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Synthesis and Structure–Activity Relationship of a Pyrimido[4,5-*d*]pyrimidine Derivative with Antidepressant Activity

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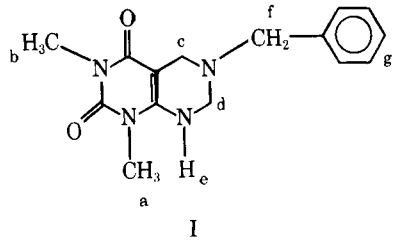
Received April 10, 1979, from **INSERM U16, Place de Verdun, 59045 Lille Cedex, France*, and the ‡*Laboratoire de Dynamique des cristaux moléculaires associé au CNRS (ERA 465), Bât. P.5, Université des Sciences et Techniques de Lille, 59650 Villeneuve d'Ascq, France.* Accepted for publication May 2, 1980.

Abstract □ The synthesis and antidepressant properties of a new pyrimido[4,5-*d*]pyrimidine are described. Spectral data determined in solution and in the solid state allowed establishment of the relationship between the activity and the conformation of the molecule. The spatial structure seems to be in accordance with a possible binding at the presynaptic α-receptor sites.

Keyphrases □ Pyrimido[4,5-*d*]pyrimidines—synthesis and evaluation for antidepressant activity, binding at presynaptic α-receptor sites, structure–activity relationships □ Structure–activity relationships—pyrimido[4,5-*d*]pyrimidines, synthesis and evaluation for antidepressant activity, binding at presynaptic α-receptor sites □ Antidepressant activity—pyrimido[4,5-*d*]pyrimidines, synthesis and evaluation for antidepressant activity, binding at presynaptic α-receptor sites, structure–activity relationships

The basic assumptions in the analysis of a structure–activity relationship are that the drug and the receptor exhibit mutual complementarity and that the study of drug conformation yields information relevant to the geometry of the binding sites of the receptors. As a continuation of studies on 1,3-dimethyl-2,4-dioxo-6-substituted octahydropyrimido[4,5-*d*]pyrimidines (1), the particular behavior of the 6-benzyl derivative (I) was evaluated. This compound appears to possess significant antidepressant activity. Antidepressant drugs containing the pyrimido[4,5-*d*]pyrimidine ring system have not been described. In addition, the apparent importance of the benzyl moiety[1] in inducing such properties prompted the establishment of relationships between three-dimensional molecular structure and pharmacological response.

The mechanism of action of antidepressant drugs has been studied recently (2). It is generally accepted that the therapeutic effect of antidepressant agents may be a consequence of an increased availability of norepinephrine at postsynaptic sites. A 3-week administration of tricyclic antidepressant drugs gradually decreased the sensitivity of the presynaptic α-receptor (3), which would explain the delay in the onset of the clinical effect. On the other hand,



I

the feedback inhibition of brain norepinephrine neurons by tricyclic antidepressants was due to an α-receptor mediation (4). This finding would explain the increase of the availability of norepinephrine at postsynaptic receptor sites after an antidepressant treatment. A correlation may be postulated between antidepressant activity and the action of the drug at the α-adrenergic receptors.

EXPERIMENTAL[2]

1,3-Dimethyl-2,4-dioxo-6-benzyl-1,2,3,4,5,6,7,8-octahydropyrimido[4,5-*d*]pyrimidine (I)—To an ethanolic solution of 9 g (0.06 mole) of 1,3-dimethyl-6-aminouracil were added successively 8.5 g (0.08 mole) of benzylamine and 12 ml of 35% aqueous formaldehyde (0.12 mole) solution. The mixture was stirred and heated under reflux for 2 hr and then concentrated *in vacuo* to dryness. The residual solid was washed with acetone and recrystallized from ethanol to give 14 g (81%) of white crystals. Further recrystallization from ethanol gave an analytical sample, mp 160°; IR(KBr pellets): 1630 and 1690 (C=O), 3150 (NH), and 2750 and 2810 (CH_2) cm^{-1}; PMR (dimethyl sulfoxide-d_6): δ 3.23 (s, 3H, CH_3a), 3.36 (s, 3H, CH_3b), 3.75 (s, 2H, CH_2c), 4.15 (d, 2H, CH_2d), 7.48 (t, 1H, J_{d-e} = 3 Hz, NHe), 3.56 (s, 2H, CH_2f), and 7.56 (s, 5H, phenyl g) ppm.

Anal.—Calc. for $C_{15}H_{18}N_4O_2$: C, 62.92; H, 6.34; N, 19.56. Found: C, 62.88; H, 6.33; N, 19.56.

Pharmacological Assays—Compound I was subjected to the classical tests for psychopharmacological effects. The central nervous system (CNS) activity was evaluated first as described by Irwin (5). Graded doses were given to groups of five mice, and the animals were observed con-

[1] Other pyrimido[4,5-*d*]pyrimidines with a 6-alkyl or aryl group were devoid of such activity (1).

[2] Melting points were determined with a Büchi capillary melting-point apparatus and are uncorrected. PMR spectra were obtained with a Jeol-MH 60 spectrometer using tetramethylsilane as the internal standard. IR spectra were recorded on a Perkin-Elmer 177 spectrometer. Crystallographic data were collected on a Philips PW 1100 diffractometer with graphite monochromated *M* kα radiation at room temperature. Elemental analyses were performed with a Perkin-Elmer CHN 240 instrument.

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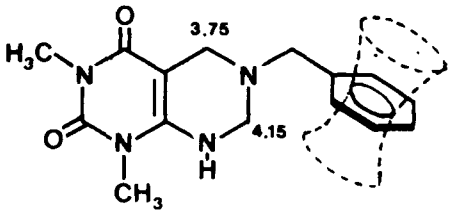

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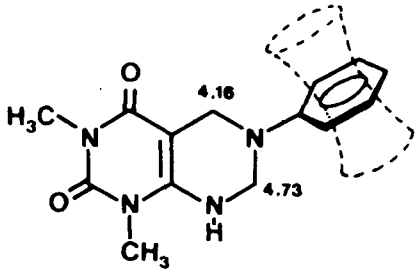

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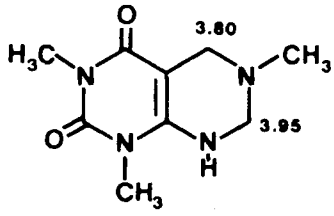

III

Figure 1—*Computer drawn perspective view of I.*

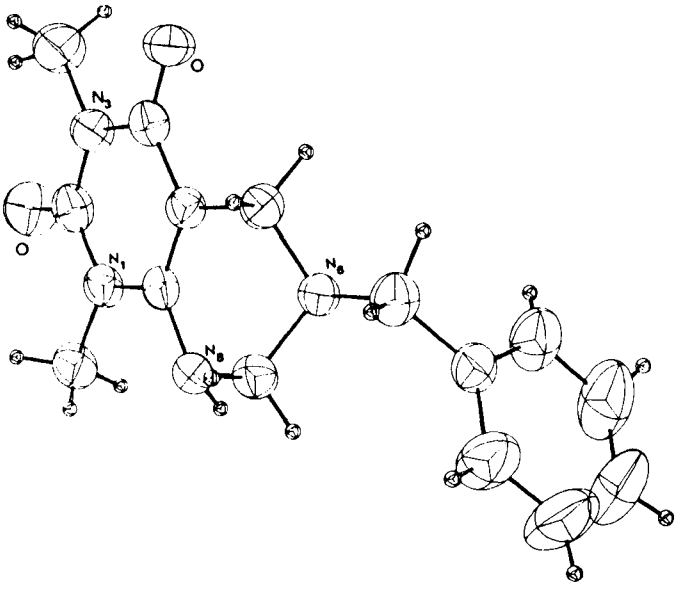

Figure 2—*Comparison between the chemical shifts of the* c *proton (and* d *proton) for different 8-substituted pyrimidopyrimidines.*

tinuously over 6 hr. Lethality and delayed effects were noted 24 hr after drug administration.

Tests for Neurological Deficit—*Activity Chambers (6)*—Sedative activity was measured in groups of 10 mice housed singly in photobeam cages. The number of beam interruptions occurring during the 60-min periods immediately prior to and after dosing was recorded.

Traction Test (7)—The percentage of mice unable to climb onto a wire and balance themselves within 5 sec after being suspended by the forepaws was determined 60 min after administration of the test compound.

Forced Motor Activity (8)—This activity was evaluated by the use of a rod revolving at 14 rpm. The number of mice remaining on the rod for >1 min was used as the measure.

Anticonvulsant Activity—*Convulsions from Pentylenetetrazol (9)*—Pentylenetetrazol was administered in a convulsifying dose (125 mg/kg ip) to groups of 10 mice pretreated for 1 hr with the test compound. Animals that did not have any tonic–clonic attack for 30 min after the pentylenetetrazol were considered to be protected.

Convulsions from Electroshock (10)—The effect of the compound on the maximal electroshock seizure was investigated in mice after intraperitoneal administration. The stimulation was carried out by means of corneal electrodes at 30 mamp for 0.2 sec. The percentage of mice protected from the tonic phase of the seizure was recorded.

Antipsychotic Activity (11)—The ability to reverse a conflict of motivation between exploration and punishment was assessed. The floor of the cage was divided into four plates. When a mouse went from one plate to another, it was subjected to an electrical shock.

Prevention of Prochlorperazine-Induced Catalepsy (12)—This measurement was used as an index of antidepressant activity. Groups of five rats were pretreated with prochlorperazine (10 mg/kg sc); 5.5 hr later, they were treated with the test compound having anticataleptic activity. The test compound was given again 0.5, 1.5, and 2.5 hr thereafter.

***d*-Amphetamine-Induced Lethality (13)**—*d*-Amphetamine-induced lethality was used to evaluate antipsychotic activity. Mice were medicated orally with test drugs 0.5 hr before intraperitoneal injection of *d*-amphetamine (20 mg of the free base/kg). Deaths were recorded during the following 4 hr.

RESULTS

The Irwin test (5) showed that I was not toxic up to 300 mg/kg po. No peculiar symptomatology was observed. Compound I had no effect on the motor activity for mice at a dose of 100 mg/kg (6). At the same dose,

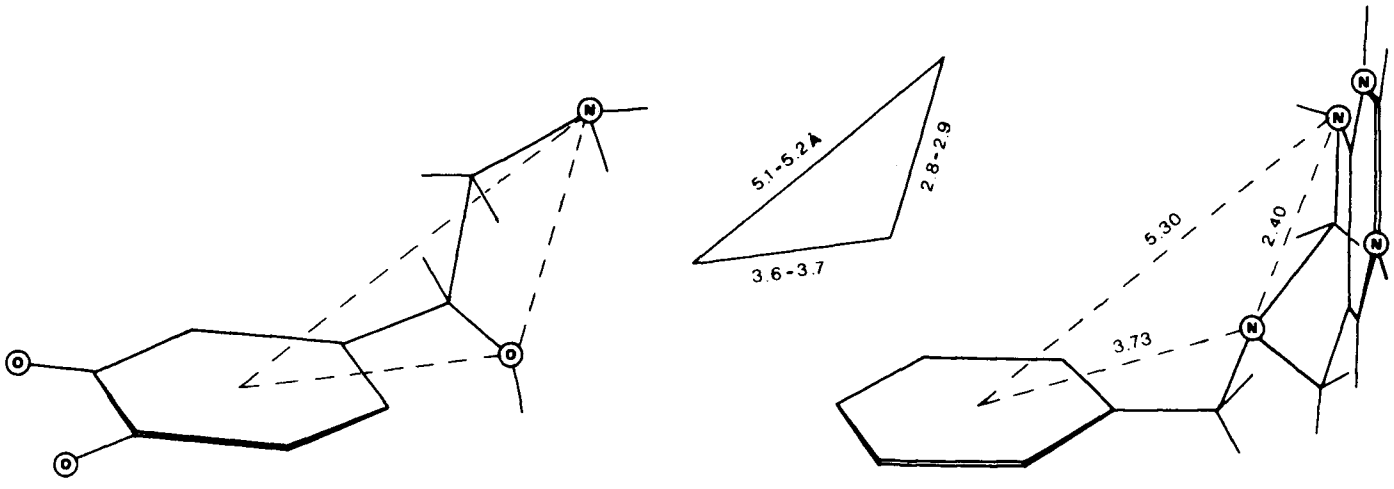

Figure 3—*Proposed binding mode at α-adrenergic receptor sites for norepinephrine and I.*

traction (7) and equilibration (8) reflexes were not changed, and pentylenetetrazol- or electroshock-induced convulsive effects (9, 10) were not antagonized. No anxiolytic activity was observed until 30 mg/kg was administered (11). No central anticholinergic action was noted at 100 mg/kg by the classical antipilocarpine test in rats (14).

Prochlorperazine-induced catalepsy (13) was inhibited at the ED_{50} value of 30 mg/kg ($p \leq 0.05$, Student t test). The amphetaminic group toxicity effect was potentiated at 50 mg/kg (13). Thus, the results reported here suggest that I may be a significant and selective antidepressant compound.

Structural Determinations—The crystal data were: space group P2/C; a = 7.109(3), b = 12.766(3), and c = 16.110(4) Å; β = 97.52(5); V = 1395.2 Å^3; Z = 4; D_c = 1.33; and R = 0.052.

The distances between the center of the phenyl ring and N-6 and N-8 atoms are 3.73 and 5.30 Å, respectively (Fig. 1). The interatomic distance[3] between N-6 and N-8 is 2.40 Å.

PMR Spectrographic Results—The shifts (expressed in δ parts per million using deuterated dimethyl sulfoxide as the solvent) (Fig. 2) corresponding to the methylene protons (c and d) of I were designed unambiguously and compared to shifts reported previously for other compounds (II and III) (1).

The chemical shifts of protons c and d unequivocally indicate that the anisotropic cone of the phenyl ring is closed to proton d (deshielding effect) and that, in solution, I exhibits a privileged conformation in accordance with that observed in the solid state.

DISCUSSION

A number of binding sites at the receptor are known to be important for α-adrenergic activity: the catechol moiety, the β-hydroxyl group, and the ammonium function (Fig. 3). The extended Hückel theory (16) and the perturbative configuration interaction using localized orbitals (17, 18) calculations have permitted postulation of the distance between the center of the aryl ring and the oxygen atom (3.6–3.7 Å), the center of the ring and the nitrogen atom (5.1–5.2 Å), and the nitrogen–oxygen distance (2.8–2.9 Å) as the main characteristics of the molecule binding the α-adrenergic receptor sites.

The spatial configuration of I fits this proposed model.

It can be concluded that I displays structural features making it suitable for binding the presynaptic receptor sites. Nevertheless, I cannot activate them; the bulky group attached to the nitrogen atom accounts for this inhibition. As such, when it partially occupies the site, it hinders the feedback inhibition of norepinephrine, increasing its release in the synaptic cleft. This effect could account for its pharmacological properties and provides information on the activity of certain antidepressant drugs.

[3] Full details concerning the crystallographic data (interatomic distances, angles, and coordinates) are available (15).

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Effect of Sodium Oleate on Salicylic Acid Binding to Human Serum Albumin

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Abstract □ The pharmacological activity of salicylates is related to the nonprotein-bound fraction of drug in the plasma. Free fatty acids have been shown to displace bound drug and to increase the serum levels of salicylates. Continuous ultrafiltration was used to measure unbound salicylic acid at 37°. A nonlinear analysis of the ultrafiltration data using whole number values for the number of binding sites indicates that sodium oleate displaces the salicylic acid competitively at both binding sites. Increased concentrations of fatty acids due to disease or the infusion of fatty acid emulsions perhaps may produce toxic levels of salicylic acid.

Keyphrases □ Sodium oleate—effect on binding of salicylic acid to human serum albumin □ Salicylic acid—binding to human serum albumin, effect of sodium oleate □ Protein binding—effect of sodium oleate on binding of salicylic acid to human serum albumin

Since the pharmacological activity of many drugs is related to the non-protein-bound fraction of the drug in plasma (1–3), any decrease in the fraction of protein-bound drug may be expected to result in an increased pharmacological effect. Free fatty acids bind rapidly to albumin at more than one site (4–6), have a higher affinity for albumin than do most drugs (7, 8), and increase the concentration of unbound drug *in vivo* by displacement of

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