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ORIGINAL ARTICLE

Synthesis of pyrazolo-enaminones, bipyrazoles and pyrazolopyrimidines and evaluation of antioxidant and antimicrobial properties

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KEYWORDS

Intramolecular cyclization;
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Abstract A novel pyrazolo-enaminones, bipyrazoles and bipyrazolopyridines from 1-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)butane-1,3-dione and 4-methyl-2-phenyl-2*H*-pyrazolo[3,4-*b*] pyridine-3,6(3*aH*,7*H*)-dione have been synthesized by assisted heating with microwave radiation without any catalyst. The pyridine and pyrazole ring formation has been developed from easily accessible enamino keto esters by formylation followed by intramolecular cyclization. The general applicability for the synthesis of the important pyrazolo-enaminones, bipyrazoles and pyrazolo-pyridines heterocycles was attributed to simplicity of operation, synthesis without catalyst, energy efficiency (shorter reaction time under microwave irradiation), good yields, more environmentally friendly and more cost-effective procedure. The antioxidant activity of new heterocyclic compounds

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was evaluated by free radical scavenging by DPPH assay. Several of these compounds showed good activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria.

1. Introduction

Pyrazolo(3,4-b)pyridines and related fused derivatives are considered important class of pharmaceutical agents (Zhang et al., 2010; El-Sayed, 2009; Misra et al., 2003; Nagender et al., 2014; El-Borai et al., 2012), which includes compounds that have antitumor properties (Stepanenko et al., 2011); antidepressant (Kunstmann et al., 1984), and cytotoxic activity (Manpadi et al., 2007). Furthermore, these heterocyclic systems are part of the chemical structure of glycogen synthase kinase-3 (GSK-3) and cyclin-dependent kinases (CDKs) inhibitors (Beutne et al., 2009), and others that have activity against Alzheimer's disease (AD) (Bhat et al., 2003). Likewise, the pyrazolo-enaminones, bipyrazoles and bipyrazolopyridines have a great importance in medicinal chemistry, possessing anti-inflammatory, antimicrobial and cytotoxic activities (Kumari and Venkatarao, 2020).

Conjugated nitrogenous systems in addition to therapeutic applications can also be used as semiconductors (Wang et al., 2021; Wu et al., 2012; Li et al., 2012; Gu et al., 2016). These derivatives also have been used to prevent various diseases induced by active oxygen and as trapping free radicals' agents. Due to the interest of this type of compounds, various synthetic methods have been developed that their authors have reported previously (Datta et al., 2011).

Recently, interest in these heterocyclic compounds has increased considerably due to their potential application in both organic and medicinal chemistry. Several syntheses of these derivatives have been carried out using different catalysts, which have proven to be extremely effective. Especially in the reaction of primary amines with β-dicarbonyl compounds to obtain pyrazolo-enaminones with high yields, iodine (Gogoi et al., 2005), copper nanoparticles (Figueiredo and Kascheres, 1997) and silica (SiO_2) were used (Mohammadizadeh et al., 2009). The synthesis of pyrazolo [3,4-*b*]pyridine derivatives was carried out by a cyclocondensation reaction in one pot assisted by microwave irradiation or by conventional heating (Bennamane et al., 2008).

Badr et *al*. prepared a series of pyrazoles, bipyrazoles and polysubstituted pyranopyrazoles that were evaluated for their cytotoxic properties (Badr and Abd El Razik, 2018). Also, Kumar et *al*. reported the synthesis of a series of 1*H*-pyrazolo[3,4-*b*]pyridines from the corresponding β-ketoester in four steps (Kumar et al., 2016).

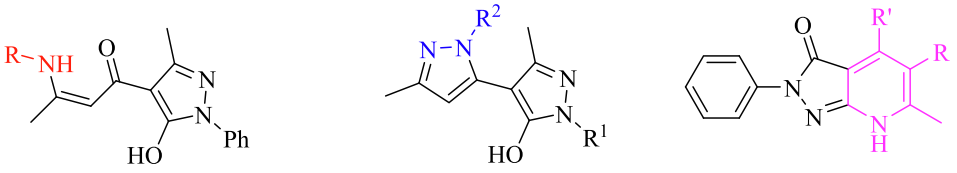
Preparation of a mixture of pyrazolo[3,4-*b*]pyridines and pyrazolo[4,3-*c*]pyridines from 3-acylpyridine-*N*-oxide of tosylhydrazones by reaction with electrophilic reagents in the presence of amines as base with high yields have been described (Lominac et al., 2012). The 1*H*-pyrazolo[3,4-*b*]pyridines were also prepared by condensation of amino-pyrazole and α,β-unsaturated ketones in ionic liquid without catalyst (Narsaiah et al., 2010). More recently, new compounds possessing skeletons characterized by a common pyrazole ring fused to a nitrogenous heterocycle entity to six membered were synthesized (Shekarrao et al., 2014). Recent works also describes the preparation of new pyrazoles (Tian et al., 2020) and chemical reactions involving the formation of enaminones (Guodong et al., 2020).

In previous work, we have developed a new method for the synthesis of pyrazolo[3,4-*b*]pyridine-3-ones and [1,5-*a*] pyrimidine-2(1*H*)-ones based on the reaction of 2-chloronicotinonitrile with some hydrazines at reflux of xylene or ethanol (Fadel et al., 2011). This work becomes the continuation of previous work of our research group, which studied the condensation of amino-pyrazole with α,β-unsaturated compounds. To develop a new and fast route with better performance by minimizing the reaction time of the condensation of the ethyl acetoacetate with 3-amino-1-phenyl-1*H*-pyrazol-5 (4*H*)-one to synthesis of 4-methyl-2-phenyl-2*H*-pyrazolo[3,4-*b*]pyridine-3,6(3a*H*,7*H*)-dione (Akhramez et al., 2016). The main objective of this work consists in the efficient preparation of suitably substituted mono-pyrazoles, bipyrazoles and pyrazolo-pyrimidines that allow us to know the structural requirements that determine the antioxidant and antibacterial activities of these pyrazole derivatives (Scheme 1).

2. Results and discussion

2.1. Chemistry

A general strategy was developed to obtain pyrazolo-enaminones from DHA. This procedure begins with condensation of commercially available DHA (dehydroacetic acid or 3-acetyl-4,6-dimethyl-2*H*-pyran-2-one) with phenylhydrazine to give the 1,3-dicarbonyl compound (**2**) in two steps, according to the procedure described in the literature with modifications (Bendaas et al., 1999). The condensation



mono-pyrazoles *bi-pyrazoles* *pyrazolo-pyridines*

Scheme 1 Pyrazole derivatives synthesized in this word.

of the pyrazolo-diketone (**2**) with selected anilines leads to pyrazolo-enaminones **3** (Scheme 2).

The addition of the 3-bromoaniline to the diketo-*N*-phenyl-pyrazole (**2**) and heating at reflux of the solvent indicated in the Table 1 during the time indicated in h in the same Table 1 allows obtaining the pyrazolo-enaminone **3a** with the indicated yields (Table 1, entries 1-11).

The addition of AcONa as a base and using ethanol as solvent leads to moderate yields (Table 1, entries 1 and 2). While the addition of acetic acid (AcOH) in ethanol allows to increase the yields (entries 3 and 4). Also, good results will be achieved by maintaining acid medium in methanol (Table 1, entries 6 and 7) or dichloromethane (Table 1, entry 9). These results show that the presence of acid or base is not essential for this reaction (Table 1, entries 5, 8, 10 and 11). The studies carried out indicate that heating for short reaction times (2 h) lead to better results than long times stirring at room temperature (18-20 h) (Table 1, compare entries 1 and 2; 3 and 4; 6 and 7).

Optimized conditions for **3a** (using acetic acid in ethanol and reflux for 2 h) have been applied to the condensation of diketo-*N*-phenyl-pyrazole (**2**) with different anilines to obtain pyrazolo-enaminones **3b-j** in yields ranging from 68 to 89% (Scheme 3).

In general, the presence of electron donor or withdrawing substituents on aromatic nucleus of anilines does not affect the yield of these amination.

The synthesis of 3,4′-bipyrazol-5′-ols from 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one (dehydroacetic acid, DHA) and hetero-aryls hydrazines was previously reported in the literature (Parshad et al., 2014). In this work the treatment of **2** with hydrazines gave 3;4′bipyrazol-5′-ol heterocycles **4a-g** in good yields (Scheme 3). The different electrophilicity of the two carbonyl groups of diketones compared to hydrazines explains the regioselectivity of this condensation reaction. The NH_2– group of the hydrazine reacts with the less hindered carbonyl and the intermediate from this condensation undergoes another condensation, this time between the second hydrogen of the hydrazine and the benzyl carbonyl which, followed by dehydration, gives rise to the formation of the pyrazole derivative. Despite condensing the diketone with a monosubstituted hydrazine, only the presence of the indicated regioisomer **4** was detected. The experimental results proved that the structure of the [3,4′]-bipyrazoles is indicated in the Scheme 3. Then the reaction of pyrazolyl-1,3-butanedione **2** with an equimolar amount of 5-amino-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **6**, in refluxing ethanol leads exclusively to the 4-(5-hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-6-methyl-2-phenyl-2*H*-pyrazolo[3,4-*b*]pyridin-3(3α*H*)-one **5**, with high yield (92%) (Scheme 4).

The condensation of the aminopyrazolone **6** with ethyl acetoacetate has been carried out under two different conditions (**A** and **B**) (Scheme 5).

The treatment of 5-amino-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **6** with 5 equivalents of ethyl acetoacetate without solvent and catalyst gives regioselectively 4-methyl-2-phenyl-2*H*-pyrazolo[3,4-*b*]pyridine-3,6(3a*H*,7*H*)-dione (**7**) in good yield (*Condition A*). While after several attempts to improve the yield of **8**, this was obtained with 54% when treating **6** with acetoacetate in toluene at 120 °C overnight (Scheme 5, *Condition B*).

Previously, the pyrazolopyridine **8** was prepared with a complicated method in only 42% of yield (Akhramez et al., 2016).

The condensation reaction of aminopyrazolone **6** with ethyl acetoacetate to give **7** was carried out with three different methods and each method several conditions were tested to determine the reaction type that gives the best yield, so we brought in different solvents, different catalysts and changes of the amount of ethyl acetoacetate as described in the (Table 2).

The amino group of the pyrazolone undergoes nucleophilic addition to the ester and gives the intermediate keto-amide that was involved in an intramolecular cyclisation followed by dehydration giving the pyrazolo-pyridine **7**. A plausible mechanism for the formation of **7** is detailed in the Scheme 6.

The utilization of polar protic solvents decreased the yields (EtOH, Table 2 entries 3–8 and MeOH, entries 9–11), but lipophilic solvents such as toluene significantly lowered the yields, fact attributable to high lipophilicity that difficult the solubility of starting material (Table 2, entries 12–13).

Without solvent, catalyst free and starting from 5 equivalents of acetoacetate ester, they were the best conditions (Table 2, entry 1). The microwave irradiation assistance was used to improve the yields and to reduce the reaction time (Table 2, entries 1, 2, 5, 6, 7, 8 and 9).

Using the conditions **B**, a mixture of the pyrazolo-pyrimidines **7** and **8** was obtained. The results of the NMR spectra as well as the results of the X-ray diffraction allowed to confirm the proposed structure for compound **8** (to see ORTEP, Fig. 1) (Akhramez et al., 2016). It is important to highlight that the X-ray diffraction results confirm that the **8** isomer corresponds to the represented structure and not as a pyridine isomer as indicated in previous scientific works (Fadel et al., 2011).

A *retro*-Claisen of the β-keto ester (ethyl acetoacetate) facilitates the acetylation of the aminopyrazole (**6**) followed by the condensation of this acetyl group with ethyl acetoacetate giving the corresponding unsaturated β-keto ester. Subsequently the intra-molecular condensation of the ketone function with the methylene group of the pyrazolone takes place yielding the pyrazolo-pyridine **8**. A plausible mechanism for the formation of **8** from the aminopyrazole **6** is indicated in Scheme 7.

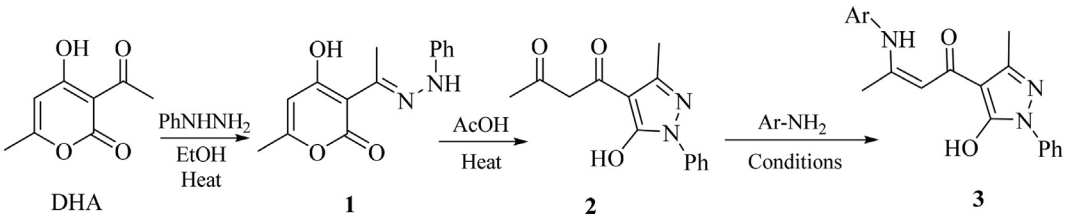
2.2. Potential biological results

Many compounds containing pyrazole heterocycle are found to have potent antibacterial and antioxidant properties (Anush et al., 2018).

In this work and in accordance with the foreseen objectives, the antioxidant and antibacterial activity of selected compounds from each series was evaluated to know the effect of the skeleton of these heterocyclic structures on the activity according to validated procedures.

2.2.1. ***Antioxidant activity*** *(Kedare and Singh, 2011)*

The free radical scavenging potentials of synthesis compounds and ascorbic acid (Vitamin C) at different concentrations were evaluated by DPPH test. Antioxidants react with DPPH, which is a nitrogen-centered radical with a characteristic absorption at 517 nm and convert to 1,1-diphenyl-2-picryl hydrazine.



Scheme 2 Synthesis of pyrazolo-enaminone **3** from DHA.

The degree of discoloration indicates the scavenging potentials of the antioxidant compounds as shown in Fig. 2, in which all representative pyrazole derivative compounds tested as well as ascorbic acid (Vitamin C) (positive control), have reduced the DPPH radical in dose dependent manner. Radical scavenging activity was expressed as the concentration (μg/mL) of compound that reduces DPPH (40 μg/mL) by 50% (IC_{50}).

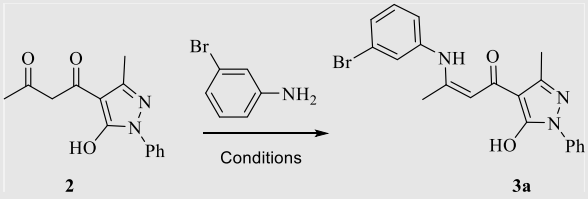
All these newly synthesized compounds exhibited significantly antioxidant activity, with an IC_{50} ranged between 5.5 and 180 mg/mL (Table 3).

When analyzing the results, no correlation was found between antioxidant power and polarity (log P). Thus, diketone **2**, which presents the lowest value of log P (1.42) of the compounds in the series of monopyrazoles, shows an antioxidant activity 16 times lower than that of **3h** (Table 3, see **2** and **3h** compounds). It should be noted that the aromatic hydroxyl group is essential for antioxidant activity but so is the position on the aromatic nucleus. The situation of the hydroxyl group in position *para* of the substituted aniline favors the antioxidant activity, as confirmed by the fact that compound **3h** has an antioxidant activity four times higher than the pyrazoloenaminone **3e** that has the hydroxyl group in position 3.

The compound **3h** has been found to be the strongest of this series (IC_{50} = 5.5 μg/mL), it shows the best antioxidant activity and of the same order as vitamin C (ascorbic acid, IC_{50} = 5-μg/mL) used as a reference (standard antioxidant). This excellent free radical scavenging activity with the DPPH method can be attributed to the hydroxyl groups of pyrazolo-enaminone **3h**.

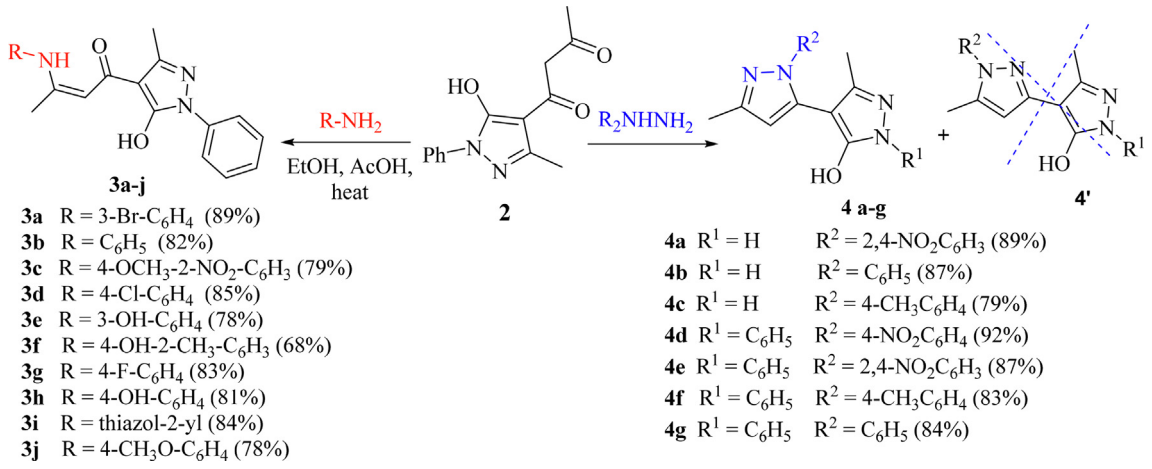
The presence of the 4-hydroxyphenylamino group can be oxidized to the corresponding quinonimine, manifesting a high antioxidant power. The same functional phenol group was possessed by compound **3e**, which show interesting activity (IC_{50} = 20 μg/mL), but less than **3h** as we have indicated, a fact attributable to the position of the hydroxyl group which, being in the *metha* position, does not allow the formation of quinonimines. Compound **3j** (IC_{50} = 90 μg/mL), which is equivalent to the methoxylated derivative of **3h**, has an antioxidant activity 18 times less than **3h**. This reduction in activity is attributed to the fact that the methoxyl group prevents the formation of the intermediate quinonimine responsible for the antioxidant power. The presence of halogens on the substituted aniline maintains the activity (see **3a** and **3d**). Among the bipyrazoles, it should be noted that **4a** is the one that shows the highest antioxidant activity (IC_{50} = 20 μg/mL). Among the bipyrazoles, it should be noted that **4a** is the one that shows the highest antioxidant activity. This activity seems to be related to the presence of nitro groups as substituents of the benzene ring. It is notable that **4a** loses 50% of its activity

Table 1 Optimization of the condensation reaction of diketo-*N*-phenylpyrazole with 3-bromoaniline.

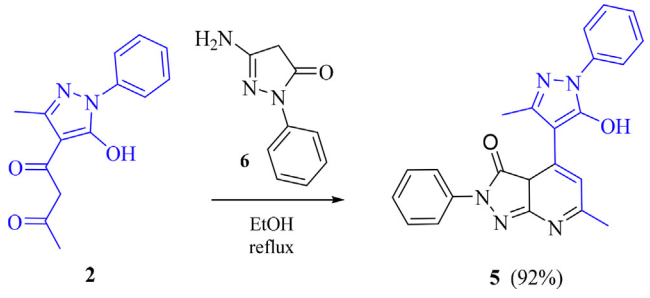


Entry	Temperature	Cat.	Solvent	Time (h)	Yield (%)
1	a	AcONa	EtOH	2	62
2	b	AcONa	EtOH	20	44
3	**a**	**AcOH**	**EtOH**	**2**	**89**
4	b	AcOH	EtOH	18	63
5	**a**	**–**	**EtOH**	**4**	**81**
6	a	AcOH	MeOH	2	77
7	b	AcOH	MeOH	20	58
8	a	–	Acetone	2	68
9	a	AcOH	CH_2Cl_2	2	70
10	a	–	$CHCl_3$	2	68
11	a	–	Acetonitrile	2	65

a: 80 °C; b: room temperature. These reactions were carried out with 1 mmol of **2** and 1 mmol of 3-bromoaniline in 5 mL of the chosen solvent.



Scheme 3 Synthesis of pyrazoloenaminones and bipyrazoles.



Scheme 4 Preparation of pyrazolo-pyrimidine **5.**

when a phenyl group is introduced on the nitrogen atom (**4e**, (IC_{50} = 40 µg/mL). The bipyrazole derivative **4d** (IC_{50} = 18 0 µg/mL), which has only one nitro group, has its antioxidant activity reduced by 4 times compared to **4e**, which has two nitro groups in its structure.

Finally, pyrazolopyridines **7** and **8** also have antioxidant properties that in this case do correlate with the polarity of the structure, that is, compound **7** with the lowest log P is the one with the highest antioxidant power (Table 3).

These results reveal that these compounds can be inhibiting or preventing the consequences of the oxidative stress. The ability of some compounds to scavenge free radicals in DPPH test does not mean that these compounds will perform readily where complex mechanisms are operating such as those in physiological substrates.

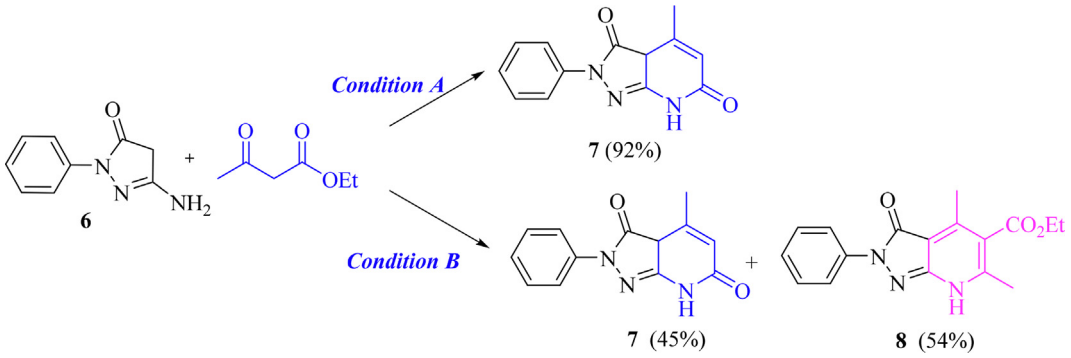
For this reason, there is need to verify the antioxidant effect in scavenging specific species such as superoxide anion radical ($O2^{\cdot -}$), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2). These oxygen species are often generated in organisms by diverse cellular processes, such as the electron transport chain in mitochondria, in microsomes and through enzymes like xanthine oxidase and NADPH oxidase or from exogenous factors. Studies are currently being carried out to determine the mechanism of action of the antioxidant effect of these compounds.

2.2.2. Antibacterial activity

Antibacterial properties of selected compounds were determined *in vitro* using a micro-dilution method (Balouiri et al., 2016) at different concentrations against both Gram-Negative (Escherichia coli ATCC 25922) (Table 4) and Gram-Positive (*Staphylococcus aureus* ATCC 25923) (Table 5) bacteria.

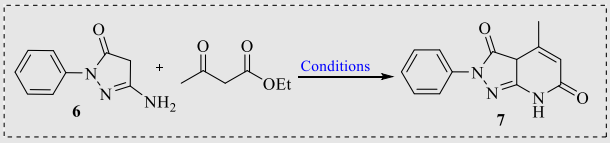
The results are expressed by the halo diameter of inhibition (mm), which signifies the sensitivity of the strains to the molecules tested.

The results obtained were compared with those obtained from commercial antibiotics (C (Chloramphenicol), CN (Gentamicin), TE (Tetracycline), AMC (Amoxicillin-Clavulanic acid)).



Scheme 5 *Condition A*: ethyl acetoacetate 5 eq, solvent free, 180 °C, 4 h. *Condition B*: ethyl acetoacetate 2 eq, toluene, 120 °C, overnight.

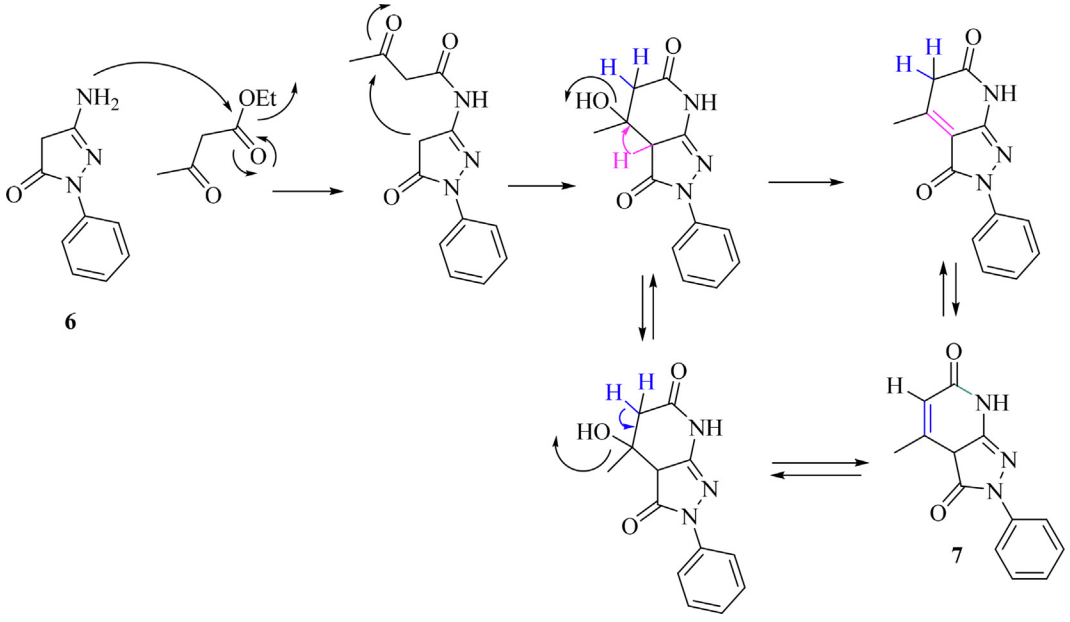
Table 2 Study of reaction conditions to synthesis of **7.**



					Conventional Heating		MW Irradiation		Sealed Tube	
Entry	*Cond. **A** or **B***	Solvent	ketoester ratio (eq)	Catalyst	Yield (%)	Time (h)	Yield (%)	Time (min)	Yield (%)	Time (h)
1	**A**	–	**5**	–	**92**	**4**	**95**	**10**	**92**	**2**
2	**A**	–	2	–	75	4	80	10	72	2
3	**C**	EtOH	1	–	63	6	66	15	60	3
4	**C**	EtOH	1.5	–	71	6	79	15	77	3
5	**C**	EtOH	1	APTS	72	6	80	15	66	3
6	**C**	EtOH	1	Acetic acid	76	6	83	15	67	3
7	**C**	EtOH	1.2	$NaNO_2/I_2$	43	8	44	15	39	4
8	**C**	EtOH	1	HCl	77	6	83	15	73	3
9	**C**	MeOH	1	–	64	6	73	15	59	3
10	**C**	MeOH	2	–	74	6	81	15	79	3
11	**C**	MeOH	1.5	APTS	75	6	81	15	77	3
12	**B**	Toluene	1	–	45	8	48	20	37	4
13	**B**	Toluene	1	APTS	51	8	54	20	41	4

A: 180 °C, 4 h. **B**: 120 °C, overnight. **MW**: 100 °C. **C**: other conditions.

All the compounds studied possessed some antibacterial activity against *E. coli* (Table 4). Growth of *E. coli* was significantly inhibited in the presence of **4b**, the most active compound. The compounds **3d**, **5** and **3b** also show interesting antibacterial activity superior to amoxicillin. The antibacterial activity is related to polarity / lipophilicity, the most active compounds are neither the most polar nor the most lipophilic (**4b** (log P = 3.07); **3d** (log P = 3.72); **5** (log P = 3.48); **3b** (log P = 3.16). Regarding the structure, bipyrazoles like **4b** show greater activity against Gram-negative bacteria (*E. coli*) than monopyrazole derivatives such as **3d** and **3b**. With regard to the substituents on the aromatic ring, the presence of electron donating (**4c**, **4f**) or electron-withdrawing substituents (**4a**, **4d**, **4e**) leads to a decrease in antibacterial activity. While in the series of enanimones, the presence of chlorine as a substituent in the 4-position of aniline (**3d**) presents activity of the same order as that derived with aniline without substituents (**3b**). However, the presence of hydroxyl groups (**3h**, **3e**) leads to a decrease in activity against *E. coli*.



Scheme 6 A plausible mechanism for the formation of **7** from the aminopyrazole **6.**

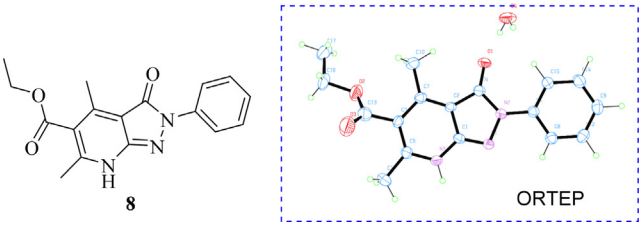


Fig. 1 ORTEP diagram of the pyrazolo-pyridine **8** (Lominac et al., 2012).

From these results, compounds **3e**, **3d**, **3j** and **4a** were more active than other compounds against *Staphylococcus aureus* (Table 5). To these must be added **4b**, which has an important antibacterial activity against both Gram (+) (Table 5) and Gram (−) (Table 4). The presence of 4-chlorophenyl and 3-hydroxy groups as well as 4-nitrophenyl enhances the antibacterial activity of pyrazolo-enaminones, and 4-nitrophenyl enhances the activity of bipyrazole compounds. With regard to enaminones, the presence of a hydroxyl substituent in the *metha* position of the aniline (**3e**) is more favorable than in the *para* position (**3h**). While in the case of halogens, the substitution in *para* (**3d**) is more appropriate than in *metha* position (**3a**). Furthermore, bipyrazolopyridines (**5**) and bipyrazoles (**7**) have often exhibited good activity. From the results of the antioxidant activity, compound **3h** appears to be the most promising, while **3e** shows the best inhibitory effect against *S. aureus* and bipyrazole **4b** satisfactorily inhibit the growth of *E. coli*. Due to the serious problems of resistance to the antibacterials currently used for the treatment of infections, it is of interest and the search for new antimicrobial agents is vital.

3. Conclusion

A series of heterocyclic enaminones (**3a-j**) has been synthesized with good yields under classical conditions. This preparation was based on a condensation of diketo-*N*-phenyl-pyrazole with different substituted anilines in the presence of catalytic amount of acid. Subsequently, bipyrazole structures (**4a-g**) were obtained by condensing the diketo-*N*-phenyl-pyrazole (**2**) with different phenyl-hydrazines. Furthermore, it was carried out, a new synthesis of bi-pyrazolopyridine (**5**) by a fast one-step condensation of diketo-*N*-phenyl-pyrazole (**2**) with 3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**6**). Several of this nitrogenated compounds have shown an interesting antibacterial activity against *E. coli* (**3b**, **3d**, **3e** and **4d**) or *S. aureus* (**3e** and **4a**). In addition, some compounds have presented good antioxidant results, especially the enaminone **3h** with results of the same order as vitamin C. Finally, it should be noted that the not inconsiderable activity of compounds **3h** (antioxidant), **3b** (antibacterial activity against *E. coli*), and **3e** (antibacterial activity against *S. aureus*), turns them into leads that, through new modifications, can optimize both their activity and their pharmacokinetics.

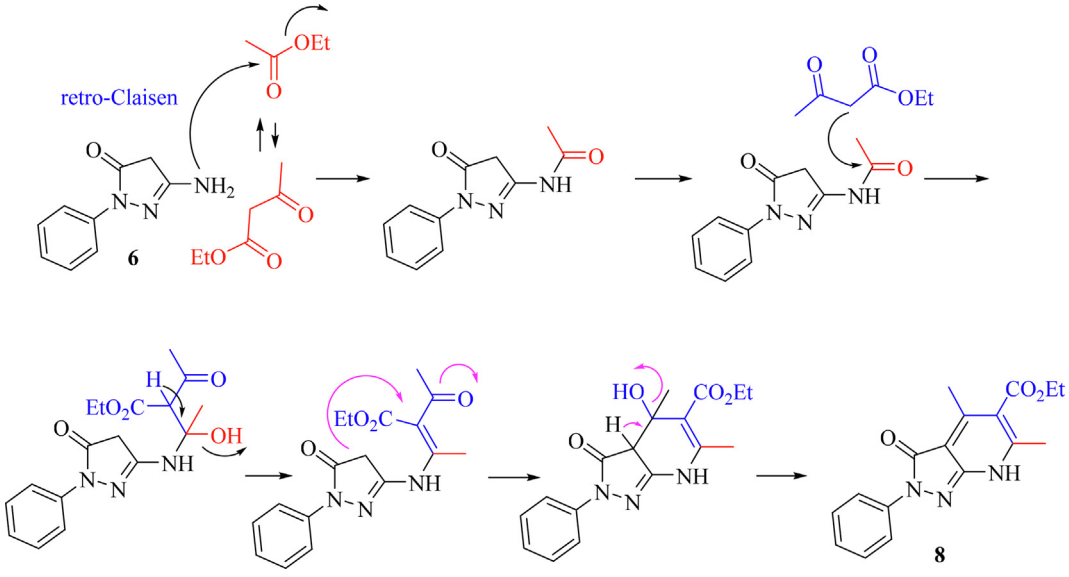
4. Experimental section

4.1. Chemistry

4.1.1. General

Microwave-assisted reactions were carried out in a CEM Discover LabMate Microwave synthesis instrument in a glass tube (10 mL) sealed with a silicon septum under magnetic stirring and an IR sensor measured the temperature of vessel surface. Fixed temperature of 100 °C with a variable MW power (maximum 300 W) was used. The reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel (60 F254, Merck) plates. Compounds were visualized by UV irradiation.

^{1}H and ^{13}C NMR spectra were recorded with a Bruker spectrometer at 300 MHz (^{13}C, 75.5 MHz). Chemical shifts are given in parts per million (ppm) from tetramethylsilane (TMS) as internal standard in $CDCl_3$, and the residual peak of DMSO in DMSO d_6. Melting points (mp [°C]) were taken



Scheme 7 Proposed mechanism for the formation of **8.**

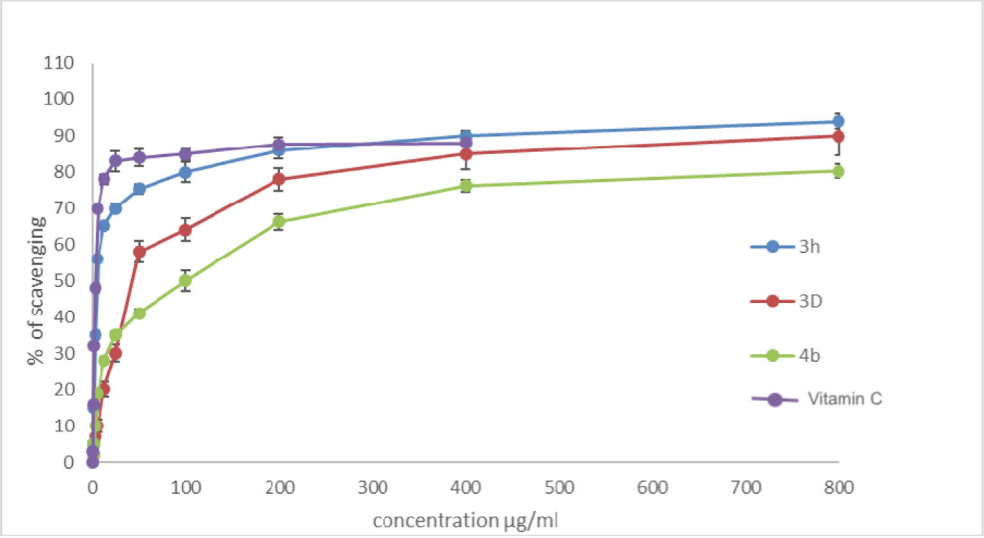


Fig. 2 Antioxidant activity of pyrazole-derivatives evaluated by DPPH method.

on samples in open capillary tubes and are not corrected. The following abbreviations are used for the ^{1}H NMR spectra multiplicities: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet. Coupling constants (*J*) are reported in Hertz [Hz]. Elemental analyzes have been performed on a LECO TruSpec CHN Elemental Analyzer. ESI Mass Spectrums have been carried out with Agilent LC/MSD/ToF mass spectrophotometer (CCITUB, Chemical Faculty, University of Barcelona).

Compounds **1, 2, 4d, 4e** and **4g** were reported previously by Singh**,** S. P. Naithani, R.; Aggarwal, R.; Prakash, O. C-C Bond Cleavage Studies in Bipyrazoles: A Convenient Synthesis of Pyrazolo-5-ols *Synth. Commun.* **2005**, *35*, 611–619.

Compound **3b** was previously described by Bendaas, A.; Hamdi, M.; Sellier, N. Synthesis of bipyrazoles and pyrazoloisoxazoles from 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one. *J. Heterocycl. Chem.* **1999**, 35, 611–619.

Compound **3j** was previously reported by Asiri, A. M.; Al-Youbi, O. A.; Faidallah, H. M.; Weng, S.; Tiekinkc, R. T. (2*Z*)-1-(5-Hydroxy-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-(4-methoxyanilino)-but-2-en-1-one. *Acta Cryst., Section E*: Structure Reports Online, **2011**, 67, o2353.

Compound **3g** was previously reported by Asiri, Abdullah M. *Acta Cryst., Section E*: Structure Reports Online, **2012**, 68, o764.

Compound **3d** was previously reported by Asiri, Abdullah M. *Acta Cryst., Section E*: Structure Reports Online, **2011**, 67, o2157.

4.1.2. General procedure for the synthesis of (E)-4-hydroxy-6-methyl-3-(1-(2-phenylhydrazono)ethyl)-2H-pyran-2-one ***(1)***

Phenylhydrazine (17.8 mmol, 1.75 mL) was added to a solution of dehydroacetic acid (17.9 mmol, 3.02 g) in hot ethanol (20 mL). After heating for 5 min under reflux, the yellow precipitate formed was collected by filtration and the solid obtained is recrystallized from ethanol to give (**1**) as yellow crystalline product, mp 205–207 °C; 95% yield (4.370 g, 0.017 mol). ^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) 16.1 (s, 1H, OH), 7.77 (d, J = 7.5, 2H) 7.19 (t, J = 8.1, 2H) 7.03 (t, J = 8.4, 1H), 6.43(s, 1H, NH), 5.81 (s, 1H, H), 2.53 (s, 3H, CH_3), 2.10 (s, 3H, CH_3). ^{13}C-RMN (75.5 MHz, $CDCl_3$): δ (ppm)181.0; 169.5; 163.7; 163.6; 145.0; 130.1; 122.5; 113.8; 105.8; 96.6; 20.3; 16.4.

4.1.3. Synthesis of 1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)butane-1,3-dione ***(2)***

A solution of *N*-2-phenylhydrazone (**1**) (2.6 g, 10.2 mmol) in glacial acetic acid (20 mL) is refluxed for 2 h, and then the crude reaction was extracted with CH_2Cl_2/water. After evapo-

Table 3 Results of the antioxidant activity.

Compound	IC_{50} µg/mL	Log P	Compound	IC_{50} µg/mL	Log P
2	80 ± 5.9	1.42	**4b**	100 ± 6.5	3.07
3a	18 ± 1.9	3.99	**4d**	180 ± 9.0	4.02
3b	34 ± 1.5	3.16	**4e**	40 ± 3.0	4.25
3c	21 ± 2.0	3.22	**4f**	80 ± 5.0	4.85
3d	40 ± 2.5	3.72	**4g**	40 ± 2.2	4.97
3e	20 ± 1.7	3.85	**5**	48 ± 4.0	3.48
3h	5.5 ± 0.3	2.77	**7**	21 ± 1.3	1.07
3j	90 ± 5.0	3.03	**8**	48 ± 2.0	1.83
4a	20 ± 1.4	4.06	Vitamin C	5 ± 0.7	−2.15

Table 4 Antibacterial activity on (Escherichia coli ATCC 25922).

Compound	inhibition area (mm)				
	2 mg/mL	4 mg/mL	5 mg/mL	6 mg/mL	8 mg/mL
2	12 ± 0.2	13 ± 0.3	15 ± 0.1	15 ± 1.4	16 ± 0.6
3a	6 ± 0.1	7 ± 0.4	7 ± 0.2	7 ± 1.1	7 ± 0.7
3b	15 ± 0.4	14 ± 0.7	14 ± 0.5	17 ± 0.1	17 ± 0.1
3c	13 ± 0.1	9 ± 0.4	7 ± 0.4	7 ± 0.6	6 ± 0.2
3d	16 ± 0.3	14 ± 0.7	15 ± 07	17 ± 0.3	19 ± 0.8
3e	10 ± 0.2	27 ± 0.8	23 ± 2.1	16 ± 0.6	9 ± 0.4
3h	6 ± 0.1	9 ± 0.1	8 ± 0.9	8 ± 0.4	10 ± 0.5
3j	9 ± 0.1	18 ± 0.2	15 ± 1.1	12 ± 0.7	10 ± 0.9
4a	10 ± 0.4	8 ± 0.3	8 ± 0.1	6 ± 0.7	7 ± 0.1
4b	19 ± 0.4	18 ± 0.8	9 ± 0.7	11 ± 0.3	12 ± 1.2
4d	10 ± 0.3	11 ± 0.4	11 ± 0.3	13 ± 0.1	14 ± 0.6
4e	12 ± 0.5	8 ± 0.2	9 ± 0.1	9 ± 0.8	1 ± 0.5
4f	10 ± 0.8	5 ± 0.4	6 ± 0.6	8 ± 0.4	7 ± 0.4
4g	9 ± 0.2	10 ± 0.7	10 ± 0.7	12 ± 0.6	17 ± 0.5
5	15 ± 0.8	16 ± 0.6	17 ± 0.1	14 ± 0.8	16 ± 0.9
7	10 ± 0.3	11 ± 0.6	11 ± 0.3	10 ± 0.3	12 ± 0.7
8	7 ± 0.5	7 ± 0.5	8 ± 0.6	7 ± 0.4	8 ± 0.7
C	22 ± 0.9	–	–	–	–
CN	26 ± 0.7	–	–	–	–
TE	15 ± 0.1	–	–	–	–
AMC	7 ± 0.1	–	–	–	–

ration of the solvent, the crude product (2.0 g, 7.740 mmol) was recrystallized from acetonitrile to give **2** as a yellow solid, mp 89–91 °C; 75% yield (1.970 g, 7.630 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ(ppm) 14.70 (br, 1H, OH), 11.33 (s, 1H, OH), 8.11 (d, J = 7.5, 2H, Ar), 7.12 (t, J = 8.1, 2H, Ar), 6.90 (t, J = 8.1, 1H, Ar), 5.66 (s,1H), 2.47 (s, 3H, CH_3), 1.79 (s, 3H, CH_3); ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm) 188.6; 171.2; 163.5; 144.5; 139.9; 128.4 (x 2); 123.4; 117.6 (x 2); 95.2; 58.6; 23.0; 15.6.

*4.1.4. General procedure for the synthesis of pyrazolo-enaminones **3a-j***

To a solution of 1-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)butane-1,3-dione (**2**) (258 mg, 1 mmol) in absolute

Table 5 Antibacterial activity on *Staphylococcus aureus* ATCC 25923).

Compound	Inhibition area (mm)				
	2 mg/mL	4 mg/mL	5 mg/mL	6 mg/mL	8 mg/mL
2	9 ± 1.0	10 ± 1.2	12 ± 1.1	10 ± 1.0	10 ± 1.0
3a	12 ± 1.0	8 ± 0.98	8 ± 1.0	8 ± 1.0	7 ± 1.0
3b	12 ± 1.0	8 ± 1.0	7 ± 1.0	11 ± 1.0	11 ± 1.0
3c	13 ± 1.0	12 ± 1.0	8 ± 1.0	7 ± 1.0	10 ± 1.0
3d	16 ± 1.1	12 ± 1.0	12 ± 1.2	10 ± 0.8	11 ± 1.0
3e	19 ± 1.2	18 ± 1.0	17 ± 1.0	16 ± 1.1	18 ± 1.1
3h	11 ± 1.0	11 ± 1.0	10 ± 0.8	10 ± 1.0	10 ± 1.0
3j	14 ± 1.1	10 ± 1.0	10 ± 1.0	13 ± 1.0	13 ± 1.0
4a	15 ± 1.1	12 ± 1.0	16 ± 1.0	14 ± 0.9	12 ± 1.0
4b	14 ± 1.1	11 ± 0.9	12 ± 1.0	14 ± 1.1	16 ± 1.0
4d	9 ± 1.0	10 ± 1.0	10 ± 1.0	14 ± 1.0	12 ± 1.0
4e	9 ± 1.0	9 ± 0.96	13 ± 1.0	16 ± 1.1	12 ± 1.0
4f	11 ± 1.0	10 ± 1.0	13 ± 1.0	13 ± 0.8	15 ± 1.0
4g	7 ± 1.0	9 ± 1.0	8 ± 1.0	6 ± 1.0	5 ± 1.0
5	12 ± 1.0	9 ± 1.0	9 ± 1.0	10 ± 1.0	10 ± 1.0
7	12 ± 1.0	9 ± 1.0	11 ± 1.0	9 ± 1.0	8 ± 1.0
8	9 ± 1.0	13 ± 1.0	8 ± 1.0	8 ± 1.0	10 ± 1.0
C	23 ± 1.0	–	–	–	–
CN	15 ± 1.0	–	–	–	–
TE	21 ± 1.0	–	–	–	–
AMC	22 ± 1.0	–	–	–	–

ethanol (5 mL), a suitable amine (1 mmol) was added with a few drops of acetic acid. The crude reaction is refluxed under magnetic stirring. Once the reaction was judged complete, the expected product was isolated by chromatography on silica gel (eluent: hexane/ethyl acetate: 7/3).

(Z)-3-((3-Bromophenyl)amino)-1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)but-2-en-1-one ***3a***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a yellow solid. mp 176–178 °C; 89% yield (0.370 g, 0.897 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ(ppm) 11.98 (s, OH); 7.94 (s, 1H, NH); 7.91–6.56 (m, 9H, ArH); 5.74 (s, 1H, CH); 2.41 (s, 3H, CH_3); 2.01 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm) 191.5; 160.7; 149.3; 145.8; 136.4; 131.7; 129.9; 129.1 (x 2); 126.9; 126.1; 122.0 (x 2); 121.1; 112.8; 103.5; 97.4; 24.2; 14.1. MS *m*/*z* (%): M^+ = 412.09 (100%). *Anal*. Calcd. for $C_{20}H_{18}BrN_3O_2$: C, 58.38; H, 4.40; N, 10.20. Found: C, 58.30; H, 4.31; N, 10.17.

(Z)-1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(phenylamino)but-2-en-1-one ***3b***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a yellow solid. mp 170–172 °C; 82% yield (0.270 g, 0.809 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ(ppm) 11.97 (s, OH); 7.68–7.40 (m, 5H, ArH); 7,39 (s, 1H, NH); 7,23–6,74 (m, 5H, ArH); 5,66 (s, 1H, CH); 2,70 (s, 3H, CH_3); 2,25 (s, 3H, CH_3). ^{13}C-RMN (75.5 MHz, DMSO d_6): δ(ppm) 192.8; 159.7; 149.1); 138.1; 134.5; 130.6 (x 2); 129.7 (x 2); 128.9 (2 x); 128.4; 126.1; 122.8 (x 2); 122.4; 102.4; 98.9; 22.7; 14.0. MS *m*/*z* (%): M^+ = 343.01 (100%). *Anal*. Calcd. for $C_{20}H_{19}N_3O_2$: C, 72.05; H, 5.74; N, 12.60. Found: C, 71.93; H, 5.61; N, 12.59.

(Z)-1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-((4-methoxy-2-nitrophenyl)amino)but-2-en-1-one ***3c***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a pink solid. mp 178–180 °C; 79% yield (0.322 g, 0.788 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ(ppm) 11.98 (s, OH); 7.65–7.42 (m, 5H, ArH); 7.35 (s, 1H, ArH); 7.24 (s, 1H, NH); 7.13 (d, J = 9.3, 1H, ArH); 6.96 (d, J = 9.3, 1H, ArH); 5.79 (s, 1H, CH); 3.70 (s, 3H, OCH_3); 2.65 (s, 3H, CH_3); 2.29 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm) 191.3; 160.2; 155.6; 149.5; 138.5; 137.2; 132.5; 130.3 (x 2); 128.4 (x 2); 126.5); 122.8 (x 2); 121.4; 118.9; 105.4; 101.3; 99.7; 55.9; 23.8; 14.5. MS *m*/*z* (%): M^+ = 409.11 (100%). *Anal*. Calcd. for $C_{21}H_{20}N_4O_5$: C, 61.74; H, 4.90; N, 13.72. Found: C, 61.63; H, 4.81; N, 13.58.

(Z)-3-((4-Chlorophenyl)amino)-1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)but-2-en-1-one ***3d***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a yellow solid. mp 191–193 °C; 85% yield (0.312 g, 0.848 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 11.96 (s, OH); 7.68 (d, J = 8 Hz, 2H, ArH); 7.49 (t, J = 8 Hz, 2H, ArH); 7.36 (d, J = 8 Hz, 2H, ArH); 7.24–7.26 (m, 1H, Ar); 7.12 (d, J = 8 Hz, 2H, ArH); 5.78 (s, 1H, CH); 2.50 (s, 3H, CH_3); 2.25 (s, 3H, CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ(ppm) 185.2; 160.1; 146.2; 138.1; 136.1; 135.4; 135.1; 132.6; 129.8 (x 2); 128.9 (x 2); 126.8 (x 2); 122.2 (x 2); 101.5; 97.7; 22.6; 15.2. MS *m*/*z* (%): M^+ = 368.02 (100%). *Anal*. Calcd. for $C_{20}H_{18}ClN_3O_2$: C, 65.37; H, 4.90; N, 11.44. Found: C, 65.28; H, 4.78; N, 11.29.

(Z)-1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-((3-hydroxyphenyl)amino)but-2-en-1-one ***3e***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as an orange solid. mp 228–230 °C; 78% yield (0.272 g, 0.778 mmol). **^{1}H NMR** (400 MHz, $CDCl_3$): δ(ppm) 11.92 (s, OH); 7.88 ((d, J = 8 Hz, 2H, ArH); 7.45 (t, J = 8 Hz, 2H, ArH); 7.27 (t, J = 8 Hz, 1H, ArH); 6.65–6.72 (m, 3H, ArH); 5.70 (s, 1H, CH); 2.51 (s, 1H, CH_3); 2.18 (s, 1H, CH_3). **^{13}C NMR** (100.6 MHz, $CDCl_3$): δ(ppm) 187.5; 157.2; 149.0; 140.3; 130.9; 128.9 (x 2); 126.5; 122.9 (x 2); 115.6; 109.9; 100.0; 99.7; 98.2; 20.8; 15.6. *Anal*. Calcd. for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found: C, 68.30; H, 5.31; N, 12.17.

(Z)-3-((4-Hydroxy-2-methylphenyl)amino)-1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)but-2-en-1-one ***3f***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a yellow solid. mp 235–237 °C; 68% yield (0.246 g, 0.677 mmol). **^{1}H NMR** (300 MHz, DMSO d_6): δ(ppm) 11.96 (s, OH); 7.59–7.45 (m, 5H, ArH); 7.22 (s, 1H, NH); 6.59 (s, 1H, ArH); 6.43 (d, J = 9.3, 1H, ArH); 6.38 (d, J = 9.3, 1H, ArH); 5.72 (s, 1H, CH); 2.58 (s, 3H, CH_3); 2.36 (s, 3H, CH_3); 2.24 (s, 3H, CH_3). **^{13}C NMR** (75.5 MHz, DMSO d_6): δ (ppm) 189.2; 160.3; 153.6; 149.3; 138.5; 132.8; 130.3 (x 2); 128.2; 124.5 (x 2); 121.0; 114.7; 105.1; 88.7; 20.8; 19.1; 13.7. *Anal*. Calcd. for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.33; H, 5.93; N, 11.58.

(Z)-3-((4-Fluorophenyl)amino)-1-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)but-2-en-1-one ***3g***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a yellow solid. mp 180–182 °C; 83% yield (0.291 g, 0.828 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm) 11.98 (s, OH); 7.78 (d, J = 8 Hz, 2H, ArH); 7.45 (t, J = 8 Hz, 2H, ArH); 7,35 (t, J = 8 Hz, 2H, ArH); 7.22 (d, J = 7.8, 2H, ArH); 7.12 (d, J = 7.8, 2H, ArH); 5.45 (s, 1H, CH); 2.54 (s, 3H, CH_3); 2.16 (s, 3H, CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$)): δ(ppm) 185.1; 162.2; 161.0; 160.2; 146.3; 138.3; 137.5; 133.9; 130,1 (x 2); 129.1; 127.4 (x 2); 125.9 (x 2); 121.3; 120.2 (x 2); 115.7; 104.6; 96.9; 20.2; 15.3. MS *m*/*z* (%): M^+ = 352.08 (100%). *Anal*. Calcd. for $C_{20}H_{18}FN_3O_2$: C, 68.35; H, 5.16; N, 11.96. Found: C, 68.30; H, 5.02; N, 11.80.

(Z)-1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-((4-hydroxyphenyl)amino)but-2-en-1-one ***3 h:*** Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a yellow solid. mp 226–228 °C; 81% yield (0.283 g, 0.809 mmol). **^{1}H NMR** (400 MHz, $CDCl_3$): δ(ppm) 11.55 (s, OH); 7.87 (d, J = 8 Hz, 2H, ArH); 7.48 (t, J = 8 Hz, 2H, ArH); 7.27 (t, J = 8 Hz, 2H, ArH); 7.12 (d, J = 8 Hz, 2H, ArH); 6.58 (d, J = 8 Hz, 2H, ArH); 5.38 (s, 1H, CH); 2.46 (s, 3H, CH_3); 2.11 (s, 3H, CH_3). **^{13}C NMR** (100.6 MHz, $CDCl_3$): δ(ppm) 185.7; 170.0; 160.2; 151.9; 150.4; 138.5; 136.4; 129.2 (x 2); 125.1; 122.8 (x 2); 116.2; 114.0; 101.5; 98.6; 21.5; 14.1. *Anal*. Calcd. for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found: C, 68.99; H, 5.42; N, 11.98.

(Z)-1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-(thiazol-2-ylamino)but-2-en-1-one ***3i***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a purple solid. mp 173–175 °C; Yield 84%; **NMR** (300 MHz, DMSO d_6): δ (ppm) 8.55 (d, J = 5.1 Hz, 1H, CH-N); 8.03 (dd, J_1 = 8.7 Hz, J_2 = 1.2 Hz, 2H, CH-$Ar_{(ortho)}$; 7.97 (d,

J = 4.8 Hz, 1H, CH-S); 7.42 (s, 1H, CH); 7.32 (dt, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 2H, CH-$Ar_{(meta)}$); 7.03 (dt, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H, CH-$Ar_{(para)}$); 2.58 (s, 3H, CH_3); 2.31 (s, 3H, CH_3). **^{13}C NMR** (75.5 MHz, DMSO d_6): δ(ppm) 164.0; 163.8; 162.6; 149.7; 147.1; 140.7; 129.9; 128.8 (x 2); 123.2; 118.4 (x 2); 116.1; 112.7; 94.2; 24.4; 16.1. *Anal.* Cald.$C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46. Found: C, 60.04; H, 4.88; N, 16.61.

(Z)-1-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-((4-methoxyphenyl)amino)but-2-en-1-one ***3j****:* Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a yellow solid. mp 222–224 °C; 78% yield (0.283 g, 0.778 mmol). **^{1}H NMR** (400 MHz, $CDCl_3$): δ(ppm) 11.62 (s, OH); 7.80 (d, J = 8 Hz, 2H, ArH); 7.48 7.24 (t, J = 8 Hz, 3H, ArH); 7.22 (d, J = 8 Hz, 2H, ArH); 7.12 (d, J = 8 Hz, 2H, ArH); 5.46 (s, 1H, CH); 3.73 (s, 3H, OCH_3); 2.49 (s, 3H, CH_3); 2.17 (s, 1H, CH_3); 2.05 (H_2O). **^{13}C NMR** (100.6 MHz, $CDCl_3$): δ(ppm) 187.2; 159.1; 155.6; 149.5; 137.2; 132.5; 129.3 (x 2); 127.3 (x 2); 126.3; 122.8 (x 2); 121.4; 117.5 (x 2); 102.2; 99.7; 53.8; 22.6; 17.0. *Anal.* Calcd. for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.49; H, 5.79; N, 11.48.

4.1.5. General procedure for the synthesis of 3,4′-bipyrazol-5′-oles ***4a-g***

A mixture of pyrazolylbutane-1,3-dione (**2**) (258 mg, 1 mmol) and an aromatic hydrazine (1 mmol) in absolute ethanol (5 mL) was refluxed for 2 h. The precipitate obtained from the hot solution is collected by filtration and washed several times with diethyl ether and recrystallized from ethanol.

2-(2,4-Dinitrophenyl)-3′,5-dimethyl-1′H,2H-[3,4′-bipyrazol]-5′-ol ***4a***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a yellow solid. mp 262–264 °C; 89% yield (0.307 g, 0.89 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ(ppm) 12.01 (s, 1H, exchangeable OH); 8.66 (s, 1H, ArH); 8.21 (d, J = 7.4, 1H, ArH); 8.16 (s, 1H, NH); 7.84 (d, J = 7.3, 1H, ArH); 6.14 (s, 1H, CH); 2.31 (s, 3H, CH_3); 1.81 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm) 161.2; 150.1; 147.8; 144.7; 142.3; 140.1; 137.8; 128.3; 124.9; 119.6; 111.2; 106.3; 13.6; 12.1. MS m/z (%): M^+ = 345.12 (100%). *Anal.* Calcd. for $C_{14}H_{12}N_6O_5$: C, 48.84; H, 3.51; N, 24.41. Found: C, 48.67; H, 3.42; N, 24.29.

3′,5-Dimethyl-2-phenyl-1′H,2H-[3,4′-bipyrazol]-5′-ol ***4b***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as an yellow solid. mp 253–255 °C; 87% yield (0.221 g, 0.869 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ(ppm) 11.91 (s, 1H, exchangeable OH); 8.10 (s, 1H, NH); 7.69 (d, J = 8,2, 2H, ArH); 7.41 (t, J = 8.4, 2H, ArH); 7.25 (t, J = 8.4, 1H, ArH); 6.09 (s, 1H, CH); 2.29 (s, 3H, CH_3); 1.84 (s, 3H, CH_3). ^{13}C NMR (δ-ppm): 159.8; 152.8; 142.7; 140.0; 138.8; 129.7 (x 2); 127.2; 123.8 (x 2); 106.7; 104.1; 13.1; 12.7. MS m/z (%): M^+ = 255.10 (100%). *Anal.* Calcd. for $C_{14}H_{14}N_4O$: C, 66.13; H, 5.55; N, 22.03. Found: C, 65.98; H, 5.44; N, 21.93.

3′,5-Dimethyl-2-(p-tolyl)-1′H,2H-[3,4′-bipyrazol]-5′-ol ***4c***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as an orange solid. mp 267–269 °C; 79% yield (0.345 g, 0.919 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ (ppm) 12.20 (s, 1H, exchangeable OH); 8.23 (s, 1H, NH); 7.61 (d, J = 7.1, 2H, ArH); 7.45 (d, J = 7.1, 2H, ArH); 6.39 (s, 1H, CH); 2.39 (s, 3H, CH_3); 2.32 (s, 3H, CH_3); 1.84 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm) 157.9; 148.3; 141.4; 138.7; 137.8; 137.1; 129.0; 124.6; 109.2; 105.0; 21.4; 14.1; 13.6. MS m/z (%): M^+ = 269.22 (100%). *Anal.* Calcd. for $C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 66.95; H, 5.84; N, 20.83.

3′,5-Dimethyl-2-(4-nitrophenyl)-1′H,2H-[3,4′-bipyrazol]-5′-ol ***4d***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as an orange solid; mp 239–241 °C; 92% yield (0.345 g, 0.919 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ (ppm) 11.99 (s, 1H, exchangeable OH); 8.24 (d, J = 9.3, 2H, ArH); 7.65 (m, 3H, ArH); 7.41 (t, J = 7.5, 2H, ArH); 7.24 (t, J = 7.2, 1H, ArH); 6.38 (s, 1H, CH); 2.28 (s, 3H, CH_3); 1.93 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm) 150.5; 149.2; 147.3; 145.9; 145.2; 138.3; 135.5; 129.3 (x 4); 125.9; 125.0; 123.2 (x 2); 120.9 (x 2); 111.4; 99.1; 13.9; 12.9. MS m/z (%): M^+ = 376.10 (100%). *Anal.* Calcd. for $C_{20}H_{17}N_5O_3$: C, 63.99; H, 4.56; N, 18.66. Found: C, 66.80; H, 4.44; N, 18.53.

2-(2,4-Dinitrophenyl)-3′,5-dimethyl-1′-phenyl-1′H,2H-[3,4′-bipyrazol]-5′-ol ***4e***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as an orange solid; mp 230–232 °C; 87% yield (0.365 g, 0.868 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ(ppm) 12.10 (s, 1H, exchangeable OH); 9.31 (s, 1H, ArH); 8.80 (d, J = 9.6, 1H, ArH); 8.29 (d, J = 9.6, 1H, ArH); 7.62–7.41 (m, 5H, ArH); 6.68 (s, 1H, CH); 2.44 (s, 3H, CH_3); 1.89 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm)150.7; 148.4; 146.2; 145.6; 143.1; 139.9; 137.9; 136.8; 129.4 (x 2); 127.7; 126.1; 124.5; 121.0 (x 2); 120.2; 108.7; 97.1; 14.4; 13.6. MS m/z (%): M^+ = 350.26 (100%). *Anal.* Calcd. for $C_{20}H_{16}N_6O_5$: C, 57.14; H, 3.84; N, 19.99. Found: C, 57.60; H, 3.37; N, 19.93.

3′,5-Dimethyl-1′-phenyl-2-(p-tolyl)-1′H,2H-[3,4′-bipyrazol]-5′-ol ***4f***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a brown solid. mp 235–237 °C; 83% yield (0.285 g, 0.827 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ (ppm) 12.21 (s, 1H, exchangeable OH); 7.68 (d, J = 8.7, 2H, ArH); 7.49 (t, J = 8.4, 2H, ArH); 7.44 (d, J = 8.2, 2H, ArH); 7.35 (d, J = 8.1, 2H, ArH); 7.24 (t, J = 7.2, 1H, ArH); 6.24 (s, 1H, CH); 2.31 (s, 3H, CH_3); 2.26 (s, 3H, CH_3); 1.74 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm) 152,3; 149.7; 148.4; 138.4; 136.3; 134.5; 130.4 (x 2); 129.7 (x 2); 129.2 (x 2); 126.3; 125.7; 122.6 (x 2); 109.6; 95.4; 20.9; 13.9; 12.9. MS m/z (%): M^+ = 345.04 (100%). *Anal.* Calcd. for $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27. Found: C, 72.99; H, 5.70; N, 16.13.

3′,5-Dimethyl-1′,2-diphenyl-1′H,2H-[3,4′-bipyrazol]-5′-ol ***4 g***: Starting from 1 mmol (258 mg) of **2** and following the general procedure indicated above, the desired compound was obtained as a white solid. mp 228–230 °C; 84% yield (0.277 g, 0.838 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ (ppm) 12.07 (s, 1H, exchangeable OH); 7.66–7.44 (m, 10*H*, ArH); 6.33 (s, 1H, CH); 2.29 (s, 3H, CH_3); 1.94 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ(ppm) 165.4; 160.2; 152.7; 150.0; 146.0; 141.6; 140.6; 128.9 (x 2); 128.8 (x 2); 123.3; 122.8; 118,3 (x 2); 102.6; 101.3; 91.9; 14.2; 13.1. MS m/z (%): M^+ = 330.15 (100%). *Anal.* Calcd. for

$C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.05; H, 5.30; N, 16.86.

*4.1.6. 4-(5-Hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-6-methyl-2-phenyl-2,3a-dihydro-3H-pyazolo[3,4-b]pyridine-3-one (**5**)*

A mixture of 1-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)butane-1,3-dione **2** (258 mg, 1 mmol) and 3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)-one (1 mmol) in ethanol (4 mL) was refluxed for 4 h. The precipitate, obtained from the hot solution, was collected and washed several times with ethanol, the pure product was recrystallized from ethanol. Orange solid; mp 182–184 °C; 92% yield (0.365 g, 0.918 mmol). ^{1}H NMR (300 MHz, DMSO d_6): δ (ppm) 14.08 (s, 2H, OH); 8.19–7.03 (m, 10*H*, ArH); 6.11 (s, 1H, CH); 2.36 (s, 3H, CH_3); 2.06 (s, 3H, CH_3). ^{13}C NMR (75.5 MHz, DMSO d_6): δ (ppm) 165.3; 160.2; 152.7; 150.0; 146.3; 146.0; 141.5; 140.6; 128.9 (x 2); 128.7 (x 2); 123.3; 122.8; 118.3 (x 4); 102.6; 91.8; 31.1; 17.9; 17.5. MS *m*/*z* (%): M^+ = 398.10 (100%). *Anal*. Calcd. for $C_{23}H_{19}N_5O_2$: C, 69.50; H, 4.82; N, 17.62. Found: C, 69.39; H, 4.69; N, 17.57.

*4.1.7. 4-Methyl-2-phenyl-2H-pyrazolo[3,4-b]pyridine-3,6(3aH,7H)-dione **(7)***

4.1.7.1. Conventional heating. In a 100 mL flask was dissolved (0.875 g, 5 mmol) of 3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)–one **6** in a suitable volume of ethyl acetoacetate (3.19 mL, 25 mmol). The reaction mixture was heated at 180 °C for 4 h, after the reaction is judged complete by TLC plate, filtered immediately and washed with cold diethyl ether, to give a gray precipitate of compound **7**.

4.1.7.2. Microwave irradiation. A mixture of (5 mmol; 0,875 g) of 3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)–one (**6**) in a suitable volume of ethyl acetoacetate (3.19 mL, 25 mmol) was introduced to microwave oven at 100 °C. After the completion of the reaction, the solid product was collected by filtration and crystallized from diethyl ether to give the corresponding compound **7**.

4.1.7.3. Sealed tube. The mixture of (0.875 g, 5 mmol) of 3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)–one (**6**) in a suitable volume of ethyl acetoacetate (3.19 mL, 25 mmol) was placed in a sealed tube in a sand bath (180–185 °C). After the reaction was complete, the reaction mixture was cooled and filtered. The precipitate obtained was washed thoroughly with diethyl ether to give the pure compound **7**.

Gray powder; mp 276–278 °C; 92% yield (1.11 g, 4.601 mmol); R_f = 0.22 (Ethyl Acetate–Hexane, 2:3). ^{1}HNMR (300 MHz, DMSO d_6): δ(ppm) 2.41 (s, 3H, CH_3); 6.02 (s, 1H, H); 7.20 (t, *J* = 7.4 Hz, 1H, Ar-H); 7.45 (t, *J* = 8.0 Hz, 2H, Ar-H); 7.74 (d, *J* = 7.7 Hz, 2H, Ar-H); 11.57 (s, 1H, NH); ^{13}C NMR (75.5 MHz, DMSO d_6): δ 17.6; 108.9; 119.6 (x 2); 124.9; 129.3 (x 2); 138.2; 149.9; 155.0; 159.8; 166.6. MS *m*/*z* (%): M^+ = 242.06 (100%). *Anal*. Calcd. for $C_{13}H_{11}N_3O$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.59; H, 4.49; N, 17.37.

*4.1.8. Ethyl 4,6-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carboxylate **(8)***

A solution of 3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**6**) (350 mg, 2 mmol) and ethyl acetoacetate (3.19 mL, 25 mmol) was refluxed in a sand bath (120 °C) under stirring overnight. The orange crystals formed (compound **8**) were collected by filtration and washed with diethyl ether.

Orange crystal; mp 260–262 °C; 54% yield (0.336 g, 1.079 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ(ppm) 1.39 (t, *J* = 5.9 Hz, 3H), 2.28 (s, 3H, CH_3), 2.77 (s, 3H, CH_3), 4.40 (q, *J* = 5.9 Hz, 2H, CH_2), 7.21 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.40 (t, *J* = 7.5 Hz, 2H, t, Ar-H), 7.85 (d, *J* = 7.6 Hz, 2H, Ar-H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ(ppm) 14.6; 22.2; 61.7; 109.0; 122.3 (x 2); 125.7; 129.1 (x 2); 137.4; 150.9; 153.7; 157.3; 159.2; 167.3. MS *m*/*z* (%): M^+ = 312.09 (100%). *Anal*. Calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.29; H, 5.34; N, 13.35.

4.2. Antioxidant activity determination

The antioxidant activity of the synthesized compounds was measured following the Kedare procedure (Kedare and Singh, 2011). The antioxidant activity of the synthesized compounds was measured using more stable free radicals. DPPH (2,2-diphenyl-1-picrylhydrazyl free radical) is a very stable free radical in the crystalline state and in solution, with a violet coloration. The antioxidant activity of this method is the ability of antioxidants to act as scavengers of free radicals. They act by transferring a hydrogen atom, which leads to the disappearance of the DPPH during the reaction and to a change of color in the initial solution. The progress of the reaction was followed by the spectrophotometric method at 517 nm.

The standard solution of vitamin C (positive control) was 1 mg / 100 µL of methanol. A solution of DPPH (1 mg / 25 mL of methanol) was prepared as a control. In 96 well plates, the products were solubilized in a suitable volume of DMSO to have a concentration of 20 mg / mL. In 96 well plates, 16 µL of the solution of each product and 16 µL of vitamin C (10 mg / mL) were added to methanol to have a final volume of 200 µL. Then 100 µL was transferred to the next well containing 100 µL of methanol (1/2 dilution).

The same procedure was repeated to perform the cascade 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128 dilutions. After which, 150 µL of DPPH (1 mg / 25 mL methanol) were added to the wells containing 100 µL of the mixture and then incubated for 30 min in the dark at 37 °C.

Absorbance was measured by a spectrophotometer at 517 nm. The white test is a mixture of methanol and the DPPH reagent under the same experimental conditions. The results are expressed as the percentage of anti-radical activity. The value of the IC_{50} determined from the graphical plot of scavenging activity according to the different concentrations of each product, was defined as the concentration of the antioxidant necessary to reduce the initial concentration of DPPH to 50%. The results obtained for the compounds evaluated were compiled in Table 3.

4.3. Antibacterial activity determination

The antibacterial activity of the synthesized molecules **2, 3a, 3b, 3j, 3d, 3e, 3h, 4a, 4b, 4d, 4e, 4f, 4g, 5, 6a** and **6b** was tested on two Gram + bacterial strains (*Staphylococcus aureus ATCC 25923*) and Gram-negative (*Escherichia coli ATCC 25922*) following the applied disc diffusion method (Balouiri et al., 2016). This method is based on the migratory power of molecules on

a solid medium inside a Petri dish. It allows us to demonstrate the antibacterial effect of the molecules to be tested, as well as the determination of the resistance or sensitivity of these bacteria to the molecules.

(a) Preparation of bacterial suspensions

For each test bacteria, an inoculum was produced from a culture of 24 h at 37 °C, suspended in liquid Luria-Bertani medium (10 g of peptone; 5 g of yeast extract; 10 g of NaCl) sterile so as to obtain an optical density between 0.07 and 0.08 (approximately 10^8 mL^{-1} CFU). Antibacterial tests are carried out using young cultures in the exponential growth phase.

(b) Preparation of discs

The disks were obtained from Wattman paper, with a diameter of 6 mm by the punch. They were autoclaved and then stored at room temperature.

(c) Seeding

A volume of 15 mL of Muller Hinton's agar (Infusion of beef: 300 mL; casein peptone: 17.5 g; corn starch: 1.5 g; agar: 17.0 g and pH = 7.4.) was poured in Petri dishes. After solidification of the culture medium, 0. 2 mL of the bacterial suspension to be tested (10^8 CFU. mL^{-1}) was spread on the surface, then the excess was removed.

(d) Deposit of discs

Under aseptic conditions and using sterile forceps, the Wattman paper discs are placed on the Muller Hinton culture medium beforehand, seeded at the surface with the target microorganisms, the concentration of which is 10^8 CFU. mL^{-1} then soaked with 10 µL of each molecule to be studied. Previously inoculated with the chosen microorganism, the dishes are kept at 4 °C for 1 h so that the molecule can diffuse and subsequently incubated in the oven at 37 °C for 24 h. The study is done by measuring the diameter of the zone of inhibition around the disc using a caliper or a ruler graduated in millimeters (mm). The results obtained for the compounds evaluated have been detailed in Tables 4 and 5.

4.3.1. Partition coefficient (log P)

It has been determined theoretically by the program ChemDraw Professional 20. ChemDraw: PerkinElmer Inc. Addlink Software Científic. Creative Commons (CC), CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/deed.es). https://www.addlink.es/productos/chembiodraw

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103527.

References

Akhramez, S., Hafid, A., Khouili, M., Mentre, O., Ketatni, E.M., 2016. Ethyl 4,6-dimethyl-3-oxo-2-phenyl-3,7-dihydro-2*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxyl-ate monohydrate. IUCrData 1,. https://doi.org/10.1107/S2414314616016199/xu4017sup1.cif x161619.

Anush, S.M., Vishalakshi, B., Kalluraya, B., Manju, N., 2018. Synthesis of pyrazole-based Schiff bases of Chitosan: Evaluation of antimicrobial activity. Biomac. https://doi.org/10.1016/j.ijbiomac.2018.07.129.

Badr, M.H., Abd El Razik, H.A., 2018. 1,4-Disubstituted-5-hydroxy-3-methylpyrazoles and some derived ring systems as cytotoxic and DNA binding agents. Synthesis, in vitro biological evaluation and in silico ADME study. Med. Chem. Res. 27, 442. https://doi.org/10.1007/s00044-017-2071-y.

Balouiri, M., Sadiki, S.M., Ibnsouda, K., 2016. Antibacterial Activity of a New Ready-To-Use Calcium Silicate-Based Sealer. J. Pharm. Anal. 6, 71–79. https://doi.org/10.1590/0103-6440202003870.

Bendaas, A., Maamar, H., Seiller, N., 1999. Synthesis of bipyrazoles and pyrazoloisoxazoles from 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one. J. Heterocycl. Chem. 36, 1291–1294. https://doi.org/10.1002/jhet.5570360529.

Bennamane, N., Nedjar-Kolli, B., Bentarzi, Y., Hammal, L., Geronikaki, A., Eleftheriou, F., Lagunin, A., 2008. Synthesis and in silico biological activity evaluation of new *N*-substituted pyrazolo-oxazin-2-one systems. Bioorg. Med. Chem. 16, 3059–3066. https://doi.org/10.1016/j.bmc.2007.12.033.

Beutne, G.L., Kuethe, J.T., Kim, M.M., Yasuda, N., 2009. Expedient Synthesis of 3-Alkoxymethyl- and 3-Aminomethyl-Pyrazolo[3,4-*b*]piridines. J. Org. Chem. 74, 789–794. https://doi.org/10.1021/jo802363q.

Bhat, R., Xue, Y., Berg, S., Hellberg, S., Ormö, M., Nilsson, Y., Radesáter, A.C., Jerning, E., Borgegàrd, T., Nylöf, M., Giménez-Cassina, A., Hernández, F., Lucas, J.L., Díaz-Nido, J., Avila, J., 2003. Structural Insights and Biological Effects of Glycogen Synthase Kinase 3-specific Inhibitor AR-A014418. J. Biol. Chem. 278, 45937–45945. https://doi.org/10.1074/jbc.M306268200.

Datta, B., Madhusudana Reddy, M.B., Pasha, M.A., 2011. Molecular Iodine-Catalyzed Mild and Effective Synthesis of β-Enaminones at Room Temperature. Synth. Commun. 41, 2331–2336. https://doi.org/10.1080/00397911.2010.502997.

El-Borai, M.A., Rizk, H.F., Abd-Aal, M.F., El-Deeb, I.Y., 2012. Synthesis of pyrazolo[3,4-*b*]pyridines under microwave irradiation in multi-component reactions and their antitumor and antimicrobial activities – Part 1. Eur. J. Med. Chem. 48, 92–96. https://doi.org/10.1016/j.ejmech.2011.11.038.

El-Sayed, T.A., 2009. Synthesis of some novel pyrazolo[3,4-*b*]pyridine and pyrazolo[3,4-*d*]pyrimidine derivatives bearing 5,6-diphenyl-1,2,4-triazine moiety as potential antimicrobial agents. Eur. J. Med. Chem. 44, 4385–4392. https://doi.org/10.1016/j.ejmech.2009.05.031.

Fadel, S., Hamri, S., Hajbi, Y., Suzenet, F., Hafid, A., Rakib, E.M., Khouili, M., Pujol, M.D., Guillaumet, G., 2011. Synthesis of new pyrazolo[3,4-*b*]pyridin-3-ones and pyrazolo[1,5-*a*]pyrimidin-2(1*H*)-one and study of the pyrazolic nitrogen reactivity. Arkivoc 240–251. https://doi.org/10.3998/ark.5550190.0012.219.

Figueiredo, L.J.O., Kascheres, C., 1997. Quinone Diazides and Enaminones as a Source of New Azo Compounds with Potential

Nonlinear Optical Properties. J. Org. Chem. 62, 1164–1167. https://doi.org/10.1021/jo9604907.

Gogoi, S., Bhuyan, R., Barua, N.C., 2005. Iodine-catalyzed conversion of β-dicarbonyl compounds into β-enaminones within a minute under solvent-free conditions. Synth. Commun. 35, 2811–2818. https://doi.org/10.1080/00397910500290557.

Gu, P.Y., Wanga, Z., Zhang, O., 2016. Azaacenes as active elements for sensing and bio Applications. J. Mater. Chem. B 4, 7060–7074. https://doi.org/10.1039/C6TB02052F.

a) Guodong, W., Yanhui, G., Jieping, W., 2020. Base-Promoted, Metal- and Oxidant-Free C = C Bond Cleavage in Enaminones for Ambient Synthesis of NH_2-Amidines. Chin J Org Chem. 40, 645-650. https://doi.org/10.6023/cjoc201912018 b) Hu, D., Lu Yanga, L., Wan, J.-P., 2020. Biaryl and atropisomeric biaryl aldehyde synthesis by one-step, metal-free benzannulation of aryl enals and propiolates. Green Chem. 22, 6773-6777. https://doi.org/10.1039/D0GC02806A

Kedare, S.B., Singh, R.P., 2011. Genesis and development of DPPH method of antioxidant assay. J. Food Sci. Technol. 48, 412–422. https://doi.org/10.1007/s13197-011-0251-1.

Kumar, N.R., Poornachandra, Y., Swaroop, D.K., Jitender, D.G., Kumar, C.G., 2016. Synthesis of novel ethyl 2,4-disubstituted 8-(trifluoromethyl)pyrido[2′,3′:3,4]pyrazolo[1,5-*a*]pyrimidine-9-carboxylate derivatives as promising anticancer agents. Bioorg. Med. Chem. Lett. 26, 5203–5206. https://doi.org/10.1016/j.bmcl.2016.09.062.

Kumari, M.A., Venkatarao, C., 2020. A Review on Recent Trends in the Bioactive Studies of Pyrazole Derivatives. Asian J Research Chem. 13, 383–394.

Kunstmann, R., Lerch, U., Gerhards, H., Leven, M., Schacht, U., 1984. 2,3,4,4a,5,9b-Hexahydro-1*H*-indeno[1,2-*b*]pyridines: potential antidepressants. J. Med. Chem.. 27, 432–439. https://doi.org/10.1021/jm00370a004.

Li, G., Duong, H.M., Zhang, Z., Xiao, J., Liu, L., Zhao, Y., Zhang, H., Huo, F., Li, S., Ma, J., Wudl, F., Zhang, Q., 2012. Approaching a stable, green twisted heteroacene through "clean reaction" strategy. Chem. Commun. 48, 5974–5976. https://doi.org/10.1039/C2CC32048G.

Lominac, W.J., D'Angelo, M.L., Smith, M.D., Ollison, D.A., Hanna, J.M., 2012. Construction of pyrazolo[3,4-*b*]pyridines and pyrazolo [4,3-*c*]pyridines by ring closure of 3-acylpyridine *N*-oxide tosylhydrazones. Tetrahedron Lett. 53, 906–909. https://doi.org/10.1016/j.tetlet.2011.12.055.

Manpadi, M., Uglinskii, P.Y., Rastogi, S.K., Cotter, K.M., Wong, Y. S.C., Anderson, L.A., Ortega, A.J., Slambrouck, S., Steelant, W.F. A., Rogelj, S., Tongwa, P., Antipin, M.Y., Magedov, I.V., Kornienko, A., 2007. Three-component synthesis and anticancer evaluation of polycyclic indenopyridines lead to the discovery of a novel indenoheterocycle with potent apoptosis inducing properties. Org. Biomol. Chem. 5, 3865–3872. https://doi.org/10.1039/B713820B.

Misra, R.N., Xiao, H., Rawlin, D.B., Shan, W., Kellar, K.A., Mulheron, J.G., Sack, J.S., Tokarski, J.S., Kimbally, S.D., Webster, K.R., 2003. 1*H*-Pyrazolo[3,4-*b*]pyridine inhibitors of cyclin-dependent kinases: highly potent 2,6-Difluorophenacyl analogues. Bioorg. Med. Chem. Lett. 13, 2405–2408. https://doi.org/10.1016/S0960-894X(03)00381-0.

Mohammadizadeh, M.R., Hasaninejad, A., Bahramzadeh, M., Khanjarlou, Z.S., 2009. P_2O_5/SiO_2 as a New, Efficient, and Reusable Catalyst for Preparation of β-Enaminones Under Solvent-Free Conditions. Synth Comm. 39, 1152–1165. https://doi.org/10.1080/00397910802513052.

Nagender, P., Reddy, G.M., Kumar, R.N., Poornachandra, Y., Kumar, C.G., Narsaiah, B., 2014. Synthesis, cytotoxicity, antimicrobial and anti-biofilm activities of novel pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives. Bioorg. Med. Chem. Lett. 24, 2905–2908. https://doi.org/10.1016/j.bmcl.2014.04.084.

Narsaiah, B., Shi, D.Q., Zhou, Y., Liu, H., 2010. Construction of pyrazolo[3,4-*b*]pyridines and pyrazolo[4,3-*c*]pyridines by ring closure of 3-acylpyridine *N*-oxide tosylhydrazones. Synth. Commun. 40, 3660–3668. https://doi.org/10.1080/00397910903471796.

(a) Parshad, M., Kumar, D., 2014. Design, synthesis and characterization of isomeric 3,4'-bipyrazol-5'-ols and their antifungal activity. Chem. Biol. 4, 100-110. (b) Gelin, S., Chantegrel, B., Ilah-Nadi, A., 1983. Synthesis of 4-(acylacetyl)-1-phenyl-2-pyrazolin-5-ones from 3-acyl-2*H*-pyran-2,4(3*H*)-diones. Their synthetic applications to functionalized 4-oxopyrano[2,3-*c*]pyrazole derivatives. J Org Chem. 48, 4078-4082. https://doi.org/10.1021/jo00170a041

Shekarrao, K., Kaishap, P.P., Saddanapu, V., Addlagatta, A., Gogoi, S., Boruah, R.C., 2014. Microwave-assisted palladium mediated efficient synthesis of pyrazolo[3,4-*b*]pyridines, pyrazolo[3,4-*b*] quinolines, pyrazolo[1,5-*a*]pyrimidines and pyrazolo[1,5-*a*]quinazolines. RSC Adv. 4, 24001–24006. https://doi.org/10.1039/c4ra02865a.

(a) Stepanenko, I.N., Novak, M.S., Mühlgassner, G., Roller, A., Hejl, M., Arion, V.B., Jakupec, M.A., Keppler, B.K., 2011. Organometallic 3-(1*H*-Benzimidazol-2-yl)-1*H*-pyrazolo[3,4-*b*] pyridines as Potential Anticancer Agents. Inorg Chem. 50, 11715-11728. https://doi.org/10.1021/ic201704u (b) Manetti, F., Schenone, S., Bondavalli, F., Brullo, C., Bruno, O., Ranise, A., Mosti, L., Menozzi, G., Fossa, P., Trincavelli, M.L., Martini, C., Martinelli, A., Tintori, C., Botta, M., 2005. Synthesis and 3D QSAR of New Pyrazolo[3,4-*b*]pyridines: Potent and Selective Inhibitors of A1 Adenosine Receptors. J Med Chem. 48, 7172-7185. https://doi.org/10.1021/jm050407k

a) Tian, L., Wan, J. P., Sheng, S., 2020. Transition Metal-free C−H Sulfonylation and Pyrazole Annulation Cascade for the Synthesis of 4-Sulfonyl Pyrazoles. ChemCatChem. 2533-2537. https://doi.org/10.1002/cctc.202000244 b) Arrousse, N., Salim, R., Kaddouri, y., Zarrouk, A., Zahri, D., El Hajjaji, F., Touzani, R., Taleb, M., Jodeh, S., 2020. The inhibition behaviour of two pyrimidine-pyrazole derivatives against corrosion in hydrochloric solution: Experimental, surface analysis and in silico approach studies. Arabian J. Chem. 13, 5949-5965. https://doi.org/10.1016/j.arabjc.2020.04.030.

Wang, Z., Chen, B., Zhao, J., Zhang, Q., Lin, Z., Weng, J., Huang, W., 2021. Recent progress in 1,4-diazafluorene-cored optoelectronic materials: A review. Dyes Pigments 191,. https://doi.org/10.1002/asia.201000659 109365.

Wu, Y., Yin, Z., Xiao, J., Liu, J., Wei, F., Tan, K.J., Kloc, C., Huang, L., Yan, Q., Hu, F., Zhang, H., Zhang, Q., 2012. Crystal Structure and Phototransistor Behavior of *N*-Substituted Heptacence. ACS Appl. Mater. Interf. 4, 1883–1886. https://doi.org/10.1021/am3003389.

Zhang, X., Lin, Q., Zhong, P., 2010. A Facile One-pot Synthesis of 1-Arylpyrazolo[3,4-*d*]pyrimidin-4-ones. Molecules 15, 3079–3086. https://doi.org/10.3390/molecules15053079.