α_1-Adrenoceptor Reagents. Synthesis of Some 5,6,11,11a-Tetrahydro-1*H*-imidazo[1′,5′ : 1,6]pyrido and 5,6,11,11b-Tetrahydro-1*H*-imidazo-[1′,5′ : 1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-diones

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Several 2-substituted 5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′ : 1,6]pyrido (1) and 5,6,11,11b-tetrahydro-1*H*-imidazo[1′,5′ : 1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-diones (2) were synthesized and studied by 2D-NMR spectroscopy and difference nuclear Overhauser effect experiments. All the compounds were evaluated for *in vitro* α_1 adrenoceptor affinity by radioligand receptor binding assays. The most active derivative in displacement of [^{3}H]prazosin from rat cortical membranes was 1b (K_i = 219 nM). At 1 μM concentration, compounds 1 and 2 had no effect on the benzodiazepine or 5-HT$_{1A}$ receptor. The biological activity profile of 1b makes it a possible lead compound for the design of new selective α_1 adrenoceptor ligands.

Key words β-carboline–hydantoin; *N*-phenylpiperazine: nuclear Overhauser effect; α_1-adrenergic receptor

Adrenoceptors (AR) are a complex family of receptors that includes several subtypes within each of the classes, α_1, α_2 and β. The α-adrenergic receptors play an important role in the regulation of a variety of physiological processes, particularly within, though not limited to, the cardiovascular system. These include, for instance, the observation that centrally located α-adrenoceptors are the main target for the development of antihypertensive agents. The variety of origins and pathologies of hypertension requires several antihypertensive drugs to be clinically available, since it is difficult to control all types of hypertension through the use of a single drug, and each antihypertensive agent has particular side effects. Several selective ligands for the α_1AR are known (*e.g.* prazosin, WB4101), but the fact that their chemical structures are apparently unrelated has left the nature of the binding site uncertain.

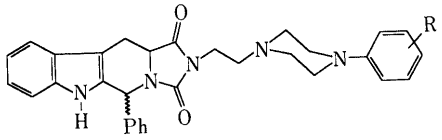
With a view to preparing new selective ligands acting on the α_1AR and clarifying how some of the ligand's structural features influence the fit with the receptor protein, we synthesized a series of tetrahydro-1*H*-imidazo[1′,5′ : 1,6]pyrido and [1′,5′ : 1,2]pyrido[3,4-*b*]-indole-1,3(2*H*)-dione derivatives **1** and **2**.

The quinazoline-2,4-dione derivative SGB-1543 (**3**)[1,2] is a potent α_1AR ligand with antagonist properties. A variety of structural analogs to SGB-1543 with different heterocycles,[3-6] such as pyrimido[5,4-*b*]indole (**4**) derivatives,[7] have been proven effective in lowering blood pressure by antagonizing the α_1-adrenoceptor. On the other hand, CI-926 (**5**) is an orally effective antihypertensive agent in several animal models of hypertension,[8] and the profile of CI-926 suggests that its antihypertensive effect is due to blockade of α_1-adrenergic receptors.

In the compounds synthesized in this study, the β-carboline–hydantoin system can be considered a structure analogous to pyrimido[5,4-*b*]indole-2,4-dione, with the pyrimidine-2,4-dione being replaced by a hydantoin ring and the indole nucleus by a β-carboline system. Substitutions at position 2 are represented by several (phenylpiperazinyl)ethyl side chains, which are considered to be an essential moiety for the lowering of blood pressure.[9]

Chemistry

Compounds **1a**—**e** were synthesized as shown in Chart 1. The starting (±)-*cis*-1-phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**6**) was prepared by reaction of



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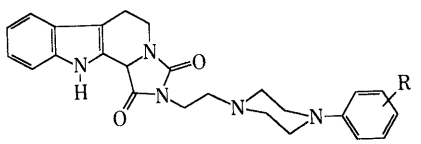

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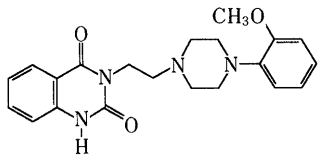

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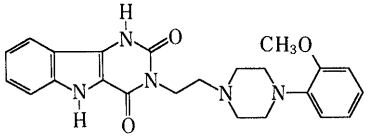

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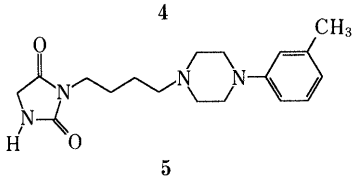

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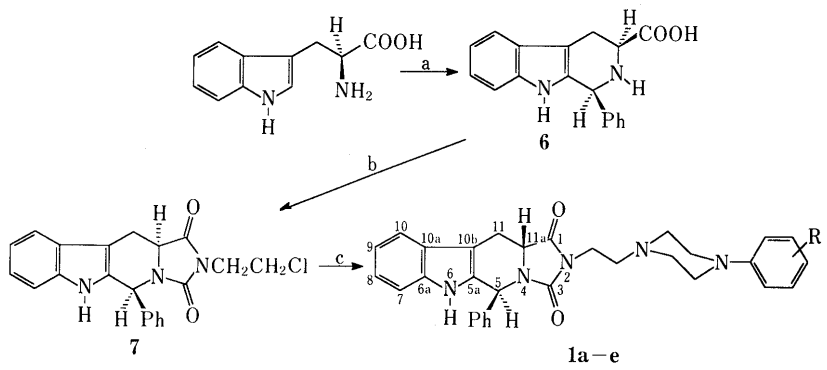


a) $PhCHO/H^+$; b) $ClCH_2CH_2NCO$/acetone, N_2; c) *N*-phenylpiperazines/acetonitrile

Chart 1

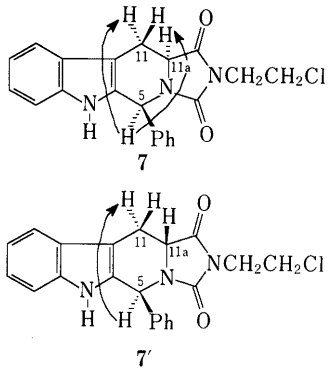


Chart 2. Schematic Illustration of NOE for **7** and **7′**

L-tryptophan with benzaldehyde.[10] Treatment of **6** with 2-chloroethyl isocyanate in refluxing acetone gave *cis*-2-(2-chloroethyl)-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (**7**).

The carbon signals attributable to the indole ring were assigned on the basis of the distortionless enhancement by polarization transfer (DEPT) method and reference data.[10,11] The stereochemical assignment of **7** was determined by ^{13}C-NMR at C-5 and C-11a and confirmed by nuclear Overhauser effect (NOE) experiments. In a preceding paper,[10] we have demonstrated that in analogous structures the *cis* isomer could be completely converted into the *trans* isomer on heating in refluxing acetonitrile in the presence of sodium carbonate, while the *trans* isomer remained unaffected when treated under analogous conditions. Therefore, the *cis* isomer **7** [($CDCl_3$) δ: 56.7 (C-11a), 57.8 (C-5); (DMSO-d_6) δ: 55.9 (C-11a), 57.7 (C-5)] was converted into the *trans* isomer **7′** [($CDCl_3$) δ: 51.8 (C-11a), 53.0 (C-5); (DMSO-d_6) δ: 51.7 (C-11a), 53.0 (C-5)]. Irradiation of **7** at δ 5.73 (H-5) decreased the signal intensity of the H-11a methine proton at δ 4.38 and one of the methylene protons (H-11α) at 3.47 (Chart 2). This confirmed that compound **7** was the *cis* isomer. In the difference NOE spectrum of **7′**, irradiation at δ 6.32 (H-5) enhanced the signal intensity of one of the methylene protons (H-11α) at δ 3.53, but that of the H-11a methine proton at δ 4.35 remained unchanged. These results confirmed that compound **7′** was the *trans* isomer.

The reaction of **7** with *N*-phenylpiperazines in refluxing acetonitrile in the presence of Na_2CO_3 afforded the corresponding *trans*-2-[2-(4-phenylpiperazin-1-yl)ethyl]-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]-pyrido[3,4-*b*]indole-1,3(2*H*)-diones (**1a—e**) (Chart 1). The physical properties of these compounds are summarized in Table 1, and all of them have been characterized on the basis of their ^{1}H- and ^{13}C-NMR data (see Experimental). The carbon signals were assigned on the basis of the DEPT method.

Chart 3 shows the synthetic pathway for preparing the 2-[2-(4-phenylpiperazin-1-yl)ethyl]-5,6,11,11b-tetrahydro-1*H*-imidazo[1′,5′:1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-diones (**2a—e**) (Table 2). The starting (±)-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid[12] (**8**) was treated with 2-chloroethyl isocyanate to give **9**.[13] The third step in preparing the final compounds **2a—e** was to treat **9** with *N*-phenylpiperazines in refluxing acetonitrile in the presence of sodium carbonate.

The structures of these compounds have been characterized on the basis of their ^{1}H- and ^{13}C-NMR data, and all of them gave satisfactory elemental analyses. For example, the IR spectrum of **2a** exhibited a band at 3360 cm^{-1} (NH) and absorptions at 1780, 1720 cm^{-1} (carbonyl groups). The ^{1}H-NMR spectrum ($CDCl_3$) showed in the aromatic region the characteristic patterns of the indole and phenyl rings. All the aliphatic protons could be assigned using ^{1}H–^{1}H correlation spectroscopy (COSY) (see Experimental). Our attention was drawn to the absence of the signal due to the methine proton H-11b, suggesting that compound **2a** exists in the enol form in $CDCl_3$. This was confirmed by examination of the ^{13}C-NMR spectrum, in which the signal of the 11b-carbon appears at 87.1 ppm, whereas in the case of **9**, this carbon resonates at 56.3 ppm. The carbon signals attributable to the aliphatic region were analyzed by the application of 2D heteronuclear correlation (HETCOR) spectroscopy.

These results suggest that internal hydrogen bonding stabilizes the enol form in the derivatives **2**, where a seven-membered ring can be formed. This hydrogen bonding is not possible in the structure **9**. A similar result was obtained in the hydrolysis of **9** to give the 2-hydroxyethyl derivative.[13]

Biological Results and Discussion

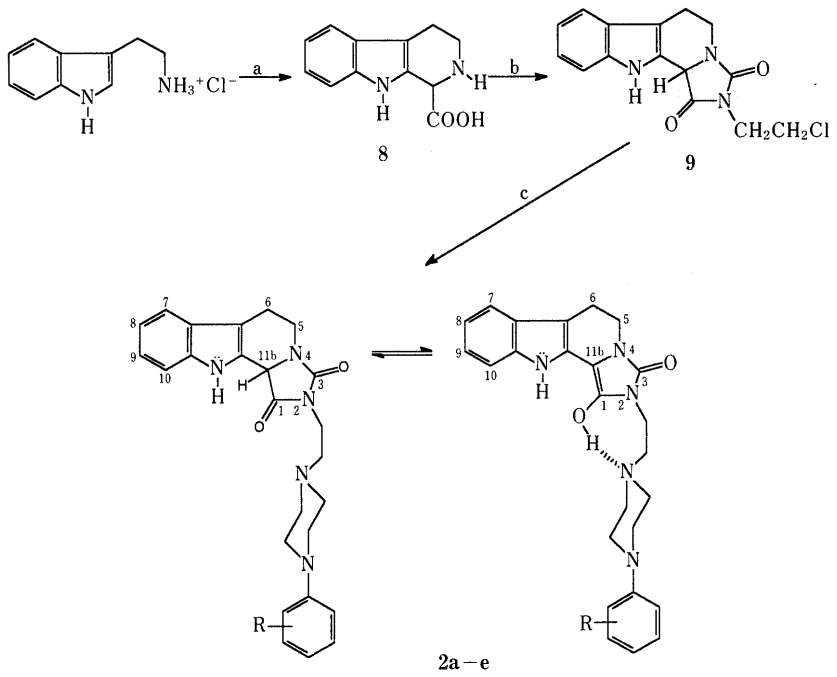
The compounds were all tested for the ability to interact *in vitro* with the central benzodiazepine, α_1-adrenergic and

Table 1. Physical Properties of 2-[2-(4-Phenylpiperazin-1-yl)ethyl]-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-diones (**1a—e**)

No.	R	mp (°C)	Recryst. solv.	Yield (%)	Formula	Analysis (%) Calcd			Found		
						C	H	N	C	H	N
1a	H	129—131	EtOH	60	$C_{31}H_{31}N_5O_2$	73.63	6.18	13.85	73.72	6.15	13.42
1b	*o*-OCH_3	96—98	MeOH–H_2O	41	$C_{32}H_{33}N_5O_3$	71.75	6.21	13.07	71.39	6.20	12.98
1c	*m*-Cl	98—100	MeOH–H_2O	54	$C_{31}H_{30}ClN_5O_2$	68.94	5.60	12.97	68.83	5.70	12.62
1d	*m*-CF_3	116—118	MeOH–H_2O	44	$C_{32}H_{30}F_3N_5O_2$	67.00	5.27	12.21	66.80	5.27	11.98
1e	*p*-NO_2	203—204	EtOH–H_2O	52	$C_{31}H_{30}N_6O_4$	67.61	5.49	15.26	67.50	5.70	14.98

Table 2. Physical Properties of 2-[2-(4-Phenylpiperazin-1-yl)ethyl]-5,6,11,11b-tetrahydro-1*H*-imidazo[1′,5′:1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-diones (**2a—e**)

No.	R	mp (°C)	Recryst. solv.	Yield (%)	Formula	Analysis (%) Calcd			Found		
						C	H	N	C	H	N
2a	H	172—173	EtOH–H_2O	35	$C_{25}H_{27}N_5O_2$	69.90	6.34	16.30	70.01	6.17	16.48
2b	*o*-OCH_3	104—106	EtOH–H_2O	40	$C_{26}H_{29}N_5O_3$	67.95	6.36	15.25	67.82	5.93	15.34
2c	*m*-Cl	209—210	EtOH–H_2O	69	$C_{25}H_{26}ClN_5O_2$	64.71	5.65	15.10	64.70	5.12	15.57
2d	*m*-CF_3	120—122	EtOH–H_2O	37	$C_{26}H_{26}F_3N_5O_2$	62.76	5.27	14.08	62.90	5.12	13.93
2e	*p*-NO_2	192—194	EtOH–H_2O	34	$C_{25}H_{26}N_6O_4$	63.27	5.52	17.72	63.12	5.35	17.58



a) HOOC-CHO/H_2O; b) $ClCH_2CH_2NCO$/acetone, N_2; c) *N*-phenylpiperazines/acetonitrile

Chart 3

5-HT_{1A} serotoninergic receptors. At 1 μM concentration, compounds **1d** and **2a,d,e** had no effect on the α_1-adrenergic receptor, while **1e**, **2c**, **1c**, **2b** and **1a** displaced [^{3}H]prazosin specific binding by 11, 13, 27, 30 and 58%, respectively. Compound **1b** (R = *o*-OCH_3) showed a nM range interaction at the α_1-adrenergic receptor ($K_i = 219 \pm 24$ nM, mean ± S.E. of three experiments). At 1 μM concentration, **1** and **2** had no effect on the benzodiazepine or 5-HT_{1A} receptor.

Analysis of the data from this study reveals that compounds **1** have higher *in vitro* affinity at the α_1-adrenergic receptor than the analogs **2**. Compound **1b** binds with moderate affinity and good selectivity to α_1AR. The structural feature which seems to be necessary for receptor binding in these molecules, is the presence of an *ortho*-methoxy substituent on the phenyl ring at the N-4 position of the piperazine moiety of the side chain.

This is in agreement with previous studies suggesting that the introduction of an *o*-methoxy group on the phenylpiperazine moiety gives the highest affinity for

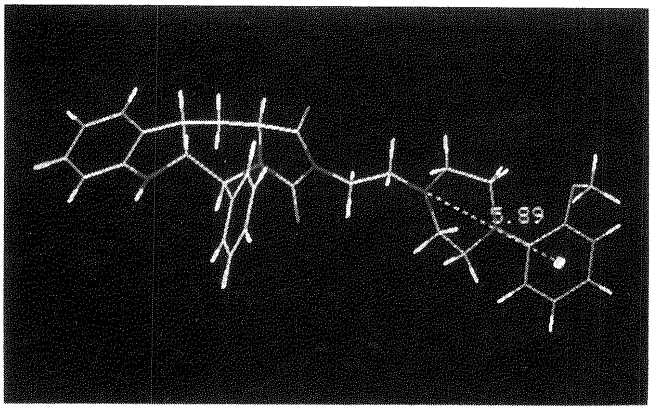


Fig. 1. Minimum Energy Conformation for Compound **1b**

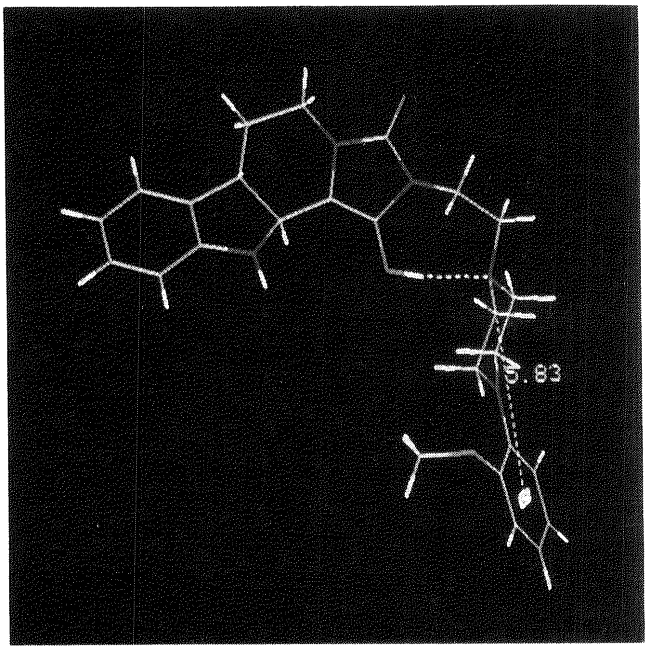


Fig. 2. Minimum Energy Conformation for Compound **2b**

α_1-adrenoceptor.[6,7,14] These changes in affinity could be partially explained by steric effects. Probably *meta* or *para* substituents create steric bulk in a forbidden volume of the receptor site, while *ortho* substituents arrange the phenyl ring and the piperazine group into a conformation which better fits the binding site. Electronic effects may also be involved.

Interestingly, compound **1b** was significantly more potent than **2b**. Molecular modeling studies for these analogs were performed on a Silicon Graphic IRIS workstation with the DISCOVER module of the INSIGHT II program.[15] The structures were minimized using the steepest descent method and a conformational search around flexible bonds was performed through a 360° range in increments of 30°, reaching RMS (root mean square) values of 0.00012 for **1b** and 0.00010 for **2b**.

In the more stable conformations of **1b** and **2b** (Figs. 1 and 2), the mean distance between the center of the phenyl ring and the nitrogen was 5.89 and 5.83 Å, respectively (suitable distance for binding to the receptor protein). However, conformer **2b** showed an intramolecular hydrogen bond between the enol form and the nitrogen of the piperazine (1.81 Å). This would cause a decrease of the basicity of this nitrogen and could explain the low affinity of **2b** compared to **1b**.

In conclusion, these results suggest that compound **1b** is a possible lead compound for the design of new selective α_1 adrenoceptor ligands.

Experimental

Melting points were determined using a capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin–Elmer 781 spectrophotometer. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were taken on a Varian VXR-300S spectrometer, with tetramethylsilane as an internal standard. Optical rotations were measured on a Perkin–Elmer 141 polarimeter. Elemental analysis was carried out on a Perkin–Elmer 2400 CHN elemental analyzer. Solvents were purified and dried prior to use by distillation from an appropriate drying agent. Analytical thin-layer chromatography (TLC) was performed on precoated TLC plates (Silica gel 60 F_{254}, layer thickness 0.2 mm). Column chromatography was performed with silica gel (70—230 mesh).

The following intermediates were prepared according to the literature: (±)-*cis*-1-phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**6**).[10] (±)-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (**8**),[12] and 2-(2-chloroethyl)-5,6,11,11b-tetrahydro-1*H*-imidazo[1′,5′ : 1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (**9**).[13]

***cis*-2-(2-Chloroethyl)-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo-[1′,5′ : 1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (7)** 2-Chloroethyl isocyanate (890 mg, 8.5 mmol) was added to a suspension of **6** (2.5 g, 8.5 mmol) in dry acetone (50 ml). The reaction mixture was refluxed under N_2 for 2 h, then evaporated to dryness to give 2.2 g (67%) of **7**, mp 202—203 °C (methanol/water). IR (KBr): 3340, 1770, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.02 (1H, ddd, J=14.9, 11.3, 2.1 Hz, H-11), 3.47 (1H, ddd, J=14.9, 4.5, 1.2 Hz, H-11), 3.59—3.82 (4H, m, NCH_2CH_2Cl), 4.38 (1H, dd, J=11.3, 4.5 Hz, H-11a), 5.74 (1H, s, H-5), 7.07—7.29 (8H, m, H-9, H-8, H-7 indole and phenyl), 7.50 (1H, d, J=8.3 Hz, H-10 indole), 7.55 (1H, s, NH). ^{1}H-NMR (DMSO-d_6) δ: 2.98—3.09 (1H, m, H-11), 3.36 (1H, dd, J=14.6, 4.2 Hz, H-11), 3.66—3.76 (4H, m, NCH_2CH_2Cl), 4.55 (1H, dd, J=11.3, 4.3 Hz, H-11a), 5.88 (1H, s, H-5), 6.99—7.07 (2H, m, H-9, H-8 indole), 7.21—7.36 (6H, m, H-7 indole and phenyl), 7.54 (1H, d, J=7.3 Hz, H-10 indole), 10.76 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 22.4 (C-11), 39.8, 40.2 (NCH_2CH_2Cl), 56.7 (C-11a), 57.8 (C-5), 106.7 (C-10b), 111.1 (C-7), 118.4 (C-10), 120.1 (C-9), 122.7 (C-8), 126.0 (C-10a), 133.1 (C-5a), 136.6 (C-6a), 127.6, 128.6, 128.8, 138.3 (phenyl), 153.9 (C-3), 171.3 (C-1). ^{13}C-NMR (DMSO-d_6) δ: 22.0 (C-11), 39.6, 41.1 (NCH_2CH_2Cl), 55.9 (C-11a), 57.7 (C-5), 105.0 (C-10b), 111.4 (C-7), 118.3 (C-10), 118.9 (C-9), 121.5 (C-8), 126.0 (C-10a), 134.8 (C-5a), 136.8 (C-6a) 127.3, 127.4, 128.2, 140.7 (phenyl), 153.8 (C-3), 171.7 (C-1). *Anal.* Calcd for $C_{21}H_{18}ClN_3O_2$: C, 66.40; H, 4.77; Cl, 9.33; N, 11.06. Found: C, 66.36; H, 4.95; Cl, 9.66; N, 10.95.

***trans*-2-(2-Chloroethyl)-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo-[1′,5′ : 1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (7′)** A suspension of **7** (0.5 g, 1.3 mmol) and sodium carbonate (0.3 g, 2.6 mmol) in acetonitrile (20 ml) was refluxed for 2 h. After filtration and evaporation of the filtrate under reduced pressure, the residual oil was purified by silica gel column chromatography with *n*-hexane–ethyl acetate (1 : 1) to give 0.44 g (90%) of **7′**, mp 204—205 °C (ethanol–water). IR (KBr): 3340, 1770, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.94 (1H, ddd, J=15.4, 11.1, 1.8 Hz, H-11), 3.52 (1H, dd, J=15.1, 5.5 Hz, H-11), 3.72—3.92 (4H, m, NCH_2CH_2Cl), 4.34 (1H, dd, J=10.9, 5.5 Hz, H-11a), 6.31 (1H, s, H-5), 7.14—7.40 (8H, m, H-9, H-8, H-7 indole and phenyl), 7.56 (1H, d, J=7.4 Hz, H-10 indole), 7.87 (1H, s, NH). ^{1}H-NMR (DMSO-d_6) δ: 2.83 (1H, ddd, J=14.8, 11.8, 2.0 Hz, H-11), 3.41 (1H, dd, J=14.9, 5.7 Hz, H-11), 3.75—3.82 (4H, m, NCH_2CH_2Cl), 4.64 (1H, dd, J=10.6, 5.6 Hz, H-11a), 6.25 (1H, s, H-5), 6.99—7.12 (2H, m, H-9, H-8 indole), 7.28—7.38 (6H, m, H-7 indole and phenyl), 7.55 (1H, d, J=7.6 Hz, H-10 indole), 10.92 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 23.2 (C-11), 39.7, 40.2 (NCH_2CH_2Cl), 51.8 (C-11a), 53.0 (C-5), 107.3 (C-10b), 111.0 (C-7), 118.1 (C-10), 119.7 (C-9), 122.5 (C-8), 125.8 (C-10a), 130.0 (C-5a), 136.4 (C-6a), 127.8, 128.6, 128.8, 138.9 (phenyl), 153.9 (C-3), 172.4 (C-1). ^{13}C-NMR (DMSO-d_6) δ: 22.8 (C-11), 39.9, 41.2 (NCH_2CH_2Cl), 51.7 (C-11a), 53.0 (C-5), 106.0 (C-10b), 111.5 (C-7), 118.3 (C-10), 119.0 (C-9), 121.8 (C-8), 125.8 (C-10a), 131.2 (C-5a), 136.8 (C-6a), 127.9, 128.3, 128.8, 140.1 (phenyl), 154.0 (C-3),

172.6 (C-1).

***trans*-2-[2-(4-Phenylpiperazin-1-yl)ethyl]-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-diones (1a—e). General Procedure** An *N*-phenylpiperazine (13 mmol) was added to a suspension of **7** (2.5 g, 6.5 mmol) and sodium carbonate (1.4 g, 13 mmol) in acetonitrile (70 ml), and the reaction mixture was refluxed for 80 h. The precipitate was removed by filtration and the filtrate was evaporated under reduced pressure to give an oil, which was purified by column chromatography (silica gel, ethyl acetate–hexane, 1 : 1). The title compounds were each obtained as a solid.

***trans*-2-[2-(4-Phenylpiperazin-1-yl)ethyl]-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (1a)** IR (KBr): 3340, 1780, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.57—2.96 (11H, m, $4CH_2$-piperazine, CH_2–N, H-11), 3.15 (1H, dd, J=15.3, 5.8 Hz, H-11), 3.65 (2H, t, J=5.5 Hz, N–CH_2), 4.09 (1H, dd, J=11.0, 5.8 Hz, H-11a), 6.35 (1H, s, H-5), 6.73—6.82 (4H, m, ArH), 7.04—7.26 (9H, m, ArH), 7.39 (1H, d, J=7.3 Hz, H-10 indole), 8.80 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 22.5 (C-11), 35.5 (N–CH_2), 48.3 ($2CH_2$-piperazine), 51.5 (C-11a), 52.6 (C-5), 52.8 ($2CH_2$-piperazine), 55.3 (CH_2–N), 107.8, 110.9, 115.7, 118.4, 119.4, 119.7, 122.6, 125.9, 127.9, 128.7, 128.8, 128.9, 129.9, 136.4, 139.0, 150.7 (aromatic carbons), 154.5 (C-3), 172.7 (C-1).

***trans*-2-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]-5-phenyl-5,6,-11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (1b)** IR (KBr): 3320, 1780, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.65—2.87 (11H, m, $4CH_2$-piperazine, CH_2–N, H-11), 3.19 (1H, dd, J=15.0, 5.7 Hz, H-11), 3.64 (2H, t, J=5.5 Hz, N–CH_2), 3.78 (3H, s, OCH_3), 4.11 (1H, dd, J=11.1, 5.7 Hz, H-11a), 6.33 (1H, s, H-5), 6.70—6.97 (4H, m, ArH), 7.12—7.27 (8H, m, ArH), 7.41 (1H, d, J=7.2 Hz, H-10 indole), 8.87 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 22.5 (C-11), 35.5 (N–CH_2), 49.9 ($2CH_2$-piperazine), 51.5 (C-11a), 52.6 (C-5), 53.0 ($2CH_2$-piperazine), 55.1, 55.3 (CH_2–N, OCH_3), 107.7, 110.9, 111.0, 118.0, 118.3, 119.6, 120.7, 122.5, 122.6, 125.9, 127.9, 128.5, 128.8, 129.9, 136.4, 139.0, 140.7, 151.9 (aromatic carbons), 154.4 (C-3), 172.7 (C-1).

***trans*-2-[2-[4-(3-Chlorophenyl)piperazin-1-yl]ethyl]-5-phenyl-5,6,11,-11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (1c)** IR (KBr): 3340, 1780, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.61—2.93 (11H, m, $4CH_2$-piperazine, CH_2–N, H-11), 3.13 (1H, dd, J=15.3, 5.7 Hz, H-11), 3.66 (2H, t, J=5.4 Hz, N–CH_2), 4.10 (1H, dd, J=11.4, 5.7 Hz, H-11a), 6.37 (1H, s, H-5), 6.61 (1H, d, J=8.1 Hz, H-6 *m*-Cl-phenyl), 6.67 (1H, s, H-2 *m*-Cl-phenyl), 6.76 (1H, d, J=8.1 Hz, H-4 *m*-Cl-phenyl), 7.10 (1H, t, J=8.1 Hz, H-5 *m*-Cl-phenyl), 7.14—7.27 (8H, m, H-9, H-8, H-7 indole and 5H phenyl), 7.39 (1H, d, J=7.8 Hz, H-10 indole), 8.81 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 22.7 (C-11), 35.8 (N–CH_2), 48.1 ($2CH_2$-piperazine), 51.8 (C-11a), 52.8 (C-5), 52.9 ($2CH_2$-piperazine), 55.7 (CH_2–N), 108.3, 111.2, 113.9, 115.7, 118.8, 119.2, 120.0, 123.0, 126.2, 128.3, 129.0, 129.2, 130.1, 130.3, 134.9, 136.6, 139.2, 152.1 (aromatic carbons), 154.8 (C-3), 173.0 (C-1).

***trans*-2-[2-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]ethyl]-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (1d)** IR (KBr): 3320, 1770, 1720 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.40—3.08 (11H, m, $4CH_2$-piperazine, CH_2–N, H-11), 3.40—3.52 (1H, m, H-11), 3.58—3.83 (2H, m, N–CH_2), 4.54—4.59 (1H, m, H-11a), 6.29 (1H, s, H-5), 7.00—7.23 (4H, m, ArH), 7.28—7.40 (8H, m, ArH), 7.54 (1H, d, J=7.2 Hz, H-10 indole), 10.88 (1H, s, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.9 (C-11), 35.5 (N–CH_2), 47.7 ($2CH_2$-piperazine), 51.6 (C-11a), 52.3 (C-5), 52.9 ($2CH_2$-piperazine), 54.4 (CH_2–N), 106.2, 110.9, 111.5, 114.6, 118.3, 118.7, 118.9, 121.8, 123.9 (q, CF_3, $^1J_{C-F}$=272.5 Hz), 125.9, 127.9, 128.1, 128.7, 129.9, 130.2, 131.3, 136.8, 140.1, 151.3 (aromatic carbons), 154.4 (C-3), 172.6 (C-1).

***trans*-2-[2-[4-(4-Nitrophenyl)piperazin-1-yl]ethyl]-5-phenyl-5,6,11,-11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (1e)** IR (KBr): 3370, 1770, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.48—2.72 (7H, m, $2CH_2$-piperazine, CH_2–N, H-11), 3.10—3.25 (4H, m, $2CH_2$-piperazine), 3.30 (1H, dd, J=15.5, 5.8 Hz, H-11), 3.67—3.72 (2H, m, N–CH_2), 4.21 (1H, dd, J=11.0, 5.8 Hz, H-11a), 6.36 (1H, s, H-5), 6.69 (2H, d, J=9.3 Hz, H-2 and H-6 *p*-NO_2-phenyl), 7.12—7.31 (8H, m, H-9, H-8, H-7 indole and 5H phenyl), 7.46 (1H, d, J=7.2 Hz, H-10 indole), 8.06 (2H, d, J=9.1 Hz, H-3 and H-5 *p*-NO_2-phenyl), 8.46 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 22.8 (C-11), 35.4 (N–CH_2), 46.3 ($2CH_2$-piperazine), 51.7 (C-11a), 52.2 (C-5), 52.9 ($2CH_2$-piperazine), 55.0 (CH_2–N), 107.9, 111.0, 112.4, 118.4, 119.9, 122.8, 125.7, 125.9, 128.0, 128.7, 128.9, 130.0, 136.4, 138.2, 139.0, 154.5 (aromatic carbons), 154.6 (C-3), 172.7 (C-1).

2-[2-(4-Phenylpiperazin-1-yl)ethyl]-5,6,11,11b-tetrahydro-1*H*-imidazo-[1′,5′:1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-diones (2a—e). General Procedure An *N*-phenylpiperazine (6 mmol) was added to a suspension of **9** (1 g, 3 mmol) and sodium carbonate (0.64 g, 6 mmol) in acetonitrile (30 ml), and the reaction mixture was refluxed for 80 h. The precipitate was removed by filtration and the filtrate was evaporated under reduced pressure to give an oil, which was purified by column chromatography (silica gel, ethyl acetate–hexane, 7 : 3). The title compounds were each obtained as a solid.

2-[2-(4-Phenylpiperazin-1-yl)ethyl]-5,6,11,11b-tetrahydro-1*H*-imidazo-[1′,5′:1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (2a) IR (KBr): 3360, 1780, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.82—3.03 (6H, m, 2H-6, $2CH_2$-piperazine), 3.36—3.58 (5H, m, H-5a, $2CH_2$-piperazine), 3.70 (2H, t, J=6.0 Hz, CH_2–N), 3.87 (2H, t, J=6.0 Hz, N–CH_2), 4.48 (1H, dd, J=13.8, 4.8 Hz, H-5b), 6.84—6.89 (3H, m, H-2, H-4 and H-6 phenyl), 7.13 (1H, td, J=7.8, 1.2 Hz, H-8 indole), 7.22—7.29 (3H, m, H-3 and H-5 phenyl, H-9 indole), 7.39 (1H, d, J=8.1 Hz, H-10 indole), 7.52 (1H, d, J=7.5 Hz, H-7 indole), 8.57 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 20.7 (C-6), 35.8 (C-5), 40.1, 40.2 (–NCH_2CH_2N–), 47.9, 48.2 ($2CH_2$-piperazine), 56.1, 57.4 ($2CH_2$-piperazine), 87.1 (C-11b), 111.2, 111.7, 116.0, 119.2, 119.9, 120.1, 123.9, 124.0, 125.5, 129.0, 136.7, 150.2 (aromatic carbons), 155.4 (C-3), 169.4 (C-1).

2-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]-5,6,11,11b-tetrahydro-1*H*-imidazo[1′,5′:1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (2b) IR (KBr): 3350, 1780, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.75—2.97 (6H, m, 2H-6, $2CH_2$-piperazine), 3.39—3.56 (5H, m, H-5a, $2CH_2$-piperazine), 3.66 (2H, t, J=5.7 Hz, CH_2–N), 3.73 (2H, t, J=5.7 Hz, N–CH_2), 3.84 (3H, s, OCH_3), 4.58 (1H, dd, J=13.8, 5.1 Hz, H-5b), 6.84—6.94 (3H, m, H-3, H-4 and H-6 phenyl), 6.99—7.02 (1H, m, H-5 phenyl), 7.14 (1H, td, J=8.1, 1.2 Hz, H-8 indole),7.26 (1H, td, J=8.1, 1.2 Hz, H-9 indole), 7.41 (1H, d, J=8.4 Hz, H-10 indole), 7.52 (1H, d, J=7.8 Hz, H-7 indole), 8.85 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 20.5 (C-6), 35.7 (C-5), 40.1, 40.4 (–NCH_2CH_2N–), 49.2, 49.4 ($2CH_2$-piperazine), 55.2 (OCH_3), 56.4, 57.7 ($2CH_2$-piperazine), 87.1 (C-11b), 111.0, 111.5, 111.7, 118.1, 119.2, 119.9, 120.8, 123.5, 123.9, 124.1, 125.5, 136.7, 140.7, 152.2 (aromatic carbons), 155.4 (C-3), 169.5 (C-1).

2-[2-[4-(3-Chlorophenyl)piperazin-1-yl]ethyl]-5,6,11,11b-tetrahydro-1*H*-imidazo[1′,5′:1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (2c) IR (KBr): 3390, 1780, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.76—2.92 (6H, m, 2H-6, $2CH_2$-piperazine), 3.08—3.34 (5H, m, H-5a, $2CH_2$-piperazine), 3.68 (2H, t, J=5.7 Hz, CH_2–N), 3.77 (2H, t, J=6.6 Hz, N–CH_2), 4.52 (1H, dd, J=13.5, 4.8 Hz, H-5b), 6.65—6.73 (2H, m, H-4 and H-6 phenyl), 6.75 (1H, s, H-2 phenyl), 7.08 (1H, t, J=7.8 Hz, H-5 phenyl), 7.18—7.22 (2H, m, H-8, H-9 indole), 7.34 (1H, d, J=8.4 Hz, H-10 indole), 7.43 (1H, d, J=8.4 Hz, H-7 indole), 8.62 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 20.3 (C-6), 39.7 (C-5), 40.4, 40.6 (–NCH_2CH_2N–), 46.6, 49.0 ($2CH_2$-piperazine), 56.3, 57.5 ($2CH_2$-piperazine), 88.2 (C-11b), 111.6, 111.8, 114.0, 115.9, 118.9, 119.6, 120.2, 123.6, 123.9, 125.4, 129.9, 134.8, 136.4, 151.8 (aromatic carbons), 156.3 (C-3), 170.6 (C-1).

2-[2-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]ethyl]5,6,11,11b-tetrahydro-1*H*-imidazo[1′,5′:1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (2d) IR (KBr): 3300, 1790, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.76—3.00 (6H, m, 2H-6, $2CH_2$-piperazine), 3.31—3.53 (5H, m, H-5a, $2CH_2$-piperazine), 3.65 (2H, t, J=5.7 Hz, CH_2–N), 3.81 (2H, t, J=6.0 Hz, N–CH_2), 4.42 (1H, dd, J=13.5, 5.1 Hz, H-5b), 6.94—7.09 (3H, m, H-2, H-4 and H-6 phenyl), 7.17—7.29 (3H, m, H-5 phenyl, H-8, H-9 indole), 7.33 (1H, d, J=8.1 Hz, H-10 indole), 7.46 (1H, d, J=8.1 Hz, H-7 indole), 8.51 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 20.7 (C-6), 35.8 (C-5), 40.2, 40.6 (–NCH_2CH_2N–), 47.4, 47.7 ($2CH_2$-piperazine), 55.8, 57.1 ($2CH_2$-piperazine), 87.1 (C-11b), 111.7, 112.1, 113.2, 116.1, 118.7, 119.2, 120.1, 123.8, 124.1, 124.2 (q, CF_3, $^1J_{C-F}$=272.6 Hz), 125.4, 129.5, 131.4, 136.7, 150.3 (aromatic carbons), 155.4 (C-3), 169.4 (C-1).

2-[2-[4-(4-Nitrophenyl)piperazin-1-yl]ethyl]-5,6,11,11b-tetrahydro-1*H*-imidazo[1′,5′:1,2]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (2e) IR (KBr): 3350, 1780, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.75—2.93 (6H, m, 2H-6, $2CH_2$-piperazine), 3.23—3.44 (5H, m, H-5a, $2CH_2$-piperazine), 3.69 (2H, t, J=6.0 Hz, CH_2–N), 3.78 (2H, t, J=6.3 Hz, N–CH_2), 4.50 (1H, dd, J=13.8, 4.9 Hz, H-5b), 6.69 (2H, dd, J=9.3, 1.5 Hz, H-2 and H-6 phenyl), 7.10 (1H, t, J=7.8 Hz, H-8 indole), 7.22 (1H, t, J=8.0 Hz, H-9 indole), 7.34 (1H, d, J=8.1 Hz, H-10 indole), 7.46 (1H, d, J=8.1 Hz, H-7 indole), 8.01 (2H, dd, J=9.6, 2.1 Hz, H-3 and H-5 phenyl), 8.69 (1H, s, NH). ^{13}C-NMR ($CDCl_3$) δ: 20.8 (C-6), 36.2 (C-5), 40.3, 40.5 (–NCH_2CH_2N–), 46.7, 47.5 ($2CH_2$-piperazine), 56.2, 57.8 ($2CH_2$-piperazine), 87.1 (C-11b), 111.9, 112.5, 113.0, 119.2, 119.4, 123.9, 124.2, 125.7, 126.0, 136.9, 138.9, 154.7 (aromatic carbons), 156.7 (C-3), 170.9 (C-1).

Binding Assays For receptor-binding assays, male Sprague–Dawley rats (*Rattus norvegicus albinus*), weighing 180—200 g, were used in all cases. Labeling of benzodiazepine binding sites with [^{3}H]flunitrazepam was performed as previously described.[16] The rat brain was homogenized (Polytron) in 25 mM potassium phosphate (KPi) buffer (pH 7.4). Homogenate fractions (100 μl, about 100 μg protein) were added to tubes which contained 900 μl of 25 mM KPi, providing 0.25 nM [^{3}H]flunitrazepam (Amersham, 37 Ci/mmol), alone (for total binding determination) or in the presence of 2 μM diazepam (for determining nonspecific binding) or 1 μM of the compound to be investigated. Incubation lasted for 90 min at 0—4 °C.

α_1-Adrenergic binding was measured by using [^{3}H]prazosin (New England Nuclear, 18 Ci/mmol).[17] Rat cerebral cortex membranes were obtained in assay buffer (50 mM Tris·HCl pH 7.4, with 2.5 mM $MgCl_2$). Membrane fractions (100 μl, about 130 μg protein) were added to tubes which contained 900 μl of assay buffer, providing 0.2 nM [^{3}H]prazosin, alone (total binding) or in the presence of 10 μM phentolamine (unspecific binding) or 1 μM of the compound to be investigated. Incubation was performed at 25 °C for 30 min.

Binding to the 5-HT_{1A} receptor was performed as reported earlier.[18] Rat hippocampal membranes were obtained in homogenization buffer (50 mM Tris·HCl pH 7.4 at 37 °C, 0.5 mM Na_2EDTA, 10 mM $MgSO_4$). Membranes (100 μl, about 120 μg protein) were added to tubes containing 900 μl of incubation buffer (homogenization buffer containing 10 μM pargyline and 0.1% ascorbic acid), providing 1 nM [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]OH-DPAT, New England Nuclear 141 Ci/mmol), alone (total binding) or in presence of 10 μM 5-hydroxytryptamine (nonspecific binding) or 1 μM of the compound to be investigated. Incubation proceeded at 37 °C for 15 min.

For all three binding assays, incubation was terminated by rapid filtration under vacuum, using a Brandel harvester. After drying, the filters were processed for radioactivity determination. Proteins were determined by the method of Lowry *et al.*,[19] with bovine serum albumin as the standard.

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