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AQUEOUS MEDIUM SYNTHESIS, CHARACTERIZATION AND EVALUATION OF THE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF NEW PYRIMIDINE- AND PURINE-LIGATED PYRROLE

Chaima Adaika[1], Mohamed Dehamchia[1*], Nawal Khier[2], Samir Bayou[3], Fatheddine Boukhallout[1], Abdelhafeez M. A. Mohammed[4] and Zine Regainia[5]

[1]Laboratory of Applied Chemistry and Environment, Department of Chemistry, Faculty of Exact Sciences, University of El Oued, PO Box 789, 39000, Algeria
[2]Laboratory of Bioinformatics, Applied Microbiology and Biomolecules, University of Boumerdès, 35000 Bumerdes, Algeria
[3]Laboratory of Macromolecular Synthesis and Thio-Organic Macromolecular (LSMTM), Department of Macromolecular Chemistry, Faculty of Chemistry, USTHB BP 32 el Alia, Algiers, 16111, Algeria
[4]Department of Chemistry, Alzaiem Alazhari University, PO Box 1432, Khartoum North, 13311, Sudan
[5]Laboratory of Applied Organic Chemistry (LAOC), Faculty of Sciences, Department of Chemistry, Badji Mokhtar University PO Box 12 Annaba-Algeria

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ABSTRACT. A new pyrimidine- and purine-ligated pyrrole were synthesized through the Hantzsch multicomponent reaction using nucleobases (cytosine, adenine or guanine) as amine nucleophiles in water as solvent. The structures of the synthesized compounds were elucidated through proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) and Fourier-transform infrared (FT-IR) spectroscopy, mass spectrometry, and elemental analysis. The total antioxidant capacity (TAC) of the products was evaluated by the phosphomolybdenum assay. The *in vitro* antioxidant activity of the target compounds was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2′-azino-bis(3-ethylenzothiazoline-sulfonic acid) diammonium salt (ABTS$^{\bullet+}$) free radicals. Compound **2c** displayed the highest antioxidant activity against DPPH with IC_{50} of 6.55 µg/mL, and **3c** exhibited the highest antioxidant activity against ABTS with IC_{50} of 18.37 µg/mL. Furthermore, the *in vitro* antibacterial properties of these compounds was assessed against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 13883 (Gram-negative: G^-), and *Staphylococcus aureus* ATCC 25932 (Gram-positive: G^+) bacterium, and compared with Gentamicin used as reference antibiotic. Compound **2a** exhibited good antibacterial activity against *Klebsiella pneumonia* with inhibition zone of 17 mm. Compound **3a** also showed a good antibacterial effect against *E. coli* taxon with inhibition zone of 18.5 mm relative to Gentamicin (20 mm).

KEY WORDS: Antibacterial activity, Antioxidant activity, Hantzsch synthesis, Nitrogenous bases, Pyrrole

INTRODUCTION

The pyrrole moiety is an attractive five-membered heterocyclic structure showing enormous and diverse types of biological and pharmacological properties [1-3]. Substituted pyrroles have been shown to possess a wide variety of biological activities, which include anticancer [4], antifungal [5], antileishmanial [6], antimicrobial [7], anticonvulsant [8], antiviral [9], antioxidant [10], and anti-inflammatory [11]. Furthermore, the pyrrole core structure is essential to the structure of many pharmaceuticals that are sold commercially as well as naturally occurring substances including tolmetin [12], zomepirac [13], atorvastatin [14], vitamin B_{12}, and bile pigments like bilirubin and biliverdin [15].

*Corresponding authors. E-mail: mohchar5@yahoo.fr; dehamchia-mohamed@univ-eloued.dz

The development of new biological and pharmacological entities has focused a lot of emphasis on pyrrole fused with heterocycles that contain nitrogen [16, 17]. Among them, pyrrolo-pyrimidine and pyrrolo-purine are of particular importance [18, 19]. Some of their derivatives have antitumor activity [20], anti-cancer activity [21], and human A3 adenosine receptor antagonists [21].

Over the past few decades, the bacterial infections have remained one of the leading causes of death worldwide. In 2019, approximately 8 million deaths around the world are associated with 33 types of bacteria, with a half of these fatalities attributed *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumonia*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* [22]. These microorganisms have shown resistance to a large number of commercial antibiotics. This difficult situation is a great challenge for researchers to design and develop new therapeutic agent in order to prevent from this bacterial infections damage [23-25].

Due to the importance of pyrrole for various biological applications, several methods for the synthesis of polysubstituted pyrroles have attracted the attention of organic chemists and varieties of synthetic strategies have been reported in the literature [26, 27]. In this case, Hantzsch multicomponent reaction (MCR) is widely used as practical and convenient one-pot method for the synthesis of various biologically active pyrrole derivatives [28, 29].

In the literature, numerous reports about the Hantzsch pyrrole synthesis have been reported [28-30]. These include solid-phase synthesis [31], organocatalytic synthesis [32], green synthesis [33] and solvent-free synthesis, using the high-speed vibration milling (HSVM) technique [34]. Our research team recently published a study on the synthesis of certain new fused heterocyclic compounds using nitrogenous bases (nucleobases), such as adenine, guanine, and cytosine, as amine donors [35-37].

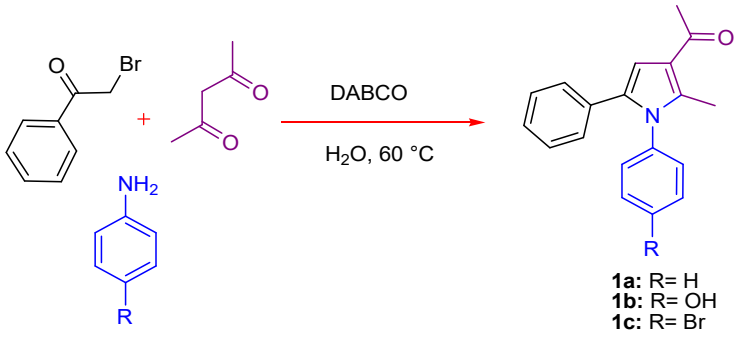
Given the aforementioned results, and as part of our ongoing research on the synthesis of biologically active heterocycles, we report here the synthesis of new pyrimidine- and purine-linked with pyrrole moiety compounds by Hantzsch MCR using nitrogenous bases (cytosine, adenine, or guanine). Furthermore, we evaluated the antibacterial and the antioxidant activities of synthesized compounds.

RESULTS AND DISCUSSION

Chemical synthesis

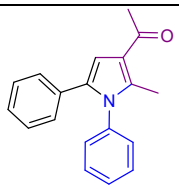
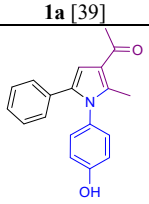
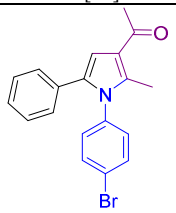
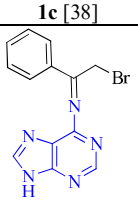
Our current study started from the synthesis of the *N*-aryl-pyrrole derivatives (**1a–c**) *via* Hantzsch multicomponent reaction according to the previously reported method [38, 39]. The reactions were carried out by condensation of an aromatic amine (aniline, *p*-aminophenol, or *p*-bromoaniline), acetylacetone, and α-bromoacetophenone. As indicated in Scheme 1, the reactions were performed in water as solvent at 60 °C using 5 mol% of 1,4-diazabicyclo[2.2.2]octane (DABCO) as catalyst. All the products were isolated as yellow oil in yields ranged between 82% and 89%, and the results are summarized in Table 1.

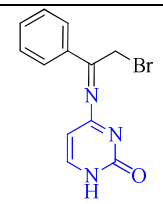
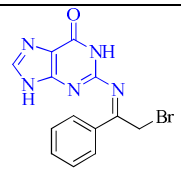
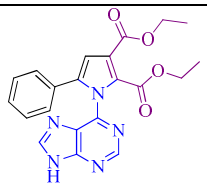
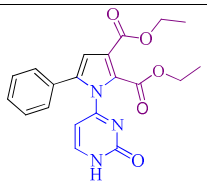
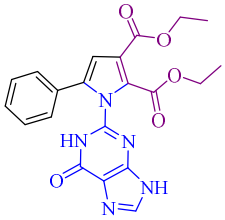
In order to show the generality of this reaction and to investigate the effect of other parameters on the reaction yields and products, nucleobases, which have an amino (NH_2) group such as cytosine, adenine and guanine, were employed as amine reagents. As shown in Scheme 2, three novel Schiff-bases (azomethine imines) derivatives **2a-c** were synthesized by condensation of nucleobase, α-bromoacetophenone and acetylacetone without catalyst. All reactions were performed in H_2O at 60 °C and the products **2a-c** were obtained by filtration in 60-97% yields. The results are summarized in Table 1. All products **2a-c** were characterized by their spectroscopic and analytical data.



Scheme 1. Synthesis of 1,2,3,5-substituted-4*H*-pyrroles (**1a–c**).

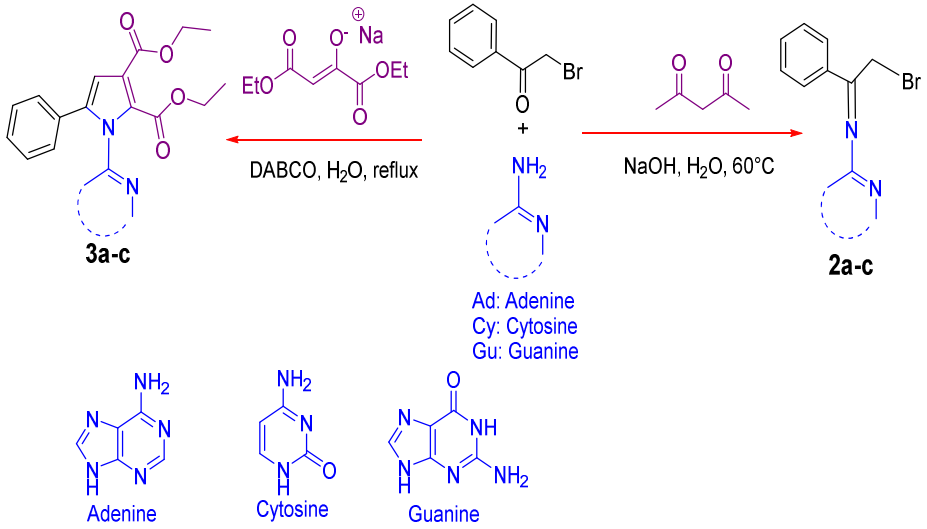
Table 1. Reaction conditions and isolated yields of synthesized compounds (**1a-c**), (**2a-c**), and (**3a-c**).

Product	1,3-Dicarbonyle	Amine	Reaction conditions	Yield (%)	m.p. (°C)
 **1a** [39]	Acetylacetone	Aniline	1.5-2 h[(a)]	87	Yellow oil
 **1b** [38]		4-Aminophenol	1.5-2 h[(a)]	89	Yellow oil
 **1c** [38]		4-Bromoaniline	2-3 h[(a)]	90	Yellow oil
 **2a**		Adenine	5-6 h[(a)]	97	271-273

 **2b**		Cytosine	5-6 h[(a)]	77	196-198
 **2c**		Guanine	5-6 h[(a)]	40	>300
 **3a**	Sodium diethyl oxalacetate	Adenine	7-8 h[(b)]	23	274-176
 **3b**		Cytosine	7-8 h[(b)]	36	224-226
 **3c**		Guanine	7-8 h[(b)]	74	298-300

[(a)]: H_2O, 60 °C; [(b)]: H_2O, DABCO, reflux.

In addition, we evaluated the replacement of acetylacetone by sodium diethyloxaloacetate as activated 1,3-dicarbonyle reagent. In this case, pyrimidine- and purine-ligated pyrrole derivatives (**3a**–**c**) were prepared by the Hantzsch multicomponent condensation of sodium diethyloxaloacetate, nucleobase, and 2-bromoacetophenone in water as solvent using 5 mol% of DABCO as catalyst (Scheme 2). The products **3a**, **3b**, and **3c** were obtained with 23, 36, and 74% yields, respectively (Table 1).



Scheme 2. Synthesis of Schiff-bases (**2a-c**) pyrimidine- and purine-ligated pyrrole (**3a**–**c**).

The structure of pyrimidine- and purine ligated-pyrrole (**3a-c**) were elucidated using ^{1}H- and ^{13}C NMR, FT-IR, mass spectroscopy and Elemental analyses. The Fourier transform (FT-IR) spectra of the Hantzsch products **3a-c** showed a strong absorption band over 1700 cm^{-1}, which corresponds to C=O ester groups. In addition, all IR spectra showed a characteristic band over 1600 cm^{-1}, which corresponds to aromatic ring stretch in all products. The proton nuclear magnetic resonance (^{1}H NMR) spectra of compounds **3a-c** show two triplets of 6 protons at 1.10-1.20 ppm region belong to methyl group (2CH_3) of ethyl carboxylate groups. The two methylene (2CH_2) of ester groups appear as two quartets at 4.10-4.20 ppm. Another signal at $\delta \approx$ 7-8 ppm belongs to the proton of the aromatic rings. The electrospray ionization (ESI) mass spectra of synthesized compounds showed peaks at m/z = $[M+1]^+$, which correspond to the molecular mass of the ion $[M+H]^+$.

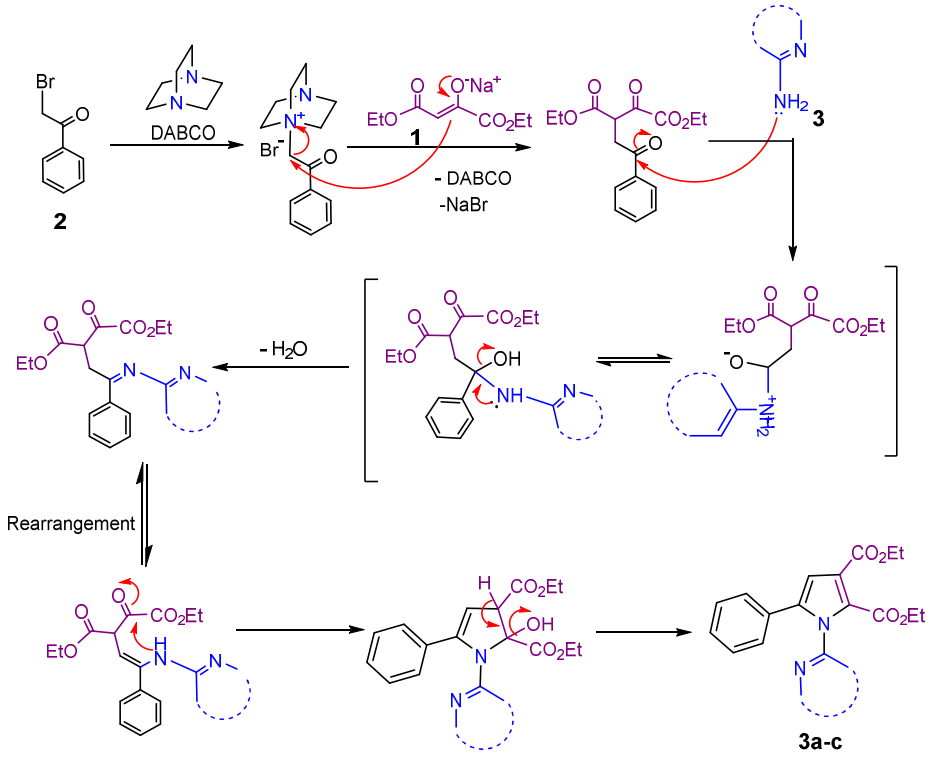
The suggested mechanism for the synthesis of pyrimidine- and purine-ligated pyrrole catalyzed by DABCO is shown in Scheme 3.

Bioactivities

To discover the beneficial effects of the synthesized compounds in the treatment of different diseases, it is essential to study for their *in vitro* biological activity. In particular, for the nitrogen-containing heterocycles, it is important to evaluate their antioxidant, antibacterial and antimicrobial activity.

In vitro antibacterial activity

The newly prepared compounds **2a-c** and **3a-c** were screened for their *in vitro* antibacterial effects against Gram-positive bacterial taxon *S. aureus,* ATCC 25923 and Gram-negative bacterial strains *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *K. pneumonia* (ATCC 13883) at a concentration of 15 mg/mL using the disk diffusion method [40, 41]. The inhibition zones were given in mm and were compared to Gentamicin, which was used as reference drugs at a concentration of 15 mg/mL. DMSO was used as negative control. The results of the bioassays expressed as diameters of the inhibition zones in mm and are reported in Table 2.



Scheme 3. Suggested Mechanism for the synthesis of pyrimidine- and purines ligated-pyrrole **3a-c**.

Table 2. In vitro antibacterial activity of synthesized compounds (**2a-c**) and (**3a-c**) against Gram-negative and Gram-positive bacteria.

Compounds	Inhibition zones (mm) at 15 mg/mL			
	E. coli	*P. aeruginosa*	*K. pneumoniae*	*S. aureus*
DMSO	-	-	-	-
2a	8	15	17	-
2b	13	11	12	-
2c	-	10	13	-
3a	18.5	-	12	12.5
3b	8	12	15	-
3c	10.5	12	12	-
Gentamicin	20	19	21	22
(-): No activity was observed				

The observed values in Table 2 show that compounds derived from adenine (**2a** and **3a**) are compared with the activity of selected antibiotic gentamicin against *E. coli* and *K. pneumonia* at 15 mg/mL. These compounds expressed antibacterial activity against both bacterial species with zones of inhibition of 17 and 18.5 mm, respectively. Against *S. aureus,* the tested compounds **2a-c**, **3b-c** did not express antibacterial effect at the same concentration (15 mg/mL), but compound **3a** displayed low activity with inhibition zone value of 12.5 mm.

In vitro antioxidant activity

The *in vitro* antioxidant properties of compounds **2a-c** and **3a-c** were screened using 1,1-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) [42, 43] and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt ($ABTS^{+\bullet}$) [44] assays. The total antioxidant activity was obtained using the phosphomolybdenum method.

*DPPH scavenging activity of synthesized compounds **2a-c** and **3a-c***

The radical scavenging activity (RSA) of DPPH was calculated as a percentage inhibition using the following equation:

$$\%\ \text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Ascorbic acid (vitamin C) was used as standard agent. The DPPH reducing potentials of synthesized compounds **2a-c** and **3a-c** were reported in IC_{50} values. The results obtained in this assay are summarized in Table 3 and the percentage inhibition (I %) are clarified in Figure 1(A).

Table 3. *In vitro* antioxidant property of **2a-c** and **3a-c** using the DPPH assay.

Compound	Concentration µg/mL							
	5	25	50	75	100	125	150	IC_{50}
2a	27.7	43.81	46.08	46.46	48.35	51.64	52	112.79
2b	51.6	53.1	46.06	56.4	53.1	52	67	35.43
2c	36.3	59	64.4	72	88.7	89.4	97.2	17.29
3a	44	46	49.2	50	50.02	53	55	75.11
3b	41.1	42	47	53.53	54.54	55.02	54.67	60.71
3c	34.67	57	58	57.6	67	74.2	82.5	18.56
Vitamin C	61.6	72.92	74.36	74.69	75.03	75.36	75.36	9.97

The IC_{50} values correspond to the concentration of a sample, which has capability to quench 50% of DPPH radical present in the reaction mixture. Low IC_{50} values indicate a high antioxidant activity of the sample compound.

As shown in the Table 3 and Figure 1(A), all synthesized molecules 2a**-c** and **3a-c** have free radical scavenging activity in the DPPH assay. The IC_{50} values in the DPPH assays were ranged between 17.29 and 112.79 µM. Compounds derived from guanine **2c and 3c**, which have a (NH) and (CO) groups, showed the highest antioxidant activity (17.29 and 18.56 µM). Whereas, shift base derived from adenine **2a** showed lower antioxidant activity (112.79 µM). These results revealed that the presence of the guanine ring contributed differently to the radical scavenging capacity.

*ABTS scavenging activity of synthesized compounds **2a-c** and **3a-c***

The ability of the synthesized compounds to scavenge ABTS radical was expressed as percent (%) RSA and calculated using the formula:

$$\%\ \text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of the ABTS radical cation in methanol and A_{sample} is the absorbance of the ABTS radical cation in the sample solution. Ascorbic acid (vitamin C) was used

as positive control. The radical scavenging activities of synthesized compounds **2a-c** and **2a-c** in the ABTS assay are summarized in Table 4 and the percentage inhibition at different concentrations are presented in Figure 1(B).

Table 4. *In vitro* antioxidant activity of compounds **2a-c** and **3a-c** using ABTS assay.

Compound	Concentration µg/mL									
	5	12.5	25	37.5	50	100	150	200	250	IC_{50}
2a	11.86	27.43	32	46.29	64.29	70.14	73.86	80.14	82.86	40.34
2b	5.42	7.93	8.9	10.29	26.43	30.46	40.89	48.96	58.14	205.26
2c	1.97	17.8	22.67	45.76	54.52	58.14	60.08	69.4	75.8	43.76
3a	11.82	12.93	45.34	65.77	68.99	75.10	82.89	76.08	76.63	28.15
3b	12.28	18.86	22.71	30.14	47.14	70.71	73.71	75.57	77.57	55.71
3c	58	55.22	44.51	34.35	27.12	11.54	7.37	6.95	1.4	18.37
Vitamin C	9.78	10.46	14.54	30.84	43.61	63.18	77.85	84.24	91.03	66.72

As shown in Table 4 and Figure 1(B), the significant antioxidant properties against $ABTS^{+\bullet}$ radical cation were observed for the compounds **3c** and **3a** with IC_{50} values 18.37 µg/mL and 28.15 µg/mL, respectively. The lowest antioxidant activity was observed for compound **2b** with IC_{50} value 205.26 µg/mL. In addition, the results in Table 4 indicated that the antioxidant activities of the synthesized compounds **2a**, **2c**, **3a-c** are more than that of vitamin C used as a standard antioxidant.

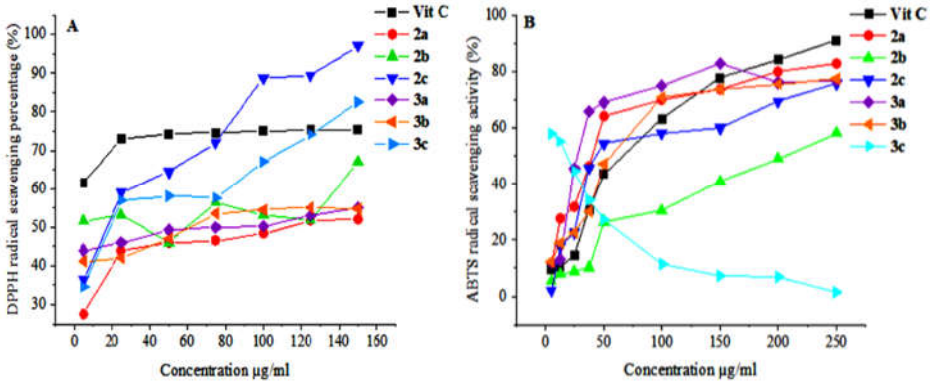


Figure 1. Radical scavenging activity of the synthesized compounds (**2a-c**) and (**3a-c**) at various concentrations against DPPH (**A**) and ABTS (**B**) radicals at 517 and 734 nm.

Total antioxidant capacity (TAC) assessment

The total antioxidant capacity (TAC) of the synthesized library was estimated by the phosphomolybdenum assay described by Prieto *et al.* [45]. This method is based on the reduction of Mo(VI) to Mo(V) by the formation of a green phosphate/Mo(V) complex at an acidic pH. The results are expressed in milligrams (mg) of Gallic acid or ascorbic acid per gram (g) of tested sample and are depicted in Table 5.

Table 5. Total antioxidant capacity (TAC) of the tested compounds using the phosphomolybdenum method.

Total antioxidant capacity (TAC)		
Comp	TAC (mg GAE/g dry compounds)	TAC (mg AAE/g dry compounds)
2a	372.9±0.073	677.1±0.0761
2b	524.3±0.096	1106.3±0.0474
2c	502±0.055	1043±0.0271
3a	397.7±0.096	747.5±0.0320
3b	463.5±0.079	933.9±0.0753
3c	550.4±0.061	1180.2±0.0211
Linear equation	$y = 12.891x - 0.1015$	$y = 4.5483x + 0.0065$

AAE: Ascorbic acid equivalent. GAE: Gallic acid equivalent.

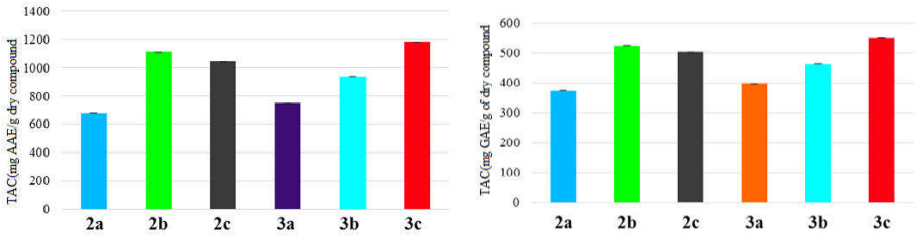


Figure 2. Total antioxidant capacity of synthesized compounds by phosphomolybdenum assay.

As we can see from Table 5 and Figure 2, the TAC values of the synthesized compounds **2a-c** and **3a-c** were ranged from 372.9 ± 0.073 to 550.4±0.061 mg (GAE)/g sample, and from 677.1±0.0761 to 1180.2±0.0211 mg (AAE)/g sample, respectively. All tested compounds **2a-c** and **3a-c** exhibited antioxidant capacity. Compound **3c** showed the highest antioxidant capacity in the two methods with TAC values of 550.4±0.061 mg (GAE)/g sample and 1180.2±0.0211 mg (AAE)/g of sample. In addition, the lowest potent level of activity was observed in compound **2a** with TAC values of 372.9±0.073 mg (GAE)/g of dry compound and 677.1±0.0761 mg (AAE)/g of dry compound.

EXPERIMENTAL

Materials and methods

Solvents, reagents and free radicals (DPPH and ABTS) were commercially available and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at Scientific and Technical Research Center in Physicochemical Analysis *(*CRAPC*)*. The chemical shifts, δ, are reported in parts per million (ppm) and were measured in DMSO-d_6 relative to tetramethylsilane (TMS) as internal standard. FT-IR spectra (in KBr, $\bar{\upsilon}$ cm^{-1}) were recorded on an Agilent Cary 630 FT-IR spectrometer. High-resolution mass spectra (HRMS-ESI) were obtained on Bruker micro TOF spectrometer using methanol as the spray solvent. Melting points (m.p.) were measured using Cole-Parmer Stuart MP-200 instrument and are uncorrected. Absorbance measurements for the antioxidant assays were conducted using a SECOMAM PRIMLIGHT reader. TLC was performed on Merck Kiesel gel 60 F254 aluminum backed plates. The spots were detected by ninhydrin using UV irradiation. The G^+ and G^- bacterial species used in this study were sourced from the Pasteur Institute, Ministry of health, Algeria.

Chemical synthesis

*General procedure **A** for the multicomponent synthesis of pyrroles (**1a-c**) in aqueous medium* [38, 39]

A mixture containing α-bromoacetophenone (1 mmol), acetyl acetone (1 mmol), amine (1 mmol), and DABCO (5 mol%) was stirred in 5 mL of water at 60°C for the specified duration (Figure 1). Once the reaction was complete, as confirmed by TLC, the mixture was diluted with ice water and extracted twice with ethyl acetate (2×10 mL). The combined organic layers were dried over magnesium sodium sulfate ($MgSO_4$) or calcium chloride ($CaCl_2$). The solvent was then evaporated under reduced pressure to concentrate the crude product, which was subsequently purified by silica gel column chromatography eluted with a mixture of ethyl acetate and n-hexane (20:80). These compounds were synthesized according to the literature procedure [38, 39].

*General procedure **B** for the synthesis of Schiff-bases N-(2-bromo-1-phenylethylidene)arylamine derivatives (**2a-c**)*

The Schiff-bases **2a-c** were obtained by reacting equimolar quantities of nucleobase (adenine, cytosine, or guanine), acetylacetone and 2-bromoacetophenone in water in the presence of one equivalent of NaOH. The mixture was heated for about 5–6 hours at 60 °C. The progress of the reaction was monitored using TLC. Once the reaction was complete, the mixture was allowed to cool to room temperature, diluted with water and a solid product was formed. The precipitate was separated by filtration, washed with water, then with petroleum ether several times. The obtained solid was recrystallized in EtOH at low temperature yielded the pure product.

*N-(2-Bromo-1-phenylethylidene)-9H-purin-6-amine (**2a**)*. This compound was prepared according to the general procedure **B** using adenine (0.34 g, 2.5 mmol), 2-bromoacetophenone (0.5 g, 2.5 mmol), acetylacetone (0.25 g, 2.5 mmol) and NaOH (0.1 g, 2.5 mmol). Compound **2a** was obtained as a white solid in 97% yield; m.p. 271-273 °C. FT-IR (KBr, $\bar{\upsilon}$ cm^{-1}): 1593 $(C=N)_{aliphatic}$, 1676-1697 $(C=N)_{purine}$, 1222 (C-N), 646 (C-Br); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.13 (s, 1H, $NH_{imidazole}$), 7.74 (m, 1H, Ar), 7.62 (m, 4H, H-Ar), 7.31 (s, 2H, CH_{purine}), 5.88 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm) δ 156.45, 152.97, 150.42, 142.10, 134.72, 134.60, 129.52, 128.59, 118.82, 49.88. HRMS (ESI): m/z = 315.9937 $[M+H]^+$ (calculated for $[C_{13}H_{10}BrN_5+H]^+$: 316.0198). Elemental analysis, calculated for $C_{13}H_{10}BrN_5$ (316.16): C, 49.39; H, 3.19; N, 22.15. Found: C, 49.70; H, 3.42; N, 22.37%.

*N-(2-Bromo-1-phenylethylidene)-1H-pyrimidin-2-one-4-amine (**2b**)*. This compounds was prepared according to the general procedure **B** using cytocine (0.28 g, 2.5 mmol), α-bromoacetophenone (0.5 g, 2.5 mmol), acetylacetone (0.25 g, 2.5 mmol), and NaOH (0.1 g, 2.5 mmol). Compound **2b** was obtained as a white solid in 77% yield; m.p: 196-198 °C. FT-IR (KBr, $\bar{\upsilon}$ cm^{-1}): 3063 (N-H), 1676 (C=O), 1609 (C=N), 1355 (C-N), 685 (C-Br); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.62 (s, 1H, NH), 7.99 (d, 2H, H-Ar), 7.42 (m, 3H, H-Ar), 6.62 (d, 1H, CH_{ethyl}), 5.68 (d, 1H, CH_{ethyl}), 5.23 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 156.34, 147.19, 146.58, 146.54, 134.02, 130.32, 129.15, 128.49, 128.29, 108.23, 30.47. HRMS (ESI): m/z = 292.0023 (calculated for $[C_{12}H_{10}BrN_3O+H]^+$: 292.0085. Elemental analysis, calculated for $C_{12}H_{10}BrN_3O$ (292.13): C, 49.34; H, 3.45; N, 14.38. Found: C, 49.20; H, 3.48; N, 14.18%.

*N-(2-Bromo-1-phenylethylidene)-1H-purin-6(9H)-one-2-amine (**2c**)*. This compounds was prepared according to the general procedure **B** using guanine (0.34 g, 2.5 mmol), α-bromoacetophenone (0.5 g, 2.5 mmol), acetylacetone (0.25 g, 2.5 mmol), NaOH (0.1 g, 2.5 mmol). Compound **2c** was obtained as a white solid in 40% yield; m.p. >300 °C. FT-IR (KBr, $\bar{\upsilon}$

cm^{-1}): 3059(N-H)$_{imidazole}$, 3318 (N-H)$_{amide}$, 1662 (C=O), 1558 (C=N), 1373 (C-N), 686 (C-Br). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 12.33 (s, 1H, NH)$_{imidazole}$, 10.52 (s, 1H, NH $_{pyrimidine}$), 7.98 (m, 2H, H-Ar), 7.61 (s, 1H, CH $_{imidazole}$), 7.49 (m, 2H, H$_{arom}$), 7.37(m,1H, H$_{arom}$), 6.30 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 136.74, 134.84, 134.51, 133.63, 129.59, 129.38, 129.12, 128.73, 128.42, 126.76, 33.14. HRMS (ESI): m/z = 333.0228 $[M+H]^+$ (calculated for $[C_{13}H_{10}BrN_5O+H]^+$: 333.0219. Elemental analysis, calculated for $C_{13}H_{10}BrN_5O$ (332.16): C, 47.01; H, 3.03; N, 21.08. Found: C, 47.03; H, 3.04; N, 21.11%.

*General procedure **C** for the synthesis of pyrimidine- and purine-ligated pyrrole **3a-c** using DABCO as catalyst*

An equimolar mixture of sodium diethyloxalacetate (2.5 mmol), nucleobase (adenine, cytosine, or guanine) (2.5 mmol), 2-bromoacetophenone (2.5 mmol) and 5 mol% of DABCO in 20 mL of water as solvent was stirred for 5-6 hours at reflux. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature, diluted with water, and the precipitate was separated by filtration. Recrystallization from ethanol/water (50/50) at low temperature to obtain the corresponding pyrimidine- and purine ligated-pyrrole **3a-c**.

*Diethyl 1-(9H-purin-6-yl)-5-phenyl-1H-pyrrole-2,3-dicarboxylate (**3a**)*. The compounds was prepared according to the general procedure **C** using adenine (0.34 g, 2.5 mmol), 2-bromoacetophenone (0.5 g, 2.5 mmol), and sodium diethyloxalacetate (0.5 g, 2.5 mmol). Compound **3a** was obtained as a yellowish-white solid in 23% yield; m.p. 274-276 °C. FT-IR (KBr, $\bar{\upsilon}$ cm^{-1}): 3235 (NH$_{imidazo}$), 3093 (C-H)$_{aroma}$, 2926 (C-H)$_{aliphatic}$, 1692 (C=O), 1569 (C=N), 1302 (C-N), 1222 (C-O). ^{1}H NMR (600 MHz, DMSO-d_6, δ ppm): 9.22 (s, 1H, NH), 8.52 (s, 1H, CH $_{imidazole}$), 7.99 (s, 1H, CH $_{pyrimidine}$), 7.56 – 7.52 (m, 5H, H-Ar), 6.16 (s, 1H, CH $_{pyrrole}$), 4.03 – 3.99 (m, 4H, 2CH_2), 1.06 (t, J = 7.1 Hz, 3H, CH_3), 0.86 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6, δ ppm): 203.67, 203.44, 160.69, 160.54, 152.43, 144.85, 144.79, 144.79, 139.91, 139.77, 139.66, 139.52, 138.80, 138.73, 138.66, 138.58, 128.97, 128.73, 60.04, 58.63, 15.32, 14.87, 14.41, 13.87. HRMS (ESI): m/z= 406.1506 $[M+H]^+$ (calculated for $[C_{21}H_{19}N_5O_4+H]^+$: 406.1510). Elemental analysis, calculated for $C_{21}H_{19}N_5O_4$ (405.41): C, 62.22; H, 4.71; N, 17.27. Found: C, 62.14; H, 4.73; N, 17.30%

*Diethyl 1-(2-oxo-1,2-dihydropyrimidin-4-yl)-5-phenyl-1H-pyrrole-2,3-dicarboxylate (**3b**)*. The compounds was prepared according to the general procedure **C** using cytocine (0.28 g, 2.5 mmol), 2-bromoacetophenone (0.5 g, 2.5 mmol), and sodium diethyloxalacetate (0.5 g, 2.5 mmol). Compound **3b** was obtained as a yellowish-white solid in 36% yield; m.p. 224-226 °C. IR (KBr, $\bar{\upsilon}$ cm^{-1}): 3352 (N-H)$_{amide}$, 3050 (C-H)$_{aromatic}$, 2938 (C-H)$_{aliphatic}$, 1697 (C=O)$_{amide}$, 1739 (C=O)$_{ester}$, 1613 (C=C), 1653 (C=N), 1369 (C-N), 1215-1180 (C-O); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 11.63 (s, 1H, NH), 7.67 – 7.48 (m, 5H, H-Ar), 6.81 (d, 1H, J = 7.6 Hz, CH$_{ethyl}$), 6.63 (d, 1H, J = 7.5 Hz, CH$_{ethylen}$), 5.24 (s, 1H, CH $_{\boldsymbol{pyrrole}}$), 4.34 – 3.70 (m, 4H, 2CH_2), 1.43 – 0.82 (m, 6H, 2CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 194.44, 193.19, 147.20, 143.87, 135.06, 134.78, 133.50, 130.30, 129.39, 128.57, 128.37, 128.28, 125.96, 108.71, 108.24, 97.74, 55.61, 55.07. HRMS (ESI): m/z = 382.1406 $[M+H]^+$ (calculated for $[C_{20}H_{19}N_3O_5+H]^+$: 382.1397). Elemental analysis, calculated for $C_{20}H_{19}N_3O_5$ (381.38): C, 62.99; H, 5.02; N, 11.02. Found: C, 62.82; H, 4.98; N, 11.10%.

*Diethyl 1-(6-oxo-6,9-dihydro-1H-purin-2-yl)-5-phenyl-1H-pyrrole-2,3-dicarboxylate (**3c**)*. The compounds was prepared according to the general procedure **C** using guanine (0.34 g, 2.5 mmol), 2-bromoacetophenone (0.5g, 2.5 mmol), and sodium diethyloxalacetate (0.5 g, 2.5 mmol). Compound **3c** was obtained as a yellowish-white solid in 74% yield; m.p. 298-300 °C. IR (KBr,

$\bar{\upsilon}$ cm^{-1}): 3318 (N-H)$_{amide}$, 3010 (C-H)$_{aromatic}$, 3107 (N-H)$_{imidazole}$, 2909 (C-H)$_{aliphatic}$, 1670 (C=O)$_{amide}$, 1690 (C=O)$_{ester}$, 1558 (C=N), 1467 (C=C), 1373 (C-N), 1208 (C-O); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.55 (s, 1H, NH), 8.00 (s, 1H, NH), 7.68 (s, 1H, CH$_{imidazo}$), 7.60 – 7.53 (m, 5H, H), 6.08 (s, 1H, CH$_{pyrrole}$), 4.27 – 4.17 (m, 4H, 2CH_2), 1.07 (t, J = 7.1 Hz, 3H, CH_3), 0.85 (t, J =$_{arom}$ 7.1 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 200.24, 197.66, 160.31, 144.31, 136.19, 134.20, 133.24, 129.30, 128.90, 128.83, 128.62, 128.42, 125.93, 103.31, 60.97, 14.15, 14.08. HRMS (ESI): m/z= 422.1457 $[M+H]^+$ (calculated for $[C_{21}H_{19}N_5O_5+H]^+$: 422.1459). Elemental analysis, calculated for $C_{21}H_{19}N_5O_5$ (421.41): C, 59.85; H, 4.54; N, 16.62. Found: C, 59.84; H, 4.55; N, 16.62%.

Biological effectiveness

Protocol for the in vitro antibacterial assessment

The *in vitro* antibacterial activity of the target prepared compounds was screened against the strains of gram-positive (*S. aureus* ATCC 25923 and gram-negative (*E. Coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *K. pneumonia* ATCC 13883) by the agar disc diffusion test [38-39].

All the products were dissolved in dimethyl sulfoxide (DMSO), which has no inhibitory activity for dilution in order to prepare stock solutions and two daughter solutions (15-3.25 mg/mL). The Whattman filter papers were sterilized in an autoclave for 1 hour at 140 °C. The agar plates were uniformly inoculated on the surface with a fresh culture broth of G^+ bacteria including the impregnated discs with 60 µL placed on the agar plates, and then incubated at 30 °C for 1 h to allow good diffusion. They were then transferred to an incubator at 37±2 °C for 24 hours before to examine the inhibition zones causing by tested compounds on the bacterial strains. The inhibition zones were recorded by measuring the zones surrounding the disk at the mm scale, and the results were compared with gentamicin as standard antibiotic.

In vitro antioxidant evaluation

The *in vitro* antioxidant capacity of the synthesized compounds was tested through their ability to reduce the 1,1-diphenyl-1-picrylhydrazyl (DPPH$^•$) and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt ($ABTS^{+•}$) free radicals. The total antioxidant activity was evaluated by phosphomolybdenum method. Ascorbic acid (vitamin C), butylated hydroxy toluene (BHT), and gallic acid (GA) were used as standard agents.

Protocol for the DPPH radical scavenging assay [40]

The antioxidant activity of the prepared compounds was determined from the bleaching of the purple-colored methanolic solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH) according to the previously described protocol [41, 42]. Different concentrations (10, 25, 50, 100, 200, 250 and 500 µg/mL) of the tested compounds were prepared, and 1 mL of varying concentrations were mixed with 2 mL of a 0.004% (w/v) solution of DPPH in methanol. The mixture was incubated for 30 min in the dark at room temperature. Next, 100 µL of each solution were placed into 96-well plates, and the absorbance (A) of the mixture was recorded at 517 nm.

Protocol for the ABTS radical scavenging assay

Free radical scavenging activity of compounds **2a-c** and **3a-c** was determined by ABTS radical cation decolonization assay [43]. $ABTS^{•+}$ radical cation was prepared by reaction of two stock solutions of ABTS (7 mM) and potassium persulfate (2.4 mM). The mixture was kept in the dark

at room temperature for 16 h to give the free radicals. The solution of ABTS radical was then diluted to obtain an absorbance of 0.701 ± 0.005 units at 734 nm using a spectrophotometer.

To determine the ABTS scavenging effect, 990 µL $ABTS^{\bullet+}$ radical cation was reacted with 10 µL of each sample and was incubated at room temperature for 7 min. After incubation, the absorbance was recorded at 734 nm using a PRIMLIGHT, SECOMAM reader.

Evaluation of total antioxidant activity (TAC) by phosphomolybdenum (PM) assay

Using micropipette, 0.1 mL of each compound (62.5 µg/mL) in methanol was mixed with 1 mL of reagent solution (containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes containing the mixture were incubated at 95 °C in a water bath for 90 min. After incubation, the mixture was cooled to room temperature and the absorbance of the sample solution was read at 695 nm using a PRIMLIGHT, SECOMAM reader against the blank. All experiments were carried out in triplicate. The total antioxidant capacity of the sample was expressed as the number of gallic acid equivalent (GAE) or ascorbic acid equivalent (AAE).

CONCLUSION

New pyrimidine- and purine-ligated pyrrole derivatives were synthesized using nucleobases (cytosine, adenine or guanine) by Hantzsch multicomponent reaction in the presence of DABCO as a catalyst. The *in vitro* antioxidant activity of all the newly compounds was investigated by examining their scavenging effects on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylenzothiazoline-sulfonic acid) diammonium salt (ABTS) free radicals, in comparison with vitamin C and gallic acid. The results show that compounds **2c** and **3c** exhibit significant antioxidant activity against DPPH and ABTS free radicals. Additionally, the total antioxidant capacity (TAC) of the synthesized compounds was checked using the phosphomolybdenum method. The *in vitro* antibacterial activities of these compounds have been evaluated against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumonia* ATCC 13883 (Gram-negative: G^-) and *Staphylococcus aureus* ATCC 25932 (Gram-positive: G^+) bacterium using gentamicin as reference antibiotic. Compounds **2a** and **3a** show significant inhibitory effects and could be considered as the most potent antibacterial agents.

Conflict of interest

There are no conflict to declare.

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