A Versatile Synthesis, PM3-Semiempirical, Antibacterial, and Antitumor Evaluation of Some Bioactive Pyrazoles

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Received August 8, 2010
DOI 10.1002/jhet.806
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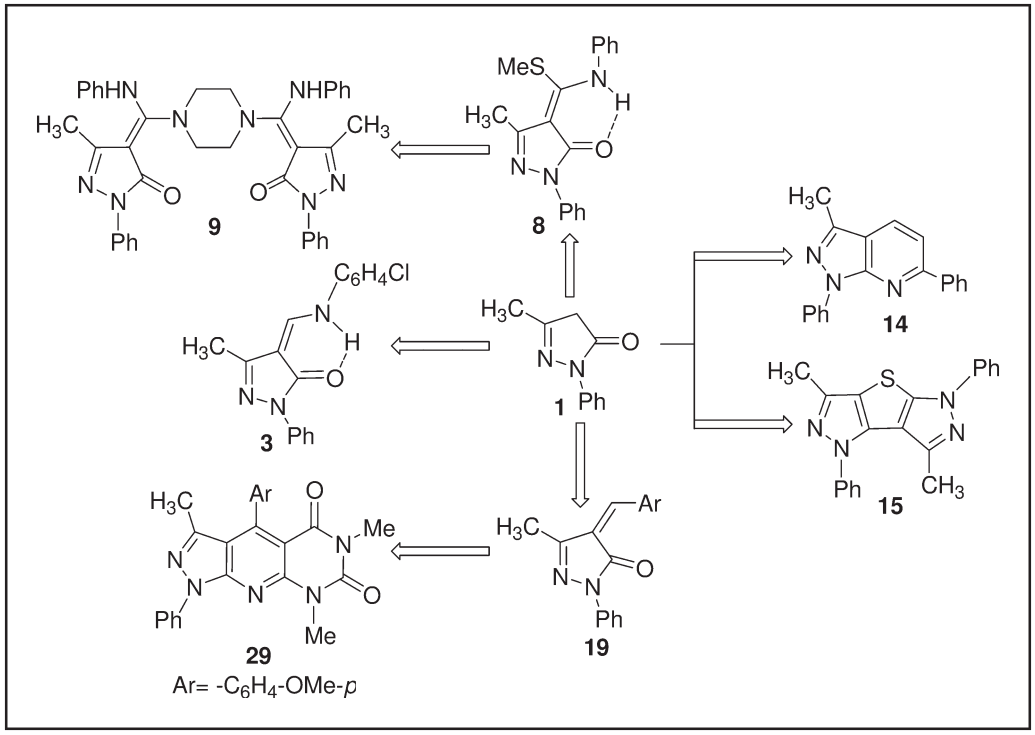


This work describes the syntheses and biological activities of some novel isolated or fused heterocyclic ring systems incorporated pyrazolone moiety, for example; enaminones containing pyrazolone ring photochromic functional unit, 4-[(4-chlorophenylamino)methylene]-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**3**) and some analogous derivatives **4**, **9**, and **10**, also as pyrazolo[3,4-*b*]pyridine, pyrazolo[3,4-*b*]quinoline, pyrazolo[3′,4′:4,5]thieno[2,3-*c*]pyrazoline and pyrazolo[3,4-*c*]pyrazole were synthesized and characterized. Newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data and quantum mechanical calculations. Selected products were tested for their antibacterial and antitumor agents.

J. Heterocyclic Chem., **49**, 543 (2012).

INTRODUCTION

Pyrazoles are key structures in numerous compounds of therapeutic importance [1]. Compounds containing this ring system are known to display diverse pharmacological activities such as antibacterial, antifungal [2], anti-inflammatory, analgesic [3], and antipyretic [3]. 3-Alkyl-4-arylmethylpyrazol-5-ones are reported to exhibit potent antihyperglycemic activity [4], 1-phenyl-3-tetrafluoroethylpyrazol-5-one is an anxiolytic [5] and 4,4-dichloro-1-(2,4-dichlorophenyl)-3-methyl-5-pyrazolone as a potent catalytic inhibitor of human telomerase [6], telomerase is a large ribonucleoprotein complex of reverse transcriptase enzyme, because it suppresses the telomerase activity in tumor cells it can be an encouraging strategy of anticancer therapy [7]. Pyrazoles are important in treatment of ophthalmological diseases [8], dye stuffs [9], and antidepressant activity [10]. The number of investigations for organic photochromic materials has increased considerably in recent years because of their potential commercial applications in several areas, such as high-density optical storage media, eye-protecting glasses and optical switching devices [11]. So far, though numerous organic photochromic molecular systems or photoactive devices have been explored, these systems which have been classified according to the photochromic

structural units mainly belong to a small number of families, such as spiropyrans, spiroxazine, diarylethenes, Schiff bases [12], and pyrazolone derivatives [13]. Its photochromic properties are due to intermolecular proton transfer (PT) [14]. PT Reaction is central to several fields of chemistry and biochemistry and plays a key role in pharmaceutical action, enzyme activity and the stabilization of base pairs in duplex DNA.

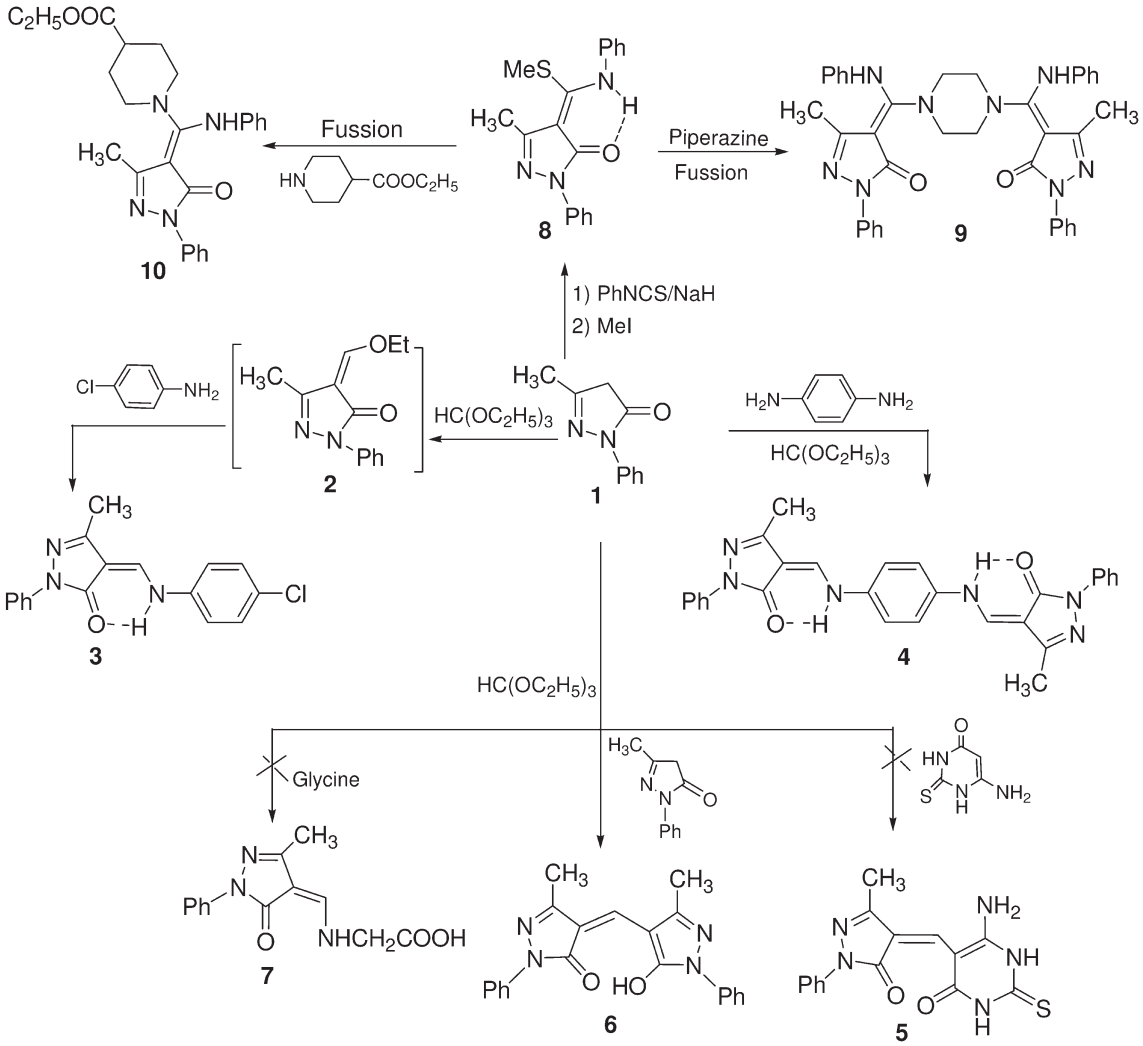
Schiff bases are an interesting class of compounds possessing O—H⋯N hydrogen bond and the PT reaction can proceed through it. These compounds are often thermo or photochromic behavior, undergoing reversible PT in the solid state. However, until recently no NH form had been structurally characterized [15]. Chemotherapeutically valuable compounds in this class such as 4-(arylmethylene)-2,3-dihydropyrazol-3-ones (as neoplastic lesion inhibitors) are reported as patent in literature [16].

RESULTS AND DISCUSSION

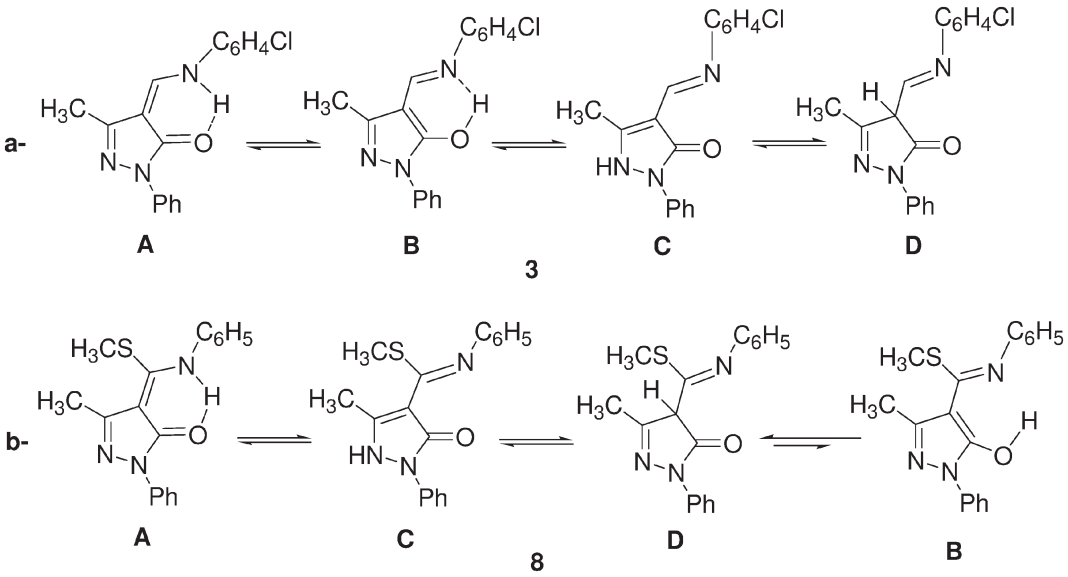
In this study, enaminocarbonyl compounds containing pyrazolone ring were synthesized, by reaction of 1-phenyl-3-methylpyrazol-5-one (**1**) [17] with triethyl orthoformate to give the intermediate **2**, which transformed with *N*-nucleophiles such as *p*-chloroaniline in *n*-butanol *via* addition-elimination mechanism to give 4-[(4-chlorophenylamino)methylene]-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)one (**3**) in good yield (Scheme 1).

The exact tautomeric form of compound **3** [Scheme 2 (a)] was established by the quantum mechanical calculations using the PM3 semiempirical molecular orbital

Scheme 1. Reactions involving active methylene group of 1-phenyl-3-methylpyrazol-5-one (**1**) with different reagents.



Scheme 2. Tautomeric forms of 4-[(4-chlorophenylamino)methylene]-3-methyl-1-phenyl-1*H*-pyrazol-5(4H)one (**3**) and (*E*)-3-methyl-4-(methylthio (phenylamino)methylene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**8**).



method (Table 1), the tautomeric form A was found to have the lower total energy.

The structure of **3** was established on the basis of its analytical and spectral data. The IR spectrum showed bands at 3419, 3061, and 592 cm^{-1} corresponding to (NH), (=C—H) and (C—Cl) groups, respectively, its ^1H NMR spectrum exhibited three singlet signals at δ 2.29, 7.61, and 8.48 ppm due to methyl, methine and (NH) protons, respectively. The results of quantum mechanical molecular orbital calculations using the PM3-semiempirical molecular quantum mechanical method (Table 1) indicated that (*Z*) configuration was found to have the lower binding energy than the (*E*) configuration which indicates the global stability of the (*Z*) configuration (Fig. 1) as (4*Z*)-4-[(4-chlorophenylamino)methylene]-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)one (**3**). The distance of H-bond in tautomer

Table 1

Quantum mechanical data obtained from PM3-semiempirical MO calculations of the configurations of different compounds.

Cpd.	Configuration	Total energy (Kcal/mol)	Binding energy (Kcal/mol)	Twist angle (°)	Dipole moment (Debye)
3	Z, A[a]	−76677.5	−4005.1	5.0	4.02
	B[a]	−76674.2	−4002.0	44	1.6
	E, C[a]	−76672.3	−3999.9	176	3.42
	D[a]	−76670.2	−3997.8	5.0	1.6
4	Z, Z	−120938.7	−6727.7	3.3	1.07
	E, Z	−120935.8	−6724.8	179.5	3.75
	E, E	−120936.0	−6725.0	178, 178	1.14
6	Z	−92203.7	−5026.3	0.0	2.83
	E	−92204.3	−5027.2	178	1.90
8	E, A[a]	−77455.1	−4351.2	178.8	4.57
	E, B[a]	−77452.13	−4348.3	101.3	1.94
	C[b]	−77455.46	−4351.7	89.7	3.37
	E, D[a]	−77455.95	−4352.1	122.7	4.18
9	Z, Z	−159982.2	−9233.3	4.1	2.86
	E, Z	−159981.5	−9232.6	174.8	5.66
	E, E	−159981.8	−9232.8	176, 175	3.14
10	Z	−113505.1	−6380.1	4.0	1.76
	E	−113507.2	−6382.5	171	3.07
18	Z	−88744.1	−4735.0	10	2.85
	E	−88745.3	−4736.2	179	

[a]Tautomeric forms.
[b]Perpendicular to each other [angle nearly 90°].

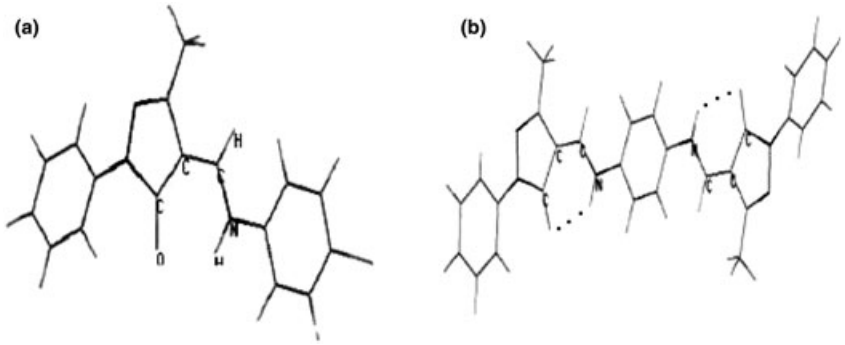


Figure 1. Optimum geometries of (a) compound **3** and (b) compound **4**.

A is 2.4 Å (H·····O) stronger than that of tautomer B, 2.5 Å (H·····N).

A considerable number of *bis*-heterocyclic compounds exhibited various biological activities including antibacterial, fungicidal, tuberculostatic and plant growth regulative properties [18]. Also, it was reported that *bis*-heterocyclic compounds display much better antibacterial activity than mono-hetrocyclic compounds [19]. These observation promoted us to synthesize the 1,4-*bis*(3-methyl-1-phenyl-(4*Z*)-4-methyleneamino-1*H*-pyrazolo-5-(4*H*)-one)benzene (**4**) as *bis*-enamino containing *bis*-pyrazolone ring by reaction of **1** with *p*-phenylenediamine. Its constitution was supported by elemental analysis, IR, ^{1}H NMR and ms spectra. The mass spectrum gave a molecular ion peak at *m*/*z* 476 (M^+, 33%). The results of quantum mechanical molecular orbital calculations (Table 1) indicated that the (*Z,Z*) configuration was found to have the lower binding energy than (*E,Z*) configuration or (*E,E*) configuration which indicates the global stability of the (*Z,Z*) configuration [Fig. 1(b)] as 1,4-*bis*(3-methyl-1-phenyl-(4*Z*)-4-methyleneamino-1*H*-pyrazolo-5-(4*H*)-one)benzene (**4**). The UV-spectra of compounds **3** and **4** were measured in the range from 200 to 450 nm for **3** and to 500 nm for **4**. The UV spectra were observed to be complicated and composed of different overlapped absorption peaks in this range of wavelengths. The different peaks in this range were subjected to Gaussian analysis followed by least square fitting to detect the individual peaks in each spectra using PKFIT Ver 2.0 software. The analyzed spectra were found to compose of the absorption peaks in (Table 2).

From these data it was found that, both compounds **3** and **4** have good optical absorption characters, but compound **4** was better than **3** due to its high proton transfer ability.

Because of the enhanced biological activity of pyrimidine moiety [20], it was of interest to introduce a substituent at position 5 of this moiety, whereby a highly conjugated derivative **5** can be synthesized. Treatment of **1** with triethylorthoformate and 6-aminothioruacil or glycine afforded methylidene-dipyrazolinone **6**. Alternatively, the structure **6** was also formed *via* intermolecular condensation of intermediate **2** by heating with another molecule of **1**. The structure of **6** was proved by its identical melting point with that reported in literatures [21] besides its IR and mass spectra which showed the molecular ion peak at *m*/*z* 358 (M^+) as a base peak.

Table 2

Influence of DMF on λ_{max} (nm) of compounds **3** and **4**.

Compound No.	Wavelength λ_{max} (nm)	Absorbance	Log ϵ	Wavelength λ_{max} (nm)	Absorbance	Log ϵ
3	219	0.316	4.13	344	0.653	4.45
	235	0.211	3.96	364	0.087	3.57
	247	0.123	3.72	378	0.179	3.89
	264	0.413	4.25	392	0.180	3.89
	299	0.009	2.59	415	0.204	3.94
4	218	0.319	4.60	390	0.097	4.08
	229	0.279	4.54	410	0.307	4.59
	244	0.138	4.24	433	0.093	4.07
	254	0.192	4.38	442	0.087	4.04
	270	0.257	4.51	454	0.111	4.14
	342	0.167	4.32	468	0.046	3.76
	364	0.087	4.04	482	0.085	4.03
	376	0.152	4.28			

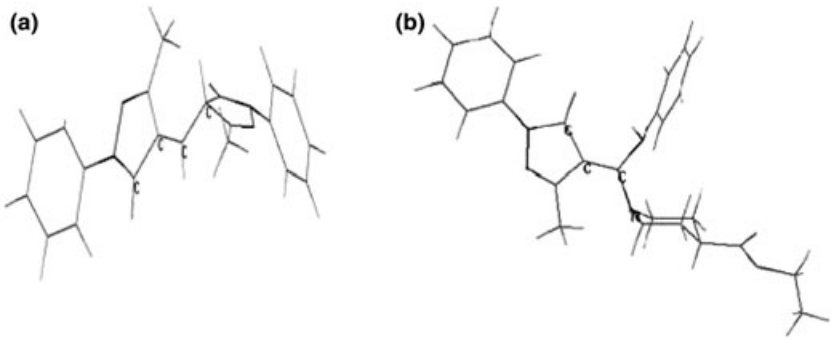


Figure 2. Optimum geometries of (a) compound **6** and (b) compound **10**.

The results of quantum mechanical molecular orbital calculations (Table 1) indicated that the (*E*) configuration was found to have the lower binding energy than the (*Z*) configuration which indicates the global stability of the (*E*) configuration. (4*E*)-4-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)methylene]-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**6**) [Fig. 2(a)].

Furthermore, a diamine fragment is incorporated into the heterocyclic core of a variety of drug classes (e.g., cardizem as calcium channel blocker, ciprofloxacin as antibacterial and clozapine as antipsychotic) were reported in literature [22]. Also, piperazinyl derivatives were reported as antipsychotic agents [23]. This persuaded us to synthesize compound **9** through the reaction of the pyrazolone derivative **1** with phenylisothiocyanate in the presence of sodium hydride a strong base followed by methylation with methyl iodide to afford the thiocarbamoyl **8** [24]. Compound **8** was fused with secondary amines namely; piperazine or ethyl piperidine-4-carboxylate to afford the corresponding *bis*-*N*-pyridyl and *N*-pyridyl **9** and **10**, respectively (Scheme 1). Its structure was confirmed by elemental analysis, IR, ^{1}H NMR and mass spectra.

The exact tautomeric form of compound **8** [Scheme 2 (b)] was established by the quantum mechanical calculations using the PM3 semi-empirical molecular orbital method (Table 1), the tautomeric forms **A**, **B** and **D** were the tautomeric form **A**, **C** and **D** was found in equilibrium at room temperature since the difference in binding energy between them does not exceed 1K cal/mol.

The results of quantum mechanical molecular orbital calculations (Table 1) indicated that the (Z,Z) configuration was found to have the lower binding energy than the (*E,Z*) configuration and (*E,E*) configuration which indicates the global stability of the (*Z,Z*) configuration as 1,4-*bis*(3-methyl-1-phenyl-(4*Z*)-4-methylenylanilino-1*H*-pyrazolo-5-(4*H*)-one)piperazine (**9**) (Fig. 3).

The replacement of the (S-Me) group with amines has been reported [25]. 1,4-Dihydropyridine systems show exceptional properties as calcium antagonists, as powerful arteriolar vasodilators and also as inhibitors of dihydrofolate reductase [26], This enthused us to replace the (S-Me) group of compound **8** with ethyl isonipicotate to give compound **10** (Scheme 1). Its constitution was proved by its IR, ^{1}H NMR and mass spectra. The results of quantum mechanical molecular orbital calculations (Table 1) indicated that the (*E*) configuration was found to have the lower binding energy than the (*Z*) configuration, which indicates the global stability of the (*E*) configuration as ethyl(3-methyl-1-phenyl-(4*E*)-4-metylenylanilino-1*H*-pyrazolo-5-(4*H*)-one) piperidine-4-carboxylate (**10**) [Fig. 2(b)].

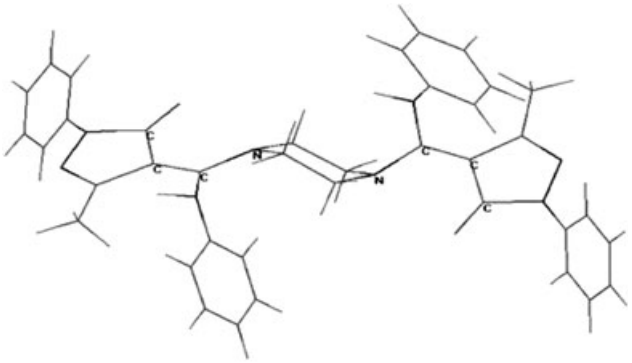


Figure 3. Optimum geometry of compound **9**.

Ketonic Mannich bases are β-bifunctional reactive synthons. These compounds have been widely used as good alkylating agents [27]. C-alkylation reaction of ketonic Mannich bases w\ith active hydrogen derivatives has been reported [28]. Accordingly, the reaction of phenylalkanone Mannich base hydrochloride **11** with **1** in aqueous methanol gave 1,5-diketone **12**. It was characterized by spectral data and elemental analyses. The IR spectrum of **12** exhibited bands at 1738 and 1686 cm^{-1} for (C=O) and (O=C—N—Ph) groups. The ^{1}H NMR spectrum showed a singlet signal at δ 2.04 of (CH_3—C=N), quartet at δ 2.25 of ($\underline{CH_2}$—CH_2CO, J = 4.0 Hz), triplet, at δ 2.75 of (CH_2—$\underline{CH_2}CO$, J = 4.0 Hz), triplet at δ 3.34 of ($\underline{CH}CH_2CH_2CO$, J = 4.0 Hz) and multiplet at δ 7.20–8.00 ppm for aromatic protons. The mass spectrum showed the molecular ion peak at *m/z* 306 (28%).

Compound **12** can be used as a key intermediate for the construction of 3-methyl-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]

pyridine (**14**) through the reaction of **12** with $(NH_4)_2CO_3$ in presence of acetic acid. Formulation of structure **14** was based on elemental analysis, IR and mass spectra which showed the molecular ion peak at *m*/*z* 285 (5%).

Carticaine [29] and carticaine analogues [30] were proved to be local anesthetics and antiarrhythmic agents. Therefore, the thiophene ring of carticaine was replaced by the pyrazolone moiety to prepare a carticaine analogue in an attempt to assess and enhance its biological activity. Therefore, attempted synthesis of the target compound **16** according to the Gewald method in our previous work [26] by reaction of **1** with ethyl cyanoacetate and sulfur in the presence of triethylamine, was unsuccessful. Instead of tricyclic system, 3,7-dimethyl-1,5-diphenyl-1*H*,5*H*-pyrazolo[3′,4′:4,5]thieno[2,3-*c*]pyrazoline (**15**) was unequivocally obtained. The constitution of **15** was ascertained by its IR, ^{1}H NMR and mass spectra which showed the molecular ion peak at *m*/*z* 344 [M^+].

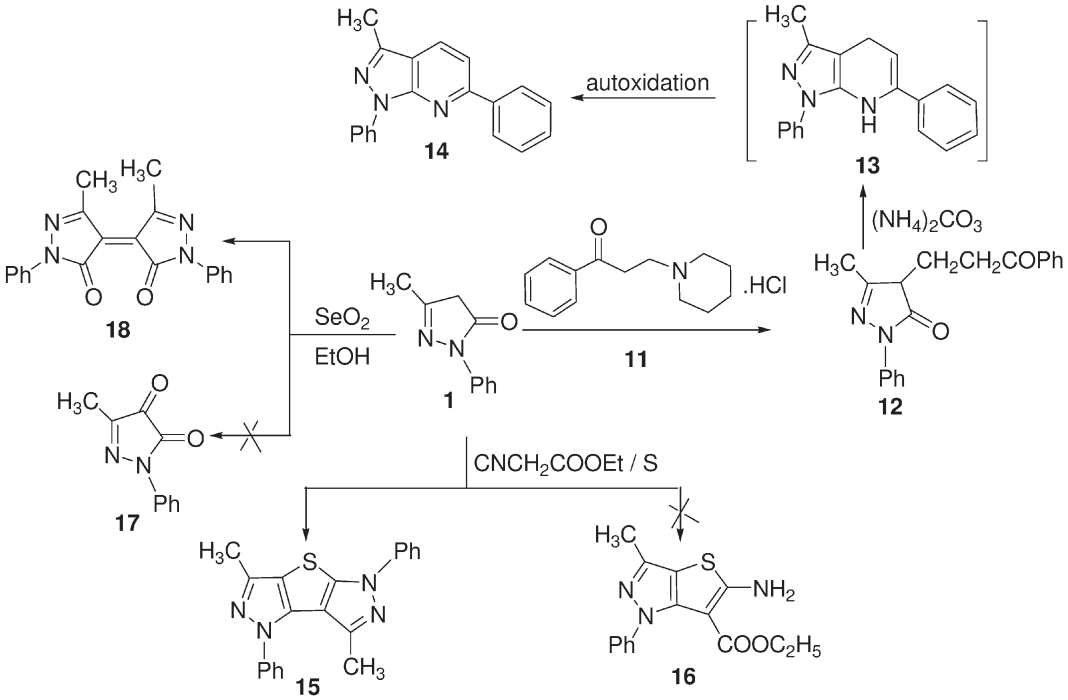
The synthetic potentialities of 1,2-diketone promoted us to oxidize **1** by selenium oxide to obtain **17** but the condensation product **18** was isolated as a sole product instead of of **17** (Scheme 3). Compatible analytical and spectroscopic evidences were gained for structure **18**. Accordingly, its ^{1}H NMR spectrum showed a singlet signal at δ 2.15 due to two methyl groups and multiplet at δ 7.16–7.90 ppm for aromatic protons.

On the other hand, investigation of the reaction of 4-(4-methoxy-benzylidene) pyrazolinone (**19**) [31] with cyanoacetamide in methanol containing triethylamine, the pyridine derivative **23** was the isolated product. The probable mechanism for the formation of the pyridine derivative **23** is that the 4-(4-methoxybenzylidene) pyrazolinone **19** undergoes Michael condensation with one mole of cyanoacetamide to give a Michael adduct **20** which eject *p*-methoxybenzylidine cyanoacetamide molecule. The later compound was then condensed with another cyanoacetamide molecule with subsequent loss of water to afford the pyridine derivative **23** (Scheme 4).

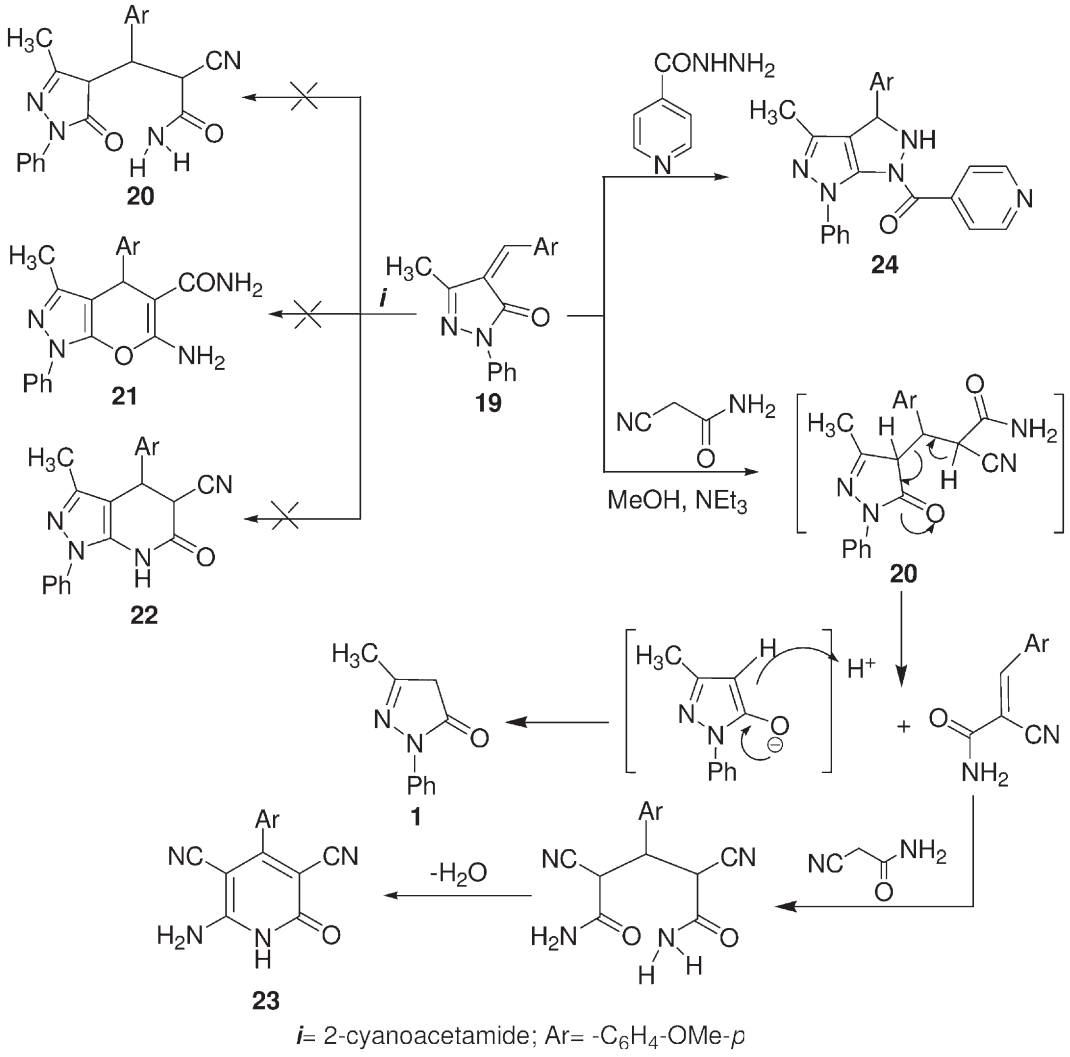
Moreover, compound **23** was isolated in good yield by the reaction of *p*-methoxybenzaldehyde with cyanoacetamide (1:1) molar ratio in methanol catalyzed by triethylamine. When this reaction was carried out in a (1:2) molar ratio the same product was obtained with higher yield. The structure **23** was ascertained by elemental analysis, IR, ^{1}H NMR, and mass spectra as well as its identical melting point with that reported in literature [32]. The formation of pyridine derivative **23** is in accordance with the mechanistic proposal in literature [33].

Substituted bicyclic pyrazolones were used as inhibitors of tumor necrosis factor-α-(TNF-α) production [34]. Also, α,β-unsaturated ketones were used as synthons to construct different heterocycles [35]. Therefore, when a mixture of **19** and nicotinic hydrazide was refluxed in methanol catalyzed by triethylamine, 2,3-dihydro-3-(4-methoxyphenyl)-4-methyl-6-phenyl-pyrazolo[3,4-*c*]pyrazol-1(2*H*)-yl)(pyridin-4-yl)methanone (**24**) was obtained. The product was

Scheme 3. Synthesis of pyrazolo[3,4-*b*]pyridine (**14**), thieno[3,2-*c*]pyrazole-6-carboxylate (**15**), and *bis*-pyrazolone (**18**) derivatives.



Scheme 4. Synthesis of 6-amino-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (**23**) and (3-(4-methoxyphenyl)-4-methyl-6-phenyl-2,3-dihydropyrazolo[3,4-c]pyrazol-1(6*H*)-yl) (pyridin-4-yl)methanone (**24**).

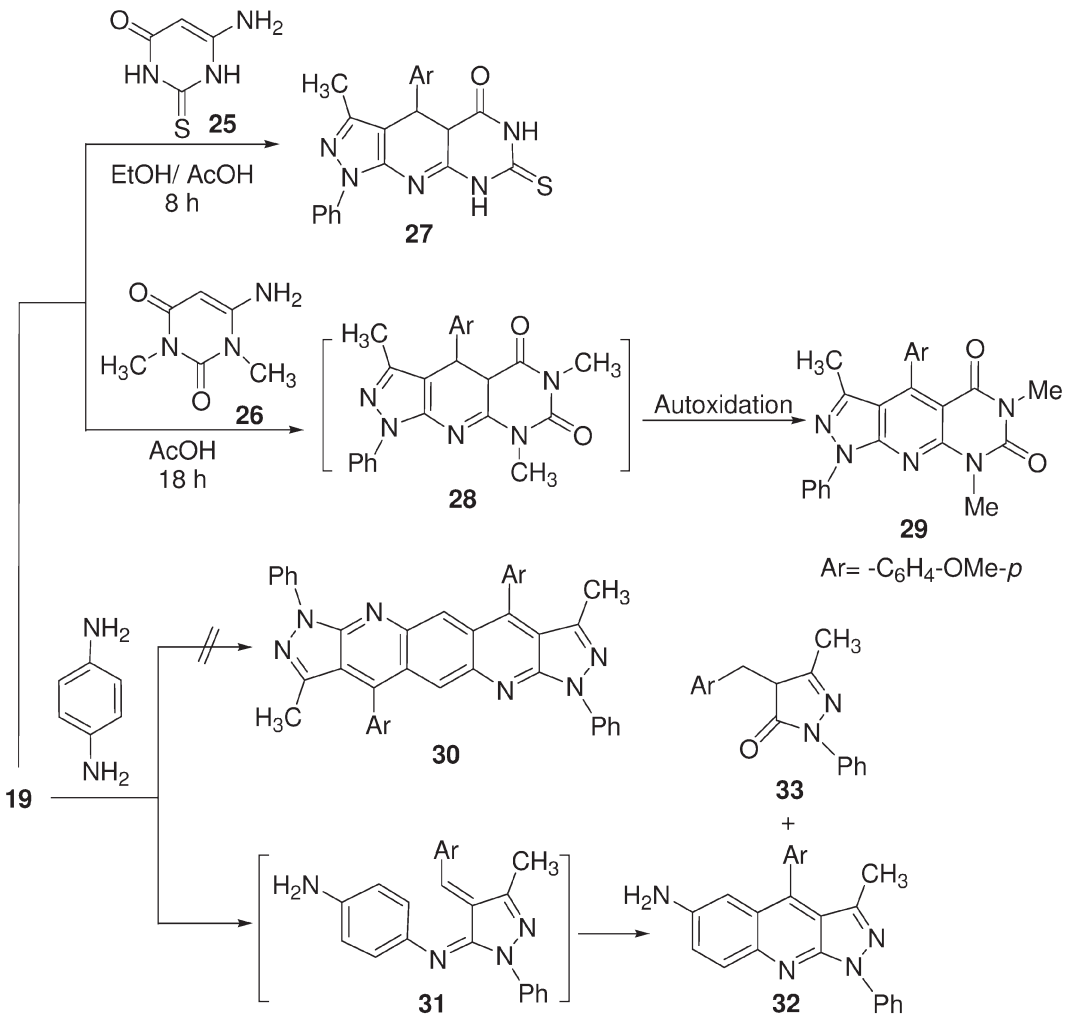


characterized by its spectroscopic and analytical data. The ^{1}H NMR spectrum exhibited characteristic singlet signals at δ 8.41 and 8.79 ppm for the methine and amino protons, respectively. The mass spectrum showed the molecular ion peak at *m/z* 411 (M^+, 5%).

Pyrazolopyridopyrimidine derivatives are reported as antimicrobial [36], potent and selective PDE5 inhibitors [37]. In view of these reports and as continuation of our recent studies [38], persuaded us to synthesize compounds **27** and **29** *via* cycloaddition of 4-(4-methoxybenzylidene) pyrazolinone derivative **19** with 6-amino-thiouracil (**25**) in ethanol and acetic acid mixture to afford **27** and with 6-amino-1,3-dimethyluracil (**26**) in acetic acid to afford **29** through auto-oxidation (Scheme 5). The products **27** and **29** were characterized by their elemental analysis and spectral data (ir, ^{1}H NMR and mass spectra). The ^{1}H NMR spectrum of **27** displayed a singlet signal at δ 2.50 ppm corresponding to methyl protons, doublets at δ 2.69 and 2.85 ppm due to (CH—CO, J = 12.8 Hz) and (CH, J = 12.8 Hz), respectively, and other three singlet signals at δ 3.69, 5.28, and 7.93 ppm due to (OCH_3), (NH), and (NH), (NH, exchangeable with D_2O), respectively.

Attempting to synthesize the *bis*(pyrazolopyridino)benzene derivative **30** by reaction of **19** with *p*-phenylenediamine in ethylene glycol at 180°C was unsuccessful and compound **32** was formed instead of **30**. Compound **32** was achieved through the oxidation effect upon the intermediate 4-anisylidine-5-aryliminopyrazoline derivative **31**, formed from condensation of **19** with *p*-phenylenediamine (Scheme 5). The mechanism of this reaction is in line with those

Scheme 5. Reactions of arylidine derivative **19** with different amines.



reported in literature [39]. The constitution of **32** was proved by IR, mass spectrum and elemental analysis.

Antibacterial activity. The antibacterial activity evaluated by using agar plate method. In this method *E. coli* (Gram -ve) and *B. subtillias* (Gram +ve) were used as test organism and Ampicillin was used as reference drug. The results of antibacterial activities of some synthesized compounds were shown in Table 3. Test solutions of these compounds were tested at concentration of 100 ppm. It should be mentioned that both DMF and DMSO did not show any inhibition on bacterial growth. Based on the diameter of inhibition zones, the antibacterial activity of the tested compound on the growth of *E. coli* and *B. subtillias* can be ranked descending as compounds **10** > **18** > **27** > **29** = **14** = **3** = **9** = **4** =**12** > **15** = **24** = **6** = **32** > **23**. This finding may suggest the possible use of the compounds that showed antibacterial activity in the field of chemotherapy as antimicrobial agents.

Antitumor studies. The standard method used to separate, identify, and purify DNA fragments is electrophoresis [40] through agarose gels, the technique is simple, rapid to perform, and capable of resolving mixtures of DNA fragments that cannot be separated adequately by other sizing procedures, such as density gradient centrifugation. Furthermore, the location of DNA within the gel can be determined directly bands of DNA in the gel are stained with low concentration of the fluorescent dye, ethidium bromide. The method used to detect strand breakage is based up on the different tail mobility of different forms of bacterial DNA. Compounds **3**, **4**, **10**, **15**, **27** and **29** have strong degredative effect on DNA while other compounds has slight effect on DNA, these effect may be attributed to the hydrolysis of glycosidic bond in DNA. The hydrolytic effect of these compounds on the DNA may be useful in treatment and these compounds can be used as antitumor agents after some *in vitro* studies.

Table 3

Diameter of inhibition zone in mm as a criterion of antibacterial activity of some synthesized compounds at concentration 100 ppm.

Compound No.	Inhibition zone (mm)	
	Gram positive bacteria *Bacillus subtilis*	Gram negative bacteria *Escherichia coli*
10	36	40
18	30	35
27	25	30
29	22	28
14	22	27
3	22	27
9	22	25
4	20	24
12	20	23
15	17	23
24	16	22
6	15	20
32	14	18
23	12	14
Ampicillin	19	21

CONCLUSION

Fourteen novel compounds incorporated pyrazolone or pyrazole moieties were synthesized and characterized by elemental analyses and spectral data. The compounds characterization was extended to include the quantum mechanical molecular orbital calculations (Table 1) for compounds **3**, **4**, **6**, **8**, **9**, **10** and **18**. Compounds **3** and **8** have four tautomer structures **A-D** in which the most stable structure is tautomer **A** in both cases due to the H-bond formation and in view of the less energy structures and also other ene compounds were also studied. The tested compounds were screened for their antibacterial and antitumor activities. The tested compounds exhibited in high antibacterial activity activities also, compounds **3**, **4**, **10**, **15**, **27** and **29** have strong degredative effect on DNA.

EXPERIMENTAL

All melting points (uncorrected) were determined on a Gallenkamp electric melting point apparatus. Elemental microanalyses were carried out at Microanalytical Unit, Faculty of Science (Cairo University) and the found values were within ±0.4% of the theoretical values. The IR spectra were recorded using KBr disc with a Mattson 5000 FTIR spectrometer (Faculty of Science, Cairo University). The ^{1}H NMR and ^{13}C NMR spectral data were measured in $CDCl_3$ or DMSO-d_6 on a Varian XL 200, 300 MHz instruments using TMS as internal standard. Chemical shifts were reported in ppm (δ) downfield from internal TMS and coupling constants are expressed in hertz. The Mass spectra were recorded on GC-MS QP-1000 EX. Shimadzu Instrument (Faculty of Science, Cairo University). The UV-vis spectral absorptions were carried out with Unicam UV_2-100 UV/Visible spectrometer V3.32. Follow-up of the reactions and checking the homogeneity of the compounds were monitored by thin layer chromatography (TLC) using EM science silica gel coated plates with visualization by irradiation with ultraviolet lamp λ 254 nm. (*Z*)-3-Methyl-4-(methylthio(phenylamino) methylene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**8**) and 4-(4-methoxybenzyl)-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**33**) were reported previously in the literature [24,39].

Synthesis of enaminopyrazolones 3, 4 and 6. To a warm solution of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**1**) (0.5 g, 2.87 mmol) in *n*-butanol (15 mL), triethylorthoformate (0.48 mL, 2.87 mmol) was added drop wise with stirring, then added 4-chloroaniline (0.37 g, 2.87 mmol), *p*-phenylenediamine (0.31 g, 2.87 mmol) or 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (0.5 g, 2.87 mmol). The reaction mixture was heated at 100°C for 1 h and for 2 h in case of reaction with 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one, left to cool, the formed precipitate was collected by filtration and recrystallized from methanol to afford **3**, **4** and **6**, respectively.

(4*Z*)-4-[(4-Chorophenylamino)methylene]-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)one (3). (85%), mp 143–145°C; yellow crystals; UV-vis spectral measurements: solvent (DMF), concentration, (2.33×10^{-5}M): λ_{max} = 219, 235, 247, 264, 299, 344, 364, 378, 392, 415; IR (KBr) ′υ (cm^{-1}), 3419, 3061, 2980, 2914, 1672, 1625, 1588, 1004, 753, 592; ^{1}H NMR (200 MHz, DMSO-d_6): δ, 2.29 (s, 3H), 7.10–7.52 (m, 5H), 7.61 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 8.48 (s, 1H); ms: (m/z, %): 313 (M^+ +2, 4), 312 (M^++1, 3), 311 (M^+, 12), 185 (33), 128 (5), 126 (14), 109 (16), 56 (26), 57 (100). *Anal.* Calcd. for $C_{17}H_{14}ClN_3O$ (311.77): C 65.49, H 4.53%. Found: C 65.57, H 4.59%.

1,4-*Bis*(3-methyl-1-phenyl-(4*Z*)-4-methyleneamino-1*H*-pyrazolo-5-(4*H*)-one)benzene (4). (87%) mp 240°C; orange-yellow crystals; UV-vis spectral measurements: solvent (DMF), concentration (7.98×10^{-6}M): λ_{max} = 218, 229, 244, 254, 270, 342, 364, 376, 390, 410, 433, 442, 454, 468, 482; IR (KBr) ′υ (cm^{-1}), 3416, 3330, 3044, 2916, 1664, 1623, 1593, 1003, 753; ^{1}H NMR (200 MHz, DMSO-d_6): δ, 2.25 (s, 6H), 6.62 (d, *J* = 8.4 Hz, 2H), 7.07–7.42 (m, 10H), 7.66 (s, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 8.48 (s, 2H); ms: (m/z, %): 476 (M^+, 33), 185 (100), 109 (8), 108 (6), 77 (21). *Anal.* Calcd. for $C_{28}H_{24}N_6O_2$ (476.53): C 70.57, H 5.08%. Found: C 70.37, H 5.10%.

(4*E*)-4-[(4,5-Dihydro-3-methyl-5-oxo-1-phenyl-1*H*-pyrazolo-4-yl)methylene]-3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (6). (45%); mp 178–180°C; pale yellow crystals; IR (KBr) ′υ (cm^{-1}), 3006, 2989, 2957, 1621, 1589, 1548, 1005, 754; ms: (m/z, %): 358 (M^+, 100), 315 (7), 266 (10), 225 (6), 197 (7), 128 (12), 91 (32), 77 (88). *Anal.* Calcd. for $C_{21}H_{18}N_4O_2$ (358.39): C 70.38, H 5.06%. Found: C 70.46, H 5.13%.

1,4-*Bis*(3-methyl-1-phenyl-(4*Z*)-4-methylenylanilino-1*H*-pyrazolo-5-(4*H*)-one)piperazine (9) and (*Z*)-ethyl 1-((3-methyl-5-oxo-1-phenyl-1*H*-pyrazol-4(5*H*)-ylidene)(phenylamino) methyl)piperidine-4-carboxylate (10). A mixture of **8** [24] (0.4 g, 1.23 mmol) and piperazine (0.06 g, 0.62 mmol) or ethyl isonipicotate (0.72 g, 0.41 mmol) were fused in an oil bath at 120–140°C for 3 h. The mixture was cooled and treated with cold water (30 mL) or methanol (20 mL), the solid product that formed was collected by filtration, dried and recrystallized from DMF and methanol to afford **9** and **10**, respectively.

9; (62%); mp 250°C; yellow crystals; IR (KBr) ′υ (cm^{-1}), 3389, 3241, 3040, 2978, 2915, 1632, 1593, 1550, 1003, 758; ^{1}H NMR (200 MHz, DMSO-d_6): δ, 2.18 (s, 6H), 2.24–2.87 (m, 8H), 6.88–8.1 (m, 20H), 9.67 (s, 2H); ^{13}C NMR (200 MHz, DMSO-d_6): δ, 170.2, 165.1, 147.8, 144.4, 137.9, 129.1, 124.4, 121.7, 118.6, 118.5, 116.9, 75.4, 48.3; ms: (m/z, %): 605 (M^+, 4), 551 (22), 523 (17), 339 (24), 313 (61), 236 (56), 129 (68), 97 (100). *Anal*. Calcd. for $C_{38}H_{36}N_8O_2$ (636.74):C 71.68, H 5.70%. Found: C 71.52, H 5.64%.

10; (40%); mp 122–124°C; pale yellow crystals; IR (KBr) ′υ (cm^{-1}), 3420, 3057, 2926, 1729, 1925, 1595, 1561, 1005, 757; ^{1}H NMR (200 MHz, $CDCl_3$): δ, 1.26 (t, *J* = 4.8 Hz, 3H), 1.84 (q, *J* = 8.4 Hz, 4H), 2.09 (s, 3H), 2.48 (q, 1H), 3.18 (t, *J* = 8.4 Hz, 4H), 4.14 (q, *J* = 4.8 Hz, 2H), 4.74 (s, 1H), (NH, exchangeable with D_2O), 7.06–7.91 (m, 10H); ms: (m/z, %): 432 (M^+, 6), 339 (5), 313 (7), 275 (34), 213 (10), 157 (10), 97 (23), 57 (100). *Anal*. Calcd. for $C_{25}H_{28}N_4O_3$ (432.51):C 69.42, H 6.53%. Found: C 69.40, H 6.60%.

3-Methyl-4-(3-oxo-3-phenylpropyl)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (12). A mixture of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**1**) (0.34 g, 1.95 mmol) and **11** (0.5 g, 1.95 mmol) in methanol (20 mL) was refluxed over a steam bath for 3 h. The reaction mixture was left to cool, filtered and the filtrate was basified. The formed precipitate was filtered, dried and recrystallized from ethanol to afford **12** (65%); mp 79°C; white crystals; IR (KBr) ′υ (cm^{-1}), 3303, 3061, 2911, 1738, 1686, 1651, 1594, 1573, 1001, 751; ^{1}H NMR ($CDCl_3$): δ, 2.04 (s, 3H), 2.25 (q, *J* = 4.0 Hz, 2H), 2.75 (t, *J* = 4.0 Hz, 2H), 3.34 (t, *J* = 4.0 Hz, 1H), 7.2–8.0 (m, 10H); ms: (m/z, %): 306 (M^+, 28), 187 (30), 186 (100), 105 (22), 149 (28), 125 (13), 83 (38), 57 (43). *Anal*. Calcd. for $C_{19}H_{18}N_2O_2$ (306.36): C 74.49, H 5.92%. Found: C 74.52, H 6.02%.

3-Methyl-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine (14). A mixture of **12** (0.5 g, 1.63 mmol) and ammonium carbonate (0.16 g, 1.63 mmol) in acetic acid (10 mL) was refluxed for 8 h, the solvent was evaporated, the formed residue was neutralized with diluted ammonium hydroxide, the solid precipitate was filtered off and recrystallized from ethanol to give **14** (60%); mp 150°C; red crystals; IR (KBr) ′υ (cm^{-1}), 3060, 2921, 1595, 1498, 1003, 755; ms: (m/z, %): 285 (M^+, 4), 245 (21), 244 (100), 213 (13), 186 (37), 129 (25), 105 (62), 91 (20), 77 (72). *Anal*. Calcd. for $C_{19}H_{15}N_3$ (285.34): C 79.98, H 5.30%. Found: C 79.89, H 5.28%.

Ethyl 5-amino-3-methyl-1-phenyl-1*H*-thieno[3,2-*c*]pyrazole-6-carboxylate (15). A mixture of **1** (0.5 g, 2.87 mmol), ethylcyanoacetate (0.31 g, 2.87 mmol), sulfur (0.09 g, 2.87 mmol) and triethylamine (0.03 mL, 2.87 mmol) in ethanol (20 mL) was stirred for 3 h at 40–50°C, the formed precipitate was filtered, dried and recrystallized from ethanol to afford **15** (45%); mp 120°C; pale yellow crystals; IR (KBr) ′υ (cm^{-1}), 3153, 2919, 1608, 1535, 1000, 764; ^{1}H NMR (200 MHz, DMSO-d_6): δ, 2.3 (s, 6H), 7.26–7.73 (m, 10H); ms: (m/z, %): 346 (M^++2, 3), 345 (M^++1, 4), 344 (M^+, 11), 314 (8), 239 (2), 211 (4), 174 (34), 91 (43), 77 (12), 76 (100). *Anal*. Calcd. for $C_{20}H_{16}N_4S$ (344.43):C 69.74, H 4.68%. Found: C 69.65, H 4.66%.

4(*Z*)-3-Methyl-4-(3-methyl-5-oxo-1-phenyl-1*H*-pyrazolo-4(5*H*)-ylidene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one (18). A mixture of **1** (0.5 g, 2.87 mmol) and selenium oxide (0.33 g, 2.87 mmol) in ethanol (20 mL) was refluxed for 4 h, the formed black powder was filtered off and the filtrate was left to cool. The solid precipitate was filtered, dried and recrystallized from methanol to give **18** (45%); mp 208–210°C; white crystals; IR (KBr) ′υ (cm^{-1}), 3397, 3063, 2919, 2790, 1624, 1594, 1476, 1004, 757; ^{1}H NMR (200 MHz, DMSO-d_6): δ, 2.15 (s, 6H), 7.16–7.90 (m, 10H); ms: (m/z, %): 346 (M^++2, 10), 345 (M^++1, 16), 344 (M^+, 51), 315 (43), 239 (9), 211 (14), 174 (71), 132 (10), 91 (47), 77 (100). *Anal*. Calcd. for $C_{20}H_{16}N_4O_2$ (344.37): C 69.76, H 4.68%. Found: C 69.82, H 4.59%.

6-Amino-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (23). A mixture of arylidine **19** (0.5 g, 1.7 mmol), cyanoacetamide (0.14 g, 1.7 mmol) and TEA (0.4 mL) in ethanol (20 mL) were refluxed for 15 min then left to stand at room temperature for two day, the solid precipitate was filtered, dried and recrystallized from ethanol to give **23** (65%); mp 205–207°C, [Lit. [32], 205°C (dec)]; white crystals; IR (KBr) ′υ (cm^{-1}), 3446, 3365, 3303, 3172, 2982, 2940, 2207, 1697, 1582, 1509, 1042, 727; ^{1}H NMR ($CDCl_3$): δ, 3.89 (s, 3H), 6.96–7.26 (m, 4H), 7.8 (s, 2H), 8.26 (s, 1H); ms: (m/z, %): 266 (M^+, 10), 203 (13), 202 (100), 158 (18), 89 (8), 77 (1). *Anal*. Calcd. for $C_{14}H_{10}N_4O_2$ (266.25): C 63.15, H 3.79%. Found: C 63.35, H 3.68%.

(3-(4-Methoxyphenyl)-4-methyl-6-phenyl-2,3-dihydropyrazolo[3,4-*c*]pyrazol-1(6*H*)-yl) (pyridin-4-yl)methanone (24). A mixture of the arylidine **19** (0.5 g, 1.7 mmol), nicotinic hydrazide (0.24 g, 1.7 mmol) and triethylamine (0.4 mL) were heated for 15 min then left for two day at room temperature, the solid precipitate was filtered, dried and recrystallized from ethanol to furnish **24** (70%); mp 157–159°C; pale yellow crystals; IR (KBr) ′υ (cm^{-1}), 3445, 3043, 2993, 1658, 1602, 1551, 1025, 1548, 1408, 1328; ^{1}H NMR (200 MHz, DMSO-d_6): δ, 2.48 (s, 3H), 3.82 (s, 3H), 7.02–7.83 (m, 13H), 8.41 (s, 1H), 8.79 (s, 1H); ms: (m/z, %): 412 (M^++1, 1), 411 (M^+, 5), 276 (1), 275 (1), 185 (4), 135 (5), 133 (100), 106 (26), 78 (17). 106 (26), 78 (17). *Anal*. Calcd. for $C_{24}H_{21}N_5O_2$ (411.46): C 70.06, H 5.14%. Found: C 69.84, H 5.09%.

5,5a-Dihydro-5-(4-methoxyphenyl)-8-phenyl-1,3,6-trimethylpyrazolo[4′,3′:5,6]pyrido [2,3-*d*]thiopyrimidine-4(3*H*)one (27). A mixture of arylidine **19** (0.5 g, 1.7 mmol), with 6-aminothiouracil (**25**) (0.22 g, 1.7 mmol) was refluxed in ethanol (20 mL) in presence of catalytic amount of glacial acetic acid for 8 h, left to stand at room temperature, water basified by ammonium hydroxide was added. The solid precipitate was collected by filtration and crystallized from ethanol to give **27** (60%); mp 258–260°C; white crystals; IR (KBr) ′υ (cm^{-1}), 3378, 2961, 2924, 1661, 1600, 1542, 1228, 1031, 771; ^{1}H NMR (200 MHz, DMSO-d_6): δ, 2.50 (s, 3H), 2.69 (d, *J* = 12.8 Hz, 1H), 2.85 (d, *J* = 12.8 Hz, 1H), 3.69 (s, 3H), 5.28 (s, 1H), 6.76–7.00 (m, 9H), 7.93 (s, 1H), (NH, exchangeable with D_2O); ms: (m/z, %): 419 (M^++2, 3), 385 (6), 278 (4), 263 (100), 262 (8), 233 (2), 232 (12), 108 (9), 107 (3), 77 (16). *Anal*. Calcd. for $C_{22}H_{19}N_5O_2S$ (417.48): C 63.29, H 4.59%. Found: C 63.12, H 4.70%.

5-(4-Methoxyphenyl)-8-phenyl-1,3,6-trimethylpyrazolo[4′,3′:5,6]pyrido[2,3-*d*] pyrimidine-2,4(1*H*,3*H*)dione (29). A mixture of arylidine **28** (0.5 g, 1.7 mmol) with 1,3-dimethyl-6-aminouracil (**26**) (0.27 g, 1.7 mmol) was heated in glacial acetic acid (15 mL) on steam bath for 18 h, the reaction mixture was left to cool, the formed solid precipitate was collected by filtration, dried and recrystallized from acetic acid to give **29** (65%); mp 240–242°C; white crystals; IR (KBr) ′υ (cm^{-1}), 3058, 3002, 2918, 1698, 1599, 1503, 1030, 756; ms: (m/z, %): 428 (M^++1, 27), 339 (3), 292 (6),

272 (100), 215 (13), 155 (12), 136 (8), 57 (29). *Anal*. Calcd. for $C_{24}H_{21}N_5O_3$ (427.46): C 67.44, H 4.95. Found: C 67.24, H 5.02%.

4-(4-Methoxyphenyl)-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]quinolin-6-amine (32). A mixture of arylidine **19** (1.0 g, 1.7 mmol) and *p*-phenelendiamine (0.19 g, 1.71 mmol) were heated for 4 h in ethylene glycol (20 mL) at 180°C. After cooling the solution was digested with methanol (20 mL) and refrigerated. Insoluble dark red residue was filtered off and recrystallized from toluene to give **32** (32%); mp 260°C; colorless crystals; IR (KBr) ′υ (cm^{-1}), 3435, 3051, 2927, 2836, 1606, 1593, 1028, 752; ms: (m/z, %): 380 (M^+, 60), 318 (9), 316 (41), 196 (3), 180 (5), 165 (7), 125 (16), 114 (16), 113 (100), 112 (14), 107 (16), 77 (44), 55 (56). *Anal*. Calcd. for $C_{24}H_{20}N_4O$ (380.44): C 75.77, H 5.30%. Found: C 75.43, H 5.51%.

The methanolic filtrate was basified with sodium hydroxide (10%) then, extracted two times with toluene (25 mL). The aqueous solution was boiled with charcoal, filtered and acidified with hydrochloric acid (10%). The precipitate was filtered, dried and recrystallized from ethanol to yield **33** [39].

Quantum mechanical calculation. The molecular total energy and preference geometry of the different configurations of the different synthesized compounds were determined by carrying out geometry optimization process of the concerned configuration using the PM3-semi-empirical MO quantum mechanical method. The software package is Hyperchem 8.04 (Beto version) which accommodated on Pentium IV-2–8 MHz personal computer was employed.

***In vitro* antimicrobial activity.** The tested compounds were evaluated by the agar diffusion technique [41] using a 100 ppm solution in DMSO. The test organisms were two bacterial strains: *E. coli* (Gram -ve) and *B. subtillias* (Gram +ve). Agar nutrient medium was prepared, autoclaved and poured into sterilized petri-dishes; few drops of dense bacterial suspension were gently spread over the medium surface using a sterilized spatula. The bacterial smear was left to dry and then a number of pores were made on agar-nutrient medium using a sterilized crock porer. For screening the antibacterial activities, solution of the tested compounds (100 ppm) were transferred separately into the pores without overflow. The tested compounds were dissolved in DMF or DMSO therefore, DMF and DMSO were included as references for comparison. The test was carried out under completely aseptic conditions. The plates were then incubated at 32°C ± 2°C for 24 h. The antibacterial activity was expressed as the diameter (mm) of inhibition zone.

Antitumor activity. The standard method used to separate, identify, and purify DNA fragments is electrophoresis through agarose gels, the technique is simple, rapid to perform, and capable of resolving mixtures of DNA fragments that cannot be separated adequately by other sizing procedures, such as density gradient centrifugation. Furthermore, the location of DNA within the gel can be determined directly bands of DNA in the gel are stained with low concentration of the fluorescent dye, ethidium bromide. The method used to detect strand breakage is based up on the different tail mobility of different forms of bacterial DNA. Compounds **3**, **4**, **10**, **15**, **27**, and **29** have strong degredative effect on DNA while other compounds has slight effect on DNA, these effect may be attributed to the hydrolysis of glycosidic bond in DNA. The hydrolytic effect of these compounds on the DNA may be useful in treatment and these compounds can be used as antitumor agents after some *in vitro* studies.

Further investigations UV stabilizer for polymers and therapeutic importance; as drugs, dyestuffs and light screening agents of the compounds prepared in this work are still in progress and will be published separately.

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