Supplementary informations B

Synthesis of 2-aminopyridine lactones and studies of their antioxidant, antibacterial and antifungal properties.

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Antibacterial-antifungal

The 2-aminopyridines and bis-2-aminopyridines are one of nitrogen containing heterocyclics that known for their very important therapeutic and biological proprietés 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.*

Material and methods

Microorganisms, inoculums and antifungal assay:

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.

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 minutes, the upper homogeneous suspension is transferred to a sterile tube and mixed with a vortex mixer for 15 seconds. These suspensions were diluted 1:50 in the RPMI medium. The suspensions were mixed for 15 second 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.

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 x (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.

Microorganisms, inoculums and antibacterial assay:

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 hours 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.

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 hours 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 hours, 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).

Results and Discussion

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, figure1). 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 (figure1) has a variable activities between bacterial strains.

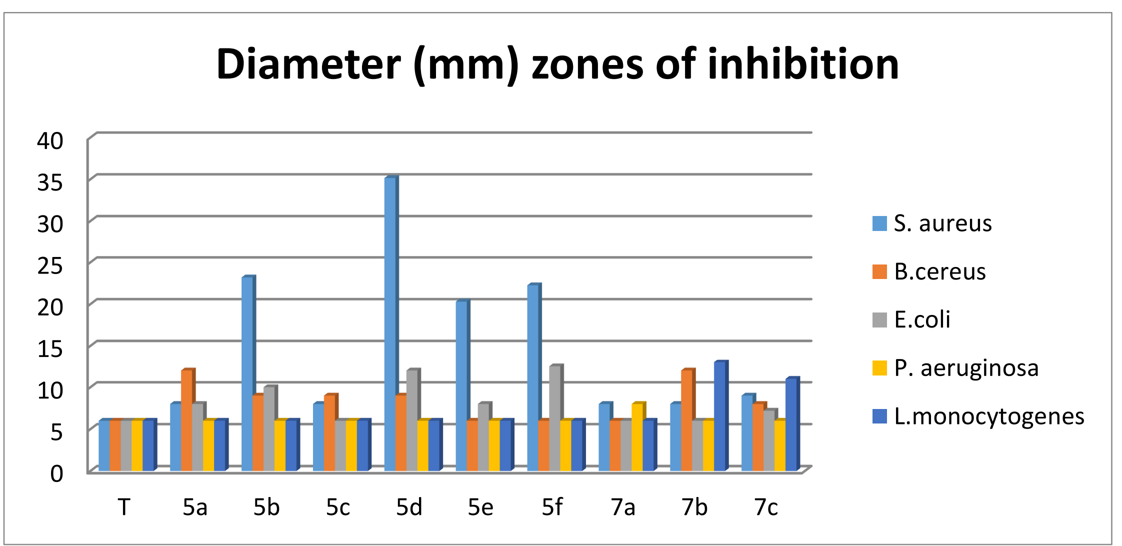
The morphology of the cell membrane may be a main issue that affects the activity of antimicrobial agents. The cell membrane of the bacteria consists of peptidogly can which is thicker in the gram positive bacteria and is usually posses a barrier to the degree of diffusion of antimicrobial agents into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

Table1: 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

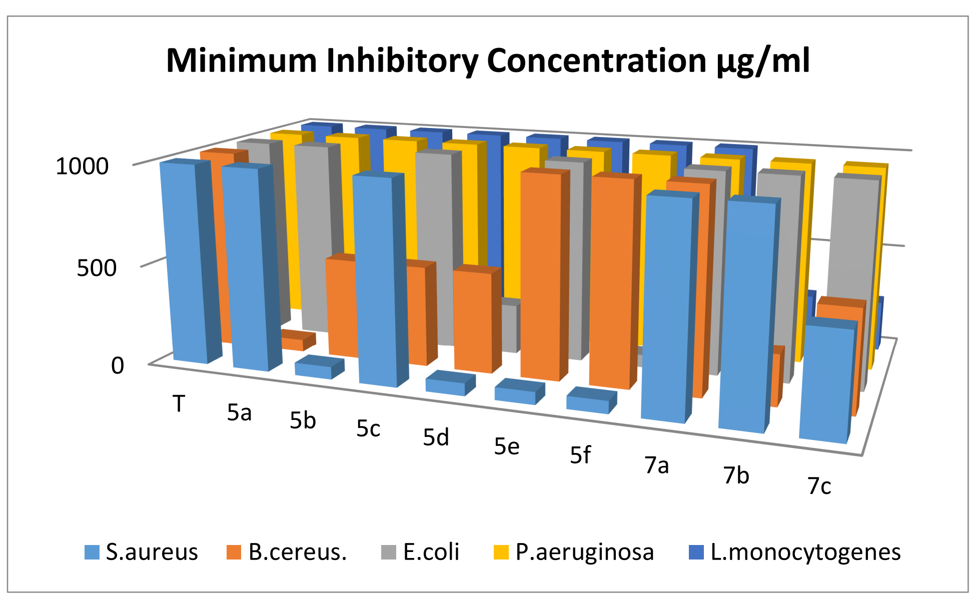
5c	8	9	6	6	6
5d	35.15	9	12	6	6
5e	20.3	6	8	6	6
5f	22.2	6	12.5	6	6
7a	8	6	6	8	6
7b	8	12	6	6	13
7c	9	8	7.2	6	11

Figure1 : 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 antibacterial activity against *S. aureus* with a diameters of inhibition zones between 8 and 9mm. 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 13mm ,11 mm for *L.monocytogenes* respectively. Also, *B.cereus* revealed a moderate sensivity 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 (figure2), 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*.

Antifungal activity

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

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.

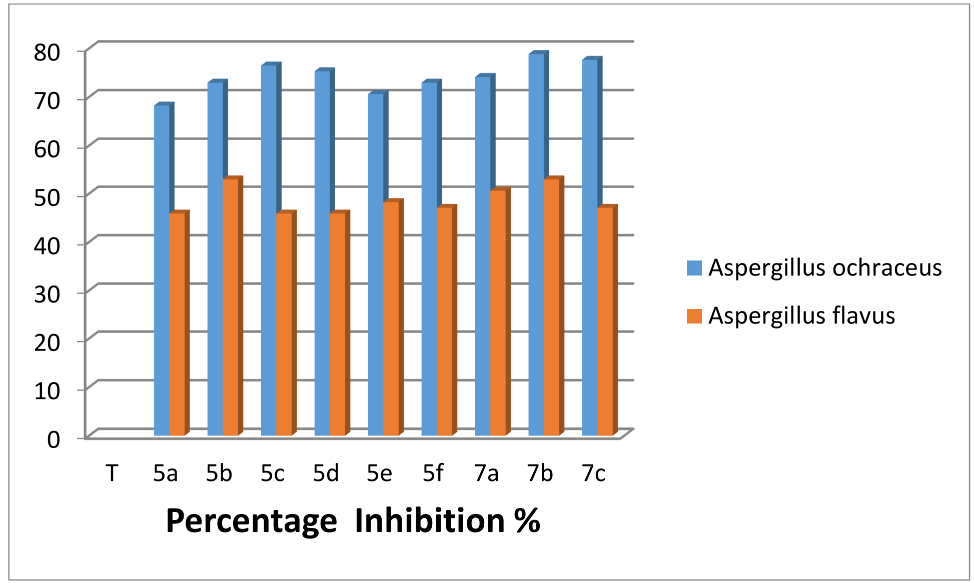
Evaluation of the antifungal activity of the synthesized compounds showed that the strain *Aspergillus ochraceus* was highly sensitive compared to *Aspergillus flavus* to all compounds. The compounds 7c and 7b showed strong inhibition of 78%, (the diameter of the inhibition zone was in 18-19 mm) and 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 46mm. 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*.



Conclusion

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

References

1. Kibou, K., Cheikh, N., Choukchou-Braham, N., Mostefa-kara, B., Benabdellah , M., Villemin, D. *J. Mater. Environ. Sci*, **2011**, 2 (3) 293-298.

2. Villemin, D.;Belhadj, Z.; Cheikh, N.; Choukchou-Braham.; Bar, N.; Lohier, J.*Tetrahedron letters.* **2013**, *54*, 1664–1668.
3. Cheikh, N.; Villemin, D.; Bar, N.; Lohier, J. F.; Choukchou-Braham, N.; Mostefa-Kara, B.;Sopkova J. *Tetrahedron*. **2013**, *69*, 1234-1247.
4. (a) Gholap, A. R.; Toti, K. S.; Shirazi, F.; Kumari, R.; Bhat, M. K.; Deshpande, M. V.; Srinivasan, K. V. *Bioorg. Med. Chem*. **2007**, 15, 6705–6715; (b) Manna, F.; Chimenti, F.; Bolasco, A.; Bizzarri, B.; Filippelli, W.; Filippelli, A.; Gagliardi, L. G. *Eur. J. Med. Chem*. **1999**, 34, 245–254; (c) Altundas, A.; Ayvaz, S.; Logoglu, E. *Med. Chem. Res*. **2011**, *20*, 1–8.
5. Ann J.; Ki Y.; Yoon S.; Kim MS.; Lee JU.; Kim C.; et al. *Bioorg Med Chem.* **2016**, *24*,1231-40.
6. Alaa, A.-M. ABdel-Aziz; Hussein, I. El-Subbagh; Takehisa, K. *Bioorg*. &*Med. Chem*. **2005**, *13*, 4929-4935.
7. Valenciano AL.; Ramsey AC.; Santos WL.; Mackey ZB. *Bioorg Med Chem*. **2016**, *24*, 4647-51.
8. Kolodziej K.; Romanowska J.; Stawinski J.; Boryski J.; Dabrowska A.; Lipniacki A.; et al. *Eur J Med Chem*. **2015**, *100*, 77- 88.
9. Z, Qing-Wei; L, Jian-Qi. Bull. *Korean Chem. Soc*. **2012**, 33, *2*. 535
10. Maria-Joao, Q. R. P.; Isabel, F. C. F. R.; Ricardo, C. C.; Letıcia, E. M. *Bioorg. Med. Chem.* **2007**,*15*, 1788-1794
11. Sharma, S.; Sharma, A, K.; Singh, O.; Singh, U. K. *International Journal Of Pharma Professional's Research*. **2011**, *2*(3), 351-354
12. Crémieux, A. *Res. Microbiol.***1995**, *146* , 73-83
13. Sener, E, A.; Arpacı, O, T.; Yalcın, I.; Altanlar, N. *Il Farmaco*, **2000**, *55*, 397-405
14. Gupta ,V., Singh, S ., Gupta, Y.K. *Res. J. Chem. Sci.***2013**, *3*(9) 26-29
15. Marinescu, M. *Int J Pharm Bio Sci.* **2017**, 8(2), 338-355
16. Nagashree, S., Mallu, P., Mallesha, L., Bindya.,S. *J.Chemistry*. **2013**,pp 5.
17. Shrikant, V. H., Rahul, D. K., Pratima, P. M., Shital, S. K., Madhav, J. H., Ajay, N. A., Bhaskar, S. D. *J .Sch. Res.Lib*. **2015**, *7* (4), 249-256.
18. Bijo, M., Githa, E. M., Nirmal, M., Vijayabaskaran, M. *J .Sch. Res.Lib.***2010**, *2*(6), 238-242
19. Shweta Singh1, Pooja Chawla2, Viney Chawla1 and Shailendra K. Saraf. *RASAYAN J .Chem* .**2013**, *6* (3), 196-200.
20. Parmar. K. C., Vora. J. J.., Vasava. S. B. *J. Chem. Phar. Res*, **2014**, *6(*4), 1259-1263

21. Kumar, S., Sharma, N., Maurya, I. K., Verma, A., Kumar, S Bhasin., K.k ., Sharma, R. K. *New J. Chem.* **2017**, *41*(8), 2919-2926
22. Yuoh, A. C.B., Agwara, M. O., Yufanyi,D.M ., Conde, M. A.,Jagan, R., Eyong. K.O. *Inter. J. Inorg. Chem*. **2015**, 1-9.
23. Alaghaz, Abdel-Nasser M. A. *J. Mol. Struct*. **2014**, *1068*, 27-42
24. Gupta, Anand Kumar S.; Barhate, V. D. *Res. J. Pharm., Biol. Chem. Sci*. **2012**, *3*(3), 1013-1026.
25. Peng, Z; Liu, T; Shen, X; Liu, Z; Peng, X; Xiao, Z. *Huaxue Shiji*. **2011**, *33*(8), 689-692.
26. Patel, N. B.; Patel, A. L. *Indian J. Chem.* B. **2009**, 48B(5), 705-711
27. Kaya, I.; Cihangiroglu, N. *J. Heterocycl. Chem*. **2004**, 11(1), 37-42.
28. Fioravanti, R.; Biava, M.; Porretta, G. C.; Landolfi, C.; Simonetti, N.; Villa, A.; Conte, E.; Porta-Puglia, A. *Eur. J. Med. Chem. **1995***, 30(2), 123-32.
29. Clinical Laboratory Standards Institute (formerly NCCLS). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi-approved standard,. 2nded. CLSI document M38-A2, USA: Wayne PA; 2008.
30. Remmal A. et al., 1993. Improved method for determination of antimicrobial activity of essential oils in agar medium. *J. Essent. Oils Res*., **5**(2), 179-184.
31. Satrani B. et al. *Ann. Falsif. Expert. Chim.*, **2001, 94**(956), 241-250.
32. Treki, A.S., Merghem, R. et Dehimat, L. *Sciences & Technologie*, **2009**, 29,25-29.
33. Mims, C., Dockrell, H.M., Goering, R.V., Roitt, I., Wakelin, D., Zuckerman M, *Medical Microbiology, Elsevier Mosby, updated 3rd Edition*, **2004**, 11-12