IL FARMACO, **49** (4), 259-265; 1994

SYNTHESIS AND CNS ACTIVITIES OF PYRIDOPYRAZINONE AND PYRIDODIAZEPINONE DERIVATIVES (*) ([1])

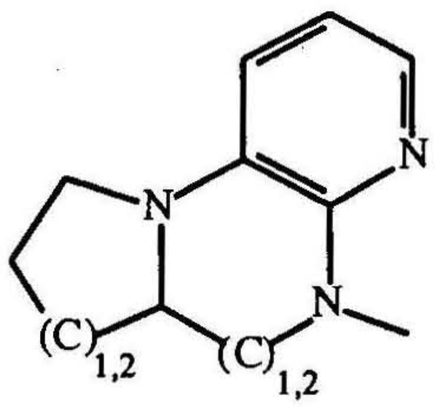
FRANCESCO SAVELLI (**), ALESSANDRO BOIDO
Istituto di Scienze Farmaceutiche, Università di Genova
Viale Benedetto XV 3, 16132 Genova (Italy)

MARGHERITA SATTA, ALESSANDRA PEANA
Cattedra di Farmacognosia, Università di Sassari
Via Muroni 23, 07100 Sassari (Italy)

CRISTINA MARZANO
Dipartimento di Scienze Farmaceutiche, Centro di studio sulla Chimica del Farmaco e dei Prodotti biologicamente attivi del C.N.R. Via Marzolo 5, 35131 Padova (Italy)

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[2,3] that may show biological activities, we described in preceding papers pyrido-pyrazine[4] and pyrido-diazepine derivatives[5] 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 policyclic 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.



A

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 **3** 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***[6,7]. 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-

(*) Part III of the series "Heterotricyclic systems"
(**) To whom correspondence should be addressed.

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.

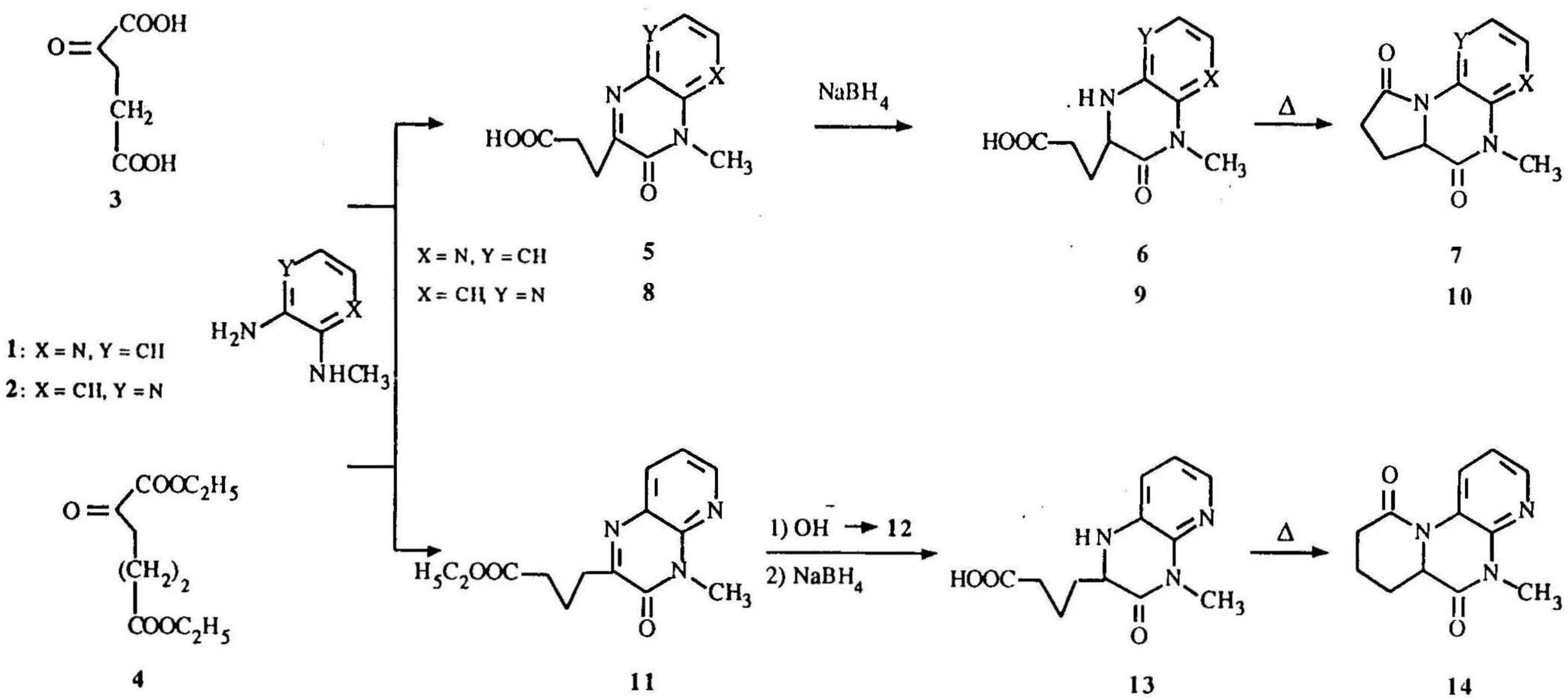
The tricyclic compounds **7**, **10** and **20a,b** were investigated for CNS activities and tested for their capacity to inhibit DNA synthesis in Ehrlich ascites tumor cells.

EXPERIMENTAL SECTION

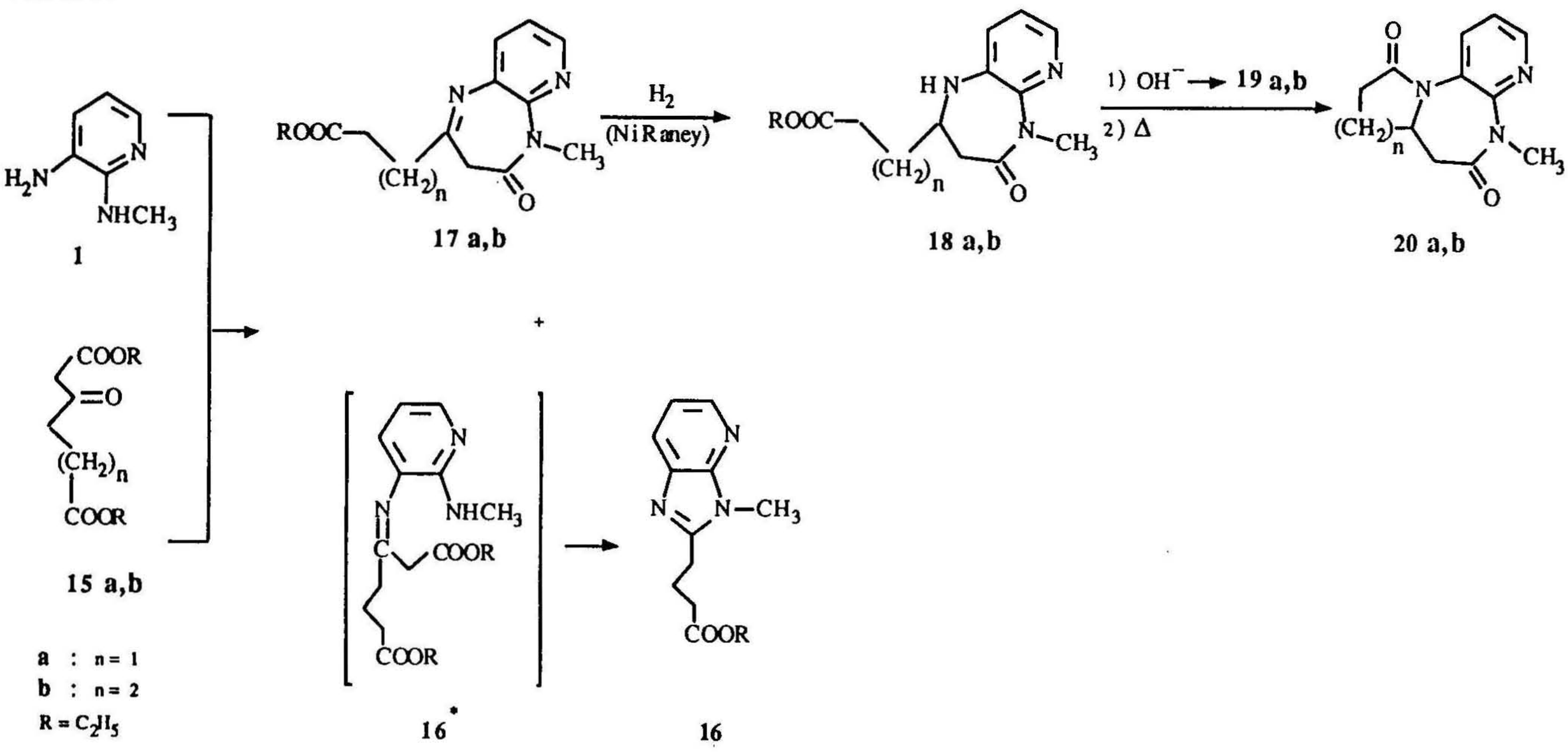
A) Chemistry

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.

SCHEME 1



SCHEME 2



IR spectra were recorded on a Perkin-Elmer Model 297 spectrophotometer and ^{1}H-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 aminopyridines and α-ketoacids

a) 2-(2-Carboxyethyl)-4-methyl-pyrido[2,3-b]pyrazin-3(4H)-one ***5*** - To a suspension of 2-ketoglutaric acid **3** (2.95 g, 20 mmoles) in ethanol (15 ml) was added an ethanol solution (20 ml) of 3-amino-2-methylaminopyridine **1** (2.5 g, 20 mmoles), 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 recrystallized from ethanol. mp 194-196 °C (yield 88%). UV: λ_{max} nm (log ε): 220 (4.41), 322 sh (4.06), 331 (4.07); IR (KBr): 3190, 1740, 1635 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.93 (t, J = 6.8 Hz, 2H), 3.30 (t, J = 6.8 Hz, 2H), 3.82 (s, 3H), 7.30 (dd, J = 7.9 Hz, pyr β-H), 8.09 (dd, J = 7.9 Hz, pyr γ-H), 8.56 (dd, J = 4.6 Hz, pyr α-H), 10.10 (br s, OH, exchangeable).

Anal. ($C_{11}H_{11}N_3O_3$) C,H,N.

b) 3-(2-Carboxyethyl)-4-methyl-pyrido[2,3-b]pyrazin-2(1H)-one ***8*** - 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: λ_{max} nm (log ε): 222 (4.28), 328 sh (3.92), 331 (3.95); IR (KBr): 3400, 1710, 1660 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.78 (t, J = 6.4 Hz, 2H), 3.14 (t, J = 6.4 Hz, 2H), 3.62 (s, 3H), 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. ($C_{11}H_{11}N_3O_3$) C,H,N.

c) 2-(2-Ethylcarboxypropyl)-4-methyl-pyrido[2,3-b]pyrazin-3(4H)-one ***11*** - Starting from **1** and diethyl 2-oxo-adipate **4**[8] and following the same procedure described above, **11** was obtained in 85% yield, mp 117-119 °C (ethanol). IR ($CHCl_3$): 1725, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.26 (t, J = 7.2 Hz, 3H), 2.17 (m, 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, 2H), 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. ($C_{14}H_{17}N_3O_3$) C,H,N.

7,8-Dihydro-5-methyl-pyrido[3,2-e]pyrrole[1,2-a]pyrazin-6,9(5H,6aH)-dione **7**

Sodium borohydride (1.0 g) was added to a solution of **5** (2 g, 9 mmoles) in 2N NaOH (10 ml) and the mixture was allowed to stand at room temperature for 24 h. The mixture was acidified with 10% solution of tartaric acid, extracted several times with dichloromethane, the combined extracts were washed with water, dried (Na_2SO_4) and evaporated to give an oily residue which was let to stand *in vacuo* at 150 °C for 1 h. After cooling, the residue was triturated with ethanol and filtered off to give a solid which was suspended in a 5% solution of $NaHCO_3$ with stirring for 15 minutes. Compound **7** was obtained in 46% yield, mp 141-144 °C (ethanol). UV: λ_{max} nm (log ε): 224 (4.42), 293 (4.11); IR ($CHCl_3$): 1705, 1690 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ2.62 (m, 4H), 2.54 (s, 3H), 4.41 (t, J = 7.0 Hz, 1H), 7.08 (dd, J = 8.0 Hz, pyr β-H), 8.19 (dd, J = 4.2 Hz, pyr γ-H), 8.41 (dd, J = 7.3 Hz, pyr α-H).

Anal. ($C_{11}H_{11}N_3O_2$) C,H,N.

6a,7,8,9-Tetrahydro-5-methyl-5H-dipyrido[1,2-a:2,3-e]pyrazin-6,10-dione **14**

Compound **11** (2 g, 7 mmoles) was suspended in 2N NaOH (10 ml) and stirred for 6 h at room temperature. The mixture was washed with diethyl ether, neutralized with 10% solution of tartaric acid and extracted with dichloromethane to give in 80% yield 2-(3-carboxypropyl)-4-methyl-pyrido[2,3-b]pyrazin-3(4H)-one **12**, mp 201-203 °C (ethanol). IR ($CHCl_3$): 1710, 1660 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ1.97 (q, J = 7.1 Hz, 2H), 2.40 (t, J = 7.3 Hz, 2H), 2.88 (t, J = 7.3 Hz, 2H), 3.67 (s, 3H), 7.42 (dd, J = 7.6 Hz, pyr β-H), 8.22 (dd, J = 7.9 Hz, pyr γ-H), 8.61 (dd, J = 4.7 Hz, pyr α-H), 12.07 (OH, exchangeable).

Anal. ($C_{12}H_{13}N_3O_3$) C,H,N.

Compound **12** was reduced to **13** with sodium borohydride, as reported for **7**. The reaction mixture was neutralized (10% tartaric acid solution), saturated with NaCl and extracted several times with dichloromethane. The organic solution was dried (Na_2SO_4) and evaporated to give 2-(3-carboxypropyl)-1,2-dihydro-4-methyl-pyrido[2,3-b]pyrazin-3(4H)-one **13** in yields not higher than 38%, mp 118-121 °C (ethanol). IR (Kbr): 3350, 1690, 1630 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ1.62 (m, 4H), 2.24 (m, 2H), 3.38 (s, 3H), 3.92 (m, 1H), 6.40 (br s, NH exchangeable), 6.88 (dd, J = 7.8 Hz, pyr β-H), 7.08 (dd, J = 8.0 Hz, pyr γ-H), 7.70 (dd, J = 2.4 Hz, pyr α-H), 12.02 (br s, OH, exchangeable).

Anal. ($C_{12}H_{15}N_3O_3$) C,H,N.

Compound **13** was cyclized to **14** in 65% yield by fusion under the condition described for **10**, mp 110-112 °C (ethanol). UV: λ_{max} nm (log ε): 287 (3.97); IR ($CHCl_3$): 1685 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ1.92 (m, 2H), 2.22 (m, 1H), 2.58 (m, 3H), 3.51 (s, 3H), 4.08 (t, J = 6.2 Hz, 1H), 7.08 (dd, J = 8.8 Hz, pyr β-H), 7.38 (dd, J = 9.0 Hz, pyr γ-H), 8.30 (dd, J = 4.8 Hz, pyr α-H).

Anal. ($C_{12}H_{13}N_3O_2$) C,H,N.

7,8-Dihydro-5-methyl-pyrido[3,2-e]pyrrole[1,2-a]pyrazin-6,9(5H,6aH)-dione **10**

Starting from **8**, 3-(2-carboxyethyl)-3,4-dihydro-1-methyl-pyrido[2,3-b]pyrazin-2(1H)-one **9** was obtained in 52% yield by reduction with sodium borohydride, mp 163-164 °C (ethanol). λ_{max} nm (log ε): 213 (4.61), 265 (3.48); 316 (4.03); IR (KBr): 3250, 1720, 1690 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ1.88 (m, 2H), 2.34 (m, 2H), 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. ($C_{11}H_{13}N_3O_3$) C,H,N.

Compound **9** gave, by heating *in vacuo* at melting point for 1 h, a solid residue which was dissolved in dichloromethane and extracted with a 5% $NaHCO_3$ solution. The organic solution was washed with water, dried (Na_2SO_4) and evaporated to give **10** in 58% yield as needles, mp 175-177 °C (ethanol). UV: λ_{max} nm (log ε): 224 (4.20), 269 (3.88), 294 (3.88); IR ($CHCl_3$): 1725, 1690 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ2.28-2.70 (m, 4H), 3.38 (s, 3H), 4.62 (t, J = 12 Hz, 1H), 7.31 (dd, J = 8.0 Hz, pyr β-H), 7.67 (dd, J = 8.9 Hz, pyr γ-H), 8.30 (dd, J = 4.6 Hz, pyr α-H).

Anal. ($C_{11}H_{11}N_3O_2H_2O$) C,H,N.

Reaction of 3-Amino-2-methylaminopyridine with β-Ketoesters

A solution of **1** (2.5 g, 20 mmoles) and diethyl 2-oxopimelate **15b** (5.0 g, 22 mmoles) 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-ethylcarboxypropyl)-3-methyl-imidazo[2,3-b]pyridine **16** was obtained, mp 70-71 °C (diethyl ether). UV: λ_{max} nm (log ε): 251 (3.65), 286 (4.08); IR ($CHCl_3$): 1725 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ1.26 (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. ($C_{13}H_{17}N_3O_2$) C,H,N.

The liquid residue obtained by evaporation of diethyl ether was chromatographed on basic alumina. By elution with dichloromethane, 3,5-dihydro-2-(ethylcarboxypropyl)-5-methyl-4H-pyrido[2,3-b][1,4]diazepin-4-one **17b** was collected as an oil (52% yield). UV: λ_{max} nm (log ε): 294 (3.93); IR ($CHCl_3$): 1725, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ1.76 (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. ($C_{15}H_{19}N_3O_3$) C,H,N.

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

The reaction of **1** with diethyl 3-oxo-adipate **15a**[9], carried out as described above, afforded, 3,5-dihydro-2-(ethylcarboxyethyl)-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: λ_{max} nm (log ϵ): 295 (3.90); IR ($CHCl_3$): 1725, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ1.24 (t, J = 7.0 Hz, 3H), 2.74 (t, J = 6.2 Hz, 2H), 2.96 (t, J = 6.2 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. ($C_{14}H_{17}N_3O_3$) C,H,N.

Reduction of **17a,b** to **18a,b**

An ethanol suspension of **17a** (2.75 g, 10 mmoles) and Raney nickel (3 g) was shaken at room temperature in a Parr apparatus under 60 psi of hydrogen. After 12 h the uptake of hydrogen ceased, the catalyst was filtered off and washed with ethanol. The filtrate was concentrated to dryness under reduced pressure affording a crude semisolid product which was triturated with diethyl ether, filtered and the solid residue was purified either by chromatography on neutral alumina or by crystallization from ethanol.

1,2,3,5-tetrahydro-2(2-ethylcarboxyethyl)-5-methyl-4H-pyrido[2,3-b][1,4]diazepin-4-one **18a** was obtained, in 65% yield, as an oil. UV: λ_{max} nm (log ϵ): 258 (3.69), 308 (3.74); IR ($CHCl_3$): 1725, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ1.25 (t, J = 7.0 Hz, 3H), 1.91 (m, 2H), 2.30-2.70 (m, 4H), 3.41 (s, 3H), 3.68 (s, NH, exchangeable), 3.95 (m, 1H), 4.14 (q, J = 7.0 Hz, 2H), 6.98 (dd, J = 8.0 Hz, pyr β-H), 7.16 (dd, J = 8.6 Hz, pyr γ-H), 8.11 (dd, J = 4.2 Hz, pyr α-H).

Anal. ($C_{14}H_{19}N_3O_3$) C,H,N.

From **17b**, by an identical procedure, 1,2,3,5-tetrahydro-2(3-ethylcarboxypropyl)-5-methyl-4H-pyrido[2,3-b][1,4]diazepin-4-one **18b**, was obtained in 55% yield as a microcrystalline powder; mp 108-109 °C. UV: λ_{max} nm (log ϵ): 261 (3.88), 307 (3.77); IR ($CHCl_3$): 1725, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ1.26 (t, J = 7.0 Hz, 3H), 1.69 (m, 4H)), 2.38 (m, 3H), 2.61 (dd, J = 13.0 Hz, 1H), 3.49 (s, 3H), 3.60 (br s, NH, echangeable), 3.89 (m, 1H), 4.16 (q, J = 7.0 Hz, 2H), 6.89 (dd, J = 6.4 Hz, pyr β-H), 7.19 (dd, J = 6.4 Hz, pyr γ-H), 8.11 (dd, J = 4.2 Hz, pyr α-H).

Anal. ($C_{15}H_{21}N_3O_3$) C,H,N.

It was often difficult to purify the reduced products **18a** and **18b** from unreacted **17a** and **17b**. In this case we submitted the unresolved mixture to the next reaction, because the unreacted **17a,b** cannot cyclize to **20a,b**.

7,7a,8,9-Tetrahydro-5-methyl-5H-pyrido[2,3-b]pyrrole[1,2-d][1,4]diazepin-6,10-dione **20a** and 7a,8,9,10-Tetrahydro-5-methyldipyrido[2,3-b: 1,2-d][1,4]diazepin-6,11(5H,7H)-dione **20b**.

Compound **18a** (2.5 g, 10 mmoles) was suspended in 2N NaOH (10 ml). After stirring at room temperature for 6 h, the alkaline solution was washed with dichloromethane, neutralized with 10% solution of tartaric acid and extracted with dichloromethane. The solution was dried (Na_2SO_4) and evaporated to dryness to give 1,2,3,5-tetrahydro-2-(2-carboxyethyl)-5-methyl-4H-pyrido[2,3-b][1,4]diazepin-4-one **19a** as an oil which was triturated with diethyl ether and recrystallized from ethanol, mp 143-145 °C. IR (KBr): 3310, 1710, 1625 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ1.70 (m, 2H), 2.30 (m, 3H), 3.23 (s, 3H), 3.38 (m, 1H), 3.78 (m, 1H), 5.46 (br s, NH, exchangeable), 7.08 (dd, J = 7.9 Hz, pyr β-H), 7.38 (dd, J = 7.1 Hz, pyr γ-H), 7.79 (dd, J = 4.5 Hz, pyr α-H), 12.12 (br s, OH, exchangeable).

Anal. ($C_{12}H_{15}N_3O_3$) C,H,N.

Compound **19a** was kept up at melting temperature under reduced pressure (10^{-1} mm Hg) for one hour. After cooling the residue was suspended in dichloromethane and washed with 5% solution of $NaHCO_3$ solution. The organic solution was dried (Na_2SO_4) and evaporated to dryness to give an oily residue which was chromatographed on neutral alumina eluting with dichloromethane, whereby **20a** was collected (69% yield), mp 139-141 °C (ethanol-diethyl ether). UV: λ_{max} nm (log ϵ): 283 (3.85); IR ($CHCl_3$): 1700, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ2.25 (m, 2H), 2.60 (m, 3H), 2.93 (dd, J = 16.0 Hz, 1H), 3.41 (s, 3H), 4.38 (m, 1H), 7.23 (dd, J = 10.0 Hz, pyr β-H), 7.72 (dd, J = 9.5 Hz, pyr γ-H), 8.46 (dd, J = 5.0 Hz, pyr α-H).

Anal. ($C_{12}H_{13}N_3O_2$) C,H,N.

Starting from **18b** and following the above 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}; ^{1}H-NMR (DMSO-d_6): δ1.56 (m, 4H), 2.28 (m, 3H), 3.38 (m, 3H + 1H), 3.78 (m, 1H), 5.42 (br s, NH, exchangeable), 7.08 (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. ($C_{13}H_{17}N_3O_3$) C,H,N.

Compound **19b** was converted to **20b** (76% yield) by fusion *in vacuo*, mp 138-139 °C; UV: λ_{max} nm (log ϵ): 288 (3.86); IR ($CHCl_3$): 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ2.03 (m, 4H), 2.50 (m, 3H), 2.81 (dd, J = 14.0 Hz, 1H), 3.42 (s, 3H), 4.14 (m, 1H), 7.22 (dd, J = 9.8 Hz, pyr β-H), 7.69 (dd, J = 9.0 Hz, pyr γ-H), 8.42 (dd, J = 4.8 Hz, pyr α-H).

Anal. ($C_{13}H_{15}N_3O_2$) C,H,N.

B) Pharmacology

The following investigation have been worked out on the heterotricyclic compounds **7,14,20a** and **20b**: explorative, muscle relaxing, spontaneous motor, antinociceptive activities and influence on the narcotic effect of Evipan.

Material and methods

For all the above tests, male Swiss albino mice were used. The animals, weighing 18-22 g, were housed at constant temperature (20-22 °C). The test compounds were administered s.c. at the dose of 1/5 mmole dissolved in 10 ml/kg of PEG 200, 30 min before tests.

Explorative activity

It was detected with the Boissier and Simon test[10] 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).

TABLE 1 - EXPLORATIVE ACTIVITY

Compound	Dose (mg/kg)	Number of explored holes	±S.E.	Var. %
Controls		24.4	4.66	
Diazepam	5	4.8**	0.86	-80
7	43	4.6**	1.6	-81
14	46	7.6**	1.91	-69
20 a	46	11.4*	1.32	-53
20 b	49	10.6*	1.21	-57

Values are expressed as mean ± S.E. of animals. For statistical analysis "t" test was used. *$p < 0.05$; ** $p < 0.01$; n. 5 for each substance.

Motor coordination (muscle relaxing activity)

This activity was evaluated with the Kinnard and Carr[11] 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 incoordinate. Diazepam (5 mg/kg s.c.) was used as a reference drug (Table 2, Fig. 2).

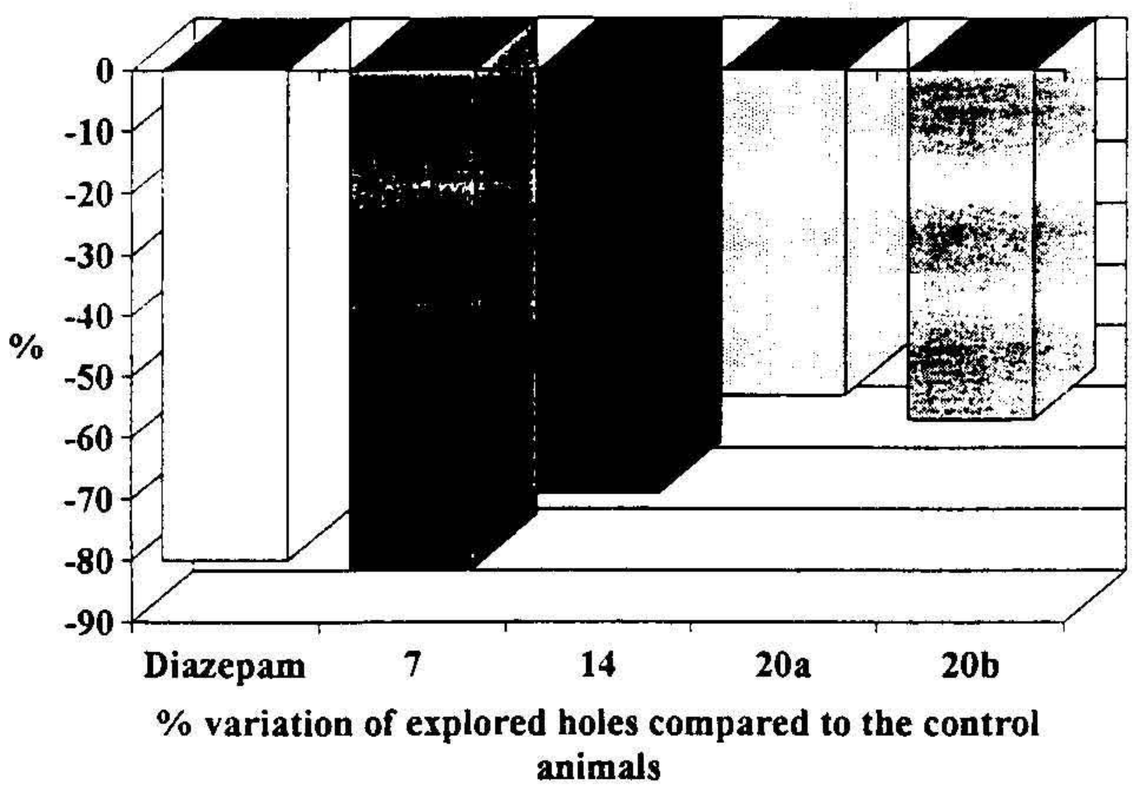


Fig. 1 - Explorative activity.

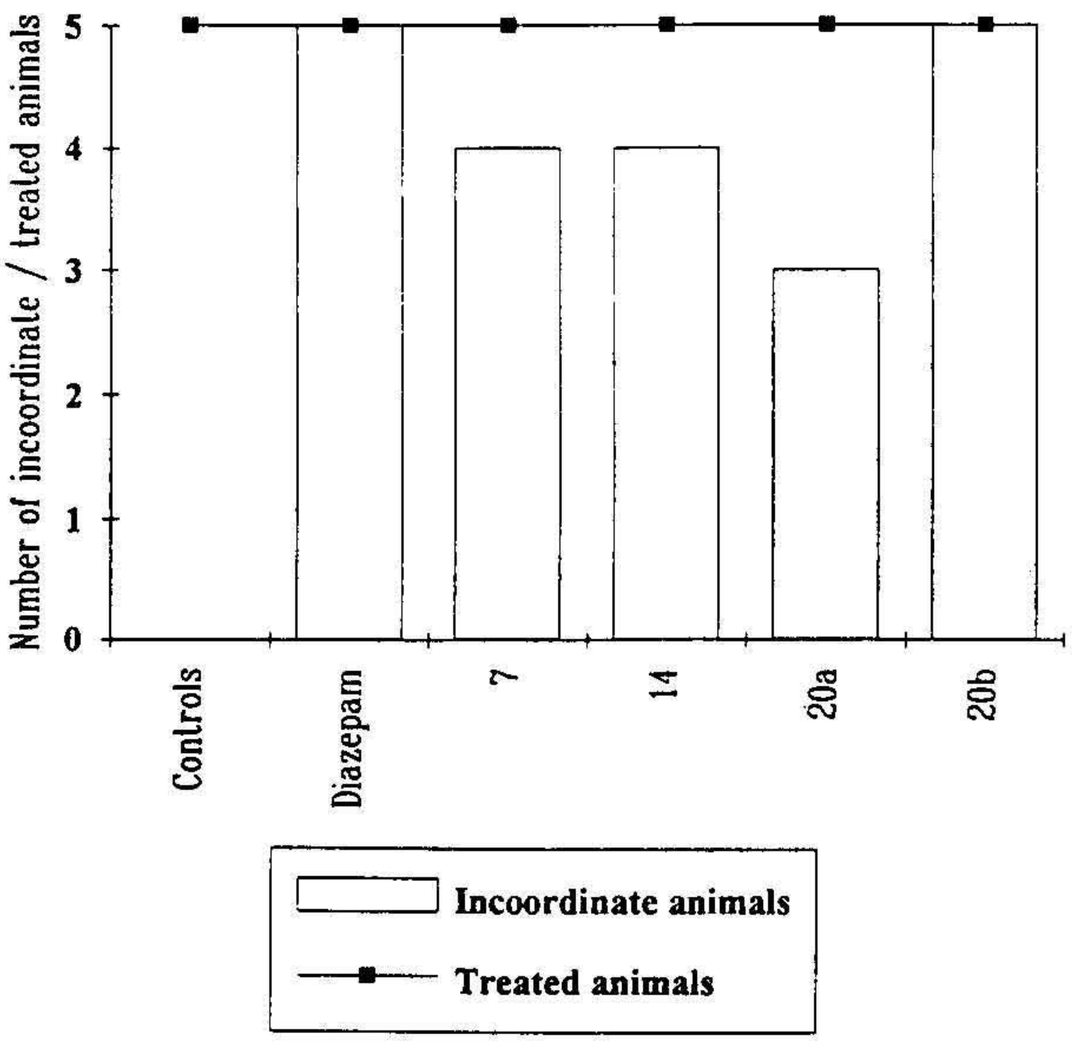


Fig. 2 - Muscle relaxing activity.

TABLE 2 - MUSCLE RELAXING ACTIVITY

Compoud	Dose mg/kg	Number of animals *Incoordinate*	*Treated*
Controls		0	5
Diazepam	5	5	5
7	43*	4	5
14	46*	4	5
20 a	46*	3	5
20 b	49*	5	5

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

SPONTANEOUS MOTOR ACTIVITY

Cages similar to those used by Raphaelson and Rabin[12] 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).

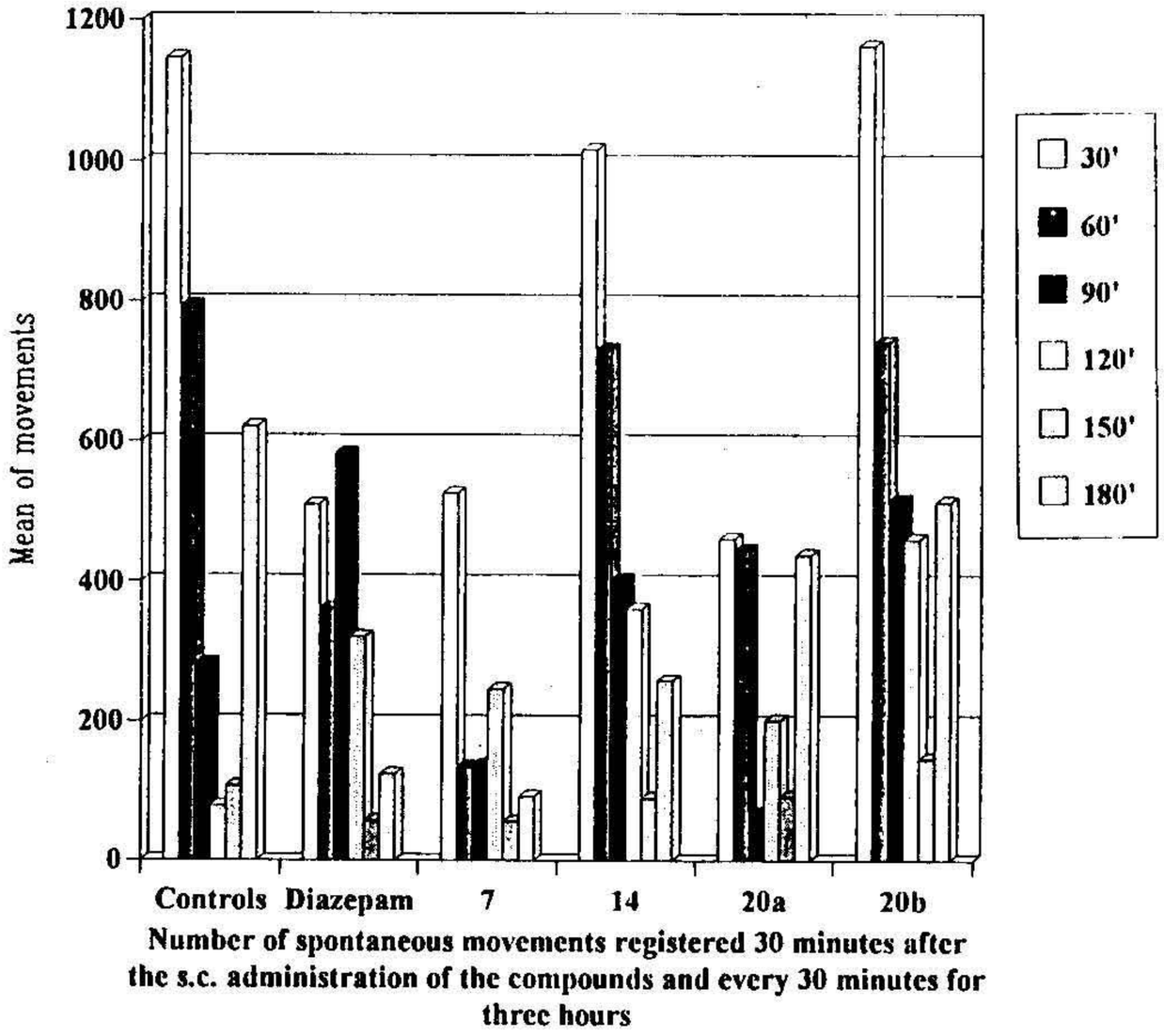


Fig. 3 - Spontaneous motor activity.

ANTINOCICEPTIVE ACTIVITY (HOT PLATE TEXT)

The method of Woolfe - McDonald[13] 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[14]. 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).

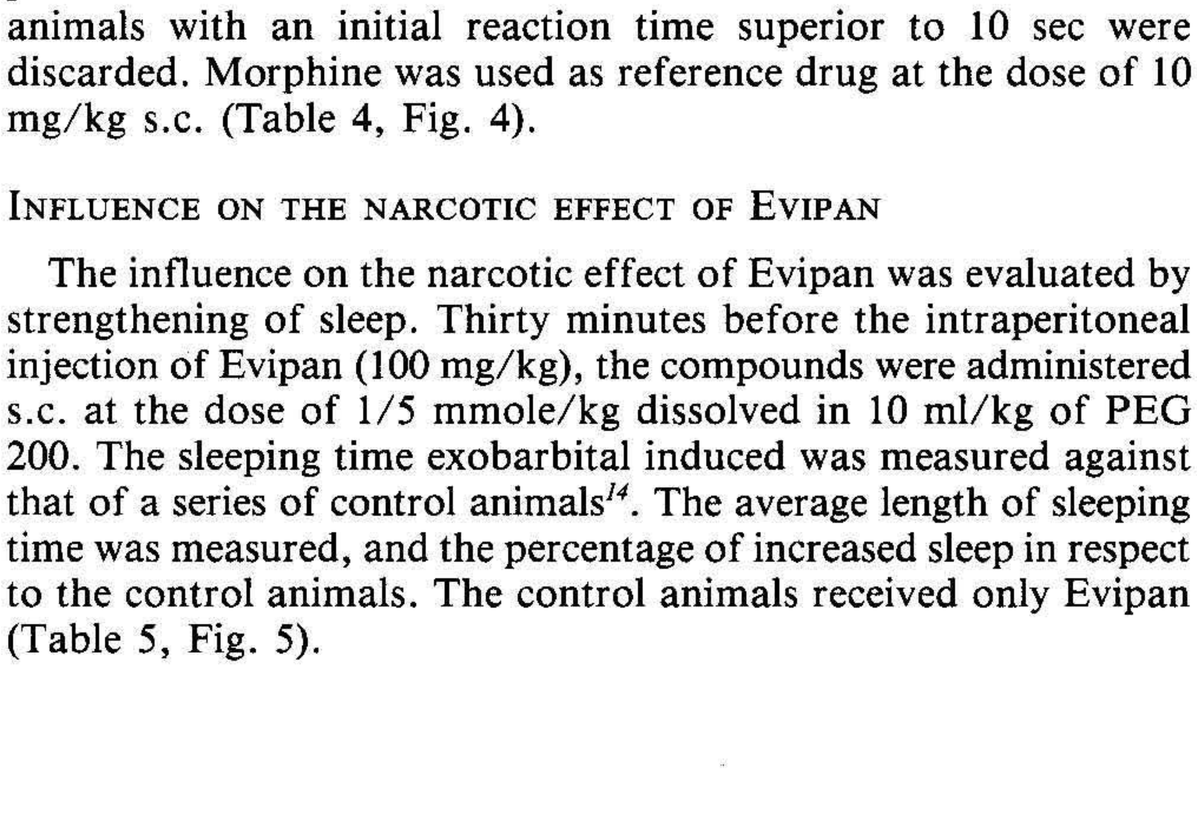


Fig. 4 - Analgesic activity - Hot plate test.

TABLE 3 - SPONTANEOUS MOTOR ACTIVITY.

Number of spontaneous movements every 30 minutes for three hours after the administration									
Compound	Dose (mg/kg)	30'	60'	90'	120'	150'	180'	Average	% Change
Controls		1146	793	284	79	108	621	505	
Diazepam	5	509	363	583	322	58	125	327	-35
7	43*	524	136	139	247	57	93	199	-61
14	46*	1017	731	404	361	90	259	477	-5
20a	46*	460	444	69	202	93	437	284	-44
20b	49*	1163	741	515	459	147	512	589	17

* : 0.2 mmoles/Kg / 10 ml

TABLE 4 - ANTINOCICEPTIVE ACTIVITY - HOT PLATE TEST.

Mean reaction time in seconds (± S.E.) after the dosing (% Change relative to 0 value)										
Compound	Dose mg/Kg	0	30'	%	60'	%	120'	%	180'	%
Morphine	10	7.03±0.65	11.63±1.75	65	13.52±1.18	92	17.20±1.79**	145	13.45±1.85	91
7	43	6.42±0.86	10.18±1.95	59	8.98±1.08	40	11.35±1.23**	108	8.55±1.48	33
14	46	7.37±0.73	9.68±1.70	31	11.80±1.89	60	13.15±1.82*	78	10.33±1.51	40
20a	46	7.97±0.67	7.25±0.56	-9	11.72±1.75	47	13.50±2.17*	69	18.88±0.79	36
20b	49	8.40±0.56	10.88±1.18	29	14.20±1.96	69	13.72±2.14*	63	10.08±1.33	20

Statistical analysis was performed using "t"test for paired data, at second hour
*** : $p < 0.05$; ** : $p < 0.01$; n = 6 for each substance**

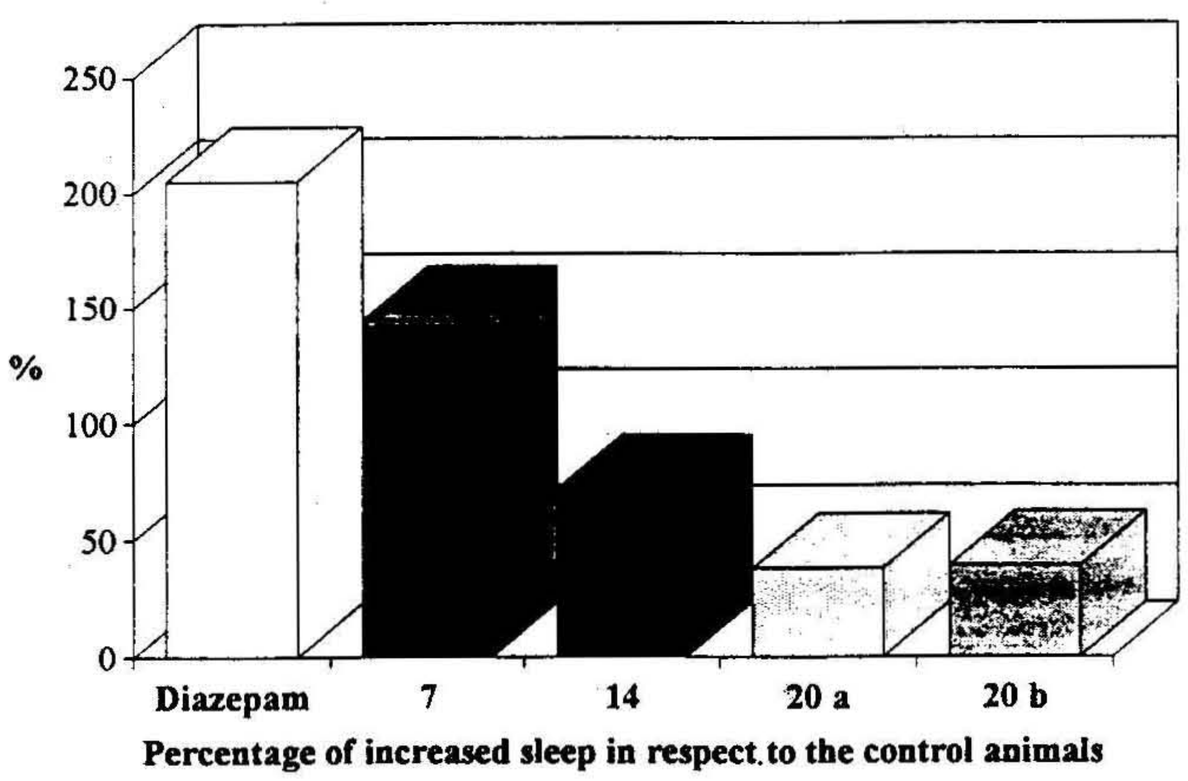


Fig. 5 - Influence on the narcotic effect of evipan (Sleep strengthening).

TABLE 5 - INFLUENCE ON THE NARCOTIC EFFECT OF EVIPAN.

Compound	Dose (mg/kg)	Increase %
Diazepam	2.5	205**
7	43	145**
14	46	72**
20a	46	38*
20b	49	39*

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

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%.

C) Biological activity

Ehrlich cells[15]

Ehrlich ascites tumor (Lettrè strain from Heidelberg) was routinely transferred by injecting intraperitoneally 2×10^6 cells per animal into NCL mice. For the experiments, the tumor cells, collected on the 6^{th}-7^{th} day after the transplant, were suspended (2×107 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 ^{3}H-thymidine

(4.77 TBq mM^{-1}; from Amersham International LTd, UK) in a small volume of the same medium were added and the cells were further incubated for 30 min at 37 °C. The acid-insoluble fraction was precipitated by adding 5% ice-cold trichloroacetic acid and filtered on Whatman GF/C filters (2.5 cm in diameter). After several washing with cold 1% trichloroacetic acid the filters were dried and counted by a Packard A 300 CD liquid scintillation spectrometer. The filtrations were carried out with a Sample Manifold apparatus (Millipore Corporation, Bedford, USA).

The results were calculated as percentage of radioactivity incorporated into DNA of untreated control cells (about 3-6 MBq); the ID_{50}, that is the drug concentration, expressed in $\mu g/ml$, which induces a 50% inhibition of DNA synthesis, was then calculated by probit analysis.

TABLE 6 - DNA SYNTHESIS INHIBITION IN EHRLICH CELLS.

Compound	ID_{50}	Standard deviation
7	93.66	1.77
14	Not detectable	
20a	20.91	1.6
20b	Not detectable	

ID50 ± Standard deviation expressed $\mu g/kg$.

RESULTS AND DISCUSSION

The results of the pharmacological screening on pyridopyrazinones **7,14** and pyridodiazepinones **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 **7** 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 pyridopirazinone cyclohomologues **7** and **14** and support our interest on these tricyclic structures whose potentialities will be further investigated.

Received October 6, 1993; accepted November 10, 1993.

REFERENCES

(1) Presented in part at XVII Congresso Nazionale della Società Chimica Italiana, Genova 25-30 Oct. (1992) and supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica.
(2) F. Savelli, A. Boido, *J. Heterocycl. Chem., 29,* 529 (1992).
(3) F. Savelli, A. Boido, *Boll. Chim. Farm., 131,* 309 (1992).
(4) G. Pirisino, M.C. Alamanni, F. Savelli, F. Sparatore, P. Manca, M. Satta, *Il Farmaco, Ed. Sci., 38,* 350 (1983).
(5) F. Savelli, A. Boido, G. Pirisino, L. Piu, M. Satta, P. Manca, *Il Farmaco, Ed. Sci., 42,* 833 (1987).
(6) M. Israel, L.C. Jones, *J. Heterocycl. Chem., 10,* 201 (1973).
(7) F. Savelli, A. Boido, A. Mulè, L. Piu, M.C. Alamanni, G. Pirisino, M. Satta, A. Peana, *Il Farmaco, Ed. Sci., 44,* 1250 (1989).
(8) H. Goult, *Bull. Soc. Chim. Fr., 11,* 382 (1912).
(9) B. Riegel, W.M. Lilienfeld, *J. Am. Chem. Soc., 67,* 1273 (1945).
(10) I.R. Boissier, P. Simon, *Arch. Int. Pharmacol., 147,* 372 (1964).
(11) W.J. Kinnard, C. Carr, *J. Pharmacol. Exp. Ther., 121,* 354 (1957).
(12) A.C. Raphaelson, B.M. Rabin, *Amer. J. Psychol., 77,* 493 (1964).
(13) J. Woolfe, A.D. Mc Donald, *J. Pharmacol., 80,* 300 (1944).
(14) R. Lisciani, A. Baldini, B. Silvestrini, *Arzneim. Forsch., 32,* 674 (1982).
(15) M. Ferlin, G. Chiarelotto, F. Baccichetti, F. Carlassare, L. Toniolo, F. Bordin, *Il Farmaco, 47,* 1513 (1992).