New Pyrimido[5,4-*b*]indoles as Ligands for α_1-Adrenoceptor Subtypes

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A new series of compounds were designed as structural analogues of the α_1-AR ligand RN5 (**4**), characterized by a tricyclic 5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione system connected through an alkyl chain to a phenylpiperazine (PP) moiety. These compounds were synthesized and tested in binding assays on human α_{1A}-AR, α_{1B}-AR, and α_{1D}-AR subtypes expressed in HEK293 cells. Several structural modifications were performed on the PP moiety, the tricyclic system, and the connecting alkyl chain. Many of the new molecules showed a preferential affinity for the α_{1D}-AR subtype. Some compounds, including **39** and **40**, displayed substantial α_{1D}-AR selectivity with respect to α_{1A}-AR, α_{1B}-AR, serotonergic 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and dopaminergic D_1 and D_2 receptors. Two conformationally rigid analogues of **4**, useful for studying the architecture of the receptor/ligand complex, were also prepared and tested. A subset of the new compounds was then used to evolve a preliminary pharmacophore model for α_{1D}-AR antagonists, based on a more generalized model we had developed for α_1-AR antagonists. This new model rationalized the relationships between structural properties and biological data of the pyrimido[5,4-*b*]indole compounds, as well as other compounds.

Introduction

α_1-Adrenergic receptors (α_1-AR) belong to the seven-transmembrane-domain (7-TM) receptor superfamily and mediate many physiological effects of the catecholamines epinephrine and norephineprine.[1] Three different native α_1-AR subtypes have been cloned and are referred to as α_{1A}-AR, α_{1B}-AR, and α_{1D}-AR.[2−5] These subtypes are expressed in many human tissues including liver, brain, myocardium, and vascular smooth muscle. Each α_1-AR subtype has a distinct pharmacology and shows a discrete tissue distribution.[6] Unlike the α_{1B}-AR and α_{1D}-AR subtypes, several splice variants of α_{1A}-ARs have been isolated from human heart, prostate, and hippocampus.[7,8] Four of these present a typical 7-TM receptor structure, differing only in their distal C-terminal sequences, and show apparently identical pharmacological profiles. Other isoforms appear to be prematurely truncated and are unable to bind ligand or activate functional responses. At present, however, the physiological and pathological roles of full-length and truncated α_1-ARs remain to be clarified.

In the past decades, several non-subtype-selective α_1-AR antagonists, such as prazosin and doxazosin, have been used effectively for treatment of hypertension and benign prostatic hyperplasia (BPH), a urologic disorder prevalent in elderly males.[9,10] Increasing evidence for the role of α_{1A}-ARs in bladder outlet obstruction in patients with BPH[11,12] has encouraged the use of α_{1A}-AR selective antagonists in symptomatic therapy of BPH. To date, several highly selective α_{1A}-AR antagonists have been synthesized, some of which are in preclinical and clinical development.[13,14]

On the other hand, the search for α_{1B}-AR or α_{1D}-AR selective ligands has been less fruitful, with only a few compounds showing even modest selectivity for these subtypes being described in the literature. Examples of such ligands are provided by (+)-cyclazosin (**1**), L-765,314 (**2**), and BMY 7378 (**3**) (Chart 1). (+)Cyclazosin, structurally related to prazosin, is the most selective α_{1B}-AR antagonist reported to date, although its α_{1B}-AR selectivity is disputed.[15,16] L-765,314 is another prazosin analogue that has shown α_{1B}-AR selectivity.[17] On the other hand, BMY 7378 has become the reference ligand for characterization of α_{1D}-ARs.[18] However, the usefulness of these molecules is restricted by their limited selectivity. Moreover, BMY 7378 (**3**) also binds with high affinity to serotonergic 5-HT_{1A} receptors, where it has partial agonist activity.[19] Hence, the discovery of new, highly selective ligands for α_{1B}-ARs and α_{1D}-ARs might represent a major advance, providing useful probes to define the functional roles of each subtype that might possibly possess unique therapeutic applications.

Recently, we were involved in developing new selective α_1-AR ligands characterized by a tricyclic 5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione system coupled, by means of an alkyl chain, to a phenylpiperazine (PP) moiety.[20−22] Among them, RN5 (**4**) (Chart 1) emerged as one of the most interesting, showing high affinity and selectivity for α_1-ARs on rat cortical membranes over α_2-AR, β_2-AR, and 5-HT_{1A} receptors.[20] Several analogues of **4** were obtained, focusing particularly on structural variations in the PP moiety. When tested on cloned α_1-

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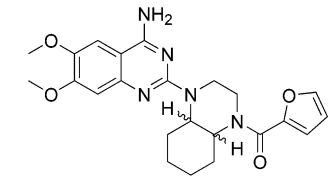
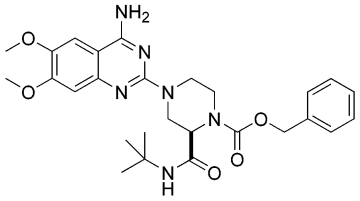
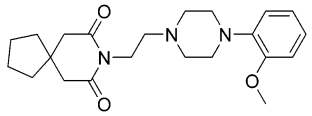
Chart 1



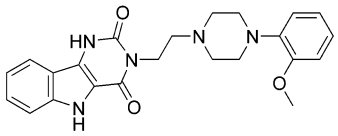
1 Cyclazosin



2 L-765,314



3 BMY 7378



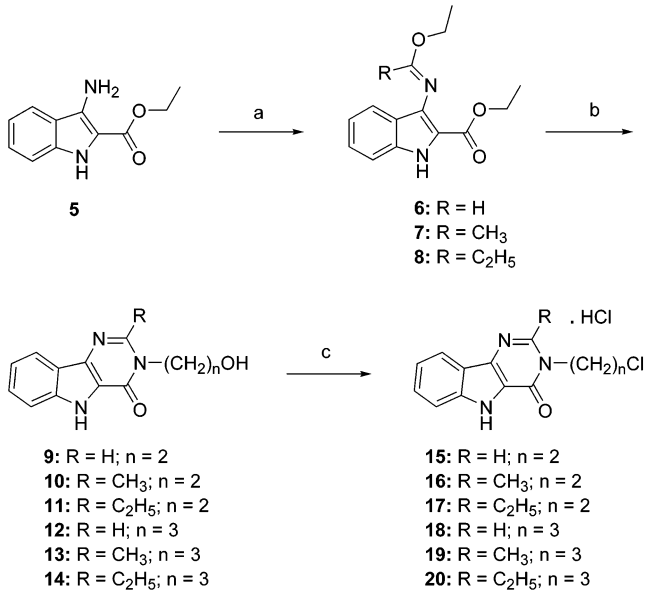
4 RN5

AR subtypes, derivatives bearing a substituent in the 4-position of the PP aromatic ring showed decreased affinity but were able to discriminate among α_1-AR subtypes with a preference for α_{1D}-ARs.[22] This suggested that α_{1D}-ARs might, unlike the other two subtypes, tolerate the steric bulk of a substituent in the 4-position. The tricyclic 5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione moiety of **4** was less thoroughly investigated, and only a few structural modifications were made.

We now report the synthesis and binding properties of a new series of pyrimido[5,4-*b*]indole derivatives structurally related to **4**. The new molecules present variations in the PP moiety, in the length of the connecting alkyl chain and in the tricyclic system as follows. (i) Substituents of increasing steric bulk in the 4-position or alkoxy group in the 2-position were inserted on the aryl ring of the PP moiety. (ii) The ethylene chain in **4** was elongated to three methylene units in some molecules. (iii) The pyrimido[5,4-*b*]indole-2,4-dione moiety was modified by deletion of the C=O group in the 2-position or substituted by the bioisosteric benzothieno[3,2-*d*]pyrimidine-2,4-dione system.

In addition, the flexibility of the [4-(2-methoxyphenyl)piperazin-1-yl]ethyl moiety in **4** allows the molecule to adopt a variety of low-energy conformations. To obtain more rigid α_1-AR ligands for studying the architecture of the receptor/ligand complex, we also

Scheme 1[a]



5

6: R = H
7: R = CH_3
8: R = C_2H_5

9: R = H; n = 2
10: R = CH_3; n = 2
11: R = C_2H_5; n = 2
12: R = H; n = 3
13: R = CH_3; n = 3
14: R = C_2H_5; n = 3

15: R = H; n = 2
16: R = CH_3; n = 2
17: R = C_2H_5; n = 2
18: R = H; n = 3
19: R = CH_3; n = 3
20: R = C_2H_5; n = 3

[a] Conditions: (a) $RC(OC_2H_5)_3$, reflux; (b) $H_2N(CH_2)_nOH$, reflux; (c) $SOCl_2$, toluene, reflux.

prepared two conformationally restricted analogues of **4** in which the *N*-(2-methoxyphenyl)piperazine moiety was substituted by the strictly related 1,2,3,4,4a,5-hexahydropyrazino[2,1-*c*][1,4]benzoxazine. The latter system can be regarded as a 1-(2-methoxyphenyl)-piperazine in which the methoxy group is connected with a carbon atom of the piperazine ring through a single bond.

Finally, because the new pyrimido[5,4-*b*]indoles showed a preferential affinity for α_{1D}-ARs, we used the program Catalyst to develop a pharmacophore model for α_{1D}-AR antagonists. The calculated model consists of a positive ionizable portion, three hydrophobic features, and two hydrogen bond acceptor groups. This model showed a good statistical significance with a correlation coefficient of 0.91 and successfully predicts the affinities of the molecules of, and external to, the training set.

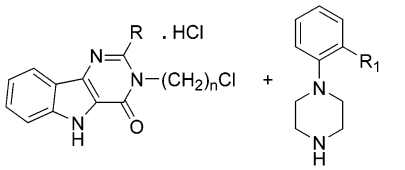
Chemistry

The synthetic pathway to the final 3-[2-[4-(2-substitutedphenyl)piperazin-1-yl]alkyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one derivatives **23**−**31** is shown in Schemes 1 and 2.

The starting aminoester **5**, which was prepared according to the Unangst's method,[23] was reacted with triethylortho esters of formic, acetic, or propionic acids to afford the corresponding iminoethers **6**−**8**. Products **6**−**8** were reacted with 2-ethanolamine or 3-amino-1-propanol to obtain tricyclic alcohols **9**−**14**. By reaction with $SOCl_2$ in toluene, alcohols **9**−**14** were converted to the corresponding alkyl chloride hydrochlorides **15**−**20** (Scheme 1). Reaction of chloro derivatives **15**−**20** with 1-(2-methoxyphenyl)piperazine (**21**) or 1-(2-ethoxyphenyl)piperazine (**22**) at 140 °C afforded final products **23**−**31** (Scheme 2).

The preparation of final compounds **39**−**46** and **48** is presented in Scheme 3. Indolylurea **32**[20] was reacted with 1-(4-substitutedphenyl)piperazines **33**−**38** to afford final products **39**−**44**. Phenylpiperazines **33**−**38** were obtained by reaction of the suitable 4-substituted aniline

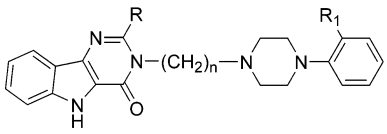
Scheme 2[a]



15 - 20

21: $R_1 = CH_3O$
22: $R_1 = C_2H_5O$

a



23: R = H; n = 2; $R_1 = CH_3O$
24: R = CH_3; n = 2; $R_1 = CH_3O$
25: R = C_2H_5; n = 2; $R_1 = CH_3O$
26: R = H; n = 3; $R_1 = CH_3O$
27: R = CH_3; n = 3; $R_1 = CH_3O$
28: R = C_2H_5; n = 3; $R_1 = CH_3O$
29: R = H; n = 2; $R_1 = C_2H_5O$
30: R = CH_3; n = 2; $R_1 = C_2H_5O$
31: R = C_2H_5; n = 2; $R_1 = C_2H_5O$

[a] Conditions: (a) 140 °C.

and bis(2-chloroethyl)amine hydrochloride in 2-butoxyethanol at reflux and in the presence of potassium carbonate.

Furthermore, compound **32**, under the same experimental conditions used for the preparation of **39**−**44**, was reacted with 1-(2-ethoxyphenyl)piperazine (**22**) or with 4-(2-methoxyphenyl)piperidine[24] to obtain **45** or **46**, respectively.

Analogously, benzothienyl urea **47**[25] was reacted with 4-[4-(1-methylethyl)phenyl]piperazine (**33**) to give the final derivative **48**.

Scheme 4 shows the synthetic pathway for the preparation of the final compounds **49**−**52**, which structurally bear a (4-substitutedphenyl)piperazine moiety and lack the carbonylic group in the 2-position of the tricyclic system. Synthesis was performed by coupling alkyl chlorides **15** or **16** (Scheme 1) with 4-[4-(1-methylethyl)-phenyl]piperazine (**33**) or 4-[4-(1,1-dimethylethyl)phenyl]-piperazine (**34**).

The synthetic pathway to tricyclic amine **62**, required to prepare final compounds **63** and **64**, is shown in Scheme 5. The preparation of **62** had been previously reported by Gupta.[26] Recently, Baxter described the synthesis of this amine, even if any spectroscopic data for intermediates and **62** were reported.[27,28] Since we had some difficulties in reproducing Gupta's procedure, Baxter's method was followed with some modifications. The starting compound was oxirane **53**, which was prepared by reaction of 2-nitrophenol with epichlorohydrin.[29] Compound **53** was reacted with phthalimide to afford the secondary alcohol **54**, which was then oxidized to the corresponding ketone **55**. This step was quite critical. After some attempts with different oxidant reagents (Jones' reagent as in Gupta's method, chromic anhydride in pyridine as in Baxter's procedure, anhydrous DMSO in acetic anhydride, pyridinium chlorochromate), Dess−Martin's reagent,[30] as modified by Geiss,[31] was preferred because it gave the best yield (87%) in the oxidized product **55.** It was then converted into the bicyclic derivate **56** through a catalytic reduction with H_2 on 10% Pd/C at atmospheric pressure, and after hydrolysis of the phthalimide system with hydrazine hydrate, amine **57** was obtained. After the protection of the primary amine group with benzyl chloroformate to give **58**, **58** was reacted with chloroacetyl chloride to give amide **59**. Cyclization of **59** to the tricyclic derivate **60** with potassium carbonate in DMSO, reduction of the amide carbonyl with the borane−THF complex, and successive deprotection of secondary amine afforded amine **62**.

Finally, reaction of **62** with indolylurea **32** or with alkyl chloride **16** gave target compounds **63** and **64**, respectively (Scheme 6).

Pharmacology

Tricyclic compounds **23**−**28**, **30**, **39**−**46**, **48**−**52**, **63**, and **64** along with RN5 (**4**), its chloro analogue **65**,[20] and the *des*-methyl analogue **66**[32] (Chart 2) were tested in binding assays on human α_{1A}-AR, α_{1B}-AR, and α_{1D}-AR subtypes stably expressed in HEK293 cells using [^{125}I]-BE 2254 as radioligand. Their affinity values were expressed as pK_i or, for compounds with low water solubility, as a percentage of inhibition of specific binding at the highest concentration tested (1 μM).

Affinities of compounds **39** and **40** for some other receptor classes, such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, D_1, and D_2 receptors, were also measured.

Moreover, to evaluate the effects of compounds **40** and RN5 (**4**) on the signal transduction pathway coupled to α_1-ARs, we measured their ability to block norepinephrine-induced stimulation of inositol phospholipid hydrolysis in rat hippocampal slices.

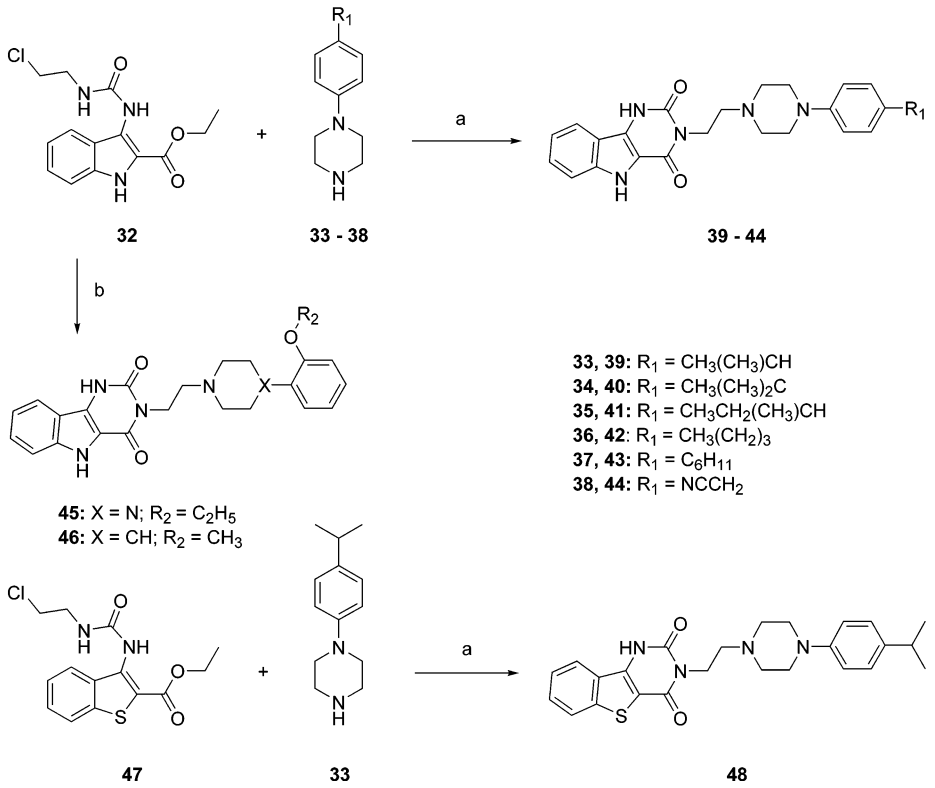
Results and Discussion

The radioligand binding data on human cloned α_1-AR subtypes of selected new tricyclic derivatives, compounds **4** (RN5) and **65** (whose synthesis and affinities on rat α_1-AR had been already reported)[20] and **66** are shown in Table 1.

As a general trend, many of tested compounds showed affinities for the three receptor subtypes in the order α_{1D}-AR ≥ α_{1A}-AR > α_{1B}-AR, and some displayed some selectivity for α_{1D}-ARs. As expected, RN5 (**4**) maintained the same high affinity for the human cloned α_1-AR subtypes as was previously observed with rat cortical α_1-AR. RN5 (**4**) displayed a slight preference for α_{1A}-ARs and α_{1D}-ARs (pK_i = 9.57 and 9.44, respectively) with respect to α_{1B}-ARs (pK_i = 8.74). Its chloro analogue **65**, with 10-fold lower affinity values, was still a good ligand showing no selectivity among subtypes.

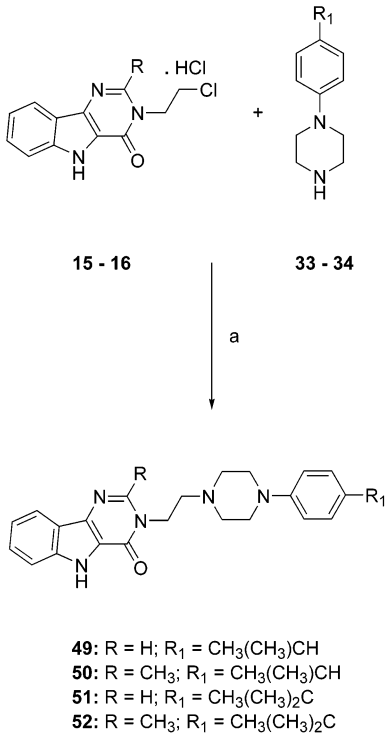
Compounds **45** and **66**, in which an ethoxy and a hydroxy group respectively replaces the methoxy group of **4**, showed a decreased affinity for all three subtypes; however, the reduction was more pronounced for α_{1A}-ARs and α_{1B}-ARs. Therefore, **45** and **66** showed a slight selectivity for α_{1D}-ARs. A similar but less pronounced reduction in affinity was observed with **46**, in which a

Scheme 3[a]



[a] Conditions: (a) 140 °C; (b) **22** or 4-(2-methoxyphenyl)piperidine, 140 °C.

Scheme 4[a]



[a] Conditions: (a) 140 °C.

4-(2-methoxyphenyl)piperidine moiety replaced the *N*-(2-methoxyphenyl)piperazine in **4**. This indicates that the piperazine nitrogen vicinal to the aryl ring is not essential for binding, although it contributes to an increased affinity.

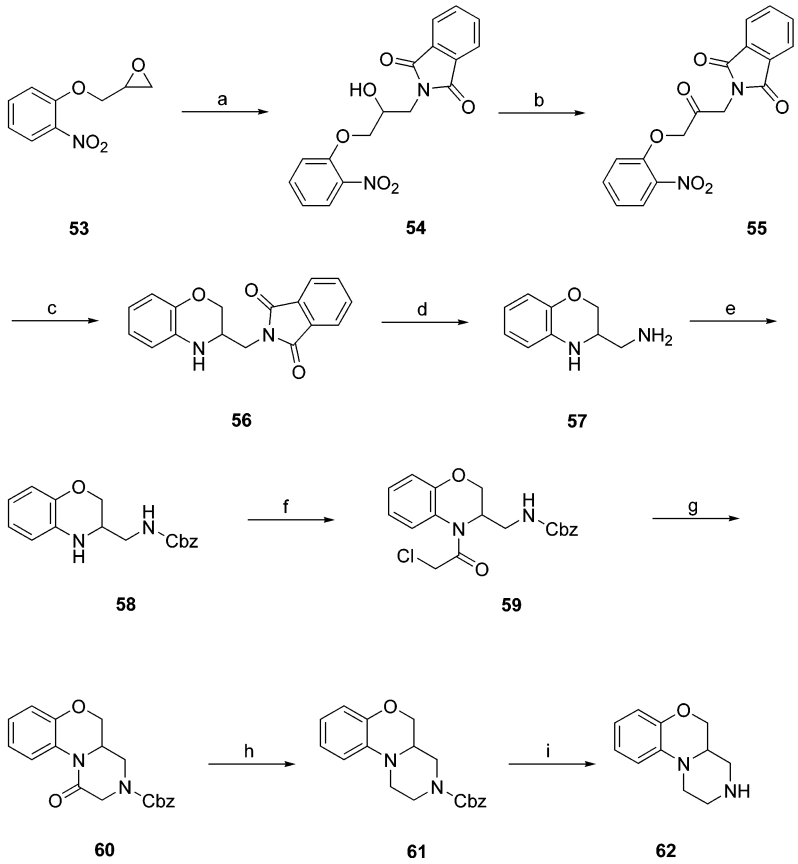
In previously reported series of pyrimido[5,4-*b*]indoles, the shift of the substituent on the phenyl ring of the 4-(2-methoxyphenyl)piperazine moiety from the 2- to the 4-position invariably produced a notable drop in affinity.[21] However, in some derivatives, it gave rise to the appearance of selectivity for α_{1D}-ARs.[22] In this study, we synthesized compounds **39**−**44** to analyze the influ-

Table 1. Binding Properties of 5*H*-Pyrimido[5,4-*b*]indole Derivatives

	pK_i (M) or [% of inhibition at 1 μM][a]		
compd	α_{1A}-AR	α_{1B}-AR	α_{1D}-AR[b]
23[c]	8.85 ± 0.08	7.86 ± 0.11	9.14 ± 0.08 (9.46)
24[d]	8.52 ± 0.03	7.68 ± 0.05	9.39 ± 0.19 (9.38)
25[c]	7.31 ± 0.13	6.61 ± 0.05	8.16 ± 0.10 (8.43)
26[c]	7.78 ± 0.17	6.79 ± 0.04	7.79 ± 0.12 (7.74)
27	8.31 ± 0.14	7.57 ± 0.08	8.59 ± 0.16
28[c]	7.97 ± 0.11	7.56 ± 0.12	7.90 ± 0.11 (7.82)
29[d]	8.01 ± 0.03	7.21 ± 0.07	8.22 ± 0.12 (7.70)
39[d]	4.89 ± 0.18	5.02 ± 0.13	7.45 ± 0.14 (5.36)
40[c]	[8 ± 3]	[2 ± 4]	7.70 ± 0.08 (6.77)
41[c]	[26 ± 4]	[4 ± 3]	7.22 ± 0.014 (7.43)
42[c]	[19 ± 6]	[6 ± 5]	6.61 ± 0.07 (6.96)
43[c]	[7 ± 2]	[18 ± 5]	[3 ± 2] (6.74)
44[c]	[48 ± 5]	[55 ± 8]	8.00 ± 0.03 (7.92)
45[c]	8.14 ± 0.07	7.84 ± 0.03	8.80 ± 0.10 (8.14)
46	8.81 ± 0.15	8.54 ± 0.14	9.03 ± 0.22
48[c]	[18 ± 7]	[4 ± 4]	6.90 ± 0.08 (7.13)
49	<5	<5	<5
50[c]	5.45 ± 0.15	5.39 ± 0.23	5.77 ± 0.11 (6.47)
51	<5	<5	<5
52[c]	6.22 ± 0.03	5.69 ± 0.08	7.01 ± 0.06 (7.33)
63[c]	5.10 ± 0.08	5.11 ± 0.10	6.90 ± 0.10 (7.06)
64[d]	<5	<5	<5 (5.91)
65[c]	8.33 ± 0.10	8.59 ± 0.10	8.44 ± 0.16 (8.82)
66[d]	8.05 ± 0.07	7.93 ± 0.10	9.20 ± 0.27 (8.32)
4[c]	9.57 ± 0.14	8.74 ± 0.14	9.44 ± 0.11 (9.48)

[a] Each value is the mean ± SE for data from three different experiments conducted in duplicate. [b] Estimated and predicted affinity values calculated by Catalyst for the training set and test set, respectively, are reported in parentheses. [c] Compound used to build the training set. [d] Compound used to build the test set.

Scheme 5[a]



[a] Conditions: (a) phthalimide, butanol, pyridine, reflux; (b) Dess−Martin reagent, CH_2Cl_2, 60 °C; (c) H_2, Pd/C, EtOH; (d) hydrazine hydrate, EtOH, reflux; (e) ClCbz, THF, −78 °C; (f) $ClCOCH_2Cl$, CH_2Cl_2, −78 °C; (g) K_2CO_3, DMSO; (h) BH_3−THF, THF, 0 °C; (i) Pd/C, 1,4-cyclohexadiene, CF_3COOH, MeOH/H_2O.

ence of bulky alkyl substituents in the 4-position of the phenyl ring on affinity and selectivity for α_1-AR subtypes. Unfortunately, many of them showed a very low water solubility, precluding their testing at concentrations higher than 1 μM in binding assays. In these cases, the percentage inhibition of specific binding at the highest concentration tested (1 μM) was measured and is reported in Table 1. As expected, compounds **39**−**44** showed very low affinities, particularly for α_{1A}-AR and α_{1B}-AR subtypes. While the 4-cyclohexyl derivative **43** had no measurable affinity for any of the three subtypes, the 4-(1-methylethyl) derivative **39** and the 4-(1,1-dimethylethyl) derivative **40** displayed moderate affinities for α_{1D}-ARs ($pK_i = 7.45$ and 7.70, respectively) with no measurable affinity for α_{1A}-AR or α_{1B}-AR subtypes. In addition, when tested in radioligand assays on serotonergic 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and dopaminergic D_1 and D_2 receptors, they showed no affinity ($pK_i \leq 5$). Thus, **39** and **40** represent two interesting molecules with good selectivity for α_{1D}-ARs.

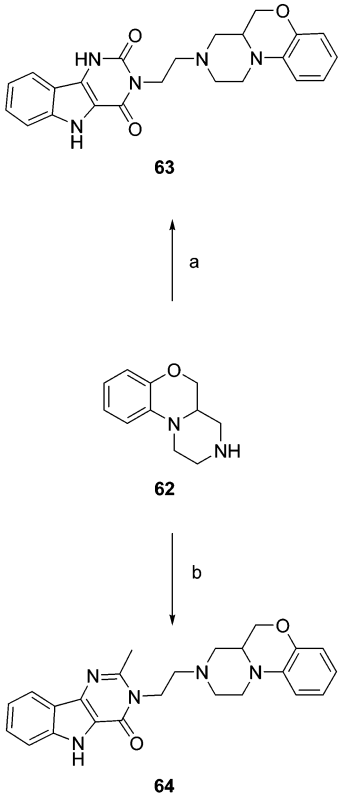
Some other structural variations were made on the pyrimido[5,4-*b*]indole-2,4-dione moiety of RN5 (**4**). We had already reported that the replacement of the indole nucleus of **4** with a benzothieno system leads to α_1-AR ligands retaining high affinity.[21] We applied the same strategy to compound **48**, which can be considered the benzothienyl analogue of **39**. However, this variation was detrimental for selectivity, since **48** showed a lower affinity for α_{1D}-ARs than **39**.

The role of the carbonyl group in the 2-position of the pyrimido[5,4-*b*]indole-2,4-dione system was examined by preparing compounds **23**−**30**, in which C═O group was replaced by a hydrogen, methyl, or ethyl group. The overall effect of this modification was a reduction in affinity, particularly for α_{1A}-AR and α_{1B}-AR subtypes. With respect to α_{1D}-ARs, derivatives with a methyl group in the 2-position were the most interesting. In fact, among compounds **23**−**25**, which present an ethylene chain connecting the tricyclic system with the PP moiety, **24** showed the highest affinity ($pK_i = 9.39$) and, as opposed to **4**, a slight selectivity for the α_{1D}-AR. The elongation of the connecting alkyl chain in compounds **26**−**28** to three methylene units led to a further decrease in affinity. Again, the methyl derivative **27** was the best of the three compounds.

Compound **30** bears a 2-ethoxy group on the phenyl ring of the PP moiety. As already noted for **45**, it showed lower affinity values for all three subtypes compared to its methoxy analogue **24**, indicating that the increased bulk of the ethoxy group is not well tolerated in α_1-AR binding sites.

Further structural modifications led to compounds **49**−**52**, which present a 4-(1-methylethyl) or a 4-(1,1-dimethylethyl) substituent on the phenyl ring in the PP moiety and lack the C═O group (replaced with a hydrogen or methyl group) in the 2-position of the pyrimido[5,4-*b*]indole system. Almost all these compounds showed no significant affinity or selectivity for α_1-AR subtypes, although methyl derivatives **50** and **52** displayed a minimal degree of affinity, as expected, whereas the unsubstituted analogues **49** and **51** were completely inactive.

Scheme 6[a]



[a] Conditions: (a) **32**, 140 °C; (b) **16**, 140 °C.

Within this series, RN5 (**4**) and **24** are the compounds showing the highest affinity for α_{1D}-ARs. Both compounds bear the flexible [4-(2-methoxyphenyl)piperazin-1-yl]ethyl moiety, which allows them to adopt a variety of low-energy conformations. Derivatives **63** and **64** can be regarded as conformationally restricted analogues of **4** and **24**, respectively, bearing a rigid 1,2,3,4,4a,5-hexahydropyrazino[2,1-*c*][1,4]benzoxazine in place of the *N*-(2-methoxyphenyl)piperazine moiety. When tested in the binding assay, both **63** and **64** showed a dramatic loss in affinity for all the α_1-AR subtypes. The loss of affinity was at least 3 and, in some cases, over 4 orders of magnitude (see **4** vs **63** for α_{1A}-ARs). The only exception was **63** and α_{1D}-ARs, where there was a smaller (350-fold) decrease. These results indicate that the rigid 1,2,3,4,4a,5-hexahydropyrazino[2,1-*c*][1,4]-benzoxazine is not recognized by α_1-AR binding sites, and its planar conformation is probably not adopted by the flexible *N*-(2-methoxyphenyl)piperazine moiety of **4** and **24** in binding to the receptor.

RN5 (**4**) and the selective α_{1D}-AR compound **40** were also tested to evaluate their effects on α_1-AR-coupled transduction pathways. Compound **4** was able to completely antagonize norepinephrine-stimulated inositol phospholipid hydrolysis in rat hippocampal slices at concentrations of 1 μM, thus showing full antagonistic properties (Table 2). On the other hand, **40** only partially reduced (25%) norepinephrine-stimulated inositol phosholipid hydrolysis. One explanation for this partial reduction is that **40** is α_{1D}-AR selective and therefore blocks only some of the hippocampal α_1-ARs. Among the three α_1-AR subtypes, the α_{1D}-AR is the least efficiently coupled to inositol phospholipid hydrolysis.[33] It should also be pointed out that **40** has a lower affinity for α_{1D}-ARs than **4**, and the 1 μM concentration tested may not be sufficient to competitively antagonize the effects of 100 μM norepinephrine.

Chart 2

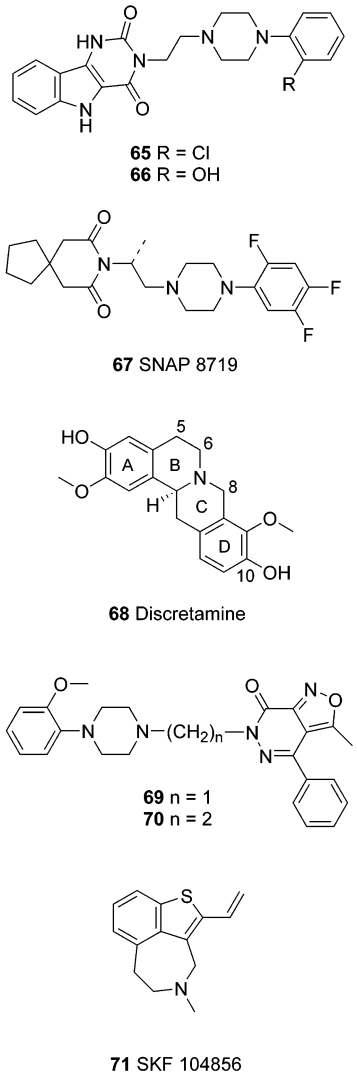


Table 2. Stimulation of [^{3}H]Inositol Phosphate Formation by Norepinephrine (100 μM) in the Presence of **4** or **40** (1 μM) in Rat Hippocampal Slices

	[^{3}H]inositol monophosphate,[a] dpm/mg of protein	
compd	control	100 μM norepinephrine
none	3656 ± 281	12484 ± 375
4	3471 ± 167	3987 ± 96[b]
40	3968 ± 62	10390 ± 343[b]

[a] Values are the mean ± SEM of at least four determinations. [b] $P < 0.01$ when compared with value obtained with norepinephrine in the absence of test compounds.

There have been many recent efforts to rationalize the antagonist selectivity of α_1- and α_2-ARs at the molecular level. In this context, some of us have developed a pharmacophore hypothesis for α_1-AR antagonists[34] (hereafter referred to as the "old model") by means of the software Catalyst.[35] The old model consists of five features (three hydrophobic, a hydrogen bond acceptor, and a positive ionizable group; Figure 1A) and is in good agreement with a previous model for α_1-AR antagonists reported by De Marinis[36] and other groups.[37]

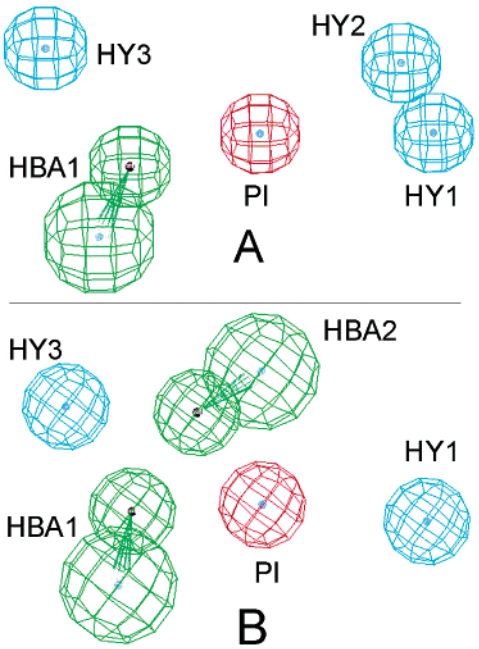


Figure 1. Comparison between the five-feature pharmacophore model for α_1-AR antagonists (A, referred to as the "old model" in the text) and the HipHop-generated pharmacophore model for α_{1D}-AR antagonists (B) shows four common features (i.e. HY1, PI, HBA1, and HY3). Pharmacophore features are color-coded as follows: blue for hydrophobics (HY); red for positive ionizable groups (PI); green for hydrogen bond acceptors (HBA).

However, it should be emphasized that ligands that selectively recognize only one of several closely related subtypes could represent very useful pharmacologic tools. Consequently, the search for subtype selective α_1-AR ligands has dramatically increased. Similarly, both ligand- and receptor-based approaches have been used to decipher the molecular features responsible for affinity and selectivity among α_1-AR subtypes.

The biological data obtained with the title compounds indicate that with the exception of **4**, **28**, and **65**, all compounds showed preferential affinity for the α_{1D} subtype. Assuming that all these compounds interact with the similar binding sites at each α_1-AR subtype, a selection of the new pyrimido[5,4-*b*]indole compounds was submitted to a computational protocol to improve the old pharmacophore model for α_1-AR antagonists and to identify a new model specifically for α_{1D}-AR antagonists.

The first pharmacophore reported for α_{1D}-ARs was generated by Bremner and co-workers[38] by application of the Catalyst HypoGen routine to three compounds with high affinity for α_{1D}-ARs and an α_{1A}/α_{1D} selectivity of at least 10-fold. This model, consisting of a hydrogen bond acceptor, a positive ionizable group, and a hydrophobic moiety, unfortunately showed no correlation ($r = -0.14$) between biological data and structural properties of the studied compounds.[38] Alternatively, we used the Catalyst HipHop routine (also referred to as common feature hypothesis generation) to analyze these three compounds: BMY 7378 (**3,** Chart 1), SNAP 8719 (**67**), and discretamine (**68**) (Chart 2). Because of the limited number of compounds and their small affinity range (Table 3), the HipHop method was preferred over HypoGen. HipHop generates models by identification

Table 3. Actual and Predicted Binding Affinities for Compounds Collected from the Literature Used To Validate the Proposed Pharmacophoric Model for α_{1D}-AR Antagonists

	K_i (nM)				
compd	α_{1A}	α_{1B}	α_{1D}[a]	α_{1A}/α_{1D}	ref
BMY 7378, **3**	251	631	6.3 (1.9)	39.8	44
SNAP 8719, **67**	294	191	1.6 (6.2)	183.7	38
discretamine, **68**	616	360	25 (14000)[c]	24.6	38
69			315.8 (1200)		37
70	1.4	31.1	1.5 (1.6)	0.9	37
SKF 104856, **71**	36	23	1.6 (86000)[b]	22.5	45

[a] Predicted affinity values calculated by Catalyst for the test set compounds are reported in parentheses. [b] This compound was able to map only three pharmacophoric features. [c] This compound was able to map only five pharmacophoric features.

of the common chemical features shared by the molecules and their relative alignment to the common feature set without considering biological data. On the other hand, the HypoGen method is usually applied to develop three-dimensional pharmacophore models from molecules with a wide range of diversity in both structure and activity, with the latter spanning at least 4 orders of magnitude. Thus, during common feature hypothesis generation, affinity data were not taken into consideration and **67**, the most selective α_{1D}-AR compound so far discovered,[39] was considered as the reference structure (see Experimental Section).

The results showed that all hypotheses generated by the HipHop program consisted of five features: four of them (one hydrophobic (HY), a positive ionizable (PI) group, and two hydrogen bond acceptors (HBA)) were always found, while the sole difference between hypotheses consisted in an aromatic ring versus an additional hydrophobic group. This last pharmacophore hypothesis (two HYs, two HBAs, and one PI, hereafter referred to as the HipHop model; Figure 1B) was evaluated further. In fact, a comparison between the HipHop model and our old model of α_1-AR (Figure 1A) shows that four features of each model (HY1, PI, HBA1, and HY3) are located at almost identical spatial positions. Only HY2 of the old model and HBA2 of the new model lie in unoccupied regions of space. Thus, the process of identifying common chemical features shared by selective α_{1D}-AR antagonists suggests that an additional feature (HBA2 of the HipHop model) should be added to account for the affinity and selectivity of α_{1D}-AR-selective antagonists and to rationalize the relationships between structure and biological data of such compounds.

To test this hypothesis, we generated another model based on a selected set of pyrimido[5,4-*b*]indole derivatives and their α_{1D}-AR affinities. Sixteen molecules of the new compounds (Table 1) were used to build a training set following the Catalyst guidelines. pK_i (M) values of these compounds ranged from 9.44 (**4**) to 5.77 (**50**), spanning about 3.5 orders of magnitude. Compound **43**, which was inactive, was also included with a pK_i value arbitrarily set at 5.70. Conformational models within a range of 20 kcal/mol with respect to the global minimum were generated for all compounds by means of a molecular mechanics approach based on the use of the CHARMm force field[40] and the poling algorithm.[41] The training set comprising biological data and conformational models was submitted to the HypoGen routine,

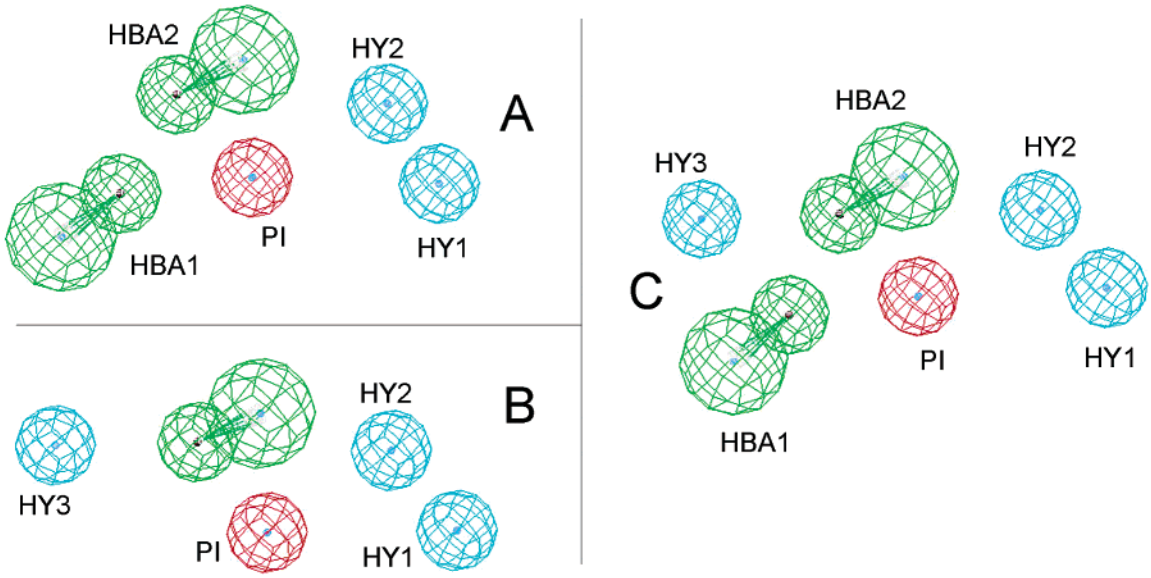


Figure 2. Pharmacophore hypotheses 1−3 (A) and 4 (B) generated by means of HypoGen from a training set of 16 α_{1D}-AR antagonists. (C) The final pharmacophore model for α_{1D}-AR antagonists obtained by merging hypotheses 1 and 4 into a new six-feature pharmacophore hypothesis. Features are color-coded as follows: blue for hydrophobics (HY); red for positive ionizable groups (PI); green for hydrogen bond acceptors (HBA).

forcing the program to find pharmacophore hypotheses characterized by at least five features.

On the basis of the summary of the computational run and a hierarchical cluster analysis of the hypotheses generated, a selection was made among the 10 pharmacophores generated. In particular, hypotheses 1−3 showed high similarity in 3D spatial shape and were therefore considered to be equivalent (Figure 2A). In contrast, hypothesis 4, belonging to a different cluster, showed a diverse composition in terms of chemical features compared to hypotheses 1−3 (Figure 2B). Moreover, it was shown for many compounds of the training set that the same conformer of each compound mapped both hypotheses 1 and 4 as the best fit. Superposition of these hypotheses led to identification of four pairs of chemical features located at the same spatial positions. Both hypotheses showed two hydrophobics (HY1 and HY2) accommodating the ortho-substituted phenyl ring bound to the piperazine and a positive ionizable feature (PI) able to fit the N1 nitrogen atom of the same heteroring. Also, in the two hypotheses, the hydrogen bond acceptor group HBA2 is located almost exactly at the same coordinates. The fixed and null costs of this run were found to be 68.6 and 123.3, respectively. For the first four five-feature hypothesis models, the total costs were close to the fixed cost and ranged from 70.9 (hypothesis 1) to 76.0 (hypothesis 4).

Finally, the two complementary five-feature hypotheses were merged into a new six-feature model (referred to as the final pharmacophore, Figure 2C). This model is depicted in Figure 3 (with compound **4** superposed as a representative example), while its geometric parameters (namely, distances and angles between pharmacophoric features) are reported in Table 4. Although the final model has an additional feature (HBA2) compared to the old model, in agreement with the HipHop calculations, its features are all matched by the chemical groups of compound **4**. Thus, the *o*-methoxyphenyl moiety maps both HY1 and HY2; the N1 nitrogen atom of the piperazine ring corresponds to the PI group; the two carbonyl groups are the HBAs; and the condensed phenyl ring of the terminal heterocyclic portion of the molecule maps HY3. This model also accurately estimates the pK_i value for **4** (9.48 versus an experimental value of 9.44) and was submitted to the Regress Hypothesis routine of Catalyst to allow it

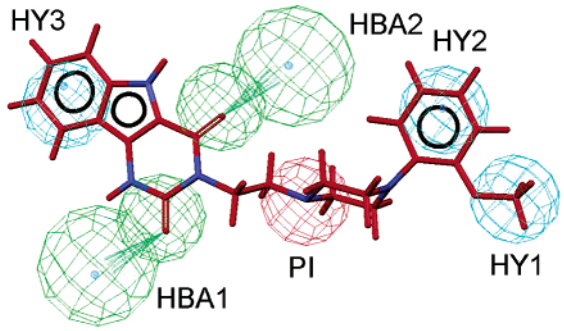


Figure 3. Final pharmacophore model for α_{1D}-AR antagonists with compound **4** bound. Pharmacophore features are color-coded as follows: blue for hydrophobics (HY); red for positive ionizable groups (PI); green for hydrogen bond acceptors (HBA).

Table 4. Geometric Parameters (Distances and Angles between Features) of the Final Pharmacophoric Model for α_{1D}-AR Antagonists

feature	distance, Å	feature	angle, deg
HY1−HY2	4.1	HY1−HY2−PI	94.6
HY1−PI	7.5	HY2−PI−HBA2	97.8
HY1−HBA2	11.0	PI−HBA2−HBA1	69.8
HY1−HBA1	12.6	HBA2−HBA1−HY3	62.3
HY1−HY3	16.5	HY2−PI−HY3	124.4
HY2−PI	5.9	HY2−HBA2−HBA1	117.4
HY2−HBA2	8.0		
HY2−HBA1	10.8		
HY2−HY3	13.7		
PI−HBA2	4.6		
PI−HBA1	5.2		
PI−HY3	9.4		
HBA2−HBA1	4.5		
HBA2−HY3	5.7		
HBA1−HY3	5.5		

to be used to estimate affinities of the training set, as well as to predict affinities of compounds external to the training set. In fact, a good correlation between estimated and measured affinity values of the entire training set was obtained, with a correlation coefficient of 0.91.

In addition, the new model accounts for major structure−activity relationships of the compounds in several ways. The first is that conformationally constrained compounds, where the (2-methoxyphenyl)piperazine moiety was transformed into a tricyclic system, were predicted to have low affinity due to the conformational rearrangement of the phenyl ring relative to the piperazine. In fact, our previous work in this field,[42] in agreement with literature reports, demonstrated that twisted or orthogonal conformations of the piperazine relative to the phenyl ring of the arylpiperazinyl moiety of the ligands are important for α_1-AR antagonism. As a consequence, affinities of compounds **63** and **64** were estimated and predicted to be 7.06 and 5.91, respectively, mainly due to their lack of ability to map one of the HY1 and HY2 features.

Second, transformation of the methoxy substituent of **4** into larger alkoxy groups, as well as hydroxy or chloro, led to a decreased affinity accounted by the model as a partial fit into the HY1−HY2 system. In fact, lengthening the 2-methoxy substituent of the arylpiperazinyl moiety to an ethoxy group led to decreased affinity for compounds **45** and **30** (calculated to be 8.14 and 7.70, respectively) on the basis of a partial match between the ethyl group and the HY1 feature of the model, compared to a perfect fit of the methoxy substituent. Similarly, compound **65** with a chlorine atom at the ortho position instead of the methoxy group of **4** was predicted to have an affinity of 8.82 and an experimental value of 8.44, mainly due to the inability of the *o*-chlorophenyl moiety to fit well both HY1 and HY2. Finally, a slightly decreased affinity of **66** relative to **4** was found experimentally (9.20 versus 9.44, respectively). Although the hydrophilic hydroxy group of **66** lies within the sphere representing HY1, this interaction is not considered profitable by the program because of the opposing hydrophobic/hydrophilic character of the feature and the substituent. Therefore, HY1 is viewed as a missing feature, leading to a predicted affinity of 8.32 for **66** versus the observed value of 9.20. These findings confirm that the methoxy substituent at the ortho position of the phenylpiperazine moiety optimizes interaction with α_{1D}-ARs, as demonstrated generally for all α_1-ARs.

Third, transformation of a pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione to a pyrimido[5,4-*b*]indole-(3*H*)4-one system did not affect receptor affinity, as evidenced for compounds **23** and **24** with respect to **4**. Such compounds showed an orientation in the model very similar to that of **4**, with a good fit into all the pharmacophore features. In fact, the carbonyl moiety and the unsubstituted heterocyclic nitrogen atom of **23** and **24** correspond to the HBA1 and HBA2 features of the model, respectively. Calculated affinities for **23** and **24** were 9.46 and 9.38 versus experimental values of 9.14 and 9.39, respectively. Compound **25** showed similar interactions with the pharmacophore and an estimated affinity of 8.43 versus an experimental affinity of 8.16. This decreased affinity is a consequence of partially obscuring the N4 atom (corresponding to HBA1) by the ethyl substituent on the heteroring, with the program predicting that the nitrogen atom is not sufficiently uncrowded to have a perfect fit with HBA1.

Fourth, the length of the polymethylene chain linking the arylpiperazine moiety and the terminal heterocyclic fragment influenced receptor affinity, with an ethyl spacer being the optimal length to bring the chemical features of compounds to the appropriate distance for interaction with the pharmacophore elements. Compounds with a propyl spacer (**26**−**28**) showed lower fit values for the model, accounting for their decreased affinity relative to their ethyl counterparts (Table 1).

However, the affinity of compounds bearing an *N*-(4-substituted phenyl)piperazine moiety was difficult to rationalize on the basis of our pharmacophore model, which allowed for a classification of such compounds in two subclasses based on the structural properties of the para substituent. As examples, compounds **41** and **42** with alkyl chains of four carbon atoms, similar to **4**, could match HY2 with their phenyl ring, while a C-shaped conformation of the alkyl chain allowed the terminal methyl moiety to correspond to HY1. Such an orientation was possible on the basis of the conformational freedom of the piperazinylalkyl moiety, which underwent a conformational rearrangement, while the most basic nitrogen atom of the piperazine ring remained fixed in three-dimensional space. Affinity values of these compounds were estimated to be 7.43 and 6.96, in good agreement with the experimentally observed values of 7.20 and 6.61, respectively. On the other hand, compounds bearing bulky para substituents, such as **40** (*p*-*tert*-butyl-substituted), or branched and relatively short alkyl chains, such as **39** (*p*-isopropyl-substituted), were underestimated by the pharmacophore model. Calculated affinities for **39** and **40** were 5.36 and 6.77, while experimental values of 7.45 and 7.70 were observed, respectively. Such a discrepancy may be due to a partial fit of the *p*-substituted phenyl ring into the HY1/HY2 system, suggesting that while the current pharmacophore model accounts for a large majority of the SARs of our new compounds, it is yet unable to rationalize variation in affinity due to different substituents at the para position.

To further assess the validity and predictive power of the pharmacophore hypothesis, we used molecules outside the training set along with their reported affinities for α_{1D}-ARs. In addition to several pyrimido-[5,4-*b*]indoles described above, compounds of this "test set" were taken from the literature. Their predicted affinities, calculated by the program Catalyst on the basis of the final six-feature pharmacophore model for α_{1D}-AR antagonists, are reported in Table 3.

In particular, compound **70** (Chart 2) showed molecular fragments fulfilling all features of the pharmacophore model. In fact, the 3-methyl group, the nitrogen atom at the 5-position, and the 7-carbonyl group of the isoxazolo[3,4-*d*]pyridazin-7(6*H*)-one moiety matched HY3, HBA1, and HBA2, respectively. Moreover, the PI group was represented by the N1 nitrogen atom of the piperazine ring, and the 2-methoxyphenyl substituent of the molecule corresponded to the HY1 and HY2 features of the model. In this orientation, the affinity of **70** for

the α_{1D}-AR, expressed as K_i, was predicted to be 1.6 nM and was in good agreement with the experimental value of 1.5 nM.[37] The shortening of the ethyl chain to a methylene spacer led to a partial superposition of **69** into the pharmacophoric model, with the methyl group of the isoxazole ring being unable to reach HY3. Thus, the predicted affinity of **69** was 1200 nM versus a measured value of 316 nM.[37]

Finally, the model was able to predict K_i values of BMY 7378 (**3**) and SNAP 8719 (**67**) (1.9 and 6.2 nM) in good agreement with their experimentally observed values of 6.3 and 1.6 nM, respectively.

In contrast, both SKF 104856 (**71**) and discretamine (**68**) showed a much lower predicted affinity than that reported in the literature. This may be due to the reduced overall size of these compounds compared to the others studied, resulting in their inability to reach all the predicted pharmacophore features. For example, discretamine mapped only five features in its best orientation into the model. While ring A and its methoxy substituent corresponded to HY1 and HY2, the two oxygen atoms at positions 9 and 10 (ring D) were HBA1 and HBA2 of the model, respectively. However, while the PI feature was filled by the nitrogen atom, HY3 was completely absent, causing a predicted affinity (14 000 nM) much lower than the experimental value (25 nM). Such an orientation was in partial agreement with a theoretical model of α_{1D}-ARs recently reported by Carotti and co-workers[43] describing a hydrophobic interaction between aromatic ring A of discretamine and Phe^{358}, a weak hydrogen bond (or a polar interaction) between the hydroxy group at C10 and Cys^{240}, and finally, a salt bridge involving the nitrogen atom of discretamine and Asp^{170}.

In a similar manner, SKF 104856 (**71**) mapped HY1, HY2, and PI of the model with its unsaturated side chain, thiophene ring, and basic nitrogen atom, respectively. Both the hydrogen bond acceptors (HBA1 and HBA2) and HY3 features of the model were omitted, however, leading to a predicted affinity of 86 000 nM versus an experimental value of 1.6 nM.

In summary, computational results from HipHop and HypoGen calculations on compounds with widely varying affinities and selectivities toward α_{1D}-ARs led to the development of a pharmacophore model characterized by a three-feature system accommodating the substituted phenylpiperazine moiety, as well as an additional three-feature system interacting with the terminal heterocyclic moiety of these compounds. Moreover, the polymethylene chain appeared to serve only as a spacer, bringing the two molecular domains in the correct spatial orientation to profitably interact with these receptors.

Conclusions

A new series of pyrimido[5,4-*b*]indole derivatives was prepared and tested in binding assays on the three human cloned α_1-AR subtypes. Most of the new compounds showed a preferential affinity for the α_{1D}-ARs and some of them, such as **39** and **40**, displayed a good α_{1D}-AR selectivity with respect to the other two α_1-AR subtypes, as well as some other serotonergic and dopaminergic receptors.

A structure−affinity relationship analysis based on fitting a preliminary pharmacophore model for α_{1D}-AR antagonists identified structural features important for affinity and selectivity of the new compounds. In particular, (i) transformation of the (2-methoxyphenyl)-piperazine moiety into a tricyclic system led to a decrease in affinity predicted by the model to be due to a conformational rearrangement of the phenyl ring relative to the piperazine nucleus. (ii) Two hydrogen bond acceptor groups are required for α_{1D}-AR binding properties. Such substituents, represented by the carbonyl moieties of the pyrimidindione ring or by both the carbonyl and the unsubstituted nitrogen atom of the pyrimidinone ring, matched HBA1 and HBA2 of the pharmacophore model. (iii) The distance between the phenylpiperazine and the terminal heterocyclic fragment is crucial for α_{1D}-AR affinity. An ethyl spacer is the optimal chain to bring domains within the appropriate distance to interact with the features of the pharmacophore model. (iv) Although the pharmacophore model is characterized by a good correlation coefficient ($r = 0.91$) and is able to rationalize the major SARs of the new pyrimido[5,4-*b*]indole compounds, it was unable to accurately account for structure−affinity relationships based on different para substituents on the phenyl ring bound to the piperazine. Consequently, the pharmacophore hypothesis for α_{1D}-AR antagonists reported here should be considered as a preliminary model. We are currently carrying out additional studies to further refine this model and to better define the influence of the substituents and substitution pattern on the phenyl ring linked to the piperazine nucleus.

Moreover, it can be anticipated that the improvement of this model is ongoing by incorporation of other α_{1D}-AR antagonists into the training set to develop a common pharmacophoric model for all the structural classes of compounds that proved to be α_{1D}-AR antagonists.

Finally, additional efforts have been planned to highlight which pharmacophoric features are peculiar for the α_{1D}-AR recognition with respect to the other α_1-AR subtypes.

Experimental Section

Chemistry. Melting points were determined in a Gallenkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer in KBr disks. Elemental analyses for C, H, N, and S were within ±0.4% of theoretical values and were performed on a Carlo Erba elemental analyzer model 1108 apparatus. ^{1}H NMR spectra were recorded on a Varian Inova Unity 200 spectrometer (200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR) in DMSO-d_6 solution. Chemical shifts are given in δ values (ppm), using tetramethylsilane as the internal standard; coupling constants (J) are given in hertz. Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), sp (septet), m (multiplet), br (broad signal). All the synthesized compounds were tested for purity on TLC (aluminum sheet coated with silica gel 60 F_{254}, Merck) and visualized by UV (λ = 254 and 366 nm). All chemicals and solvents were reagent grade and were purchased from commercial vendors.

Ethyl 3-[(Ethoxymethyliden)amino]-1*H*-indole-2-carboxylate (6). 2-Ethoxycarbonyl-3-aminoindole **5** (2.0 g, 9.79 mmol) was dissolved in 10 mL of triethyl orthoformate, and the reaction mixture was heated under reflux for 2 days. After the mixture was cooled, the precipitate was filtered, washed with cyclohexane, and dried. Recrystallization from cyclohexane gave **6** (1.2 g, 47%) as a white powder: mp 146−148 °C; IR (KBr) cm^{-1} 3324 (NH), 1677 (C=O); ^{1}H NMR (DMSO-d_6) δ

1.30 (t, $J = 7.0$ Hz, 3 H, CH_3), 1.37 (t, $J = 7.2$ Hz, 3 H, CH_3), 4.26 (q, $J = 7.0$ Hz, 2 H, CH_2), 4.37 (q, $J = 7.2$ Hz, 2 H, CH_2), 6.98−7.07 (m, 1 H, indole), 7.22−7.31 (m, 1 H, indole), 7.34−7.41 (m, 1 H, indole), 7.48−7.54 (m, 1 H, indole), 8.03 (s, 1 H, N=CH), 11.32 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{14}H_{16}N_2O_3$) C, H, N.

Ethyl 3-[(Ethoxyethyliden)amino]-1*H*-indole-2-carboxylate (7). The same procedure, as described for the synthesis of **6**, was followed using triethyl orthoacetate. Recrystallization from cyclohexane afforded **7** (77%) as a white powder: mp 138−139 °C; IR (KBr) cm^{-1} 3318 (NH), 1666 (C=O); ^1H NMR (DMSO-d_6) δ 1.28 (t, $J = 7.2$ Hz, 3 H, CH_2CH_3), 1.34 (t, $J = 7.2$ Hz, 3 H, CH_2CH_3), 1.74 (s, 3 H, CCH_3), 4.24 (q, $J = 7.2$ Hz, 2 H, CH_2CH_3), 4.30 (q, $J = 7.2$ Hz, 2 H, CH_2CH_3), 6.93−7.04 (m, 1 H, indole), 7.20−7.40 (m, 3 H, indole), 11.25 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{15}H_{18}N_2O_3$) C, H, N.

Ethyl 3-[(Ethoxypropyliden)amino]-1*H*-indole-2-carboxylate (8). The same procedure, as described for the synthesis of **6**, was followed using triethyl orthopropionate. Recrystallization from cyclohexane afforded **8** (94%) as a white powder: mp 162−164 °C; IR (KBr) cm^{-1} 3310 (NH), 1666 (C=O); ^1H NMR (DMSO-d_6) δ 0.92 (t, $J = 7.6$ Hz, 3 H, CCH_2CH_3), 1.27 (t, $J = 7.0$ Hz, 3 H, OCH_2CH_3), 1.34 (t, $J = 7.0$ Hz, 3 H, OCH_2CH_3), 2.06 (q, $J = 7.6$ Hz, 2 H, CCH_2CH_3), 4.23 (q, $J = 7.0$ Hz, 2 H, OCH_2CH_3), 4.31 (q, $J = 7.0$ Hz, 2 H, OCH_2CH_3), 6.95−7.03 (m, 1 H, indole), 7.20−7.39 (m, 3 H, indole), 11.24 (br s, 1H, NH which exchanges with D_2O). Anal. ($C_{16}H_{20}N_2O_3$) C, H, N.

3-(2-Hydroxyethyl)-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (9). Compound **6** (1.2 g, 4.6 mmol) was dissolved in 5 mL of 2-ethanolamine, and the reaction mixture was heated under reflux for 7 h. After cooling, the reaction mixture was poured into water (50 mL). The solid was filtered off, washed with water, and dried. Recrystallization from EtOH gave **9** (0.8 g, 80%): mp 296−298 °C; IR (KBr) cm^{-1} 3437 (OH), 3315 (NH), 1669 (C=O); ^1H NMR (DMSO-d_6) δ 3.70 (t, $J = 5.4$ Hz, 2 H, CH_2OH), 4.16 (t, $J = 5.4$ Hz, 2 H, NCH_2), 4.95 (br s, 1 H, OH which exchanges with D_2O), 7.18−7.28 (m, 1 H, indole), 7.41−7.57 (m, 2 H, indole), 7.97−8.04 (m, 1 H, indole), 8.21 (s, 1 H, N=CH), 12.11 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{12}H_{11}N_3O_2$) C, H, N.

3-(2-Hydroxyethyl)-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (10). The same procedure, as described for the synthesis of **9**, was followed starting from **7**. Recrystallization from EtOH afforded **10** (72%): mp 251−252 °C; IR (KBr) cm^{-1} 3158 (broad, OH + NH), 1671 (C=O); ^1H NMR (DMSO-d_6) δ 2.72 (s, 3 H, CH_3), 3.65−3.77 (m, 2 H, CH_2OH), 4.22 (t, $J = 5.6$ Hz, 2 H, NCH_2), 5.02 (t, $J = 5.8$ Hz, 1 H, OH which exchanges with D_2O), 7.14−7.24 (m, 1 H, indole), 7.37−7.54 (m, 2 H, indole), 7.94−8.00 (m, 1 H, indole), 11.89 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{13}H_{13}N_3O_2$) C, H, N.

3-(2-Hydroxyethyl)-2-ethyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (11). The same procedure, as described for the synthesis of **9**, was followed starting from **8**. Recrystallization from toluene afforded **11** (77%): mp 208−209 °C; IR (KBr) cm^{-1} 3152 (broad, OH + NH), 1660 (C=O); ^1H NMR (DMSO-d_6) δ 1.33 (t, $J = 7.4$ Hz, 3 H, CH_3), 3.05 (q, $J = 7.4$ Hz, 2 H, CH_2CH_3), 3.66−3.73 (m, 2 H, CH_2OH), 4.23 (t, $J = 6.2$ Hz, 2 H, NCH_2), 5.02 (t, $J = 5.6$ Hz, 1 H, OH which exchanges with D_2O), 7.15−7.25 (m, 1 H, indole), 7.38−7.54 (m, 2 H, indole), 7.95−8.02 (m, 1 H, indole), 11.93 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

3-(3-Hydroxypropyl)-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (12). The same procedure, as described for the synthesis of **9**, was followed starting from **6** and 3-amino-1-propanol. Recrystallization from EtOH afforded **12** (50%): mp 233−234 °C; IR (KBr) cm^{-1} 3444 (broad, OH), 1660 (C=O); ^1H NMR (DMSO-d_6) δ 1.78−2.02 (m, 2 H, $CH_2CH_2CH_2$), 3.47 (t, $J = 6.2$ Hz, 2 H, CH_2OH), 4.17 (t, $J = 7.2$ Hz, 2 H, NCH_2), 4.68 (br s, 1 H, OH which exchanges with D_2O), 7.18−7.28 (m, 1 H, indole), 7.42−7.59 (m, 2 H, indole), 7.98−8.04 (m, 1 H, indole), 8.30 (s, 1 H, N=CH), 11.85 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{13}H_{13}N_3O_2$) C, H, N.

3-(3-Hydroxypropyl)-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (13). The same procedure, as described for the synthesis of **9**, was followed starting from **7** and 3-amino-1-propanol. Recrystallization from toluene afforded **13** (80%): mp 220−222 °C; IR (KBr) cm^{-1} 3440 (broad, OH), 3189 (NH), 1677 (C=O); ^1H NMR (DMSO-d_6) δ 1.77−1.93 (m, 2 H, $CH_2CH_2CH_2$), 2.70 (s, 3 H, CH_3), 3.36−3.56 (br m, 2 H, CH_2OH), 4.21 (t, $J = 7.2$ Hz, 2 H, NCH_2), 4.71 (br s, 1 H, OH which exchanges with D_2O), 7.15−7.25 (m, 1 H, indole), 7.38−7.55 (m, 2 H, indole), 7.95−8.01 (m, 1 H, indole), 11.93 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

3-(3-Hydroxypropyl)-2-ethyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (14). The same procedure, as described for the synthesis of **9**, was followed starting from **8** and 3-amino-1-propanol. Recrystallization from toluene afforded **14** (77%): mp 183−184 °C; IR (KBr) cm^{-1} 3151 (broad, OH + NH), 1671 (C=O); ^1H NMR (DMSO-d_6) δ 1.36 (t, $J = 7.6$ Hz, 3 H, CH_3), 1.75−1.93 (m, 2 H, $CH_2CH_2CH_2$), 2.98 (q, $J = 7.6$ Hz, 2 H, CH_2CH_3), 3.47−3.55 (m, 2 H, CH_2OH), 4.22 (t, $J = 7.6$ Hz, 2 H, NCH_2), 4.70 (t, $J = 7.2$ Hz, 1 H, OH which exchanges with D_2O), 7.15−7.25 (m, 1 H, indole), 7.38−7.54 (m, 2 H, indole), 7.95−8.02 (m, 1 H, indole), 11.89 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{15}H_{17}N_3O_2$) C, H, N.

3-(2-Chloroethyl)-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one Hydrochloride (15). Alcohol **9** (0.7 g, 3.00 mmol) was dissolved in 20 mL of toluene, and $SOCl_2$ (0.73 g, 6.1 mmol) was added. The reaction mixture was heated under reflux for 2 h. Then it was concentrated under reduced pressure. Cyclohexane (20 mL) was added to the residue, and the mixture was stirred for 30 min. The solid was filtered off, washed with cyclohexane, and dried to give **15** (0.8 g, 89%). This crude product was used without further purification: mp 240−242 °C; IR (KBr) cm^{-1} 3198 (NH), 1674 (C=O); ^1H NMR (DMSO-d_6) δ 4.02 (t, $J = 5.8$ Hz, 2 H, CH_2Cl), 4.46 (t, $J = 5.8$ Hz, 2 H, NCH_2), 7.20−7.32 (m, 1 H, indole), 7.40−7.58 (m, 2 H, indole), 7.98−8.10 (m, 1 H, indole), 8.33 (s, 1 H, N=CH), 12.21 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{12}H_{11}Cl_2N_3O$) C, H, N.

3-(2-Chloroethyl)-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one Hydrochloride (16). The same procedure, as described for the synthesis of **15**, was followed starting from alcohol **10**. Crude **16** (88%) was used without further purification: mp 224 °C; IR (KBr) cm^{-1} 3226 (NH), 1710 (C=O); ^1H NMR (DMSO-d_6) δ 2.86 (s, 3 H, CH_3), 3.98 (t, $J = 6.6$ Hz, 2 H, CH_2Cl), 4.51 (t, $J = 6.6$ Hz, 2 H, NCH_2), 7.22−7.32 (m, 1 H, indole), 7.45−7.59 (m, 2 H, indole), 8.15−8.21 (m, 1 H, indole), 12.37 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{13}H_{13}Cl_2N_3O \cdot 0.5H_2O$) C, H, N.

3-(2-Chloroethyl)-2-ethyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one Hydrochloride (17). The same procedure, as described for the synthesis of **15**, was followed starting from alcohol **11**. Crude **17** (92%) was used without further purification: mp 243−245 °C; IR (KBr) cm^{-1} 3158 (NH), 1728 (C=O); ^1H NMR (DMSO-d_6) δ 1.37 (t, $J = 7.6$ Hz, 3 H, CH_3), 3.17 (q, $J = 7.6$ Hz, 2 H, CH_2CH_3), 3.98 (t, $J = 6.8$ Hz, 2 H, CH_2Cl), 4.53 (t, $J = 6.8$ Hz, 2 H, NCH_2), 7.22−7.32 (m, 1 H, indole), 7.45−7.60 (m, 2 H, indole), 8.20−8.27 (m, 1 H, indole), 12.34 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{14}H_{15}Cl_2N_3O$) C, H, N.

3-(3-Chloropropyl)-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one Hydrochloride (18). The same procedure, as described for the synthesis of **15**, was followed starting from alcohol **12**. Crude **18** (85%) was used without further purification: mp 219−222 °C; IR (KBr) cm^{-1} 3218 (NH), 1692 (C=O); ^1H NMR (DMSO-d_6) δ 2.18−2.36 (m, 2 H, $CH_2CH_2CH_2$), 3.75 (t, $J = 6.4$ Hz, 2 H, CH_2Cl), 4.28 (t, $J = 7.2$ Hz, 2 H, NCH_2), 7.24−7.34 (m, 1 H, indole), 7.46−7.62 (m, 2 H, indole), 8.10−8.18 (m, 1 H, indole), 8.76 (s, 1 H, N=CH), 12.46 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{13}H_{13}Cl_2N_3O$) C, H, N.

3-(3-Chloropropyl)-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one Hydrochloride (19). The same procedure, as described for the synthesis of **15**, was followed starting from alcohol **13**. Crude **19** (92%) was used without further purification: mp 251−254 °C; IR (KBr) cm^{-1} 3221 (NH), 1692 (C=O);

^{1}H NMR (DMSO-d_6) δ 2.13−2.29 (m, 2 H, $CH_2CH_2CH_2$), 2.96 (s, 3 H, CH_3), 3.83 (t, J = 6.4 Hz, 2 H, CH_2Cl), 4.31 (t, J = 7.2 Hz, 2 H, NCH_2), 7.25−7.36 (m, 1 H, indole), 7.50−7.64 (m, 2 H, indole), 8.38−8.45 (m, 1 H, indole), 12.66 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{14}H_{15}Cl_2N_3O$) C, H, N.

3-(3-Chloropropyl)-2-ethyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one Hydrochloride (20). The same procedure, as described for the synthesis of **15**, was followed starting from alcohol **14**. Crude **20** (92%) was used without further purification: mp 220−225 °C; IR (KBr) cm^{-1} 3212 (NH), 1689 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.40 (t, J = 7.2 Hz, 3 H, CH_3), 2.13−2.28 (m, 2 H, $CH_2CH_2CH_2$), 3.18 (q, J = 7.2 Hz, 2 H, CH_2CH_3), 3.84 (t, J = 6.2 Hz, 2 H, CH_2Cl), 4.31 (t, J = 7.6 Hz, 2 H, NCH_2), 7.24−7.34 (m, 1 H, indole), 7.47−7.62 (m, 2 H, indole), 8.30−8.38 (m, 1 H, indole), 12.50 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{15}H_{17}Cl_2N_3O$) C, H, N.

3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (23). A mixture of compound **15** (0.5 g, 1.76 mmol) and 1-(2-methoxyphenyl)piperazine (**21**) (1.7 g, 8.80 mmol) in a 10 mL flask was heated in an oil bath at 140 °C for 30 min. After the mixture was cooled, the solid mass was suspended in EtOH (10 mL) and then water (5 mL) was added. The solid was filtered off, washed with water, and dried. Recrystallization from EtOH gave **23** (0.5 g, 71%) as a white powder: mp 219−220 °C; IR (KBr) cm^{-1} 3154 (NH), 1665 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.51−2.74 (m, 6 H, NCH_2), 2.58−3.01 (m, 4 H, NCH_2), 3.76 (s, 3 H, OCH_3), 4.25 (t, J = 5.6 Hz, 2 H, $CONCH_2$), 6.84−6.98 (m, 4 H, aromatic), 7.19−7.29 (m, 1 H, indole), 7.41−7.58 (m, 2 H, indole), 8.00−8.04 (m, 1 H, indole), 8.28 (s, 1 H, NCH), 12.13 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{23}H_{25}N_5O_2$) C, H, N.

3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (24). The same procedure, as described for the synthesis of **23**, was followed starting from **16**. Recrystallization from toluene afforded **24** (48%) as a white powder: mp 234−236 °C; IR (KBr) cm^{-1} 3182 (NH), 1669 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.63−2.76 (m, 6 H, NCH_2), 2.74 (s, 3 H, CH_3), 2.95−2.97 (m, 4 H, NCH_2), 3.72 (s, 3 H, OCH_3), 4.29 (t, J = 6.6 Hz, 2 H, $CONCH_2$), 6.85−6.96 (m, 4 H, aromatic), 7.15−7.25 (m, 1 H, indole), 7.39−7.55 (m, 2 H, indole), 7.95−8.00 (m, 1 H, indole), 11.94 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{24}H_{27}N_5O_2$) C, H, N.

3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]-2-ethyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (25). The same procedure, as described for the synthesis of **23**, was followed starting from **17**. Recrystallization from MeOH/water afforded **25** (64%) as a white powder: mp 195−196 °C; IR (KBr) cm^{-1} 3184 (NH), 1664 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.37 (t, J = 7.4 Hz, 3 H, CH_2CH_3), 2.55−2.78 (m, 6 H, NCH_2), 2.95−3.10 (m + q, 4 H + 2 H, $ArNCH_2 + CH_2CH_3$), 3.77 (s, 3 H, OCH_3), 4.30 (t, J = 7.0 Hz, 2 H, $CONCH_2$), 6.87−6.94 (m, 4 H, aromatic), 7.16−7.25 (m, 1 H, indole), 7.44−7.55 (m, 2 H, indole), 7.97−8.04 (m, 1 H, indole), 11.94 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{25}H_{29}N_5O_2$) C, H, N.

3-[3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl]-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (26). The same procedure, as described for the synthesis of **23**, was followed starting from **18**. Recrystallization from EtOH/water afforded **26** (36%) as a white powder: mp 148−150 °C; IR (KBr) cm^{-1} 3180 (broad, NH), 1673 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.81−2.03 (m, 2 H, $CH_2CH_2CH_2$), 2.25−2.60 (m, 6 H, NCH_2), 2.72−3.03 (m, 4 H, $ArNCH_2$), 3.75 (s, 3 H, OCH_3), 4.15 (t, J = 7.0 Hz, 2 H, $CONCH_2$), 6.72−6.97 (m, 4 H, aromatic), 7.18−7.28 (m, 1 H, indole), 7.39−7.56 (m, 2 H, indole), 7.97−8.04 (m, 1 H, indole), 8.32 (s, 1 H, N=CH), 12.10 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{24}H_{27}N_5O_2$) C, H, N.

3-[3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl]-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (27). The same procedure, as described for the synthesis of **23**, was followed starting from **19**. Recrystallization from MeOH/water afforded **27** (40%) as a white powder: mp 195−197 °C; IR (KBr) cm^{-1} 3183 (NH), 1654 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.78−2.01 (m, 2 H, $CH_2CH_2CH_2$), 2.35−2.63 (m, 6 H, NCH_2), 2.71 (s, 3 H, CCH_3), 2.79−3.04 (m, 4 H, $ArNCH_2$), 3.76 (s, 3 H, OCH_3), 4.20 (t, J = 7.0 Hz, 2 H, $CONCH_2$), 6.81−6.94 (m, 4 H, aromatic), 7.15−7.24 (m, 1 H, indole), 7.39−7.51 (m, 2 H, indole), 7.93−8.00 (m, 1 H, indole), 11.91 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{25}H_{29}N_5O_2$) C, H, N.

3-[3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl]-2-ethyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (28). The same procedure, as described for the synthesis of **23**, was followed starting from **20**. Recrystallization from MeOH/water afforded **28** (65%) as a white powder: mp 164−165 °C; IR (KBr) cm^{-1} 3149 (NH), 1664 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.37 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.81−2.02 (m, 2 H, $CH_2CH_2CH_2$), 2.36−2.65 (m, 6 H, NCH_2), 2.76−3.14 (m + q, 4 H + 2 H, $ArNCH_2 + CH_2CH_3$), 3.77 (s, 3 H, OCH_3), 4.22 (t, J = 7.0 Hz, 2 H, $CONCH_2$), 6.81−6.93 (m, 4 H, aromatic), 7.16−7.25 (m, 1 H, indole), 7.40−7.55 (m, 2 H, indole), 7.97−8.04 (m, 1 H, indole), 11.93 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{26}H_{31}N_5O_2$) C, H, N.

3-[2-[4-(2-Ethoxyphenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (29). The same procedure, as described for the synthesis of **23**, was followed starting from **15** and 1-(2-ethoxyphenyl)piperazine **22**. Recrystallization from EtOH/water afforded **29** (40%) as a white powder: mp 174−175 °C; IR (KBr) cm^{-1} 3150 (NH), 1662 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.33 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 2.50−2.77 (m, 6 H, NCH_2), 2.82−3.12 (m, 4 H, $ArNCH_2$), 4.00 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 4.22 (t, J = 6.0 Hz, 2 H, $CONCH_2$), 6.75−6.97 (m, 4 H, aromatic), 7.18−7.28 (m, 1 H, indole), 7.40−7.58 (m, 2 H, indole), 7.97−8.05 (m, 1 H, indole), 8.28 (s, 1 H, N=CH), 12.12 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{24}H_{27}N_5O_2$) C, H, N.

3-[2-[4-(2-Ethoxyphenyl)piperazin-1-yl]ethyl]-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (30). The same procedure, as described for the synthesis of **23**, was followed starting from **16** and **22**. Recrystallization from dimethylformamide afforded **30** (60%) as a white powder: mp 246−248 °C; IR (KBr) cm^{-1} 3155 (NH), 1668 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.34 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 2.50−2.82 (m + s, 6 H + 3 H, $NCH_2 + N=CCH_3$), 2.86−3.15 (m, 4 H, $ArNCH_2$), 4.01 (q, J = 7.0 Hz, 2 H, OCH_2CH_3), 4.29 (t, J = 6.2 Hz, 2 H, $CONCH_2$), 6.75−7.01 (m, 4 H, aromatic), 7.13−7.27 (m, 1 H, indole), 7.37−7.58 (m, 2 H, indole), 7.92−8.02 (m, 1 H, indole), 11.94 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{25}H_{29}N_5O_2$) C, H, N.

3-[2-[4-(2-Ethoxyphenyl)piperazin-1-yl]ethyl]-2-ethyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (31). The same procedure, as described for the synthesis of **23**, was followed starting from **17** and **22**. Recrystallization from dimethylformamide/water afforded **31** (60%) as a white powder: mp 218−220 °C; IR (KBr) cm^{-1} 3187 (NH), 1663 (C=O); ^{1}H NMR (DMSO-d_6) δ 1.34 (t, J = 7.2 Hz, 3 H, $N=CCH_2CH_3$), 1.38 (t, J = 6.8 Hz, 3 H, OCH_2CH_3), 2.55−2.82 (m, 6 H, NCH_2), 2.86−3.17 (m + q, 4 H + 2 H, $ArNCH_2 + N=CCH_2CH_3$), 4.00 (q, J = 6.8 Hz, 2 H, OCH_2CH_3), 4.30 (t, J = 6.2 Hz, 2 H, $CONCH_2$), 6.76−7.00 (m, 4 H, aromatic), 7.13−7.25 (m, 1 H, indole), 7.37−7.57 (m, 2 H, indole), 7.92−8.03 (m, 1 H, indole), 11.94 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{26}H_{31}N_5O_2$) C, H, N.

3-[2-[4-[4-(1-Methylethyl)phenyl]piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (39). This procedure is presented as an example for the synthesis of compounds **39**−**44**. 4-(1-Methylethyl)aniline (13.5 g, 100 mmol), bis(2-chloroethyl)amine hydrochloride (17.8 g, 100 mmol), and potassium carbonate (13.8 g, 100 mmol) were added to 2-butoxyethanol (60 mL), and the mixture was vigorously stirred and refluxed for 30 h. After the mixture was cooled, water (100 mL) was added and the aqueous layer was separated from the organic one. Ethyl acetate (100 mL) was added to the latter, and the resulting solution was washed with 4 M aqueous NaOH (100 mL) and then with brine (3 × 70 mL). Successively, the organic layer was dried over sodium sulfate and the solvents were evaporated in vacuo. The resulting crude oil, 4-[4-(1-methylethyl)phenyl]piperazine (**33**) (18.7 g), was successively used for the synthesis of **39** without further purification. However, a sample of **33** was converted to fumarate salt

and characterized. A small portion (1.0 g) of the crude oil was added to a saturated solution of fumaric acid in 2-propanol (20 mL). A white solid precipitated, and after collection by filtration, it was washed with cold 2-propanol (5 mL) and dried. The precipitated salt was recrystallized from EtOH to give **33**·0.5(fumaric acid) as white crystals (0.9 g): mp 203−205 °C dec; IR (KBr) cm^{-1} 3100, 2965, 2860, 2710 (broad bands, NH_2^+), 1640, 1620 (C═O); ^1H NMR (D_2O) δ 1.02 (d, J = 6.9 Hz, 6 H, CH_3), 2.71 (sp, J = 6.9 Hz, 1 H, C*H*CH_3), 3.15−3.31 (m, 8 H, CH_2), 6.33 (s, 1 H, fumarate CH), 6.88−7.20 (m, 4 H, aromatic). Anal. ($C_{13}H_{20}N_2$·0.5$C_4H_4O_4$) C, H, N.

A mixture of *N′*-(2-chloroethyl)-*N′*-[3-(2-ethoxycarbonyl)-indolyl]urea **32** (1.0 g, 3.23 mmol) and 3.3 g of the crude oil **33** previously obtained was heated in an oil bath at 140 °C for 2 h. After being cooled, the reaction mixture was treated with warm EtOH (20 mL). The crude solid was filtered off, washed with EtOH and successively with water, and dried. Recrystallization from dimethylformamide afforded **39** as a white powder (0.8 g, 57%): mp >300 °C; IR (KBr) cm^{-1} 3160 (NH), 1705, 1625 (C═O); ^1H NMR (DMSO-d_6) δ 1.13 (d, J = 6.8 Hz, 6 H, CHC*H*$_3$), 2.50−2.68 (m, 6 H, NCH_2), 2.75 (sp, J = 6.8 Hz, 1 H, C*H*CH_3), 2.93−3.16 (m, 4 H, ArNCH_2), 4.09 (t, J = 6.5 Hz, 2 H, $CONCH_2$), 6.72−6.90 (m, 2 H, aromatic), 6.94−7.18 (m, 1 H + 2 H, indole + aromatic), 7.25−750 (m, 2 H, indole), 7.85−7.95 (m, 1 H, indole), 11.75 (br s, 1 H, NH which exchanges with D_2O), 11.90 (br s, 1 H, NH which exchanges with D_2O); ^{13}C NMR (DMSO-d_6) δ 24.10, 32.46, 37.37, 48.62, 52.89, 55.28, 112.75, 113.46, 114.74, 115.49, 119.48, 120.54, 125.87, 126.58, 126.93, 138.06, 138.77, 149.20, 150.97, 156.46. Anal. ($C_{25}H_{29}N_5O_2$) C, H, N.

3-[2-[4-[4-(1,1-Dimethylethyl)phenyl]piperazin-1-yl]-ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (40). Compound **40** was prepared according to the procedure presented for compound **39**. Starting 4-(1,1-dimethylethyl)-aniline and bis(2-chloroethyl)amine hydrochloride were reacted to obtain intermediate 1-[4-(1,1-dimethylethyl)phenyl]piperazine (**34**) as a crude oil. Reaction between **32** and **34** gave a solid that was recrystallized from dioxane to afford **40** as a pure product (76%): mp >300 °C; IR (KBr) cm^{-1} 3163, 3102 (NH), 1707, 1634 (C═O); ^1H NMR (DMSO-d_6) δ 1.22 (s, 9 H,-CCH_3), 2.48−2.52 (m, 6 H, NCH_2), 3.00−3.10 (m, 4 H, NCH_2), 4.11 (t, J = 6.6 Hz, 2 H, $CONCH_2$), 6.80−6.86 (m, 2 H, aromatic), 7.06−7.23 (m, 2 H + 1 H, aromatic + indole), 7.35−7.46 (m, 2 H, indole), 7.91−7.97 (m, 1 H, indole), 11.76 (br s, 1 H, NH which exchanges with D_2O), 11.95 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{26}H_{31}N_5O_2$) C, H, N.

3-[2-[4-[4-(1-Methylpropyl)phenyl]piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (41). Compound **41** was prepared according to the procedure presented for compound **39**. Starting 4-(1-methylpropyl)aniline and bis-(2-chloroethyl)amine hydrochloride were reacted to obtain intermediate 1-[4-(1-methylpropyl)phenyl]piperazine (**35**) as a crude oil. Reaction between **32** and **35** gave a solid that was recrystallized from dioxane to afford **41** as a pure product (69%): mp >300 °C; IR (KBr) cm^{-1} 3162, 3102 (NH), 1709, 1624 (C═O); ^1H NMR (DMSO-d_6) δ 0.61(t, J = 7.6 Hz, 3 H, CH_2CH_3), 0.99 (d, J = 7.0 Hz, 3 H, CHC*H*$_3$), 1.27−1.44 (m, 2 H, C*H*$_2$CH$_3$), 2.26−2.48 (m, 7 H, NCH_2 + CH), 2.75−3.10 (m, 4 H, NCH_2 piperazine), 3.98 (t, J = 6.8 Hz, 2 H, $CONCH_2$), 6.63−6.75 (m, 2 H, aromatic), 6.80−6.91 (m, 2 H, aromatic), 6.93−7.03 (m, 1 H, indole), 7.18−7.33 (m, 2 H, indole), 7.75−7.85 (m, 1 H, indole), 11.64 (br s, 1 H, NH which exchanges with D_2O), 11.84 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{26}H_{31}N_5O_2$) C, H, N.

3-[2-[4-(4-Butylphenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (42). Compound **42** was prepared according to the procedure presented for compound **39**. Starting 4-butylaniline and bis(2-chloroethyl)amine hydrochloride were reacted to obtain intermediate 1-(4-butylphenyl)-piperazine (**36**) as a crude oil. Reaction between **32** and **36** gave a solid that was recrystallized from dioxane to afford **42** as a pure product (83%): mp >300 °C; IR (KBr) cm $^{-1}$ 3162, 3103 (NH), 1715, 1622 (C═O); ^1H NMR (DMSO-d_6) δ 0.77 (t, J = 6.8 Hz, 3 H, CH_3), 1.12−1.20 (m, 2 H,C*H*$_2$CH$_3$), 1.32−1.42 (m, 2 H, C*H*$_2$CH$_2$CH$_3$), 2.29−2.59 (m, 8 H, C*H*$_2$CH$_2$CH$_2$-CH_3 + NCH_2), 2.90−3.00 (m, 4 H, NCH_2), 4.00 (t, J = 6.8 Hz, 2 H, $CONCH_2$), 6.65−6.74 (m, 2 H, aromatic), 6.87−6.93 (m, 2 H, aromatic), 7.00−7.06 (m, 1 H, indole), 7.20−7.35 (m, 2 H, indole), 7.80−7.87 (m, 1 H, indole), 11.67 (br s, 1 H, NH which exchanges with D_2O), 11.87 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{26}H_{31}N_5O_2$) C, H, N.

3-[2-[4-(4-Cyclohexylphenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (43). Compound **43** was prepared according to the procedure presented for compound **39**. Starting 4-cyclohexylaniline and bis(2-chloroethyl)amine hydrochloride were reacted to obtain intermediate 1-(4-cyclohexylphenyl)piperazine (**37**). Reaction between **32** and **37** gave a solid that was recrystallized from dimethylformamide to afford **43** as a pure product (53%): mp >300 °C; IR (KBr) cm^{-1} 3160, 3100 (NH), 1717, 1624 (C═O); ^1H NMR (DMSO-d_6) δ 1.00−1.51 (m, 5 H, cyclohexane), 1.55−1.89 (m, 5 H, cyclohexane), 2.26−2.45 (m, 1 H, cyclohexane), 2.59−2.61 (m, 6 H, NCH_2), 3.03−3.15 (m, 4 H, NCH_2), 4.14 (t, J = 8.0 Hz, 2 H, $CONCH_2$), 6.79−6.85 (m, 2 H, aromatic), 6.99−7.16 (m, 2 H + 1 H, aromatic + indole), 7.32−7.45 (m, 2 H, indole), 7.90−7.97 (m, 1 H, indole), 11.58 (br s, 1 H, NH which exchanges with D_2O), 11.95 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{28}H_{33}N_5O_2$) C, H, N.

3-[2-[4-[4-(Cyanomethyl)phenyl]piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (44). Compound **44** was prepared according to the procedure presented for compound **39**. Starting 4-aminobenzyl cyanide and bis(2-chloroethyl)amine hydrochloride were reacted to obtain intermediate 1-[4-(cyanomethyl)phenyl]piperazine (**38**) as a crude oil. Reaction between **32** and **38** gave a solid that was recrystallized from dimethylformamide/water to afford **44** as a pure product (58%): mp >300 °C; IR (KBr) cm^{-1} 3160, 3102 (NH), 2250 (CN), 1714, 1622 (C═O); ^1H NMR (DMSO-d_6) δ 2.47−2.53 (m, 6 H, NCH_2), 3.05−3.15 (m, 4 H, NCH_2), 3.87 (s, 2 H, CH_2CN), 4.11 (t, J = 6.6 Hz, 2 H, $CONCH_2$), 6.82−6.88 (m, 2 H, aromatic), 6.89−7.19 (m, 2 H + 1 H, aromatic + indole), 7.31−7.44 (m, 2 H, indole), 7.90−7.97 (m, 1 H, indole), 11.76 (br s, 1 H, NH which exchanges with D_2O), 11.95 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{24}H_{24}N_6O_2$) C, H, N.

3-[2-[4-(2-Ethoxyphenyl)piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (45). A mixture of compound **32** (0.4 g, 1.3 mmol) and 1-(2-ethoxyphenyl)-piperazine (1.3 g, 6.50 mmol) **22** in a 10 mL flask was heated in an oil bath at 140 °C for 30 min. After the mixture was cooled, the solid mass was suspended in EtOH (5 mL). The solid was filtered off, washed with water, and dried. Recrystallization from dimethylformamide/water gave **45** (0.2 g, 36%) as a white powder: mp 288−290 °C; IR (KBr) cm^{-1} 3156 (NH), 1713, 1627 (C═O); ^1H NMR (DMSO-d_6) δ 1.34 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 2.50−2.78 (m, 6 H, NCH_2), 2.81−3.15 (m, 4 H, ArNCH_2), 4.00 (q, J = 7.0 Hz, 2 H, O*C*H$_2$CH$_3$), 4.10 (t, J = 6.8 Hz, 2H, $CONCH_2$), 6.75−6.98 (m, 4 H, aromatic), 7.03−7.18 (m, 1 H, indole), 7.28−7.50 (m, 2 H, indole), 7.90−8.01 (m, 1 H, indole), 11.77 (br s, 1 H, NH which exchanges with D_2O), 11.97 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{24}H_{27}N_5O_3$) C, H, N.

3-[2-[4-(2-Methoxyphenyl)piperidin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (46). A mixture of compound **32** (0.3 g, 0.87 mmol) and 4-(2-methoxyphenyl)-piperidine (0.83 g, 4.35 mmol) in a 10 mL flask was heated in an oil bath at 140 °C for 30 min. After the mixture was cooled, the solid mass was suspended in EtOH (5 mL). The solid was filtered off, washed with water, and dried. Recrystallization from dimethylformamide/water gave **46** (0.21 g, 52%) as a white powder: mp 278−280 °C; IR (KBr) cm^{-1} 3157, 3103 (NH), 1711, 1625 (C═O); ^1H NMR (DMSO-d_6) δ 1.50−1.74 (m, 4 H, ArCHC*H*$_2$), 1.98−2.20 (m, 2 H, NCH_2), 2.57 (t, J = 7.0 Hz, 2 H, $CONCH_2$C*H*$_2$), 2.75−2.96 (m, 1 H, ArCH), 2.98−3.16 (m, 2 H, NCH_2), 3.77 (s, 1 H, OCH_3), 4.09 (t, J = 7.0 Hz, 2H, $CONCH_2$CH$_2$), 6.82−6.98 (m, 2 H, aromatic), 7.06−7.22 (m, 2 H + 1 H, aromatic + indole), 7.33−7.48 (m, 2 H, indole), 7.89−

8.99 (m, 1 H, indole), 11.75 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{24}H_{26}N_4O_3$) C, H, N.

3-[2-[4-[4-(1-Methylethyl)phenyl]pyperazin-1-yl]ethyl]-benzothieno[3,2-d]pyrimido-(1*H*,3*H*)2,4-dione (48). A mixture of *N*′-(2-chloroethyl)-*N*′-[3-(2-ethoxycarbonyl)benzothienyl]-urea **47** (0.5 g, 1.53 mmol) and 1.6 g of the crude oil **33**, prepared in the synthesis of compound **39**, was heated in an oil bath at 140 °C for 2 h. After being cooled, the reaction mixture was treated with warm EtOH (20 mL). The crude solid was filtered off, washed with EtOH and successively with water, and dried. Recrystallization from dimethylformamide/water afforded **48** as a pure product (0.6 g, 88%): mp >300 °C; IR (KBr) cm^{-1} 3196 (NH), 1709, 1637 (C═O); ^1H NMR (DMSO-d_6) δ 1.13 (d, J = 7.2 Hz, 6 H, CH_3), 2.51−2.65 (m, 6 H, NCH_2), 2.76 (sp, J = 7.2 Hz, 1 H, C*H*CH_3), 2.95−3.15 (m, 4 H, NCH_2), 4.08 (t, J = 6.6 Hz, 2 H, $CONCH_2$), 6.78−6.85 (m, 2 H, aromatic), 7.01−7.08 (m, 2 H, aromatic), 7.40−7.67 (m, 2 H, aromatic), 8.06−8.12 (m, 1 H, aromatic), 8.34−8.40 (m, 1 H, aromatic), 12.54 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{25}H_{28}N_4O_2S$) C, H, N, S.

3-[2-[4-[4-(1-Methylethyl)phenyl]piperazin-1-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (49). A mixture of **15** (0.3 g, 1.21 mmol) and 1.5 g of the crude oil **33**, prepared in the synthesis of compound **39**, was heated in an oil bath at 140 °C for 30 min. After being cooled, the reaction mixture was treated with EtOH (10 mL). The crude solid was filtered off, washed with EtOH, and dried. Recrystallization from EtOH afforded **49** as a pure product (0.3 g, 60%): mp 254−257 °C; IR (KBr) cm^{-1} 3180 (NH), 1662 (C═O); ^1H NMR (DMSO-d_6) δ 1.15 (d, J = 7.0 Hz, 6 H, $CHCH_3$), 2.07−2.82 (m + sp, 6 H + 1 H, NCH_2 + C*H*CH_3), 3.02−3.15 (m, 4 H, NCH_2), 4.25 (t, J = 6.6 Hz, 2 H, $CONCH_2$), 6.80−6.87 (m, 2 H, aromatic), 7.03−7.10 (m, 2 H, aromatic), 7.22−7.28 (m, 1 H, indole), 7.45−7.60 (m, 2 H, indole), 7.99−8.03 (m, 1 H, indole), 8.29 (s, 1 H, NCH), 12.13 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{25}H_{29}N_5O$) C, H, N.

3-[2-[4-[4-(1-Methylethyl)phenyl]piperazin-1-yl]ethyl]-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (50). A mixture of **16** (0.5 g, 1.68 mmol) and 1.7 g of the crude oil **33**, prepared in the synthesis of compound **39**, was heated in an oil bath at 140 °C for 30 min. After being cooled, the reaction mixture was treated with EtOH (10 mL). The crude solid was filtered off, washed with EtOH, and dried. Recrystallization from toluene afforded **50** as a pure product (0.3 g, 42%): mp 259−261 °C; IR (KBr) cm^{-1} 3152 (NH), 1662 (C═O); ^1H NMR (DMSO-d_6) δ 1.14 (d, J = 6.8 Hz, 6 H, $CHCH_3$), 2.59−2.81 (m + s + sp, 6 H + 3 H + 1 H, NCH_2 + CH_3 + C*H*CH_3), 3.01−3.10 (m, 4 H, NCH_2), 4.29 (t, J = 6.6 Hz, 2 H, $CONCH_2$), 6.81−6.87 (m, 2 H, aromatic), 7.03−7.10 (m, 2 H, aromatic), 7.14−7.24 (m, 1 H, indole), 7.38−7.53 (m, 2 H, indole), 7.93−7.99 (m, 1 H, indole), 11.92 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{26}H_{31}N_5O$) C, H, N.

3-[2-[4-[4-(1,1-Dimethylethyl)phenyl]piperazin-1-yl]-ethyl]-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (51). A mixture of **15** (0.4 g, 1.61 mmol) and 1.7 g of the crude oil **34**, prepared in the synthesis of compound **40**, was heated in an oil bath at 140 °C for 30 min. After being cooled, the reaction mixture was treated with EtOH (10 mL). The crude solid was filtered off, washed with EtOH, and dried. Recrystallization from EtOH afforded **51** as a pure product (0.4 g, 46%): mp 262−264 °C; IR (KBr) cm^{-1} 3181 (NH), 1673 (C═O); ^1H NMR (DMSO-d_6) δ 1.23 (s, 9 H, CCH_3), 2.55−2.72 (m, 6 H, NCH_2), 3.03−3.07 (m, 4 H, NCH_2), 4.26 (t, J = 6.6 Hz, 2 H, $CONCH_2$), 6.81−6.87 (m, 2 H, aromatic), 7.18−7.28 (m, 2 H + 1 H, aromatic + indole), 7.45−7.57 (m, 2 H, indole), 7.99−8.03 (m, 1 H, indole), 8.29 (s, 1 H, NCH), 12.12 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{26}H_{31}N_5O$) C, H, N.

3-[2-[4-[4-(1,1-Dimethylethyl)phenyl]piperazin-1-yl]-ethyl]-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(3*H*)4-one (52). A mixture of **16** (0.3 g, 1.00 mmol) and 1.1 g of the crude oil **34**, prepared in the synthesis of compound **40**, was heated in an oil bath at 140 °C for 30 min. After being cooled, the reaction mixture was treated with EtOH (10 mL). The crude solid was filtered off, washed with EtOH, and dried. Recrystallization from EtOH afforded **52** as a pure product (0.3 g, 67%): mp 275−277 °C; IR (KBr) cm^{-1} 3187 (NH), 1665 (C═O); ^1H NMR (DMSO-d_6) δ 1.23 (s, 9 H, CCH_3), 2.63−2.75 (m, 6 H, NCH_2), 2.74 (s, 3 H, CH_3), 3.05−3.09 (m, 4 H, NCH_2), 4.31 (t, J = 6.6 Hz, 2 H, $COCH_2$), 6.83−6.90 (m, 2 H, aromatic), 7.19−7.25 (m, 2 H + 1 H, aromatic + indole), 7.39−7.54 (m, 2 H, indole), 7.94−8.00 (m, 1 H, indole), 11.93 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{27}H_{33}N_5O$) C, H, N.

2-[2-Hydroxy-3-(2-nitrophenoxy)propyl]isoindole-1,3-dione (54). A mixture of 2-(2-nitrophenoxymethyl)oxirane (**53**) (24.3 g, 0.12 mol), phthalimide (18.2 g, 0.12 mol), and pyridine (1.2 mL) in 1-butanol (75 mL) was heated under reflux for 16 h. After being cooled, the suspension was decanted and the solid was suspended in EtOH (70 mL) and stirred for 1 h. Then the residue was filtered, washed with EtOH, and dried. Recrystallization from EtOH afforded **54** as a pure product (16.4 g, 39%): mp 128−129 °C; IR (KBr) cm^{-1} 3529 (OH), 1710 (C═O); ^1H NMR (DMSO-d_6) δ 3.65−3.87 (m, 2 H, NCH_2), 4.10−4.31 (m, 2 H + 1 H, OCH_2CHOH), 5.45 (d, J = 5.4 Hz, 1 H, CHO*H* which exchanges with D_2O), 7.05−7.15 (m, 1 H, aromatic), 7.30−7.39 (m, 1 H, aromatic), 7.58−7.73 (m, 1 H, aromatic), 7.75−7.93 (m, 4 H + 1 H, isoindole + aromatic). Anal. ($C_{17}H_{14}N_2O_6$) C, H, N.

2-[3-(2-Nitrophenoxy)-2-oxypropyl]isoindole-1,3-dione (55). A solution of alcohol **54** (9.6 g, 28.04 mmol) in dichloromethane (390 mL) was added, dropwise at 60 °C, to a solution of Dess−Martin periodinane (27.2 g, 64.13 mmol) in anhydrous DMSO (20 mL). After 3 h at 60 °C, the reaction mixture was stirred at room temperature overnight. Then a solution of sodium thiosulfate (72.3 g) in 5% aqueous $NaHCO_3$ (900 mL) and chloroform (900 mL) were added. The organic layer was separated, washed with $NaHCO_3$ (2 × 600 mL) and water (600 mL), and dried over anhydrous sodium sulfate. The solvents were eliminated in vacuo and the residue was recrystallized from EtOH to afford **55** as a pure product (8.3 g, 87%): mp 160−161 °C; IR (KBr) cm^{-1} 1722 (C═O); ^1H NMR (DMSO-d_6) δ 4.81 (s, 2 H, NCH_2), 5.34 (s, 2 H, OCH_2), 7.07−7.22 (m, 1 H, aromatic), 7.23−7.37 (m, 1 H, aromatic), 7.58−7.75 (m, 1 H, aromatic), 7.80−8.05 (m, 4 H + 1 H, isoindole + aromatic). Anal. ($C_{17}H_{12}N_2O_6$) C, H, N.

2-(3,4-Dihydro-2*H*-benzo[1,4]oxazin-1-ylmethyl)isoindole-1,3-dione (56). To a well-stirred suspension of ketone **55** (8.3 g, 24.33 mmol) in absolute EtOH (1200 mL) under N_2, 10% Pd/C (2.4 g) was added carefully. Then H_2 was fluxed in the reaction mixture, which was stirred at room temperature for 24 h. Successively, the catalyst was filtered off and the solvent was evaporated in vacuo. The light-yellow residue was suspended in cyclohexane (80 mL) and stirred for 12 h. Then the solid was filtered off and recrystallized from EtOH to give **56** as a pure product (5.8 g, 81%): mp 137−139 °C; IR (KBr) cm^{-1} 3348 (NH), 1713 (C═O); ^1H NMR (DMSO-d_6) δ 3.55−3.80 (m, 2 H + 1 H, NCH_2CHNH), 3.93−4.15 (m, 2 H, OCH_2-CH), 6.10 (br s, 1 H, NH which exchanges with D_2O), 6.40−6.57 (m, 2 H, aromatic), 6.59−7.75 (m, 2 H, aromatic), 7.75−7.97 (m, 4 H, isoindole). Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

***C*-(3,4-Dihydro-2*H*-benzo[1,4]oxazin-3-yl)methanamine (57).** Compound **56** (5.8 g, 19.88 mmol) was dissolved in warm EtOH (150 mL). Then hydrate hydrazine (3.1 g, 61.63 mmol) was added and the reaction mixture was heated under reflux and stirred for 3 h. After the mixture was cooled, the solvent was evaporated in vacuo and the solid residue was suspended in chloroform and stirred overnight. Then the suspension was filtered and the chloroform was evaporated to afford a light-yellow oil, which slowly solidified. This residue (3.2 g, 98%) was used for the succesive step without further purification: mp 79−82 °C; IR (KBr) cm^{-1} 3363 (broad, NH_2, NH); ^1H NMR (DMSO-d_6) δ 2.60 (d, J = 6.2 Hz, 2 H, $CHCH_2$-NH_2), 3.09−3.26 (m, 1 H, C*H*CH_2NH_2), 3.30 (br s, 2 H, NH_2 which exchanges with D_2O), 3.81 (dd, ^2J = 10.6 Hz, ^3J = 7.0 Hz, 1 H, $OCH_AH_BCHCH_2NH_2$), 4.15 (dd, ^2J = 10.6 Hz, ^3J = 2.4 Hz, 1H, $OCH_AH_BCHCH_2NH_2$), 5.78 (br s, 1 H, NH which exchanges with D_2O), 6.36−6.50 (m, 1 H, aromatic), 6.53−6.72 (m, 3 H, aromatic); ^1H NMR ($CDCl_3$) δ 1.51 (br s, 3 H, NH_2 + NH which exchanges with D_2O), 2.70 (dd, ^2J = 12.6 Hz, ^3J =

8.0 Hz, 1 H, $OCH_AH_BCHCH_AH_BNH_2$), 2.90 (dd, ^2J = 12.6 Hz, ^3J = 4.8 Hz, 1 H, $OCH_AH_BCHCH_AH_BNH_2$), 3.28−3.42 (m, 1 H, $OCH_AH_BCHCH_AH_BNH_2$), 3.95 (dd, ^2J = 10.8 Hz, ^3J = 6.6 Hz, 1 H, $OCH_AH_BCHCH_AH_BNH_2$), 4.19 (dd, ^2J = 10.8 Hz, ^3J = 2.8 Hz, 1 H, $OCH_AH_BCHCH_AH_BNH_2$), 6.56−6.68 (m, 2 H, aromatic), 6.70−6.81 (m, 2 H, aromatic). Anal. ($C_9H_{12}N_2O$) C, H, N.

Benzyl (3,4-Dihydro-2*H*-benzo[1,4]oxazin-3-ylmethyl)-carbamate (58). A solution of benzyl chloroformate (4.0 g, 23.45 mmol) in anhydrous THF (100 mL) was added, under N_2, dropwise at −78 °C to a well-stirred solution of amine **57** (3.2 g, 19.49 mmol) and triethylamine (2.9 g, 29.23 mmol) in anhydrous THF (100 mL). Then the reaction mixture was stirred at room temperature for 3 days. Successively, a saturated solution of aqueous $NaHCO_3$ (150 mL) and the mixture was extracted with chloroform (4 × 100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo to afford **58** as a brown oil (5.8 g, 99%), which was used for the successive step without further purification: IR (KBr) cm^{-1} 3347 ((NH), 1702 (C═O); ^{1}H NMR (DMSO-d_6) δ 2.85−3.22 (m, 2 H, $OCH_AH_BCHCH_2NHCO$), 3.25−3.43 (m, 1 H, $OCH_AH_BCHCH_2NHCO$), 3.84 (dd, ^2J = 10.8 Hz, ^3J = 5.4 Hz, 1 H, $OCH_AH_BCHCH_2NHCO$), 4.05 (dd, ^2J = 10.8 Hz, ^3J = 2.6 Hz, 1 H, $OCH_AH_BCHCH_2NHCO$), 5.03 (s, 2 H, $COOCH_2$), 5.86 (br s, 1 H, ArNH which exchanges with D_2O), 6.39−6.51 (m, 1 H, aromatic), 6.54−6.73 (m, 2 H, aromatic), 7.22−7.43 (m, 5 H, aromatic), 7.48 (br t, J = 5.6 Hz, 1 H, CH_2NHCO which exchanges with D_2O). Anal. ($C_{17}H_{18}N_2O_3$) C, H, N.

Benzyl [4-(2-Chloroacetyl)-3,4-dihydro-2*H*-benzo[1,4]-oxazin-3-methyl]carbamate (59). A solution of chloroacetyl chloride (2.6 g, 23.02 mmol) in dichloromethane (100 mL) was added dropwise at −78 °C, under N_2, to a solution of the amine **58** (5.8 g, 19.44 mmol) and triethylamine (3.3 g, 32.61 mmol) in dichloromethane (300 mL), and the reaction mixture was stirred at room temperature for 24 h. Then water (500 mL) was added and the organic layer was washed with 1 M HCl (2 × 100 mL), dried over anhydrous sodium sulfate, and evaporated in vacuo. Recrystallization of the brown solid residue from toluene afforded **59** as a pure product (6.6 g, 90%): mp 157−158 °C; IR (KBr) cm^{-1} 3351 (NH), 1697, 1652 (C═O); ^{1}H NMR (DMSO-d_6) δ 2.95−3.30 (m, 2 H), 4.05−4.17 (m, 1 H), 4.27−4.77 (m, 4 H), 4.97 (s, 2 H, $COOCH_2$), 6.82−6.98 (m, 2 H, aromatic), 7.00−7.15 (m, 1 H, aromatic), 7.22−7.43 (m, 5 H, aromatic), 7.62 (br s, 1 H, CH_2NHCO which exchanges with D_2O), 7.75−7.96 (m, 1 H, aromatic). Anal. ($C_{19}H_{19}ClN_2O_4$) C, H, N.

Benzyl 1,2,3,4,4a,5-Hexahydro-1*H*-pyrazino[2,1-*c*][1,4]-benzoxazine-1-one-3-carboxylate (60). A suspension of **59** (6.6 g, 17.71 mmol) and anhydrous potassium carbonate (7.7 g, 55.71 mmol) in anhydrous DMSO (400 mL) was stirred under N_2 for 2 days at room temperature. Then water (1000 mL) was added and the mixture was extracted with ethyl acetate (4 × 100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. The brown oil residue was purified by flash chromatography using ethyl acetate/cyclohexane (1:1, v/v) as eluent. The homogeneous fractions were evaporated in vacuo to afford **60** as a pale-yellow solid product (2.9 g, 49%): mp 111−113 °C; IR (KBr) cm^{-1} 1691, 1668 (C═O); ^{1}H NMR (DMSO-d_6) δ 3.20−3.42 (m, 1 H, OCH_2CHCH_2NCOO), 3.82−4.25 (m, 4 H), 4.27−4.52 (m, 2 H), 5.13 (s, 2 H, $COOCH_2$), 6.85−6.98 (m, 2 H, aromatic), 7.00−7.16 (m, 1 H, aromatic), 7.23−7.47 (m, 5 H, aromatic), 8.10−8.21 (m, 1 H, aromatic). Anal. ($C_{19}H_{18}N_2O_4$) C, H, N.

Benzyl 1,2,3,4,4a,5-Hexahydro-1*H*-pyrazino[2,1-*c*][1,4]-benzoxazine-3-carboxylate (61). Borane−THF complex (1 M solution in THF, 42.9 mL) was added dropwise at 0 °C and under N_2 to a solution of lactam **60** (2.9 g, 8.63 mmol) in anhydrous THF (150 mL). Then the reaction mixture was stirred at room temperature for 24 h. Successively, the reaction mixture was cautiously poured in ice/water (500 mL), acidified to pH 3.5 with 1 M HCl, and stirred for 30 min. The suspension was basified to pH 10 with potassium carbonate and extracted with chloroform (4 × 100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. The oily residue **61** (2.6 g, 93%) slowly solidified and was used for the successive step without further purification: mp 88−90 °C; IR (KBr) cm^{-1} 1701 (C═O); ^{1}H NMR (DMSO-d_6) δ 2.55−2.81 (m, 2 H), 2.88−3.17 (m, 2 H), 3.75−4.16 (m, 4 H), 4.25−4.40 (m, 1 H), 5.12 (s, 2 H, $COOCH_2$), 6.57−6.98 (m, 4 H, aromatic), 7.23−7.47 (m, 5 H, aromatic). Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

1,2,3,4,4a,5-Hexahydro-1*H*-pyrazino[2,1-*c*][1,4]benzoxazine (62). A mixture of 10% Pd/C (2.6 g), 1,4-cyclohexadiene (6.4 g, 80.24 mmol), and trifluoroacetic acid (2.7 g, 24.03 mmol) was added under N_2 to a cold (at 0 °C) suspension of carbamate **61** (2.6 g, 8.01 mmol) in a MeOH/water (9:1, v/v) (300 mL) mixture. The reaction mixture was slowly heated at room temperature and stirred under N_2 for 24 h. After, the catalyst was filtered off and the solvents were evaporated in vacuo. Then 10% aqueous sodium carbonate (150 mL) was added to the residue and the mixture was extracted with chloroform (5 × 60 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The oily residue **62** (1.1 g, 73%) slowly solidified and was used for the successive steps without further purification: mp 182−185 °C; IR (KBr) cm^{-1} 3307 (NH); ^{1}H NMR (DMSO-d_6) δ 2.15−2.36 (m, 1 H, $OCH_AH_BCHCH_AH_BNH$), 2.38−2.54 (m, 1 H, $ArNCH_AH_BCH_AH_BNH$), 2.55−2.78 (m + br s, 1 H + 1 H, $ArNCH_AH_BCH_AH_BNH$ + $ArNCH_AH_BCH_AH_BNH$ which exchanges with D_2O), 2.80−3.06 (m, 1 H + 1 H + 1 H, $ArNCH_AH_BCH_AH_BNH$ + $OCH_AH_BCHCH_AH_BNH$), 3.50−3.66 (m, 1 H, $ArNCH_AH_BCH_AH_BNH$), 3.83 (dd, ^2J = 10.6 Hz, ^3J = 8.8 Hz, 1 H, $OCH_AH_BCHCH_AH_BNH$), 4.18 (dd, ^2J = 10.6 Hz, ^3J = 2.8 Hz, 1 H, $OCH_AH_BCHCH_AH_BNH$), 6.55−6.91 (m, 4 H, aromatic); ^{13}C NMR (DMSO-d_6) δ 45.07 (CH_2), 45.96 (CH_2), 46.28 (CH_2), 52.46 (NCH), 67.14 (OCH_2), 112.99 (CH), 115.83 (CH), 118.88 (CH), 121.13 (CH), 135.92 (C), 144.62 (C). Anal. ($C_{11}H_{14}N_2O$) C, H, N.

3-[2-[1,2,3,4,4a,5-Hexahydro-1*H*-pyrazino[2,1-*c*][1,4]-benzoxazin-3-yl]ethyl]-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)-2,4-dione (63). A mixture of **32** (0.2 g, 0.80 mmol) and amine **62** (0.5 g, 2.89 mmol) was heated in an oil bath at 140 °C for 15 min. After being cooled, the reaction mixture was treated with EtOH (15 mL). The crude solid was filtered off, washed with water, and dried. Recrystallization from dimethylformamide/water afforded **63** as a pure product (0.1 g, 45%): mp >300 °C; IR (KBr) cm^{-1} 3151 (NH), 1701, 1636 (C═O); ^{1}H NMR (DMSO-d_6) δ 1.70−1.92 (m, 1 H, $OCH_AH_BCHCH_AH_BN$), 2.28−2.33 (m, 1 H, $ArNCH_AH_BCH_AH_BN$), 2.45−2.72 (m, 1 H + 2 H, $ArNCH_AH_BCH_AH_BN$ + NCH_2CH_2NCO), 2.86−3.21 (m, 1 H + 1 H + 1 H, $ArNCH_AH_BCH_AH_BN$ + $OCH_AH_BCHCH_AH_BN$), 3.58−3.77 (m, 1 H, $ArNCH_AH_BCH_AH_BN$), 3.79−3.98 (m, 1 H, $OCH_AH_BCHCH_AH_BNH$), 4.04−4.33 (m, 1 H + 2 H, $OCH_AH_BCHCH_AH_BN$ + NCH_2CH_2NCO), 6.55−6.91 (m, 4 H, aromatic), 6.99−7.21 (m, 1 H, indole), 7.27−7.50 (m, 2 H, indole), 7.86−8.01 (m, 1 H, indole), 11.78 (br s, 1 H, NH which exchanges with D_2O), 11.97 (br s, 1 H, NH which exchanges with D_2O); ^{13}C NMR (DMSO-d_6) δ 37.27 (CH_2), 45.46 (CH_2), 51.70 (NCH), 52.28 (CH_2), 53.48 (CH_2), 55.17 (CH_2), 66.94 (CH_2), 112.77 (CH), 113.10 (CH), 113.47 (C), 114.74 (C), 115.73 (CH), 118.91 (CH), 119.52 (CH), 120.55 (CH), 121.03 (CH), 125.89 (C), 126.97 (CH), 135.28 (C), 138.06 (C), 144.44 (C), 150.98 (CO), 156.48 (CO). Anal. ($C_{23}H_{23}N_5O_3$) C, H, N.

3-[2-[1,2,3,4,4a,5-Hexahydro-1*H*-pirazino[2,1-*c*][1,4]-benzoxazin-3-yl]ethyl]-2-methyl-5*H*-pyrimido[5,4-*b*]indole-(1*H*,3*H*)2,4-dione (64). A mixture of **16** (0.1 g, 0.32 mmol) and amine **62** (0.2 g, 1.17 mmol) was heated in an oil bath at 140 °C for 15 min. After being cooled, the reaction mixture was treated with EtOH (2 mL). The crude solid was filtered off, washed with EtOH, and dried. Recrystallization from dimethylformamide/water afforded **64** as a powder (0.04 g, 40%): mp 278−280 °C dec; IR (KBr) cm^{-1} 3181 (NH), 1664 (C═O); ^{1}H NMR (DMSO-d_6) δ 1.79−2.01 (m, 1 H, $OCH_AH_BCHCH_AH_BN$), 2.19−2.35 (m, 1 H, $ArNCH_AH_BCH_AH_BN$), 2.47−2.71 (m, 1 H + 2 H, $ArNCH_AH_BCH_AH_BN$ + NCH_2CH_2NCO), 2.74 (s, 3 H, CH_3), 2.94−3.20 (m, 1 H + 1 H + 1 H, $ArNCH_AH_BCH_AH_BN$ + $OCH_AH_BCHCH_AH_BN$), 3.62−3.80 (m, 1 H, ArN-

$CH_AH_BCH_AH_BN$), 3.81−3.96 (m, 1 H, $OCH_AH_BCHCH_AH_BNH$), 4.12−4.40 (m, 1 H + 2 H, $OCH_AH_BCHCH_AH_BN + NCH_2CH_2$-NCO), 6.58−6.91 (m, 4 H, aromatic), 7.13−7.28 (m, 1 H, indole), 7.36−7.58 (m, 2 H, indole), 7.92−8.04 (m, 1 H, indole), 11.93 (br s, 1 H, NH which exchanges with D_2O). Anal. ($C_{24}H_{25}N_5O_2$) C, H, N.

Binding Experiments on Human Cloned α_1AR Subtypes. Transfection and Cell Culture. HEK293 cells were transfected with the constitutively active pRSVICAT vectors containing the human α_{1A}AR,[4] α_{1B}AR,[3] or α_{1D}AR[5] cDNA by calcium phosphate transfection.[46] Cells were propagated for several weeks in the presence of 400 μg/mL gentamycin, and subclones were screened by radioligand binding for high receptor expression. Transfected HEK293 cells were propagated in 75 cm^2 flasks at 37 °C in a humified 5% CO_2 incubator in Dulbecco's modified Eagle's medium containing 4.5 g/L glucose, 1.4% glutamine, 20 mM HEPES, 100 mg/L streptomycin, 10^5 units/L penicillin, and 10% calf serum. The cells were detached by trypsinization and subcultured at a ratio of 1:4 upon reaching confluency.

Radioligand Binding. Confluent 100 mm plates were washed with phosphate-buffered saline (20 mM $NaPO_4$, 154 mM NaCl, pH 7.6) and harvested by scraping. Cells were collected by centrifugation and homogenized with a Polytron. Cell membranes were collected by centrifugation at 30000*g* for 10 min and resuspended by homogenization. Receptor density was determined by saturation analysis of the α_1-AR specific antagonist radioligand [^{125}I]BE 2254 (20−800 pM).[33] For analysis of competition by selective drugs, 50 pM radioligand was used. Curves were analyzed by nonlinear regression analysis using GraphPad Prism.[47] Nonspecific binding was determined in the presence of 10 μM phentolamine.

Binding Experiments on 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, D_1, and D_2 Receptors. Binding assays were performed on male CRL:CD(SD)BR-COBS rats weighing about 150 g. The animals were killed by decapitation, and their brains were rapidly dissected (hippocampus for 5-HT_{1A}; striatum for 5-HT_{1B}, D1, D2; cortex for 5-HT_{2A}), frozen, and stored at −80 °C until the day of assay.

Tissue was homogenized in about 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) using an Ultra Turrax TP-180 (2 × 20 s) and centrifuged at 50000*g* for 10 min (Beckman model J-21B refrigerated centrifuge). The pellet was resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min, and centrifuged again at 50000*g* for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The pellet was then resuspended in the appropriate incubation buffer: 50 mM Tris-HCl (pH 7.7) for 5-HT_{2A} receptors; same buffer with the addition of 10 μM pargyline for the other receptors; with 4 mM $CaCl_2$ for 5-HT_{1A} receptors; with 4 mM $CaCl_2$ and 0.1% ascorbic acid for 5-HT_{1B} and 5-HT_{2C} receptors; 50 mM Tris-HCl, pH 7.1, containing 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% ascorbic acid for D_1 and D_2 receptors.

Binding assays were done as described previously.[48] Briefly, the following incubation conditions were used: for 5-HT_{1A}, [^{3}H]-8-OH-DPAT (specific activity of 157 Ci/mmol, NEN) final concentration of 1 nM, 30 min at 25 °C (nonspecific binding, 5-HT 10 μM); for 5-HT_{1B}, [^{3}H]-5-HT (specific activity 14.6 Ci/mmol, NEN) final concentration of 2 nM, 30 min at 25 °C (nonspecific binding, 5-HT 10 μM); for 5-HT_{2A}, [^{3}H]ketanserin (specific activity of 60 Ci/mmol, Amersham) final concentration of 0.7 nM, 15 min at 37 °C (nonspecific binding, methysergide 1 mM); for D_1, [^{3}H]SCH23390 (specific activity of 71 Ci/mmol, NEN) final concentration of 0.4 nM, 15 min at 37 °C (nonspecific binding, (−)-*cis*-flupentixol 10 μM); for D_2, [^{3}H]spiperone (specific activity of 19 Ci/mmol, NEN) final concentration of 0.2 nM, 15 min at 37 °C (nonspecific binding, (−)-sulpiride 100 μM).

Incubations were stopped by rapid filtration under vacuum through GF/B filters which were then washed with 12 mL (4 × 3 times) of ice-cold 50 mM Tris-HCl buffer (pH 7.4) using a Brandel M-48R apparatus and counted in 4 mL of Filter Count (Packard) in a LKB 1214 RACKBETA liquid scintillation spectrometer. Dose−inhibition curves were analyzed by the "Allfit"[49] program to obtain the concentration of unlabeled drugs that inhibited ligand binding by 50%. The K_i values were derived from the IC_{50} values.[50]

Estimation of Inositol Phospholipid Hydrolysis. Sprague−Dawley rats (about 200 g) were killed by decapitation. The brains were rapidly removed and dissected on ice. Hippocampi were sliced (350 μm × 350 μm) with a McIlwain tissue chopper, and the slices were immediately suspended in Krebs−Hensleit buffer (118 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM K_2HPO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 11.7 mM glucose, equilibrated with 95% O_2/5% CO_2 to raise the pH to 7.4) and incubated at 37 °C for 30 min with three intermediate changes of the buffer. Forty microliters of gravity-packed slices were then transferred to 3 mL vials containing 0.3 μM *myo*-[2-^{3}H]inositol (New England Nuclear, specific activity of 16.5 Ci/mmol) in a final volume of 275 μL. After 60 min of incubation, LiCl (7 mM) was added, followed by 100 μM norepinephrine 10 min later. When present, test compounds were added 5 min prior to norepinephrine. After 60 min, the slices were washed three times with buffer and the reaction (cleavage of inositol phosphates from membrane phospholipids) was stopped by addition of 0.9 mL of chloroform/methanol (1/2, v/v). The [^{3}H]inositol monophosphates present in the aqueous phase were extracted by anion exchange chromatography and measured as described previously.[51]

Computational Methods. Calculations and graphic manipulations were performed on a Silicon Graphics Octane workstation by means of the software Catalyst 4.6.

Compounds reported in the paper were built using the two- and three-dimensional sketcher of the program. A representative family of conformations was generated for each molecule using the poling algorithm and the "best quality conformational analysis" method, based on the CHARMm force field.

Conformations were collected that fell within a 20 kcal/mol range above the lowest-energy conformation that was found.

BMY 7378 (**3**), SNAP 8719 (**67**), and discretamine (**68**) with their associated conformational models were submitted to common feature hypothesis generation (Catalyst HipHop) with the aim of producing pharmacophore models by generating alignments of common chemical features. SNAP 8719 (**67**), the most active α_{1D}-AR ligand and selective α_{1A}/α_{1D} antagonist of this series, was considered as the "reference compound" (except for this classification, biological data in the analysis were not used) specifying a "Principal" value of 2 and a "MaxOmitFeat" value of 0 during hypotheses generation. HipHop uses these values to determine which molecule should be considered to build hypothesis space and how many features in the final hypotheses must map the chemical feature in each compound, respectively. If Principal is set to 2, the chemical feature space of the conformers of such a compound is used to define the initial set of potential hypotheses, while a MaxOmitFeat value of 0 associated with the reference compound forces it to map all the features of each pharmacophore hypothesis generated.

On the other hand, 16 pyrimido[5,4-*b*]indole derivatives (namely, **4**, **23**, **25**, **26**, **28**, **40**−**45**, **48**, **50**, **52**, **63**, and **65**), with their associated conformational models and α_{1D}-AR affinity values, were submitted to Catalyst HypoGen with the aim of building a potential pharmacophore model for this α_1-AR subtype.

The chemical functions included in both HipHop and HypoGen calculations were the hydrogen bond acceptor lipid (HBA), hydrogen bond donor (HBD), positive ionizable (PI), aromatic ring (RA), and hydrophobic (HY) features.

The program was forced to keep only hypotheses with at least five features and to include a positive ionizable group (reported to be a critical key for α_1-AR antagonistic activity) in the composition of hypotheses generated by both HipHop and HypoGen.

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