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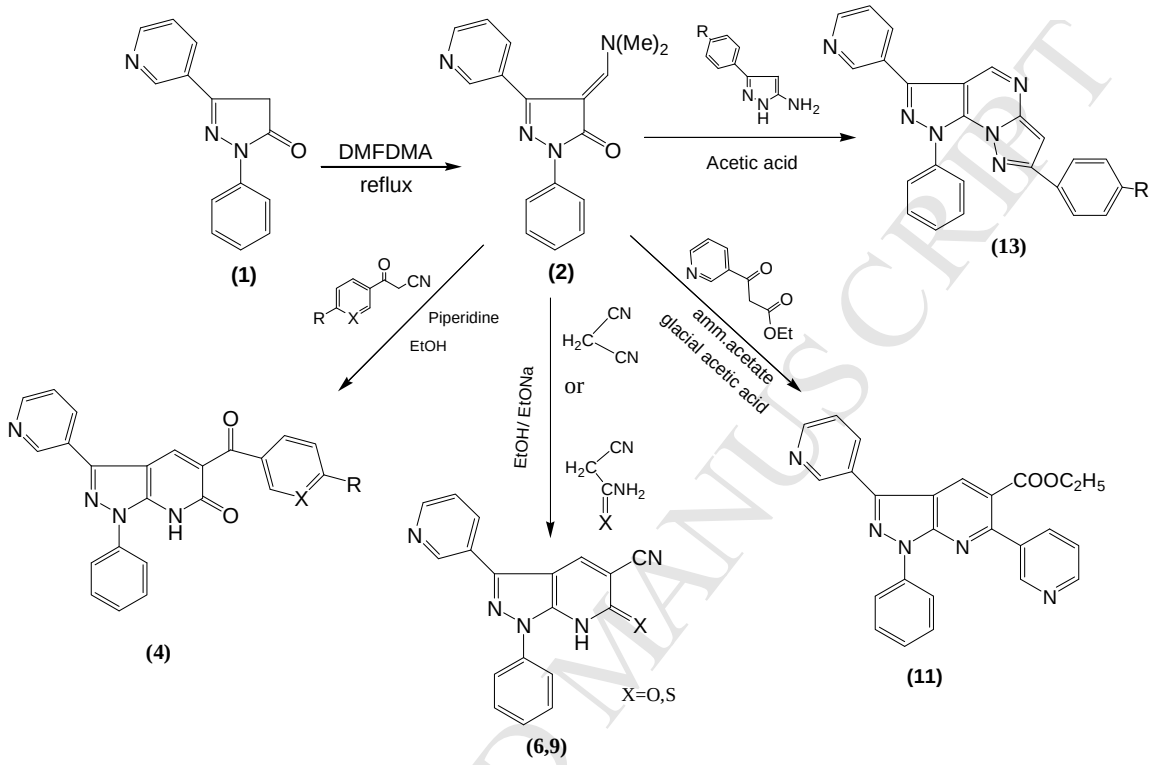
New pyrazolopyridine compounds were prepared by conventional heating and microwave irradiation technique. The synthesized compounds were screened for antioxidant, antitumor and antimicrobial activities.



Microwave- assisted synthesis of some new pyrazolopyridines and their antioxidant, antitumor and antimicrobial activities.

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ABSTRACT

The chemical behavior of 4-(dimethylaminomethylene)-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-5(4*H*)-one (enaminone) (**2**) towards some active methylene reagents has been reported to give pyrazolopyridine derivatives. All the reactions were carried out by conventional heating and microwave irradiation technique. The antioxidant activity of the prepared compounds was studied using 1, 1-phenyl-2-picrylhydrazyl (DPPH) assay. Compounds **(4c)** and **(4d)** showed the highest activity. The antitumor activity against liver and breast cell lines was tested. Compounds **(6), (9)** and (**11)** showed the highest activity for liver cell line while compounds (**6)** and **(9)** showed the highest activity for breast cell line. Compounds **(4a-d)** were screened for their antibacterial activity against Gram-positive, Gram-negative bacteria and antifungal activity.

*Keywords***:** enaminones, microwave technology, antioxidant, antitumor, antimicrobial activities.

1. Introduction

The chemistry of the enaminones is potentially an area of considerable scope and has recently received a very important attention. They have multiple electrophilic and nucleophilic centers and undergo a variety of cycloaddition and self condensation reactions. For these reasons they considered versatile synthetic intermediates [1-5]. Pyrazolo[3,4-b]pyridine skeleton had proven to be interesting class of heterocycles due to diverse biological properties including antitubercular, antibacterial and antioxidant activities [6- 15]. Recently, the pyrazolo pyridine compounds found a great importance in the synthesis of some fluorescence dyes [16] and as anti corrosion protection of stainless steel in aggressive media [17].

Microwave irradiation has broken new grounds in synthetic organic chemistry not only in term of the reduction of reaction time, but also simplicity of reaction work up, besides energy saving, high efficiency and environmental benign procedures [18-20]. In this paper, we have continued seeking the development of new and simple methods for the synthesis of pyrazolopyridine derivatives.

2. Results and Discussion

2.1. Chemistry

In regards to our previous work [21] targeting to explore the synthesis of new pyrazolopyridine derivatives because of their role as pharmacophores of considerable importance [8]. We like to report here the condensation of dimethylformamide-dimethylacetal (DMFDMA) with the active methylene group in the pyrazolone system in dry condensation to give 4-(dimethylamino methylene)-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-5(4*H*)-one (enaminone) (**2**) (Scheme 1). This interesting compound (**2)** enables us to continue our research for the synthesis of new series of pyrazolopyridine derivatives.

This reaction normally producing two stereoisomers (**2*E***) and (**2*Z***), the formation of isomer (**2*Z*)** was not observed (TLC and ^{1}H NMR spectrum), which revealed a singlet at 7.15 ppm characteristic of the exocyclic (C=CH) proton for isomer (*E*), while this proton was reported to appear at 6.90 ppm for isomer (***Z***) [22].

Of noteworthy observation the reaction of pyrazolone with aromatic aldehydes always giving the corresponding arylidenes in the isomer (***E*)** [23].

< **Scheme 1** >

The reaction of enaminone (**2**) with some *β*- ketonitriles **(3a-d)** in ethanolic medium in the presence of piperidine as catalyst gave 5-substituted aroyl-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*] pyridin-6(7*H*)-one derivatives (**4a-d**) (Scheme 2). The IR spectra of compounds (**4a-d**) showed absorption at ν 1597-1663 cm^{-1} for exocyclic carbonyl group and carbonyl of pyridinone nucleus and ν 3389-3432 cm^{-1} for imine or hydroxyl group.

< **Scheme 2** >

The reaction of enaminone (**2**) with malononitrile in refluxing ethanolic sodium ethoxide solution may give two possible products: 6-imino-1-phenyl-3-(pyridin-3-yl)-1,6-dihydropyrano[2,3-c]pyrazol-5-carbonitrile (**5)** or 6-oxo-1-phenyl-3-(pyridin-3-yl)-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridin-5-carbonitrile (**6**) (Scheme 3). Structure of compound **(6**) was ruled out based on IR and ^{1}H NMR spectral data. The IR spectrum revealed two absorption bands at ν 1651 and 2198 cm^{-1} for the amidic carbonyl and C≡N groups respectively. The ^{1}H NMR spectrum of compound 6, showed the absence of imine signal. It is worth noting that the reaction of enaminone (**2**) with cyanoacetamide gave the same structure **(6)** obtained by the reaction of **(2)** with malononitrile. This is clear by (TLC, m.p., mixed m.p. and spectral data) (see Experimental). In a similar behavior the reaction of enaminone (**2**) with cyanothioacetamide gave 1-phenyl-3-(pyridin-3-yl)-6-thioxo-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridin-5-carbonitrile **(9)** not compound (**8**) ruled out by IR spectral data which revealed two absorption bands at ν 1373 and 2202 cm^{-1} corresponding to amidic thione and cyano groups respectively. These results are in accordance with that previously reported work [15]. The reaction of pyrazolopyridone (**6**) with phosphorous pentasulphide in boiling pyridine gave compound (**9**) (TLC, m.p., mixed m.p and spectral data).

< **Scheme 3** >

The reaction of enaminone **(2)** with ethyl-3-oxo-3-(pyridin-3-yl)propanoate **(10)** in boiling acetic acid and ammonium acetate gave ethyl -1-phenyl-3,6-di(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-5-carboxylate **(11)** (Scheme 4). The IR spectrum of compound **(11)** showed absorption band at ν 1615 cm^{-1} for the carbonyl of ester group.

< **Scheme 4** >

The reaction of enaminone **(2)** with 3-substituted-5-amino-1H-pyrazoles **(12 a-b)** in boiling acetic acid gave 7- aryl-3-(pyridin-3-yl)-1*H*-bispyrazolo [3,4-e:2',3'-a] pyrimidine derivatives **(13a-c)** (Scheme 5). This reaction was accomplished by the nucleophilic attack of amino group to the double bond via Michael addition reaction beginning of elimination of the dimethylamino group followed by dehydration to give **(13)**.

< **Scheme 5** >

All the prepared compounds (Schemes 2, 3, 4 and 5) were done by conventional heating and microwave irradiation technique. From the comparison of the results cited in **(Table 1)**, it is clear that the reaction time of is reduced from (8-15 hr) to (15-25 min) and the yield is increased from (60-80%) to (82-93%).

< **Table 1**>

3. Pharmacology

3.1 Antioxidant evaluation

Considerable progress has been made in recent years in relating ageing to oxidation in biological cells. The reactive oxygen species (ROS), cause of oxidation in biological cells are basically involved in detoxification of invading organism and chemicals, ROS also initiate lipid peroxidation in healthy cells leading to diverse pathologies such as Alzheimer's disease, atherosclerosis, diabetes, Parkinson's disease, etc. [24]. Thus reduction of the rate of these life-linacting metabolic processes by use of chemicals [25, 26] has been a subject of current research.

An imbalance between antioxidants and ROS result in oxidative stress, leading to cellular damage. Several authors demonstrated that antioxidant intake is inversely related to mortality from coronary heart disease and to incidence of heart attack [27-29]. Antioxidant defense system of our body, which includes vitamin C (ascorbic acid), vitamin E (α - tocopherol, γ tocopherol), carotenoids (β- carotene, α- carotene, β- cryptoxathin, butein, zeaxanthin, lycopene and several polyphenolic compounds including flavonoids (catechins, flavonals, flavones and iso flavonoids) [30- 32]. Dietary can also impede carcinogenesis by scavenging oxygen radicals as interfering with the binding of carcinogens to DNA.

3. 1. 2 Materials and methods

DPPH radical scavenging assay

The antioxidant activities of prepared compounds were assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to Ardestani and Yazdanparast with ascorbic acid as standard [33]**.** Each tested sample and ascorbic acid (reference compound) (50 µg) was dissolved in 1 ml DMSO. The dissolved sample (250 µl) was added to 1 ml DPPH/DMSO solution (6 mg/50 ml) and the total volume was adjusted to 3 ml with DMSO. An equal amount of DMSO was used as a control. After vortexing the mixture was incubated for 30 min in dark at room temperature. Absorbance was measured using a spectrophotometer at 517 nm.

DPPH radical scavenging % = [1 – (A sample/ A control)] x 100.

Serial dilutions (5-50 µg/ml) of each compound were measured by the same assay to obtain EC50 (the amount of compound that gives half-maximal scavenging response) according to Brand-Williams et al [34].

3.1.2 Results and discussion

Radical scavenging activities of the ten compounds were measured. The results were summarized in Table 2, Figure 1 and Figure 2. Compound **(4c)** has the most potent antioxidant activity that was found to be very close to the values of standard ascorbic acid due to the presence of fluorine group in the aromatic ring. Compound **(11)** has no antioxidant activity. The other eight compounds were considerably less effective radical scavengers in the order **4d > 13c > 4b > 4a > 13b > 9 > 13a > 6**.

< **Table 2**>

<**Figure 1**>

<**Figure 2**>

3.2 Antitumor evaluation

The search for novel drugs is still a priority goal for cancer therapy, due to the rapid development of resistance to multiple chemotherapeutic drugs. In addition, the high toxicity usually associated with cancer chemotherapy drugs and their undesirable side effects increase the demand for novel antitumor drugs active against untreatable tumors with fewer side effects and/or with greater therapeutic efficiency [35]. Breast cancer is a major health problem worldwide [36]. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 [37]. Liver cancer rapidly reduces quality of life and typically causes death six months - one year from diagnosis [38]. Globally, it is the fifth leading cause of cancer and the third leading cause of cancer death. This cancer varies widely in incidence throughout the world, with rising incidence in Egypt [39].

3. 2. 1 Antitumor activity

The anticancer activities of prepared compounds were investigated on two human cancer cell lines, HepG-2 (liver cancer) and MCF7 (breast cancer) according to SRB assay [40].

3.3.2 Results and discussion

Antitumor activities of the tested compounds were measured used SRB assay for breast cancer (Figure 3) and liver cancer (Figure 4). These results were tabulated in Table 3. The overall results indicated that, some compounds have antitumor activity through induction of apoptosis in MCF7 breast cancer cell line, suggesting that they might be a potential alternative agent for human breast cancer therapy. The ten

compounds can be ordered according to their anti-breast cancer as **9> 6> 4d> 11> 4c>4b> 4a> 13c> 13b> 13a**. Some compounds have antitumor activity through induction of apoptosis in HEPG2 cancer cell line, suggesting that they might be a potential alternative agent for human hepatic cancer therapy. The ten compounds can be ordered according to their anti-hepatic cancer as **6> 11> 9> 4c> 4b> 4d> 4a> 13c> 13b> 13a**.

< **Table 3**>

<**Figure 3**>

<**Figure 4**>

3.3 Antimicrobial evaluation

Antimicrobial compounds could be used as antibacterial, antifungal and antiviral agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics [41, 42].

3. 3. 1 *Antibacterial assay*

The microorganisms used in this study included Gram-positive bacteria (*Bacillus*), Gram-negative bacteria (*E. coli*, *E. cloaca*, *serratia*) in addition to some fungal species (*F. oxysporum*, *P. expansum*). The strains under study were obtained from the culture collection of Microbiology Unit (Faculty of Science, Tanta University, Tanta, Egypt). Bacteria were cultured on nutrient agar and the fungus was cultured on sabaroud agar slopes. Antimicrobial activities of the synthesized compounds were tested in vitro on nutrient agar at 30ºC after 24 h by the cut-plug method according to Pridham et al. [43].

3.3.2. Results and discussion

The results of the antimicrobial activities are summarized in (**Table 4**). Antimicrobial activities of compounds **(4a-d)** showed negative results for the *Bacillus* and *F. oxysporum* strains. Only compound (**4b)** showed a good antimicrobial activity for *P. expansum* while other three compounds gave negative results. On the other hand, the four compounds gave relatively good antibacterial activities for *Serratia* in the order

of **4c > 4b, 4d > 4a** and an excellent activities for *E. coli* and *E. cloaca* in the order of **4d > 4b > 4c > 4a** and **4c > 4d > 4b > 4a**, respectively.

<**Table 4 >**

4. Experimental

4.1. General

Melting points were recorded on a Gallenkamp melting point apparatus and are reported uncorrected. The infrared spectra were recorded on Perkin–Elmer FTIR 1430 spectrophotometer using KBr disk technique. The ^{1}H NMR spectra were recorded at 25^{0} C in DMSO-d_6 with TMS as an internal standard. Chemical shifts are reported in ppm and results are expressed as δ values on a Bruker AC spectrometer (300 MHz). Reactions were conducted under microwave irradiation in closed vessels under magnetic stirring by using Synthos 3000 dual magnetrons system and with maximum power of 1000 W. Reaction progress was monitored by thin layer chromatography (TLC) using benzene/acetone (2/1 by volume) as eluent. Mass spectra were measured on a Finnigan MAT 8222 EX mass spectrometer at 70 eV. Absorbance was measured using a Jenway 6305 spectrophotometer. Microanalyses were preformed on Perkin.Elemer 2400 Elemental Analyzer at Microanalytical center at Cairo University.

Compounds **(3a-d**) were prepared according literature procedures [44, 45], compound **(10)** [46] and compounds **(12a-c)** [47].

4.2. 4-(Dimethylaminomethylene)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-5(4H)-one (2).

A mixture of 1 (2.99 g, 12.6 mmol) and DMFDMA (2.519 ml) was heated under reflux for 10 h. The reaction mixture was triturated with ethanol to give a solid product. Filtration and recrystallized from ethanol to give faint green crystals in yield (69%); m.p. 220-222^{o} C; IR (KBr) ν_{max} / cm^{-1}= 1400 (C-N), 1602 (C=N $_{pyrazole\ ring}$), 1671 (C=O), 2918 (Aliph.-H), 3013 (Arom.-H). ^{1}HNMR (DMSO- d_6): δppm = 3.35 (s, 6H, 2CH_3), 7.13 (s, 1H, CH), 7.33- 7.74 (m, 5H, Ar-H), 8.12-8.94 (m, 4H, pyridine ring). MS m/z (%): M = 292 (74%), 248 (77), 222(11), 194(24), 159 (35), 116 (89), 77 (100), 63 (24), 51 (44).

Anal.; For $C_{17}H_{16}N_4O$ (292.34) Calcd.: C 69.85 ; H 5.52; N 19.17% Found: C 70.27 ; H 5.79; N 19.42%.

*4.3. General procedure for the synthesis of 5-aroyl-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridin-6(7H)-one (**4a-d**).*

4.3.1. Method (A):

To a solution of 2 (0.49 g, 1.7 mmol) in ethanol (20 ml) and piperidine (0.5 ml) as a catalyst was added substituted β- ketonitriles **3a-d** (1.7 mmol). The reaction mixture was heated under reflux for (8- 10) h and the progress of the reaction was monitored by TLC using benzene/acetone (2:1) as eluent. The solvent was evaporated under reduced pressure; the oil residue was treated with petroleum ether 40-60^0 C (3 x 10 ml) and recrystallized from ethanol to give (**4a-d**).

4.3.2. Method (B):

The procedure was similar to that described in Method (A) except that the mixture was capped in closed vessels and irradiated in a microwave oven at 130 ^{0}C for 20 min using Synthos 3000 (500 W). The reaction was worked as usual and the obtained solid was recrystallized from ethanol to give **(4a-d)**.

4.3.3. 5-Nicotinoyl-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridin-6(7H)-one **4a**

Brown crystals in yield (75%); m.p. 290-292^o C; IR (KBr) ν_{max} / cm^{-1}= 1663 (C=O), 2195 (Aliph.-H), 3074 (Arom.-H), 3389 (NH or OH). ^{1}HNMR (DMSO- d_6): δppm = 7.33- 7.74 (m, 5H, Ar-H), 8.15-8.77 (m, 8H, pyridine ring), 8.92 (s, 1H, broad NH), 8.97 (s, 1H, pyridone ring). MS m/z (%): M = 392 (29%), 332(98), 289(31), 277(91), 264(100), 242(31), 218(38), 207(42), 181(38), 152(40), 95(64), 50(38). Anal.; For $C_{23}H_{15}N_5O_2$ (393.44) Calcd.: C 70.22; H 3.84; N 17.80 Found: C 70.44 H 4.01; N 17.99%.

4.3.4. 5-(4-Methylbenzoyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridin-6(7H)-one **4b**

Buff crystals in yield (67%); m.p. 276-277° C; IR (KBr) ν_{max} / cm^{-1}= 1639 (C=O), 2193 (Aliph.-H), 3076 (Arom.-H), 3432 (NH or OH). ^{1}HNMR (DMSO- d_6): δppm = 2.25 (s, 3H, CH_3), 7.31- 7.66 (m, 9H, Ar-H), 8.22-8.83 (m, 4H, pyridine ring), 8,93 (s, 1H, broad NH), 8.98 (s, 1H, pyridone ring). MS m/z (%): M = 406 (99%), 392(35), 379(49), 351(75), 324(51), 230(44), 207(58), 195(100), 169(61), 124(43), 110(74), 92(76), 68(59). Anal.; For $C_{25}H_{18}N_4O_2$ (406.44) Calcd.: C 73.88; H 4.46; N 13.78% Found: C 74.11; H 4.65; N 13.95%.

4.3.5 5-(4-Fluorobenzoyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridin-6(7H)-one **4c**

Faint brown crystals in yield (70%); m.p. 300-302° C; IR (KBr) ν_{max} / cm^{-1}= 1597 (C=O), 2196 (Aliph.-H), 3046 (Arom.-H), 3430 (NH or OH). ^{1}HNMR (DMSO- d_6): δppm = 7.33- 7.68 (m, 9H, Ar-H), 8.15-8.77 (m, 4H, pyridine ring), 8.90 (s, 1H, broad NH), 8.98 (s, 1H, pyridone ring). MS m/z (%): M = 410(68%), 394(43), 380(46), 363(73), 308(60), 260(77), 233(92), 207(46), 183(78), 127(46), 95(100), 77(95), 65(55), 53(51). Anal.; For $C_{24}H_{15}FN_4O_2$ (410.40) Calcd.: C 70.24; H 3.68; F 4.63; N 13.65, % Found: C 70.63; H 3.89; F 4.73 N 13.89%.

4.3.6. 5-(4-Chlorobenzoyl)-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridin-6(7H)-one **4d**

Faint brown crystals in yield (62%); m.p. 304-306° C; IR (KBr) ν_{max} / cm^{-1}= 1659 (C=O), 2188 (Aliph.-H), 3036 (Arom.-H), 3423 (NH or OH). ^{1}HNMR (DMSO- d_6): δppm = 7.33- 7.66 (m, 9H, Ar-H), 8.15-8.77 (m, 4H, pyridine ring), 8.93 (s, 1H, broad NH), 8.96 (s, 1H, pyridone ring). MS m/z (%): M^{+2} = 428 (20%), 426 (60), 362(52), 350(76), 324(58), 320(67), 289(80), 236(72), 206(78), 193(60), 170(67), 138(67), 107(74), 97(79), 60(100). Anal.; For $C_{24}H_{15}ClN_4O_2$ (426.85) Calcd.: C 67.53; H 3.54; Cl 8.31; N 13.13% Found: C 67.85; H 3.75; Cl 8.63; N 13.46%.

4.4 Synthesis of 6-oxo-1-phenyl-3-(pyridin-3-yl)-6,7-dihydro-1H-pyrazolo[3,4-b]pyridin-5-carbonitrile (**6**)

4.4.1. Method (A):

To a solution of 2 (0.49 g, 1.7 mmol) in ethanolic sodium ethoxide solution (0.1 g of sodium metal in 10 ml absolute ethanol) malononitrile or cyanoactamide (1.7 mmol) was added. The

reaction mixture was heated under reflux for 12 h. The solvent was evaporated under reduced pressure, then washed with diethyl ether (2 x 10 ml) and recrystallized from ethanol to give compound (**6**).

4.4.2. Method (B)

The procedure was similar to that described in Method (A) except that the mixture was capped in closed vessels and irradiated in a microwave oven at 120 ^{o}C for 25 min using Synthos 3000 (500 W). The reaction was worked as usual and the obtained solid was recrystallized from ethanol to give **(6)** as Buff crystals in yield (63%) m.p. 290-293^{o} C; IR (KBr) ν_{max} / cm^{-1}= 1651 (C=O), 2198 (C≡N), 3077 (Arom.-H). ^{1}HNMR (DMSO- d_6): δppm = 7.33- 7.66 (m, 5H, Ar-H), 8.15-8.77 (m, 4H, pyridine ring & 1H, pyridone ring), 8.90 (s, 1H, broad NH). MS m/z (%): M = 313 (50%), 301(48), 279(73), 227(73), 209(60), 165(55), 127(68), 105(100), 80(61), 63(85). Anal.; For $C_{18}H_{11}N_5O$ (313.31) Calcd.: C 69.00; H 3.54; N 22.35% Found: C 69.40; H 3.69; N 22.68%.

*4.5. Synthesis of 1-phenyl-3-(pyridin-3-yl)-6-thioxo-6,7-dihydro-1H-pyrazolo[3,4-b]pyridin-5-carbonitrile (**9**)*

4.5.1. Method (A):

To a solution of 2 (0.49 g, 1.7 mmol) in ethanolic sodium ethoxide solution (0.1 g of sodium metal in 10 ml absolute ethanol) was added cyanothioactamide (0.17 g, 1.7 mmol). The reaction mixture was heated under reflux for 12 h. The solvent was evaporated under reduced pressure, then washed with diethyl ether (2 x 10 ml) and recrystallized from ethanol to give (**9**).

4.5.2. Method (B):

A mixture of 6 (0.51 g, 1.5 mmol) and phosphorous pentasulphide (1.02 g, 2.2 mmol) in 20 ml pyridine was refluxed for 10 h. The solvent was evaporated under reduced pressure, washed with diethyl ether (2 x 10 ml) and crystallized from ethanol to give (**9**) with the same m.p. and mixed m.p. as mentioned in method (A)

4.5.3. Method (C):

The procedure was similar to that described in Method (A) and Method (B) except that the mixture was capped in closed vessels and irradiated in a microwave oven at 120 ^{0}C for 25 min using Synthos 3000 (500 W). The reaction was worked as usual and the obtained solid was recrystallized from ethanol to give **(9)** as faint brown crystals in yield (64%); m.p. 283-286^o C; IR (KBr) ν_{max} / cm^{-1}= 1373 (C=S), 2202 (C≡N), 3085 (Arom.-H). ^{1}HNMR (DMSO- d_6): δppm = 7.30- 7.56 (m, 5H, Ar-H), 8.25-8.74 (m, 4H, pyridine ring & 1H, pyridone ring), 8.88 (s, 1H, broad NH). MS m/z (%): M = 329(56%), 220(61), 203(83), 193(68), 169(61), 143(62), 130(69), 115(100), 96(73), 71(86), 64(52), 53(62). Anal.; For $C_{18}H_{11}N_5S$ (329.38) Calcd.: C 65.64; H 3.37; N 21.26; S 9.74% Found: C 65.96; H 3.59; N 21.60; S 9.97%.

*4.6. Synthesis of ethyl-1-phenyl-3,6-di(pyridin-3-yl)-1H-pyrazolo[3,4-b]pyridin-5-carboxylate (**11**)*

4.6.1. Method (A):

To a solution of 2 (0.49 g, 1.7 mmol) in 20 ml glacial acetic acid in the presence of ammonium acetate (0.15 g) was added ethyl-3-oxo-3-(pyridin-3-yl)propanoate (**10**) (0.32 g, 1.7 mmol). The reaction mixture was heated under reflux for 8 h. The reaction mixture was monitored by TLC using benzene/acetone (2:1) as eluent. The solvent was evaporated under reduced pressure, washed with diethyl ether (2 x 10 ml) and recrystallized from methanol to give (**11**).

4.6.2. Method (B):

The procedure was similar to that described in Method (A)except that the mixture was capped in closed vessels and irradiated in a microwave oven at 120 ^{o}C for 15 min using Synthos 3000 (500 W). The reaction was worked as usual and the obtained solid was recrystallized from ethanol to give **(11)** as faint yellow crystals in yield (71%); m.p. 273-275^o C; IR (KBr) ν_{max} / cm^{-1}= 1615 (C=O), 3017 (Arom.-H). ^{1}HNMR (DMSO- d_6): δppm = 1.32 (t, *J* **=** 7.0 Hz, 3H, CH_3), 4.40 (q, *J* **=** 7.1 Hz, 2H, CH_2), 7.33- 7.74 (m, 5H, Ar-H), 8.32-8.94 (m, 9H, pyridine ring). MS m/z (%): M = 421 (54%), 392(50), 376(53), 348(49), 322(49), 259(100), 242(73), 206(50), 164(58), 127(87), 105(71),

77(96). Anal.; For $C_{25}H_{19}N_5O_2$ (421.45) Calcd.: C 71.25; H 4.54; N 16.62 Found: C 71.60; H 4.78; N 16.98%.

4.7. General procedure for the synthesis of 7-aryl-3-(pyridin-3-yl)-1H-dipyrazolo [4,3-e:2',3'-a] pyrimidine (**13a-c**)

4.7.1. Method (A):

To a solution of 2 (0.49 g, 1.7 mmol) in acetic acid (10 ml) was added 3-substituted-5-amino-1*H*-pyrazole (1.6 mmol). The mixture was heated under reflux for (8-10) h. The mixture was poured into ice cold water; the formed solid was collected by filtration, dried and crystallized from DMF to give (**13a-c**).

4.7.2. Method (B):

The procedure was similar to that described in Method (A) except that the mixture was capped in closed vessels and irradiated in a microwave oven at 130 ^{0}C for 20 min using Synthos 3000 (500 W). The reaction was worked as usual and the obtained solid was recrystallized from DMF to give **(13a-c)**.

4.7.3. 7-Phenyl-3-(pyridin-3-yl)-1H-dipyrazolo[4,3-e:2',3'-a]pyrimidine (**13a**)

Yellow crystals in yield (80 %) ; m.p. 304-305 ^{0}C; IR (KBr) ν_{max} / cm^{-1}= 1617 (C=N), 3062 (Arom.-H). ^{1}HNMR (DMSO- d_6): δppm = 7.24 (s, 1H, CH of pyrazole), 7.25-7.79 (m, 10H, Ar-H), 8.22-9.13(m, 4H, pyridine ring & 1H, pyrimidine ring). MS m/z (%): M = 388 (17%), 329(37), 312(59), 248(100), 234(18), 220(22), 191(13), 115(12), 100(15), 77(52). Anal.; For $C_{24}H_{16}N_6$ (388.42) Calcd.: C 74.21; H 4.15; N 21.64% Found: C 74.62; H 4.32; N 21.99%.

4.7.4. 7-(4-Chlorophenyl)-3-(pyridin-3-yl)-1H-dipyrazolo[4,3-e:2',3'-a]pyrimidine (**13b**)

Yellow crystals in yield (74 %); m.p. 310-312 ^{0}C; IR (KBr) ν_{max} / cm^{-1}= 1659 (C=N), 3065 (Arom.-H). ^{1}HNMR (DMSO- d_6): δppm = 7.24 (s, 1H, CH of pyrazole), 7.22-7.78(m, 9H, Ar-H), 8.22-9.11(m, 4H, pyridine ring & 1H, pyrimidine ring). MS m/z (%): M = 423 (45%), 335(63), 315(40), 296(40), 273(48), 237(54), 193(54), 164(58), 146(55), 127(64),

119(53), 90(52), 71(42), 54(100).. Anal.; For $C_{24}H_{15}ClN_6$ (422.87) Calcd.: C 68.17; H 3.58; Cl 8.38; N 19.87% Found: C 68.60; H 3.72; Cl 8.60; N 20.26%.

4.7.5. 7-(4-Toloyl -3-(pyridin-3-yl)-1H-dipyrazolo[4,3-e:2',3'-a]pyrimidine (**13c**)

Deep yellow crystals in yield (60 %); m.p. 320-322 ^{0}C; IR (KBr) ν_{max} / cm^{-1}= 1615 (C=O), 2991 (Aliph.-H), 3060 (Arom.-H). ^{1}HNMR (DMSO- d_6): δppm = 2.42 (s, 3H, CH_3), 7.22 (s, 1H, CH of pyrazole), 7.23-7.73(m, 9H, Ar-H), 8.00-9.11(m, 4H, pyridine ring & 1H, pyrimidine ring). MS m/z (%): M = 402 (25%), 396(67), 342(24), 314(100), 286(48), 248(13), 199(29), 173(62), 149(37), 119(62), 97(51), 77(78). Anal.; For $C_{25}H_{18}N_6$ (402.45) Calcd.: C 74.61; H 4.51; N 20.88% Found: C 74.89; H 4.69; N 21.22%.

5. Conclusion

The objective of this work was established by testing antioxidant, anticancer and antimicrobial activities for the new synthesized compounds. The results demonstrate that compounds **(4c**) and **(4d)** showed the highest antioxidant activity, compounds **(6**) and **(9)** showed the highest anticancer activity while **(4d)** exhibited the strong activity against most of the tested organisms.

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Figure 1: Assessment of the antioxidant activities of the compounds in comparison with the ascorbic acid.

Figure 2: Scavenging antioxidant percentage of the ten compounds tested

Figure 3: IC50 of the ten compounds tested on MCF7 for breast cancer

Figure 4: IC50 of the ten compounds tested on HEPG2 for liver cancer.

Scheme 1. Synthesis of 4-(dimethylaminomethylene)-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-5(4*H*)-one (2).

Scheme 2. Synthesis of 5-aroyl-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo [3,4-*b]*pyridin-6(7*H*)-one **(4a-d).**

Scheme 3. Synthesis of 6-oxo-1-phenyl-3-(pyridin-3-yl)-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridin-5-carbonitrile **(6)**

and 1-Phenyl-3-(pyridin-3-yl)-6-thioxo-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridin-5-carbonitrile **(9**)

Scheme 4. Synthesis of ethyl-1-phenyl-3,6-di(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-5-carboxylate (**11)**

Scheme 5. Synthesis of 7-aryl-3-(pyridin-3-yl)-1*H*-dipyrazolo [4,3-*e*:2',3'-*a*] pyrimidine **(13a-c)**

Table 1: Comparison between conventional heating and microwave irradiation

Table 2: Decrease of DPPH absorbance (%) by the synthesized compounds

Table3: IC50 of the compounds tested on breast and liver cancer

Table 4: Diameters of inhibition zones (mm) of the compounds against different tested microorganisms

Table 1: Comparison between conventional heating and microwave irradiation methods.

Compound no.	X	R	Time		Yield (%)	
			Microwave irradiation	Conventional heating	Microwave irradiation	Conventional heating
4a	N	H	130 ^{o}C, 20 minutes	Refluxing 8 hours	90	75
4b	CH	CH_3	130 ^{o}C, 20 minutes	Refluxing 10 hours	83	67
4c	CH	F	130 ^{o}C, 20 minutes	Refluxing 8 hours	82	70
4d	CH	Cl	130 ^{o}C, 20 minutes	Refluxing 10 hours	87	62
6	-	-	120 ^{o}C, 25 minutes	Refluxing 12 hours	90	63
9	-	-	120 ^{o}C, 25 minutes	Refluxing 12 hours	92	64
11	-	-	120 ^{o}C, 15 minutes	Refluxing 8 hours	90	71
13a	-	H	130 ^{o}C, 20 minutes	Refluxing 10 hours	93	80
13b	-	Cl	130 ^{o}C, 20 minutes	Refluxing 8 hours	88	74
13c	-	CH_3	130 ^{o}C, 20 minutes	Refluxing 10 hours	85	60

Table 2: Decrease of DPPH absorbance (%) by the synthesized compounds

Compound no.	**Decrease of DPPH absorbance % mean ± SD (*n* = 3)**	**EC50 (µg/ml)**
4a	35.98 ± 1.60	38.64
4b	38.89 ± 1.15	54.76
4c	72.34 ± 1.61	4.21
4d	63.63 ± 2.14	8.97
6	1.14 ± 0.53	> 100
9	17.04 ± 2.67	92.85
11	-ve effect	-ve effect
13a	9.47 ± 1.60	> 100
13b	29.35 ± 0.79	80.50
13c	39.59 ± 1.17	53.76
Ascorbic acid (standard)	82.77 ± 1.37	3.22

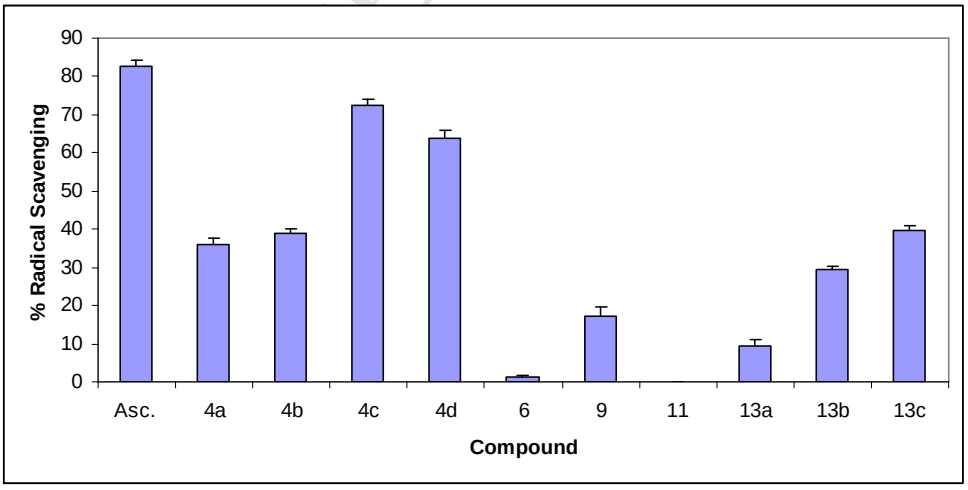
Table3: IC50 of the compounds tested on breast and liver cancer

Compound no	**IC50 (µg/ml)**	
	breast cancer	**liver cancer**
4a	10.10	11.00
4b	9.90	8.85
4c	8.70	7.80
4d	6.45	9.00
6	3.00	3.30
9	2.70	3.90
11	6.90	3.75
13a	15.90	17.30
13b	14.70	16.20
13c	10.50	12.90
Standard-DOX	**MCF7-DOX** (4.40)	**HEPG2-DOX** (3.10)

Table 4: Diameters of inhibition zones (mm) of the compounds against different tested microorganisms

Compound no.	*Bacillus*	*Echerechia coli*	*Enterobacter cloaca*	*Serratia*	*Fusarium oxysporum*	*Penicillium expansum*
4a	-ve	10.5	11.5	12.5	-ve	-ve
ab	-ve	17	12.5	17	-ve	10
4c	-ve	15	21	17.5	-ve	-ve
4d	-ve	19	19	17	-ve	-ve
Chloram-phenicol	21	18	17	25.8	12	10

The concentration used was 10 mg/ml. Control discs were performed in DMSO (dimethylsulfoxide) and no an inhibitions zones of were observed (-ve resistance).



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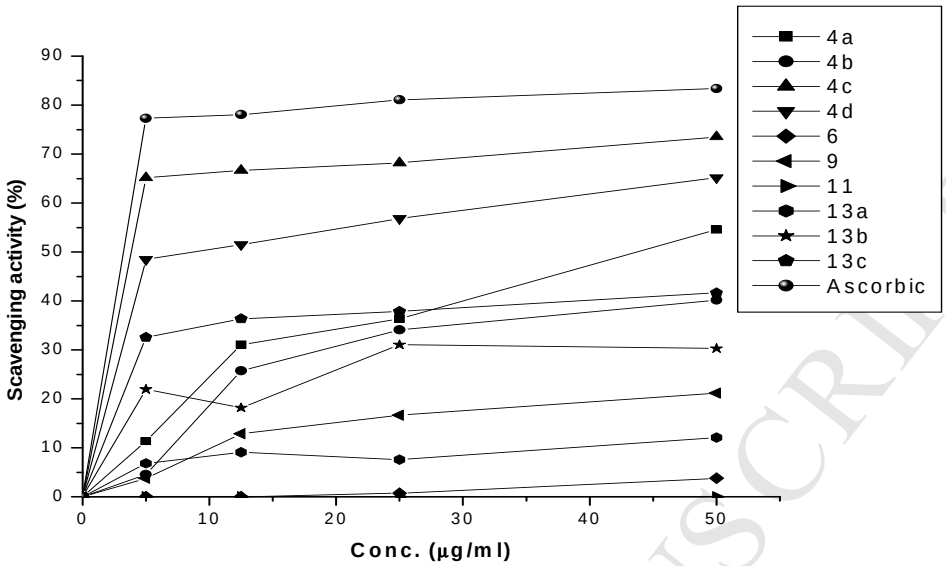


Figure 2: Scavenging antioxidant percentage of the tested compounds

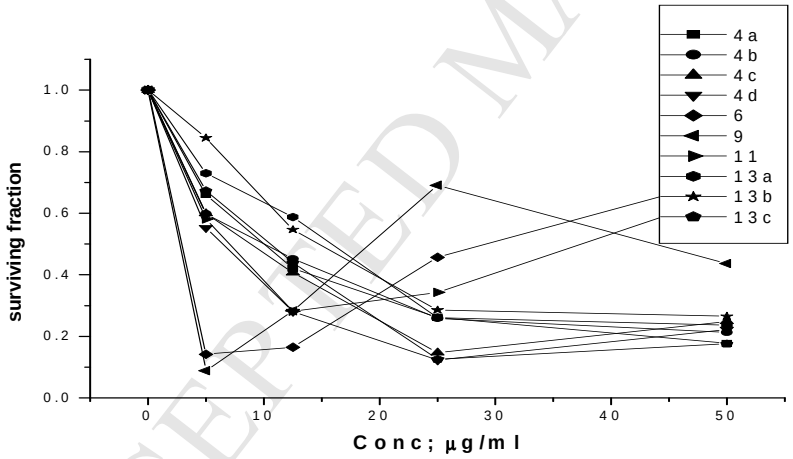


Figure 3: IC50 of the ten compounds tested on MCF7 for breast cancer

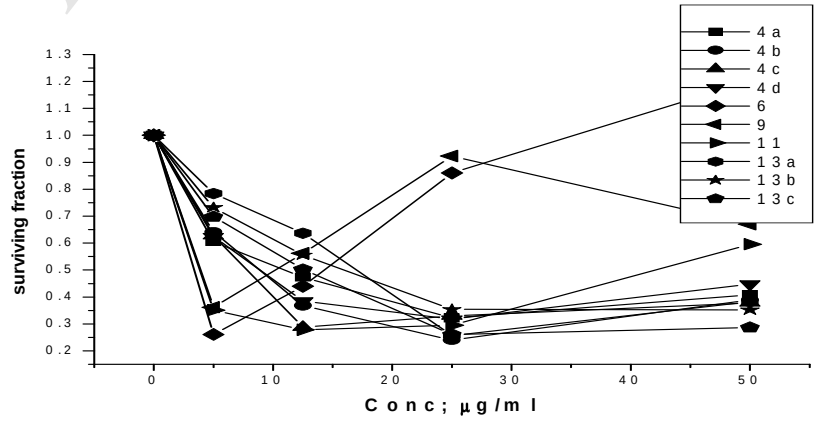
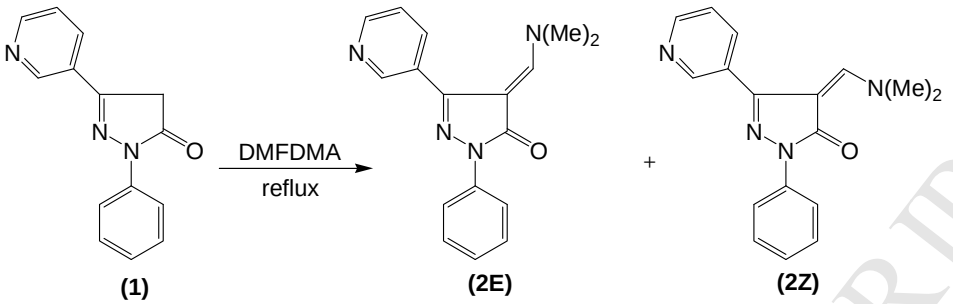


Figure 4: IC50 of the ten compounds tested on HEPG2 for liver cancer.



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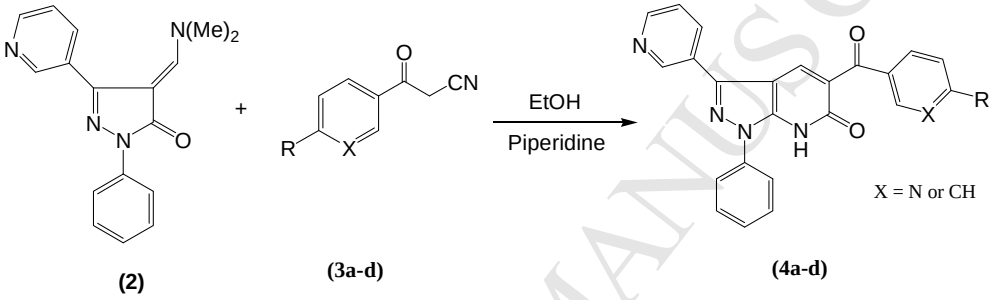

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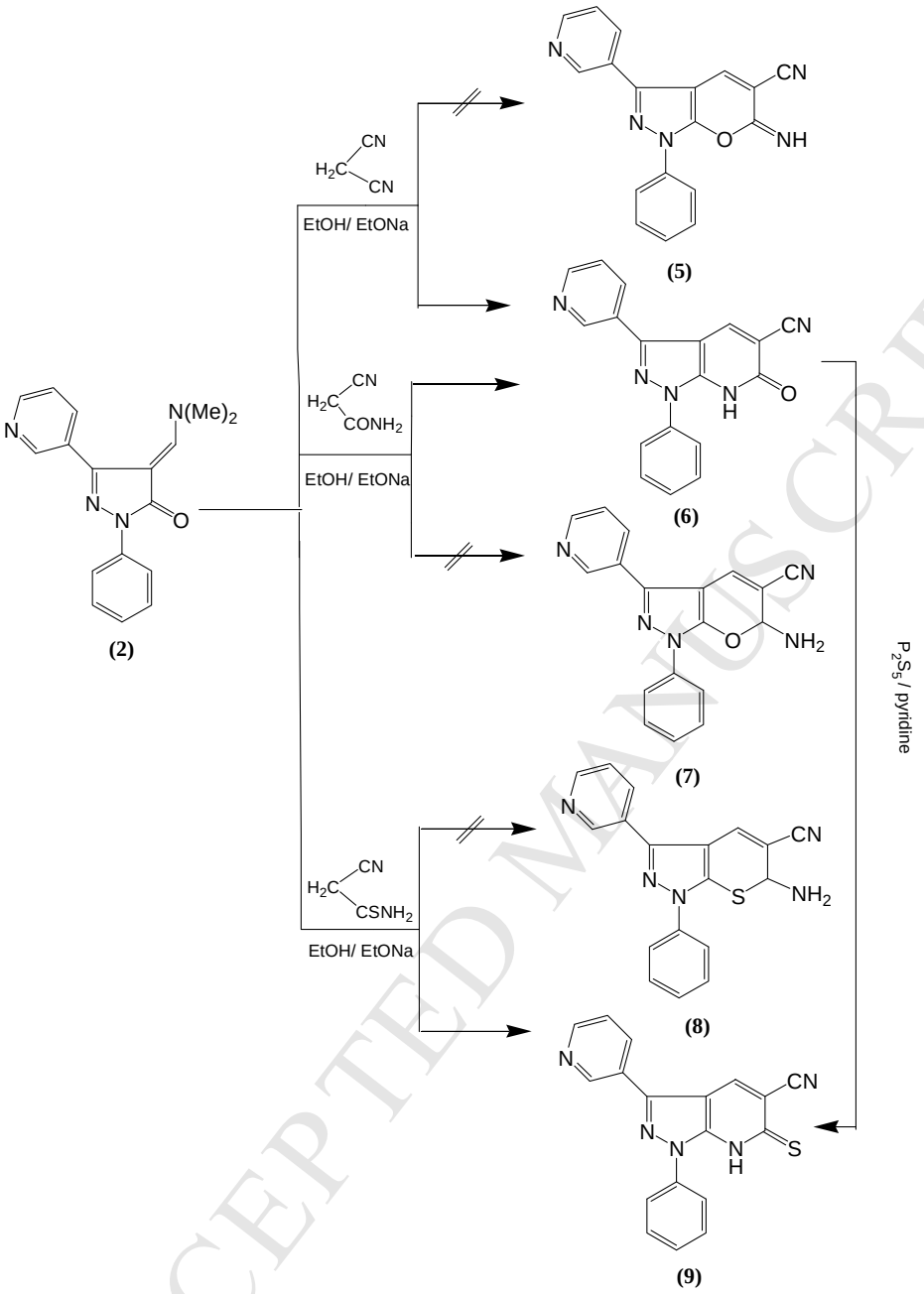
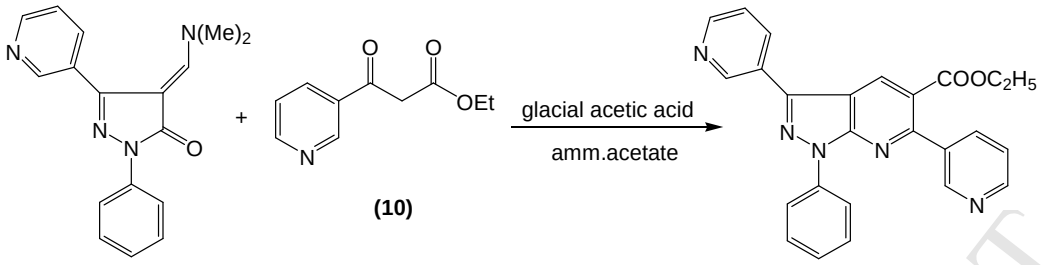
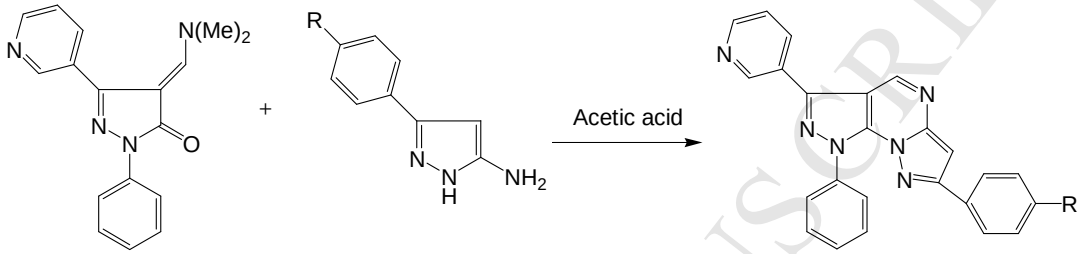


Scheme 3. Synthesis of 6-oxo-1-phenyl-3-(pyridin-3-yl)-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridin-5-carbonitrile **(6)** and 1-Phenyl-3-(pyridin-3-yl)-6-thioxo-6,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridin-5-carbonitrile **(9**)



(2) **(10)** **(11)**

Scheme 4. Synthesis of ethyl-1-phenyl-3,6-di(pyridin-3-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-5-carboxylate (**11)**



(2) **(12 a-c)** **(13 a-c)**

Scheme 5. Synthesis of 7-aryl-3-(pyridin-3-yl)-1*H*-dipyrazolo [4,3-*e*:2',3'-*a*] pyrimidine **(13a-c)**

> 4-(dimethylaminomethylene)-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazol-5(4*H*)-one (enaminone) was reported.> The chemical behavior of enaminone with some active methylene reagent was examined.> The reactions were carried out by conventional heating and microwave irradiation.> Most of the synthesized compounds were screened for antioxidant, antitumor and antimicrobial activities