Proceedings Paper

Synthesis of 2-aminopyridine Lactones and Studies of Their Antioxidant, Antibacterial and Antifungal Properties

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Abstract: In the present work, the synthesis and biological activities of substituted 2-aminopyridine δ-lactone derivatives were achieved. 4,6,6-trimethyl-2-oxo-5,6-dihydro-2H-pyran-3-carbonitrile was synthesised from 4-hydroxy-4-methylpentan-2-one, followed by its transformation in enaminonitrile with DMFDMA. The antioxidant effects of substituted 2-aminopyridine δ-lactone derivatives were evaluated through DPPH assay and revealed a great antioxidant capacity. The antifungal and antibacterial activities were investigated by disc diffusion method against clinical Gram-negative bacteria and against clinical fungi. The study shows moderate to very good antibacterial and antifungal activities for the new substituted 2-aminopyridine δ-lactone derivatives.

Keywords: 2-aminopyridines; bis-2-aminopyridines; antioxidant; DPPH; radical scavenger; antibacterial activity; antifungical activity

1. Introduction

Substituted 2-aminopyridine δ-lactone derivatives were achieved. 4,6,6-trimethyl-2-oxo-5,6-dihydro-2H-pyran-3-carbonitrile was synthesised from 4-hydroxy-4-methylpentan-2-one[], followed by its transformation in enaminonitrile with DMFDMA[].
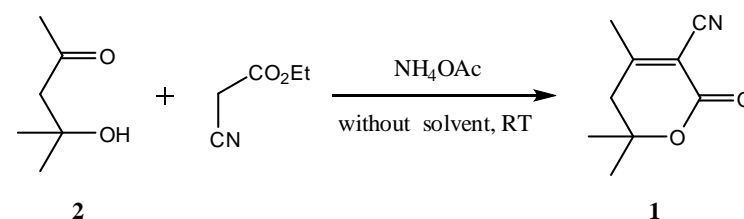
The compound **3** was prepared by the reaction of δ-lactone nitrile «4,6,6-trimethyl-2-oxo-5,6-dihydro-2H-pyran-3-carbonitrile » **1** with dimethylformamide dimethylacetal DMFDMA in stoichiometric amounts. The reaction was performed at room temperature during 24 h and afforded good overall yield (72%) [1].
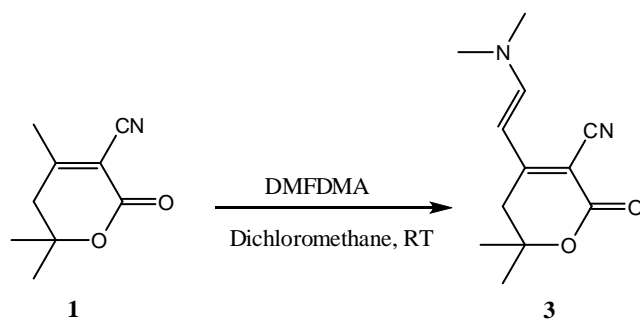
The reaction of enaminolactone nitrile **3** and primary amines **4a–f** in refluxed DMF according to our previous work [1] results in new substituted 2-aminopyridines **5a–f.**

Table 1. Synthesis of 2-aminopyridine lactones.
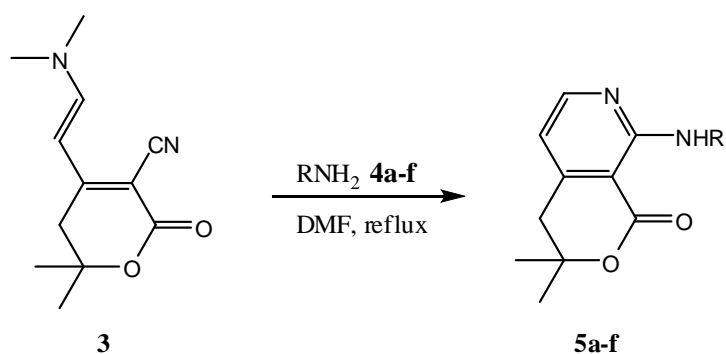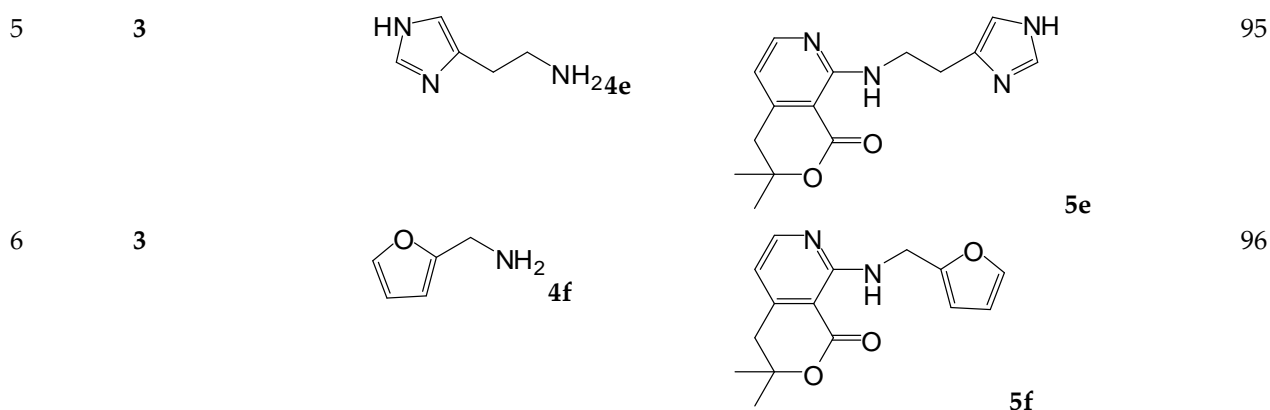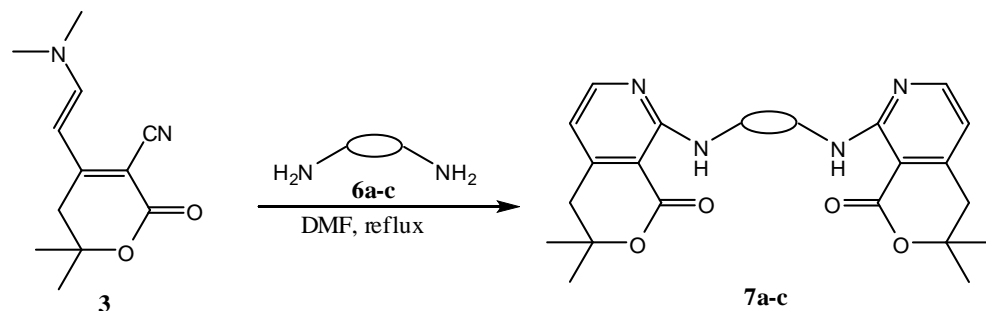
Entry	Enaminolactone	RNH₂	Product	Yield (%)
1	**3**	**4a**	**5a**	95
2	**3**	**4b**	**5b**	87
3	**3**	**4c**	**5c**	92
4	**3**	**4d**	**5d**	96

| 5 | **3** | 4e | 5e | 95 |
| 6 | **3** | 4f | 5f | 96 |

The structure of substituted 2-aminopyridine δ-lactones characterised by spectroscopic methods (IR, ¹H NMR, ¹³C NMR and MS).

The reactions between 1 equiv of diamines **6a–c** with 2 equiv of enaminolactone nitrile **3**. The mixture was refluxed in DMF during 6 h. After removing of the solvent and purification by column chromatography, we afforded the new original bis-(2-aminopyridines) **7a–c** in moderate to good yields (Table 2)

The structure of the compounds **7a–c** was confirmed by spectral data (IR, ¹H NMR and ¹³CNMR).

Table 2. Synthesis of bis-2-aminopyridine lactones.

Entry		RNH₂	Product	Yield (%)
1	**3**	6a	7a	57
2	**3**	6b	7b	60
3	**3**	6c	7c	89

2. Antioxidant Effects

The antioxidant effects of substituted 2-aminopyridine δ-lactone derivatives were evaluated through DPPH assay and revealed a great antioxidant capacity.

For initial screening of antioxidant activity DPPH on TLC was employed [2]. After the qualitative confirmation of antioxidant potential, spectroscopic measurements were made through DPPH assay. The antioxidant proprieties were measured and evidenced in terms of their efficient concentration IC_{50}, as well as their reduction kinetics. [3] Evaluation of the antioxydant activity by the test of DPPH, revealed a great antioxydant capacity for the most of compounds tested with a variation of IC_{50} between 1.30–3.61 mg/mL and times of reaction of 30 min.

3. Antifungal and Antibacterial Activities

The antifungal and antibacterial activities of 2-aminopyridines and bis-2-amino-pyridines were investigated in vitro in order to evaluate their efficacy. The antibacterial activity of the compounds was determined by the disc diffusion method [4,5] against clinical Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and Gram-positive bacteria: *Staphylococcus aureus, Listeria monocytogenes* and *Bacillus cereus*. The antifungal activity of the compounds was determined by using a direct-contact and agar diffusion test [6] against clinical fungi *Aspergillus flavus* and *Aspergillus ochraceus.* The compounds showed moderate to very good antibacterial and antifungal activities, that the **5b**, **5d**, **5e** and **5f** presents a best minimal inhibitory concentration (MIC) with 62.5 μg/mL. The *Aspergillus ochraceus* strain revealed a stronger sensitivity than *Aspergillus flavus* to all compounds tested, While that the **7c** and 7**b** showed a braod-spectrum antifungal activity again pathogenic *Aspergillus ochraceus* with an inhibition percentage of 77% and 78%, respectively. Based our results, the compounds of 2-aminopyridines and bis-2-amino-pyridines can be considered as a source of novel antibiotic and antifungal.

4. Experimental

Described in Supplementary Informations.

5. Conclusions

The study shows moderate to very good antibacterial and antifungal activities for the new substituted 2-aminopyridine δ-lactone derivatives.

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Supplementary Information A

Synthesis

According to your previously paper:

Fadhila Salhi, Nawel Cheikh, Didier Villemin, Bachir Mostefa-Kara, Nathalie Bar, Karine Jarsalé, and Nourredine Choukchou-Braham, **Catalyzed reaction of enaminonitrile with primary amines by SbF₃: synthesis of new 2-aminosubstituted-pyridine-fused δ-lactones.** Arkivok, *V, 64-74 (2018);* doi :10.24820/ark.5550190.p010.495

https://www.arkat-usa.org/get-file/63574/and   https://www.arkat-usa.org/get-file/63569

Antioxydant

1. Introduction

Currently, nitrogen-containing heterocyclics have attracted people's attention for their unique physicochemical characteristics and bioactivity [1]. As one of the most abundant compound, The 2-aminopyridine are an important privileged structure for the development of antimicrobial agents, as compounds containing this scaffold exhibits diversified biological and pharmaceutical activities [2–4], including anti-inflammatory [5,6], antitumoral [7], analgesic [8], antipyretic [9], antitubercular [10]. and antiviral [11]. More recently, it is also recognized their antioxydant properties that there are a lot number of reports which show that natural and synthetic 2-aminopyridines derivatives possess antioxidant activity [12–21].

In this context, the aim of the present study was to evaluate the antioxidant activity of our 2-aminopyridines synthesized for their valorization as antioxidants by the method of DPPH• scavenging.

The search for new more effective antioxidants has become an axis of global research as evidenced by the ever increasing number of articles in this topic because of their role in the prevention of chronic diseases such as diseases of the heart, cancer, diabetes, hypertension, and Alzheimer's disease by combating oxidative stress [22–24]. Furthermore understanding the phenomenon involved in the mechanisms of action of these molecules is an essential standard in order to imagine new more active structures. The chemical synthesis enables in some cases to improve the activity thereof by structural modifications.

Oxidation, caused by reactive oxygen species (ROS), is a pervasive biological process in physiology and metabolism of many organisms [25]. Reactive oxygen species (ROS) are normally generated in the human body with high potential to damage almost all types of cellular constituents and scavenged by antioxidant defenses system when ROS remains at physiological concentrations [26], which explains their involvement in the induction and/or amplification of a number of human pathologies. It is essential to preserve the endogenous antioxidant defense systems and normal cell functions when ROS remains at physiological concentrations. Therefore, the body can have the capacity to avoid many harmful damages [27]. However, these systems are insufficient to prevent the harm entirely [28]. It is reported that free radicals, including superoxide anion, hydroxyl radical, and hydrogen peroxide can cause various pathological damages like atherosclerosis, coronary heart disease, and many other diseases associated with aging to the organism, and lead to harmful alterations in foods and pharmaceutical industries [29–31]. Therefore, it is urgent to develop exogenous antioxidant supplements to help the human body reduce oxidative scratch and to fight these harmful species.

The antioxidant activity of a compound corresponds to its ability to resist oxidation. The most known natural antioxidants are the β-carotene (provitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E) [32]. Indeed, The ability of most synthetic or naturally

occurring antioxidants to scavenge free radicals such as hydroxyl radicals (OH•) and superoxide (O2•) should be attributed to their different contents of amino groups in their structures [32].

Several methods are used to evaluate in vitro and in vivo antioxidant activity by scavenging different radicals [33], such as peroxide ROO• methods by ORAC (Oxygen Radical Absorbance Capacity) and TRAP (Total Radical-Trapping Antioxidant Parameter) [34], ferric ions method FRAP (Ferric Reducing Antioxidantion Parameter) [35], or the radical ABTS•(ammonium salt of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) [36]and the method using the free radical DPPH•(2,2-diphenyl-picrylhydrazyl).

2. **Experimental Section**

2.1. General Information

The UV–vis absorbance of the tested mixture was measured with a Unicam UV 300 spectrophotometer.

2.2. Initial Screening of Antioxidant Potential

Antioxidant activity was determined using qualitative and quantitative analysis:

2.2.1. DPPH assay on TLC

DPPH assay with TLC was used to measure the antioxidant activity of our synthesis compounds and was carried out according to a modified version of the method described by Bektas [37]. A methanolic solution of 1 mg/mL of each compound was prepared. Five microliters each of this solutions was applied on the TLC plate. Plate was developed by methanol. The plate was dried in the fumehood. Then the plate was sprayed with 0.2% of DPPH reagent in methanol, as an indicator [38] and stayed for 30 min at room temperature. Purple color of DPPH reagent bleaching by yellow spots on TLC plate sprayed is the indication of positive antioxidant activity.

2.2.2. DPPH Radical Scavenging Activity

DPPH radical scavenging activity of each compound was determined according to the method of Shen [39]. Briefly, a solution of DPPH (4%) in methanol as the free radical source was prepared and 1.9 mL of this solution was added to 100 μL of the solution of all samples in methanol at different concentration (1, 0.5, 0.25, 0.125 & 0.0625 mg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. The decrease of solution absorbance due to proton donating activity of components of each samples was determined at 517 nm using a UV-VIS spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The negative control contains only the DPPH solution and the positive control is represented by standard antioxidant solutions; Vitamin C whose absorbance was measured in the same conditions as the test sample. Three replicates for each sample concentration were tested and the DPPH radical scavenging activity was calculated using the following formula: DPPH Radical Scavenging Activity (%) = [(A0 − A1/A0) × 100], where A0 is the absorbance of the control, and A1 is the absorbance standard sample and references. All the tests were performed in triplicates and the results were averaged.

3. **Results and Discussion**

3.1. DPPH Assay on TLC

Various assays have been used to test for antioxidant activity but the mostly widely used methods are those that involve generation of free radical species which are then neutralized by antioxidant compounds [40]. In qualitative analysis of antioxidant activity, the 2, 2,diphenyl-1-picrylhydrazyl (DPPH) assay on TLC plates was used as a screening test for the radical scavenging ability of the different 2-aminopyridines.

The DPPH method measures electron-donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Any molecule that can donate an electron or hydrogen to a mixture will react with the DPPH. DPPH is reduced from a purple compound to a light yellow compound by electrons from oxidant compounds. Reaction of DPPH with hydroxyl groups involves a homolytic substitution of one of the phenyl rings of DPPH yielding 2-(4-hydroxyphenyl)-2-phenyl-1- picryl hydrazine as a major product whilst 2-(4nitrophenyl)-2phenyl-1-picrylhydrazine is also formed via a series of secondary processes. The concentration of DPPH at the end of a reaction will depend on the concentration and structure of the compound being scavenged [41].

The TLC-DPPH screening method indicated the presence of antioxidant compounds in all of the compounds tested, with **5b**, **5d**, **5e**, **5f** and **7a** showing the most prominent antioxidant activity (Figure 1). Whereas, **5a, 5c, 7b** and **7c** apparently showed active compounds with less activity compared to Vitamin C. Purple color of DPPH reagent was bleached by yellow spots was the indication of positive antioxidant activity.
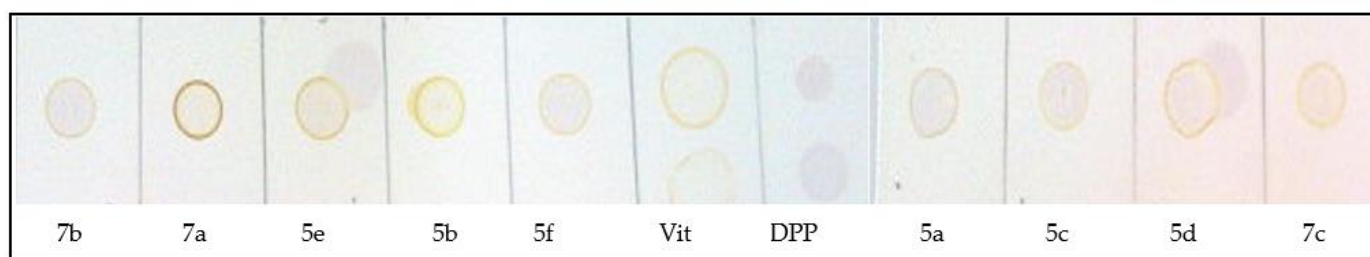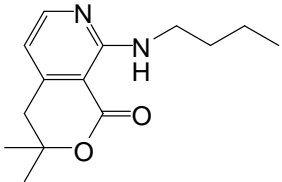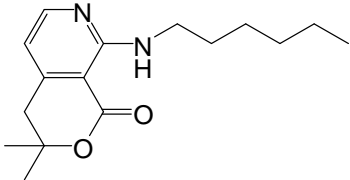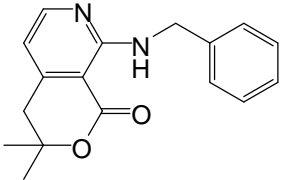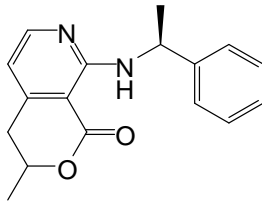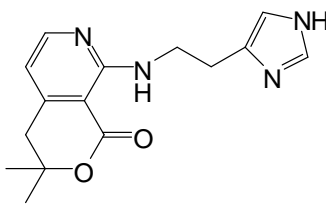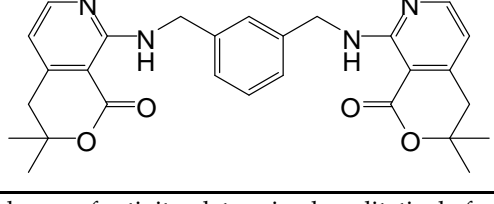

Figure 1. DPPH assay on TLC of synthesized 2-aminopyridines and bid 2-aminopyridines.

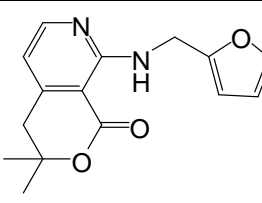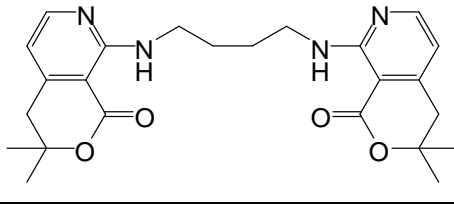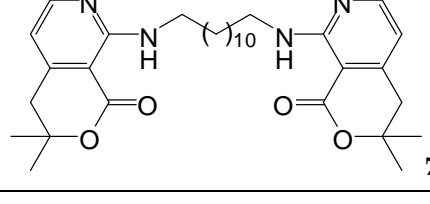The degree of activity of all the samples tested was determined qualitatively from observation of the yellow colour intensity (Table 1).The most of samples tested showed activity that are a moderate to good candidate to isolate antioxidant compounds. From this results of Table 1 we can conclude that all the 2-aminopyridine and bis-2-aminopyridine tested have a antioxidant activity.

Table 1. Qualitative DPPH assay on TLC of synthesized 2-aminopyridines.

2-aminopyridine/bis 2-aminopyridine	The Degree of Activity
5a	++
5b	+++
5c	++

5d +++

5e +++

5f +++

7a +++

7b ++

7c +++

The degree of activity, determined qualitatively from observation of the yellow color intensity: weak (+), moderate (++), strong (+++) and no activity (−).

3.2. DPPH Assay

The extent of decrease in the absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant. These scavenging activities might be due to the presence of different NH contents.

The antioxidant activity is dependent on the mobility of the hydrogen atom of the amino groups of 2-aminopyridines. In the presence of a free radical DPPH, the H atom is transferred onto the latter to obtain a stable molecule of DPPH, this causes a decrease in the concentration of free radicals and also the absorbance during the reaction time to the depletion of the hydrogen donor antioxidant capacity.

The results obtained in measuring test of the percentage inhibition of DPPH free radical scavenging activity are recorded in Figures 2 and 3. It seems that the percentage inhibition of the free radical increases with increasing concentration for Vitamin C is or for all 2-aminopyridines and bis 2-aminopyridines tested.
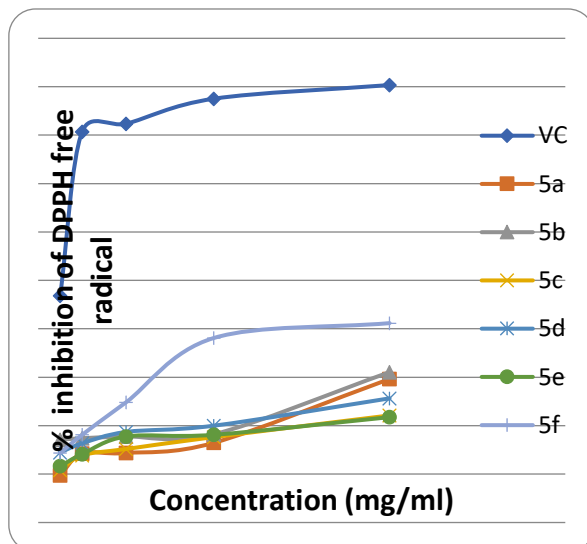

Figure 2. Inhibition of DPPH (%) for the 2-aminopyridines and vitamin C as a function of concentration.
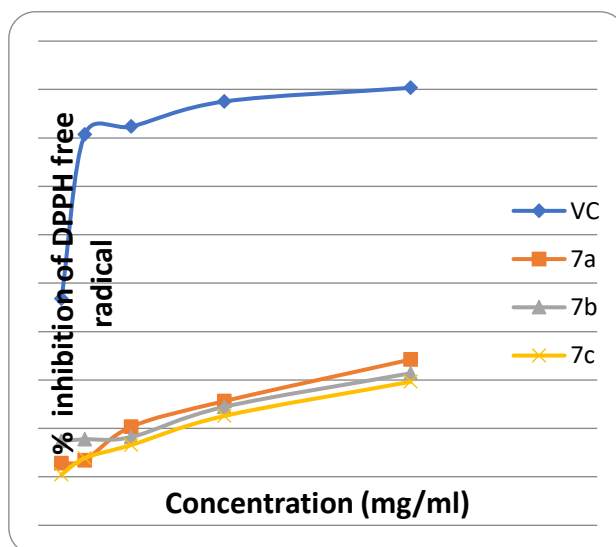

Figure 3. Inhibition of DPPH (%) for the bis 2-aminopyridines and vitamin C as a function of concentration.

It is noted that the percentage inhibition of the free radical to all 2-aminopyridines is less than that of vitamin C for all concentrations used (Figures 2 and 3). To overcome the influence of the concentration, in the majority of studies, the reactivity is estimated by the effective concentration IC50 (or the inverse 1/IC50) of the antioxidant, which corresponds to a 50% reduction activity (absorbance) of DPPH• in the reaction medium. The antioxidant capacity of a compound is even higher than its IC50 is small. The index shows the IC50 concentrations of antioxidant are necessary to decrease the initial concentration of DPPH• with 50% (expressed in mol of Antioxidant/mol of DPPH• or milligrams of Antioxidant/gram of DPPH• in the reaction medium), but does not take into account the influence of the concentration on the reaction time [37].

The IC50 values for the 2-aminopyridines, bis-2-aminopyridines and vitamin C are shown in Figure 4. All the 2-aminopyridines tested could bring the stable free radical 2.2-diphenyl-1 picrylhydrazyl (DPPH) in diphenyl-picrylhydrazine yellow-colored with an IC50 1.308, 1.959, 2.260, 3.042, 3.314 and 3.616 mg/mL of **5f**, **5a**, **5b**, **5d**, **5c** and **5e** respectively showing antioxidant activity with uneven values lower than that of vitamin C 0.130 mg/mL. While bis -2 aminopyridines show a more effective inhibition compared to 2-aminopyridines with an IC50 1.690, 1.995 and 2.244 mg/mL of **7a**, **7c** and **7b** respectively.
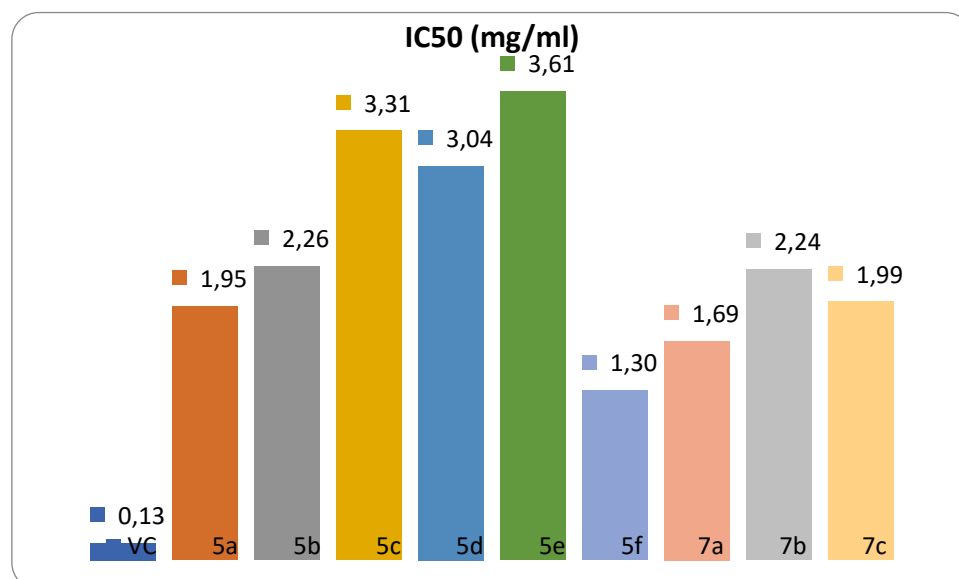

Figure 4. The IC50 of the 2-aminopyridines, bis 2-aminopyridines and vitamin C.

It appears from these results that bis -2 aminopyridines show a more effective antioxidant activity than 2 aminopyridines compared to vitamin C that is the most effective antioxidant.

Finally, most of the derivatives containing two amino groups have a better scavenging effect than that of derivatives with one amino group, which is in accord with the conclusion that the aminated derivatives are more potent as a scavenger of hydroxyl radicals. [14,32] The amino group in pyridine may play an important role to act as an electron donor to quench free radicals by providing an electron, conceivably via an electron attack on the free radicals [15,42]. The stronger electron-donating groups tend to donate more electrons to quench more reactive free radicals, which may help stabilize the free radicals' form [15,43]. It would be reasonable to presume that the amino group of 2-aminopyridine ring should be an important factor that influences the scavenging activity against DPPH radicals. Besides, the enhanced scavenging capability against DPPH radicals may be affected by the number of amino groups of pyridine ring. The results further confirm that the number of amino groups could influence the antioxidant activity of 2-aminopyridine and bis-2-aminopyridine.

4. Conclusions

In summary, the study of antioxidant activity of 2-aminopyridine and bis-2-aminopyridine, by trapping method free radical DPPH showed that the most of these compounds have a moderate antioxidant activity but less effective as compared to vitamin C. More, most of the derivatives containing two amino groups have a better scavenging effect than that of derivatives with one amino group, that why it would be reasonable to presume that the number and the position of amino group on pyridine could influence the antioxidant property of these aminopyridines derivatives. Further comprehensive investigation to ascertain this hypothesis on antioxidant and structure- activity relationships

should be carried out. Moreover, economically it is desirable to conduct a thorough analysis of the mechanisms of action and a more advanced search on the synergy of these basic compounds.

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Supplementary Information B

1. Antibacterial-Antifungal

The 2-aminopyridines and bis-2-aminopyridines are one of nitrogen containing heterocyclics that known for their very important therapeutic and biological propriétés such as, anti-inflammatory [4,5], analgesic [4,5], antipyretic [6], antiparasitic [7] and antiviral [8] antitumoral [9], antioxydant [10], antitubercules [11]. More recently, it is also recognized their antimicrobial properties [12–28].

In this context, the main objectives of this work were to investigate antibacterial and antifungal activities of the 2-aminopyridines and bis-2-aminopyridines to determine their Minimum Inhibitory Concentration againt clinical gram positive and gram negative bacteria and finding out their effacacy against two standart fungal strains *Aspergillus ochraceus* and *Aspergillus flavus.*

2. Material and Methods

2.1. Microorganisms, Inoculums and Antifungal Assay

2.1.1. Microorganisms and Cultural Methods

In the presents study, a standard fungal strains: *Aspergillus ochraceus* and *Aspergillus flavus* isolated from the dates by the laboratory team of valorization of vegetal resource and food security in semi-arid Areas, south west of Algeria, (University of Bechar). The tested organism was selected according to their ease of availability and pathogenicity to human, animals and plants. The isolates of organisms were subculture once onto potato dextrose agar (PDA) (Merck, Darmstadt, Germany) and incubated for 48 to 72 h at 35 °C.

2.1.2. Inocula Preparation

Inocula was prepared by growing the fungi on PDA for 48 to 72 h at 35 °C and then until 7th day at 25 °C as described by the reference method M38-A2 recommended by NCCLS guidelines [29]. The Inocula was prepared by flooded colonies with approximately 5 mL of sterile 0.85% saline. Tween 20 (0.01 mL) was added to facilitate the preparation of fungal strains inocula. The resulting mixture is transferred to a sterile tube. After the settling of the larger and heavy particles for 4 to 5 min, the upper homogeneous suspension is transferred to a sterile tube and mixed with a vortex mixer for 15 s. These suspensions were diluted 1:50 in the RPMI medium. The suspensions were mixed for 15 s to ensure homogeneity and subsequently diluted to adjust the turbidity of a 0.5 McFarland standard (0.4×10^4 to 5×10^4 CFU/mL). This density was read using a spectrophotometer (UV-VIS 1650 Shimatzu, Japan) and matched to an optical density (OD) for strain.

2.1.3. Assay for Antifungal Activity

The antifungal activity was evaluted by the method of dilution in a solid medium reported by Remmal et al. (1993) and Satrani et al. (2001) with modification [30,31].

10% solution of DMSO in water was prepared. 1.5 mL of each of compounds tested dissolved in this solution was added to 13.5 mL of a medium Potato dextrose agar PDA so as to obtain 1 mg /L concentration of the compound in the medium. After homogenization, the mixture was poured into petri dishes. Witnesses, containing the culture medium and the Potato dextrose agar solution alone are also prepared.

Seeding is done by injection. Petri dishes (control and test) were incubated for 7 days at 27 °C. The growth of filaments is recorded daily. A measure diameters of colonie is performed at the end to calculate the inhibition rate (I%) [15] using the following formula: I'(%) =100 × (dC-dE)/dC, where I'(%) = Inhibition percentage rate, dC = diameter of colony in the petri dishes « positifs control » and dE = diameter of colony in the petri dishes containing compounds tested.

2.2. Microorganisms, Inoculums and Antibacterial Assay

2.2.1. Inocula Preparation

Four reference strains ATCC, from the laboratory of valorization of vegetal resource and food security in semi-arid Areas, south west of Algeria, (University of Bechar) are tested: Gram-negative bacteria: *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853) and Gram-positive bacteria: *Staphylococcus aureus* (ATCC25923) and *Bacillus cereus* (ATCC11778).

The different bacterial strains were pricked by the striations method and then incubated at 37 °C for 18 to 24 h to obtain a young culture and isolated colonies were used subsequently to prepare the inoculum by soaking in solution tubes of sterile distilled water to have an initial cell density or turbidity adjacent to the 0.5 McFarland.

2.2.2. Antibacterial Assay

The antibacterial activity of the compounds was carried out by disc diffusion method cited in (Treki et al., 2009) [32].

After adjusting the turbidity of the suspension used inoculum, a swab was dipped in the suspension and the whole surface was plated with Mueller Hinton agar MHA to the three times. After each application, the petri box was turned approximately 60° to ensure an homogeneous distribution of the inoculum. Finally, it was swabbed all around the edge of the agar surface.

Discs of sterile Whatman paper (6 mm in diameter) was impregnated in each with concentrations (1, 0.5, 0.25, 0.125, 0.0625 mg/mL) of solids taken up in solution of DMSO 10% in water and applied by means of a clamp on the surface of the MHA medium. All the petri dishes were incubated for 24 h at 37 ° C. An uninoculated petri dishes medium with solvent was incubated to serve as a negative growth control under the same conditions. After 24 h, all of petri dishes were compared to control negative petri dishe. The antibacterial activity was determined by measuring using a rule the diameter of the inhibition zone. The lowest concentration of the compounds that inhibits growth of the organism was determined as the Minimum Inhibitory Concentration (MIC).
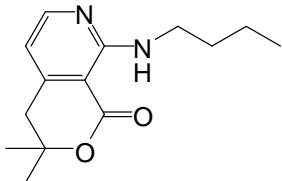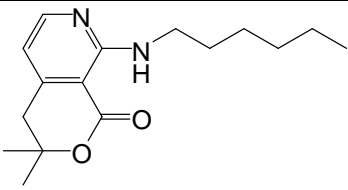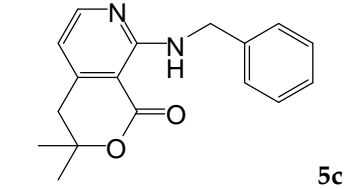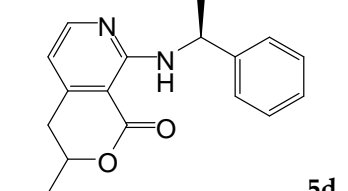
3. Results and Discussion
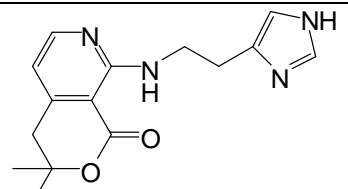
3.1. Antibacterial Activity

The diameters of the inhibition zones of 2-aminopyridines and bis-2-aminopyridines with a conccentration of 1 mg/mL against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram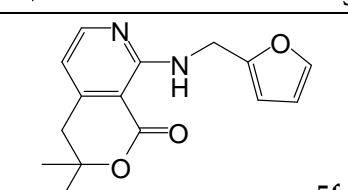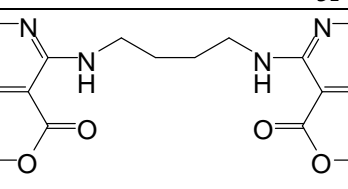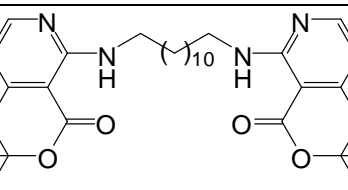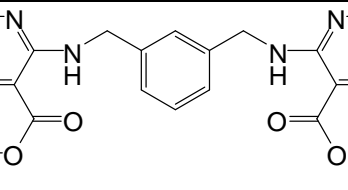-positive bacteria (*Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*) shown in (Table 1, Figure 1). The Disk diffusion method allowed us to bring out the antibacterial power 2-aminopyridines and bis-2-aminopyridines against the five bacterial strains, that the histogram (Figure 1) has a variable activities between bacterial strains.

The morphology of the cell membrane may be a main issue that affects the activity of antimicrobial agents. The cell membrane of the bacteria consists of peptidogly can which is thicker in the gram positive bacteria and is usually posses a barrier to the degree of diffusion of antimicrobial agents into the enzyme [33]. The activity can be enhanced or reduced by the combination depending upon interactions between the compounds.

Table 1. Diameter (mm) zones of inhibition of 2-aminopyridines and bis-2-aminopyridines against bacteria.

2-aminopyridine/ bis 2-aminopyridine	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*L. monocytogenes*
T	6	6	6	6	6
5a	8	12	8	6	6

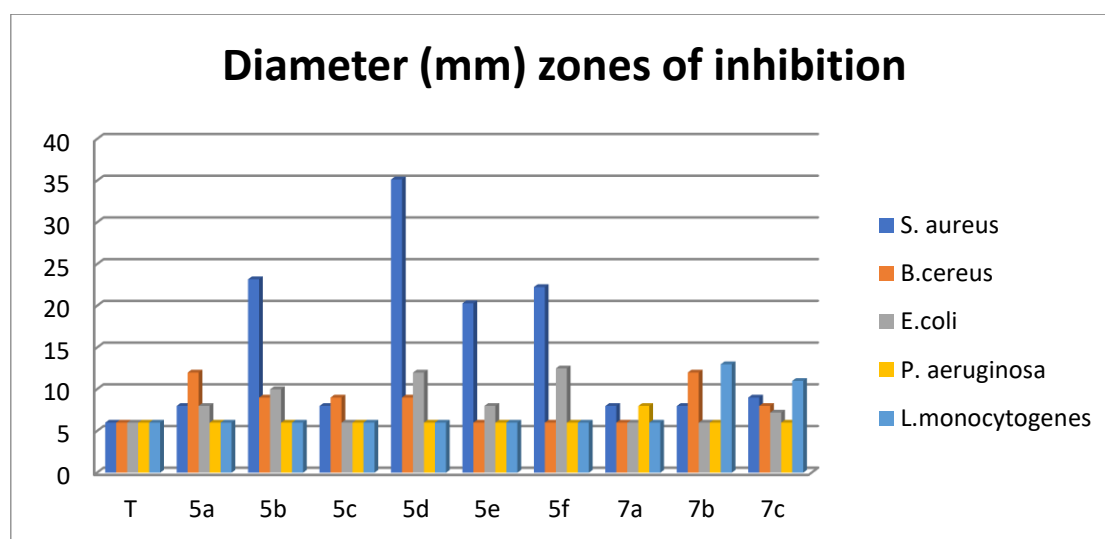|					
5b	23.19	9	10	6	6
5c	8	9	6	6	6
5d	35.15	9	12	6	6
5e	20.3	6	8	6	6
5f	22.2	6	12.5	6	6
7a	8	6	6	8	6
7b	8	12	6	6	13
7c	9	8	7.2	6	11

Figure 1. Diameter (mm) zones of inhibition of 2-aminopyridines and bis-2-aminopyridines against bacteria.

It is observed that the different studied bacterial strains react differently to the tested compounds, that some bacterial strains show a moderate to good sensitivity against 2-aminopyridines. The bacterial strain *Pseudomonas aeruginosa* showed no sensitivity against all the compounds tested except the compound **7a** revealed a weak inhibition of 8 mm. The best zones of inhibition are obtained by mono aminopyridines **5b**, **5d**, **5e** et **5f** against the two bacterial strains *S. aureus* and *Escherichia coli* in the range of 20.3–35.15 mm and 8–12.5 mm respectively. While the compounds **5a, 5c**, **7a**, **7b**, ans **7c** showed moderate anti-bacterial activity against *S. aureus* with a diameters of inhibition zones between 8 and 9 mm. The compound **7a, 7b** showed no inhibition against *Escherichia coli*. Most synthetic compounds are not effective against *P. aeruginosa* and *L. monocytogenes* except the compound **7a** with a weak inhibition of 8 mm for *P. aeruginosa* and compounds **7b, 7c** with a moderate inhibition of 13 mm, 11 mm for *L. monocytogenes* respectively. Also, *B. cereus* revealed a moderate sensity against **5a**, **5b**, **5d**, **5f** and **7c**.
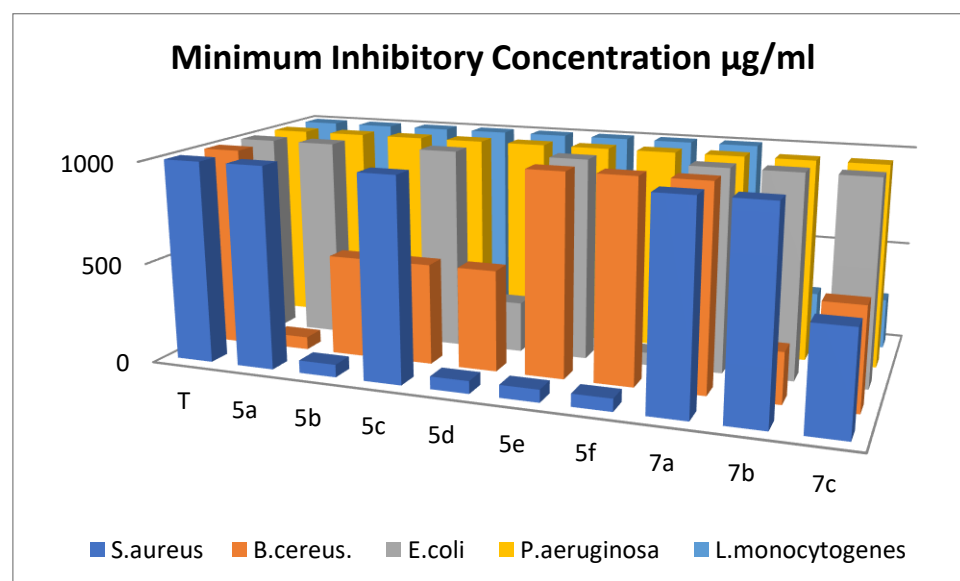

Figure 2. MIC (µg/mL) of 2-aminopyridines and bis-2-aminopyridines against bacteria.

The MIC was determined by disc diffusion method. Based on the histogram (Figure 2), it was observed that the derivatives of the 2-aminopyridines and bis-2- aminopyridines show a significant MIC to the order of 62.5 and 500 µg/mL, as well as the product **7a** had

a low sensitivity against bacterial strain *Pseudomonas aeruginosa* with a MIC of 1000 µg/mL. This compound have a low-dose antibacterial activity. The lowest and the best MIC of 2-aminopyridine was observed by the **5b**, **5d**, **5e** and **5f** compounds against bacterial strain *Statphylocoque aureus* with a MCI of 62.5 µg/mL. A sensitivity was noted for *B. cereus* with the compounds **5a**, **5b**, **5c**, **5d**, and **7c** in the concentration of 62.5–500 µg/mL. Moreover, this effect was also observed for *E. coli* with the compounds **5f**, **5d**, and **5b** in the concentration of 62.5, 250 and 500 µg/ mL, respectively. Also, the compound **7b** and **7c** showed a good MIC of 250 µg/mL against *L. monocytogenes*.

3.2. Antifungal Activity

The results of the antifungal activity of 2-aminopyridines and bis-2-aminopyridines tested are summarized in Table 2. The action of the compounds is determined by the diameter of the radial growth of a fungal strain and their percentage inhibition strain compared to a control. The results of the antifungal screening data revealed that all the tested compounds showed considerable and varied activity against the two fungal strains used.

Table 2. antifungal activity synthesized 2-aminopyridines and bis-2-aminopyridines.

2-aminopyridine/ bis 2-aminopyridine	*Aspergillus ochraceus*	*Aspergillus flavus*
T	85	85
5a	27	46
5b	23	40
5c	20	46
5d	21	46
5e	25	44
5f	23	45
7a	22	42
7b	18	40
7c	19	45

Evaluation of the antifungal activity of the synthesized compounds showed that the strain *Aspergillus ochraceus* was highly sensitive compared to *Aspergillus flavus* to all compounds. The compounds 7c and 7b showed strong inhibition of 78%, (the diameter of the inhibition zone was in 18–19 mm) and the compounds 5a, 5b, 5d, 5f, and 7c showed a higher inhibition rate in range of 72–76% (the diameter of the inhibition zone was in 20–23 mm) against *Aspergillus ochraceus*. Also, the compounds 5a and 5e showed an important antifungal activity with a inhibition rate of 68% and 70%, respectively.

Aspergillus flavus was moderately sensitive to all compounds as compared with *Aspergillus ochraceus*. The compounds with the most pronounced antifungal activity were 5b and 7b with 52% of inhibition of *Aspergillus flavus* (the diameter of the inhibition zone was 40 mm). Moreover, the compound 7a revealed an inhibition percentage of 50% (the diameter of the inhibition zone was 42 mm).

The compounds 5f, 7c and 5e also showed antifungal activity against *A. flavus* at a percentage from 47% to 48%, whereas the compounds 5a, 5b and 5d showed the lowest inhibition against this fungus (45%) with a diameter of 46 mm. The biological activity of 2-aminopyridines and bis-aminopyridines is to be related to its chemical composition, the functional group of compounds (amine). Thus, the nature of chemical structures that constitutes it, but their proportion ply a determinant role.
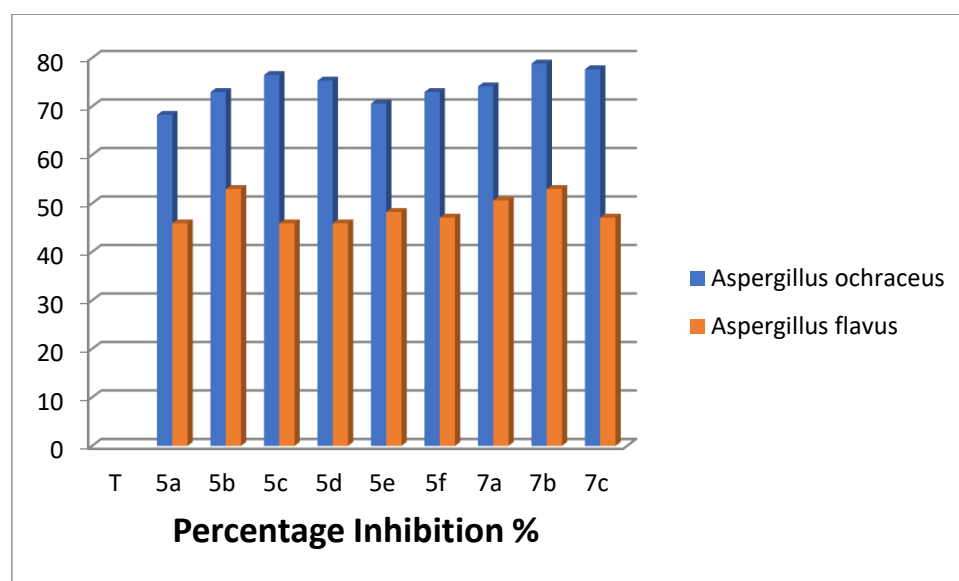
Figure 2. Inhibition percentage of 2-aminopyridines and bis 2-aminopyridines against *Aspergillus flavus* and *Aspergillus ochraceus*.

4. Conclusions

As part of this work, we studied the biological activity of some 2-aminopyridine compounds one antifungal activity and other antibacterial activity.In general the results reveled that majority of the tested compounds exhibited moderate to good antibacterial activity against bacteria and encouraging antifungal activity againt fungal strain tested. This study reveal that the 2-aminopyridine based compounds have a broad range of biological properties, even if it is a simpler structure without any other heteroring in molecule, or is a more complex molecule with more hetero-rings. 2-Aminopyridines can be very good drugs for treating several diseases.

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