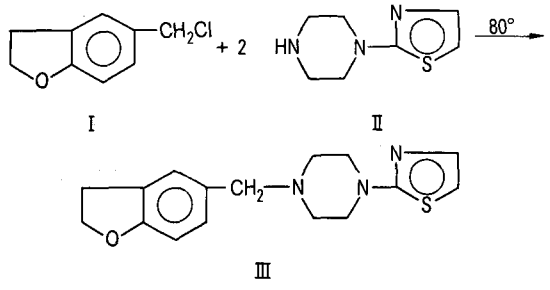
A New Central Direct Dopaminergic Stimulant: 1-(Coumaran-5-yl Methyl)-4-(2-Thiazolyl) Piperazine Hydrochloride (S 3608)

In 1971, CORRODI, FUXE and UNGERSTEDT[1] described the potent dopamine receptor stimulating properties of Piribedil (ET 495) previously studied as a peripheral vasodilator[2]. The chief metabolite of the latter, is a catecholic compound (S 584)[3]. This catecholic grouping, which is also found in dopamine and apomorphine molecules, would be responsible in part for the dopaminergic activity of Piribedil, as it has been shown by COSTALL and NAYLOR's experiments[4]. These results prompted us to synthesize new compounds in which the methylenedioxy group of ET 495's molecule would be replaced by a moiety which could not be metabolized into a catecholic derivative. This research has led to the title compound III (S 3608), which was synthesized according to the following scheme:



[5-chloromethyl coumaran I (bp 109–110 °C under 0.6 mm) n_D^{25} 1,5710[5] was heated with an excess of 1-(2-thiazolyl) piperazine II (bp 111–113 °C under 0,5 mm), at 80° for 1 h. 1-(coumaran-5-yl-methyl)-4-(2-thiazolyl) piperazine (S 3608) III, has mp 97 °C (EtOH). The hydrochloride of III has mp 216–218 °C (EtOH); UV λ_{max}^{MeOH} nm (log ε):237 (3,96) 276,5 (4,02); pka 25° (H_2O):6,7].

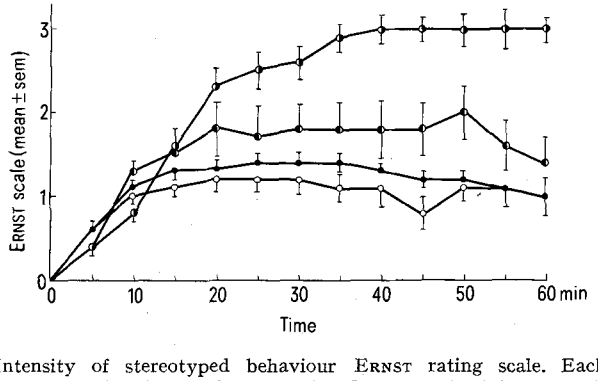
S 3608 is a new neuropharmacological agent inducing a potent activation of central dopaminergic receptors in the rat. Contrary to Piribedil, S 3608 is devoid of potent cardiovascular properties. The LD_{50} (mice) of III (hydrochloride) were found 450 (385–527) mg/kg i.p. and above 500 mg/kg p.o. Its action was studied using its stimulating properties on the extrapyramidal tract in the rat.

Methods. Stereotyped behaviour. Charles River CD ♂ rats (300 g) were used. The ERNST's rating scale[6] was chosen. The animals of different experimental groups received different doses of S 3608 (20–40–80 mg/kg), by s.c. route, in water solution, or in other groups, different doses of apomorphine HCl (Boyer) (0.3, 0.6, 1.25 and 2.5 mg/kg). Each experimental group consisted of 6 animals. The effects of each dose, for the two compared substances, were successively studied on isolated rats (Makrolon cages: 25 × 16 × 14 cm), and also on grouped animals (3 rats in a Makrolon cage: 36 × 22 × 18 cm).

A piece of corrugated paper was placed on each cage's floor. In all experiments, animals were individually scored at 5 min intervals, by the same experimenter, during 1 h.

Unilateral nigro-neostriatal lesions. Charles River CD ♂ rats (150 g) were used and UNGERSTEDT's[7] method was employed. After a pentobarbital (50 mg/kg i.p.) injection, the head of the anesthetized rat was immobilized in a LPC stereotaxic device. The needle tip of an Hamilton syringe (5 µl) was inserted into the left substantia nigra site, using the following coordinates: AP = 2.42 mm; H = 2.5 mm; L = 1.5 mm (KÖNIG and KLIPPEL[8]); 4 µl of a 6-OH dopamine (Aldrich) solution (8 µg) were injected in 4 min 30 sec. Lesionned rats were employed after a 20 days delay, and treated with Piribedil[9] or S 3608. Each rat was introduced in a "Rotometer" and the turning behaviour towards the right side was then measured during 30 min after i.p. administration of 25 mg/kg of Piribedil or S 3608. In another experiment, the total mean number of turns was determined during 3 h after the i.p. injection of 6 mg/kg of the compared compounds. Each experimental group consisted of 6 animals.

Influence on morphine-induced rigidity. 5 groups of 8 rats were used for the calculation of the catatonigenic ED_{50} of morphine HCl. The following doses of morphine were 5, 7.5, 11.2, 16.8 and 25.3 mg/kg (i.p. route). Animals are individually scored 15 and 30 min after injection. The total number of rats in complete catatonia of each experimental group was obtained by addition of the two measurements[10]. The catatonigenic ED_{50} of the analgesic was determined by Litchfield and Wilcoxon's method. In drug interaction experiments, analgesic doses were identical to those administered in control experiments, but all rats were previously treated with S 3608 (8 mg/kg i.p.), 30 min prior to morphine injections. The catatonigenic ED_{50} of morphine modified by combination with 3608 was then calculated. In other comparative experi-



Intensity of stereotyped behaviour ERNST rating scale. Each experimental point is the mean (± SEM) obtained from 6 animals, during 60 min experiments. ◐, Apomorphine (1.25 mg/kg, s.c. isolated rats. ◑, Apomorphine (1.25 mg/kg, s.c.) grouped rats. ○, S 3608 (80 mg/kg, s.c.) isolated rats. ●, S 3608 (80 mg/kg, s.c.) grouped rats.

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ments, rats were pretreated with ergocornine (methanesulfonate: 5 mg/kg i.p.), CB 154 (2-Br α-ergocryptine mesylate: 5 mg/kg i.p.)[11], or yohimbine HCl (Merck) (1 mg/kg i.p.), 20 min before morphine injections.

Results. Stereotyped movements. S 3608 induced automatic movements similar to those produced by apomorphine (sniffing, licking), but less important (biting reduced) (Figure). The stimulating effects of apomorphine appeared dose dependent. In grouped animals, the apomorphine effects (1.25 mg/kg) was greatly enhanced compared to that obtained in isolated rats. On the contrary, S 3608 at 80 mg/kg s.c. never induced a high intensity component of the stereotyped activity, as reported with Piribedil[12,13]. The intensity of stereotypy was not enhanced by increasing the dosage over 40 mg/kg, or by grouping animals. Furthermore, S 3608 even at 80 mg/kg did not induce agressivity in grouped rats, contrary to apomorphine at 2.5 mg/kg (Figure).

Rotations. S 3608 and Piribedil (25 mg/kg i.p.) induced a turning behaviour, contralateral to the left lesionned nigro-neostriatal tract. The total number of turns/rat ± SE were respectively in 30 min = 222 ± 92 and 214 ± 84 ($n = 6$). The same compounds administered at 6 mg/kg i.p., induced in 3 h a total number of rotations of 1151 (±370) and 957 (±261) for S 3608 and Piribedil, respectively ($n = 6$). The effect of the two substances appeared similar on the rotation model and was of a long duration.

Interaction with morphine catatonia. The catatonigenic ED_{50} of morphine alone was 8.2 mg/kg (6–11). After a previous treatment with S 3608 (8 mg/kg), the morphine induced rigidity was modified, and the ED_{50} value was 27.3 mg/kg (15–47) ($p < 0.05$). On the other hand, ergocornine, CB 154 and yohimbine gave evidence of antagonistic properties.. In these experiments, the new catatonigenic ED_{50} of morphine were respectively, 38 mg/kg (18–79), 23.5 mg/kg (13–40) and 23.3 mg/kg (12–43) ($p < 0.05$).

Conclusion. The central stimulating effect of S 3608 in the rat induced a stereotyped behavioural response, similar to that of apomorphine. The intensity of stimulation was self-limited and increasing the dosage of S 3608 above 40 mg/kg did not modify the response. The stereotyped response was only enhanced in higher doses in the case of apomorphine. On the contrary, these changes were not observed with S 3608 indicating the central stimulant effect is different in nature or intensity. S 3608 and Piribedil induced turning in rats, contralateral to a lesion in the substantia-nigra. The 2 compounds have a sustained effect, the central dopaminergic stimulant potency being comparable. S 3608 exerted a delay on the onset of morphine catatonia, modifying the ED_{50} of the analgesic. Ergocornine and CB 154 behaved as powerful antagonists on morphine catatonia, the former compound inducing peripheral neurovegetative signs, but the antagonistic effect of CB 154 was observed without any vegetative signs. Yohimbine modified the morphine rigidity, indicating that a block of the central sympathetic activity may be detected in the morphine model, as well as a strong direct dopaminergic stimulant effect.

The data support the hypothesis that S 3608 is a new direct central dopamine stimulant, different from apomorphine, qualitatively and quantitatively. In the rotation model, the potency of stimulation is similar to that of Piribedil. Further experiments are in progress to elucidate the mechanism of action of S 3608 on central dopaminergic and noradrenergic mechanisms.

Résumé. Le S 3608 [1-(coumaran-5-yl méthyl)-4-(2-thiazolyl) pipérazine] produit une important activation des récepteurs centraux de dopamine chez le rat. Les résultats permettent de formuler l'hypothèse d'un mécanisme d'action direct aux doses faibles.

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Ultrastructural Changes in the Neural Lobe of the Rat Pituitary Induced by Reserpine Treatment

Since 1952[1] and 1955[2] there have been reports on the inhibitory role of amines on the release of antidiuretic hormone. The histochemically demonstrable aminergic innervation of the neurosecretory cells in the supraoptic nucleus[3,4] or the direct aminergic innervation of the neural lobe[5] are both likely sites for influencing neuronal control. The observation that reserpine depletes neurosecretory granules from the neurons of supraoptic nucleus[4] led us to study further the effect of reserpine on the ultrastructure of the neural lobe.

The dosages of reserpine, Serpasil® (Ciba), were those generally used for depletion of amines from the brain tissue. Serpasil® was injected i.p. in 10 mg/kg, 5 mg/kg, 2.5 mg/kg, and 1 mg/kg dosages 24 h before killing the rats. 28 male albino rats of Sprague-Dawley strain, weighing about 200 g, were used for the present study. Gomori's chrom-alum haematoxylin staining[6] for neurosecretory material was performed on the neural lobes of 2 rats in every dosage group and on 4 controls. The other rats were perfused via the left ventricle with 2.5% glutaraldehyde 0.1 *M* phosphate buffer solution for 15 min. The neural lobes were then excized and immersion fixed for 4 h in the same fixative, postfixed in 1% OsO_4 for 1 h, dehydrated and embedded in Epon-Araldite. The ultrathin sections were stained with lead citrate[7] and uranyl acetate[8].

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