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Research Article

Evaluation of antibacterial and antifungal activities of N-benzylthienopyrimidinone derivatives

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Abstract

This study is part of the biological investigation of the chemical library of molecules already described by the Laboratory of Organic Chemistry and Therapeutic Chemistry of the University of Bordeaux. The main objective was to explore the contribution of a thienyl moiety attached to the pyrimidinone nucleus, in the expression of an antimicrobial activity.

The structural modifications mainly concerned the conservation or not of the benzo fragment attached to the thienyl, the saturation or not in position-1,2 of the pyrimidinone ring, the substitution on N-benzyl with more or less lipophilic units, the modification of the orientation of the thienyl fragment with, on the one hand, the compounds in which the sulfur is located near the N1 nitrogen (series of thieno[2,3-d]pyrimidin-4-ones) and on the other hand, compounds in which the sulfur is located near the ketone group (series of thieno[3,2-d]pyrimidin-4-ones).

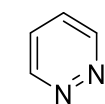
In general, thienyl fragment with sulfur located near the ketone group and the unsaturated pyrimidinone nucleus in the 1,2-position, seem to promote a broad spectrum of antibacterial activity, with compound **9c** which is active on both Gram + bacteria and Gram - bacteria studied. The same pattern was observed for antifungal activity, which is maximum with the compounds of the thieno[3,2-d]pyrimidin-4-ones series for an MIC of 31.25 µg/ml on the strains of *Candida albicans* and *Candida kruzei* studied.

Keywords: Thienopyrimidinones, antibacterial activity, antifungal activity.

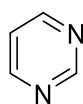
INTRODUCTION

Heterocyclic compounds are very abundant in nature and are of great importance for life because their structural subunits exist in many natural products such as nucleic acids, vitamins, hormones and antibiotics^{1,2,3}.

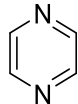
We were particularly interested in nitrogen heterocycles including diazines among which we can mention the pyrimidine, which is an aromatic heterocyclic nitrogen molecule, close to pyridine and containing two nitrogen atoms. It is also a position isomer of the other diazines, pyridazine and pyrazine (Figure 1).



Pyridazine
(1,2-diazine)



Pyrimidine
(1,3-diazine)



Pyrazine
(1,4-diazine)

Figure 1: Molecular structure of diazines

It is the most present in nature because of its participation in the basic structure of nucleic acids including DNA and RNA but also purines and uric acid. Indeed, pyrimidines have a long history that extends from the days of their discovery as important constituents of these nucleic acids to their current use in AIDS chemotherapy⁴. Derivatives possessing this synthon, particularly polycondensed derivatives, have attracted much interest in the design and discovery of new physiologically and pharmacologically active compounds⁵. The biologic activities recognized for them are diverse: anticancer^{6,7,8,9,10,11,12}, antivirals^{4,13,14}, antibiotics¹⁵, antifungals^{16,17}, antiparasitics^{18,19,20,21,22,23,24,25,26,27}...

In our recent work, we have been particularly interested in benzopyrimidine or quinazoline (Figure 2) compounds, including benzylquinazolinones and their reduced derivatives which have shown significant antiplatelet, antiplasmodic and trypanocidal profiles with a favorable index of selectivity²⁸. The results of this previous work, had suggested that the tetrahydroquinazoline nucleus N3 substituted by a benzyl moiety, would constitute a potentially useful basis for

developing new pharmacologically active molecules. To complete the pharmacomodulation study of pyrimidine derivatives, the study of the influence of the aromatic system fused to the pyrimidinic heterocycle was envisaged.

Futhermore, antibiotic resistance in pathogenic bacteria is a global public health problem. Despite the development of new effective drugs, the speed of emergence of this drug resistance is more important than the frequency of discovery of new pharmacologically active molecules²⁹. To challenge this

problem, new investigations into the antimicrobial activity of synthetic molecules could contribute to strengthening the therapeutic arsenal to be used against emerging diseases. This study, as part of biological investigation of the chemical library of molecules already described by the Laboratory of Organic and Therapeutic Chemistry of the University of Bordeaux, had as its main objective to explore the contribution of a thienyl moiety attached to the pyrimidinone nucleus (Figure 2), in the potentiation of antimicrobial activity.

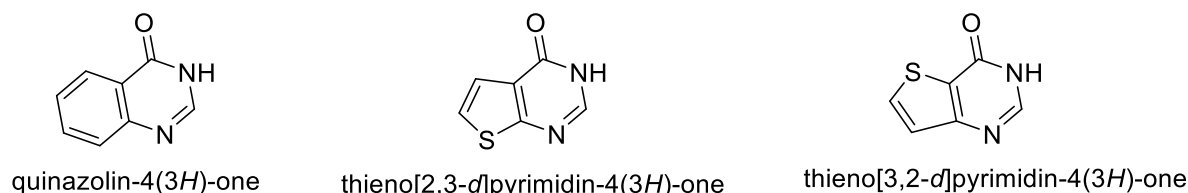


Figure 2: Structures of quinazolinones and thienopyrimidinones

MATERIAL AND METHODS

Material

The compounds tested in this study and shown in Figure 4, were synthesized by the team of the Laboratory of Organic Chemistry and Therapeutic Chemistry of the University of Bordeaux. Some of them have already been tested for

thrombolytic activity³⁰, antiproliferative³¹... These thienopyrimidine-patterned structures continue to attract a lot of interest due to their wide range of biological activities³²

As part of this study, ten N3-substituted benzylthienopyrimidinone derivatives were selected from the chemical library of the Laboratory of Organic Chemistry of the University of Bordeaux.

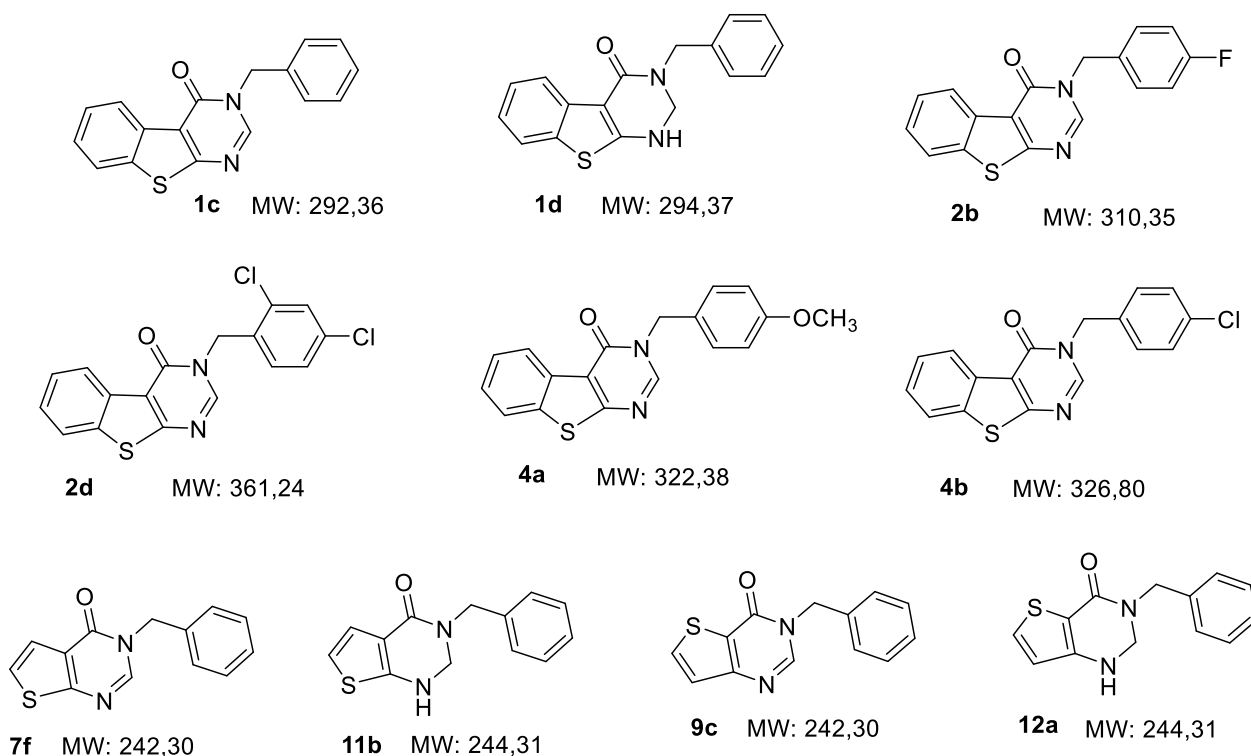


Figure 3: Structures of the tested compounds

Methods:

Antimicrobial activity tests were carried out on reference strains from the Laboratory of Bacteriology and Virology at Aristide Le Dantec Hospital. These are the strains of:

- *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) chosen for Gram + bacteria.

- *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 27853) chosen for Gram – bacteria.

- *Candida albicans* (ATCC 24433) and *Candida kruzei* (ATCC 14243) chosen for fungi.

The bacterial strains were transplanted into a nutrient medium and incubated at 37°C for 24 hours. The sensitivity tests were performed *in vitro* by the Kirby Bauer method on

Mueller Hinton medium³³. The sensitivity of the strains was determined by measuring the diameter of the inhibition zone around the discs. For each strain, a 10% aqueous solution of DMSO was used as a control and three trials were performed with a target concentration of 2 mg/ml. Ceftazidime, a third-generation cephalosporin, was used as a reference molecule for the evaluation of activity on Gram-negative bacteria and vancomycin, which is a glycopeptide, as the reference molecule for Gram-positive bacteria. Amphotericin B has been used as the antifungal reference.

The Minimum Inhibitory Concentration (MIC) was determined by the micro-broth dilution method using the Cation Adjusted Mueller Hinton Broth (CAMHB) medium with microplates containing 96 wells providing positive and negative control³⁴. A clear appearance of the well was interpreted as the absence of growth and wells showing a cloudy appearance were considered positive due to the growth of germs.

RESULTS AND DISCUSSION

The pharmacomodulation study (Figure 4) focused on:

- Conservation of the benzo moiety attached to thienyl (compounds **1c**, **1d**, **2b**, **2d**, **4a** and **4b**)
- Saturation or not, in the 1,2-position of the pyrimidinone ring (compounds **1d**, **11b** and **12a**)

- Substitution on N-benzyl with more or less lipophilic units (compounds **2b**, **2d**, **4a** and **4b**)
- Removal of the benzo moiety attached to a pyrimidinone nucleus (compounds **7f**, **9c**, **11b** and **12a**)
- The modification of the orientation of the thienyl fragment (compounds of the "inverted" series) with the sulfur located near the ketone group (compounds **9c** and **12a**)

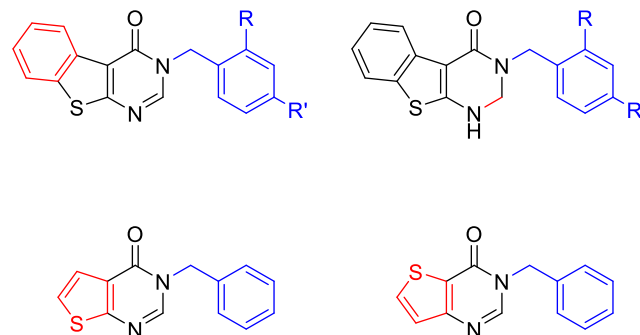


Figure 3 : Main structural modifications

Table I presents the results of the study of the sensitivity of bacteria and fungi to the thienopyrimidinones tested.

Table I: Sensitivity study of compounds on Gram-negative bacteria, Gram-positive bacteria, and fungi

Compounds tested	Measurement of the inhibition zone diameter in mm					
	Gram-positive bacteria		Gram negative bacteria		Fungi	
	<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>C. krusei</i>
	2mg/ml		2mg/ml		2mg/ml	
1c	6	6	6	6	10	7
1d	6	6	6	6	8	10
2b	6	6	8	6	10	10
2d	6	6	6	6	7	7
4a	6	6	6	7	12	-
4b	6	6	6	6	-	10
7f	6	6	7	6	10	11
9c	7	8	8	6	8	10
11b	6	6	7	11	-	12
12a	6	6	8	6	11	8
Ceftazidime	-	-	25	23	-	-
Vancomycine	16	18	-	-	-	-
Amphotericin B	-	-	-	-	20	21

The results from the determination of minimum inhibitory concentrations (MICs) of compounds tested on strains of bacteria and fungi are summarized in Table II.

Table II: Minimum inhibitory concentrations of the compounds tested

Compounds tested	CMI en µg/ml					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>C. krusei</i>
1c	-	-	-	-	125	2000
1d	-	-	-	-	125	250
2b	-	-	500	-	250	
2d	-	-	2000	-	2000	2000
4a	-	-	-	-	125	-
4b	-	-	-	2000	-	2000
7f	-	-	-	-	125	125
9c	250	500	250	-	31,25	31,25
11b	-	-	-	-	31,25	62,5
12a			2000	500	250	125

For the sensitivity, the presence of a benzo moiety attached to the thienopyrimidinone nucleus, does not seem to improve antimicrobial activity on the strains studied. Indeed, the elimination of this moiety seems to potentiate the antibacterial activity observed both with compounds in which sulfur is located near the N1 nitrogen (series of thieno[2,3-d]pyrimidin-4-ones) or "normal" series compounds (**7f**, **11b**) as well as with compounds in which the sulfur is located near the ketone group (thieno[3,2-d]pyrimidin-4-ones series) named "inverted" series compounds (**9c**, **12a**). Kanawade et al. obtained with thienopyrimidinones without benzo moiety attached to thienyl comparable MICs (from 200 to 250 µg/ml) on strains of Gram-negative bacteria³⁵. However, the antibacterial activity profile obtained with our compounds is greater than that observed with thieno[2,3-d]pyrimidine-2,4-dithiones derivatives obtained by Hafez et al.³⁶.

It should be noted that the saturation of the double bond-1,2 combined with the absence of the benzo moiety and the position of sulfur near the ketone group, contribute to the broadening of the spectrum of antimicrobial activity. Compound **9c** has the profile of a molecule with one of the lowest molecular weights of the compounds studied and is among the least lipophilic compounds. This would explain its activity on Gram-negative germs such as *E. coli*. Indeed, in the latter the penetration of hydrophilic molecules through porins is favored by the low molecular weight while for hydrophobic molecules, the lipophilic character is the factor favoring their passage by passive diffusion³⁷ through the membrane. This also seems to be the case for the sensitivity of *Pseudomonas aeruginosa* towards compound **11b**, presenting a saturation of the 1,2-double bond of the pyrimidinone nucleus.

For fungi, the strains studied (*C. albicans* and *C. krusei*) are quite sensitive to compounds tested with MICs varying for the most part from 31.25 µg/ml to 125 µg/ml (observed with 60% of the compounds tested). The most active compounds on the fungi used are compound **9c** (thienyl with the position of sulfur near the ketone group and suppression of the benzo moiety) and compound **11b** (thienyl with the position of sulfur close to the N1 nitrogen, suppression of the benzo moiety, with saturation of the 1,2-double-bond of the pyrimidinone ring). This saturation would be the determining factor on the relatively higher antifungal activity of compound

11b (with an MIC of 32.25 µg/ml) compared to that observed with compound **7f** (with an MIC of 125 µg/ml).

The presence of a methoxy (electron donating group), in the para position of the N-benzyl substituent, probably contribute to obtain a greater sensitivity of fungi, particularly observed with the strain of *C. albicans* studied.

In general, the thienyl moiety with the position of sulfur near the ketone group, attached to the pyrimidinone nucleus (unsaturation in 1,2-position) seems to promote a broad spectrum of activity, with the compound **9c** active on both Gram + bacteria and Gram - bacteria studied. The same pattern was observed with the antifungal activity, which is maximum with the compounds of the inverted series for an CMI of 31.25 µg/ml on the two strains of *Candida* studied.

CONCLUSION

The antimicrobial activity of the thienopyrimidinones studied, seems to be linked to electronic factors (presence of benzene moiety attached to the thienyl ring) as well as to configurational factors (orientation of the thienyl ring fused to the nitrogen heterocycle). In addition to the prospect of studying the selectivity of these compounds, the study of the influence of the thiophene ring and the saturation of the pyrimidine nucleus will be continued on strains of *Plasmodium falciparum* and *Trypanosoma brucei brucei* in order to carry out a comparative study with the activity of quinazolinones and their reduced analogues²⁸.

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Conflicts of interest:

The authors declare that they have no conflict of interest.

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