in the presence of potassium hydroxide the pyrazolopyrimidine derivatives 9a,b were obtained^[20] (Scheme 2).



Reagents and conditions: a) 4-OMeC₆H₄CHO, AcOH, reflux 3 h, b) C₆H₅CHO, NaOH, reflux 2 h, c) CS₂, reflux 2 h, d) CS₂, NaOH, reflux 12 h.

Scheme 2

The presence of the azo methine proton at δ 8.88 indicated the structure of the Schiff base 6. While compound 7a was approved by: its IR which devoid any band for the cyano group, ¹H-NMR which displayed peaks at δ : 1.13 (t, 3H, CH₃CH₂-O, J = 6.0 Hz), 4.26 (q, 2H, -CH₂O, J = 6.6 Hz), and MS: m/z 492 [M⁺] (1.19%).

Meanwhile, compound 1a was subjected to the reaction with cyanoacetic acid, and/or formamide, to produce cyanoacetamide and pyrazolopyrimidine derivatives 10 and 11, respectively. When compound 1b reacted with triethylorthoformate, ethyl formimidate 12 was produced (Scheme 3).

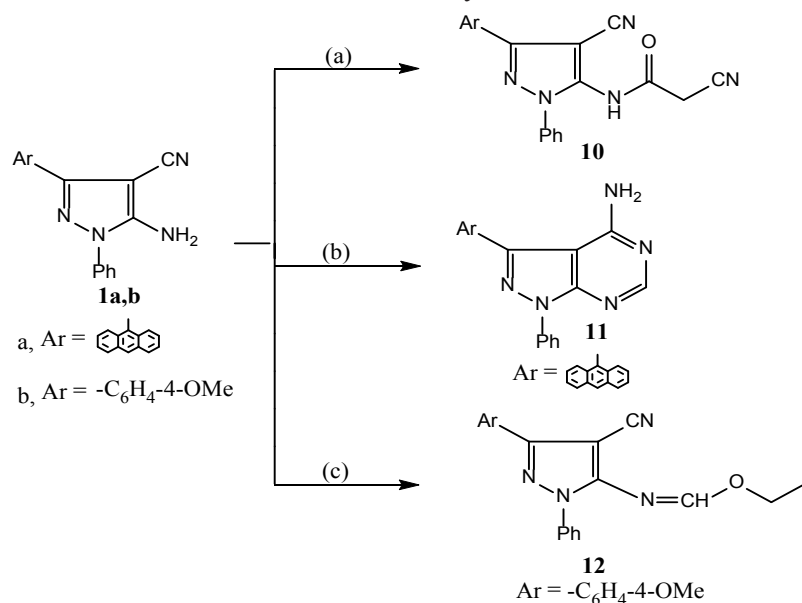
Formation of compound 10 may take place through the attack of the amino group on the carbonyl of the carboxylic acid followed by elimination of one molecule of water. The IR spectra

of 10 revealed the presence of two peaks a 2260, 2227 which are attributable to two (CN) groups, also, the ¹H-NMR showed peaks at δ : 4.50 (s, 2H, COCH₂CN), and 9.65 (s, 1H, NH, D₂O exchangeable) which confirmed the structure.

The IR spectrum of the aminopyrimidine derivative 11 was devoid of $\nu_{C\equiv N}$ and revealed the appearance of the absorption bands for NH₂ group at 3476, 3276, 3140 cm⁻¹. The mass spectrum of this product showed a peak at m/z 387 [M⁺] (7.94%), which approved the structure.

The IR spectrum of compound 12 was devoid of ν_{NH_2} , while its ¹H-NMR displayed peaks at δ 1.17 (t, 3H, CH₃CH₂-O, J = 6.4 Hz), 4.11 (q, 2H, -CH₂O, J = 6.8 Hz) indicating the presence of the ethyl moiety.

The study was extended to explore the reac-

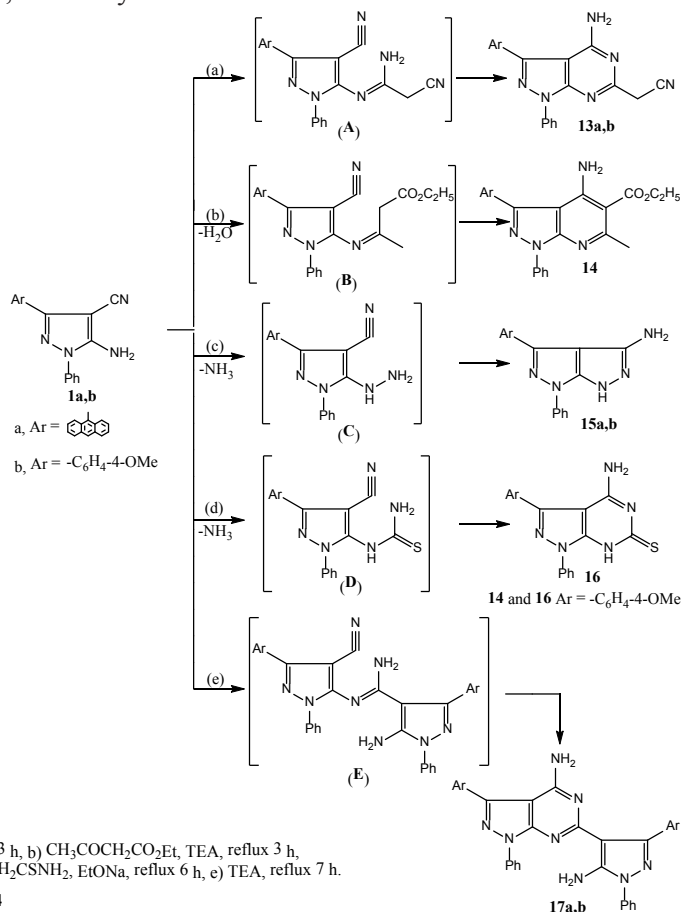


Reagents and conditions: a) CNCH_2COOH , Ac_2O , reflux 1.5 h, b) HCONH_2 , reflux 6 h, c) $\text{CH}(\text{OC}_2\text{H}_5)_3$, Ac_2O , reflux 6 h.

Scheme 3

tivity of the enaminonitriles 1a,b towards some carbon and nitrogen nucleophiles. Thus, reaction of 1a,b with malononitrile and/or ethyl acetoacetate in the presence of a base afforded the acetonitrile and aminoester derivatives 13a,b and 14, respectively. While treatment of 1a,b with hy-

drazine hydrate, thiourea in the presence of sodium ethoxide and/or refluxing with triethyl amine in ethanol, furnished the pyrazolopyrazole^[21] and pyrazolopyrimidine^[22] derivatives 15a,b, 16 and 17, respectively (Scheme 4).



Reagents and conditions: a) CNCH_2CN , EtONa , reflux 3 h, b) $\text{CH}_3\text{COCH}_2\text{CO}_2\text{Et}$, TEA, reflux 3 h, c) $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, reflux 7 h, d) NH_2CSNH_2 , EtONa , reflux 6 h, e) TEA, reflux 7 h.

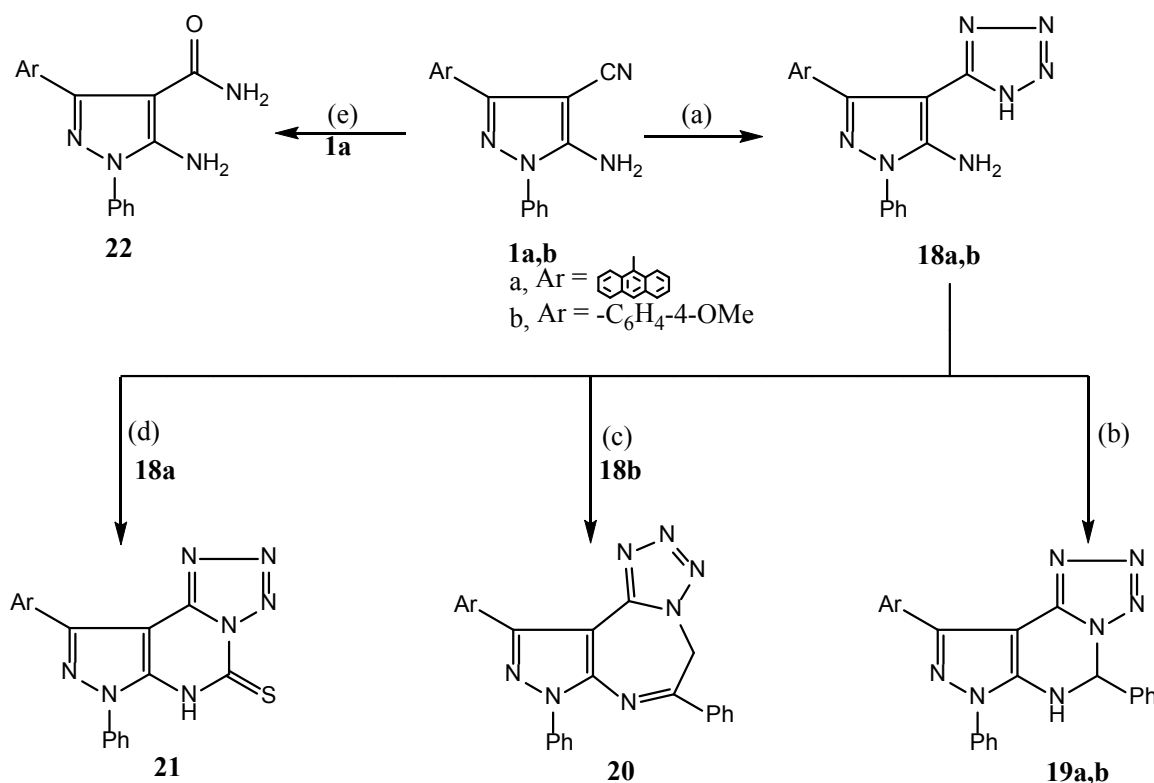
Scheme 4

Formation of the acetonitrile derivative 13 may take place through the nucleophilic attack of the amino group on the cyano group of malonitrile to form the intermediate (A) followed by ring closure. The structure of 13a was supported by its $^1\text{H-NMR}$ which displayed a peak at δ 2.16 (s, 2H, CH_2CN) and the mass spectrum which showed m/z 426 [M^+] (6.25%). Also, in compound 14, the condensation reaction between the amino group and ethyl acetoacetate produced the intermediate (B) which underwent ring closure to form the expected product. The IR of 14 showed the following peaks 3431, 3221 (NH_2) and 1708 (C=O), and the $^1\text{H-NMR}$ displayed signals at δ : 1.19 (t, 3H, $\text{CH}_3\text{CH}_2\text{-O}$, $J = 6.2$ Hz), 2.48 (s, 3H, CH_3), 4.28 (q, 2H, $-\text{CH}_2\text{O}$, $J = 6.4$ Hz) which is in accordance with the structure.

In case of formation of compound 15, hydrazine reacted with the amino group forming the intermediate (C) through the elimination of one molecule of ammonia then ring closure takes place. The structure was indicated from the IR spectra which devoid of $\nu_{\text{C=N}}$, and the $^1\text{H-NMR}$

which showed two signals at δ 8.63 (s, 2H, NH_2 , D_2O exchangeable) 9.99 (s, 1H, NH , D_2O exchangeable) for compound 15b. Compound 16 was formed in the same manner through the intermediate (D). But the formation of compound 17 takes place by the reaction of two molecules of compound 1, where the amino group of the first molecule attacks the cyano group of the second molecule producing the intermediate (E) followed by ring closure in the same manner. The structures of the products 17a,b get support from the IR spectra which devoid of $\nu_{\text{C=N}}$ and the mass spectra which displayed m/z 720 [M^+] (16.65%) and 580 [M^+] (1.02%), respectively.

Reaction of 1a,b with sodium azide in the presence of ammonium chloride in dimethylformamide gave the tetrazole derivatives 18a,b. Compounds 18a,b were treated with benzaldehyde, phenacyl bromide/sodium acetate and/or carbon disulfide/pyridine producing the tetrazolopyrimidine, tetrazolodiazepine and tetrazolopyrimidinethione derivatives 19a,b, 20 and 21, respectively (Scheme 5).



Reagents and conditions: a) NaN_3 , NH_4Cl , reflux 7 h, b) PhCHO , HCl , reflux 16 h, c) PhCOCH_2Br , AcONa , reflux 10 h, d) CS_2 , pyridine, reflux 10 h, e) H_2O_2 , NH_4OH , stirring 3 h.

Scheme 5

The absence of the cyano group in the IR spectra and the presence of a signal at δ 10.66 (s, 1H, NH, D₂O exchangeable) in the ¹H-NMR confirmed the proposed structure of product 18.

The formation of compound 20 may occur through the nucleophilic attack of the NH group (of tetrazole ring) on the carbon atom of the phenacyl bromide via S_N2 mechanism (with the elimination of HBr molecule) followed by ring closure through the condensation between the amino group and the carbonyl group.

Finally, when compound 1a was stirred with a mixture of hydrogen peroxide and ammonia solution for three hours at room temperature, a partial hydrolysis for the cyano group takes place and the amide derivative 22 was obtained^[23] (Scheme 5). The structure of the product was elucidated from its IR spectrum which devoid of $\nu_{C=N}$ and showed absorption band at 1676 attributable to C=O group.

Experimental

All melting points are uncorrected and were determined on a digital Stuart SMP3 electric melting point apparatus. Infrared (IR) spectra were measured on Perkin-Elmer 293 spectrophotometer (cm⁻¹) using KBr disks. ¹H-NMR spectra were measured on Varian Mercury 400 MHz spectrometer in DMSO-d₆ as a solvent using TMS as an internal standard. Chemical shifts (δ) are measured in ppm and coupling constants (J) in Hz. The mass spectra were recorded on a GC-2010 Shimadzu Gas chromatography instrument mass spectrometer (70 eV) using the electron ionization technique. Elemental microanalyses were performed on a Perkin-Elmer CHN-2400 analyzer and the microanalyses were within \pm 0.4% of the theoretical values. The biological evaluation of the products was carried out at Department of Pharmacology, Faculty of Pharmacy, Mansoura University, Egypt.

5-Amino-3-(anthracen-10-yl)-1-phenyl-1H-pyrazole-4-carbonitrile (1a).

m.p. 146-148 °C. Solvent (EtOH). IR (KBr) ν cm⁻¹: 3301 (NH₂), 3076, 3049 (CHar), 2228 (CN), 1620 (C=N), 1598 (C=C). ¹H-NMR (400 MHz, DMSO-d₆) δ : 6.76-8.86 (m, 14 H, Ar-H), 10.65 (s, 2H, NH₂, D₂O exchangeable). MS: m/z

360 [M⁺] (6.75%). Anal. Calcd for C₂₄H₁₆N₄ (360): C, 80.00; H, 4.44; N, 15.55. Found: C, 79.64; H, 4.11; N, 15.90.

N-Acetyl-N-(3-(anthracen-9-yl)-4-cyano-1-phenyl-1H-pyrazol-5-yl)acetamide (2).

A mixture of 1a (0.01 mol, 3.6 g) and acetic anhydride (30 ml) was refluxed for 3 h. The reaction mixture was left to cool and then poured into ice/water. The solid formed was collected by filtration, washed with water and recrystallized from benzene to produce 2.

m.p. 198-200 °C. IR (KBr) ν cm⁻¹: 3052 (CHar), 2228 (CN), 1683 (C=O), 1615 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ : 2.18 (s, 6H, 2COCH₃), 7.36-8.89 (m, 14 H, Ar-H). MS: m/z 444 [M⁺] (1.00%). Anal. Calcd for C₂₈H₂₀N₄O₂ (444.48): C, 75.66; H, 4.54; N, 12.66. Found: C, 75.30; H, 4.23; N, 12.93.

3-(Anthracen-9-yl)-6-methyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (3).

A solution of 1a (0.01 mol, 3.6 g) in mix of 10 ml of acetic anhydride and acetic acid (2:1) was refluxed for 8 h. After cooling, the reaction mixture was poured into ice/water, the solid that deposited was filtered off, washed with water, dried and recrystallized from benzene.

m.p. 240-242 °C. IR (KBr) ν cm⁻¹: 3455 (NH), 3052, (CHar), 2229 (CN), 1686 (C=O), 1613 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ : 2.53 (s, 3H, CH₃), 7.33-8.63 (m, 14 H, Ar-H), 9.11 (s, 1H, NH, D₂O exchangeable). MS: m/z 402 [M⁺] (5.01%). Anal. Calcd for C₂₆H₁₈N₄O (402.45): C, 77.59; H, 4.51; N, 13.92. Found: C, 77.98; H, 4.19; N, 14.30.

N-(3-(anthracen-9-yl)-4-cyano-1-phenyl-1H-pyrazol-5-yl)benzamide (4).

To a solution of 1a (0.01 mol, 3.6 g) in dioxane (30 ml), benzoyl chloride (0.01 mole, 1.4 ml) and triethylamine (2 ml) were added. The reaction mixture was refluxed for 8 h then left to cool. The reaction mixture was poured into ice/water, the solid so formed was collected by filtration, washed with water and recrystallized from methanol to produce 4.

m.p. 236-238 °C. IR (KBr) ν cm⁻¹: 3287 (NH),

3054, 3016 (CHar), 1654 (C=O), 1621 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ: 7.49-8.88 (m, 19 H, Ar-H), 9.65 (s, 1H, NH, D₂O exchangeable). MS: m/z 464 [M⁺] (6.75%). Anal. Calcd for C₃₁H₂₀N₄O (464.52): C, 80.15; H, 4.34; N, 11.06. Found: C, 79.86; H, 4.00; N, 12.00.

3-(Anthracen-9-yl)-5-(1,3-dioxoisindolin-2-yl)-1-phenyl-1H-pyrazole-4-carbonitrile (5).

A mixture of 1a (0.01 mol, 3.6 g) and phthalic anhydride (0.01 mole, 1.48 g) in acetic acid (30 ml) was refluxed for 3 h, left to cool, then poured into ice/water. The solid formed was collected by filtration, washed with water and recrystallized from acetone to give 5.

m.p. 200-202 °C. IR (KBr) ν cm⁻¹: 3080, 3053, 3017 (CHar), 2228 (NC), 1698 (C=O), 1622 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ: 7.61-8.89 (m, 18 H, Ar-H). MS: m/z 490 [M⁺] (7.49%). Anal. Calcd for C₃₂H₁₈N₄O₂ (490.51): C, 78.36; H, 3.70; N, 11.42. Found: C, 78.68; H, 3.87; N, 11.06.

3-(Anthracen-9-yl)-5-((4-methoxybenzylidene)amino)-1-phenyl-1H-pyrazole-4-carbonitrile (6).

To a mixture of 1a (0.01 mol, 3.6 g) and 4-methoxybenzaldehyde (0.01 mole, 1.06 ml) in ethanol (30 ml), a few drops of glacial acetic acid was added. The reaction mixture was refluxed for 8 h, left to cool, and then poured into ice/water. The solid so formed was collected by filtration, washed with ethanol and recrystallized from methanol to obtain the Schiff base 6.

m.p. 208-210 °C. IR (KBr) ν cm⁻¹: 3052, 3016 (CHar), 2228 (CN), 1620 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ: 3.78 (s, 3H, OCH₃), 7.62-8.23 (m, 18 H, Ar-H), 8.88 (s, 1H, N=CH). MS: m/z 478 [M⁺] (21.59%). Anal. Calcd for C₃₂H₂₂N₄O (478.54): C, 80.32; H, 4.63; N, 11.71. Found: C, 80.00; H, 4.71; N, 12.02.

General procedure for the synthesis of compounds 7a,b.

Compounds 1a,b (0.01 mol), ethanol (30 ml), benzaldehyde and or 4-chlorobenzaldehyde (0.01 mole) and NaOH (0.72g) were refluxed for 2 h. The reaction mixture was left to cool and then poured into ice/water, the obtained solid

was collected by filtration, washed with water and recrystallized from proper solvent to produce compounds 7a,b.

3-(Anthracen-9-yl)-4-ethoxy-1,6-diphenyl-1H-pyrazolo[3,4-d]pyrimidine (7a):

m.p. 186-188 °C (petroleum ether 60-80 °C / benzene). IR (KBr) ν cm⁻¹: 3067, 3049 (CHar), 1621 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ: 1.13 (t, 3H, CH₃CH₂-O, J = 6.0 Hz), 4.26 (q, 2H, -CH₂O, J = 6.6 Hz), 6.78-8.77 (m, 19 H, Ar-H). MS: m/z 492 [M⁺] (1.19%). Anal. Calcd for C₃₃H₂₄N₄O (492.57): C, 80.49; H, 4.91; N, 11.37. Found: C, 80.10; H, 5.12; N, 11.03.

6-(4-Chlorophenyl)-4-ethoxy-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (7b):

m.p. above 300 °C (EtOH). IR (KBr) ν cm⁻¹: 3073, 3010 (CHar). MS: m/z 456 [M⁺] (5.67%). Anal. Calcd for C₂₆H₂₁ClN₄O₂ (456.92): C, 68.43; H, 4.63; Cl, 7.76; N, 12.26. Found: C, 68.09; H, 4.81; Cl, 8.10; N, 11.93.

General procedure for the synthesis of compounds 8a,b.

To compounds 1a,b (0.01 mol), carbon disulfide (15 ml) was added dropwise at room temperature with stirring for 30 min. The reaction mixture was refluxed for 2 h, then left to cool. The solid that separated out was collected by filtration and recrystallized from suitable solvent to give 8a,b.

3-(Anthracen-9-yl)-4-imino-1-phenyl-4,7-dihydropyrazolo[3,4-d][1,3]thiazine-6(1H)-thione (8a).

m.p. 204-206 °C (benzene). IR (KBr) ν cm⁻¹: 3434, 3300 (NH), 3075, 3048, (CHar), 1621 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ: 6.77-8.76 (m, 14 H, Ar-H), 9.10 (s, 1H, NH, D₂O exchangeable), 10.65 (s, 1H, NH, D₂O exchangeable). MS: m/z 436 [M⁺] (11.09%). Anal. Calcd for C₂₅H₁₆N₄S₂ (436.55): C, 68.78; H, 3.69; N, 12.83; S, 14.66. Found: C, 69.10; H, 3.86; N, 13.16; S, 15.01.

4-Imino-3-(4-methoxyphenyl)-1-phenyl-4,7-dihydropyrazolo[3,4-d][1,3]thiazine-6(1H)-thione (8b).

m.p. 120-122°C (EtOH). IR (KBr) ν cm^{-1} : 3425, 3313 (NH), 3045, 3020, (CHar), 1596 (C=N). MS: m/z 366 [M^+] (7.22%). Anal. Calcd for $C_{18}H_{14}N_4OS_2$ (366.46): C, 58.99; H, 3.85; N, 15.29; S, 17.50. Found: C, 59.33; H, 4.04; N, 15.03; S, 17.81.

General procedure for the synthesis of compounds 9a,b.

To a mixture of 1a,b (0.01 mol) and potassium hydroxide (0.2 g) in ethanol (30 ml), carbon disulfide (3.5 ml) was added. The reaction mixture was refluxed for 12 h, left to cool, then poured into ice/water. The solid formed was collected by filtration, washed with ethanol and recrystallized from proper solvent to give compounds 9a,b.

3-(Anthracen-9-yl)-1-phenyl-1,7-dihydro-4H-pyrazolo[3,4-d]pyrimidine-4,6(5H)-dithione (9a).

m.p. 210-212°C (MeOH). IR (KBr) ν cm^{-1} : 3264 (NH), 3049, (CHar), 1619 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ : 6.70-8.97 (m, 14 H, Ar-H), 9.12 (s, 1H, NH, D_2O exchangeable), 10.61 (s, 1H, NH, D_2O exchangeable). MS: m/z 436 [M^+] (11.98%). Anal. Calcd for $C_{25}H_{16}N_4S_2$ (436.55): C, 68.78; H, 3.69; N, 12.83; S, 14.69. Found: C, 68.44; H, 3.91; N, 12.46; S, 14.99.

3-(4-Methoxyphenyl)-1-phenyl-1,7-dihydro-4H-pyrazolo[3,4-d]pyrimidine-4,6(5H)-dithione (9b).

m.p. 124-126 °C (EtOH). IR (KBr) ν cm^{-1} : 3428, 3313 (NH), 3046, 3021, (CHar), 1620 (C=N). MS: m/z 366 [M^+] (2.18%). Anal. Calcd for $C_{18}H_{14}N_4OS_2$ (366.46): C, 59.02; H, 3.82; N, 15.30; S, 17.49. Found: C, 59.38; H, 3.50; N, 15.67; S, 17.15.

N-(3-(Anthracen-9-yl)-4-cyano-1-phenyl-1H-pyrazol-5-yl)-2-cyanoacetamide (10).

A mixture of 1a (0.01mol, 3.6 g) and cyanoacetic acid (0.01mol, 1g) in acetic anhydride (30 ml) was refluxed for 1.5 h, left to cool and then poured into ice/ water with vigorous stirring. The solid that separated out was collected by filtration, washed with water and recrystallized from ethanol to give compound 10.

m.p. 180-182 °C. IR (KBr) ν cm^{-1} : 3184 (NH),

3051, 30.16 (CHar), 2260, 2227 (CN), 1686 (C=O), 1620 (C=N). $^1\text{H-NMR}$ (400 MHz, DM-SO- d_6) δ : 4.50 (s, 2H, COCH₂CN), 7.53-8.88 (m, 14 H, Ar-H), 9.65 (s, 1H, NH, D_2O exchangeable). MS: m/z 427 [M^+] (4.59%). Anal. Calcd for $C_{27}H_{17}N_5O$ (427.46): C, 75.86; H, 4.01; N, 16.38. Found: C, 76.20; H, 4.22; N, 16.76.

3-(Anthracen-9-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (11).

A solution of 1a (0.01 mol, 3.6 g) and formamide (30 ml) was refluxed for 4 h, left to cool and then poured into ice/water. The solid so formed was collected by filtration, washed with water and recrystallized from methanol to produce the pyrazolopyrimidine derivative 11.

m.p. 234-236 °C. IR (KBr) ν cm^{-1} : 3276, 3140 (NH₂), 3080, 3050, (CHar), 1640 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ : 5.32 (s, 2H, NH₂, D_2O exchangeable), 7.51-8.72 (m, 15 H, Ar-H+ pyrimidine-H). MS: m/z 387 [M^+] (7.94%). Anal. Calcd for $C_{25}H_{17}N_5$ (387.44): C, 77.50; H, 4.42; N, 18.08. Found: C, 77.24; H, 4.55; N, 17.79.

Ethyl N-(4-cyano-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5-yl)formimidate (12).

A mixture of compound 1b (0.01 mol, 2.9 g), triethyl orthoformate (3 ml) and acetic anhydride (3 ml) was refluxed for 6 h. The solid that precipitated out upon cooling was collected by filtration, washed with ethanol and recrystallized from ethanol to give the formimidate derivative 12.

m.p. 130-132 °C. IR (KBr) ν cm^{-1} : 3072, 3028, (CHar), 2222 (CN), 1605 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO- d_6) δ : 1.17 (t, 3H, CH₃CH₂-O, J = 6.4 Hz), 3.88 (s, 3H, OCH₃), 4.11 (q, 2H, -CH₂O, J = 6.8 Hz), 7.16-7.99 (m, 9 H, Ar-H), 8.37 (s, 1H, N=CH). MS: m/z 346 [M^+] (12.02%). Anal. Calcd for $C_{20}H_{18}N_4O_2$ (346.38): C, 69.35; H, 5.24; N, 16.17. Found: C, 69.01; H, 4.90; N, 15.81.

General procedure for the synthesis of compounds 13a,b.

A mixture of compounds 1a,b (0.01 mol), malononitrile (0.01 mol, 0.66 g) and sodium ethoxide (0.23 g in 20 ml ethanol) was refluxed

for 7 h. The reaction mixture was left to cool. The solid so formed was collected by filtration, washed with ethanol and recrystallized from ethanol to produce compounds 13a,b.

2-(4-amino-3-(anthracen-9-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)acetonitrile (13a).

m.p. > 300 °C. IR (KBr) ν cm^{-1} : 3302, 3267 (NH_2), 3051, (CHar), 2256 (CN), 1646 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 2.16 (s, 2H, CH_2CN), 6.78-8.77 (m, 14 H, Ar-H), 10.69 (s, 2H, NH_2 , D_2O exchangeable). MS: m/z 426 [M^+] (6.25%). Anal. Calcd for $\text{C}_{27}\text{H}_{18}\text{N}_6$ (426.47): C, 76.04; H, 4.25; N, 19.71. Found: C, 76.40; H, 4.00; N, 20.01.

2-(4-Amino-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-6-yl)acetonitrile (13b).

m.p. 190-192 °C. IR (KBr) ν cm^{-1} : 3420, 3314 (NH_2), 3042, (CHar), 2254 (CN), 1610 (C=N). MS: m/z 356 [M^+] (4.44%). Anal. Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_6\text{O}$ (356.38): C, 67.40; H, 4.53; N, 23.58. Found: C, 67.78; H, 4.26; N, 23.96.

Ethyl-4-amino-3-(4-methoxyphenyl)-6-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxylate (14).

A mixture of compound 1b (0.01 mol, 2.9 g), ethyl acetoacetate (0.01 mol, 1.13 ml) and few drops of triethyl amine in acetic acid (30 ml) was refluxed for 3 h. After cooling, the reaction mixture was poured into ice/HCl. The solid that separated out was collected by filtration, washed with water and recrystallized from ethanol to give the pure ester 14.

m.p. 122-124 °C. IR (KBr) ν cm^{-1} : 3431, 3221 (NH_2), 3030, (CHar), 1708 (C=O), 1608 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 1.19 (t, 3H, $\text{CH}_3\text{CH}_2\text{-O}$, $J = 6.2$ Hz), 2.48 (s, 3H, CH_3) 3.77 (s, 3H, CH_3O), 4.28 (q, 2H, $-\text{CH}_2\text{O}$, $J = 6.4$ Hz), 6.82-7.94 (m, 9 H, Ar-H), 9.87 (s, 2H, NH_2 , D_2O exchangeable). MS: m/z 402 [M^+] (2.76%). Anal. Calcd for $\text{C}_{23}\text{H}_{22}\text{N}_4\text{O}_3$ (402.45): C, 68.64; H, 5.51; N, 13.93. Found: C, 69.01; H, 5.22; N, 14.14.

General procedure for the synthesis of compounds 15a,b.

A mixture of 1a,b (0.01 mol) and hydrazine hydrate (0.02 mol, 1 ml) in 30 ml ethanol was refluxed for 3 h. The reaction mixture was left to cool. The solid so formed was collected by filtration, washed with ethanol and recrystallized from ethanol to give the pyrazolopyrazole derivatives 15a,b.

4-(Anthracen-9-yl)-6-phenyl-1,6-dihydropyrazolo[3,4-c]pyrazol-3-amine (15a).

m.p. 210-212 °C. IR (KBr) ν cm^{-1} : 3300 (NH_2), 3076, 3049, (CHar), 1621 (C=N). MS: m/z 375 [M^+] (5.55). Anal. Calcd for $\text{C}_{24}\text{H}_{17}\text{N}_5$ (375.43): C, 76.78; H, 4.56; N, 18.65. Found: C, 77.13; H, 4.33; N, 19.00.

4-(4-Methoxyphenyl)-6-phenyl-1,6-dihydropyrazolo[3,4-c]pyrazol-3-amine (15b).

m.p. 156-158 °C. IR (KBr) ν cm^{-1} : 3428 br. (NH_2 , NH), 3037, 3009 (CHar), 1623 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 3.83 (s, 3H, CH_3O), 7.04-7.83 (m, 9 H, Ar-H), 8.63 (s, 2H, NH_2 , D_2O exchangeable) 9.99 (s, 1H, NH, D_2O exchangeable). MS: m/z 305 [M^+] (5.03%). Anal. Calcd for $\text{C}_{17}\text{H}_{15}\text{N}_5\text{O}$ (305.33): C, 66.87; H, 4.95; N, 22.94. Found: C, 67.22; H, 5.20; N, 23.29.

4-Amino-3-(4-methoxyphenyl)-1-phenyl-1,7-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione (16).

To a mixture of compound 1b (0.01 mol, 2.9 g) and thiourea (0.01 mol, 0.72 g), sodium ethoxide (0.23 g in 20 ml ethanol) was added and the reaction mixture was refluxed for 7h. The solid that separated out after cooling was collected by filtration, washed with ethanol and recrystallized from ethanol to furnish compound 16.

m.p. 186-188 °C. IR (KBr) ν cm^{-1} : 3313, 3283 (NH_2 , NH), 3049 (CHar), 1600 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 3.78 (s, 3H, CH_3O), 6.69-7.82 (m, 9 H, Ar-H), 9.30 (s, 1H, NH, D_2O exchangeable) 10.11 (s, 2H, NH_2 , D_2O exchangeable). MS: m/z 349 [M^+] (10.13%). Anal. Calcd for $\text{C}_{18}\text{H}_{15}\text{N}_5\text{OS}$ (349.41): C, 61.87; H, 4.33; N, 20.04; S, 9.18. Found: C, 62.24; H, 4.08; N, 19.70; S, 8.88.

General procedure for the synthesis of compounds 17a,b.

A mixture of compounds 1a,b (0.02 mol) and triethylamine (2 ml) was dissolved in 30 ml ethanol and refluxed for 7 h. The reaction mixture was left to cool. The solid so formed was collected by filtration, washed with ethanol and recrystallized from ethanol to give 17a,b.

6-(5-Amino-3-(anthracen-9-yl)-1-phenyl-1H-pyrazol-4-yl)-3-(anthracen-9-yl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (17a).

m.p. 212-214 °C. IR (KBr) ν cm^{-1} : 3301 (NH_2), 3076, 3048 (CHar), 1621 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 6.78-8.77 (m, 28 H, Ar-H), 9.10 (s, 2H, NH_2 , D_2O exchangeable) 10.65 (s, 2H, NH_2 , D_2O exchangeable). MS: m/z 720 [M^+] (16.65%). Anal. Calcd for $\text{C}_{48}\text{H}_{32}\text{N}_8$ (720.82): C, 79.98; H, 4.43; N, 15.55. Found: C, 79.70; H, 4.14; N, 15.90.

6-(5-Amino-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (17b).

m.p. 166-168 °C. IR (KBr) ν cm^{-1} : 3424, 3313 (NH_2), 3045, 3020 (CHar), 1596 (C=N). MS: m/z 580 [M^+] (1.02%). Anal. Calcd for $\text{C}_{34}\text{H}_{28}\text{N}_8\text{O}_2$ (580.64): C, 70.33; H, 4.86; N, 19.30. Found: C, 70.68; H, 5.12; N, 18.98.

General procedure for the synthesis of compounds 18a,b.

Sodium azide (0.01 mol, 0.65 g) and ammonium chloride (0.01 mol, 0.53 g) were added to a solution of compounds 1a,b (0.005 mol) in DMF (15 ml). The reaction mixture was refluxed for 7 h, left to cool at room temperature, then poured into ice/water. The solid that deposited was collected by filtration, washed with water and recrystallized from petroleum ether 80-100 °C to produce pure solids of compounds 18a,b.

3-(Anthracen-9-yl)-1-phenyl-4-(1H-tetrazol-5-yl)-1H-pyrazol-5-amine (18a).

m.p. 206-208 °C. IR (KBr) ν cm^{-1} : 3300, 3265 (NH_2 , NH), 3075, 3049 (CHar), 1620 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 6.77-8.97 (m, 14 H, Ar-H), 9.09 (s, 2H, NH_2 , D_2O exchangeable) 10.66 (s, 1H, NH, D_2O exchangeable). MS: m/z 403 [M^+] (4.84%). Anal. Calcd for $\text{C}_{24}\text{H}_{17}\text{N}_7$ (403.44): C, 71.45; H, 4.25; N, 24.30. Found: C,

71.75; H, 3.99; N, 23.98.

3-(4-Methoxyphenyl)-1-phenyl-4-(1H-tetrazol-5-yl)-1H-pyrazol-5-amine (18b).

m.p. 118-120 °C. IR (KBr) ν cm^{-1} : 3427, 3313 (NH_2 , NH), 3046, 3020 (CHar), 1597 (C=N). MS: m/z 333 [M^+] (1.28%). Anal. Calcd for $\text{C}_{17}\text{H}_{15}\text{N}_7\text{O}$ (333.35): C, 61.25; H, 4.54; N, 29.41. Found: C, 60.94; H, 4.76; N, 29.08.

General procedure for the synthesis of compounds 19a,b.

To a mixture of compounds 18a,b (0.005 mol) and benzaldehyde (0.005 mol, 0.5 ml) in methanol (25 ml), HCl (1.5 ml) was added. The reaction mixture was refluxed for 16 h, left to cool, and then pour into cold solution of sodium carbonate. The solid that separated out was collected by filtration, washed with water several times and recrystallized from ethanol to give compounds 19a,b, respectively.

9-(Anthracen-9-yl)-5,7-diphenyl-6,7-dihydro-5H-pyrazolo[4,3-e]tetrazolo[1,5-c]pyrimidine (19a).

m.p. 228-230 °C. IR (KBr) ν cm^{-1} : 3266 (NH), 3048, 3024 (CHar), 1601 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 5.45 (s, 1H, CH-pyrimidine), 6.71-8.97 (m, 19 H, Ar-H), 10.62 (s, 1H, NH, D_2O exchangeable). MS: m/z 491 [M^+] (1.81%). Anal. Calcd for $\text{C}_{31}\text{H}_{21}\text{N}_7$ (491.55): C, 75.75; H, 4.31; N, 19.95. Found: C, 76.04; H, 4.00; N, 20.30.

9-(4-Methoxyphenyl)-5,7-diphenyl-6,7-dihydro-5H-pyrazolo[4,3-e]tetrazolo[1,5-c]pyrimidine (19b).

m.p. 162-164 °C. IR (KBr) ν cm^{-1} : 3308 (NH), 3055, 3025 (CHar), 1608 (C=N). MS: m/z 421 [M^+] (11.88%). Anal. Calcd for $\text{C}_{24}\text{H}_{19}\text{N}_7\text{O}$ (421.45): C, 68.40; H, 4.54; N, 23.26. Found: C, 68.78; H, 4.80; N, 22.92.

10-(4-Methoxyphenyl)-6,8-diphenyl-5,8-dihydropyrazolo[4,3-f]tetrazolo[1,5-d][1,4]diazepine (20).

A mixture of 18b (0.005 mol, 1.67 g), phenacyl bromide (0.005 mol, 1.125 g) and sodium acetate (1.85 g) was refluxed in ethanol (25 ml) for 10 h. The reaction mixture was left to cool, then

poured into ice/water. The solid so formed was collected by filtration, washed with water and recrystallized from methanol to obtain the pure diazpine derivative 20.

m.p. > 300 °C. IR (KBr) ν cm^{-1} : 3058, 3025 (CHar), 1600 (C=N). $^1\text{H-NMR}$ (400 MHz, DM-SO-d_6) δ : 3.74 (s, 3H, OCH_3), 4.21 (s, 2H, CH_2 -diazpine), 6.71-8.97 (m, 14 H, Ar-H). MS: m/z 433 [M^+] (6.09%). Anal. Calcd for $\text{C}_{25}\text{H}_{19}\text{N}_7\text{O}$ (433.46): C, 69.27; H, 4.42; N, 22.62. Found: C, 68.92; H, 4.70; N, 23.00.

9-(Anthracen-9-yl)-7-phenyl-6,7-dihydro-5H-pyrazolo[4,3-e]tetrazolo[1,5-c]pyrimidine-5-thione (21).

A mixture of 18a (0.005 mol, 2.17 g), carbon disulfide (0.37 g) in pyridine (10 ml) was refluxed for 10 h. The reaction mixture was left to cool, and then poured into ice/HCl. The solid that separated out was collected by filtration, washed with water and recrystallized from methanol to produce 21.

m.p. 188-190 °C. IR (KBr) ν cm^{-1} : 3300 (NH), 3075, 3048 (CHar), 1621 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 6.71-8.96 (m, 14 H, Ar-H), 10.65 (s, 1H, NH, D_2O exchangeable). MS: m/z 446 [$\text{M}+1$] (3.37%). Anal. Calcd for $\text{C}_{25}\text{H}_{15}\text{N}_7\text{S}$ (445.50): C, 67.40; H, 3.39; N, 22.01; S, 7.20. Found: C, 67.78; H, 3.65; N, 22.38; S, 7.57.

5-Amino-3-(anthracen-9-yl)-1-phenyl-1H-pyrazole-4-carboxamide (22).

To a solution of 1a (0.01 mol, 3.6 g) in ethanol (30 ml), ammonium solution (50 ml) and hydrogen peroxide (10 ml) were added. The reaction mixture was stirred at room temperature for 3 h. The solid that obtained was collected by filtration, washed with water and recrystallized from ethanol to obtain the amide derivative 22.

m.p. 200-202 °C. IR (KBr) ν cm^{-1} : 3432, 3301 (NH_2), 3074, 3049 (CHar), 1676 (C=O), 1620 (C=N). $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ : 6.78-8.76 (m, 14 H, Ar-H), 9.09 (s, 2H, NH_2 , D_2O exchangeable), 10.65 (s, 2H, NH_2 , D_2O exchangeable). MS: m/z 378 [M^+] (5.91%). Anal. Calcd for $\text{C}_{24}\text{H}_{18}\text{N}_4\text{O}$ (378.43): C, 76.17; H, 4.79; N, 14.81. Found: C, 75.88; H, 5.02; N, 15.18.

Biological assessment

a) Antimicrobial Activity

The antimicrobial activity of the tested compounds was determined using the disc diffusion technique [24, 25] by preparing discs containing 1.9 – 1000 $\mu\text{g/ml}$ of each compound against gram positive *Staphylococcus aureus*, *Bacillus subtilis* and gram negative *Escherichia coli*, *Pseudomonas aeruginosa*. The anti-fungal activities of the compounds were tested against two fungi *Candida albicans*, *Aspergillus flavus*. Different dilutions were prepared. The plates were incubated at 37 °C for 24 h. for bacteria and at 28 °C for 72 h for fungi. The standard antibiotic ampicillin and antifungal colitrimazole were used as references. At the end of the incubation period, the minimum inhibitory concentrations (MIC) values were recorded (Table 1) 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 17b and 22. While for bacteria, 8a, 8b, 9b, 20 and 22 for Gram negative and for 1b, 8a, 8b, 9b, 17b, 20 and 22 Gram positive. In addition, Gram negative bacteria were more sensitive to the compounds compared with Gram positive ones.

b) Cytotoxicity assay

Materials and methods

Cell line

Some of the synthesized compounds were tested against two human tumor cell lines name-

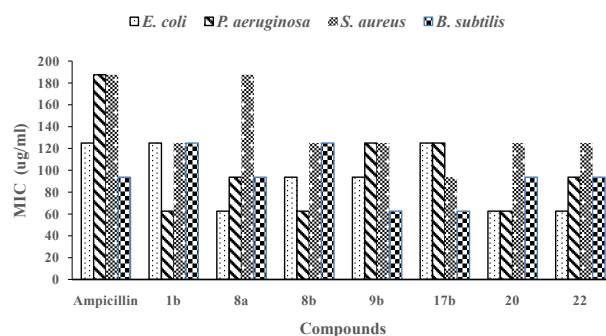


Fig 1: MIC of most potent compounds against bacteria

ly; mammary gland breast cancer (MCF-7) and human skin cancer (HFB4). The cell line were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Chemical reagents

The reagents RPMI-1640 medium , MTT , DMSO and 5-fluorouracil (sigma co., St. Louis,

USA), Fetal Bovine serum (GIBCO, UK). 5-fluorouracil was used as a standard anticancer drug for comparison.

MTT assay

The different cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay [26, 27]. This colorimetric assay is based on the

Table 1: Antimicrobial and Antimycotic Activities in terms of MIC ($\mu\text{g/mL}$).

Compound	E. coli	Pseudomonas aeruginosa	S. aureus	B. subtilis	C. Albicans	A. flavus
1a	125	93.7	187.5	187.5	11.7	7.8
1b	125	62.5	125	125	7.8	11.7
2	NA	750	NA	NA	187.5	125
3	NA	NA	NA	NA	250	125
4	NA	NA	NA	NA	187.5	93.7
5	NA	500	750	500	93.7	46.9
6	750	500	NA	750	125	93.7
7a	93.7	93.7	250	187.5	15.6	11.7
7b	NA	750	NA	500	93.7	46.9
8a	62.5	93.7	187.5	93.7	7.8	11.7
8b	93.7	62.5	125	125	15.6	11.7
9a	250	250	375	250	31.2	15.6
9b	93.7	125	125	62.5	11.7	11.7
10	250	187.5	375	250	23.4	15.6
11	500	375	750	500	62.5	31.2
12	NA	NA	750	750	125	62.5
13a	375	250	500	375	31.2	23.4
13b	250	500	375	250	62.5	31.2
14	750	375	NA	750	125	62.5
15a	250	187.5	375	250	46.9	23.4
15b	375	250	250	375	31.2	62.5
16	125	187.5	187.5	125	15.6	11.7
17a	187.5	125	250	187.5	23.4	11.7
17b	125	125	93.7	62.5	5.8	5.8
18a	187.5	187.5	250	187.5	15.6	15.6
18b	250	250	187.5	93.7	11.7	11.7
19a	375	250	500	375	46.9	23.4
19b	500	375	250	250	31.2	62.5
20	62.5	62.5	125	93.7	5.8	7.8
21	375	250	500	375	62.5	31.2
22	62.5	93.7	125	93.7	7.8	5.8
Ampicillin	125	187.5	187.5	93.7	----	----
Colitrimazole	----	----	----	----	7.8	5.8

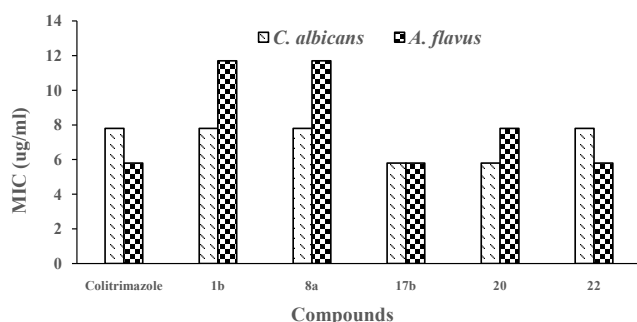


Fig 2: MIC of most potent compounds against fungi

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 100 µg/ml streptomycin at 37 C in a 5% CO₂ incubator. The cells were seeded in a 96-well plate at a density of 1.0x10⁴ cells/well [25] at 37 C for 48 h under 5% CO₂. After incubation the cells were treated with different concentration 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 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

Table 2: Cytotoxic activity of some compounds against human tumor cells

Compounds	In vitro Cytotoxicity IC ₅₀ (µg/ml)•	
	MCF7	HFB4
5-FU	5.4±0.20	8.8±0.52
1a	10.5±0.92	13.4±1.08
2	64.9±4.03	95.6±5.24
5	55.7±3.75	71.1±4.37
8b	8.2±0.67	19.4±1.53
9a	38.1±2.80	32.2±2.64
12	82.4±4.58	85.7±4.76
15a	32.8±2.44	46.8±3.15
17b	16.0±1.14	7.8±0.57
17a	20.5±1.56	26.6±2.12
21	45.8±3.11	54.3±3.79
22	5.2±0.39	9.8±0.86

• IC₅₀ (µg/ml) : 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic) , 5-FU = 5-fluorouracil.

800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/ A570 of untreated sample) X 100. The results of cytotoxic activity of the tested compounds are listed, in terms of IC₅₀ (mg/ml), in table 2.

The results in table 2 revealed that compounds 8b, 22 showed very strong cytotoxic activity and compounds 1a, 17a showed strong cytotoxic activity against (MCF7), while compounds 17b, 22 showed very strong cytotoxic activity and compounds 1a, 8b showed strong cytotoxic activity against (HFB4).

Conclusion

In this paper the enaminonitriles 1a,b were used as a key starting materials to synthesize a variety of heterocyclic ring systems containing mainly a pyrazole moiety. The newly synthesized compound were tested against gram positive *Staphylococcus aureus*, *Bacillus subtilis* and gram negative *Escherichia coli*, *Pseudomonas aeruginosa*, two fungi *Candida albicans*, *Aspergillus flavus* and two human tumor cell lines namely; mammary gland breast cancer (MCF-7) and human skin cancer (HFB4). Some of the tested compounds showed a high antimicrobial and very strong cytotoxic activities.

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