Reaction of 6-Hydroxytetrahydro-β-carboline-3-carboxylic Acids with Isocyanates and Isothiocyanates

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The reaction of (−)-(3*S*)-6-hydroxy-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (3a) with isocyanates and isothiocyanates gave the (±)-β-carboline-hydantoin (4a—d) and -thiohydantoin systems (5a—d). The treatment of (−)-(1*S*,3*S*)-6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (3b) with isocyanates yielded the (±)-*cis* diastereomer of the β-carboline-hydantoin rings (4e—h). However, the reaction of 3b with isothiocyanates provided the corresponding *trans* isomer (5e—h). These results have been confirmed by ^{13}C-NMR data and nuclear Overhauser effect (NOE) experiments. The new compounds were tested for *in vitro* binding affinity to the central-type benzodiazepine receptors.

Keywords β-carboline-hydantoin; β-carboline-thiohydantoin; isocyanate; isothiocyanate; nuclear Overhauser effect; benzodiazepine receptor

It has been shown that the benzodiazepine receptor (BzR) is part of a supramolecular complex with also contains discrete but allosterically coupled recognition sites for γ-aminobutyric acid (GABA) and barbiturates. The oligomeric units of this supramolecular complex are thought to form a drug- and transmitter-responsive chloride channel.[1-4] It is generally believed that BzR ligands elicit their pharmacological effects through modulation of this ligand-gated chloride ion channel. Only a small number of ligands chemically different from the benzodiazepines bind to the BzR with high affinity. A case in point is the group of β-carbolines, some derivatives of which appear to be related to the endogenous ligand of the BzR. Depending upon their intrinsic activity profiles, β-carbolines are classified as agonists,[5-10] inverse agonists,[11-15] antagonists,[16,17] or partial agonists.

As a part of a program directed toward the development of new drugs acting on the central nervous system (CNS), we were interested in the synthesis of new compounds combining tetrahydro-β-carboline and hydantoin or thiohydantoin skeletons (Chart 1). In the preceding papers,[18,19] we have described the preparation of such compounds (**1**: Y=H, X=O, S; **2**: X=O) and a detailed study of the stereochemistry of **1**.[20] On the basis of the biological role of 5-hydroxytryptophan, in the present work, we report the synthesis of novel tetrahydro-β-carboline derivatives **1** (Y=OH, X=O, S) in order to test their affinity for the BzR.

Results and Discussion

The tetrahydro-β-carboline-3-carboxylic acids (**3a, b**) were obtained by Pictet–Spengler condensation of L-5-hydroxytryptophan with formaldehyde and acetaldehyde in acidic media.[21] The treatment of (−)-(3*S*)-6-hydroxy-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3a**) with isocyanates and isothiocyanates in refluxing acetone/dimethylsulfoxide (DMSO) gave the corresponding (±)-β-carboline-hydantoin (**4a—d**) and -thiohydantoin systems (**5a—d**) (Chart 2). The physical properties of these compounds are summarized in Tables I and II, and all of them have been characterized on the basis of their ^{1}H- and ^{13}C-NMR data (Tables III, IV). The carbon signals attributable to the indole ring were assigned by the distortionless enhancement by polarization transfer (DEPT) method and by application of two dimensional (2D) heteronuclear correlation spectroscopy (HETCOR).

The reaction of (−)-(1*S*,3*S*)-6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**3b**) with isocyanates in refluxing acetone/DMSO provided the (±)-*cis* diastereomer (**4e—h**) (Chart 2, Table I). The stereochemical assignment at C-5 and C-11a of **4e—h** was determined by ^{13}C-NMR (Table III) using the method of Cook *et al.*[22] and confirmed by nuclear Overhauser effect (NOE) experiments (Chart 3). Irradiation at δ 1.85 (CH_3) on **4g** decreased the intensity of the H-5 methine proton signal at δ 4.92, but the intensity of the H-11a methine proton signal at 4.37 remained unchanged. This confirmed that compound **4g** was the *cis* isomer.

Treatment of **3b** with isothiocyanates in acetone/DMSO afforded the (±)-*trans* diastereomer (**5e—h**) as shown in Chart 2 (Table II). The stereochemical assignment of **5e—h** was based on the C-5 and C-11 chemical shifts (Table IV) and those of related compounds.[20] In difference NOE experiments on **5e** (Chart 3), irradiation at δ 5.66 (H-5) decreased the signal intensities of the methyl protons at δ

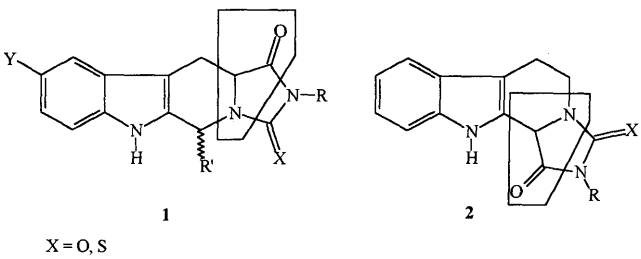


Chart 1

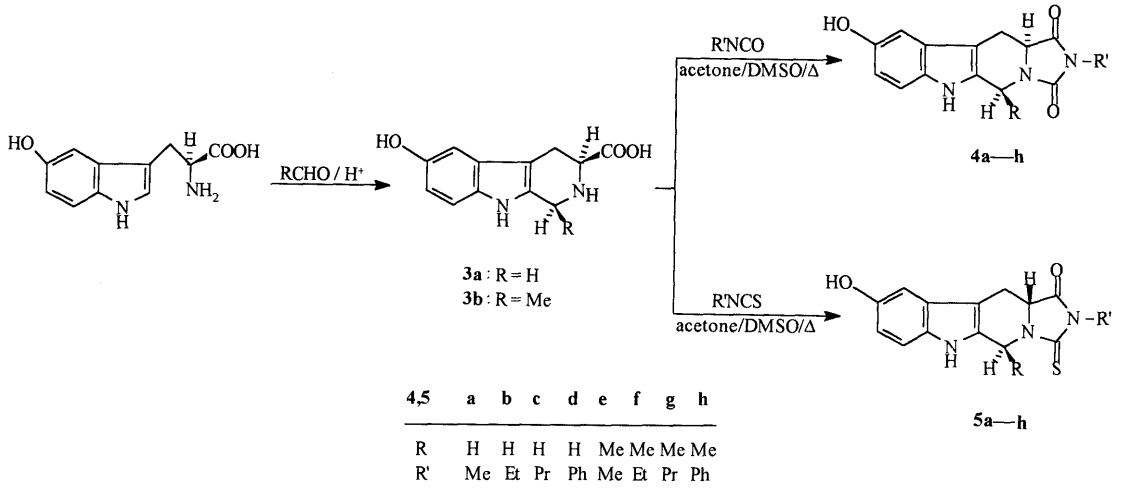


Chart 2

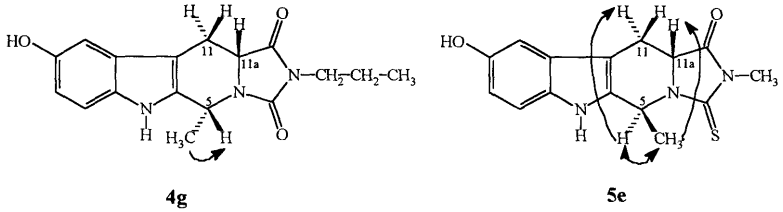


Chart 3. Schematic Illustration of NOE for **4g** and **5e**

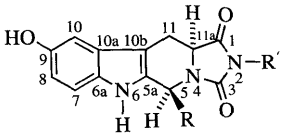
TABLE I. Physical Properties of Substituted 9-Hydroxy-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-diones (**4a—h**)

No.	mp (°C)	Recryst. solv.	Yield (%)	Formula	Analysis (%)					
					Calcd			Found		
					C	H	N	C	H	N
4a	>260	EtOH	30	$C_{14}H_{13}N_3O_3$	61.99	4.83	15.49	62.22	5.03	15.37
4b	247—248	EtOH–H_2O	58	$C_{15}H_{15}N_3O_3$	63.15	5.30	14.73	62.89	5.58	14.59
4c	>260	EtOH	40	$C_{16}H_{17}N_3O_3$	64.20	5.72	14.04	64.50	5.87	14.32
4d	>260	EtOH–H_2O	60	$C_{19}H_{15}N_3O_3$	68.46	4.54	12.61	68.69	4.80	12.37
4e	228—230	EtOH	60	$C_{15}H_{15}N_3O_3$	63.16	5.30	14.73	62.95	5.49	14.58
4f	210—212	EtOH	70	$C_{16}H_{17}N_3O_3$	64.20	5.72	14.04	64.33	5.67	14.30
4g	218—220	EtOH	80	$C_{17}H_{19}N_3O_3$	65.15	6.11	13.41	65.42	6.24	13.78
4h	222—224	EtOH	82	$C_{20}H_{17}N_3O_3$	69.15	4.93	12.10	69.42	4.63	12.15

TABLE II. Physical Properties of Substituted 9-Hydroxy-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thiones (**5a—h**)

No.	mp (°C)	Recryst. solv.	Yield (%)	Formula	Analysis (%)							
					Calcd				Found			
					C	H	N	S	C	H	N	S
5a	232 (dec.)	EtOH	65	$C_{14}H_{13}N_3O_2S$	58.52	4.56	14.62	11.16	58.61	4.54	14.69	11.07
5b	185 (dec.)	EtOH–H_2O	50	$C_{15}H_{15}N_3O_2S$	59.78	5.02	13.94	10.64	59.97	5.13	13.64	10.50
5c	220 (dec.)	EtOH	70	$C_{16}H_{17}N_3O_2S$	60.93	5.43	13.32	10.17	60.84	5.71	13.19	9.94
5d	252—254	EtOH	63	$C_{19}H_{15}N_3O_2S$	65.31	4.33	12.03	9.18	65.59	4.24	12.21	8.98
5e	217 (dec.)	EtOH	54	$C_{15}H_{15}N_3O_2S$	59.78	5.02	13.94	10.64	59.54	5.26	13.71	10.69
5f	208—210	$CHCl_3$	48	$C_{16}H_{17}N_3O_2S$	60.93	5.43	13.32	10.17	60.81	5.65	13.63	10.15
5g	196 (dec.)	EtOH–H_2O	61	$C_{17}H_{19}N_3O_2S$	61.98	5.81	12.76	9.73	61.70	5.96	12.47	9.86
5h	140 (dec.)	EtOH	45	$C_{20}H_{17}N_3O_2S$	66.09	4.72	11.56	8.82	66.02	4.79	11.39	8.97

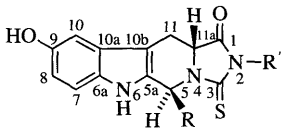
TABLE III. ^{13}C-NMR Data[a] for Compounds **4a—h**



No.	R	R′	C-1	C-3	C-9	C-6a	C-5a	C-10a	C-7	C-8	C-10b	C-10	C-11a	C-5	C-11	R	R′
4a	H	Me	173.0	155.0	150.7	130.9	130.1	127.1	111.6	111.4	104.1	102.2	55.0	37.5	22.5	—	24.4
4b	H	Et	172.6	154.5	150.5	130.6	129.9	126.9	111.4	111.2	103.8	102.0	54.6	37.4	22.4	—	32.8, 13.3
4c	H	Pr	172.9	154.7	150.5	130.6	129.9	126.8	111.4	111.2	103.8	102.0	54.6	37.4	22.5	—	41.0, 21.0, 11.0
4d	H	Ph	171.8	153.7	150.5	130.7	129.8	126.9	111.5	111.2	104.0	102.0	54.7	37.7	22.4	—	132.0, 128.7, 127.9, 126.8
4e	Me	Me	171.9	155.5	150.9	136.4	131.0	126.4	111.6	111.5	105.4	104.3	57.9	48.4	22.2	20.5	24.3
4f	Me	Et	171.6	155.1	150.6	137.3	131.2	126.3	111.4	110.8	105.2	102.3	57.6	48.3	21.9	20.5	32.9, 13.6
4g	Me	Pr	171.7	155.0	150.5	136.1	130.6	126.6	111.4	111.2	103.8	102.0	57.3	48.1	21.9	20.2	39.2, 21.0, 11.1
4h	Me	Ph	170.8	154.1	150.8	136.4	130.8	126.9	111.6	111.2	104.0	102.3	57.6	48.6	22.0	20.4	132.1, 128.8, 127.9, 126.7

a) DMSO-d_6.

TABLE IV. ^{13}C-NMR Data[a] for Compounds **5a—h**



No.	R	R′	C-3	C-1	C-9	C-6a	C-5a	C-10a	C-7	C-8	C-10b	C-10	C-11a	C-5	C-11	R	R′
5a	H	Me	180.5	173.2	150.6	130.7	129.6	126.7	111.5	111.4	103.7	102.1	57.8	41.9	22.2	—	27.4
5b	H	Et	180.1	173.1	150.8	130.9	129.8	126.8	111.9	111.7	103.8	102.3	57.9	42.1	22.5	—	35.9, 13.0
5c	H	Pr	180.4	173.3	150.8	131.0	129.9	126.9	111.8	111.7	103.9	102.4	57.9	42.3[b]	22.8	—	42.4,[b] 20.9, 11.2
5d	H	Ph	180.3	172.8	150.8	130.9	129.7	126.9	111.7	111.6	103.9	102.3	58.3	42.4	22.6	—	133.7, 129.0, 128.9
5e	Me	Me	179.6	173.3	150.6	134.4	130.6	126.5	111.8	111.5	103.1	102.2	55.3	48.4	22.4	18.9	27.3
5f	Me	Et	179.4	173.2	150.9	134.7	131.1	126.9	111.9	111.7	103.4	102.6	55.6	48.9	23.1	19.3	36.0, 13.2
5g	Me	Pr	179.6	173.6	150.8	134.7	131.0	126.7	111.8	111.7	103.3	102.5	55.5	48.7	23.0	19.2	42.3, 20.9, 11.2
5h	Me	Ph	179.4	172.9	150.8	134.5	130.9	126.7	111.7	111.6	103.3	102.4	55.8	48.8	22.9	19.1	133.5, 128.8, 128.5

a) DMSO-d_6. b) The assigments may be interchanged.

1.59 and one of the methylene protons (H-11α) at 3.19. Correspondingly, irradiation at δ 1.59 (CH_3) decreased the signal intensities of the H-11a methine proton at 4.80 and the H-5 methine proton at δ 5.66. Therefore, we confirmed that compound **5e** was the *trans* isomer.

The stereospecificity observed in the reaction of 6-hydroxytetrahydro-β-carboline-3-carboxylic acids with isocyanates and isothiocyanates is in accordance with previous results.[20] Therefore, the *trans* diastereomer is formed under thermodynamic control, while the *cis* isomer is regulated by kinetic control.

Compounds **4b—d, g** and **5a—e, g** were examined for the ability to interact *in vitro* with the central BzR. At 1 μM concentration, compound **5b** (R=H, R′=Et) displaced [^{3}H]flunitrazepam specific binding by 12%, while the displacement exerted by compound **5d** (R=H, R′=Ph) at the same concentration amounted to 16%. In conclusion, the new hybrid molecules bind very poorly to the BzR.

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 in DMSO-d_6 solution with tetramethylsilane as an internal standard on a Varian VXR-300S spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Mass spectra (MS) were obtained with a Varian MAT-711 mass spectrometer. 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).

(−)-(3*S*)-6-Hydroxy- and (−)-(1*S*,3*S*)-6-Hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acids (3a, b) These compounds were prepared according to the literature.[21]

General Procedure for the Synthesis of 4a—h Alkyl or phenyl isocyanate (9 mmol) was added to a suspension of **3** (9 mmol) in dry acetone (25 ml) and dry DMSO (5 ml). The reaction mixture was refluxed for 12 h. The solvent was evaporated off and the oil was solidified by trituration with ethyl acetate.

9-Hydroxy-2-methyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]-pyrido[3,4-*b*]indole-1,3(2*H*)-dione (4a) IR (KBr): 3500—3100 (br), 3340, 1750, 1680 cm^{-1}. ^{1}H-NMR δ: 2.70 (1H, dd, J=14.3, 11.3 Hz, H-11), 2.95 (3H, s, CH_3), 3.13 (1H, dd, J=14.7, 5.4 Hz, H-11), 4.35—4.43 (2H, m, H-5, H-11a), 4.88 (1H, d, J=16.2 Hz, H-5), 6.62 (1H, dd, J=8.6, 2.0 Hz, H-8), 6.78 (1H, d, J=2.1 Hz, H-10), 7.16 (1H, d, J=8.6 Hz, H-7), 8.73 (1H, s, OH), 10.73 (1H, s, NH). MS *m/z*: 271 (M^+), 159 ($M^+ - C_4H_4N_2O_2$, 100).

2-Ethyl-9-hydroxy-5,6-11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]-pyrido[3,4-*b*]indole-1,3(2*H*)-dione (4b) IR (KBr): 3500—3100 (br), 3340, 1740, 1680 cm^{-1}. ^{1}H-NMR δ: 1.16 (3H, t, J=7.2 Hz, CH_3), 2.67 (1H, dd, J=14.7, 11.4 Hz, H-11), 3.14 (1H, dd, J=14.7, 5.4 Hz, H-11), 3.51 (2H, q, J=7.2 Hz, CH_2), 4.35—4.43 (2H, m, H-5, H-11a), 4.89 (1H, d, J=16.2 Hz, H-5), 6.63 (1H, dd, J=8.4, 2.1 Hz, H-8), 6.80 (1H, d, J=2.1 Hz, H-10), 7.17 (1H, d, J=8.4 Hz, H-7), 8.74 (1H, s, OH), 10.73 (1H, s, NH). MS *m/z*: 285 (M^+), 159 ($M^+ - C_5H_6N_2O_2$, 100).

9-Hydroxy-2-propyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]-pyrido[3,4-*b*]indole-1,3(2*H*)-dione (4c) IR (KBr): 3500—3100 (br), 3320, 1750, 1680 cm^{-1}. ^{1}H-NMR δ: 0.90 (3H, t, J=7.5 Hz, CH_3), 1.61 (2H, sext, J=7.5 Hz, CH_2), 2.67 (1H, dd, J=14.4, 11.1 Hz, H-11), 3.15 (1H, dd, J=14.7, 5.1 Hz, H-11), 3.43 (2H, t, J=7.0 Hz, CH_2), 4.36—4.45 (2H, m, H-5, H-11a), 4.90 (1H, d, J=15.9 Hz, H-5), 6.63 (1H, dd, J=8.7, 2.1 Hz, H-8), 6.80 (1H, d, J=2.0 Hz, H-10), 7.17 (1H, d, J=8.9 Hz, H-7), 8.73 (1H, s, OH), 10.73 (1H, s, NH).

9-Hydroxy-2-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]-pyrido[3,4-*b*]indole-1,3(2*H*)-dione (4d) IR (KBr): 3400—3100 (br), 3310, 1730, 1700 cm^{-1}. ^{1}H-NMR δ: 2.93 (1H, dd, J=14.4, 11.1 Hz, H-11), 3.22 (1H, dd, J=14.7, 5.7 Hz, H-11), 4.48 (1H, d, J=16.2 Hz, H-5), 4.60 (1H, dd, J=11.1, 5.4 Hz, H-11a), 4.97 (1H, d, J=16.2 Hz, H-5), 6.66 (1H, dd, J=8.4, 2.1 Hz, H-8), 6.84 (1H, d, J=2.0 Hz, H-10), 7.20 (1H, d, J=8.7 Hz, H-7), 7.46—7.57 (5H, m, phenyl), 8.77 (1H, s, OH), 10.78 (1H, s, NH).

***cis*-9-Hydroxy-2,5-dimethyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (4e)** IR (KBr): 3400—3200 (br), 3320, 1740, 1700 cm^{-1}. ^{1}H-NMR δ: 1.92 (3H, d, J=6.7 Hz, CH_3), 2.61—2.89 (2H, m, H-11), 3.23 (3H, s, CH_3), 4.31 (1H, dd, J=11.4, 4.6 Hz, H-11a), 5.01 (1H, q, J=6.6 Hz, H-5), 6.53 (1H, dd, J=8.6, 2.0 Hz, H-8), 7.19 (1H, d, J=2.0 Hz, H-10), 7.32 (1H, d, J=8.5 Hz, H-7), 8.59 (1H, s, OH), 10.48 (1H, s, NH).

***cis*-2-Ethyl-9-hydroxy-5-methyl-5,6,11,11a-tetrahydro-1*H*-imidazo-[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (4f)** IR (KBr): 3400—3100 (br), 3300, 1750, 1700 cm^{-1}. ^{1}H-NMR δ: 1.17 (3H, t, J=7.0 Hz, CH_3), 1.88 (3H, d, J=6.5 Hz, CH_3), 2.65—2.75 (1H, m, H-11), 3.13 (1H, dd, J=14.4, 5.7 Hz, H-11), 3.48 (2H, q, J=7.0 Hz, CH_2), 4.37 (1H, dd, J=11.5, 4.7 Hz, H-11a), 4.96 (1H, q, J=6.5 Hz, H-5), 6.83 (1H, dd, J=8.7, 2.2 Hz, H-8), 7.22 (1H, d, J=2.0 Hz, H-10), 7.31 (1H, d, J=8.7 Hz, H-7), 8.74 (1H, s, OH), 10.80 (1H, s, NH).

***cis*-9-Hydroxy-5-methyl-2-propyl-5,6,11,11a-tetrahydro-1*H*-imidazo-[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (4g)** IR (KBr): 3400—3200 (br), 3340, 1740, 1690 cm^{-1}. ^{1}H-NMR δ: 0.89 (3H, t, J=7.2 Hz, CH_3), 1.59 (2H, sext, J=7.2 Hz, CH_2), 1.85 (3H, d, J=6.6 Hz, CH_3), 2.66 (1H, dd, J=14.4, 11.1 Hz, H-11), 3.09 (1H, dd, J=14.4, 4.2 Hz, H-11), 3.41 (2H, t, J=7.2 Hz, CH_2), 4.37 (1H, dd, J=11.4, 4.5 Hz, H-11a), 4.92 (1H, q, J=6.6 Hz, H-5), 6.63 (1H, dd, J=8.7, 2.1 Hz, H-8), 6.81 (1H, d, J=2.0 Hz, H-10), 7.15 (1H, d, J=8.7 Hz, H-7), 8.73 (1H, s, OH), 10.79 (1H, s, NH).

***cis*-9-Hydroxy-5-methyl-2-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo-[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (4h)** IR (KBr): 3400—3200 (br), 3360, 1760, 1700 cm^{-1}. ^{1}H-NMR δ: 1.92 (3H, d, J=6.6 Hz, CH_3), 2.81 (1H, dd, J=14.5, 11.1 Hz, H-11), 3.11 (1H, dd, J=14.5, 4.4 Hz, H-11), 4.48 (1H, dd, J=11.2, 4.5 Hz, H-11a), 4.91 (1H, q, J=6.6 Hz, H-5), 6.47 (1H, dd, J=8.6, 2.2 Hz, H-8), 6.75 (1H, d, J=2.1 Hz, H-10), 7.13 (1H, d, J=8.7 Hz, H-7), 7.35—7.48 (5H, m, phenyl), 8.59 (1H, s, OH), 10.48 (1H, s, NH).

General Procedure for the Synthesis of 5a—h Alkyl or phenyl isothiocyanate (9 mmol) was added to a suspension of **3** (9 mmol) in dry acetone (35 ml) and dry DMSO (15 ml). The reaction mixture was refluxed until complete dissolution had occurred. The solvent was evaporated off and the crude product was purified by column chromatography on silica gel (ethyl acetate–hexane, 9 : 1).

9-Hydroxy-2-methyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (5a) IR (KBr): 3500—3100 (br), 3380, 1720, 1510 cm^{-1}. ^{1}H-NMR δ: 2.83 (1H, dd, J=14.7, 11.1 Hz, H-11), 3.21 (1H, dd, J=14.7, 5.8 Hz, H-11), 3.22 (3H, s, CH_3), 4.59 (1H, d, J=16.9 Hz, H-5), 4.69 (1H, dd, J=11.1, 5.7 Hz, H-11a), 5.42 (1H, d, J=16.9 Hz, H-5), 6.66 (1H, dd, J=8.7, 2.2 Hz, H-8), 6.82 (1H, d, J=2.1 Hz, H-10), 7.20 (1H, d, J=8.6 Hz, H-7), 8.76 (1H, s, OH), 10.78 (1H, s, NH).

2-Ethyl-9-hydroxy-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]-pyrido[3,4-*b*]indole-3(2*H*)-thione (5b) IR (KBr): 3600—3100 (br), 3340, 1720, 1480, 1440 cm^{-1}. ^{1}H-NMR δ: 1.18 (3H, t, J=7.2 Hz, CH_3), 2.74 (1H, dd, J=15.0, 11.1 Hz, H-11), 3.18 (1H, dd, J=14.7, 5.4 Hz, H-11), 3.81 (2H, q, J=7.1 Hz, CH_2), 4.55 (1H, d, J=17.1 Hz, H-5), 4.66 (1H, dd, J=11.4, 5.4 Hz, H-11a), 5.37 (1H, d, J=17.1 Hz, H-5), 6.60 (1H, dd, J=8.7, 2.4 Hz, H-8), 6.77 (1H, d, J=2.4 Hz, H-10), 7.14 (1H, d, J=8.1 Hz, H-7), 8.71 (1H, s, OH), 10.74 (1H, s, NH).

9-Hydroxy-1-oxo-2-propyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (5c) IR (KBr): 3500—3200 (br), 3320, 1735, 1500, 1450 cm^{-1}. ^{1}H-NMR δ: 0.90 (3H, t, J=7.5 Hz, CH_3), 1.67 (2H, sext, J=7.5 Hz, CH_2), 2.76 (1H, dd, J=14.4, 11.4 Hz, H-11), 3.21 (1H, dd, J=14.7, 5.4 Hz, H-11), 3.74 (2H, t, J=7.2 Hz, CH_2), 4.57 (1H, d, J=16.8 Hz, H-5), 4.68 (1H, dd, J=10.8, 5.7 Hz, H-11a), 5.40 (1H, d, J=16.8 Hz, H-5), 6.63 (1H, dd, J=8.7, 2.1 Hz, H-8), 6.80 (1H, d, J=2.0 Hz, H-10), 7.17 (1H, d, J=8.7 Hz, H-7), 8.85 (1H, s, OH), 10.87 (1H, s, NH).

9-Hydroxy-1-oxo-2-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (5d) IR (KBr): 3500—3100 (br), 3380, 1735, 1500, 1450 cm^{-1}. ^{1}H-NMR δ: 3.01 (1H, dd, J=14.4, 11.4 Hz, H-11), 3.25 (1H, dd, J=14.7, 5.7 Hz, H-11), 4.63 (1H, d, J=17.1 Hz, H-5), 4.85 (1H, dd, J=11.1, 5.6 Hz, H-11a), 5.46 (1H, d, J=17.1 Hz, H-5), 6.63 (1H, dd, J=8.7, 2.4 Hz, H-8), 6.81 (1H, d, J=2.1 Hz, H-10), 7.17 (1H, d, J=8.7 Hz, H-7), 7.38—7.54 (5H, m, phenyl), 8.75 (1H, s, OH), 10.79 (1H, s, NH).

***trans*-9-Hydroxy-2,5-dimethyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (5e)** IR (KBr): 3500—3100 (br), 3340, 1745, 1500, 1450 cm^{-1}. ^{1}H-NMR δ: 1.59 (3H, d, J=6.6 Hz, CH_3), 2.78 (1H, dd, J=14.8, 11.0 Hz, H-11), 3.19 (1H, dd, J=14.8, 5.6 Hz, H-11), 3.20 (3H, s, CH_3), 4.80 (1H, dd, J=11.0, 5.7 Hz, H-11a), 5.66 (1H, q, J=6.7 Hz, H-5), 6.65 (1H, dd, J=8.7, 2.4 Hz, H-8), 6.80 (1H, d, J=2.4 Hz, H-10), 7.18 (1H, d, J=8.7 Hz, H-7), 8.78 (1H, s, OH), 10.85 (1H, s, NH).

***trans*-2-Ethyl-9-hydroxy-5-methyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (5f)** IR (KBr): 3500—3200 (br), 3350, 1730, 1460 cm^{-1}. ^{1}H-NMR δ: 1.17 (3H, t, J=6.9 Hz, CH_3), 1.57 (3H, d, J=6.6 Hz, CH_3), 2.72 (1H, dd, J=14.6, 11.0 Hz, H-11), 3.17 (1H, dd, J=14.6, 5.6 Hz, H-11), 3.80 (2H, q, J=6.9 Hz, CH_2), 4.76 (1H, dd, J=11.0, 5.6 Hz, H-11a), 5.64 (1H, q, J=6.6 Hz, H-5), 6.63 (1H, dd, J=8.7, 2.2 Hz, H-8), 6.78 (1H, d, J=2.1 Hz, H-10), 7.15 (1H, d, J=8.6 Hz, H-7), 8.67 (1H, s, OH), 10.78 (1H, s, NH).

***trans*-9-Hydroxy-5-methyl-2-propyl-1-oxo-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (5g)** IR (KBr): 3400—3100 (br), 3350, 1725, 1475, 1450 cm^{-1}. ^{1}H-NMR δ: 0.91 (3H, t, J=7.5 Hz, CH_3), 1.59 (3H, d, J=6.6 Hz, CH_3), 1.67 (2H, sext, J=7.5 Hz, CH_2), 2.74 (1H, dd, J=14.7, 11.1 Hz, H-11), 3.20 (1H, dd, J=14.7, 5.7 Hz, H-11), 3.75 (2H, t, J=7.3 Hz, CH_2), 4.84 (1H, dd, J=11.1, 6.0 Hz, H-11a), 5.66 (1H, q, J=6.6 Hz, H-5), 6.65 (1H, dd, J=8.7, 2.4 Hz, H-8), 6.80 (1H, d, J=2.1 Hz, H-10), 7.17 (1H, d, J=8.7 Hz, H-7), 8.76 (1H, s, OH), 10.86 (1H, s, NH).

***trans*-9-Hydroxy-5-methyl-1-oxo-2-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-3(2*H*)-thione (5h)** IR (KBr): 3400—3100 (br), 3350, 1750, 1500, 1470 cm^{-1}. ^{1}H-NMR δ: 1.62 (3H, d, J=6.5 Hz, CH_3), 2.90 (1H, dd, J=14.9, 10.8 Hz, H-11), 3.21 (1H, dd, J=15.0, 5.8 Hz, H-11), 4.96 (1H, dd, J=10.8, 5.8 Hz, H-11a), 5.71 (1H, q, J=6.5 Hz, H-5), 6.63 (1H, dd, J=8.5, 2.2 Hz, H-8), 6.80 (1H, d, J=2.4 Hz, H-10), 7.15 (1H, d, J=8.4 Hz, H-7), 7.37—7.54 (5H, m, phenyl), 8.71 (1H, s, OH), 10.83 (1H, s, NH).

Receptor Binding Assay [^{3}H]Flunitrazepam binding to rat brain homogenates was assayed essentially as described by Orensanz *et al.*[23] Briefly, male Sprague–Dawley rats (*Rattus norvegicus albinus*), weighing 180—200 g, were decapitated, and their brains were removed and homogenized with a Polytron PT 10 for 15 s (setting 6), in 50 volumes of 25 mM potassium phosphate (KPi) buffer, pH 7.4. For the [^{3}H]-flunitrazepam (Amersham, 37 Ci/mmol) binding assay, 100 μl (100 μg protein) fractions of homogenate were added in triplicate to tubes which contained 900 μl of 25 mM KPi, providing 0.25 nM [^{3}H]-flunitrazepam, alone or in the presence of 2 μM diazepam or 1 μM of the compound to be investigated. Incubation lasted 90 min at 0—4 °C and was terminated by rapid filtration under vacuum, using a Brandel harvester. After drying, the filters were processed for radioactivity determination. Specific binding, defined with 2 μM diazepam, represented 95% of total binding. Proteins were determinated by the method of Lowry *et al.*,[24] with bovine serum albumin as the standard.

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