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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW PYRIDAZINE DERIVATIVES

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Abstract-Five new pyridazine derivatives (1 salt and 4 pyrrolopyridazine cycloadducts) were prepared and tested *in vitro* as antimicrobial compounds. Some of them have proved to have a remarkable activity against different micro organisms (germs and fungi). The influence of microwave irradiation concerning cycloaddition reactions of pyridazinium ylides was studied. Stereo- and regiochemistry involved in these reactions are also discussed.

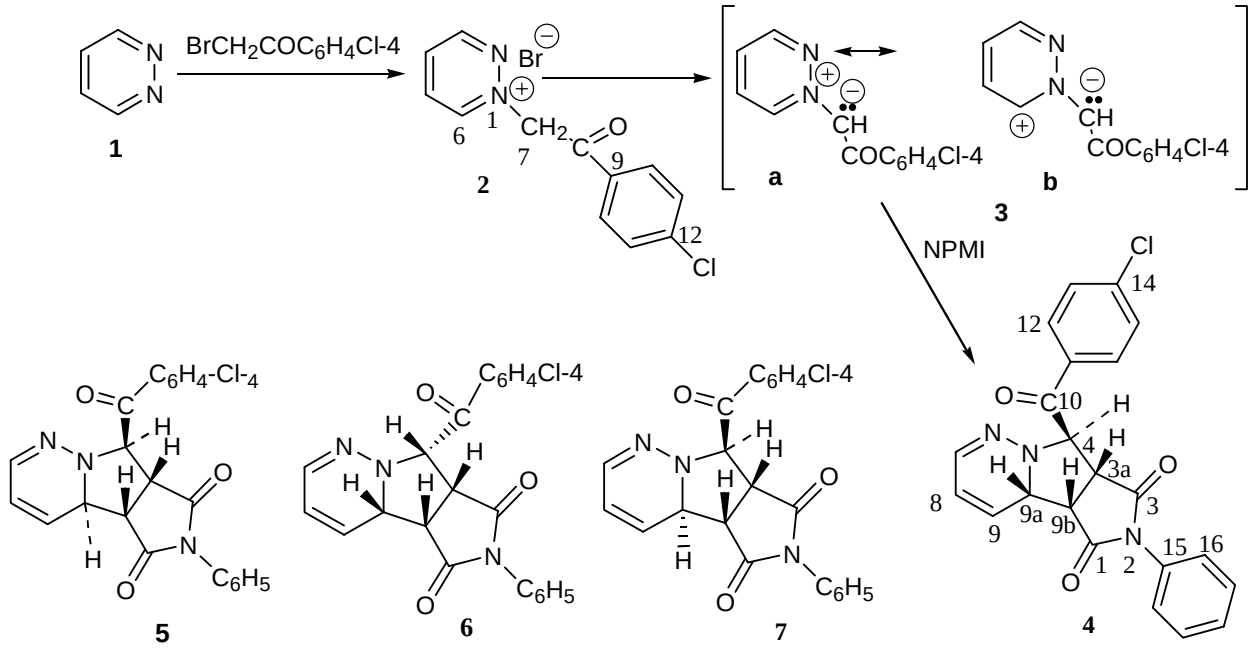
INTRODUCTION

Literature[1] describes a large variety of pyridazine compounds with different biological activities: anticancer,[2] antituberculosis,[3] antihypertensive,[4] antimicrobial,[5,6] etc. Also, the problem of stereo- and regiochemistry involved in [3+2] dipolar cycloadditions[7-11] is widely discussed, being far away to be solved. During last years the literature indications[12-15] show that microwave irradiation is a new trend in organic chemistry offering a versatile and facile pathway in a large variety of syntheses.

The emphasis of this work was, on one hand, the synthesis of new azaheterocycles with antimicrobial activity (using as intermediates ylide compounds), and, on the other, to study the chemistry involved in the cycloaddition reactions as well as the influence of microwave irradiation concerning the synthesis.

RESULTS AND DISCUSSION

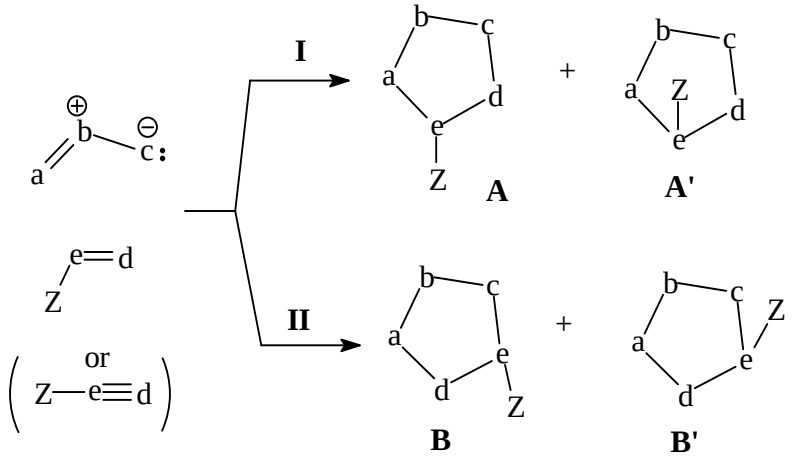
A facile way to obtain condensed pyridazines is to use ylides as intermediates. First we obtained the corresponding cycloimmonium salt (**2)** which in alkaline medium (Et_3N) generated the ylide *in situ*. *N*-Phenylmaleimide (NPMI), as a symmetrical cyclic *Z*-alkene, reacts with ylides (**3)** giving the cycloadduct (**4)**. The [3+2] cycloaddition occurs with high stereospecificity and no formation of compounds such as **5**, **6** or **7** was observed (Scheme 1).



Scheme 1. Reaction pathway of pyridazinium ylides with NPMI.

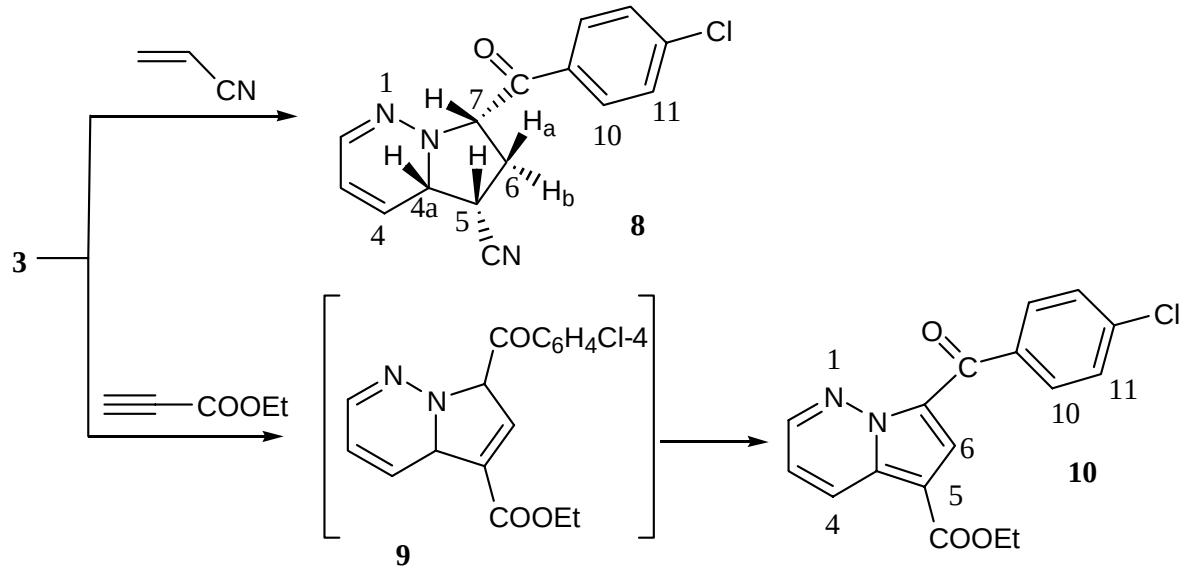
The structure of the new compound (**4)** was proved by elemental (nitrogen) and spectral analysis. The following spectral methods were used: IR, ^{1}H NMR, ^{13}C NMR, and two-dimensional experiments 2D-COSY, 2D-HETCOR (HMQC), long range 2D-HETCOR (HMBC). Taking into account the chemical shifts, the coupling constants and signal splittings in the NMR spectra, the only reasonable stereochemistry for the cycloadduct is that one presented in structure (**4)**. As the only possible manner of ring fusion at the 3a- and 9b- positions is *cis* geometry, H-3a (doublet) and H-9b (doublet of doublet) are *cis* to each other. This is confirmed in the ^{1}H NMR spectrum by coupling constants (8.0 Hz). The H-9a (doublet of doublet) has two coupling constants: one of 8.0 Hz with H-9b (which proves the *cis* vicinity) and a second one of 5.6 Hz with H-9 (which proves the *trans* vicinity). It is interesting that H-4 appear as a singlet (not as a doublet as usual) being *trans* with respect to H-3a. Steric hindrance caused by bulky neighbouring groups (4-chlorobenzoyl and NPMI ring) could be responsible. An additional evidence for the assigned structures is the extraordinarily large difference of chemical shifts between H-4 (5.92 ppm) and H-9a (4.21-4.18 ppm), H-3a (4.07-4.05 ppm) and H-9b (3.57-3.53 ppm). These differences between 2.80-2.00 ppm could be explained by the fact that, in such a configuration, the H-4 hydrogen is closely located both to the carbonyl of the fused maleimide ring and the ketone carbonyl group, suffering from a powerful anisotropic effect. This affirmation is also sustained by the ^{13}C NMR spectra in which C-4 appears at 75.90 ppm while C-9a appears at 56.61, C-9b at 51.39 and C-3a at 44.76.

The reaction of ylide (**3)** with non- symmetrical dipolarophiles such as acrylonitrile and ethyl propiolate involves additional regiochemical problems[7, 8] since alternative addition of the dipole (ylide) to the dipolarophile has often been found (Scheme 2). No matter what conditions we used (different solvents,



Scheme 2. Regiochemistry in the addition of ylides with dipolarophiles.

temperature, time), the reaction of ylide (**3)** with acrylonitrile or ethyl propiolate leads to a single isomer, the tetrahydropyrrolopyridazine adduct (**8)** or the pyrrolopyridazine adduct (**10)** (Scheme 3). This means that one bond is formed between the ylide carbon and the non-substituted carbon atom of the dipolarophile and the second bond between the positively charged carbon of the ylide and the substituted carbon of the dipolarophile (path **I**, regioisomer **(A)**, Scheme 2). This is in accordance with the usual electronic effects in dipolarophiles. Because a single regioisomer is obtained, the reaction is regiospecific being under charge control.



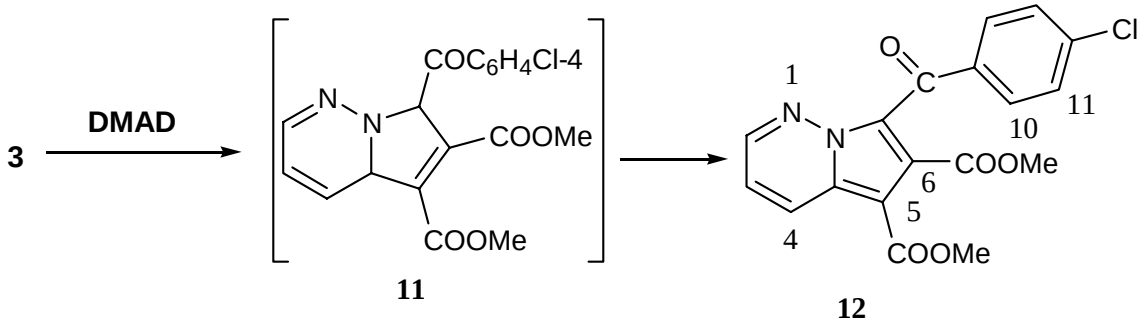
Scheme 3. Reaction pathway of pyridazinium ylides with acrylonitrile or ethyl propiolate.

The structure of the new compounds was proved by elemental (nitrogen) and spectral analysis (IR, ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC). In the ^{1}H NMR spectrum of the tetrahydropyrrolopyridazine adduct (**8)**, the most important signals are those of the H-5, H-6a, H-6b, H-7 and H-4a atoms. H-7 appears at 5.54-5.51 ppm (dd), which excludes both **B** and **B'** structures (Scheme 2). It has two different coupling

constants [*J*(7,6a)= 8.4 Hz, *J*(7,6b)= 2.8 Hz], which prove the *trans* configuration to H-6b and *cis* to H-6a. The H-6a (dtd) proton has three different coupling constants [*J*(6a,6b)=19.8 Hz, *J*(6a,7)= 8.4 Hz, *J*(6a,5)= 7.0 Hz] which proves the *cis* configuration with respect to H-7 and H-5. The H-4a (t) proton has two different coupling constants [*J*(4a,5)= 11.6 Hz, *J*(4a,4)= 6.0 Hz], which prove the *cis* configuration to H-5. An additional evidence for the assigned structures is the great chemical shifts difference between H-7 (*ca.* 5.50 ppm) and H-4a (*ca.* 4.0 ppm), H-5 (*ca.* 3.25 ppm), H-6a (*ca.* 2.80 ppm) and H-6b (*ca.* 2.15 ppm). In the ^{13}C NMR spectra carbons C-4a, C-5, C-6 and C-7 appear at chemical shifts in accordance with the saturated structure proposed for adduct (**8)** (C-4a at 55.83 ppm, C-5 at 35.35 ppm, C-6 at 26.49 ppm and C-7 at 70.70 ppm).

The main spectral data for the pyrrolopyridazine adduct (**10)** confirm the proposed structure. The H-6 proton appears at 7.81 ppm (singlet), due to the deshielding effect induced by the ethoxycarbonyl and benzoyl groups. The fact that this proton appears at a chemical shift about one ppm higher than that one of a classical pyrrolo proton is a good proof for the **(A)** type (Scheme 1) regioisomer. The H-4 proton appears at 8.69-8.66 ppm as a doublet which exclude the structure of type **(9)** (in which it should be shown as a triplet). The fact that in ^{13}C NMR spectra the carbons C-4a, C-5, C-6 and C-7 appear at high chemical shifts (C-4a at 133.62 ppm, C-5 at 105.53 ppm, C-6 at 124.81 ppm and C-7 at 126.30 ppm), are also good evidence of the aromatized structure (**10)**. As to the mechanism, the formation of compounds (**10)** could be explained by oxidative dehydrogenation of **9**.

The reaction with dimethyl acetylendicarboxylate (DMAD) leads to the aromatised pyrrolopyridazine (**12)** (Scheme 4).



Scheme 4. Reaction pathway of pyridazinium ylides with DMAD.

The same mechanism, oxidative dehydrogenation of **11**, explains the formation of **12**. The structure of the new compounds was proved by elemental and spectral analysis (IR, ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC). The lack of signals between 3.92 and 7.08 ppm in the proton spectra combined with the fact that in the ^{13}C NMR spectra the carbons C-4a, C-5, C-6 and C-7 appear at high chemical shifts (C-4a at 131.00 ppm, C-5 at 103.47 ppm, C-6 at 126.18 ppm and C-7 at 126.28 ppm), are a good evidence of the structure proposed for the aromatized pyrrolopyridazine (**12)**.

All the remaining signals from IR and NMR spectra are in accordance with the proposed structure of all compounds.

Under classical heating (refluxing in a solvent) the cycloaddition reactions present some disadvantages: the reaction time is long, the yields are not all the time satisfactory, require great amounts of solvents, etc.. Accordingly, we decided to synthesize the desired cycloadducts by microwave-heating irradiation. In Table 1 are presented the reaction results obtained under microwave heating as well as under classical conditions.

Table 1. Cycloaddition reactions of pyridazinium ylides with activated alkenes and alkynes under microwave heating and classical conditions.

Compd	Microwaves			Classical		
	Reaction time	Reaction temperature, ^{o}C	Yield %	Reaction time	Reaction temperature, ^{o}C	Yield %
4	5 min	55	89	180 min	65	81
8	5 min	55	84	180 min	65	76
10	5 min	55	87	120 min	65	83
12	5 min	55	93	120 min	65	82

As can be seen from Table 1, the microwave induces a remarkable acceleration for the [3+2] dipolar cycloaddition reaction of pyridazinium ylides to activated alkenes and alkynes, the yields are better and the amount of used solvent is half (EXPERIMENTAL) under microwave irradiation.

The *in vitro* antibacterial and antifungal activity of the newly obtained pyridazine compounds was tested having in view that our previous study had proved a certain biological activity in this respect.[3, 5, 6] 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.

The comparative analysis of the obtained data (Table 2) leads to the following conclusions concerning the relation between structure and biological activities:

- the salt (**2)** has a remarkable non selective activity against *Gram positive* germs;

Table 2. Results of *in vitro* antibacterial and antifungal activities for some pyridazine derivatives described in the text.

Strain→ Product ↓	*Staphylococcus aureus Oxford*	*Escherichia coli*	*Bacillus subtillis*	*Pseudomonas aeruginosa*	*Candida albicans*
Witness	15	14	13	17	18
2	20	24	19	18	17
4	14	18	15	16	23
8	15	16	17	-	23
10	15	22	13	18	20
12	15	20	14	17	19

- the cycloadducts have antibacterial activity only against *Escherichia coli* (moderate active for **4** and **8** and very active for **10** and **12**);
- only cycloadducts (**4)** and (**8)** are active against fungus *Candida*.

CONCLUSIONS

1. The cycloaddition reaction of pyridazinium ylides with NPMI (as activated symmetrical alkene) occurs in a highly stereospecific way.
2. As far the regiochemistry is concerned, the cycloaddition with activated non symmetrical dipolarophiles leads to a single regioisomer.
3. The microwave induces a remarkable acceleration for the [3+2] dipolar cycloaddition reaction of pyridazinium ylides to activated alkenes and alkynes and allows a general and facile way for synthesis. Also, under microwave the yields are better and the amount of used solvent is smaller.
4. The *in vitro* biological activity of the newly obtained pyridazine compounds was tested. Correlation structure-biological activity has been studied. Pyridazine salt has a remarkable non selective activity against germs but is inactive against fungus. A saturated structure for cycloadducts make them very active against fungus while an aromatized one made them inactive. As far for germs, cycloadducts seems to lack any significant activity.

EXPERIMENTAL

Melting points were determined on a MELTEMP II apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance instrument (400 MHz) downfield from an internal standard, $SiMe_4$ in DMSO-d_6 (for the compounds **2**) or in $CDCl_3$ (for the others compounds). Chemical shifts are

given in ppm (δ-scale), coupling constants (*J*) in Hz. IR spectra were recorded with a JASCO V-570 spectrometer in KBr. For the microwave irradiation we used a monomode reactor STAR-2, CHEM corporation (50 W).

1-[2-(4-Chlorophenyl)-2-oxoethyl]pyridazinium bromide (2). Pyridazine (0.80 g, 10 mmol) was suspendend in 30 mL of anhydrous benzene and, then, ω-bromo-4-chloroacetophenone (2.33 g, 10 mmol, dissolved in 20 mL of anhydrous benzene) was added. The solution was stirred for 3 h to give **2** as white crystals (no purification required). Yield 92% (2.88 g), mp 221-222 ^{o}C. Anal. Calcd for $C_{12}H_{10}N_2OBrCl$: N, 8.93. Found: 8.86. IR(cm^{-1}): 3015, 2937, 1694 (C=O), 1587, 1437, 1400. ^{1}H NMR: 6.90 (s, 2H (CH_2)), 7.51-7.47 (d, *J*=8.8, 2H (H-11)), 7.91-7.87 (d, *J*=8.8, 2H (H-10)), 8.71-8.64 (dd, *J*=11.0, *J*=5.8, 1H (H-5)), 8.91-8.86 (dd, *J*=11.0, *J*=5.0, 1H (H-4)), 9.75-9.73 (d, *J*=5.0, 1H (H-3)), 9.90-9.87 (d, *J*=5.8, 1H (H-6)). ^{13}C NMR: 69.90 (C7), 129.07 (C10), 129.62 (C12), 129.71 (C11), 131.52 (C5), 135.10 (C4), 136.67 (C3), 139.90 (C9), 150.81 (C6), 188.41 (C8, keto).

General procedure to obtain [3+2] cycloadducts (4), (8), (10) and (12). The corresponding cycloimmonium salt (**2)** (0.233 g, 1 mmol) was suspended in 10 mL of chloroform. A solution of an activated alkene or alkyne (1 mmol) and triethylamine (0.111 g, 1.1 mmol) in the same solvent (5 mL) was then added. The solution was refluxed for 2 h in the case of alkynes and for 3 h in the case of alkenes. Under microwave heating, the solution was exposed to microwave for 5 min, and the amount of solvent was half. The resulting mixture was washed thoroughly three times with water (50 mL), dried with sodium sulfate, filtered and evaporated. The crude product was crystallized from an appropriate solvent.

4-(4-Chlorobenzoyl)-2-phenyl-9a,9b,3a,4-tetrahydropyrrolo[3',4':3,4]pyrrolo[1,2-*b*]pyridazine-1,3-dione (4). White crystals from ethanol. Yield 89% (0.36 g), mp 193-195 ^{o}C. Anal. Calcd for $C_{22}H_{16}N_3O_3Cl$: N, 10.35. Found: 10.30. IR(cm^{-1}): 3063, 2973, 1779, 1713 (C=O imide), 1684 (CO keto), 1588, 1494, 1395. ^{1}H NMR: 3.57-3.53 (dd, *J*=8.0, *J*=8.0, 1H (H-9b)), 4.07-4.05 (d, *J*=8.0, 1H (H-3a)),

4.21-4.18 (dd, *J*=8.0, *J*=5.6, 1H (H-9a)), 5.92 (s, 1H (H-4)), 5.95-5.93 (m, 1H (H-8)), 6.28-6.25 (dd, *J*=9.0, *J*=5.6, 1H (H-9)), 6.86-6.85 (d, *J*=3.2, 1H (H-7)), 7.21-7.19 (d, *J*=7.2, 2H (H-16)), 7.41-7.38 (dd, *J*=7.2, *J*=3.6, 1H (H-18)), 7.49-7.45 (m, 4H (2H-13, 2H-17)), 8.16-8.13 (d, *J*=8.8, 2H (H-12)). ^{13}C NMR: 44.76 (C3a), 51.39 (C9b), 56.61 (C9a), 75.90 (C4), 118.28 (C8), 126.03 (C16), 126.49 (C9), 128.79 (C18), 129.14 (C17), 129.26 (C13), 131.07 (C12), 131.84 (C15), 132.35 (C14), 136.92 (C7), 140.71 (C11), 174.45 (C3, keto imide), 176.64 (C1, keto imide), 192.61 (C10, keto).

7-(4-Chlorobenzoyl)-4a,5,6,7-tetrahydropyrrolo[2,1-*b*]pyridazine-5-carbonitrile (8). Brown crystals from ethanol. Yield 86% (0.245 g), mp 156 ^{o}C. Anal. Calcd for $C_{15}H_{12}N_3OCl$: N, 14.71. Found: 14.65. IR(cm^{-1}): 3057, 2971, 2242 (CN), 1692 (C=O keto), 1585, 1452, 1398. ^{1}H NMR: 2.15-2.08 (seven lines, $J_{6b,6a}$=19.8, $J_{6b,7}$=2.8, $J_{6b,5}$=3.2, 1H (H-6b)), 2.80-2.74 (dtd, *J*=19.8, *J*=8.4, *J*=7.0, 1H (H-6a)), 3.26-3.20 (m, 1H (H-5)), 3.98-3.96 (dd, *J*=11.6, *J*=6.0, 1H (H4a)), 5.47-5.51 (dd, *J*=8.4, *J*=2.8, 1H (H-7)), 6.07-6.03 (dd, *J*=9.6, *J*=6.0, 1H (H-4)), 6.14-6.11 (dd, *J*=9.6, *J*=3.2, 1H (H-3)), 6.88-6.87 (d, *J*=3.2, 1H (H-2)), 7.49-7.47 (d, *J*=8.8, 2H (H-11)), 8.11-8.10 (d, *J*=8.8, 2H (H-10)). ^{13}C NMR: 27.49 (C6), 35.25 (C5), 55.83 (C4a), 70.70 (C7), 119.97 (C_3), 120.36 (C5a, CN), 124.08 (C4), 129.06 (C11), 130.85 (C10), 133.08 (C12), 136.18 (C2), 140.42 (C9), 194.39 (C9, keto).

7-(4-Chlorobenzoyl)pyrrolo[2,1-*b*]pyridazine-5-carboxylic acid ethyl ester (10). Light blue crystals from ethanol. Yield 88% (0.29 g), mp 109-110 ^{o}C. Anal. Calcd for $C_{17}H_{13}N_2O_3Cl$: N, 8.52. Found: 8.50. IR(cm^{-1}): 3095, 2954, 1705 (C=O ester), 1640 (C=O keto), 1241, 1094 (C-O-C), 1520, 1469, 1428, 1374. ^{1}H NMR: 1.43-1.39 (t, *J*=7.2, 3H (CH_3)), 4.42-4.37 (q, *J*=7.2, 2H (CH_2)), 7.19-7.15 (dd, *J*=9.2, *J*=4.0, 1H (H-3)), 7.50-7.48 (d, *J*=8.4, 2H (H-11)), 7.73 (s, 1H (H-6)), 7.86-7.84 (d, *J*=8.4, 2H (H-10)), 8.55-8.53 (d, *J*=4.0, 1H (H-2)), 8.69-8.66 (d, *J*=9.2, 1H (H-4)). ^{13}C NMR: 14.49 (CH_3), 60.53 (CH_2), 105.53 (C5), 117.81 (C3), 124.81 (C6), 126.30 (C7), 128.11 (C4), 128.77 (C11), 130.92 (C10), 133.62 (C4a), 137.31 (C12), 138.72 (C9), 144.37 (C2), 163.44 (C5a, keto ester), 183.00 (C8, keto).

7-(4-Chlorobenzoyl)pyrrolo[1,2-*b*]pyridazine-5,6-dicarboxylic acid dimethyl ester (12). White crystals from ethanol. Yield 84% (0.31 g), mp 178 ^{o}C. Anal. Calcd for $C_{18}H_{13}N_2O_5Cl$: N, 7.52. Found: 7.45. IR(cm^{-1}): 3091, 2950, 1744 (C=O ester from 5), 1707 (C=O ester from 6), 1648 (C=O keto), 1242, 1106 (C-O-C), 1588, 1500, 1452, 1389. ^{1}H NMR: 3.66 (s, 3H (CH_3, from 6)), 3.92 (s, 3H (CH_3, from 5)), 7.11-7.08 (dd, *J*=8.8, *J*=3.2, 1H (H-3)), 7.44-7.42 (d, *J*=8.4, 2H (H-11)), 7.74-7.72 (d, *J*=8.4, 2H (H-10)), 8.33-8.32 (d, *J*=3.2, 1H (H-2)), 8.63-8.60 (d, *J*=8.8, 1H (H-4)). ^{13}C NMR: 51.95 (CH_3, COOMe from 5), 52.69 (CH_3, COOMe from 6), 103.44 (C5), 117.00 (C3), 126.18 (C6), 126.28 (C7), 128.74 (C11), 128.79

(C4), 130.78 (C10), 131.00 (C4a), 136.21 (C12), 139.67 (C9), 144.71 (C2), 162.86 (C5a, keto ester), 164.52 (C6a, keto ester), 184.24 (C8, keto).

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REFERENCES

1. R. B. Silverman, "The Organic Chemistry of Drug Design and Drug Action" and the references cited therein, Academic Press, San Diego, 1992.
2. M. Rodrguez-Ciria, A. M. Sanz, M. J. Yunta, F. Gomez-Contreras, P. Navarro, I. Fernandez, M. Pardo, and C. Cano, *Bioorg. Med. Chem*., 2003, **11**, 2143.
3. C. Moldoveanu, G. Mangalagiu, G. Drochioiu, M. Caprosu, M. Petrovanu, and I. I. Mangalagiu, *An. Stiint. Univ. 'Al.I.Cuza' Iasi*, 2003, **11**, 367.
4. M. Gokçe, D. Dogruer, and M. Fethi Sahin, *Il Farmaco*, 2001, **56**, 223.
5. M. Ungureanu, I. Mangalagiu, G. Grosu, and M. Petrovanu, *Ann. Pharm. Fr*., 1997, **55**, 69.
6. St. Dima, M. Caprosu, M. Ungureanu, G. Grosu, and M. Petrovanu, *Ann. Pharm. Fr*., 1999, **57**, 415.
7. A. Padwa, "1,3-Dipolar Cycloaddition Chemistry", Vol. 2, Chapter 12, ed. by A. Padwa, pp. 277-406 and chapter 13, ed. by K. N. Houk and K. Yamaguchi, pp. 407-450 and the references cited therein, John Wiley & Sons, New York, 1984.
8. D. Klamann and H. Hagemn, "Organische Stickstoff-Verbindungen mit einer C, N-Doppelbindung", in Houben-Weyl, E_{14b}, Teil 1: Stickstoff-Ylide, ed. by P. K. Claus, pp. 1–160 and the references cited therein, Thieme, Stuttgart, New York, 1991.
9. M. Caprosu, Ghe. Zbancioc, C. Moldoveanu, and I. I. Mangalagiu, *Collect. Czech. Chem. Com.*, 2004, **69**, 426.
10. I. I. Mangalagiu, G. Mangalagiu, C. Deleanu, G. Drochioiu, and M. Perovanu, *Tetrahedron*, 2003, **59**, 111.
11. O. Tsuge, S. Kanemasa, and S. Takenaka, *Bull. Chem. Soc. Jpn*., 1985, **58**, 3137.
12. M. Lloung, A. Loupy, S. Marquend, and A. Petit, *Heterocycles*, 2004, **63,** 297.
13. H. Rodriguez, R. Perez, M. Suarez, A. Lam, N. Cabrales, and A. Loupy, *Heterocycles*, 2001, **55**, 2291.
14. St. Dima, I. I. Mangalagiu, M. Caprosu, M. Georgescu, and M. Petrovanu, *Rev. Roum. Chim.*, 2000, **45,** 555.
15. Ghe. Zbancioc, G. Mangalagiu, St. Dima, C. Moldoveanu, M. Petrovanu, and I. I. Mangalagiu, *An. Stiint. Univ. 'Al. I. Cuza' Iasi*, 2002, **10**, 146.