SYNTHESIS AND CNS ACTIVITIES OF PYRIDOPYRAZINONE AND PYRIDODIAZEPINONE DERIVATIVES (*)

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Summary — New tricyclic derivatives with cyclocondensed pyrido-pyrazine 7,10 and pyrido-diazepine 20a,20b skeletons were synthetized and biologically investigated. The compounds, preliminarily tested on explorative, muscle relaxing, antinociceptive, spontaneous motor activities and influence on the narcotic effect of Evipan, revealed interesting CNS depressant and analgesic activities. The pyrido[2,3-e]pyrrolo[1,2-a]pyrazine structure of 7 appeared the most promising for analgesic and neuroleptic activities. The above compounds were assayed also for their capacity to inhibit DNA synthesis in Ehrlich ascites tumor cells; 20a appeared to be able of inducing a significant inhibition.

In the field of our research on heteropolycyclic structures that may show biological activities, we described in preceding papers pyrido-pyrazine and pyrido-diazepine derivatives with neuroleptic activity. As an extension of this study and with the aim of determining the influence resulting from the structural differences on pharmaceutical response, we have now prepared heterotricyclic systems, corresponding to general formula A, in which the above structures are fused with pyrrolidine or piperidine rings. These compounds were tested for the above pharmacological activity. Moreover, since various polycyclic compounds can interact with DNA and/or can inhibit the activity of some important enzymes involved in DNA replication, we studied also their ability to inhibit DNA synthesis in the Ehrlich cells, a well-known tumor cell line of the mouse.

The synthetic approach to the required 7,8-dihydro-5-methylpyrido[2,3-e]pyrrole[1,2-a]pyrazin-6,9(5H,6aH)-dione 7 and 6a,7,8,9-tetrahydro-5-methyl-5H-dipyrido[1,2-a:2,3-e]pyrazin-6,10-dione 14 consisted in the condensation in boiling ethanol of 3-amino-2-methylaminopyridine 1 with 2-ketoglutaric acid 3 and diethyl 2-oxoadipate 4 respectively, as depicted in Scheme 1. The carboxy derivatives 5 and 12 were reduced with sodium borohydride in diluted sodium hydroxide solution; occasionally, during the course of the reduction, a partial cyclization from 6 to 7 occurred, which was completed by heating in vacuo, whereas the cyclization of 13 to 14 was carried out by fusion in vacuo of the isolated intermediate. The synthesis of 10 was accomplished similarly starting from 2-amino-3-methylaminopyridine 2 and was realized in order to study the structure-activity correlations with the isomer 7.

The reaction to obtain the cycloomologues 20 (Scheme 2) was carried out using the above described procedure under different conditions. Starting from 1 and diethyl 2-oxoadipate 15a in hot xylene the expected 17a was obtained, while 1 and diethyl 2-oxopimelate 15b afforded 17b, in addition to a small amount of imidazo[2,3-b]pyridine derivative 16 whose formation can be explained by the cyclization of intermediate 16**7. Compounds 17a,b were hydrogenated with Raney nickel under pressure to 18a,b, whose alkaline hydrolysis gave 19a,b without concomitant cyclization. The fusion in vacuo of 19a,b afforded smoothly 7,7a,8,9-tetrahydro-5-methyl-5H-pyrido[2,3-b]pyrrole[1,2-d] diazepin-6,10-dione 20a and 7a,8,9,10-
tetrahydro-7-methyl-5H-dipyrido[1,2-d:2,3-b]diazepin-6,11(5H,7H)-dione 20b, respectively.

The structures of all described compounds were supported by analytical and spectroscopic data. In particular reduced 9 and 13 showed a complex triplet (δ 4.0) and an exchangeable signal (δ 6.5-7.0) attributable to the methine proton and to NH, respectively. Similarly, the homologous compounds 18a,b and 19a,b exhibited the methine signal at δ 4.0, while the exchangeable peak of NH shifts to δ 3.6-5.5.

The tricyclic compounds 7, 10 and 20a,b were investigated for CNS activities and tested for their capacity to inhibit DNA synthesis in Ehrlich ascites tumor cells.

EXPERIMENTAL SECTION

A) CHEMISTRY

Melting points were determined by the capillary method on a Büchi 510 apparatus and are uncorrected. UV spectra were measured in 95% ethanol with a Perkin-Elmer Model 550S spectrophotometer.
IR spectra were recorded on a Perkin-Elmer Model 297 spectrophotometer and 1H-NMR spectra were recorded on a Varian-Gemini 200 spectrometer with TMS as internal standard. Elemental analyses for C, H, N were performed on the Carlo Erba Elemental Analyser Model 1106 at the Microanalytical Laboratory, Istituto di Scienze Farmaceutiche, Università di Genova, and were within ±0.4% of the theoretical values.

**Reactions between aminoypyridines and α-ketoacids**

a) 2-(2-Carboxyethyl)-4-methyl-pyrido[2,3-b]pyrazin-3(4H)-one 1 - To a suspension of 2-ketogluutaric acid 3 (2.95 g, 20 mmole) in ethanol (15 ml) was added an ethanol solution (20 ml) of 3-amino-2-methylaminopyridine 1 (2.5 g, 20 mmole), obtained by hydrogenation at atmospheric pressure of 2-methylamino-3-nitropyridine, and the mixture was refluxed with stirring for 90 min. The solution was evaporated under reduced pressure to give 5 as a solid which was collected by filtration and recristallized from ethanol. mp 194-196 °C (yield 88%). UV: λmax nm (log ε): 220 (4.41), 322.6 (4.06), 331.3 (4.07); IR (KBr): 3190, 1740, 1635 cm⁻¹; 'H-NMR (DMSO-d₆): 8.23 (6a, 2H), 2.48 (t, J = 7.7 Hz, 2H), 3.02 (t, J = 7.2 Hz, 2H), 3.80 (s, 3H), 4.14 (q, J = 7.1 Hz, 1H), 7.31 (dd, J = 8.0 Hz, pyr-β-H), 8.13 (dd, J = 8.0 Hz, pyr-γ-H), 8.55 (dd, J = 2.5 Hz, pyr-α-H). Anal. (C₉H₇NO₃) CH,N.

b) 3-Carboxyethyl-4-methyl-pyrido[2,3-b]pyrazin-3(4H)-one 2 - In a similar manner, starting from 2-amino-3-methylaminopyridine 2 and 3, 8 was obtained in 60% yield, mp 210-213 °C (ethanol). UV: λmax nm (log ε): 224 (4.28), 328 sh (3.92), 331.3 (3.95); IR (KBr): 3400, 1710, 1660 cm⁻¹; 'H-NMR (CDCI₃): 6.27 (t, J = 6.4 Hz, 2H), 3.62 (s, 2H), 7.62 (dd, J = 7.6 Hz, pyr-β-H), 8.15 (dd, J = 7.2 Hz, pyr-γ-H), 8.58 (dd, J = 4.3 Hz, pyr-α-H), 12.18 (s, OH, exchangeable). Anal. (C₉H₇NO₃) CH,N.

c) 2-(2-Ethoxycarbonyl)4-methyl-pyrido[2,3-b]pyrazin-3(4H)-one 11 - Starting from 1 and diethyl 2-oxopimelate 15b (5.0 g, 22 mmole) in xylene (100 ml) was refluxed for 20 h. After cooling, the solution was extracted with 2N NaOH solution. The organic solution was washed with water, dried (Na₂SO₄) and evaporated to give 11 in 58% yield as needles, mp 175-177 °C (ethanol). UV: λmax nm (log ε): 224 (4.20), 269 (3.88), 294 (3.88); IR (KBr): 3250, 1725, 1690 cm⁻¹; 1H-NMR (DMSO-d₆): 6.18 (t, 2H), 2.34 (m, 2H), 2.58 (s, 3H), 3.36 (s, 3H), 4.05 (t, J = 6.0 Hz, 1H), 6.72 (dd, J = 8.0 Hz, pyr-β-H), 7.04 (s, NH, exchangeable), 7.24 (dd, J = 8.0 Hz, pyr-γ-H), 8.30 (dd, J = 4.8 Hz, pyr-α-H). Anal. (C₁₂H₁⁵NO₂) CH,N, 78.

**Pyridopyrazinoines and diazepinones derivatives**

Starting from 8, 3-(2-carboxyethyl)-3,4-dihydro-1-methylpyrido[2,3-b]pyrazin-1(2H)-one 9 was obtained in 52% yield by reduction with sodium borohydride, mp 163-164 °C (ethanol). UV: λmax nm (log ε): 213 (4.61), 265 (3.48); 316 (4.03); IR (KBr): 3250, 1725, 1690 cm⁻¹; 1H-NMR (DMSO-d₆): 6.18 (t, 2H), 2.34 (m, 2H), 2.58 (s, 3H), 3.36 (s, 3H), 4.05 (t, J = 6.0 Hz, 1H), 6.72 (dd, J = 8.0 Hz, pyr-β-H), 7.04 (s, NH, exchangeable), 7.24 (dd, J = 8.0 Hz, pyr-γ-H), 7.41 (dd, J = 4.0 Hz, pyr-α-H), 12.12 (br s, OH, exchangeable). Anal. (C₁₂H₁⁵NO₂) CH,N.

7,8-Dihydro-5-methyl-pyrido[2,3-c]pyrrole[1,2-a]pyrazin-6,9-(5H,6H)one 10

Sodium borohydride (1.0 g) was added to a solution of 5 (2 g, 9 mmole) in 2N NaOH (10 ml) and the mixture was allowed to stand at room temperature for 24 h. The mixture was cooled, the solution was triturated with ethanol and filtered off to give a solid which was suspended in 5% NaHCO₃ solution. The solid residue was dissolved in dichloromethane and extracted with a 5% NaHCO₃ solution. The organic solution was washed with water, dried (Na₂SO₄) and evaporated to give 10 in 80% yield as needles, mp 175-177 °C (ethanol). UV: λmax nm (log ε): 224 (4.20), 269 (3.88), 294 (3.88); IR (KBr): 1725, 1690 cm⁻¹; 1H-NMR (DMSO-d₆): 6.28-7.20 (m, 4H), 3.38 (s, 3H), 4.62 (t, J = 12 Hz, 1H), 7.31 (dd, J = 8.0 Hz, pyr-β-H), 7.37 (dd, J = 8.9 Hz, pyr-γ-H), 8.30 (dd, J = 4.6 Hz, pyr-α-H). Anal. (C₁₂H₁⁵NO₂) CH,N.

**Reaction of 3-Amino-2-methylaminopyridines with β-Ketoesters**

A solution of I (2.5 g, 20 mmole) and diethyl 2-oxopimelate 15b (5.0 g, 22 mmole) in xylene (100 ml) was refluxed for 20 h. After cooling, the solution was extracted with 2N HCl, the acid solution was made alkaline with 2N NaOH and extracted with dichloromethane. The oily residue obtained after evaporation of the combined extracts was dissolved in diethyl ether (10 ml) and let to stand in a freezer for a day, whereby a small amount (0.1 g) of 2-(3-ethoxycarbonyl)3-methyl-imidazo[2,3-b]pyridine 16 was obtained, mp 70-71 °C (diethyl ether). UV: λmax nm (log ε): 251 (3.65), 286 (4.08); IR (CHCl₃): 1725 cm⁻¹; 'H-NMR (CDCl₃): 6.17 (t, J = 8.5 Hz, 3H), 2.23 (m, 2H), 2.52 (t, J = 7.0 Hz, 2H), 2.98 (t, J = 7.0 Hz, 2H), 3.84 (s, 3H), 4.14 (q, J = 8.5 Hz, 2H), 7.18 (dd, J = 8.8 Hz, pyr-β-H), 7.97 (dd, J = 9.0 Hz, pyr-γ-H), 8.32 (dd, J = 4.2 Hz, pyr-α-H). Anal. (C₁₂H₁⁵NO₂) CH,N.

The oily residue obtained by evaporation of diethyl ether was chromatographed on basic alumina. By elution with dichloromethane, 3,5-dihydro-2-(ethylcarboxyaryl)3-methyl-4H-pyrido[2,3-b][1,4]diazepin-4-one 17b was collected as an oil (52% yield). UV: λmax nm (log ε): 294 (3.93); IR (CHCl₃): 1725, 1670 cm⁻¹; 'H-NMR (CDCl₃): 6.17 (t, J = 7.2 Hz, 3H), 2.08 (q, J = 6.5 Hz, 2H), 2.42 (t, J = 2.5 Hz, 2H), 2.68 (t, J = 6.5 Hz, 2H), 3.18 (m, 2H), 3.49 (s, 3H), 4.13 (q, J = 7.2 Hz, 2H), 7.19 (dd, J = 6.8 Hz, pyr-β-H), 7.66 (dd, J = 6.8 Hz, pyr-γ-H), 8.37 (dd, J = 4.4 Hz, pyr-α-H). Anal. (C₁₂H₁⁵NO₂) CH,N.
The reaction of 1 with diethyl 3-oxo-adipate 15a, carried out as described above, afforded, 3,5-dihydro-2-(2-carboxyethyl)-5-methyl-4H-pyrido[2,3-b][1,4]diazepin-4-one 17a, as a sole product, which in a small amount was separated from the diethyl ether solution of combined extracts. A further amount (55% overall yield) was collected by chromatography on basic alumina of the oily residue obtained by evaporation of diethyl ether. White crystals, mp 60-62°C (diethyl ether); UV: λmax nm (log ε): 255 (3.88); IR (KBr): 1725, 1660 cm⁻¹; ¹H-NMR (DMSO-d₆): 8.12 (t, J = 7.0 Hz, 3H), 2.93 (dd, J = 6.4 Hz, pyr (3-H), 7.38 (dd, J = 7.1 Hz, 2H), 3.18 (m, 2H), 3.48 (s, 3H), 4.26 (q, J = 7.0 Hz, 2H), 7.18 (dd, J = 5.8 Hz, pyr β-H), 7.61 (dd, J = 6.2 Hz, pyr γ-H), 8.37 (dd, J = 4.4 Hz, pyr α-H).

Anal. (C₅H₇N₂O₂) C₉H₇N.

A further elution gave unreacted 1 (8% yield).

From 19b, by an identical procedure, 1,2,3,5-tetrahydro-2-(3-carboxypropyl)-5-methyl-4H-pyrido[2,3-b][1,4]diazepin-4-one 19b in 76% yield, mp 157-159°C (ethanol). IR (KBr): 3310, 1715, 1630 cm⁻¹; ¹H-NMR (DMSO-d₆): 8.23 (m, 4H), 2.28 (m, 3H), 3.38 (m, 3H+1H), 3.78 (m, 1H), 5.42 (br s, NH, exchangeable), 7.00 (dd, J = 6.0 Hz, pyr β-H), 7.39 (dd, J = 6.0 Hz, pyr γ-H), 7.98 (dd, J = 4.2 Hz, pyr α-H), 12.02 (br s, OH, exchangeable). Anal. (C₈H₁₂N₂O₂) C₁₀H₁₀N₂O₂.

This activity was evaluated with the Boissier and Simon test using a square board 37 cm wide with 16 equidistant holes (2.2 cm diameter), on which each animal was kept for 5 min and the explored holes were counted. Control animals received only PEG 200. Diazepam (5 mg/kg s.c.) was used as a reference drug (Table 1, Fig. 1).

**EXPLORATORY ACTIVITY**

It was detected with the Boissier and Simon test using a square board 37 cm wide with 16 equidistant holes (2.2 cm diameter), on which each animal was kept for 5 min and the explored holes were counted. Control animals received only PEG 200. Diazepam (5 mg/kg s.c.) was used as a reference drug (Table 1, Fig. 1).

**Motor Coordination (Muscle Relaxing Activity)**

This activity was evaluated with the Kinnard and Carr method, using a "Rotarod" apparatus (U. Basile, Milano) turning at 16 rpm. Six hours before dosing, animals were selected; only those remaining on the turning rod for more than 120 sec were utilized. To these animals (5 for each compound) the test substances were given 30 min before the test. Mice that remained on the turning rod for less than 2 minutes were considered incoordinated. Diazepam (5 mg/kg s.c.) was used as a reference drug (Table 2, Fig. 2).
Pyridopyrazinones and diazepinones derivatives

Fig. 1 - Explorative activity.

Fig. 2 - Muscle relaxing activity.

Fig. 3 - Spontaneous motor activity.

**ANTINOCICEPTIVE ACTIVITY (HOT PLATE TEXT)**

The method of Woolfe-McDonald" was used, employing 10 animals for each compound. The animals were placed on a stainless steel plate at 55 ±0.5 °C and the mean reaction time was determined for each group of mice just before the administration of the test compounds and 30, 60, 120 and 180 min after the dosing. The percent reaction time variations were referred to initial values. The animals with an initial reaction time superior to 10 sec were discarded. Morphine was used as reference drug at the dose of 10 mg/kg s.c. (Table 4, Fig. 4).

**INFLUENCE ON THE NARCOTIC EFFECT OF EVIPAN**

The influence on the narcotic effect of Evipan was evaluated by strengthening of sleep. Thirty minutes before the intraperitoneal injection of Evipan (100 mg/kg), the compounds were administered s.c. at the dose of 1/5 mmole/kg dissolved in 10 ml/kg of PEG 200. The sleeping time exobarbital induced was measured against that of a series of control animals. The average length of sleeping time was measured, and the percentage of increased sleep in respect to the control animals. The control animals received only Evipan (Table 5, Fig. 5).

**TABLE 2 - MUSCLE RELAXING ACTIVITY**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose mg/kg</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Incoordinate</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>43*</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>46*</td>
<td>4</td>
</tr>
<tr>
<td>20a</td>
<td>46*</td>
<td>3</td>
</tr>
<tr>
<td>20b</td>
<td>49*</td>
<td>5</td>
</tr>
</tbody>
</table>

* 1/5 mmole/kg/10 ml PEG 200.

**SPONTANEOUS MOTOR ACTIVITY**

Cages similar to those used by Raphaelson and Rabin" were utilized and the number of movements of the animals were recorded. The number of spontaneous movements were registered 30 min after the s.c. administration of the compounds and every 30 min for 3 h (Table 3, Fig. 3).
### TABLE 3 - SPONTANEOUS MOTOR ACTIVITY.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>30'</th>
<th>60'</th>
<th>90'</th>
<th>120'</th>
<th>150'</th>
<th>180'</th>
<th>Average</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td>1146</td>
<td>793</td>
<td>284</td>
<td>79</td>
<td>108</td>
<td>621</td>
<td>505</td>
<td>-35</td>
</tr>
<tr>
<td>Diazepam</td>
<td>5</td>
<td>509</td>
<td>363</td>
<td>583</td>
<td>322</td>
<td>58</td>
<td>125</td>
<td>327</td>
<td>-61</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>524</td>
<td>136</td>
<td>139</td>
<td>247</td>
<td>57</td>
<td>93</td>
<td>199</td>
<td>-5</td>
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<td></td>
<td>14</td>
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<td>404</td>
<td>361</td>
<td>90</td>
<td>259</td>
<td>477</td>
<td>-44</td>
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<tr>
<td></td>
<td>20a</td>
<td>46</td>
<td>444</td>
<td>69</td>
<td>202</td>
<td>93</td>
<td>437</td>
<td>284</td>
<td>-44</td>
</tr>
<tr>
<td></td>
<td>20b</td>
<td>49</td>
<td>741</td>
<td>515</td>
<td>459</td>
<td>147</td>
<td>512</td>
<td>589</td>
<td>17</td>
</tr>
</tbody>
</table>

* : 0.2 mmoles/Kg / 10 ml

### TABLE 4 - ANTINO CICEPTIVE ACTIVITY - HOT PLATE TEST.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose mg/Kg</th>
<th>0</th>
<th>30' %</th>
<th>60' %</th>
<th>120' %</th>
<th>180' %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>10</td>
<td>7.03±0.65</td>
<td>11.63±1.75</td>
<td>65</td>
<td>13.52±1.18</td>
<td>92</td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>6.42±0.86</td>
<td>10.18±1.95</td>
<td>59</td>
<td>8.98±1.08</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>46</td>
<td>7.37±0.73</td>
<td>9.68±1.70</td>
<td>31</td>
<td>11.80±1.89</td>
<td>60</td>
</tr>
<tr>
<td>20a</td>
<td>46</td>
<td>7.97±0.67</td>
<td>7.25±0.56</td>
<td>-9</td>
<td>11.72±1.75</td>
<td>47</td>
</tr>
<tr>
<td>20b</td>
<td>49</td>
<td>8.40±0.56</td>
<td>10.88±1.18</td>
<td>29</td>
<td>14.20±1.96</td>
<td>69</td>
</tr>
</tbody>
</table>

Mean reaction time in seconds (± S.E.) after the dosing (% Change relative to 0 value)

Statistical analysis was performed using “t” test for paired data, at second hour

* : p < 0.05; ** : p < 0.01; n = 6 for each substance

### TABLE 5 - INFLUENCE ON THE NARCOTIC EFFECT OF EVIPAN.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>2.5</td>
<td>205**</td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>145**</td>
</tr>
<tr>
<td>14</td>
<td>46</td>
<td>72**</td>
</tr>
<tr>
<td>20a</td>
<td>46</td>
<td>38*</td>
</tr>
<tr>
<td>20b</td>
<td>49</td>
<td>39*</td>
</tr>
</tbody>
</table>

For statistical analysis “t” test was used for unpaired data. *: p<0.05; **: <0.01; n. 5 for each substance

### C) BIOLOGICAL ACTIVITY

**Ehrlich cells**

Ehrlich ascites tumor (Lettré strain from Heidelberg) was routinely transferred by injecting intraperitoneally 2 x 10⁶ cells per animal into NCL mice. For the experiments, the tumor cells, collected on the 6th 7th day after the transplant, were suspended (2 x 10⁷ ml⁻¹) in Hank’s solution containing the compound to be tested and were incubated at 37 °C for 30 min; then, 40 KBq ml⁻¹ of ³H-thymidine

Fig. 5 - Influence on the narcotic effect of evipan (Sleep strengthening).

**Statistical analysis**

The data are expressed as mean values ± standard error of five or six animals and percent variation.

The statistical analyses were performed by using “t” test for unpaired data or “t” test II for paired data (hot-plate) at a significance level 5% or 1%.
of the explorative activity and that of 7 was the same of evipan (Exobarbital).

Depressant effect on CNS and good analgesic activity are illustrated in Tables 1-5 and Figures 1-5.

TABLE 6 - DNA SYNTHESIS INHIBITION IN EHRLICH CELLS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>93.66</td>
<td>1.77</td>
</tr>
<tr>
<td>14</td>
<td>Not detectable</td>
<td></td>
</tr>
<tr>
<td>20a</td>
<td>20.91</td>
<td>1.6</td>
</tr>
<tr>
<td>20b</td>
<td>Not detectable</td>
<td></td>
</tr>
</tbody>
</table>

IDS<sub>50</sub>± Standard deviation expressed μg/kg.

RESULTS AND DISCUSSION

The results of the pharmacological screening on pyridopyrazinones 7, 14 and pyridodialpine 20, 20b are illustrated in Tables 1-5 and Figures 1-5.

It is worthy to note that all compounds have marked depressant effect on CNS and good analgesic activity (central analgesia) at 1/5 mmol/kg. Compound 20b markedly reduced the spontaneous motor activity, less active 20a and diazepam, instead 20b increases it.

All compounds exhibited a appreciable depression of the explorative activity and that of 7 was the same as diazepam.

Concerning the motor coordination, 20b produced an incoordination comparable to diazepam while 7 and 14 were less active.

Compound 7 markedly potentiated the narcotic effect of evipan (Exobarbital).

Results of the hot plate test, kind of analgesia, typically overspinal showed a good central analgesic activities particularly produced for 7 and 14. 7 enhanced reaction time (+108% at 120') being the most potent one at the second hour after the administration, compared with that of morphine. Other tested compounds also showed a stronger activity at the second hour.

Compounds 14 and 20b, tested for their capacity to inhibit DNA synthesis in Ehrlich ascites tumor cells, did not induce a significant inhibition while the compound 7 and still more 20a appeared to be able of inducing a significant inhibition (Table 6).

In conclusion, from these preliminary pharmacological assays, it appears that the tested compounds revealed interesting CNS depressant and analgesic activities particularly appreciable on the pyridopyrazinone cyclohomologues 7 and 14 and support our interest on these tricyclic structures whose potentialities will be further investigated.

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