Psychosedative Agents. 2. 8-(4-Substituted 1-Piperazinylalkyl)-8-azaspiro[4.5]decane-7,9-diones

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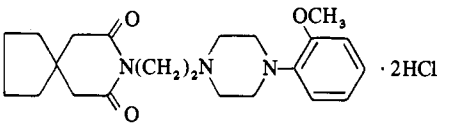
A series of 8-(4-pyridyl- and 4-pyrimidinyl-1-piperazinylalkyl)-8-azaspiro[4.5]decane-7,9-diones was synthesized for psychotropic studies. In testing for suppression of conditioned avoidance response in rats, several compds (3, 6, 8) demonstrated tranquilizing potencies and selectivities equal to or better than the corresponding properties of chlorpromazine. Their tranquilizing actions (3 and 6) were confirmed in monkeys. These compounds are outstanding in having extremely low sedative and α-adrenergic blocking side effects.

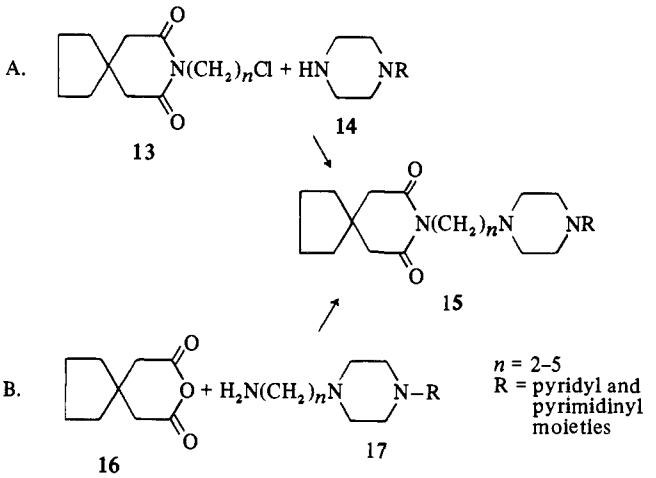
In our previous paper[1] we reported the synthesis and biological screening data of a series of *N*-(4-phenyl-1-piperazinylalkyl)-substituted cyclic imides. These compounds possess, in varying degree, psychotropic properties typical of major tranquilizers. Subsequently, we presented a preliminary report[2] on the pharmacology of a selected member, 12†, of the series. This compound produces tranquilization at dose



12

levels relatively free from side effects such as sedation and hypothermic and antiadrenergic activities. While **12** has been promising, we have continued searching for compounds with better potencies and selectivities than **12**. We replaced the Ph group in the 8-(4-phenyl-1-piperazinylalkyl)-8-azaspiro[4.5]decane-7,9-dione series with various pyridyl and pyrimidinyl moieties, and thus identified several compounds which have a marked improvement in both psychotropic potencies and selectivities over the previous substances. This paper describes such findings.

Chemistry. Two general synthetic methods were used. The starting piperazines **14** were prepared by nucleophilic



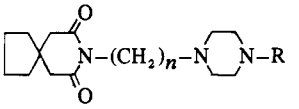
13 14 15 16 17

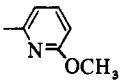
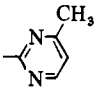
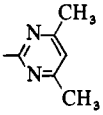
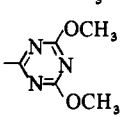
aromatic substitution. Only halides of N heteroaromatics are important substrates for this type of substitution.[3] For our present purpose, we chose halides of pyridines and pyrimidines. These piperazines, **14**, were either treated directly with *N*-(ω-chloroalkyl)imides, **13** (method A), or converted first to 1-(ω-aminoalkyl)piperazines, **17**, and followed by a condensation with the anhydride **16** (method B). The physical constants of azaspirodecanedione products are tabulated in Table I.

Biological Data. Screen for Tranquilizing Properties. We screened compounds for their tranquilizing effects by two primary tests: (1) suppression of conditioned avoidance response (CAR) in rats, and (2) antagonism of amphetamine-

†This compound is identified as **25** in ref 1.

Table I. 8-(4-Substituted 1-piperazinylalkyl)-8-azaspiro[4.5]decane-7,9-diones



No.	*n*	R	Method	% yield	Mp, °C	Formula[a]
1	2		B	59	208.5-209.5	$C_{20}H_{28}N_4O_2 \cdot HCl$
2	3		A	41	233.5-234.5	$C_{22}H_{32}N_4O_3 \cdot HCl$
3	4		B	40	172-173.5	$C_{22}H_{32}N_4O_2 \cdot HCl$
4	2		B	77	206-207	$C_{19}H_{27}N_5O_2 \cdot HCl$
5	3		A	42	214-215	$C_{20}H_{29}N_5O_2 \cdot HCl$
6	4		B	45	201.5-202.5	$C_{21}H_{31}N_5O_2 \cdot HCl$
7	5		B	17	188.5-190.5	$C_{22}H_{33}N_5O_2 \cdot HCl$
8	4		B	40	215-215.5	$C_{22}H_{33}N_5O_2 \cdot HCl$
9	3		B	26	221.5-224	$C_{22}H_{33}N_5O_2 \cdot HCl$
10	4		B	44	216.5-217.5	$C_{23}H_{35}N_5O_2 \cdot HCl$
11	4		B	28	182-183	$C_{23}H_{35}N_5O_4 \cdot HCl$

[a] Analyzed for C, H, N. Analytical results are within ±0.4% of the theoretical value.

Table II. Screening Data on Tranquilizing Properties

Compounds	ED_{50}, mg/kg ip[a]			Antagonism of amphetamine-aggregation stress in mice ED_{50}, mg/kg sc
	UER	CAR	UER/CAR[b]	
1	140.8 ± 29.4	22.6 + 1.7	6.2	>40
2	>50	>50		13.2 ± 3.1
3	33.8 ± 11.2	2.8 ± 0.32	12.1	8.4 ± 1.7
4	158.7 ± 90.4	36.9 ± 1.4	4.3	>40
5	45-50	41.0 ± 1.5	1.2	>40
6	84.9 ± 31.6	4.3 ± 0.25	19.6	9.8 ± 3.4
7	>50	37.7 ± 1.29	>1.3	23.0 ± 4.6
8	100	5.83 ± 0.59	17.2	9.3 ± 2.2
9	>50	22.7 ± 1.8	>2.2	>40
10	67.7 ± 22.5	8.1 ± 0.4	8.4	8.0 ± 1.9
11	61	35.6 ± 3.7	1.7	>40
12	26.4 ± 10.6	9.2 ± 1.2	2.9	2.2 ± 0.56
CPZ[c]	48.8 ± 14.5	4.8 ± 0.3	10.2	0.26 ± 0.06

[a] UER-ED_{50} and CAR-ED_{50} are the median effective doses for the complete suppression of the unconditioned escape response and the conditioned avoidance response, respectively, in rats. [b] The larger the UER/CAR ratio, the more selective the tranquilizing action. [c] Chlorpromazine·HCl.

Table III. Relative Sedation Potencies

	Test 1	Test 2	Test 3
Chlorpromazine·HCl	1	1	1
3	1.38	0.28	0.17
6	0.11	0.01	0.06
8	0.12	0.03	0.10

aggregation stress in mice, as outlined in our previous paper.[1] The results of the testing in terms of ED_{50}'s are listed in Table II. Compounds 3, 6, and 8 have potencies and selectivities superior to that of 12 and chlorpromazine·HCl. In accordance with out previous observations,[1] maximum potencies were obtained with compounds with a 4-C alkylene chain (3, 6, 8, 10).

Testing for Sedative Side Effects. Ideal tranquilizing agents should have as little depressive side effects as possible. To determine their sedative side effects, we subjected the selected compounds (3, 6, 8) to 3 different tests in mice: (1) spontaneous motor activity, (2) hexobarbital hypnosis potentiation, and (3) motor incoordination, as described in the Experimental Section. Compared with chlorpromazine·HCl, 3, 6, and 8 produce much less sedation as indicated by relative potencies given in Table III.

In addition, we studied the general behavioral effects of 3 and 6 in rhesus monkeys. The test compounds were administered im. The incidence of catalepsy and altered alertness was observed. Compounds 3 and 6 were much less sedative than chlorpromazine·HCl as measured by alertness. The incidence of catalepsy was similar to that induced by chlorpromazine·HCl. This is of particular interest in view of the concept that catalepsy induced by major tranquilizers is considered to be related to the CAR depression in rats.

α-Adrenergic Antagonism. α-Adrenergic receptor blocking properties are frequently found in phenothiazine-type tranquilizers. For the determination of α-adrenergic antagonism, we used two methods: (1) antagonism of the peripheral α-adrenergic, spasmogenic effect of norepinephrine in rat seminal vesicles (*in vitro* test), and (2) antagonism of the peripheral α-adrenergic action of a lethal dose of epinephrine bitartrate in the mouse (*in vivo* test).

Both the *in vitro* and the *in vivo* tests indicated that the α-adrenergic blocking activity of 6 is extremely weak, being 0.0025-0.0057 as potent as chlorpromazine·HCl. Such a low level of α-blocking activity might be expected to produce only minimal, if any, clinical side effects thought to be mediated by α-adrenergic blockade.

Acute Toxicity. The acute lethality was determined in Swiss-Webster strain male albino mice after ip dosing. Determination of median lethal dose (LD_{50}) was based on deaths occurring in the first 24 hours. The LD_{50} with 95% confidence limits for 6 is 146 (115-185) mg/kg. The corresponding value for chlorpromazine·HCl is 153 (115-204) mg/kg.

Experimental Section

Screen for Tranquilizing Properties. The screening methods for tranquilizing properties have been discussed in the previous paper.[1]

Testing for Sedative Side Effects. (1) Spontaneous Motor Activity. Mice were injected (ip) with the test compd and immediately placed into annular activity cages as described by Kissel.[4] One hour later, their log activity scores were compared with those of simultaneously tested control animals. A dose-response relationship was established and a dose of test compd which would reduce the log motor activity score to 0.3 below that of control (AED-0.3) was determined.

(2) Hexobarbital Potentiation. Mice were administered (sc) various dose levels of the test compd. At the previously detd time of peak effect, the mice are injected (ip) with a nonhypnotic dose of sodium hexobarbital (40 mg/kg). The dose that will produce loss of righting reflex in half the animals was calcd.

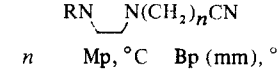
(3) Motor Incoordination. Mice were injected (ip) with the test compd, and their ability to remain walking on a rotating rod for 15 sec was detd 5, 15, 30, and 60 min after injection, essentially as described by Dunham and Miya.[5] Each test consisted of 3 trials at 1-min intervals. The ED_{50} for interruption of this coordination was then detd for each of the test intervals and for the total 60 min by pooling all readings.

α-Adrenergic Antagonism. (1) *In Vitro* **Method.** Adult rats were killed by decapitation, the seminal vesicles were removed, the coagulating gland was removed from the seminal vesicle, and each vesicle was suspended in a 10-ml tissue chamber containing oxygenated Locke-Ringer soln maintd at 38°. Contractions were recorded on a kymograph by means of an isotonic lever. Varying concns of test compd were added to the tissue chamber in order to det the concn that would reduce the response to *l*-norepinephrine base, 4 mcg/ml, by 50%.

(2) *In Vivo* **Method.** Groups of 20 male mice each, fasted 16-20 hr, were administered various doses of the test compd orally. At the previously detd time of peak activity (30, 60, or 120 min), *l*-epinephrine bitartrate (40 mg/kg ip) was injected. Deaths were recorded 24 hr later and the dose of test compd which would allow half the animals to survive (ED_{50}) was calcd.

1-(6-Methoxy-2-pyridyl)piperazine. A mixt of 2-chloro-6-methoxy pyridine (7.2 g, 0.05 m), anhyd piperazine (8.6 g, 0.1 m), anhyd Na_2CO_3 (5.3 g, 0.05 m) in 75 ml of *i*-AmOH was refluxed for 48 hr. The mixt was filtered and concd. The thick oily residue was fractionated under reduced pressure to collect 2.6 g (27%) of the product as a light colored oil, bp 110-125° (0.05 mm), $n^{25}D$ 1.5750.

Table IV



R	n	Mp, °C	Bp (mm), °C	Formula[a]
2-Pyrimidinyl	1	98-99		$C_{10}H_{13}N_5$
2-Pyrimidinyl	3	56-58	165 (0.15)	$C_{12}H_{17}N_5$
2-Pyrimidinyl	4	77-78		$C_{13}H_{19}N_5$
4-Methyl-2-pyrimidinyl[b]	3		165 (0.04)[c]	$C_{13}H_{19}N_5$
4,6-Dimethyl-2-pyrimidinyl	3	63-65	170 (0.1)	$C_{14}H_{21}N_5 \cdot HCl$[d]
2,6-Dimethoxy-4-pyrimidinyl[b]	3	70-71	190 (0.05)	$C_{14}H_{21}N_5O_2$

[a] Analyzed for C, H, N. Analytical results are within ±0.4% of the theoretical value. [b] Reaction medium: EtOH. [c] $n^{25}D$ 1.5459. [d] Mp 290-291°.

The HCl salt melted at 216° (EtOH). *Anal.* ($C_{10}H_{15}N_3O \cdot HCl$) C, H, N, Cl.

1-[4-(2,6-Dimethoxypyrimidinyl)]piperazine was prepd similarly in 50% yield, light yellow oil, bp 165° (0.1 mm), $n^{25}D$ 1.5560. The HCl salt melted at 205–207° (MeOH). *Anal.* ($C_{10}H_{16}N_4O_2 \cdot HCl$), C, H, N.

1-[4-(4-Methylpyrimidinyl)]- and 1-[4-(4,6-dimethylpyrimidinyl)]-piperazines were prepd according to Janssen.[6]

1-(ω-Cyonoalkyl)-4-pyrimidinyl piperazines were obtd by condensing equimolar amounts of ω-chloroalkylnitriles and appropriate 1-pyrimidinyl piperazines with excess anhyd Na_2CO_3 in *n*-BuOH in 68–94% yields. The physical constants of new compds are listed in Table IV.

1-(ω-Aminoalkyl)-4-pyridylpiperazines were obtd according to Mull, *et al.*[7]

1-(ω-Aminoalkyl)-4-pyrimidinylpiperazines were prepd from the corresponding ω-cyanoalkyl derivatives either by a LAH reduction or a catalytic hydrogenation at room temp under 84 kg/cm² pressure of H_2 with W-6 Raney Ni catalyst. These compds were very hygroscopic and not analyzed. They were purified by distn under reduced pressure and used immediately for the next reaction.

8-(4-Substituted 1-Piperazinylalkyl)-8-azaspiro[4.5]decane-7,9-diones. Method A. A mixt of 8-(ω-chloroalkyl)-8-azaspiro[4.5]-decane-7,9-dione (0.1 mole), 1-substituted piperazine (0.1 mole), Na_2CO_3 (0.1 mole), and *n*-BuOH was refluxed for 15 hr, and filtered. The filtrate was concd and distd to give the product. Method B. An equimolar mixt of 3.3-tetramethyleneglutaric anhydride and 1-(ω-aminoalkyl)piperazine in dry pyridine (0.1 mole/400 ml) was refluxed for 15 hr. It was concd; if the ir spectrum showed typical imide bands (1700 and 1710 cm^{-1}), the residue was purified by either distn or crystn. If the spectrum showed amide acid bands (1680, 1760, 330 cm^{-1}) instead, the residue was refluxed with 10 times its wt of Ac_2O for 15 hr. The residue obtd by removal of Ac_2O was purified either by distn or recrystn. The HCl salts were prepd by treating the free bases with an equiv amt of ethanolic HCl.

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Synthetic Thyrotropin-Releasing Factor Analogs. 3.[1,2] Effect of Replacement or Modification of Histidine Residue on Biological Activity

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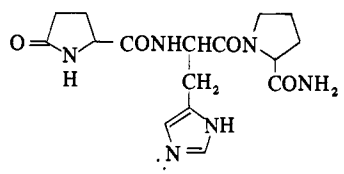
A series of analogs of TRF (thyrotropin-releasing factor, pGlu-His-Pro-NH_2) in which His was replaced by a basic, aromatic, or S-containing amino acid was synthesized and tested for TRF biological activity. pGlu-3-Me-His-Pro-NH_2 appeared to be 10 times more active biologically than the natural or synthetic TRF itself while pGlu-1-Me-His-Pro-NH_2 was almost completely inactive. [Orn^2]-, [Lys^2]-, [Arg^2]-, [Tyr^2]-TRF showed less than 0.1% of TRF activity while [Met^2]-TRF had 1% of TRF activity. All the activities were measured *in vivo*. Merrifield's solid-phase synthesis on a benzhydrylamine resin was applied for the preparation of all the compounds. pGlu-1-Me-His-Pro-NH_2 and pGlu-3-Me-His-Pro-NH_2 were also synthesized by a classical method. The final products, homogeneous in 4 different tlc systems, were characterized by means of amino acid analysis, nmr, and mass spectrometry.

A peptide molecule as structurally simple as TRF (thyrotropin-releasing factor: pGlu-His-Pro-NH_2 (**1**)), with such



TRF

high specific biological activity as well as such high specificity of action, is a model of choice for the synthesis of a series of analogs which would allow the study of relationships between specific changes in the molecular structure and modification of biological activity. Many such derivatives have already been described by Burgus,[1] Hofmann,[3a] Bowers,[3b] Chang,[3c] Nicolaides,[3d] Wilber,[3e] Gillessen,† and Vale[2] in which each of the 3 amino acid residues of the TRF molecule have been replaced or modified.

†Gillessen, *et al.*,[3f] reported the synthesis of [Lys^2]-TRF, [α,γ-diaminobutyryl^2]-TRF, [β-(3-pyrazolyl)alanine^2]-TRF, and [arginine^2]-TRF and concluded that their compounds exhibited the characteristic biological responses of TRF.

Among the analogs which have been described so far, all of the alterations of structure have resulted in a drastic reduction of specific biological activity. We now describe the synthesis of a series of 6 new tripeptides with changes of the imidazole group of histidine and substitution of histidine by a basic, aromatic, and S-containing L-amino acid; the biological assay data on these latter compounds are included.

Synthesis. The approach for the classical synthesis of pyroglutamyl-1-Me-histidylprolinamide (**1a**) and pyroglutamyl-3-Me-histidylprolinamide (**1b**) was essentially that described by Gillessen, *et al.*[4] (Scheme I). However the presence of Me acting as a protective group on the imidazole gave much better yields. *N*-Carbobenzoxypyroglutamic acid[5] (**4**) and 1-methylhistidine methyl ester (**2a**) or 3-methylhistidine methyl ester (**2b**) were first coupled by *N,N*-dicyclohexylcarbodiimide (DCI), affording the resulting dipeptides *N*-carbobenzoxypyroglutamyl-1-methylhistidine methyl ester (**3a**) and *N*-carbobenzoxypyroglutamyl-3-methylhistidine methyl ester (**3b**), respectively. Hydrogenolysis (Pd/C) yielded the 2 dipeptides pyroglutamyl-1-methylhistidine methyl ester (**5a**) and pyroglutamyl-3-methylhistidine methyl ester (**5b**), which upon treatment with hydrazine in