Synthesis of Novel 5*H*-Pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-diones as Potential Ligands for the Cloned α_1-Adrenoceptor Subtypes

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A new series of 3-[ω-[4-(4-substituted phenyl)piperazin-1-yl]alkyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)-2,4-diones (**3-10** and **12-13**) were synthesized from the *N*-(2-chloroethyl)-*N'*-[3-(2-ethoxycarbonyl)indolyl] urea (**1**) or the *N*-(3-chloropropyl)-*N'*-[3-(2-ethoxycarbonyl)indolyl] urea (**2**) and a number of 1-(4-substituted-phenyl)piperazines. 3-[2-[4-(4-Aminophenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**14**) was obtained by reduction of the parent nitro compound **8**. The obtained 5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione derivatives were tested towards cloned α_{1A}, α_{1B} and α_{1D}-adrenergic receptors subtypes in binding assays. Some compounds showed good affinity and selectivity for the α_{1D}-adrenoceptor subtype.

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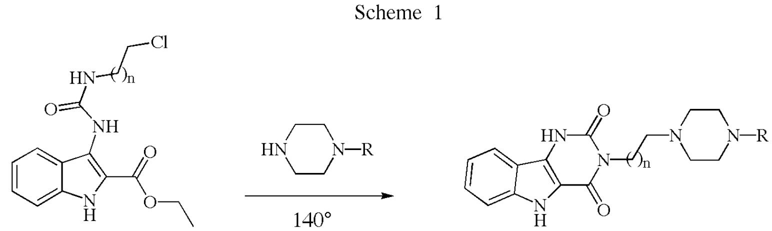
The α_1-adrenoceptor (α_1AR) belong to the superfamily of the G protein-coupled receptors and its antagonists are currently used as drugs for the treatment of hypertension and benign prostatic hypertrophy [1-3]. Recently, it was discovered that the α_1AR population is not homogeneous and three different subtypes exist, namely α_{1A}AR, α_{1B}AR and α_{1D}AR [4-7].

Over the last few years we have been interested in developing new selective α_1AR ligands as potential antihypertensive drugs. As a result of these studies, some 5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)-2,4-diones with (phenylpiperazinyl) ethyl substituents at the N-3 position showed high affinity and selectivity for the α_1AR on rat cortical membranes [8]. When these compounds were successively tested on cloned α_1AR subtypes, it was noted that derivatives with a substituent in the 4-position of the aromatic ring of the phenylpiperazine moiety showed a general decrease in affinity but they were able to discriminate between the α_{1B}AR and the α_{1A}AR/α_{1D}AR subtypes [9]. This suggested that one of the differences among the three binding sites could reside in the different capability to accomodate the steric bulk of the substituent in the 4 position. On these bases and with the aim to enlarge the knowledge on the structure-affinity relationships on this class of α_1AR ligands, we now report the synthesis of a new series of 3-[2-(4-phenylpiperazin-1-yl)ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)-2,4-diones **3-10** and **14** which contain several substituents of different nature and shape in the 4-position of the phenyl ring. Moreover, in order to assess the importance of the distance between the phenylpiperazine (PP) moiety and the tricyclic pyrimido[5,4-*b*]indole (PI) system for the

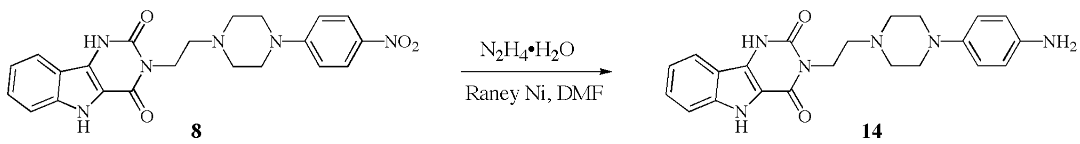
Scheme 1



1, n = 1
2, n = 2

3, n = 1; R = 4-FC$_6$H$_4$
4, n = 1; R = 4-BrC$_6$H$_4$
5, n = 1; R = 4-EtOC$_6$H$_4$
6, n = 1; R = 4-MeC$_6$H$_4$
7, n = 1; R = 4-MeCOC$_6$H$_4$
8, n = 1; R = 4-NO$_2$C$_6$H$_4$
9, n = 1; R = 4-CNC$_6$H$_4$
10, n = 1; R = 2,4-Cl$_2$C$_6$H$_3$
11, n = 1; R = C$_6$H$_5$CH$_2$
12, n = 2; R = C$_6$H$_5$
13, n = 2; R = 4-ClC$_6$H$_4$

Scheme 2



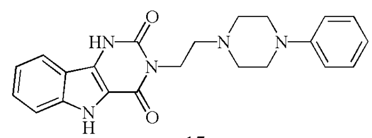
interaction of these compounds with binding sites, in compounds **12** and **13** the alkyl chain connecting the PP moiety with the PI system was extended from two to three carbon atoms.

The synthetic pathway used for the preparation of compounds **3-13** is shown in Scheme 1. The synthesis was accomplished by reaction of the *N*-(2-chloroethyl)-*N*'-[3-(2-ethoxycarbonyl)indolyl] urea (**1**) or the *N*-(3-chloropropyl)-*N*'-[3-(2-ethoxycarbonyl)indolyl] urea (**2**) with a large excess of the suitable 1-(4-substituted-phenyl)piperazine at 140° for 1 hour in the absence of solvent. Uder the same experimental conditions, reaction between urea **1** and 1-benzylpiperazine afforded compound **11**. In this step, we obtained tricyclic compounds **3-13** in moderate to good yields, *via* the closure of the pirimido-2,4-dione ring. Ureas **1** and **2** [8] were synthesized by the reaction of ethyl 3-aminoindole-2-carboxylate, prepared according to Unangst [10], with the appropriate ω-chloroalkylisothiocyanate. 3-[2-[4-(4-Aminophenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**14**) was obtained by reduction of the parent nitro compound **8** with hydrazine hydrate/Raney nickel in dimethylformamide at room temperature (Scheme 2). An evidence of this reduction is the signal corresponding to hydrogens of the NH_2 group, that, in the ^{1}H nmr spectrum of **14**, are observed as a broad singlet at δ 4.54, whose peak area is proportional to two hydrogens and which is deuterium oxide-exchangeable.

With reference to the piperazines used in the synthesis of compounds **3-13**, some of them are commercially available while others were prepared by literature methods. In particular, 1-(2,4-dichlorophenyl)piperazine [11], 1-(4-ethoxyphenyl)piperazine [12], 1-(4-bromo-phenyl)piperazine [13] and 1-(4-methylphenyl)piperazine [13] were synthesized by reaction of the corresponding 4-substituted anilines with bis(2-chloroethyl)amine hydrochloride in butoxyethanol; the reaction between 4-bromobenzonitrile and anhydrous piperazine afforded 1-(4-cyanophenyl)piperazine [15].

All the new compounds **3-14** were suitably characterized by elemental analysis and spectral data (ir, ^{1}H nmr and ^{13}C nmr), which were satisfactory for the expected structures.

5*H*-Pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-diones **3-14** were tested for their binding properties on cloned α_{1A}AR, α_{1B}AR and α_{1D}AR subtypes transiently expressed in COS-7 cells. Obtained K_i values are summarized in Table 1. Affinities of the previously described 3-[2-(4-phenylpiperazin-1-yl)ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**15**) [9], which do not bear any substituent in the 4-position of the phenyl ring, are also reported for comparison. As a general trend, the title compounds showed affinity for the three receptor subtypes in the order α_{1D}AR > α_{1A}AR > α_{1B}AR. On this last subtype, in fact, only a few of the tested compounds displayed good affinity; the most active was the 4-fluoro derivative **3** with a K_i value of 35 n*M*.



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Elongation of the connecting alkyl chain from two to three atoms and the insertion of a methylene between the phenyl ring and the piperazine represent structural modifications which seem detrimental to affinity at α_1AR subtypes (compare **15** to **11**, **12** and **13**).

With the exception of derivatives **8** and **13**, all the others showed moderate to good affinity for the α_{1D}AR. This subtype seems to be more tolerant of substitution in 4-position of the phenyl ring of ligands than the other two α_1AR receptor subclasses. Among tested molecules the 4-cyano derivative **9** is of particular interest. Although it shows moderate affinity for the α_{1D}AR, it is the most selective compound for this subtype in the series. Further pharmacological studies on **9** are in progress.

Table 1
Affinities of 5*H*-Pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-diones **3-15** for the α_{1A}AR, α_{1B}AR and α_{1D}AR Subtypes

Compound No.		K_i, n*M* [a]	
	α_{1A}AR	α_{1B}AR	α_{1D}AR
3	13.1 ± 1.0	35 ± 15	1.72 ± 0.02
4	83 ± 13	>10,000	8.8 ± 3
5	413 ± 83	>10,000	263 ± 17
6	12.4 ± 1.4	350 ± 170	2.6 ± 1.4
7	520 ± 194	>10,000	156 ± 43
8	>10,000	>10,000	>10,000
9	>10,000	>10,000	75 ± 17
10	1.2 ± 0.5	45.3 ± 20.4	0.98 ± 0.3
11	80 ± 22	1280 ± 200	31.1 ± 1
12	19.4 ± 1.0	128 ± 8	7.8 ± 3.8
13	>10,000	>10,000	>10,000
14	40 ± 12.8	260 ± 30	1.0 ± 0.5
15	0.62 ± 0.16	2.3 ± 0.33	0.17 ± 0.04

[a] The K_i binding data were calculated by the Cheng-Prusoff equation [15]. Values are means (± SEM) of three to six separate experiments.

EXPERIMENTAL

Melting points were determined in a Gallemkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. Infrared spectra (ir) were recorded on a Perkin Elmer FTIR 1600 spectrometer with KBr disks. Elemental analyses (C, H, N) are within ± 0.4% of theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1108 apparatus. Nuclear magmetic resonance spectra were recorded on a Varian instrument (200 MHz for ^{1}H nmr and 50 Mhz for ^{13}C nmr). Chemical shifts are given in δ values (ppm), using tetramethylsilane as the internal standard. All the synthesized compounds were tested for purity on TLC (aluminium sheet coated with silica gel 60 F_{254}, Merck) and visualized by UV (λ = 254 and 366 nm).

General Procedure for the Preparation of Compounds **3-11**.

A mixture of *N*-(2-chloroethyl)-*N*'-[3-(2-ethoxycarbonyl)-indolyl] urea (**1**) (1.50 g, 4.84 mmol) and the suitable piperazine (24.20 mmol) was carefully mixed and then heated in an oil bath for 1 hour at 140°. After being cooled to room temperature, the reaction mixture was suspended in warm ethanol (20 ml) and stirred for 30 minutes. The solid was isolated by filtration, washed with ethanol and successively with water and then air-dried. The product was collected and recrystallized from the appropriate solvent.

3-[2-[4-(4-Fluorophenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*, 3*H*)2,4-dione (**3**).

Recrystallization from a mixture of dimethylformamide/water (8/2, v/v) afforded **3** as a white powder (1.00 g, 51%), mp >300°; ir (potassium bromide): 3170 (NH), 1715, 1625 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.51-2.61 (m, 6H, NCH_2), 2.85-3.08 (m, 4H, $ArNCH_2$), 4.11 (t, J = 7.2 Hz, 2H, $CONCH_2$), 6.88-7.16 (m, 4H + 1H, phenyl + indole), 7.32-7.44 (m, 2H, indole), 7.91-7.97 (m, 1H, indole), 11.77 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.96 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 37.37, 48.96, 52.83, 55.24, 112.76, 113.47, 114.75, 115.20 (d, $^2J_{C,F}$ = 21.5 Hz,), 116.95 (d, $^3J_{C,F}$ = 7.6 Hz), 119.50, 120.54, 125.90, 126.94, 138.64, 147.91, 150.98, 155.89 (d, $^1J_{C,F}$ = 233.3 Hz), 156.46.

Anal. Calcd. for $C_{22}H_{22}FN_5O_2$: C, 64.84; H, 5.44; N, 17.19. Found: C, 64.64; H, 5.63; N, 17.30.

3-[2-[4-(4-Bromophenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**4**).

Recrystallization from dimethylformamide afforded **4** as a white powder (1.99 g, 88 %), mp >300°; ir (potassium bromide): 3160 (NH), 1715, 1630 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.53-2.65 (m, 6H, NCH_2), 3.00-3.10 (m, 4H, $ArNCH_2$), 4.11 (t, J = 7.0 Hz, 2H, $CONCH_2$), 6.84-6.92 (m, 2H, phenyl), 7.05-7.15 (m, 1H, indole), 7.30-7.45 (m, 2H + 2H, phenyl + indole), 7.91-7.97 (m, 1H, indole), 11.77 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.96 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 37.37, 47.89, 52.65, 55.25, 109.85, 112.73, 113.50, 114.70, 117.20, 119.54, 120.56, 125.81, 127.00, 131.44, 137.94, 150.18, 151.01, 156.46.

Anal. Calcd. for $C_{22}H_{22}BrN_5O_2$: C, 56.41; H, 4.74; N, 14.95. Found: C, 56.40; H, 4.85; N, 15.02.

3-[2-[4-(4-Ethoxyphenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*, 3*H*)2,4-dione (**5**).

Recrystallization from dimethylformamide afforded **5** as a white powder (1.05 g, 50 %), mp >300°; ir (potassium bromide): 3160 (NH), 1705, 1620 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 1.28 (t, J = 6.8 Hz, 3H, $OCH_2C\mathit{H}_3$), 2.54-2.70 (m, 6H, NCH_2), 2.85-3.05 (m, 4H, $ArNCH_2$), 3.92 (q, J = 6.8 Hz, 2H, $OC\mathit{H}_2CH_3$), 4.10 (t, J = 6.8 Hz, 2H, $CONCH_2$), 6.74-6.89 (m, 4H, phenyl), 7.07-7.16 (m, 1H, indole), 7.33-7.44 (m, 2H, indole), 7.91-7.97 (m, 1H, indole), 11.76 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.96 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 14.82, 37.42, 49.57, 52.99, 55.30, 63.11, 112.74, 113.35, 114.71, 114.85, 117.21, 119.54, 120.58, 125.83, 126.99, 137.94, 145.36, 150.94, 152.02, 156.45.

Anal. Calcd. for $C_{24}H_{27}N_5O_3$: C, 66.45; H, 6.41; N, 16.14. Found: C, 66.49; H, 6.28; N, 16.15.

3-[2-[4-(4-Methylphenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*, 3*H*)2,4-dione (**6**).

Recrystallization from dimethylformamide afforded **6** as a white powder (1.13 g, 58 %), mp >300°; ir (potassium bromide): 3160 (NH), 1705, 1625 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.19 (s, 3H, CH_3), 2.55-2.73 (m, 6H, NCH_2), 2.95-3.10 (m, 4H, $ArNCH_2$), 4.11 (t, J = 6.8 Hz, 2H, $CONCH_2$), 6.75-6.90 (m, 2H, phenyl), 6.95-7.21 (m, 2H + 1H, phenyl + indole), 7.30-7.45 (m, 2H, indole), 7.90-7.98 (m, 1H, indole), 11.78 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.97 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 18.95, 37.40, 48.70, 52.90, 55.30, 112.74, 113.35, 114.69, 115.55, 119.54, 120.57, 125.81, 127.00, 127.50, 129.35, 137.93, 148.99, 150.93, 156.45.

Anal. Calcd. for $C_{23}H_{25}N_5O_2$: C, 68.46; H, 6.25; N, 17.35. Found: C, 68.67; H, 6.41; N, 17.45.

3-[2-[4-(4-Acetylphenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**7**).

Recrystallization from dimethylformamide afforded **7** as a white powder (1.57 g, 75 %), mp >300°; ir (potassium bromide): 3170 (NH), 1710, 1660, 1625 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.44 (s, 3H, $COCH_3$), 2.53-2.70 (m, 6H, NCH_2), 3.20-3.34 (m, 4H, $ArNCH_2$), 4.12 (t, J = 6.8 Hz, 2H, $CONCH_2$), 6.92-7.01 (m, 2H, phenyl), 7.04-7.15 (m, 1H, indole), 7.30-7.46 (m, 2H, indole), 7.74- 7.87 (m, 2H, phenyl), 7.92-8.01 (m, 1H, indole), 11.78 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.97 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 26.10, 37.34, 46.65, 52.52, 55.24, 112.77, 113.03, 113.48, 114.75, 117.20, 119.52, 120.54, 125.89, 126.56, 126.97, 130.05, 138.07, 150.99, 153.85, 156.48, 195.58.

Anal. Calcd. for $C_{24}H_{25}N_5O_3$: C, 66.80; H, 5.84; N, 16.23. Found: C, 66.71; H, 5.85; N, 16.25.

3-[2-[4-(4-Nitrophenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**8**).

Recrystallization from dimethylformamide afforded **8** as a yellow powder (1.64 g, 78 %), mp >300°; ir (potassium bromide): 3150 (NH), 1710, 1620 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.52-2.71 (m, 6H, NCH_2), 3.35-3.51 (m, 4H, $ArNCH_2$), 4.12 (t, J = 6.6 Hz, 2H, $CONCH_2$), 6.96-7.18 (m, 2H + 1H, phenyl + indole), 7.31-7.45 (m, 2H, indole), 7.87-8.12

(m, 1H + 2H, indole + phenyl), 11.78 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.97 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 37.32, 46.35, 52.40, 55.15, 112.59, 112.75, 113.35, 114.67, 119.57, 120.58, 125.72, 125.87, 127.04, 136.81, 137.94, 150.93, 154.77, 156.47.

Anal. Calcd. for $C_{22}H_{22}N_6O_4$: C, 60.81; H, 5.10; N, 19.34. Found: C, 60.81; H, 5.35; N, 19.34.

3-[2-[4-(4-Cyanophenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**9**).

Recrystallization from dimethylformamide afforded **9** as a cream powder (0.76 g, 38 %), mp >300°; ir (potassium bromide): 3160 (NH), 1705, 1620 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.54-2.70 (m, 6H, NCH_2), 3.10-3.35 (m, 4H, $ArNCH_2$), 4.10 (t, J = 6.8 Hz, 2H, $CONCH_2$), 6.95-7.17 (m, 2H + 1H, phenyl + indole), 7.30-7.45 (m, 2H, indole), 7.50-7.62 (m, 2H, phenyl), 7.89-7.96 (m, 1H, indole), 11.75 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.94 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 37.32, 46.36, 52.43, 55.19, 98.14, 112.76, 113.46, 113.96, 114.73, 119.50, 120.04, 120.53, 125.88, 126.96, 133.28, 138.06, 150.98, 153.19, 156.47.

Anal. Calcd. for $C_{23}H_{22}N_6O_2$: C, 66.65; H, 5.35; N, 20.76. Found: C, 66.67; H, 5.54; N, 20.83.

3-[2-[4-(2,4-Dichlorophenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**10**).

Recrystallization from dimethylformamide afforded **10** as a white powder (1.04 g, 47 %), mp >300°; ir (potassium bromide): 3150 (NH), 1705, 1620 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.56-2.77 (m, 6H, NCH_2), 2.85-3.05 (m, 4H, $ArNCH_2$), 4.11 (t, J = 7.0 Hz, 2H, $CONCH_2$), 7.07-7.18 (m, 1H + 1H, phenyl + indole), 7.30-7.54 (m, 2H + 2H, phenyl + indole), 7.91-7.97 (m, 1H, indole), 11.77 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.96 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 37.37, 50.81, 52.95, 55.30, 112.75, 113.37, 114.71, 119.56, 120.59, 122.11, 125.88, 126.88, 127.02, 128.00, 128.47, 129.66, 137.94, 148.15, 150.95, 156.47.

Anal. Calcd. for $C_{22}H_{21}Cl_2N_5O_2$: C, 57.64; H, 4.62; N, 15.28. Found: C, 57.51; H, 4.88; N, 15.10.

3-[2-[4-(Phenylmethyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**11**).

Recrystallization from dimethylformamide afforded **11** as a white powder (1.00 g, 51 %), mp >300°; ir (potassium bromide): 3180 (NH), 1715, 1625 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.22-2.65 (m, 10H, NCH_2), 3.43 (s, 2H, $ArCH_2N$), 4.05 (t, J = 6.6 Hz, 2H, $CONCH_2$), 7.03-7.15 (m, 1H, indole), 7.19-7.45 (m, 5H + 2H, phenyl + indole), 7.90-7.97 (m, 1H, indole), 11.76 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.94 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 37.41, 52.67, 52.93, 55.29, 62.11, 112.74, 113.34, 114.68, 119.55, 120.58, 125.73, 126.89, 127.00, 128.16, 128.84, 137.92, 138.24, 150.89, 156.42.

Anal. Calcd. for $C_{23}H_{25}N_5O_2$: C, 68.46; H, 6.25; N, 17.35. Found: C, 68.26; H, 6.31; N, 17.42.

General Procedure for the Preparation of Compounds **12**-**13**.

A mixture of *N*-(3-chloropropyl)-*N*'-[3-(2-ethoxycarbonyl)-indolyl] urea (**2**) (1.00 g, 3.09 mmol) and the suitable piperazine (15.45 mmol) was carefully mixed and then heated in an oil bath for 1 hour at 140°. After cooling to room temperature, the reaction mixture was suspended in 20 ml of warm ethanol and stirred for 15 minutes. The solid was isolated by filtration, washed with ethanol and successively with water and then air-dried. The product was collected and recrystallized from the appropriate solvent.

3-[3-(4-Phenylpiperazin-1-yl)propyl]-5*H*-pyrimido[5,4-*b*]-indole-(1*H*,3*H*)2,4-dione (**12**).

Recrystallization from dimethylformamide afforded **12** as a white powder (0.40 g, 32 %), mp 294-295°; ir (potassium bromide): 3160 (NH), 1705, 1625 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 1.76-1.85 (m, 2H, $CH_2CH_2CH_2$), 2.36-2.53 (m, 6H, NCH_2), 2.98-3.08 (m, 4H, $ArNCH_2$), 4.01 (t, J = 7.4 Hz, 2H, $CONCH_2$), 6.70-6.90 (m, 3H, phenyl), 7.05-7.23 (m, 2H + 1H, phenyl + indole), 7.32-7.44 (m, 2H, indole), 7.90-7.96 (m, 1H, indole), 11.76 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.94 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 24.79, 48.22, 52.65, 55.47, 112.73, 113.48, 114.71, 115.36, 118.76, 119.50, 120.58, 125.74, 126.95, 128.89, 137.92, 150.99, 151.06, 156.58.

Anal. Calcd. for $C_{23}H_{25}N_5O_2$: C, 68.46; H, 6.25; N, 17.36. Found: C, 68.37; H, 6.31; N, 17.40.

3-[3-[4-(4-Chlorophenyl)piperazin-1-yl]-5*H*-propyl]pyrimido-[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (**13**).

Recrystallization from dimethylformamide afforded **13** as a white powder (0.77 g, 57 %), mp 296-297°; ir (potassium bromide): 3150 (NH), 1710, 1625 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 1.75-1.85 (m, 2H, $CH_2CH_2CH_2$), 2.35-2.55 (m, 6H, NCH_2), 2.96-3.10 (m, 4H, $ArNCH_2$), 4.00 (t, J = 7.0 Hz, 2H, $CONCH_2$), 6.85-6.92 (m, 2H, phenyl), 7.05-7.23 (m, 2H + 1H, phenyl + indole), 7.30-7.44 (m, 2H, indole), 7.90-7.96 (m, 1H, indole), 11.74 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.91 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 24.74, 48.02, 52.47, 55.41, 112.72, 113.48, 114.71, 116.78, 119.50, 120.56, 122.22, 125.75, 126.95, 128.57, 137.91, 149.85, 150.99, 156.58.

Anal. Calcd. for $C_{23}H_{24}ClN_5O_2$: C, 63.08; H, 5.52; N, 15.99. Found: C, 62.93; H, 5.58; N, 16.05.

3-[2-[4-(4-Aminophenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido-[5,4-*b*]indole-(1*H*, 3*H*)2,4-dione (**14**).

Compound **8** (0.35 g, 0.80 mmol) was dissolved in 10 ml of hot dimethylformamide. After cooling to room temperature, Raney nickel (0.10 g, 50 % slurry in water) and then hydrazine hydrate (3 ml) were added. The reaction mixture was stirred for 1 hour and, after elimination of the catalist by filtration, the solvent was evaporated *in vacuo*. The crude residue was suspended in ethanol (15 ml) and the solid was then collected by filtration, washed with water (20 ml) and ethanol (2 ml) and air-dried.

Recrystallization from a mixture of dimethylformamide/water (2/1, v/v) afforded **14** as an amorphous powder (0.30 g, 92 %), mp >300°; ir (potassium bromide): 3430, 3350, 3160 (NH), 1705, 1620 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 2.45-2.70 (m, 6H, NCH_2), 2.75-2.95 (m, 4H, $ArNCH_2$), 4.08 (t, J = 6.7 Hz, 2H, $CONCH_2$), 4.54 (br s, 2H, NH_2, deuterium oxide-exchangeable), 6.38-6.71 (m, 4H, phenyl), 7.07-7.13 (m, 1H, indole), 7.32-7.42 (m, 2H, indole), 7.85-8.00 (m, 1H, indole), 11.75 (broad s, 1H, NH, deuterium oxide-exchangeable), 11.93 (broad s, 1H, NH, deuterium oxide-exchangeable); ^{13}C nmr (DMSO-d_6): δ 37.44, 50.37, 53.16, 55.33, 112.76, 113.37, 114.62, 114.77, 117.82, 119.56, 120.59, 125.79, 127.01, 137.94, 141.95, 142.49, 150.94, 156.46.

Anal. Calcd. for $C_{22}H_{24}N_6O_2$: C, 65.32; H, 5.98; N, 20.78. Found: C, 65.09; H, 6.13; N, 20.62.

COS-7 Cell Expression Studies, Membrane Preparation and Radioligand Binding.

Bovine α_{1A}AR [5], hamster α_{1B}AR [4] and rat α_{1D}AR [6] were kindly donated by Dr Susanna Cotecchia (Institut de Pharmacologie et Toxicologie, Facultè de Medecine, Lausanne, Switzerland). Full lenghts cDNAs were subcloned into eukaryotic expression vectors, as pBC12BI for α_{1A}AR, pBJI for α_{1B}AR and pCMV5 for α_{1D}AR and transfected into COS-7 cells using the DEAE-dextran method. COS-7 cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum for 72 hours after transfection. Separate transfections gave expression of 4000 fmol/mg of proteins for α_{1A}AR, 6300 fmol/mg of proteins for α_{1B}AR and 620 fmol/mg of proteins for α_{1D}AR. All these receptors were not expressed in untransfected COS-7 cells. Competition binding experiments were performed as previously described [8] using 0.4 n*M* [^{3}H]prazosin. Nonspecific binding was defined by 10 m*M* phentolamine.

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