binding assay employed a standard method,[22] in triplicate, using [^{3}H]dihydromorphine or [^{3}H]naloxone; in the latter case, the medium was 0.1 M in NaCl.

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New Anticonvulsants: Schiff Bases of γ-Aminobutyric Acid and γ-Aminobutyramide

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Schiff bases of γ-aminobutyric acid (γAbu) and γ-aminobutyramide ($\gamma AbuNH_2$) were prepared and tested for anticonvulsant and γAbu mimetic activity. 4-[[(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino]butanoic acid monosodium salt (**4**) and 4-[[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino]butanamide (**5**) blocked bicuculline-induced lethality and convulsions and displaced [^{3}H]γAbu from its membrane binding sites. In the rat dorsal root sensory ganglion, compound **4** exhibited γAbu agonist properties. Compounds **4** and **5** are thus anticonvulsants and directly acting γAbu mimetics.

Under physiological conditions γ-aminobutyric acid (γAbu) poorly crosses the blood-brain barrier.[1] Of the putative γAbu mimetics prepared to date which enter the brain, only muscimol has been widely examined, and even this compound enters the brain to a very limited extent.[2] However, muscimol exhibits an unacceptable central nervous system toxicity, which prevents its wide clinical use.[3,4] From the pharmacological activities of muscimol and the present concepts on possible γAbu involvement in neuropsychiatric disorders, it is likely that a nontoxic γAbu mimetic which easily enters the brain will have useful therapeutic properties. With this in mind, we have prepared derivatives of γAbu with an imine link (Schiff base) to a lipophilic carrier[5-9] in order to facilitate the passage of γAbu across the blood-brain barrier.[10]

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Table I. Acute Lethality and Antibicuculline Activities

compd	LD_{50},[a] mg/kg ip	LD_{50},[a] mg/kg po	ED_{50},[b] mg/kg ip lethality	ED_{50},[b] mg/kg ip convulsions
4	420 ± 40	>2000	70 ± 7	15 ± 4
5	900 ± 160	>3000	65 ± 8	20 ± 3
dipropyl-acetate[c]	1000 ± 50		130 ± 22	33 ± 4
diphenyl-hydantoin	250 ± 49		50 ± 13	12.5 ± 4
muscimol	17 ± 3		>2	0.25 ± 0.1

[a] LD_{50} refers to the dose which causes death in 50% of the mice within 48 h (ip) or 7 days (po). [b] ED_{50} refers to the dose which decreases by 50% the lethality or convulsions which are induced by bicuculline in mice. [c] Refers to the sodium salt.

Table II. Displacement of [^{3}H]γAbu Binding

compd	K_i, μM rat brain	K_i, μM human cerebellum
4	0.59 ± 0.08	0.68 ± 0.09
5	14 ± 3	40 ± 7
muscimol	0.004 ± 0.001	0.011 ± 0.002
γAbu	0.025 ± 0.001	0.20 ± 0.02
dipropylacetate[a]	>100	>100
diphenylhydantoin	>100	>100

[a] Refers to the sodium salt.

Chemistry. $\gamma AbuNH_2$ reacted smoothly with the benzophenone **2** obtained from a Fries rearrangement of the ester **1**, to give compound **5** in good yield (Scheme I). The presence of the hydroxyl group on the benzophenone facilitated the formation of the imine bond.[7,8] The acid **3** was similarly obtained and converted to the sodium salt **4**, in ethanol. The IR, UV, and NMR spectra of the com-

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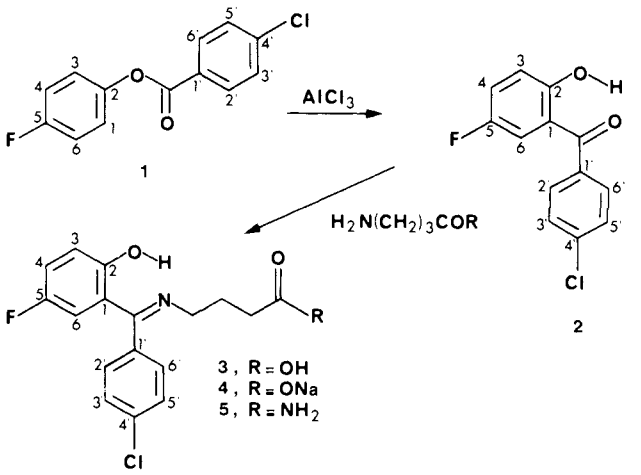
Scheme I



pounds were consistent with the proposed structures. ^{13}C NMR spectra were particularly informative due to ^{13}C–^{19}F couplings.[11,12]

Biological Results and Discussion. For antibicuculline activity, both the amide derivative **5** (SL 76 002) and the sodium salt **4** (SL 75 102) were equipotent with activities similar to that of diphenylhydantoin and twice that of sodium dipropylacetate. Both compounds showed low toxicity (Table I). In the [^{3}H]γAbu binding assay on the human cerebellar cortex preparation (without Triton pretreatment), the sodium salt **4** was 50-fold more potent than the amide **5**, exhibiting a displacement of [^{3}H]γAbu similar to that of γAbu itself (Table II). This may indicate that the amide derivative is converted, in vivo, to the acid before being active at the γAbu receptor. Indeed pharmacokinetic data indicate a rapid and sustained conversion of the amide **5** to the acid **3** in the brain. However, the conversion of **3** or **5** to free γAbu or γAbuNH$_2$ takes several hours either "in vivo" or "in vitro". This, together with the rapid onset of action of the carrier-linked forms, indicates that compounds **5** and **3** possess intrinsic γAbu mimetic activity.

In electrophysiological studies on mammalian dorsal-root ganglion neurons, γAbu but not glycine produces a chloride-dependent depolarization.[13,14] In tests on rat ganglion neurons in vitro (14 experiments), the sodium salt **4** caused a depolarization with an increase in membrane conductance similar to that observed with γAbu. The activity of the salt was, however, an order of magnitude weaker than that which was observed with γAbu. Similar responses were also produced by muscimol and (in previous in vivo experiments[13,20]) by 3-aminopropanesulfonic acid, imidazoleacetic acid, and β-guanidinopropionic acid, all of which are known γAbu agonists. Picrotoxin reduced these effects of the sodium salt **4** in parallel with the γAbu responses of the same neurons.

With regard to the ratios between toxic (LD_{50}) and anticonvulsant (ED_{50}, bicuculline lethality) doses, the amide **5** (SL 76002) has a more favorable ratio than either sodium dipropylacetate or diphenylhydantoin, two clinically useful anticonvulsants. Thus, the amide **5** (SL 76002) and the acid salt **4** (SL 75102) are relatively nontoxic anticonvulsant compounds for which the data are consistent with a γAbu mimetic action.

Experimental Section

Chemistry. Melting points were determined on a Büchi SMP 20 apparatus and are uncorrected. Differential thermic analyses (DTA) were run on a Mettler TA 2000-10 apparatus. ^1H and ^{13}C NMR spectra were recorded on a Brüker WP 80 (80 MHz) or a Brüker WP 200 (200 or 50.29 MHz) using Me_4Si as internal standard. IR spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. UV spectra were run on a Beckman C III spectrophotometer. Elemental analyses were performed on a Perkin-Elmer 240 apparatus connected to a Tektronix 31 calculator and were within ±0.3% of the calculated values.

4-Chlorobenzoic Acid, 4-Fluorophenyl Ester (1). A solution of 4-chlorobenzoyl chloride (37.6 g, 0.215 mol) in Et_2O (100 mL) was added to a stirred solution of 4-fluorophenol (24.1 g, 0.215 mol) and Et_3N (25 g, 0.25 mol) in Et_2O (500 mL) at a rate sufficient to maintain a gentle reflux. After 1 h of additional reflux the reaction mixture was filtered, and the filtrate was washed (saturated $NaHCO_3$, H_2O), dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The solid obtained was recrystallized from light petroleum (bp 60–80 °C) to give 40.9 g (76%) of **1** as a white solid: mp 94–95 °C (lit.[15] mp 145 °C); IR (KBr) 1730 (ν C=O), 1590, 1500 (aromatic ν C=C) cm^{-1}; NMR[16] (^{13}C, $CDCl_3$, 50.29 MHz) proton noise decoupled δ 116.3 (d, $^2J_{CF}$ = 23.6 Hz, C-4 and C-6), 123.1 (d, $^3J_{CF}$ = 8 Hz, C-1 and C-3), 128.0 (s, C-1′), 129.1 (s, C-3′ and C-5′), 131.6 (s, C-2′ and C-6′), 140.4 (s, C-4′), 146.8 (s, C-2), 160.5 (d, $^1J_{CF}$ = 244.1 Hz, C-5), 164.4 (s, C=O). Anal. ($C_{13}H_8ClFO_2$) C, H, Cl.

(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methanone (2). Powdered $AlCl_3$ (21.3 g, 0.16 mol) was added to a stirred melt of **1** (20 g, 0.16 mol) in a well-ventilated hood, and the mixture was heated at 200 °C for 15 min. The solid obtained on cooling was ground to a powder and added slowly to a mixture of HCl (12 N, 400 mL) and ice–water. The resulting suspension was extracted with ether, and the extracts were washed with H_2O, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The solid obtained was treated with charcoal and recrystallized from light petroleum (bp 60–80 °C) to give 12.9 g (65%) of **2** as a yellow solid: mp 65–66 °C (lit.[15] mp 174 °C). An analytical sample was obtained by column chromatography (SiO_2, $CHCl_3$): DTA mp 67.6 °C; IR ($CHCl_3$) 1635 (ν C=O), 1620 and 1595 (aromatic ν C=C) cm^{-1}; NMR (^{13}C, $CDCl_3$, 50.29 MHz) proton noise decoupled δ 118.0 (d, $^2J_{CF}$ = 23.5 Hz, C-6), 118.6 (d, $^3J_{CF}$ = 5 Hz, C-1), 120.1 (d, $^3J_{CF}$ = 7 Hz, C-3), 124.2 (d, $^2J_{CF}$ = 23.5 Hz, C-4), 129.0 (s, C-3′ and C-5′), 130.7 (s, C-2′ and C-6′), 135.9 (s, C-4′), 139.0 (s, C-1′), 154,8, (d, $^1J_{CF}$ = 239 Hz, C-5), 159.6 (s, C-2), 199.2 (s, C=O). Anal. ($C_{13}H_8ClFO_2$) C, H, Cl, F.

4-[[(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino]butanoic Acid (3). Ketone **2** (39.5 g, 0.157 mol) was added to a solution of 4-aminobutanoic acid (15 g, 0.154 mol) and NaOMe (31.7 mL of a 4.86 N solution, 0.154 mol) in EtOH (1.4 L). The solution was evaporated to near dryness at 60 °C under reduced pressure. EtOH (1.2 L) was added and the solvent evaporated as before. This procedure was repeated three times. The yellow residue obtained was taken up in water (4 L), and the solution was acidified to pH 4 by the addition of solid citric acid. Extraction with ether, followed by drying ($MgSO_4$) and evaporation, gave a yellow oil which was induced to crystallize by the addition of cold light petroleum (bp 60–80 °C). Two recrystallizations from hexane gave 31 g (60%) of **3** as a bright yellow solid: mp 98–99 °C; IR (KBr) 1695 (ν C=O), 1610 (ν C=N), 1580 and 1485 (aromatic ν C=C) cm^{-1}; UV λ_{max} (MeOH) 332 (ε 4200), 252 (11 000), 210 nm (22 000). Anal. ($C_{17}H_{15}ClFNO_3$) C, H, Cl, N.

4-[[(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino]butanoic Acid, Monosodium Salt (4). A

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solution of NaOMe in MeOH (7.69 mL of 3.11 N solution, 0.024 mol) was added to a solution of **3** (8.03 g, 0.024 mol) in EtOH (500 mL). The solution was evaporated under reduced pressure, and the residue was washed with ether and dried in vacuo to give 8.28 g (97%) of **4** as a yellow solid: mp 231 °C dec. Anal. ($C_{17}H_{14}ClFNO_3Na$ with 1.95% of H_2O) C, H, Cl N, Na.

4-[[(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)-methylene]amino]butanamide (5). The ketone **2** (15 g, 0.06 mol) was added to a solution of 4-aminobutanamide hydrochloride (9 g, 0.065 mol) and NaOMe (3.25 g, 0.06 mol) in MeOH (0.6 L). The mixture was evaporated to near dryness at 60 °C under reduced pressure. EtOH (0.4 L) was added and the solvent evaporated as before. The yellow residue obtained was dissolved in $CHCl_3$, washed (H_2O), dried ($MgSO_4$), and evaporated to dryness under reduced pressure to give a solid. Recrystallization from light petroleum (bp 60–80 °C) gave 18 g (90%) of **5** as a bright yellow solid: mp 137–140 °C. A further recrystallization from AcOEt gave 16 g (80%) of **5**, mp 139–141 °C.

DTA suggested that **5** existed in two crystalline forms: A, mp 136 °C; B, mp 142.5 °C. IR (KBr) 3400, 3200 (ν NH_2), 1655 (ν C=O), 1620 (ν C=N), 1580, 1490 (ν aromatic C=C) cm^{-1}; UV λ_{max} (MeOH) 332 (ϵ 4200), 250 (10800), 210 (24000); ^1H NMR (80 MHz, Me_2SO-d_6) δ 15.1 (s, 1, O*H*), 7.2–7.8 (m, 4, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$), 7.1–7.4 (3 d, 1, $^3J_{H_4F}$ = 8 Hz, $^3J_{H_4H_3}$ = 9 Hz, $^4J_{H_4H_6}$ = 3 Hz, H_4), 6.8–7.1 (2 d, 1, $^3J_{H_3H_4}$ = 9 Hz, $^3J_{H_3F}$ = 5 Hz, H_3), 6.7 (br, 2, $CONH_2$), 6.25–6.60 (2 d, 1, $^3J_{H_6F}$ = 10 Hz, $^4J_{H_6H_4}$ = 3 Hz, H_6), 3.3 (t, 2, $^3J_{HH}$ = 6.5 Hz, NCH_2CH_2–), 1.6–2.4 (m, 4, –$CH_2CH_2CONH_2$); ^{13}C NMR ($CDCl_3$, 50.29 MHz) proton noise decoupled δ 26.3 (s, $CH_2CH_2CH_2$), 33.0 (s, CH_2CONH_2), 51.0 (s, =NCH_2), 116.5 (d, $^2J_{CF}$ = 24.5 Hz, C-6), 199.0 (d, $^3J_{CF}$ = 8 Hz, C-3), 119.4 (d, $^3J_{CF}$ = 7.0 Hz, C-1), 119.9 (d, $^2J_{CF}$ = 23.5 Hz, C-4), 128.8 (s, C-3′ and C-5′), 129.5 (s, C-2′ and C-6′), 131.8 (s, C-1′), 135.8 (s, C-4′), 154.6 (d, $^1J_{CF}$ = 235.3 Hz, C-5), 159.3 (br s, C-2), 172.8 (d, $^4J_{CF}$ = 3.0 Hz, *C*=NCH–), 174.7 (s, $CONH_2$). Anal. ($C_{17}H_{16}ClFN_2O_2$) C, H, Cl, F, N.

Pharmacology. Acute Lethality in Mice. Compounds were administered by either the intraperitoneal or oral route to male albino mice (CD_1, Charles River, France, weighing 18–22 g), which were then returned to their home cages (10 mice per cage). The number of dead mice from each dose were noted 48 h or 7 days after intraperitoneal or oral administration, respectively. LD_{50} values (dose inducing death in 50% of the mice) were determined, by use of log-probit paper.[17]

Antagonism of Bicuculline-Induced Lethality in Mice. Compounds were injected intraperitoneally, at predetermined serial doses, 30 min before bicuculline (0.9 mg/kg, iv). The percentage of mice dead (10 mice per drug per dose and per experiment) were noted 3 h after the injection of bicuculline (controls: 100% mortality). The ED_{50} (dose protecting 50% of the mice against the lethal effect of bicuculline) was obtained for each compound by the use of log-probit paper.[17]

Antagonism of Bicuculline-Induced Clonic Convulsions in Mice. Compounds were injected intraperitoneally, at increasing doses, 30 min before bicuculline (0.45 mg/kg, iv). The percentage of mice convulsing were noted during 1 h after bicuculline (controls: 100% clonic convulsions and 10–20% tonic convulsions and death). The ED_{50} (dose protecting 50% of the animals against bicuculline clonic convulsions) was calculated for each compound using log-probit paper.[17]

Displacement of [^{3}H]γAbu Binding in Rat and Human Brains. The affinity of the compounds for the [^{3}H]γAbu binding site to membranes prepared from rat whole brains (Triton-X-100 treated) or human cerebellar cortex (no Triton treatment) was determined by the method of Enna and Snyder,[18] as modified by Lloyd et al.[19] Compounds (final concentration range 5×10^{-9} to 10^{-4} M) were incubated in the presence of 4×10^{-9} M [^{3}H]γAbu (45 Ci/mmol, Amersham Nuclear), and the displacement of [^{3}H]Abu binding was determined by scintillation spectrometry (Nuclear Chicago Mark III).

γAbu Mimetic Activity on Rat Dorsal-Root Ganglion Neurons. Compounds were administered by transient superfusion of rat dorsal-root ganglia in vitro. Single-barrel intracellular electrodes were used to record the membrane potential and also to pass constant-current transmembrane pulses, by means of which the membrane conductance could be estimated.[20] With this method of application, threshold responses (1 mV) are obtained with concentrations of 5×10^{-6} to 5×10^{-5} M γAbu. The maximum response (up to 25 mV) is obtained at concentrations greater than 10^{-4} M.

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Neuroleptic 4-Aryltetrahydropyrrolo[3,4-*b*]indoles

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7-Fluoro-4-(4-fluorophenyl)-2-[4-(4-fluorophenyl)-4-hydroxybutyl]-1,2,3,4-tetrahydropyrrolo[3,4-*b*]indole displayed neuroleptic-like activity in an animal model approximately equipotent with that of chlorpromazine but was of substantially greater duration of action. Structure–activity relationships of the series are discussed.

Previous papers from our laboratories have described the discovery of potent neuroleptic activity in a series of 5-aryl-1,2,3,4-tetrahydro-γ-carbolines[1,2] and the development of structure–activity relationships within this series[3] leading to the potent and long-acting derivative CP-36584 (I). This paper extends the previously reported work to a series of 4-aryl-1,2,3,4-tetrahydropyrrolo[3,4-*b*]indoles

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