In vitro antimicrobial activity evaluation of new nitrogen heterocycles derivates from acridine

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Abstract

The chemical synthesis of new molecules with biological activity is very interesting and opens new fields of research, important in molecular biology and pharmacology. The biological activity of some substituted dipyrido-quinolino-phenazine heptacycles derived from 2-ethoxy-6,9-diaminoacridine was investigated. The antifungal and antibacterial effects of the tested compounds depend on their molecular structure and doses.

Keywords: acridine derivatives, dipyrido-quinolino-phenazine heptacycles, biological activity

Introduction

The interest for the acridine family is the result of their various biological properties. Some of these compounds have been found to be natural compounds of marine origin, being extracted from marine mollusca, sponges and ascidians [1,7]. In fact, these types of compounds present important cytotoxic properties but specifically biological properties (inhibition of enzymatic systems, antitumoral, antiviral and anti-inflammatory activity), thus possessing antimicrobial properties against *Escherichia coli, Bacillus subtilis, Candida, Staphylococcus aureus* and *Cladosporium resinae* [8, 9]. Elaborate studies emphasized that in small concentrations some of them show interesting antimicrobial properties against *Bacillus subtilis* (6,2 μg/mL), *Trichophyton mentagrophytes (*0,8 μg/mL) and *Cryptococcus neoformans*(3,1 μg/mL) [10].

Some references concerning the antiviral activity of acridinic compounds against HSV-1 (*Herpes Simplex Virus*) and *Poliovirus* were reported [11,12].

Due to their broad spectrum of antimicrobial activity, the heterocyclic compounds were evaluated against the growth and metabolic activities of the spoilage microorganisms which are known to be responsible for the off-flavor formation and production of allergenic compounds, which often take place before the growth of fungi is notable.

The aim of this study is to evaluate the antimicrobial activity of the heterocyclic compounds synthesized from 2-ethoxy-6,9-diaminoacridine against the growth of spoilage microorganisms, such as moulds: *Aspergillus niger, Aspergillus glaucus, Aspergillus oryzae, Penicillium glaucum, Fusarium graminearum, Geotrichum candidum;* yeasts: *Candida mycoderma, Rhodotorula glutinis, Pichia membranefaciens, Saccharomyces cerevisiae;* and bacteria: *Bacillus subtilis, Bacillus cereus, Sarcina flava.*

Materials and Methods

Synthesis of heptacyclic compounds. A first step in the obtaining of the heptacycles was the synthesis of 5,6-diaminoacridine derivatives, ortho-diaminoacridine synthons. These derivatives differ by the substituents in 9th (-NH_2,-OH,-Cl, -NH-R) and 2nd (-OEt, -OH) positions [13, 14].

The next step is the obtaining of phen-5,6-dione from phenantroline, and the final condensation with the ortho-diamine by refluxing stoichiometric amounts of the two compounds in ethanol. The dipyrido[3,2-a:2_,3_-c]quinolino[2,3-h]phenazine heptacycles(eight compounds) bearing various substituents were thus obtained in good yields (64–89%). The compounds bearing amino/hydroxyl groups are more soluble in water and they are more interesting in interaction with biomolecules. So we started our study we compound which bears these groups. The purity of the obtained compounds was verified through TLC, HPLC, NMR and mass spectrometry tests.

Antimicrobial effects evaluation. The test microorganisms used were pure cultures of moulds, yeasts and bacteria isolated from the food microbiota.

The tests concerning the antimicrobial activity have been made *in vitro,* in nutritive agar growth media as: MEA (malt extract agar) for moulds and yeasts and PCA (plate count agar) for bacteria, by cultivation in Petri dishes, by using the radial diffusion method. A volume of 2 mL of cell suspension was first inoculated into fluidified and tempered agar medium and then dispersed in Petri dishes. For each tested compound 5mM solutions were prepared in different solvents, and the interactions with the microorganisms were observed using the discs' method – filter paper discs were first immersed in the solution for 5 minutes, and then placed on the surface of the solid media inoculated with test microorganisms. For each solution prepared, the solvent was used as control sample. After optimally incubating the bacteria for a period of 48 hours at 37°C, and the moulds and yeasts for 72 hours at 25°C, the evaluation of the antimicrobial effects was realized based on the diameter of the inhibition zone and the morphological characteristics of the colonies, i.e. pigmentation, sporulation intensity etc.

An inhibitory potential was calculated by reporting the diameter of the solution's inhibition area to that of the control sample (the solvent).

Results and discussions

The evolution of microorganisms was pursued from different points of view. For all of the microorganisms it was observed whether there was an area of inhibition or not. If the area of inhibition was present, it was studied whether cells grew or not in the precise area.

If the growth of microbial biomass were slowed down around the paper discs, the compound would have microbistatic properties. If there were no growth at all, the tested compound would show microbicidal properties.

In the case of moulds, it was also observed whether the tested compound would inhibit the sporulation.

The results are presented in **Table 1** and **Figures 2, 3, 4** and **5**.

Table 1 shows the inhibitory potential of the tested compounds by evaluating the diameters (in mm) of the inhibition growth zones around the paper discs.

It is observed that the antimicrobial effect is very low against bacteria, has a slow increase for the yeasts, and has high values in the case of moulds.

Table 1. The qualitative antibacterial and antifungal effects of the tested compound

Test microorganisms	Diameters of the inhibition growth zones against test microorganisms, mm		Remarks
	Solvent N-methylpyrroli-done	5mM Solution 14-ethoxy-12-hydroxy-dipyrido-quinolino-phenazine	
Aspergillus niger	22	25	
Aspergillus glaucus	15	16	
Aspergillus oryzae	0	10	The sporulation is inhibited on the surface of the solvent paper disc.
Penicillium glaucum	18	27	
Fusarium graminearum	1	6	
Geotrichum candidum	16	18	
Candida mycoderma	16	12	*
Rhodotorula glutinis	21	22	
Pichia membranefaciens	3	2	*
Saccharomyces cerevisiae	8	8	In the inhibition zone of the solvent the cellular mass is higher than the one in the inhibition zone of the solution.
Bacillus subtilis	9	0	*
Bacillus cereus	11	8	*
Sarcina flava	0	0	

*In the cases in which the solvent's inhibition zone is wider than the one of the solution, the most plausible explanation is that the tested compound has no antimicrobial properties.

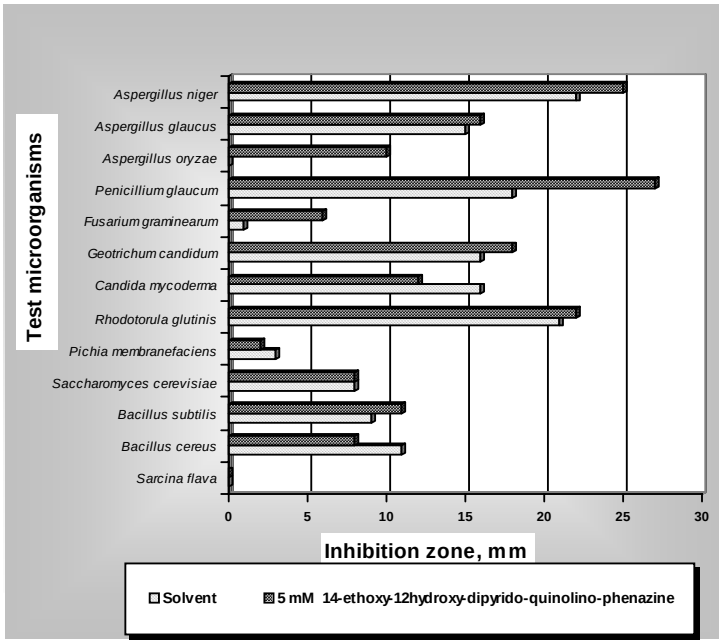


Figure 1. Comparative inhibitory potential of tested compound against spoilage microorganisms

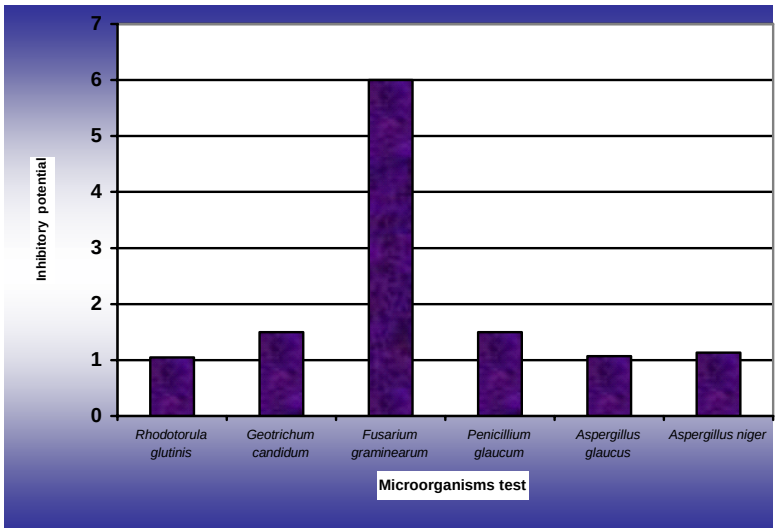
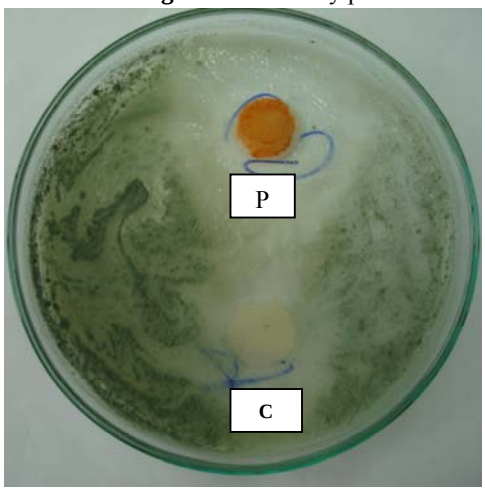
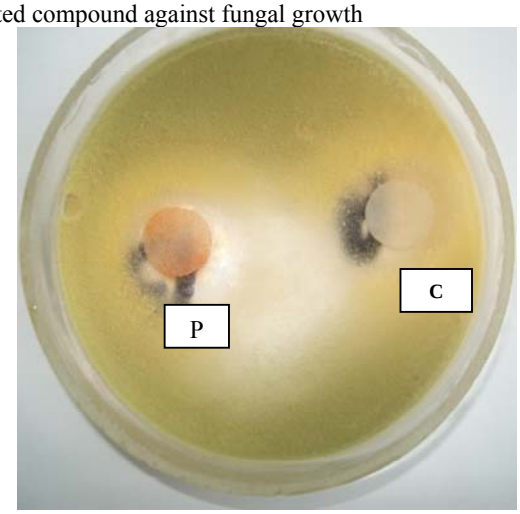


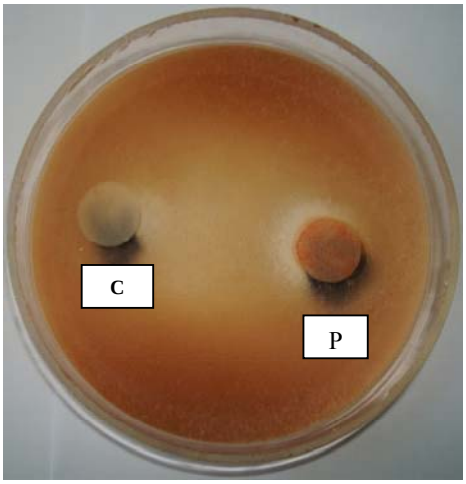
Figure 2. Inhibitory potential of tested compound against fungal growth



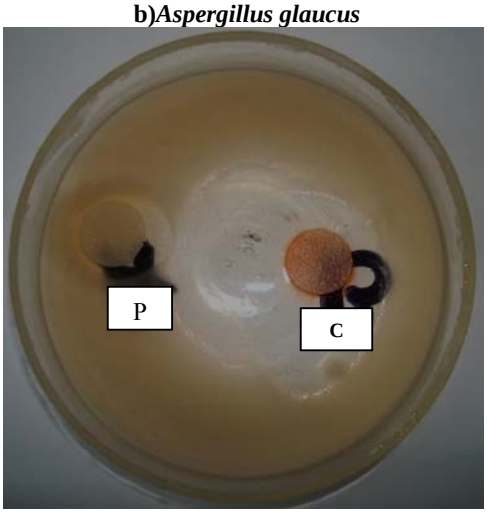
a) *Penicillium glaucum*



b)*Aspergillus glaucus*



c)*Fusarium graminearum*



d)*Geotrichum candidum*

Figure 3. Antifungal effect of 14-ethoxy-12-hydroxy-dipyrido-quinolino-phenazine, after 72 h of cultivation on solid agar media
C-control, solvent effect; P-probe, solution effect

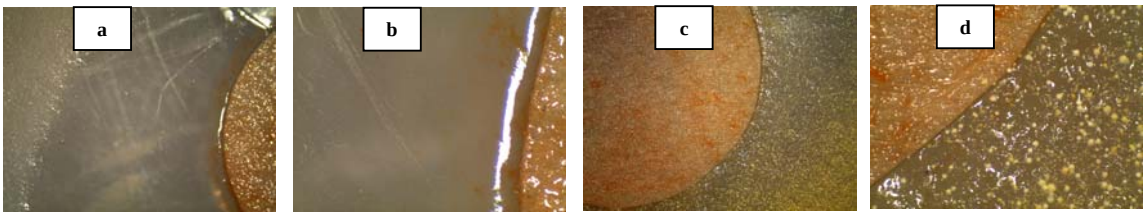


Figure 6. Stereomicroscope images of fungistatic(c,d) and fungicidal (a,b) effects of 5mM 14-ethoxy-12-hydroxy-dipyrido-quinolino-phenazine

a)*Geotrichim candidum* 8x b)*Geotrichim candidum* 35x c) *Aspergillus glaucus* 8x d)*Aspergillus glaucus* 35x

As shown the obtained data, the antimicrobial spectra of the compound with hydroxy substituent is variable against bacteria and yeasts, the inhibitory effect being the highest in the case of moulds. The antimicrobial effect of the compounds is enhanced by the presence of a hydroxy radical in the 12th position.

The compound shows microbicide properties in the case of *Geotrichum* species, and microbistatic properties for *Aspergillus niger*, *Aspergillus glaucus*, *Penicillium glaucum*, *Fusarium graminearum* and *Rhodotorula glutinis*. The highest inhibitory effect reported to the effect of the solvent was observed in the case of *Fusarium graminearum.*

The mechanism of antimicrobial action of acridinic compounds is point mutations. This type of mutations causes alterations of the DNA due to the transformation of the bases in a pair of nucleotides. There are different types of point mutation: substitution, inversion, deletion, insertion. Due to their plain aromatic structure these heterocycles can interpose between two adjoining nucleobases. These mutations take place in the course of DNA replication and can cause mutation by deletion or by insertion. In addition, the insertion of heptacycles can be on the origin of oxygen reactive species (ROS) generation which induces oxidative damages in DNA.

Conclusions

The synthesis of pentaaza heptacycles, using an efficient route, allowed the formation of a various substituted angular heterocyclic compounds derived from the corresponding phenanthroline and acridine. In the synthesis strategy, the presence of amino and/or hydroxyl groups allow the introduction of various side-chains on the intercalating heterocycles in order to improve their solubility in water and modulate their interaction with biomolecules.

The synthesis of dipyrido-quinolino-phenazine heptacycles presents practical importance in using these as disinfecting agents and as preventing agents against the contamination of the plants with phytopathogenic microorganisms (*Fusarium species*), without affecting soils microbiota with bioremediation implications or nitrogen fixation.

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References

1. YAMADA M., TANAKA Y., YOSHIMOTO Y., KIRODA S., SHIMAO I. *Synthesis of 2,9-Dichloro-1,10-phenanthroline from N,N'-Annelated Phenanthrolinedianes*, Bull. Chem. Soc. Jpn., **65,** 1006-1011, 1992.
2. MUNOZ-RUIZ P., RUBIO L., GARCIA-PALOMERO E., DORRONSORO I., MONTE-MILLAN M., VALENZUELA M.R., USAN P., BARTOLINI M., ANDRISANO V., BIDON-CHANAL A., OROZCO, LUQUE F. J., MEDINA M., MARTINEZ A., *Identification from a positional scanning peptoid library of in vivo active componds that neutralize bacterial endotoxins*, J. Med. Chem.**, 48**, 7223-7233, 2005.
3. CAVALLI, M.L. BOLOGNESI, A. MINARINI, M. ROSINI, V. TUMIATTI, M.RACANATINI, C. MELCHIORRE, *Target-directed-ligands to combat neurodegenerative diseases*, J. Med. Chem. **51**, 347-372, 2008.
4. ZEGHIDA W., DEBRAY J., CHIERICI S., DUMY P., DEMEUNYNCK M., *Concise Synthesis of 2-Amino-4(3H)-quinazolinones from Simple (Hetero)aromatic Amines*. J. Org. Chem., **6**, 2473-2475, 2008.
5. DOLPHIN G.T, CHIERICI S., OUBERAI M., DUMY P., GARCIA J., *A Multimeric Quinacrine Conjugate as a Potential Inhibitor of Alzheimer's β-Amyloid Fibril Formation,* ChemBiochem, **9**, 952-963, 2008.
6. SCHMITZ F. J., AGARWAL S. K., GUNASEKERA S. P., SCHMIDT P. G., SHOOLERY J. N., *Amphimedine, new aromatic alkaloid from a pacific sponge, Amphimedon sp. Carbon connectivity determination from natural abundance carbon-13-carbon-13 coupling constants*, J. Am. Chem. Soc., **105**, 4835-4836, 1983.
7. SKYLER D., HEATHCOCK C. H., *The Pyridoacridine Family Tree: A Useful Scheme for Designing Synthesis and Predicting Undiscovered Natural Products*, J. Nat. Prod., **65(11)**, 1573-1581, 2002.
8. CHARYULU G. A., MCKEE T. C., IRELAND C. M., *Diplamine, a cytotxic polyaromatic alkaloid from the Tunicate, Diplosoma sp*. Tetrahedron Lett., **30 (32)**, 4201-4202, 1989.
9. LINDSAY B. S., BARROWS L. R., COPP B. R., *Structural requirements for biological activity of the marine alkaloid ascididemin*, Bioorg. Med. Chem., **5 (7)**, 739-742, 1995.
10. McCarthy P. J., Pitts T. P., Gunawardana G. P., Kelly-Borges M., Pomponi S. A., *Antifungal Activity of Meridine, a Natural Product from the Marine Sponge Corticium sp.*, J. Nat. Prod, **55 (11)**, 1664-1668, 1992.
11. BISHOP M. J., CIUFOLINI M. A., *Total synthesis of kuanoniamines and dercitins*, J. Am. Chem. Soc., **114**, 10081-10082, 1992.
12. LINDSAY B. S., PEARCE A. N., COPP B. R., *Efficient and Convenient Pyridine Ring-E Formation of the Cytotoxic Marine Alkaloid Ascididemin and Related Analogues***,** Synthetic Commun., **27 (15)**, 2587-2592, 1997.
13. CHARMANTRAY F., DEMEUNYNCK M., LHOMME J., DUFLOS A., *New Route to Extended Angular Polyaza-heterocycles*, J. Org. Chem., **66**, 8222-8226, 2001.
14. DINICA R., CHARMANTRAY F., DEMEUNYNCK M., DUMY P., *Regioselective synthesis of angular nitrogen polyheterocycles:dipyrido[3,2-a:2_,3_-c]quinolino[2,3-h]phenazines* Tetrahedron Lett., **43**, 7883-7885, 2002.