Pyridazine and Phthalazine Derivatives with Potential Antimicrobial Activity

Roxana M. Butnariu,[1] Maria D. Caprosu,[1] Vasilichia Bejan,[1] Margareta Ungureanu,[2] Antonia Poiata,[2] Cristina Tuchilus,[2] Margareta Florescu[3] and Ionel I. Mangalagiu[1]*

[1] "Al. I. Cuza" University of Iasi, Organic and Biochemistry Department, Bd. Carol 11, 700506 Iasi, Romania.
e-mail: ionelm@uaic.ro
[2] University of Medicine and Pharmacy "Gr.T.Popa" Iasi, Str. Universitatii 16, 700115 Iasi, Romania.
[3] Academy of Economical Sciences, Str. Piata Romana 6, Sector 1, 010374 Bucuresti, Romania. e-mail: icefaceus@yahoo.com

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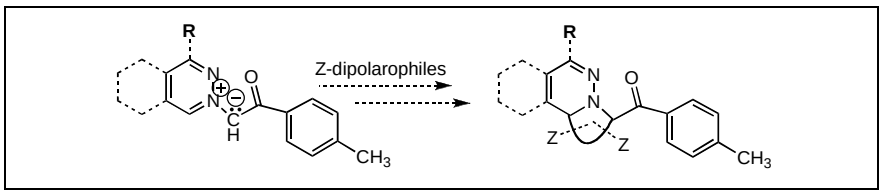

Fifteen new pyridazine and phthalazine derivatives were prepared (in good to excellent yields) and tested *in vitro* as antimicrobial compounds. All the compounds have proved to have a remarkable activity against *Gram positive* germs, the results on *Sarciria Luteea* being spectacular. Correlation structure - biological activity have been done. Stereo- and region- chemistry involved in these reactions are discussed.

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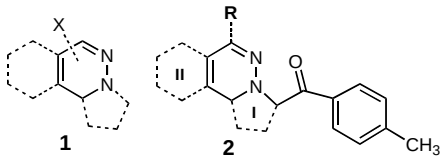
INTRODUCTION

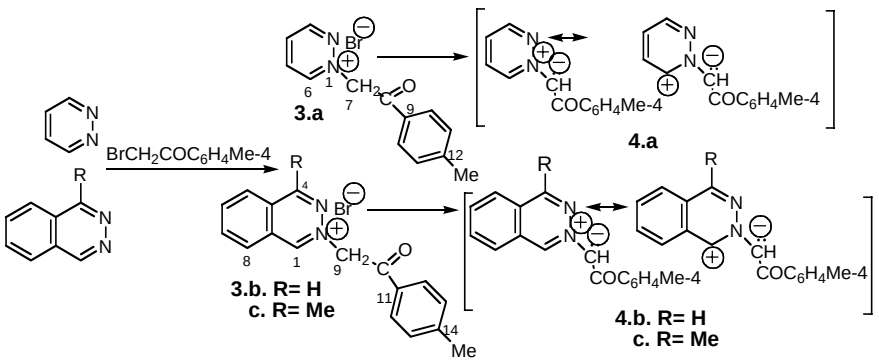
The literature describes a large variety of pyridazine and phthalazine compounds (**1**) with different biological activities: anticancer [1,2], antituberculosis [3], antimicrobial [4-6], antihypertensive [7-9], platelent aggregation inhibitor [9,10], *etc*. A facile way to obtain diazine derivatives uses cycloimmonium ylides as reactive species [11-15]. The reaction pathway involves, in the most frequent cases, a Huisgen 3+2 dipolar cycloaddition of ylides to dipolarophiles (activated alkenes and alkynes). The problems of stereo- and regiochemistry involved in these types of cycloadditions are discussed.



Considering the pyridazine – acetophenone skeleton (**2**) as the pharmacophoric group for the activity [4,9], in this work we aimed to obtain new diazine heterocycles with antimicrobial activity, having in mind three structural modifications: introduction of a pyrrolo (**I**) and/or a benzene (**II**) ring, and a substituient (**R**). In equal measure, we were interested in the chemistry of cycloaddition reactions.

In order to synthetise the desired pyridazine and phthalazine derivatives, first we obtained the corresponding cycloimmonium salt (**3**) which in alkaline medium (Et_3N) generated the ylide (**4**) *in situ*. N-Phenylmaleimide (NPMI), as a symmetrical cyclic *Z*-alkene, reacts with ylides (**4**) giving the cycloadduct (**5**).

The Huisgen 3+2 cycloaddition occurs with high stereospecificity and no formation of other isomers was observed. The reaction of ylides (**4**) with dimethyl acetylendicarboxylate (DMAD) leads to pyrrolopyridazine (**8a**) and dihydropyrrolophthalazines (**8b,c**). As to the mechanism, the formation of compounds (**8b,c**) could be explained by prototropic rearrangement of (**7b,c**) while

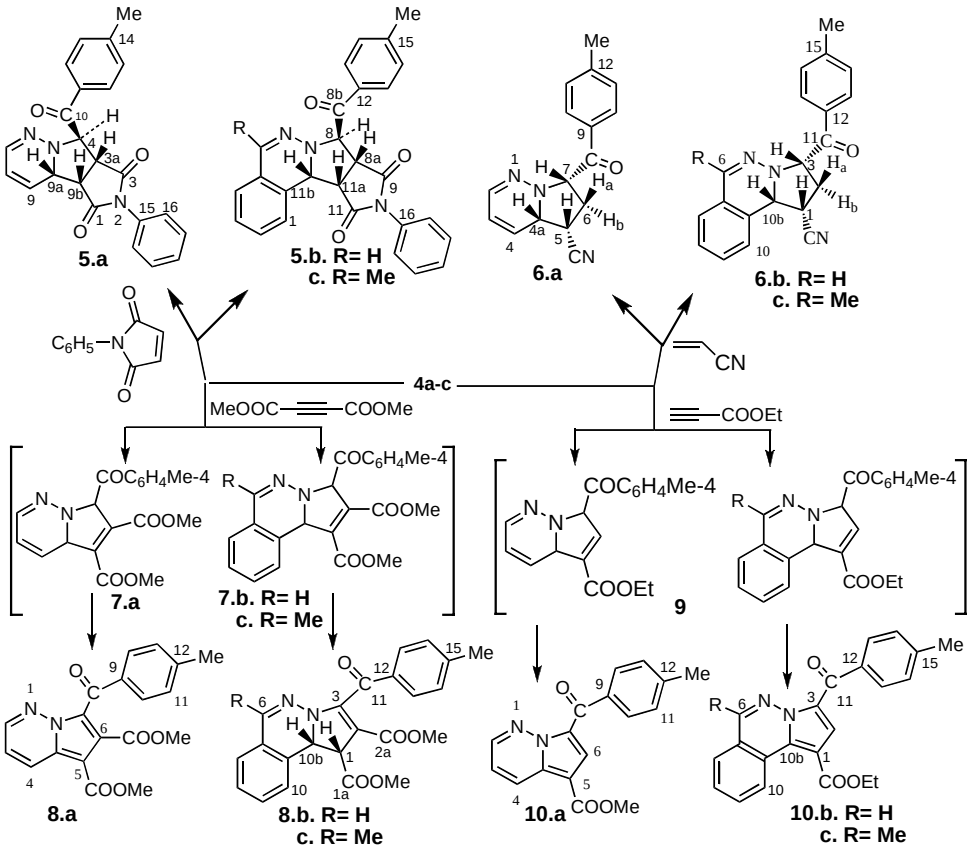


formation of (**8a**) could be explained by oxidative dehydrogenation of (**7a**).

The reaction of ylides (**4**) with non-symmetrical dipolarophiles such as acrylonitrile and ethyl propiolate involves additional regiochemical problems [11,12] since alternative addition of the dipole (ylide) to the dipolarophile has often been found. No matter what conditions we used (different solvents, temperature, time), the reaction of ylides (**4**) with acrylonitrile and ethyl propiolate leads to a single isomer, the tetrahydropyrrolo-diazine adduct (**6**) and the pyrrolo- diazine adduct (**10**), respectively. 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. 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.

The structure of the new compounds was proved by elemental 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).

The *in vitro* antibacterial and antifungal activities of the newly obtained diazine compounds were tested. The tests were performed using the diffusiometric method with rustless 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 34 °C for bacteria and at 37 °C for *Candida*. The tested substances were previously dissolved in dimethylformamide (DMF) 5% (v/v). A control solvent sample was also included. 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 1.



The comparative analysis of the data leads to the conclusions that both pyridazine and phthalazine derivatives have excellent antimicrobial activity (non selective) against *Gram positive* germs. The results on *Sarciria Luteea* are of particular note. However, pyridazine derivatives are, in most case, more active compared with the corresponding phthalazine, which means that introduction of a benzene ring (**II**) on the

Table 1

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

Strain→ Product ↓	*Staphylococcus aureus Oxford*	*Sarciria Luteea*	*Bacillus subtillis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Witness	12	10	14	17	14	16
(3a)	**26**	***52***	**28**	18	14	16
3b)	24	*51*	27	18	14	17
3c)	24	*51*	28	17	15	16
5a)	**13**	***48***	**22**	17	14	16
5b)	12	*42*	14	17	14	17
5c)	14	*43*	13	17	14	17
6a)	**25**	***35***	**26**	17	14	16
6b	24	*31*	15	18	15	17
6c	24	*31*	14	17	14	16
8a	**25**	***37***	**14**	17	14	16
8b	23	*32*	14	18	14	16
8c	22	*34*	15	17	14	17
10a	**22**	***25***	**24**	17	14	16
10b	22	*22*	14	18	15	17
10c	21	*21*	15	18	14	17

pyridazine moiety it is not beneficial for antimicrobial activity. The influence of pyrrolo (**I**) moiety and substituient (**R**) seems to be negligible.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker Avance 400 DRX spectrometer at 400 MHz. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. IR spectra (ν, cm^{-1}) were recorded with a JASCO V-570 spectrometer in KBr. Melting points were determined on a MELTEMP II apparatus and are uncorrected. The microanalyses were in satisfactory agreement with the calculated values: C, ±15; H, ±10; N, ±30.

General Procedure for Synthesis of Salts (3a-c). A solution of 2-bromo-4'-methylacetophenone (2.13 g, 10 mmol, dissolved in 15 ml of anhydrous benzene) and diazine compound (10 mmol, dissolved in 10 ml of anhydrous benzene), was stirred for 3 h at room temperature to give the corresponding cycloimmonium salt (**3**). The obtained salts were collected by filtration and dried *in vacuo*. No purification required.

2-[2-(4-Methylphenyl)-2-oxoethyl]phthalazinium bromide (3b). This compound was obtained as white crystals, 3.36 g (98%), mp 112-115°C; ir: 3051(CH), 2986(CH), 1694(C=O), 1610, 1572, 1494, 1462 (C=C, C=N); ^{1}H nmr (DMSO-d_6): δ 11.49 (s, 1H, H1), 9.90 (s, 1H, H4), 8.77-8.74 (d, 1H, H8, J=8.4), 8.60-8.58 (d, 1H, H5, J=8.0), 8.37-8.35 (t, 1H, H6, J=7.2, 8.0), 8.27-8.25 (t, 1H, H7, J=7.2, 8.4), 7.95-7.93 (d, 2H, H12, J=8.0), 7.29-7.27 (d, 2H, H13, J=8.0), 6.91 (s, 2H, CH_2), 2.39 (s, 3H, CH_3); ^{13}C nmr: δ 188.96 (C=O),154.12 (C4), 153.50 (C1), 146.53 (C14), 139.73 (C6), 136.53 (C7), 131.40 (C8), 130.87 (C11), 129.93 (C13), 128.76 (C12), 128.31 (C5), 127.81 (C4a), 127.55 (C8a), 69.15 (CH_2), 21.91 (CH_3).

General Procedure to obtain [3 + 2] cycloadducts (5 - 10). The corresponding cycloimmonium salt (**3**) (2.0 mmol) was suspended in 10 ml of chloroform. A solution of an activated alkene or alkyne (2.2 mmol) and triethylamine (0.31 g, 3.0 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, then washed thoroughly three times with water (30 ml), the chloroform layer was dried on sodium sulfate, filtered and evaporated. The residue was crystallized from an appropriate solvent.

8-(4-Methylbenzoyl)-10-phenyl-8,8a,11a,11b-tetrahydro-pyrrolo[3',4':3,4]pyrrolo[2,1-*a*]phthalazine-9,11-dione (5b). This compound was obtained as white crystals (ethanol), 0.73 g (84%), mp 232-234°C; ir: 2952 (C-H), 1772, 1721 (C=O imide), 1692 (CO keto), 1603, 1565, 1498, 1449 (C=C, C=N); ^{1}H nmr (deuteriochloroform): δ 8.02-8.00 (d, 2H, H13, J=8.0), 7.52 (s, 1H, H5), 7.49-7.31 (m, 9H, H1, H2, H3, H4, 2H14, 2H18, H19), 7.02-7.00 (d, J=7.2, 2H, H17), 5.93 (s, 1H, H8), 4.69-4.67 (d, 1H, H11b, J=8.4), 4.18-4.16 (d, 1H, H8a, J=8.0), 3.74-3.70 (dd, 1H, H11a, J=8.0, 8.4), 2.51 (s, 3H, CH_3); ^{13}C nmr: δ 193.43 (C8b, keto), 177.48 (C9, keto imide), 174.87 (C11, keto imide), 144.89 (C15), 140.61 (C5), 135.06 (C16), 132.39 (C12), 131.23 (C2), 129.50 (C13), 129.31 (C18), 129.26 (C3), 129.12 (C14), 129.01 (C1), 128.24 (C1a), 128.21 (C19), 126.60 (C17), 126.23 (C4), 123.70 (C4a), 76.42 (C8), 59.67 (C11b), 51.43 (C11a), 46.90 (C8a), 21.91 (CH_3).

3-(4-Methylbenzoyl)-1,2,3,10b-tetrahydro-pyrrolo[2,1-*a*]-phthalazine-1-carbonitrile (6b). This compound was obtained as white crystals (methanol), 0.42 g (67%), mp 172-173°C; ir: 3051 (C-H), 2951 (C-H), 2232 (CN), 1694 (CO keto), 1597, 1561, 1492, 1451 (C=C, C=N); ^{1}H nmr (deuteriochloroform): δ 8.08-8.06 (d, 2H, H13, J=8.0), 7.46 (s, 1H, H6), 7.43-7.36 (m, 2H, H9, H10), 7.29-7.25 (m, 3H, 2H14, 1H8), 7.12-7.10 (dd, 1H, H7, J=8.00, 1.6), 5.73-5.70 (dd, 1H, H3, J=2.8, 8.4), 4.51-4,50 (d, 1H, H10b, J=7.2), 3.38-3.33 (8 lines: dxdxd, 1H, H1), 2.93-2.87 (8 lines: dxdxd, 1H, H2b, J=22.8, 2.8, 4.4), 2.37-2.30 (8 lines: dxdxd, 1H, H2a, J=22.8, 4.4, 8.4), 2.49 (s, 3H, CH_3); ^{13}C nmr: δ 195.58 (C=O), 144.90 (C15), 139.87 (C6), 132.34 (C12), 131.25 (C10), 129.56 (C13), 129.41 (C9), 129.12 (C14), 128.35 (C10a), 126.66 (C7), 126.40 (C8), 124.36 (CN), 120.33 (C6a), 70.32 (C3), 59.10 (C10b), 35.53 (C1), 29.14 (C2), 21.75 (CH_3).

7-(4-Methylbenzoyl)H pyrrolo[2,1-*a*]pyridazine-5,6-dicarboxylic acid dimethyl ester (8a). This compound was obtained as yellow crystals (ethanol), 0.1 g (12%), mp 176-178°C; ir: 3071 (C-H), 2954 (C-H), 1739 (C=O ester-1), 1708 (C=O ester-

2), 1635 (C=O keto), 1604, 1537, 1504, 1452 (C=C, C=N), 1247, 1105 (C-O-C); ^{1}H nmr (deuteriochloroform): δ 8.62-8.59 (dd , 1H, H4, J=9.2, J=1.6), 8.32-8.31 (dd, 1H, H2, J=4.4, J=1.6), 7.72-7.70 (d, 2H, H10, J=8.4), 7.26-7.24 (d, 2H, H11, J=8.4), 7.07-7.04 (dd, 1H, H3, J=4.4, J=9.2), 3.92 (s, 3H, CO_2CH_3-5), 3.59 (s, 3H, CO_2CH_3-6), 2.42 (s, 3H, CH_3). ^{13}C nmr: δ 184.26 (C=O), 163.24 (C7), 144.27 (C2), 143.22 (C12), 136.57 (C9), 129.62 (C11), 129.15 (C10), 128.34 (C4), 117.49 (C3), 103.21 (C5), 101.37 (C6), 53.69 (CO_2CH_3), 51.37 (CO_2CH_3), 21.98 (CH_3)

3-(4-Methylbenzoyl)-1,10b-dihydropyrrolo[2,1-*a*]phthalazine-1,2-dicarboxylic acid dimethyl ester (8b). This compound was obtained as yellow crystals (methanol), 0.46 g (58%), mp 179-180°C; ir: 3051 (C-H), 2958 (C-H), 1745 (C=O ester-1), 1720 (C=O ester-2), 1671 (C=O keto), 1598, 1557, 1496, 1454 (C=C, C=N), 1226, 1123 (C-O-C); ^{1}H nmr (deuteriochloroform): δ 8.42-40 (d, 2H, H13, J=8.0), 7.52-7.49 (t, 1H, H8, J=7.2, 8.4), 7.43 (s, 1H, H6), 7.41-7.37 (m, 2H, H9, H10), 7.31-7.29 (d, 2H, H14, J=8.0), 7.27-7.22 (d, 1H, H7, J=8.4), 5.29-5.25 (d, 1H, H10b, J=13.2), 4.51-4.47 (d, 1H, H1, J=13.2), 3.91 (s, CO_2CH_3-2), 3.50 (s, CO_2CH_3-1), 2.42 (s, 3H, CH_3); ^{13}C nmr: δ 187.92 (C=O), 166.71 (COO-1), 164.45 (COO-2), 153.23 (C3), 145.66 (C6), 142.17 (C15), 132.62 (C12), 131.71 (C10), 130.91 (C10a), 129.71 (C14), 129.48 (C13), 128.99 (C9), 125.78 (C7), 124.89 (C6a), 123.53 (C8), 101.51 (C2), 61.75 (C10b), 53.02 (CO_2CH_3-2), 52.36 (C1), 51.36 (CO_2CH_3-1), 21.92 (CH_3).

3-(4-Methylbenzoyl)-pyrrolo[2,1-*a*]phthalazine-1-carboxylic acid ethyl ester (10b). This compound was obtained as white crystals (ethanol), 0.29 g (42%), mp 189-190°C; ir: 3058 (C-H), 2956 (C-H), 1697 (C=O ester), 1668 (C=O keto), 1596, 1562, 1498, 1458 (C=C, C=N), 1228, 1125 (C-O-C); ^{1}H nmr (deuteriochloroform): δ 9.85-9.83 (d, 1H, H10, J=8.0), 8.74 (s, 1H, H6), 7.92-7.87 (m, 4H, 2H13, H9, H8), 7.76-7.72 (d, 1H, H7, J=8.0), 7.70 (s, 1H, H2), 7.33-7.31 (d, 2H, H14, J=8), 4.44-4.38 (q, 2H, CO_2CH_2, J=7.2), 2.47 (s, 3H, CH_3), 1.43-1.39 (t, 3H, $CO_2CH_2CH_3$, J=7.2); ^{13}C nmr: δ 184.48 (C=O), 164.31 (C=O est), 146.34 (C6), 143.28 (C15), 136.33 (C12), 134.27 (C3), 132.89 (C9), 132.12 (C10b), 130.02 (C13), 129.59 (C8), 129.07 (C14), 127.56 (C10), 127.29 (C7), 126.92 (C10a), 124.12 (C2), 122.15 (C6a), 108.15 (C1), 60.71 (CO_2CH_2), 21.70 (CH_3), 14.46 ($CO_2CH_2CH_3$).

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