ANTIBACTERIAL ACTIVITY OF NEW PYRIDO[2,3-b]PYRAZINE DERIVATIVES

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Abstract: 
New series of pyrido[2,3-b]pyrazine derivatives with various side-chains were investigated for their antibacterial activity. The synthesis were achieved from 5-bromo-2,3-diaminopyridine and oxalic acid according to a three-step procedure. All the products were tested \textit{in vitro} on different bacteria strains and showed an average activity. The derivative bearing two thiocarbonyl groups exhibited good antibacterial activities against different strains \textit{Staphylococcus aureus} (0.078mg/ml), \textit{Bacillus cereus} (0.078mg/ml), and \textit{Escherichia coli} (0.625mg/ml), \textit{S.typhi} (1.25 mg/ml). The presence of side-chains on heterocyclic moiety decreased biological acivity.

Keywords: pyrido[2,3-b]pyrazine ; PTC ; antibacterial activity, minimum inhibitory concentration (MIC).

Résumé: 
L’activité antibactérienne d’une nouvelle série de dérivés de la pyrido[2,3-b]pyrazine a été étudiée. Les produits ont été préparés à partir de la 5-bromo-2,3-diaminopyridine et de l’acide oxalique. Tous les composés testés présentent une activité moyenne sur les différentes souches bactériennes. Le dérivé présentant deux groupes thiocarbonyles sur le motif hétérocyclique a montré le meilleur pouvoir inhibiteur de la croissance des souches étudiées \textit{S. aureus} (0.078mg/ml), \textit{B. cereus} (0.078mg/ml), \textit{E. coli} (0.625mg/ml), \textit{S.typhi} (1.25 mg/ml). La présence des chaines latérales sur le motif hétérocyclique s’est avérée défavorable à l’activité biologique.

Mots clés : Pyrido[2,3-b]pyrazine; CTP; Activité antibactérienne, concentration minimale inhibitrice (CMI).

INTRODUCTION: 
Resistance to antibiotherapy is a great burden from medicinal and economic point of view. It is due to the over prescription and misuse of anti-infective drugs. \textsuperscript{[1]} The increasing rate of antibiotic resistance of bacteria urges new attempts to overcome the problem. In regards of this issue, researchers focused on reinforcing the arsenal in antibiotics through the development of brand new molecules not yet affected by the resistance to antibiotherapy. Nitrogen heterocyclic derivatives appeared as a promising path to take \textsuperscript{[2]}. 

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119
The pyrido[2,3-b]pyrazine derivatives have several biological activities. Indeed, they showed pharmacological applications including antitumor [3], anti-cancer [4], anti-inflammatory [5], anti-malarial [6], anti-bacterial effects [7] and exhibit a good inhibitory action in metals corrosion [8].

In this work, a series of new pyrido[2,3-b]pyrazine derivatives were investigated as antibacterial agents against various testing strains using broth microdilution method. Thus the effect of side chains in positions 2 and 3 on the antimicrobial activity was determined.

RESULTS AND DISCUSSION

Chemistry

Pyrido[2,3-b]pyrazine derivatives 1-5 were prepared as described previously (scheme 1), by condensing 5-bromo-2,3-diaminopyridine with oxalic acid then phosphorus pentasulfide was added to obtain the dithione derivative 1. The compound thus prepared was branched with various alkyl side-chains and one aryl chain under phase transfer catalysis conditions [9]. All derivatives 1-5 were identified by the usual spectroscopic methods NMR, $^1$H, $^{13}$C and IR.

Scheme 1: synthesis of various substituted pyrido[2,3-b]pyrazine derivatives
A crystallographic study carried out on compound 5 shows that it crystallizes in monoclinic system with the space group P 121 / c 1.  

![Figure1: Ortep of compound 5](image)

**Antibacterial assay:**

1-**Products:**
1. 7-Bromo pyrido[2,3-b]pyrazine-1,4(2H,3H)dithione
2. 7-Bromo-2,3-bis(benzylsulfanyl)pyrido[2,3-b]pyrazine
3. 7-Bromo-2,3-bis (methylsulfanyl)pyrido[2,3-b]pyrazine
4. 7-Bromo-2, 3-bis(allylsulfanyl)pyrido[2,3-b]pyrazine
5. 7-Bromo-2, 3-bis[(prop-2-yn-1-yl)sulfanyl]pyrido[2,3-b]pyrazine

2-**Bacterial Strains:**
The antibacterial activity of different synthesized products was evaluated against four strains: *Escherichia coli* (ATTC 25922), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC 29213), and *Bacillus cereus*. These bacterial strains were provided by the Microbial Biotechnology Laboratory. They are maintained by transplanting on nutrient agar favorable for their growth for 24 h, in the dark and at 37 °C.

3-**Minimum inhibitory concentration determination (MIC) against bacterial strains:**
Minimum inhibitory concentration represents the lowest concentration that completely inhibits the growth of microorganisms. The MIC values were determined in 96 well-microplate using the microdilution method according to Chraibi et al. Briefly, a stock solution of each product was prepared in 10% DMSO. Then, a serial dilutions of all tested products prepared in Mueller Hinton Broth medium (MHB) at final concentrations ranged between 5 mg/mL and 0.0025 mg/mL. The 12th well was considered as growth control (freedrug control). Afterwards, 50 µL of bacterial inoculum was added to each well at a final concentration of 10⁶ CFU/mL. After incubation at 37 °C for 24 h, 10 µL of rezasurin were added to each well as bacterial growth indicator. After further incubation at 37 °C for 2 h, the bacterial growth was revealed by the change of coloration from purple to pink. Results of antimicrobial activity of synthesized products, evaluated against *S. aureus, B. cereus, E.coli* and *S.typhi* are compiled in Table1.
Table1: Antimicrobial activity of compounds 1- 5

<table>
<thead>
<tr>
<th>Products</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains (mg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.078</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>B. cereus</td>
<td>0.078</td>
<td>1.25</td>
<td>2.5</td>
<td>0.15</td>
<td>1.25</td>
</tr>
<tr>
<td>E.coli</td>
<td>0.625</td>
<td>5</td>
<td>---</td>
<td>---</td>
<td>2.5</td>
</tr>
<tr>
<td>S.typhi</td>
<td>1.25</td>
<td>5</td>
<td>5</td>
<td>---</td>
<td>2.5</td>
</tr>
</tbody>
</table>

According to our study, all products 2-5 bearing different side–chains (alkyl or aryl) on positions 2 and 3 showed an average antibacterial effect: MIC were in the range of 1.5 to 5 mg/ml.

In addition, the most potent activity was obtained with compound 1 (2,3-dithione) which presents two thione groups on the heterocyclic moiety. It inhibited both Gram-positive bacteria B.cereus and S.aureus at the lowest concentration (0.078 mg/ml). Whereas gram-negative ones, E.coli and S.thyphi were inhibited by compound 1 at the concentrations 0.625 and 1.25 mg/ml ,respectively.

These results are in agreement with several works, which state that Gram-positive bacteria are generally more sensitive than Gram-negative ones. This observation could be due to the penetration capacity of the products synthesized in the cell, and more precisely the composition of their cell membrane[13].

CONCLUSION
Our results proved that pyrido[2,3-b]pyrazine moiety could lead to promising antibacterial agents, as well as the presence of the thione function seemed to be necessary to inhibit bacteria growth. In addition, both alkyl and aryl side-chains decreased antibacterial effect.

REFERENCES


