

Synthesis And Antimicrobial Properties Of Some Pyridazin-3-Thiones Derivatives

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Abstract: Three pyridazin-3-thiones derivatives were synthesized from the 5-arylidene pyridazin-3-ones equivalent. The compounds thus prepared were characterized by their physical (TLC, M.P) and spectral data (IR and NMR). Before studying the activity, acute toxicity has been determined. Then the three compounds were screened for anti-microbial activity against strains of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*.

Key words: Pyridazin-3-thiones, Synthesis, Anti-microbial activity.

Introduction

The pyridazin derivatives are known for their therapeutic potential antihypertensive and cardiotoxic essentially. Antifungal and other properties are also reported in the literature¹.

The pyridazines-3-ones are synthesized conventionally by a condensation reaction between levulinic acid and aromatic aldehyde appropriate in the presence of hydrazine hydrate, which plays the role of agent of nucleophilic addition^{2,3,4}. The action of phosphorus pentasulfide on the lactam function of pyridazin-3-ones, allowed us to obtain pyridazin-3-thiones, which can be the starting point for the synthesis of new heterocyclic molecules of interest, by reacting the sulfur atom with several reagents (alkyl halide, hydrazine hydrate...) ^{5,6}. Pyridazines containing the group sulfur have important pharmacological activities. Thus we synthesized three derivatives for which we

studied the acute toxicity before determining their antimicrobial properties.

Materials and methods

All reactions were followed by TLC 0.25 mm silica gel plates (Hexane / chloroform: 7/3). IR spectra of the compounds were recorded on Perkin-Elmer FT-IR Spectrophotometer by using KBr discs, ¹H NMR spectra were recorded on Bruker 300 MHz. The Melting Points of the synthesized products were taken by an ordinary banc kofler apparatus.

Synthesis (Scheme 1)

Synthesis of arylidene-levulinic (I)

The mixture of levulinic acid and aromatic aldehyde is placed in an ice bath until complete dissolution. This aldol condensation requires bubbling HCl for 30 min, after 48 hours of contact

at room temperature, the product is extracted with an organic solvent, the solution is dried over calcium chloride and the solvent was evaporated to dryness. The residue obtained was recrystallized from ethanol.

Synthesis of pyridazin-3-ones (II)

The mixture of acid and hydrazine hydrate solution in ethanol was refluxed for 2h; the precipitate formed is filtered and recrystallized from a suitable solvent.

Synthesis of pyridazin-3-thiones (III)

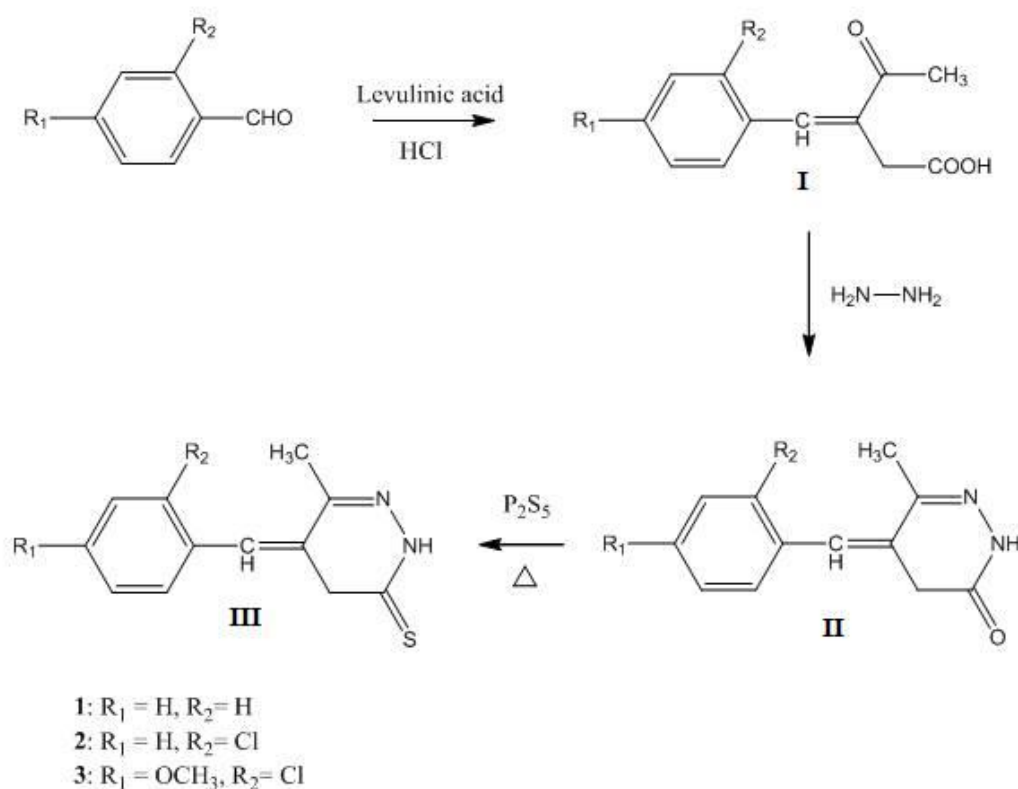
0.01 mole of pyridazin-3-one and 0.02 mole of phosphorus pentasulfide (P_2S_5) were dissolved in 40 ml of pyridine and refluxed for four hours. The solvent was evaporated under vacuum and the residue was taken up in 20 ml of boiling water. The precipitate formed after cooling was filtered, washed and recrystallized from ethanol.

Study of acute toxicity

The experiment was conducted on mice Swiss adults weighing between 20 and 30g. The administration of derivatives was performed by oral and intraperitoneal route. For the oral route, the derivatives were suspended in gum arabic (10%) and for the intraperitoneal route, the derivatives were dissolved in tween 80 (5%).

The acute toxicity was determined using the method of Lichfield and Wilcoxon, that five groups of 10 mice received orally 200, 400, 800, 1600 and 2000 $mg.kg^{-1}$ of the compounds tested. The oral administration was conducted by gavage catheter (ref. 79004 EA biomedical needles) connected to a sterile 10 ml syringe.

In parallel, a limit test at 1500 $mg.kg^{-1}$ was achieved; in fact three groups of three mice each received the synthesized derivatives by intraperitoneal route. The animals were kept under observation for 14 days.



Scheme 1 : Synthesis of pyridazine-3-thiones derivatives

Study of antimicrobial activity

The three compounds were screened for antimicrobial activity against strains of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*.

The inoculants were obtained from cultures of 18 hours at 37 ° C in Mueller-Hinton broth for bacteria, while for *Candida albicans* we used Sabouraud impregnated by chloramphenicol⁷. Then they are diluted to obtain an opacity equivalent to 0.5 on the MacFarland scale, ie approximately 10⁸ UFC.ml⁻¹.

The products synthesized were solubilized in diméthylsulfoxide (DMSO) and the resulting solutions are diluted subsequently to obtain the same concentration of 650 µg.ml⁻¹. In parallel, a control test was conducted to assess the impact of DMSO on the strains studied.

The standardized suspensions were seeded using a calibrated loop of 0.05 ml. After an incubation time of 24 h at 37 ° C, the readings are made relative to controls without test compound. The diameter of inhibition of bacterial growth is expressed in mm^{8,9}.

Results and discussion

The action of pentasulfide on ketones led to the thione function^{5,6}. Thus the reaction conducted at reflux in pyridine on pyridazin-3-ones for 4 hours lead to pyridazin-3-thiones in good yields. The structure of the derivatives **1-3** was confirmed by the spectral data of IR and ¹H NMR. The examination of infrared spectra taken in KBr, shows the disappearance of the band ν (C=O), and the appearance of the band ν (C=S) around 1080 cm⁻¹.

-5(1'-benzylidène)-6-méthyl-(2H)-pyridazin-3-thione: **1**

IR (KBr, ν max cm⁻¹) : 3150 (N-H), 1075 (C=S), 1600 (C=N), 1580-1490-1470 (C=C, Ar); ¹H NMR (300 MHz, DMSO-d₆) δ 2,30 (s, 3H, CH₃-); δ 3,90 (s, 1H, -CH₂-); δ 6,80 (s, 1H, -CH=); δ 7,20 (m, 5H, Ar); δ 14,10 (s, 1H, NH-); Melting point: 208°C. Yield 92%.

-5(2'-chloro-1'-benzylidène)-6-méthyl-(2H)-pyridazin-3-thione: **2**

IR (KBr, ν max cm⁻¹) : 3200 (N-H), 1075 (C=S), 1600 (C=N), 1590-1470-1450 (C=C, Ar). ¹H NMR

(300 MHz, DMSO-d₆) δ 2,40 (s, 3H, CH₃-); δ 3,90 (s, 1H, -CH₂-); δ 6,80 (s, 1H, -CH=); δ 7,40 (m, 4H, Ar); δ 14,30 (s, 1H, NH-); Melting point: 229°C. Yield 80%.

-5(4'-méthoxy-1'-benzylidène)-6-méthyl-(2H)-pyridazin-3-thione: **3**

IR (KBr, ν max cm⁻¹) : 3250 (N-H), 1075 (C=S), 1600 (C=N), 1530-1470-1430 (C=C, Ar); ¹H NMR (300 MHz, DMSO-d₆) δ 2,30 (s, 3H, CH₃-); δ 3,70 (s, 1H, -CH₂-); δ 6,80 (s, 1H, -CH=); δ 7,30 (m, 4H, Ar); δ 14,10 (s, 1H, NH-); Melting point: 204°C. Yield 90%.

The acute toxicity study showed that the synthesized derivatives are tolerated. Indeed, the limit dose of 1500 mg.kg⁻¹ intraperitoneally caused no lethality until 14 days. No effect was observed on groups of mice after oral administration at escalating doses. We deduce that the lethal dose 50 (LD50) is probably more than 1500 mg.kg⁻¹.

The synthesized compounds (**1-3**) were screened for antibacterial activity against certain pathogenic bacteria by disc diffusion method using both gram positive *Staphylococcus aureus*, *Bacillus subtilis*, gram negative *Escherichia coli* and antifungal activity against *Candida albicans*. The zone of inhibition was measured in mm and the results were reported on the table 1.

The test with DMSO showed a variable effect on the strains studied, this can be explained by the high concentration of DMSO in our test was 10%¹⁰.

We consider that a derivative has significant activity if the difference from the control diameter greater than 10mm¹². Thus only the derivative **2** showed significant activity against *Staphylococcus aureus* and *Escherichia coli*. The presence of an electron attractor groups on the aromatic ring as chloro group is favorable for that activity. We note that the derivative **3** containing the aromatic ring substituted by an electron donor by mesomeric effect (-OCH₃) inhibit the activity. In the case of *Candida albicans* chloro group is not necessary, since the derivatives **1** and **2** have similar activity, however the (-OCH₃) group inhibits the activity.

In conclusion, among the derivatives synthesized product **2** has a significant antimicrobial activity; in addition the acute toxicity study showed that this derivative has a low toxicity with a lethal dose 50 elevated.

Table 1: Results of antimicrobial activities of pyridazinones-thiones (in mm)

	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>C. albicans</i>
DMSO	18	14	09	16
1	23	20	13	28
2	38	28	17	30
3	18	19	10	17

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