PYRIDAZINE DERIVATIVES WITH ANTIMICROBIAL ACTIVITY

DERIVAȚI DE PIRIDAZINĂ CU ACTIVITATE ANTIMICROBIANĂ

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Abstract. *Twenty nine pyridazine derivatives (3 salts and 25 pyrrolopyridazine cycloadducts) were prepared and tested in vitro as antimicrobial compounds. Some of them have proved to have a remarkable activity against different microorganisms (germs and fungi).*
Key words: *pyrolopiridazine derivatives, 3+2 dipolar cycloadditions, biological activities.*

Rezumat. *Douăzeci și nouă de derivați de piridazină (trei săruri și douăzeci și cinci de produși de cicloadiție) au fost sintetizați și testați in vitro ca și compuși cu activitate antimicrobiană. O parte din compuși au prezentat o remarcabilă activitate asupra microorganismelor (germeni și fungi).*
Cuvinte cheie*: derivați pirolopiridazinici, cicloadiții 3+2 dipolare, activitate biologică.*

INTRODUCTION

Pyridazine derivatives have been extensively investigated because posses different biological activities: anticancer, antituberculosis, antimicrobial, antihypertensive, platelent aggregation inhibitor etc (Mangalagiu, 2011; Rodriguez-Ciria *et al.,* 2003).

In preliminary communications (Zbancioc *et al.,* 2006; Zbancioc *et al.,* 2010; Butnariu *et al.,* 2009; Tucaliuc *et al.,* 2013) is presented the synthesis and spectral analysis of pyrrolopyridazine derivatives. A simple method to obtaine fused pyridazine derivatives, involves 1,3-dipolar cycloaddition of cycloimmonium ylides to variously dipolarophiles (activated alkenes and alkynes).

MATERIAL AND METHOD

The aim of this work is to perform a thorough study concerning synthesis and activity of some azaheterocycles compounds (pyridazine derivatives).

A facile way to obtain condensed pyridazines is to use ylides as intermediates.

First, by N-alkylation of the pyridazine, we obtained the corresponding cycloimmonium salt (**5-7**) which in alkaline medium generated the ylide (***i***) *in situ*, Fig. 1:

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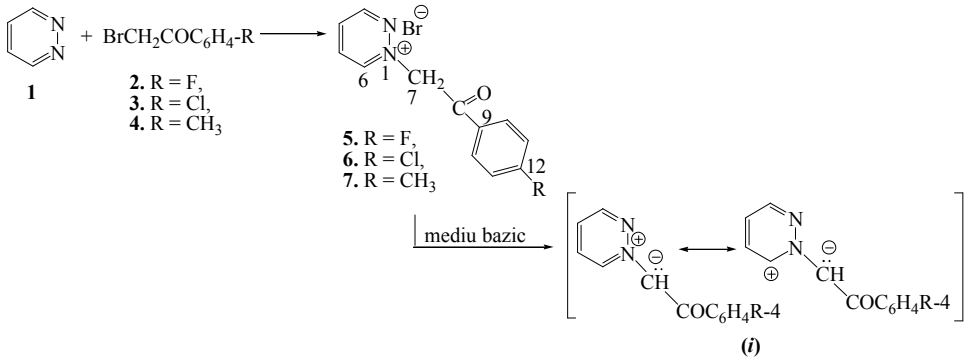


Fig. 1 - N-alkylation of the pyridazine.

The N-alkylation of the pyridazine was followed by a 3+2 dipolar cycloaddition of diazinium ylides (***i***) (generated in situ from the corresponding salts) to dipolarophiles (activated alkenes or alkynes), Fig. 2.

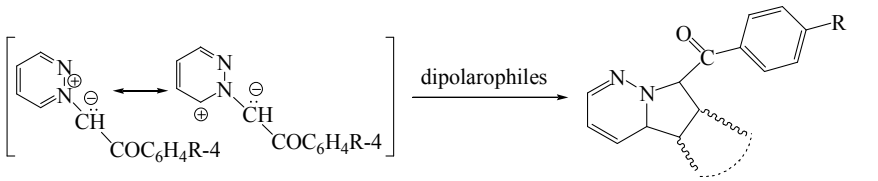
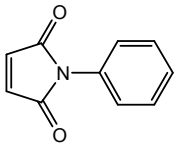
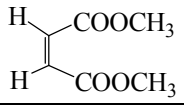
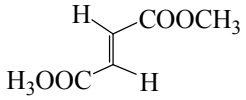


Fig. 2 - 3 +2 dipolar cycloaddition of diazinium ylides.

The dipolarophiles used in 1,3-dipolar cycloaddition are activated alkenes and alkynes and are presented in Table 1:

Table 1

Activated alkenes or alkynes (dipolarophiles used in 1,3-dipolar cycloaddition)

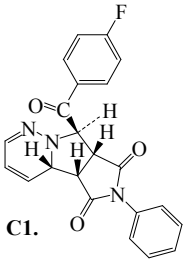
Dipolarophiles	Structure
N-Phenylmaleimide (NPMI)	
maleic ester	
fumaric ester	
acrylonitrile	$H_2C{=}CH{-}CN$
ethyl propiolate	$HC{\equiv}C{-}COOC_2H_5$

dimethyl acetylendicarboxylate (DMAD)	$H_3COOC-C\equiv C-COOCH_3$
2,2,2-trifluorocrotonate	$F_3C-CH=CH-COOC_2H_5$
2,2,2-trifluorobutinoate	$F_3C-C\equiv C-COOC_2H_5$

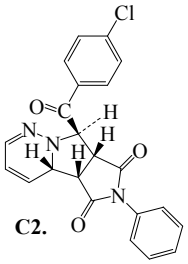
After purification, the structure of the compounds was proved by spectral analysis: the 1H NMR and 13C NMR spectra and two-dimensional experiments 2D-COSY, 2D-HETCOR(HMQC), long range 2D-HETCOR (HMBC) recorded on a Bruker Avance 400 DRX spectrometer at 400/100 MHz. Chemical shifts are given in parts per million (δ-scale), coupling constants (J) in hertz and downfield shift from internal tetramethylsilane (δ 0.00 ppm). The IR spectra were recorded on an FT-IR Shimadzu Prestige 8400s spectrophotometerin KBr. Melting points were determined using an electrothermal apparatus and are uncorrected. Flash chromatography was performed with Aldrich 230e400 mesh silica gel. TLC was carried out on Merck silica gel 60-F-254 plates.

All reagents and solvents employed were of the best grade available and were used without further purification.

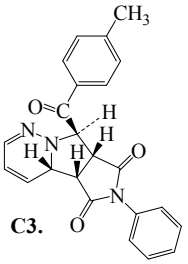
The pyridazine derivatives (**C1-C24**) have the following structures, Fig. 3:



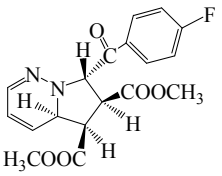
C1.



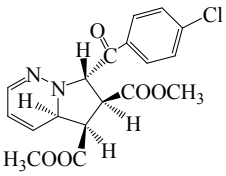
C2.



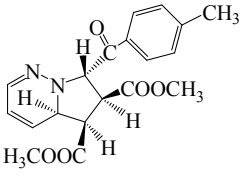
C3.



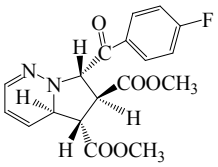
C4.



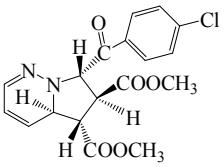
C5.



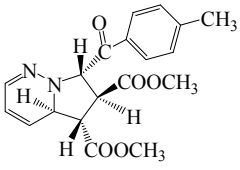
C6.



C7.



C8.



C9.

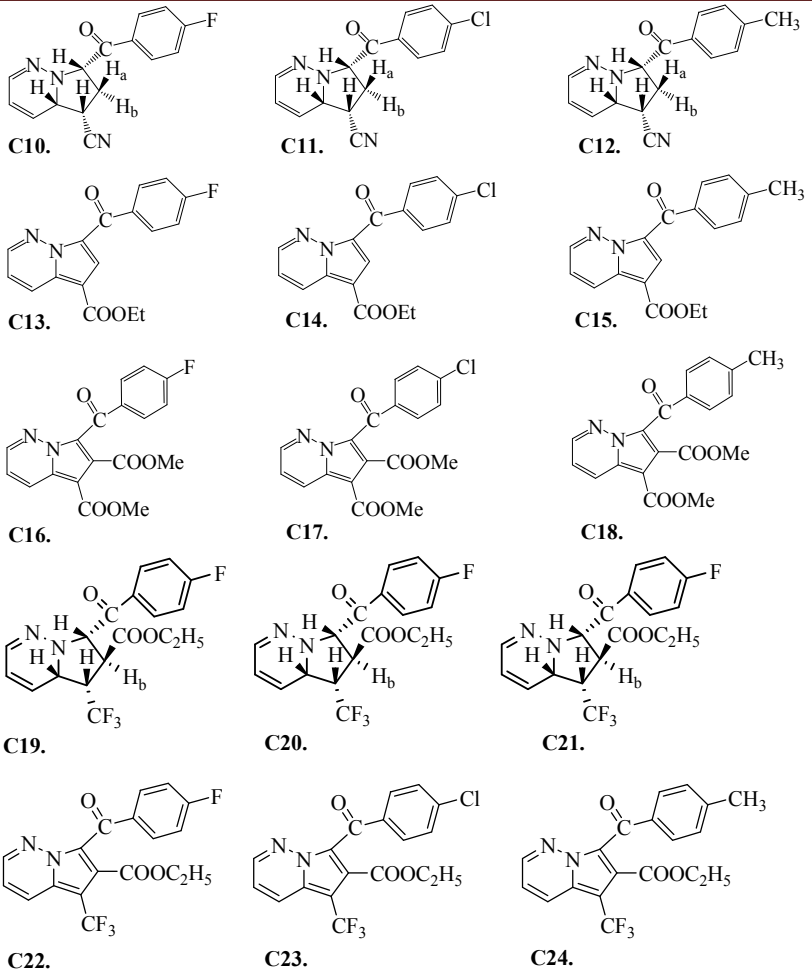


Fig. 3 - Pyridazine derivatives obtained by 3 +2 dipolar cycloaddition reaction.

The *in vitro* antibacterial and antifungal activity of the obtained pyridazine compounds was tested having in view that our previous study had proved a certain biological activity in this respect.

The test was performed using the diffusimetric method with rustles steel cylinders based on the diffusion of the tested substances on the gelose surface (for bacteria) and Sabouraud environment (for fungus *Candida albicans*). The cylinders were maintained for 24 h at thermostat, at 34 ^{o}C for bacteria and at 37 ^{o}C for *Candida*. The tested substances were previously dissolved in dimethylformamide (DMF) 5% (v/v). A witness solvent sample has been done. The inhibition diameter zone, in mm, of development of microbial strain was measured. A compound is considered active when the difference between the inhibition diameter zone of compound and witness is up to 2 mm (3-4 mm moderate active and up to 5 very active). The results are listed in Table 2.

Table 2

Results of *in vitro* antibacterial and antifungal activities for diazine derivatives described in the text.

Product	Strain					
	Staphylococcus aureus Oxford	***Sarciria luteea***	***Bacillus subtillis***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Candida albicans***
C1	27	**63**	28	16	25	25
C2	28	**54**	28	18	28	**34**
C3	31	**73**	**37**	18	24	29
C4	29	**60**	**37**	18	**35**	24
C5	33	**56**	**39**	18	**32**	25
C6	**41**	**62**	**35**	19	**37**	30
C7	29	**51**	**35**	17	**35**	27
C8	23	**54**	**36**	15	**31**	27
C9	**47**	**59**	**38**	17	**37**	29
C10	26	**50**	27	14	20	23
C11	30	**57**	29	14	28	**35**
C12	46	**60**	**39**	19	23	30
C13	27	**48**	22	20	23	22
C14	25	**48**	25	20	**33**	30
C15	**40**	**56**	**37**	18	25	28
C16	27	**52**	23	15	21	21
C17	30	**57**	26	18	**31**	24
C18	**46**	**65**	**36**	19	25	29
C19	34	**54**	29	18	26	29
C20	36	**55**	31	16	28	30
C21	39	**54**	31	16	24	28
C22	37	**62**	28	16	23	27
C23	37	**64**	30	18	28	27
C24	39	**59**	29	16	28	29

RESULTS AND DISCUSSIONS

The comparative analysis of the obtained data leads to the conclusions:

- the pyridazine derivatives have an excellent antimicrobial activity (non selective) against *Gram positive* germs, the results on *Sarciria Luteea* being spectacular;

- the cycloadducts have antibacterial activity only against *Escherichia coli* (very active for **C6** and **C9**);

- only two pyridazine derivatives (**C2** and **C11**) have a significant activity against fungus *Candida albicans*.

CONCLUSIONS

In conclusion, a study concerning synthesis, structure and biological activity of some pyridazine derivatives is reported.

The *in vitro* antibacterial and antifungal activities of the newly obtained diazine compounds were tested, some of the compounds have proved to have a remarkable activity against *Gram positive* germs, the results on *Sarciria luteea*

being spectacular. Against fungus *Candida albicans* pyridazine derivatives have no significant activity.

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