Pyrimido[5,4-*b*]indole Derivatives. 1. A New Class of Potent and Selective α_1 Adrenoceptor Ligands

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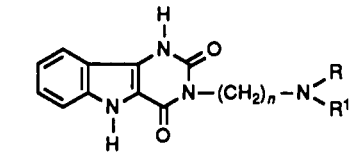
A number of 3-substituted pyrimido[5,4-*b*]indole-2,4-diones (**7–23**) were evaluated for their in vitro α_1 adrenoceptor affinity by radioligand receptor binding assays. Some compounds bearing a (phenylpiperazinyl)alkyl side chain were potent α_1 adrenoceptor ligands. The most active derivative in displacement of [^{3}H]prazosin from rat cortical membranes was 3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]pyrimido[5,4-*b*]indole-2,4-dione (**10**) (K_i = 0.21 nM). Discrete modifications in the structure resulted in higher selectivity (>10 000 times) for α_1 than α_2, β_2, and 5HT1A receptors. Some compounds also had affinity for the 5HT1A receptor. The most selective α_1 ligand was 3-[2-[4-(2-chlorophenyl)piperazin-1-yl]ethyl]pyrimido[5,4-*b*]indole-2,4-dione (**13**).

The α_1 adrenoceptor (α_1AR) is a member of the super family of G protein coupled receptors. Molecular cloning studies[1-3] have shown that these receptors have many common features which could reflect their similar mechanisms of action. The ligand binding site of these receptors involves residues within the conserved hydrophobic core formed by seven membrane-spanning clusters rich in hydrophobic amino acids.

Although several selective ligands for the α_1AR are known (e.g. prazosin, WB 4101) the fact that their chemical structures are apparently unrelated casts little light on the nature of the binding site.

With a view to preparing new selective ligands acting on the α_1AR and clarifying how some of the ligand's structural requirements influence the fit with the receptor protein, we synthesized a first series of 3-substituted pyrimido[5,4-*b*]indole-2,4-dione derivatives **1** and **2**.



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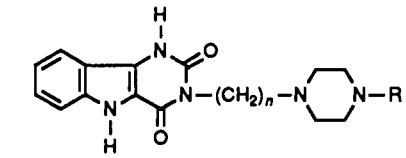

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The quinazoline-2,4-dione derivative SGB 1534[4] is a potent α_1AR ligand with antagonistic properties. In the compounds synthesized in this study, the pyrimido[5,4-*b*]indole-2,4-dione system (PI) can be considered a structure analogous to quinazoline-2,4-dione in which a pyrrole nucleus was inserted between the benzene and the pyrimidine rings. The larger aromatic plane of PI and an NH function capable of forming a hydrogen bond could be further sites for interaction with the receptor binding site. Substitutions at position 3 are represented by several (dialkylamino)alkyl or (phenylpiperazinyl)alkyl side chains which contain at least a protonatable nitrogen atom indispensable for the interaction with the receptor active site.

Mutagenesis studies[5] on the β_2AR have highlighted the importance of the binding of an aspartic residue contained in the third hydrophobic domain of the receptor protein (Asp113) which interacts with the protonated nitrogen of the ligand. An analogous residue (Asp125) with the same function is present in the primary structure of the α_1AR.[3] In the radioligand receptor binding assay some of the compounds synthetized showed notable affinity and selectivity for the α_1 receptor protein.

Table I. Physical Properties of *N*-(2-Carbethoxyindol-3-yl)-*N'*-(2-chloroalkyl)ureas

compd	*n*	% yield	mp, °C	recryst solvent	formula
4	2	88	239–240	MeOH	$C_{14}H_{16}ClN_3O_3$
5	3	82	221–222	EtOH	$C_{15}H_{18}ClN_3O_3$

Chemistry

Scheme I shows the synthetic pathway for preparing the 3-[(4-phenylpiperazinyl)alkyl]pyrimido[5,4-*b*]indole-2,4-dione derivatives **7–14**. The starting 2-carbethoxy-3-aminoindole (**3**) was reacted with ω-chloroalkyl isocyanates to give the substituted ureas **4** and **5** (Table I) (route a).

The second step to prepare the final compounds **7–14** was to react (chloroalkyl)ureas **4** and **5** with the suitable N-substituted piperazine hydrochloride. As indicated in a previous paper,[6] the reaction was carried out in THF in the presence of NaI and $NaHCO_3$. Unfortunately, only one product was isolated from this mixture identified as 2,3,5,6-tetrahydro-5-oxooxazolo[3',2':1,2]pyrimido[5,4-*b*]-indole (**6**) and not the expected N-alkylated piperazine. Compound **6** was also obtained by refluxing **4** in a solution of KOH in methanol (route b).

In a subsequent attempt, (chloroalkyl)ureas **4** and **5** were reacted at 140 °C for 2 h with a large excess of the appropriate N-substituted piperazine (as the free base) in the absence of solvent. From this reaction mixture we simultaneously obtained alkylation of the piperidine nitrogen and closure of the pyrimidine ring. The desired 3-substituted pyrimido[5,4-*b*]indole-2,4-diones **7–14** were collected in good yields (Table II) (route c).

Methylation of the two NH functions of **10** gave compound **15** (Table II) (route d). In **10** there is the possibility

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(4) SGB 1534 is the 3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]-ethyl]quinazoline-2,4-dione.
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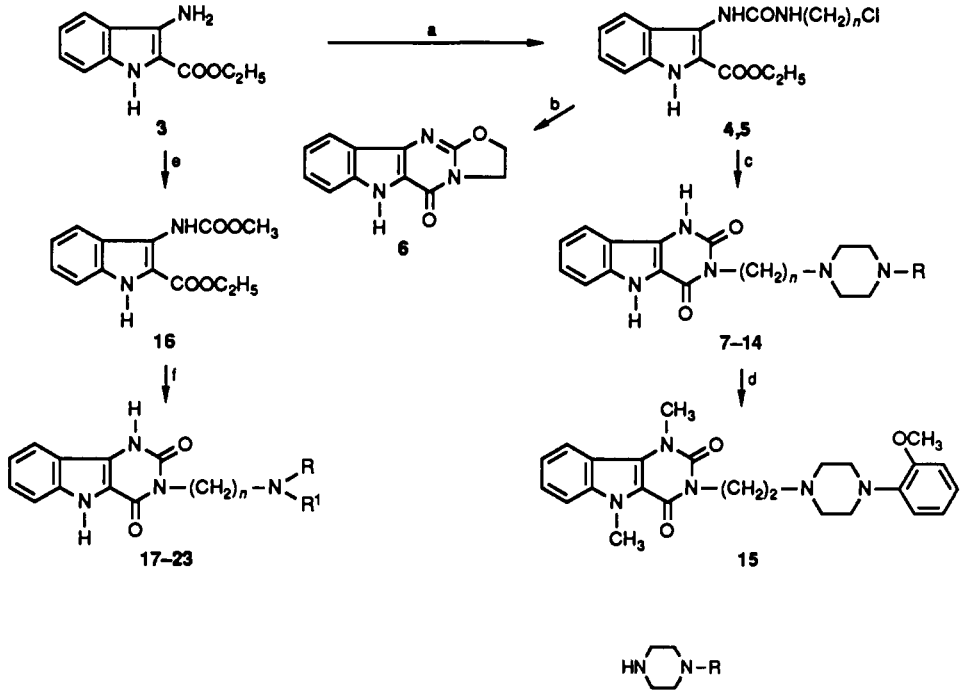
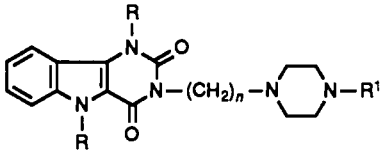
Scheme I[a]



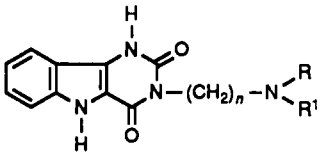
[a] Reagents: (a) toluene/$Cl(CH_2)_nNCO$/reflux; (b) MeOH/KOH; (c) HN N-R/140 °C; (d) NaH/CH_3I; (e) xylene/$ClCOOCH_3$/reflux; (f) $H_2N(CH_2)_nNRR^1$/140 °C.

Table II. Physical Properties of 3-[ω-(4-Substituted-piperazin-1-yl)alkyl]pyrimido[5,4-*b*]indole-2,4-diones



compd	*n*	R	R^1	% yield	mp, °C	recryst solvent	formula
7	2	H	H	64	>350	DMF-H_2O	$C_{16}H_{19}N_5O_2$
8	2	H	$COOC_2H_5$	64	311-312	EtOH	$C_{19}H_{23}N_5O_4$
9	2	H	C_6H_5	46	345-346	DMF-H_2O	$C_{22}H_{23}N_5O_2$
10	2	H	2-$OCH_3C_6H_4$	64	318-319	DMF-H_2O	$C_{23}H_{25}N_5O_3$
11	3	H	2-$OCH_3C_6H_4$	61	291-293	DMF-H_2O	$C_{24}H_{27}N_5O_3$
12	2	H	4-$OCH_3C_6H_4$	68	339-340	DMF-H_2O	$C_{23}H_{25}N_5O_3$
13	2	H	2-ClC_6H_4	59	328-330	DMF-H_2O	$C_{22}H_{22}ClN_5O_2$
14	2	H	4-ClC_6H_4	58	>350	DMF	$C_{22}H_{22}ClN_5O_2$
15	2	CH_3	2-$OCH_3C_6H_4$	61	175-176	EtOH-H_2O	$C_{25}H_{29}N_5O_3$

Table III. Physical Properties of 3-[(Dialkylamino)alkyl]pyrimido[5,4-*b*]indole-2,4-diones



compd	*n*	R	R^1	% yield	mp, °C	recryst solvent	formula
17	2	CH_3	CH_3	39	326-327	DMF-H_2O	$C_{14}H_{16}N_4O_2$
18	3	CH_3	CH_3	30	321-322	DMF-H_2O	$C_{15}H_{18}N_4O_2$
19	2	C_2H_5	C_2H_5	53	334-335	DMF-H_2O	$C_{16}H_{20}N_4O_2$
20	3	C_2H_5	C_2H_5	61	308-309	DMF-H_2O	$C_{17}H_{22}N_4O_2$
21	2	$-(CH_2)_4-$		62	310-312	DMF	$C_{16}H_{18}N_4O_2$
22	2	$-(CH_2)_5-$		75	335-336	DMF	$C_{17}H_{20}N_4O_2$
23	2	$-(CH_2)_2O(CH_2)_2-$		62	341-342	DMF-H_2O	$C_{16}H_{18}N_4O_3$

of O- or N-methylation on the pyrimidine ring due to tautomeric equilibrium of the cyclic amide. Analysis of the IR spectrum of **15** (two distinct carbonyl absorption bands) and of its 200-MHz ^1H NMR spectrum (sharp singlet band at δ 3.82 due to NCH_3 protons) suggests that N-methylation occurred. Under the same experimental conditions, similar heterocyclic condensed systems also gave N-methylated products.[6,7]

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Table IV. Affinity of Compounds 7–25 for Different Receptors

compd	K_i,[a] nM			
	α_1AR	5HT1A	α_2AR	β_2AR
7	>100 000	ND[b]	ND	ND
8	>100 000	ND	ND	ND
9	1.8 ± 0.2[c]	>100 000	>100 000	>100 000
10	0.21 ± 0.02[c,d]	50 ± 4.7	≥1 000	>100 000
11	13 ± 1.1	308 ± 88	55 ± 12	1 181 ± 380
12	≥5 000	>100 000	>100 000	>100 000
13	0.53 ± 0.18[c,e]	>100 000	>100 000	>100 000
14	≥10 000	>100 000	>100 000	>100 000
15	0.32 ± 0.05[c,d]	1.5 ± 0.8	2 215 ± 186	≥10 000
17	>100 000	ND	ND	ND
18	>100 000	ND	ND	ND
19	>100 000	ND	ND	ND
20	>100 000	ND	ND	ND
21	>100 000	ND	ND	ND
22	>100 000	ND	ND	ND
23	>100 000	ND	ND	ND
24	3 508 ± 821	281 ± 62	ND	ND
25	710 ± 235	226 ± 70	ND	ND

[a] The K_i binding data were calculated as described in the Experimental Section. Values are means (±SEM) of three to seven separate experiments. [b] ND, not detected. [c] Significantly different from 11 at $p < 0.01$. [d] Significantly different from 9 at $p < 0.01$. [e] Significantly different from 9 at $p < 0.05$.

For the synthesis of compounds 17–23 (Scheme I, Table III), which lack the N-substituted piperazinyl moiety, amino ester 3 was reacted with methyl chloroformate in xylene to give urethane derivative 16 (route e). This compound, at 140 °C in the presence of a large excess of the suitable (dialkylamino)alkylamine gave the 3-substituted pyrimido[5,4-*b*]indole-2,4-diones 17–23 (route f). Spectroscopic data confirmed the proposed structures. MS data were consistent with those reported in the literature for fragmentation of the pyrimido[5,4-*b*]indole-2,4-dione nucleus.[8]

Results and Discussion

The affinities of compounds 7–23 and of some phenylpiperazines such as 1-(2-methoxyphenyl)piperazine (24) and 1-(2-chlorophenyl)piperazine (25) for α_1AR and other related G protein coupled receptors are presented in Table IV. These were determined in competition binding experiments. In the present study we found some selective compounds with high affinity for α_1AR. The most important structural feature in these molecules which seemed necessary for receptor binding was the presence of a phenyl ring on the N4 atom of the piperazine moiety of the side chain. All the compounds, even those bearing a protonatable nitrogen atom in the side chain (17–23), that did not have this ring or carried a different substituent such as a carboethoxy group (8) were completely inactive in the binding assay ($K_i \gg 10^{-6}$ M). However, the piperazine ring alone was not enough to ensure binding (7) (Table IV).

Recently De Marinis[9] proposed a model of α_1AR in which one of the principal requirements of the ligand for binding to the receptor protein was the presence of an electron-rich aromatic area coupled to a protonatable nitrogen atom at a suitable distance. On this basis, we can assume that in compounds 9–15 the phenyl ring of the phenylpiperazine (PP) moiety and not the indole nucleus on the other side of the molecule resembles the required

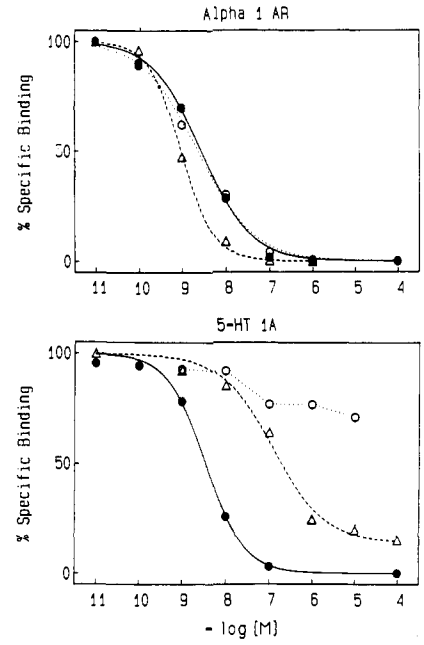

Figure 1. Competition of compounds 13 (open circle), 15 (closed circle), and 10 (triangle) for [^{3}H]prazosin binding to α_1AR (upper) and for [^{3}H]-8-OH-DPAT binding to 5HT1A receptors (lower). Membranes were incubated with the radioligand and the indicated concentrations of competing drugs for 60 min at 37 °C, then filtered. Competition curves were fitted by nonlinear regression analysis, and the computer-drawn curves are shown. For compound 13 binding to 5HT1A receptors (open circles, lower panel) the data were not fitted by the computer but a point-to-point line was drawn. The K_i values (±SEM) are reported in Table IV. See the Experimental Section for further details.

aromatic plane. This is in agreement with the fact that the PPs and many compounds in which they are part of the structure are good ligands for some G protein coupled receptors such as the α_1, α_2, 5HT1A, and D_2 receptors.

While the unsubstituted compound 9 showed a K_i of 1.8 nM for the α_1AR, the presence of a substituent (methoxy, chlorine) (12, 14) in the para position of the phenyl ring drastically reduced the affinity. Substitution of an *o*-methoxy group increased the affinity about 10-fold (K_i = 0.21 nM), making 10 the most potent compound in the study. A similar (but less pronounced) enhancement in affinity was seen when the ortho position was substituted with a chlorine atom (13) (Figure 1, upper panel). Statistical analysis showed that 10, 13, and 15 were significantly more potent ligands for α_1AR than 9. The difference in affinity among 10, 13, and 15 did not reach statistical significance. These changes in affinity could be partially explained by steric effects. Probably para substituents create a steric bulk in a forbidden volume of the active site, bringing the K_i to the micromolar level, while ortho substituents arrange the phenyl ring and the piperazine group into a conformation which better fits the binding site.[10] Electronic effects may also be involved. The substitution of methyl groups for the hydrogen atoms on the two NH functions on the pyrimido[5,4-*b*]indole-2,4-dione system had only a little effect on the affinity; the methylated compound 15 was as potent as 10, making it unlikely that the two NH functions act as hydrogen-bond donors in the α_1 protein–ligand complex.

Compound 11 was prepared so as to explore the size requirements at this site. The length of the alkyl side chain

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between the pyrimido[5,4-*b*]indole-2,4-dione system and the phenylpiperazinyl moiety seems to be critical for affinity as its extension by addition of a methylene group drastically reduced the affinity of **11** compared to **10** (100-fold).

The compounds active on the α_1AR site (**9**, **10**, **13**, **15**) were poor ligands for the α_2AR and the β_2AR ($K_i \geq \mu$M) sites. However lengthening the alkyl chain (**11**), which lowered the affinity for α_1AR, seemed to improve the activity on the α_2AR (K_i = 55 nM).

Because of their structural similarities with some potent 5HT1A agents (such as BMY 7378[11,12]), we investigated the affinity of compounds **7–15** for this receptor subtype. Compound **10**, which is the most potent at the α_1AR site, was also a potent ligand for the 5HT1A receptor (K_i = 50 nM), although 250 times weaker than on α_1AR. The *o*-chloro-substituted compound **13** was highly selective for α_1AR, but almost inactive at 5HT1A receptors (Figure 1, lower panel).

To obtain information about the role of the PP moiety of these compounds, we measured the affinity of **24** and **25** for α_1 and 5HT1A receptors. Both **24** and **25** were poor ligands for α_1AR ($K_i \geq 1$ μM) and 5HT1A receptors ($K_i \approx 200$ nM) with about 10 times greater selectivity for the latter. This suggests that PP is not the only determinant of the very high affinity (K_i < nm) of some pyrimido-[5,4-*b*]indole derivatives. The PI moiety probably contributes to the affinity, although by itself this structural type does not bind to these receptors.

Two findings suggest that receptor selectivity is at least partially determined by the PI moiety. First, **24** and **25** preferentially bound to the 5HT1A receptor while compounds **10** and **13** were selective ligands for α_1AR (ligand **13** was extremely selective). Second, methylation of the two NH functions on the PI moiety had no effect on affinity for the α_1AR, but rendered **15** the most active on the 5HT1A receptor (K_i = 1.5 nM), although not selective. The PP moiety is also obviously involved in the selectivity. In fact **9** and **13**, which possess high affinity for α_1AR, were completely unable to inhibit [^{3}H]-8-OH-DPAT binding to 5HT1A receptors.

Previous studies suggest that the *o*-methoxy substitution on PP-containing products gives the highest affinity for α_1AR[6,7] or other related receptors.[12,13] This general knowledge is confirmed by compounds **10** and **15**, which, in other hands, were also active on 5HT1A receptors. Additionally, we now present the first finding that the *o*-chloro-substituted compound **13**, which retained high affinity for α_1AR, was extremely selective, for it was almost inactive on the other receptors tested, including 5HT1A.

In conclusion, it appears that the PP moiety is the structure which leads the [(phenylpiperazinyl)alkyl]pyrimido[5,4-*b*]indole molecule to the binding site of the receptor protein. It has two major interactions which are effected via the phenyl ring and one nitrogen atom (presumably the N1 atom). The ortho substituents increase the affinity and influence selectivity for 5HT1A receptors. The alkyl chain, a spacer between the two major constituents of the molecule, can also influence affinity and selectivity. Finally the PI system binds to an accessory area and contributes to the strength and selectivity of the binding, although it is not sufficient alone for binding to the α_1AR. The [(phenylpiperazinyl)alkyl]pyrimido[5,4-*b*]indole molecule thus acts as a pharmacophore on which discrete modifications can drastically change its ability to bind to different related receptors. Hence it may serve as a useful tool for investigating the subtle differences between the binding sites of such closely related receptor proteins.

Experimental Section

Materials. Melting points were determined in a Gallenkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 281 spectrometer with KBr disks. Elemental analyses for C, H, and N were performed on a Carlo Erba Model 1106 elemental analyzer and were within ±0.4% of the theoretical values.

All the compounds synthesized were tested for purity on TLC (aluminum sheets coated with silica gel 60 F254, Merck) and visualized by UV (λ = 254 and 366 nm). The ^{1}H NMR spectra were recorded on a 90-MHz Perkin-Elmer R32 or on a 200-MHz Bruker MSL 200 instrument. Chemical shifts are given in ppm (δ) relative to tetramethylsilane and the signals were characterized as s (singlet), t (triplet), q (quartet), m (multiplet), br (broad signal).

Mass spectra (MS, *m/z*) were recorded on a Kratos MS-50 instrument fitted with a standard EI source (ionization energy 70 eV). 2-Carbethoxy-3-amino-1*H*-indole (**3**) was prepared from 2-aminobenzonitrile by a literature method,[14] mp 150–151 °C.

Route a. *N*-(2-Chloroethyl)-*N'*-[3-[2-(ethoxycarbonyl)-indolyl]]urea (4) (Table I). Amino ester **3** (2.05 g, 10 mmol) was dissolved in hot toluene (20 mL). To the solution was added 2-chloroethyl isocyanate (1.6 g, 15 mmol) and the reaction mixture was allowed to cool. The resulting precipitate was collected by filtration, washed with a small amount of cold EtOH, and recrystallized by MeOH to give **4** as white crystals (2.75 g, 88%): mp 239–240 °C; ^{1}H NMR (DMSO-d_6) δ 1.48 (t, J = 8 Hz, 3 H, CH_3), 3.35–3.95 (m, 4 H, CH_2), 4.45 (q, 2 H, OCH_2), 6.95–7.55 (m, 3 H, H5, H6, H7), 7.90 (d, J = 8 Hz, 1 H, H4), 8.28 (br s, 1 H, NH which exchanges with D_2O), 11.30 (br s, 1 H, NH which exchange with D_2O); MS *m/z* 309 (M, 18), 311 (M + 2, 6), 273 (22), 227 (55), 204 (98), 184 (28), 158 (100), 130 (53), 129 (48), 103 (36); IR (cm^{-1}) 3330 (br band), 1680, 1645. Anal. ($C_{14}H_{16}ClN_3O_3$) C, H, N.

Compound **5** was synthesized from **3** and 3-chloropropyl isocyanate by the same procedure (Table I).

Route b. 2,3,5,6-Tetrahydro-5-oxooxazolo[3',2':1,2]pyrimido[5,4-*b*]indole (6). To a hot solution of **4** (0.5 g, 1.6 mmol) in MeOH (100 mL) was added a solution of KOH (0.11 g, 2 mmol) in MeOH (3 mL). The reaction mixture was refluxed with stirring for 1 h. A white product precipitated and, once cool, was filtered off and washed with water. The crude solid was recrystallized from EtOH to give **6** as white crystals (0.33 g, 91%): mp >350 °C; ^{1}H NMR (DMSO-d_6) δ 4.30 (t, J = 8.3 Hz, 2 H, CH_2), 7.09–7.45 (m, 3 H, H7, H8, H9), 7.85 (d, J = 8.1 Hz, 1 H, H10), 11.84 (br s, 1 H, NH which exchanges with D_2O); MS *m/z* 226 (M, 60), 225 (M - 1, 100), 185 (75), 129 (64); IR (cm^{-1}) 3210 (NH), 3080 (aromatic CH), 2980, 2910 (aliphatic CH), 1690 (C=O), 1590, 1570 (C=C and C=N), 735 (aromatic 1,2 disubstituted). Anal. ($C_{12}H_9N_3O_2$) C, H, N.

Route c. 3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]-ethyl]pyrimido[5,4-*b*]indole-2,4-dione (10) (Table II). Preparation of this compound is presented as an example of the general synthesis of compounds **7–14**.

A mixture of **4** (1.54 g, 5 mmol) and 1-(2-methoxyphenyl)-piperazine (4.8 g, 25 mmol) was heated in an oil bath for 2 h at 140 °C. After cooling, the reaction mixture was treated with a small amount of EtOH and the precipitate was filtered off, washed with water, and dried. Recrystallization from DMF/water (2/1) gave **10** as white crystals (1.35 g, 64%): mp 318–319 °C; ^{1}H NMR (DMSO-d_6) δ 2.55–2.85 (br m, 6 H, NCH_2), 2.90–3.10 (br, s, 4 H, $ArNCH_2$), 3.77 (s, 3 H, OCH_3), 4.12 (t, J = 7.1 Hz, 2 H, $CONCH_2$), 6.90 (m, 4 H, ArH), 7.13–7.41 (m, 3 H, indole), 7.96 (d, 1 H, H9), 11.8 (br s, 1 H, NH which exchanges with D_2O), 12.0 (br s, 1 H,

(11) BMY 7378 is 8-[2-[4-(2-methoxyphenyl)piperazin-1-yl]-ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride.

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NH which exchanges with D_2O); MS m/z 419 (M, 15), 417 (M - 2, 5), 404 (M - 15, 3), 228 (8), 205 (100), 190 (19), 185 (9), 158 (4); IR (cm^{-1}) 3190 (br band), 1715, 1630. Anal. ($C_{23}H_{23}N_5O_3$) C, H, N.

Route d. 3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]-ethyl]-1,5-dimethylpyrimido[5,4-*b*]indole-2,4-dione (15) (Table II). Compound **10** (1.6 g, 3.80 mmol) was dissolved in DMF (50 mL) by gentle heating. Once cool, the solution was treated with 80% NaH (0.35 g, 11.40 mmol) and stirred for 20 min. After the complete evolution of hydrogen, methyl iodide (1.08 g, 7.60 mmol) was added to the reaction mixture, which was stirred for 24 h at room temperature. Then the mixture was poured into ice water and the precipitate was collected, washed with water, and recrystallized from EtOH/water (1/1) to give **15** as a white powder (1.05 g, 61%): mp 175–176 °C; ^1H NMR (DMSO-d_6) δ 2.59 (br m, 6 H, NCH_2), 2.95 (br s, 4 H, $ArNCH_2$), 3.77 (s, 3 H, OCH_3), 3.82 (s, 3 H, NCH_3), 4.07 (s, 3 H, NCH_3), 4.11 (t, J = 7 Hz, 2 H, $CONCH_2$), 6.87 (m, 4 H, ArH), 7.20–7.50 (2 t, 2 H, indole H7, H8), 7.63 (d, J = 8.7 Hz, 1 H, indole H6), 8.10 (d, J = 8.2 Hz, 1 H, indole H9); MS m/z 447 (M, 32), 445 (M - 2, 7), 432 (M - 15, 9), 285 (5), 256 (30), 229 (16), 218 (43), 205 (100), 190 (45), 158 (4); IR (cm^{-1}) 1690, 1645. Anal. ($C_{25}H_{29}N_5O_3$) C, H, N.

Route e. 2-Carbethoxy-3-[(methoxycarbonyl)amino]-1*H*-indole (16). To a hot solution of **3** (6.1 g, 30 mmol) in xylene (30 mL) was added methoxycarbonyl chloride (4.35 g, 45 mmol) and the reaction mixture was heated to reflux with stirring (3 h). After cooling, the crude precipitate was filtered, washed with cold EtOH, and recrystallized from EtOH/water (1/1) to give **16** as white crystals (6.8 g, 86%): mp 127–128 °C; MS m/z 262 (M, 35), 230 (21), 216 (20), 184 (100), 129 (22), 102 (19); IR (cm^{-1}) 3340, 3290, 3060, 2970, 1705, 1680, 1590, 730. Anal. ($C_{13}H_{14}N_2O$) C, H, N.

Route f. 3-[2-(Morpholin-4-yl)ethyl]pyrimido[5,4-*b*]-indole-2,4-dione (23) (Table III). Preparation of this compound is presented as an example of the general synthesis of compounds **17–23**.

A mixture of **16** (2.1 g, 8 mmol) and of 4-(2-aminoethyl)-morpholine (10.4 g, 80 mmol) was heated in an oil bath for 2 h at 140 °C. After cooling, the solid was filtered off and washed with cold EtOH. The crude product was recrystallized from DMF/water (2/1) to give **23** as white crystals (1.56 g, 62%): mp 341–342 °C; ^1H NMR (DMSO-d_6) δ 2.35–2.85 (br m, 6 H, NCH_2), 3.40–3.70 (br m, 4 H, OCH), 4.04 (t, 2 H, $CONCH_2$), 6.90–7.50 (m, 3 H, indole H6, H7, H8), 7.87 (d, 1 H, indole H9), 11.70 (br s, 1 H, NH which exchanges with D_2O); MS m/z 314 (M, 16), 228 (13), 201 (20), 185 (48), 158 (13), 113 (75), 100 (100); IR (cm^{-1}) 3180 (br band), 1715, 1630. Anal. ($C_{16}H_{18}N_4O_3$) C, H, N.

Binding Experiments. Competition ligand binding experiments were performed as previously described.[15,16] Briefly, α_1AR were determined by [^{3}H]prazosin (0.4 nM) specific binding to rat cortex membranes (nonspecific binding was defined by 10 μM phentolamine). β_2AR were determined by [^{125}I]pindolol (100 pM) specific binding to human peripheral blood mononuclear leukocyte membranes (nonspecific binding was defined by 1 μM (-)-propanolol). α_2AR were determined by [^{3}H]yohimbine (3.5 nM) specific binding to human platelet membranes (nonspecific binding was defined by 100 μM phentolamine). 5HT1A receptors were determined by [^{3}H]-8-OH-DPAT (1 nM) specific binding to rat hippocampus or cortex membranes (nonspecific binding was defined by 1 μM serotonin). For all the binding experiments the radioligands were incubated in the presence or absence of competing drugs for 45–60 min at 37 °C in the appropriate buffers, then rapidly filtered through Whatman GF/C glass-fiber filters under vacuum and washed with ice-chilled buffer. Competition curves were analyzed by the GRAPHPAD[17] nonlinear fitting computer program, which gives IC_{50} values. The mean values of three to seven independent experiments were used to determinate each IC_{50} (the standard error between different experiments never exceeded 10%). K_i values of competing drugs were calculated by the Cheng–Prusoff[18] equation by using the following K_D values for the different radioligands: [^{3}H]prazosin, 0.1 nM; [^{125}I]pindolol, 50 pM; [^{3}H]yohimbine, 2.5 nM; [^{3}H]-8-OH-DPAT, 0.8 nM.

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Registry No. **3**, 87223-77-6; **4**, 119704-29-9; **5**, 133399-61-8; **6**, 133399-62-9; **7**, 119704-35-7; **8**, 133399-63-0; **9**, 133399-64-1; **10**, 133399-65-2; **11**, 133399-66-3; **12**, 133399-67-4; **13**, 133399-68-5; **14**, 133399-69-6; **15**, 133399-70-9; **16**, 98792-12-2; **17**, 133399-71-0; **18**, 133399-72-1; **19**, 133399-73-2; **20**, 133399-74-3; **21**, 133399-75-4; **22**, 119704-34-6; **23**, 119704-33-5; **24**, 35386-24-4; **25**, 39512-50-0; 2-chloroethyl isocyanate, 1943-83-5; 3-chloropropyl isocyanate, 13010-19-0; methyl chloroformate, 79-22-1; piperazine, 110-85-0; 1-(ethoxycarbonyl)piperazine, 120-43-4; 1-phenylpiperazine, 92-54-6; 1-(2-methylphenyl)piperazine, 35386-24-4; 1-(4-methoxyphenyl)piperazine, 38212-30-5; 1-(2-chlorophenyl)piperazine, 39512-50-0; 1-(4-chlorophenyl)piperazine, 38212-33-8; 2-(dimethylamino)ethylamine, 108-00-9; 3-(dimethylamino)propylamine, 109-55-7; 2-(diethylamino)ethylamine, 100-36-7; 3-(diethylamino)propylamine, 104-78-9; 1-(2-aminoethyl)pyrrole, 7154-73-6; 1-(2-aminoethyl)piperidine, 27578-60-5; 4-(2-aminoethyl)morpholine, 2038-03-1.

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