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Discovery and Pharmacological Profile of New 1*H*-Indazole-3-carboxamide and 2*H*-Pyrrolo[3,4-*c*]quinoline Derivatives as Selective Serotonin 4 Receptor Ligands

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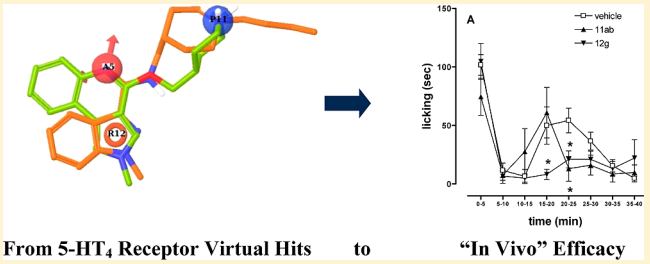
Supporting Information

ABSTRACT: Since the discovery of the serotonin 4 receptor (5-HT$_4$R), a large number of receptor ligands have been studied. The safety concerns and the lack of market success of these ligands have mainly been attributed to their lack of selectivity. In this study we describe the discovery of *N*-[(4-piperidinyl)methyl]-1*H*-indazole-3-carboxamide and 4-[(4-piperidinyl)methoxy]-2*H*-pyrrolo[3,4-*c*]quinoline derivatives as new 5-HT$_4$R ligands endowed with high selectivity over the serotonin 2A receptor and human ether-a-go-go-related gene potassium ion channel. Within these series, two molecules (**11ab** and **12g**) were identified as potent and selective 5-HT$_4$R antagonists with good in vitro pharmacokinetic properties. These compounds were evaluated for their antinociceptive action in two analgesia animal models. **12g** showed a significant antinociceptive effect in both models and is proposed as an interesting lead compound as a 5-HT$_4$R antagonist with analgesic action.



From 5-HT$_4$ Receptor Virtual Hits to "In Vivo" Efficacy

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a major neurotransmitter involved in a vast number of processes in both the central and peripheral nervous systems. Its pharmacological action is mediated by a set of specific receptors. Seven families of 5-HT receptors (5-HTRs) have been identified so far that have been cloned and coded from 5-HT$_1$ to 5-HT$_7$.[1−3] With the exception of 5-HT$_3$, all 5-HTRs belong to the superfamily of G-protein-coupled receptors and have the typical heptahelical structure of a transmembrane protein monomer. 5-HT$_4$ receptor (5-HT$_4$R) is positively coupled to adenylate cyclase by G$_s$ protein. After its activation, 5-HT$_4$R induces an increase in intracellular levels of cyclic adenosine monophosphate (cAMP), activating protein kinase A (PKA), which in turn results in the modulation of a series of ionic cellular currents.[4,5] 5-HT$_4$Rs are widely distributed in both the central and peripheral tissues and are involved in several neuronal functions.[6,7] Thus, since its discovery, 5-HT$_4$R has been considered as a potential therapeutic target, and a large number of 5-HT$_4$R ligands have been described and studied for the treatment of a number of disease indications in the past 15 years.[8,9] The most significant results have been obtained for the treatment of irritable bowel syndrome (IBS) (cisapride (**1**), tegaserod (**2**), and prucalopride (**3**)) and in the treatment of heart failure, with piboserod (**4**) producing significant improvement in left ventricular function during clinical trials on patients with symptomatic heart failure (Chart 1).[10−13] Further studies led to the discovery of 5-HT$_4$R antagonists such as compounds **5**−**9** (Chart 1).[9,14,15] Moreover, the interest in 5-HT$_4$R ligands increased when the 5-HT$_4$R was proposed to be involved in the mechanism of nociception. The first example of this involvement was demonstrated when three nonselective 5-HT$_4$R agonists, **1**, *endo*-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride (BIMU 1), and *endo*-*N*-(8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-2,3-dihydro-3-(1-methylethyl)-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride (BIMU 8), showed an antinociceptive effect in animal models.[16] Afterward, several studies on 5-HT$_4$R antagonists such as **5**, **6**, and SDZ-205557 (**10**) or partial agonists such as **2** have suggested that the

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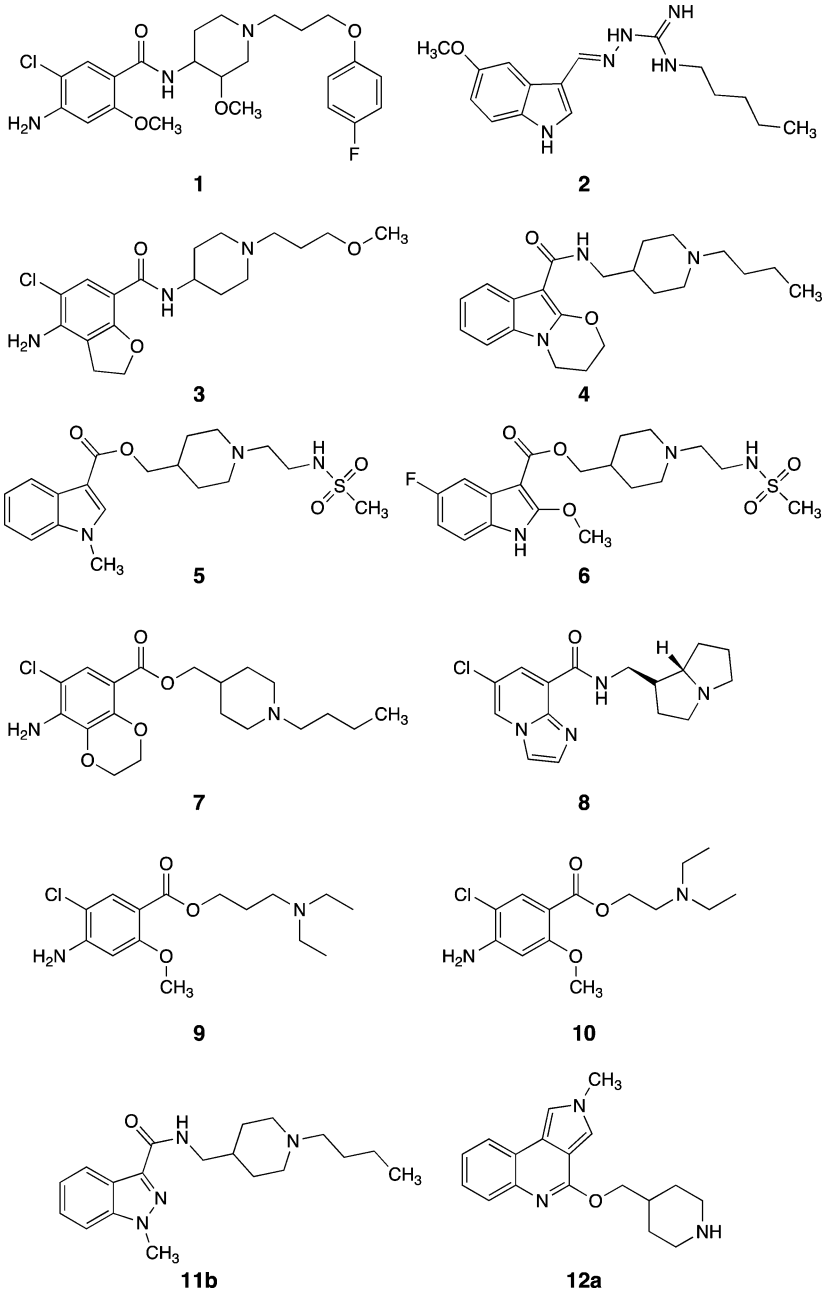
Chart 1. 5-HT$_4$R Ligands Currently Used in Clinical Practice or Trials, 1−10, Including Compounds 4−9 Used To Develop Pharmacophore Models, and Hit Compounds 11b and 12a Found in the Present Study by a VS Approach



inactivation of this receptor may be an effective method to treat some types of pain (Chart 1).[17−21]

Unfortunately, most drugs active on the serotoninergic system show side effects consistent with low selectivity toward other serotoninergic receptors or interaction with ion channels such as the human ether-a-go-go-related gene (hERG) potassium channel.[22−26]

The interaction with these channels is associated with an adverse cardiac QT-interval prolongation and caused the withdrawal of **1** from the market in 2000.[27] Nowadays, hERG inhibition is routinely investigated in the early phase of the drug discovery process to decrease the risk of subsequent cardiac safety concerns.[28]

Because of the lack of structural data on 5-HT$_4$R, there is insufficient information to perform a target-based drug design program. Thus, we decided to apply a virtual screening (VS) approach on our in-house chemical library including around 9500 heterocyclic compounds.

Studies on 5-HT$_4$R antagonists described in the literature in the past two decades have provided crucial insights into the structural motifs required for a ligand to have a strong interaction with this receptor.[9,14,15,29,30] Therefore, we built a 3D pharmacophore model based on the literature antagonists and used it for the VS of our in-house library. A total of 71 virtual hits were identified that were then tested in vitro in a human recombinant 5-HT$_4$R binding assay using **4** as a reference compound. Two hit compounds were identified, *N*-[(1-butyl-4-piperidinyl)methyl]-1*H*-indazole-3-carboxamide (**11b**) and 4-(4-piperidinylmethoxy)-2*H*-pyrrolo[3,4-*c*]-quinoline (**12a**), and derivatives of **11b** and **12a** were

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synthesized to define the structure−activity relationships (SARs) in these series. In this paper we report the activities of the newly synthesized compounds (**11a−ab** and **12a−k**) on 5-HT_4R, as well as on relevant off-target proteins such as 5-HT_{2A} receptors, and inhibition of the hERG potassium ion channel. In vitro absorption, distribution, metabolism, and excretion (ADME) properties and antinociceptive efficacy of the most promising compounds **11ab** and **12g** in two different animal models are also described.

RESULTS AND DISCUSSION

Ligand Design. A collection of 16 5-HT_4 antagonists characterized by different chemical scaffolds were taken from the literature,[9,14,15] and among them, six compounds (**4−9**) were used to define a data set to generate several 3D pharmacophore models (Chart 1). Only three models (referred to as APR29, HPR45, and AAP29) were selected for the validation step on the basis of (i) the highest score obtained and (ii) the largest diversity of electronic features to match chemical groups with different properties (e.g., hydrogen bond acceptor, hydrophobic, positive charge, and aromatic ring) in different combinations. Each model was used to perform a hit search on a database of 1000 druglike ligand decoys from Schrödinger and 10 known active compounds, seeded to have a random hit rate of 1%. The enrichment factor (EF) was calculated after each VS process, and these values were compared to determine the best performing model. The EF is the measure of how many active compounds are found within a defined "early recognition" fraction of the ordered list relative to a random distribution and is calculated as follows:

$$\mathrm{EF} = N^{x\%}{}_{\mathrm{exptl}}/(N_{\mathrm{active}} \times x\%) \quad (1)$$

where $N^{x\%}{}_{\mathrm{exptl}}$ is the number of experimentally found active structures in the top x% of the sorted database and N_{active} is the total number of active structures in the database.

The best outcome for each VS protocol is 100% (10 out of 10) at the top 1%. Figure 1 shows that the APR29 pharmacophore gave the best result compared to the other pharmacophores, showing the maximum EFs at the top 5% of the data set.

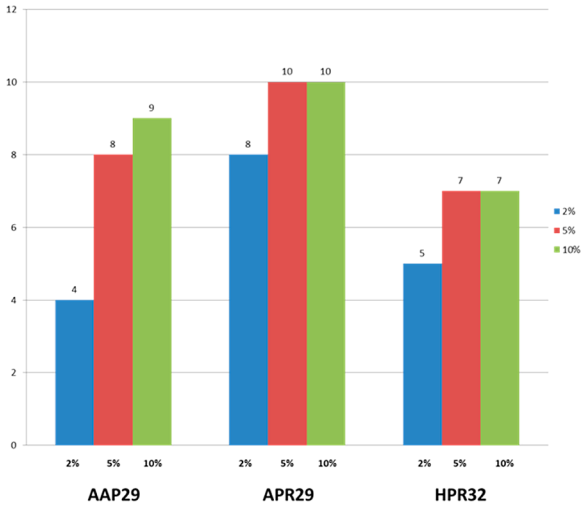

Figure 1. Enrichment for the top 2%, 5%, and 10% of the data set for the AAP29, APR29, and HPR32 hypotheses.

APR29 contained three structural features: a hydrogen bond acceptor, an aromatic ring, and a positively charged group distributed as shown in Figure 2 overlaid with reference compounds **7** and **8** (Chart 1, Figure 2).

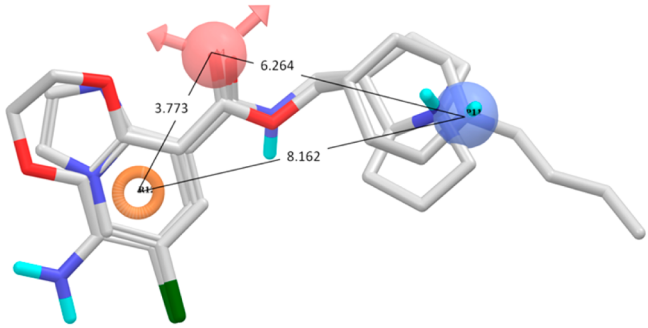

Figure 2. Pharmacophore model APR29 for 5-HT_4 antagonists **7** and **8**: aromatic ring (R12, brown), positive ionizable group (P11, blue), and acceptor H-bond group (A4, red).

APR29 was then used to carry out a virtual hit search in a multiconformer version of our proprietary database (Angelini corporate database). The hits retrieved from the pharmacophore search were filtered for non-drug-like groups and properties, giving 71 compounds characterized by several different chemical scaffolds. These molecules were submitted to a single concentration binding assay, using the human recombinant 5-HT_4R and **4** as a reference compound (data not shown). From this, compounds **11b** and **12a** (Chart 1) emerged as promising hits, showing good 5-HT_4R binding affinity, with binding inhibition values of 98% and 48%, respectively, at a concentration of 1 μM compared to **4**, which showed 100% inhibition at the same concentration. The two hit compounds matched all three pharmacophoric features of APR29, proving the validity of this pharmacophore approach for identifying novel scaffold hits (Figure 3).

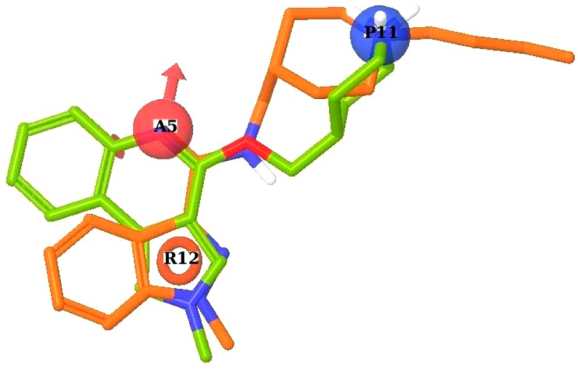

Figure 3. Match of compounds **11b** (orange) and **12a** (green) with the three pharmacophore features of the APR29 hypothesis.

On the basis of its ease of chemical manipulation, we first decided to study the chemical space around hit compound **11b** (*N*-[(1-butyl-4-piperidinyl)methyl]-1*H*-indazole-3-carboxamide), focusing our initial attention on the following substitution sites: (i) the piperidine nitrogen, (ii) the indazole nitrogens, and (iii) the 5-position of the indazole ring. We subsequently applied the SAR from this series to design and synthesize a focused library of 2*H*-pyrrolo[3,4-*c*]quinoline derivatives based on **12a**.

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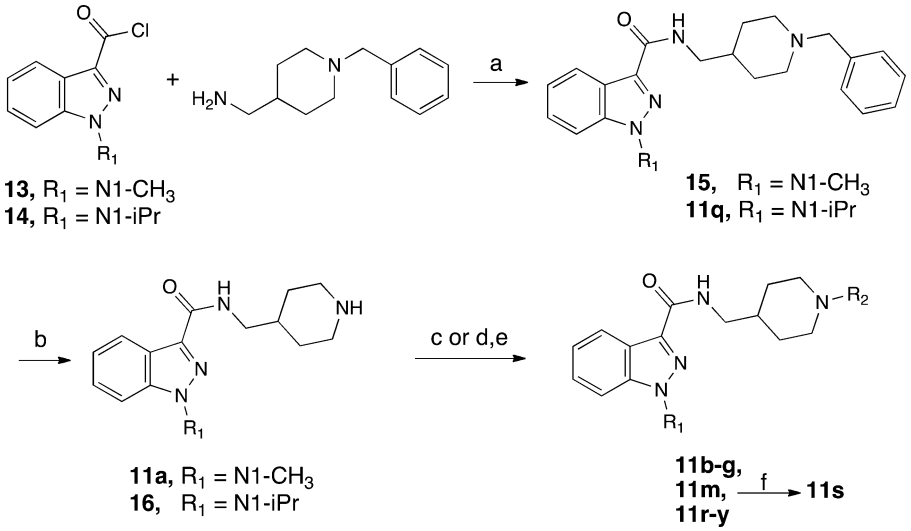
Scheme 1. Synthetic Route to Compounds 11a−g,m,q−y[a]



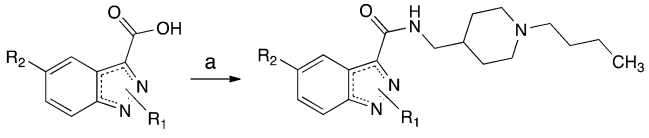
[a]Reagents and conditions: (a) toluene, room temperature; (b) H_2, 10% Pd/C, CH_3CO_2H; (c) alkyl bromide, K_2CO_3, EtOH, reflux (or DMF, 80 °C); (d) 2-vinylpyridine, CH_3CO_2H, H_2O, reflux → room temperature; (e) CH_3I, CH_3COCH_3, room temperature; (f) CH_3I, acetone, room temperature.

Synthesis. The *N*-(4-piperidinylmethyl)-1*H*-indazole-3-carboxamides **11a−g,m,q−y** were prepared as shown in Scheme 1 (see Tables 1−3 for the actual structures). The appropriate *N*-alkyl-1*H*-indazole-3-carbonyl chlorides (**13** or **14**[31]) were reacted with 1-[1-(phenylmethyl)-4-piperidinyl]methylamine to afford compounds **11q** and **15**, which were then hydrogenated to give *N*-unsubstituted derivatives **11a** and **16**, respectively. Compounds **11b−d,f,g,m,t−y** were obtained by nucleophilic substitution of **11a** and **16** with the appropriate alkyl halide or by reaction with 2-vinylpyridine for **11e** and **11r** (Scheme 1). Finally, **11s** was obtained by methylation of piperidine **11m**.

The 5-substituted *N*-[(1-butyl-4-piperidinyl)methyl]-1(2)-methylindazole-3-carboxamides **11h−k** were obtained by transformation of the 5-substituted-1(2)-methylindazole-3-carboxylic acids **17−20**[32,33] into the corresponding acyl chlorides followed by amidation with 1-(1-butyl-4-piperidinyl)-methylamine (Scheme 2) (see Table 1 for the actual structures).

Scheme 3 shows the synthetic pathway to obtain compounds **11l,n−p** (see Table 2 for the actual structures). Indazole-3-carbonyl chloride was first reacted with 1-[1-(2-phenylethyl)-4-piperidinyl]methylamine to furnish **11l**. Subsequent alkylation with the appropriate alkyl bromide or acetylation with acetic anhydride afforded indazole-3-carboxamides **11n−p** (Scheme 3).

Scheme 2. Synthetic Route to Compounds 11h−k[a]



17, R_1 = N1-CH_3, R_2 = CH_3
18, R_1 = N1-CH_3, R_2 = OCH_3
19, R_1 = N1-CH_3, R_2 = Cl
20, R_1 = N2-CH_3, R_2 = H

[a]Reagents and conditions: (a) (i) $SOCl_2$, toluene, reflux; (ii) 1-(1-butyl-4-piperidinyl)methylamine, toluene, room temperature.

Derivatives **11z−ab** were obtained as shown in Scheme 4 (see Table 3 for the actual structures). Compound **16** was alkylated under basic conditions with 1-(2-bromoethyl)-4-nitrobenzene or ethyl 4-(2-bromoethyl)benzoate[34] to afford **11z** and **21**, respectively. Then **11z** was reduced with hydrogen in the presence of Pd to afford amine **11aa**, while basic hydrolysis of **21** gave the corresponding carboxylic acid **11ab** (Scheme 4).

4-(4-Piperidinylmethoxy)-2*H*-pyrrolo[3,4-*c*]quinolines **12a−e,i−k** were prepared starting from nitrocinnamate **22**, which underwent annulation with (4-tolylsulfonyl)methyl isocyanide (TosMIC) in the presence of sodium hydride to afford pyrrole **23** (Scheme 5) (see Table 4 for the actual structures).[35]

The latter compound was transformed into 2*H*-pyrrolo[3,4-*c*]quinolin-4(5*H*)-one (**24**) in a two-step, one-pot reaction carried out with iron powder in glacial acetic acid at 85 °C.[35] Substitution at the 2-position of the pyrroloquinoline ring was easily achieved by alkylation of intermediate **24** with the appropriate alkyl halide in the presence of K_2CO_3 to achieve **25** and **26**.

Substitution at the 4-position was achieved via a two-step process involving a $POCl_3$ chlorination and subsequent nucleophilic displacement of the resultant chlorides **27** and **28**, with the appropriate (1-alkyl-4-piperidinyl)methanol derivative to afford **12b−d** and **29**. Benzyl derivative **29** was deprotected by catalytic hydrogenation to give intermediate **12a**, which was alkylated under basic conditions to give **12e,i−k**.

Compounds **12f−h** were obtained as shown in Scheme 6 (see Table 4 for the actual structures). Alkylation of piperidine derivative **12a** with 1-(2-bromoethyl)-4-nitrobenzene gave nitro derivative **30**, which was reduced to amino compound **12f**. Esters **31** and **32** were obtained by alkylation of **12a** with the appropriate ethyl[36] or methyl (**35**) (haloethyl)benzoate and were subsequently hydrolyzed to generate the final products **12g** and **12h** (Scheme 6).

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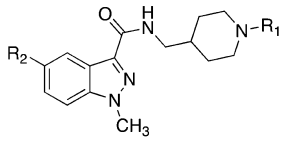
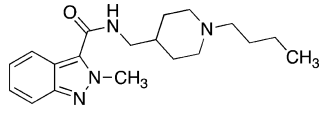
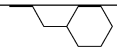
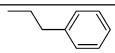
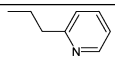
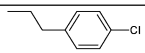
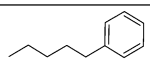
Table 1. Binding Properties of Derivatives 11a−k for the Human 5-HT$_4$R and Functional Inhibition of the hERG Potassium Ion Channel



11a-j

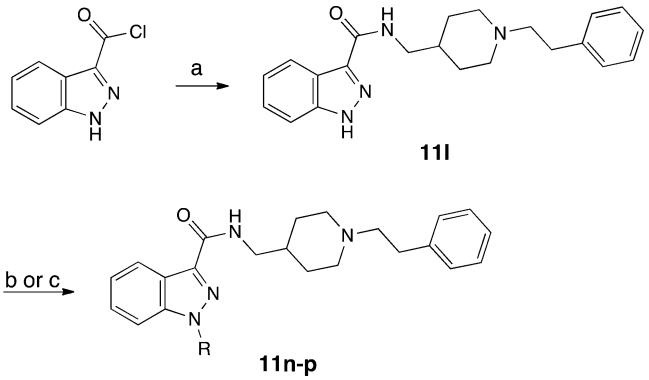


11k

compd	R_1	R_2	% inhibition 5-HT$_4$[a]			% inhibition hERG[b]
			10^{-7} M	10^{-8} M	10^{-9} M	10^{-6} M
11a[c]	H	H	33	5		
11b[d]	$CH_2CH_2CH_2CH_3$	H	88	37	12	16
11c[d]		H	93	64	20	
11d[d]		H	100	84	28	42
11e[d]		H	97	66	16	62
11f[d]		H	105	80	31	
11g[e]		H	101	64	22	
11h[d]	$CH_2CH_2CH_2CH_3$	CH_3	43	5		
11i[e]	$CH_2CH_2CH_2CH_3$	CH_3O	-9			
11j[d]	$CH_2CH_2CH_2CH_3$	Cl	40	9		
11k[c]	-	-	0			
4			97	95	73	67

[a]Percent displacement of the [^{3}H]**5** ligand from the recombinant human 5-HT$_4$R, mean values of duplicate measurements. [b]See ref 38. [c]Tested as maleic salt. [d]Tested as hydrochloride salt. [e]Tested as oxalic salt.

Scheme 3. Synthetic Route to Compounds 11l,n−p[a]



[a]Reagents and conditions: (a) 1-[1-(2-phenylethyl)-4-piperidinyl]-methylamine, toluene, reflux; (b) alkyl bromide, DMF, NaH, 0 °C → room temperature; (c) $(CH_3CO)_2O$, CH_2Cl_2, room temperature.

Biological Activities. *5-HTR Affinity in Vitro Assays.* The newly synthesized compounds **11a−ab** and **12a−k** were tested for their activity on 5-HT$_4$R in competition binding assay, at different concentrations, using [^{3}H]**5** as a radioligand and **4** as a reference compound. Preliminary data on the binding profile of several hits (**11b,d,m**) suggested low selectivity over all the 5-HT subtype receptors and transporter (Supporting Information); therefore, the affinities of the above compounds versus some off-target proteins were also tested. The screening panel demonstrated significant activity on the 5-HT$_{2A}$R, so we also decided to screen the most interesting compounds in a multiconcentration binding assay for the 5-HT$_{2A}$R to determine the activity at this target. These assays were performed in vitro using [^{3}H]ketanserin as a radiologand and methysergide as a reference compound (Tables 1−4).

5-HT$_4$R Affinity in Vitro Assays. 1H-Indazole-3-carboxamide Series. The effect of substituents at the piperidinyl nitrogen (R_1) was studied by replacement of the butyl chain (**11b**) with several alkyl-, aryl-, and heteroarylalkyl groups (Table 1). The unsubstituted derivative **11a** showed reduced

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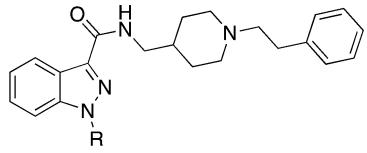
Table 2. Binding Properties of Derivatives 11d,l−p for the Human 5-HT$_4$ and 5-HT$_{2A}$ Receptors and Functional Inhibition of the hERG Potassium Ion Channel

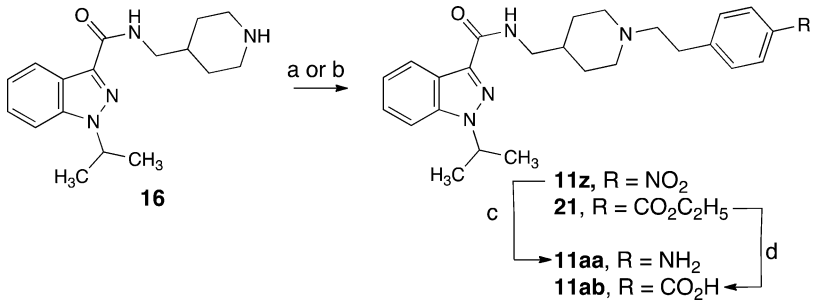


11d,l-p

		inhibition (%) of 5-HT$_4$[a]			
compd	R	10^{-8} M	10^{-9} M	inhibition (%) of 5-HT$_{2A}$[b] (10^{-7} M)	inhibition (%) of hERG[c] (10^{-6} M)
11d[d]	CH_3	84	29	79	42
11l[d]	H	56	15		
11m[d]	$CH(CH_3)_2$	108	88	80	54
11n[d]	$CH(CH_3)CH_2CH_3$	88	49	85	
11o[d]	$CH_2CH_2CH(CH_3)_2$	14			
11p[d]	$COCH_3$	90	46		
4		95	73		67
methysergide				97	

[a]Precentage of displacement of the [^{3}H]**5** ligand from the recombinant human 5-HT$_4$R, mean values of duplicate measurements. [b]Precentage of displacement of [^{3}H]ketanserin ligand from the recombinant human 5-HT$_4$R, mean values of duplicate measurements. [c]See ref 38. [d]Tested as hydrochloride salt.

Scheme 4. Synthetic Route to Compounds 11z,aa,ab[a]



[a]Reagents and conditions: (a) 4-(2-bromoethyl)-1-nitrobenzene, EtOH, K_2CO_3, reflux; (b) ethyl 4-(2-bromoethyl)benzoate,[34] KI, triethylamine, 2-butanone, reflux; (c) 10% Pd/C, H_2, EtOH, room temperature; (d) 1 N NaOH, THF, EtOH, room temperature.

affinity compared to **11b**, and binding was markedly increased by larger groups such as cyclohexylethyl, phenylethyl, 2-pyridinylethyl, (4-chlorophenyl)ethyl, and phenylbutyl (compounds **11c−g**, 64−84% inhibition at 10^{-8} M). Elongation of the alkyl linker between the piperidine nitrogen and the phenyl group from two to four carbon atoms gave a slight reduction of binding inhibition (compare **11d** and **11g**).

The effect of substitution on the indazole ring was also studied; in particular, we chose to study the 5-position due to its structural similarity with 5-HT. Chloro, methoxy, and methyl groups were selected as substituents as they have different sizes and electronics. The influence of these substituents on the 5-HTR affinity is usually dependent on the 5-HTR subtype, and in particular, 5-methoxytriptamine has been reported as a 5-HT$_4$R agonist comparable to 5-HT.[37] Introduction of a methyl group (**11h**) or a chlorine atom (**11j**) was detrimental for binding inhibition, with values of 43% and 40%, respectively, at 0.1 μM compared to 88% for the unsubstituted compound **11b** at the same concentration. A methoxy group in the same position (**11i**) totally depleted any activity even at the highest concentration tested (−9% at 0.1 μM). This drop in binding in the indazole series has not previously been shown for the 5-HT$_4$ receptor.[29] The same drop of activity was also observed when the methyl group of **11b** was shifted from the 1- to 2-position of the indazole ring (**11b** and **11k**).

A small series of derivatives were also designed to study the influence of substitution at the 1-position of the indazole ring on binding. Thus, we synthesized and tested a few derivatives of compound **11d**, in which the methyl group on N1 was removed or replaced with larger alkyl groups or with an acetyl (Table 2).

Desmethyl compound **11l** showed a lower inhibition for 5-HT$_4$R, while the larger isopropyl derivative **11m** was more active than the parent compound **11b** (88% at 1 nM). A further increase in the size of the alkyl group, such as for 1-methylpropyl (**11n**) and isopentyl (**11o**) derivatives, had a detrimental effect on binding inhibition. Interestingly, the 1-acetyl analogue **11p** showed better activity than methyl derivative **11d**. Unfortunately, its chemical instability possibly due to hydrolytic degradation prevented further development.

Finally, the SAR around the basic site of the molecule was investigated, keeping the isopropyl group on the 1-position and the unsubstituted benzene of the indazole ring constant as these substitution patterns both gave good affinity. Therefore, the *N*-[(1-substituted-4-piperidinyl)methyl]-1-isopropyl-1*H*-indazole-3-carboxamides **11m,q−ab** were prepared and assayed for their ability to bind 5-HT$_4$R (Table 3).

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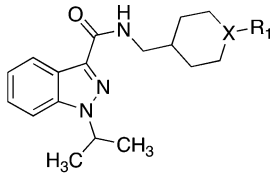
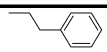
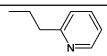
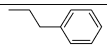
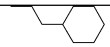
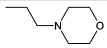
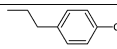
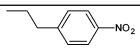
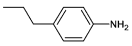
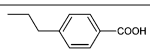
Table 3. Binding Properties of Derivatives 11m,q−ab for the Human 5-HT$_4$ and 5-HT$_{2A}$ Receptors, Functional Inhibition of the hERG Potassium Ion Channel, and Calculated log *D* at pH 7.4



11m,q-ab

compd	R_1	X	5-HT$_4$[a] p*K*i	5-HT$_{2A}$[b] p*K*i	% inhibition hERG[c] 10^{-6} M	log$D_{(7.4)}$[d]
11m[e]		N	10.1	7.5	54	3.57
11q[e]		N	9.2[f]	<6		3.67
11r[e]		N	10.0	7.0	50	2.78
11s[g]		$N^+(CH_3)$	7.5[f]	<6		0.87
11t[e]		N	9.8[f]	<6	60	4.29
11u[h]		N	9.4[f]	<6	13 (IC_{50}: 32.5 μM)	1.13
11v[i]	$(CH_2)_3N(CH_3)_2$	N	9.1	<6	52	0.30
11w[e]	$CH_2CH_2NHSO_2CH_3$	N	9.5	<6	27 (IC_{50}: 2.63 μM)	1.87
11x[e]	$CH_2CH_2CONHCH_3$	N	9.3			1.63
11y[e]		N	9.6	8.0	70	2.84
11z[j]		N	8.9	6.0	85	3.63
11aa[h]		N	9.7	7.1	20 (IC_{50}: 22.6 μM)	2.22
11ab		N	9.1	<5	1 (IC_{50}: >100 μM)	1.90
4			9.8		67	
Methysergide				8.8		

[a]Binding affinity for the human recombinant 5-HT$_4$ receptor, displacement of the [^{3}H]**5** ligand expressed as pK_i, mean values of duplicate measurements. Confidence intervals of 95% for K_i are not greater than 10% of K_i. [b]Binding affinity for the human recombinant 5-HT$_{2A}$ receptor, displacement of the [^{3}H]ketanserin ligand expressed as pK_i, mean values of duplicate measurements. Confidence intervals of 95% for K_i are not greater than 10% of K_i. [c]See ref 37. [d]log $D_{(7.4)}$ values calculated by ACDLabs 12.0. [e]Tested as hydrochloride salt. [f]Extrapolated values from three-concentration assay. [g]Tested as iodide salt. [h]Tested as dihydrochloride salt. [i]Tested as dimaleic salt. [j]Tested as oxalic salt.

When 2-pyridinylethyl or cyclohexylethyl groups were linked to the piperidine nitrogen, excellent affinities were obtained, with compounds **11r** and **11t** showing pK_i values comparable to that of compound **11m** (10.0, 9.8, and 10.1, respectively).

Shortening of the linker to one carbon atom between the phenyl and the basic site of **11m** led to benzyl derivative **11q**, which was about 10 times less potent than the parent compound.

Quaternarization of the basic tertiary nitrogen atom (**11s**) was totally detrimental to activity, showing a pK_i value about 400 times lower than that of the desmethylated counterpart **11m**. This drop in activity demonstrates the importance of steric and electronic requirements for the basic site of the molecule for its efficient binding with the receptor.[39]

Reasonably potent 5-HT$_4$ receptor ligands were obtained when polar alkyl chains were introduced as substituents on the piperidine nitrogen. Morpholine **11u**, amine **11v**, sulfonamide **11w**, and amide **11x** all showed pK_i values ranging from 9.1 to 9.5.

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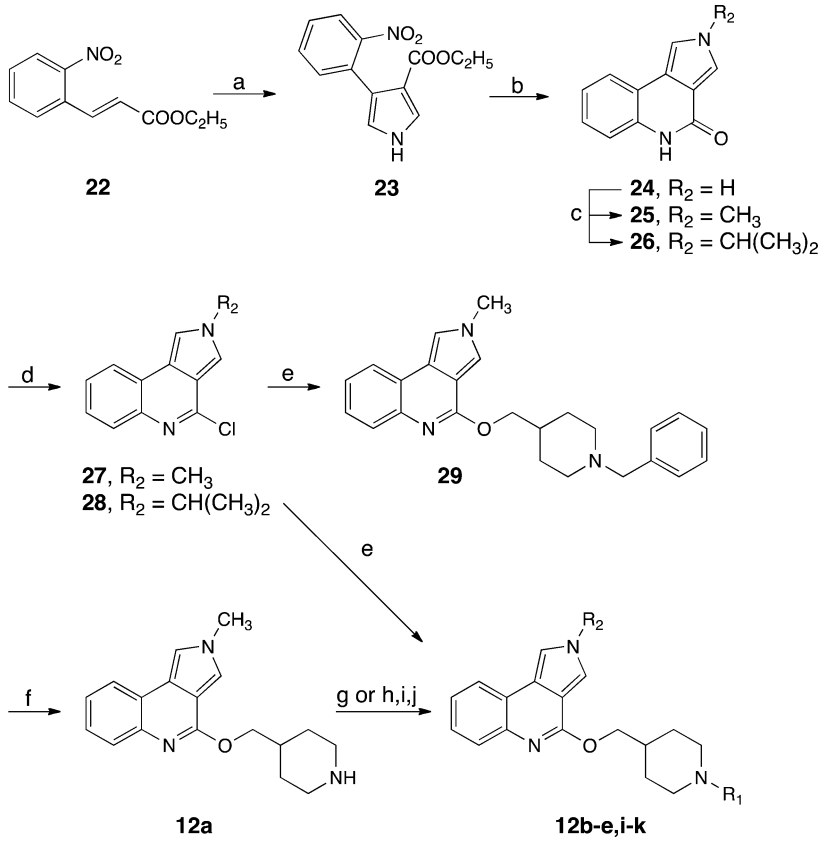
Scheme 5. Synthetic Route to Compounds 12a−e,i−k[a]



[a]Reagents and conditions: (a) TosMIC, NaH, DMSO, Et_2O, room temperature; (b) Fe, CH_3CO_2H, 85 °C; (c) XR_2, K_2CO_3, DMF, 90 °C; (d) $POCl_3$, Et_3N, 120 °C; (e) (1-alkyl-4-piperidinyl)methanol, NaH, DMF, 146 °C; (f) H_2, 10% Pd/C, EtOH; (g) XR_1, K_2CO_3, EtOH, reflux; (h) XR_1, EtOH, $NaHCO_3$, reflux; (i) XR_1, NaI, Et_3N, 2-butanone, reflux; (j) 4-(2-bromoethyl)benzyl alcohol (**33**) or [4-(methoxymethyl)phenyl]ethyl bromide (**34**), Et_3N, 2-butanone, reflux.

A study of the effect of the substitution on the 4-position of the phenyl ring of **11m** was also performed. Binding affinity values in the subnanomolar range were found for 4-hydroxy (**11y**) and 4-amino (**11aa**) derivatives (pK_i = 9.6 and 9.7, respectively). A slight decrease in affinity was found when the hydroxyl or amino groups were replaced by nitro or carboxylic moieties (**11z** and **11ab** have pK_i = 8.9 and 9.1, respectively). All these results support the hypothesis that the 5-HT_4R has a large pocket around the interaction point with the basic site of the ligand, which can accommodate both large hydrophobic moieties and polar terminal chains.[40]

2H-Pyrrolo[3,4-c]quinoline Series. A focused series of 2*H*-pyrrolo[3,4-*c*]quinoline derivatives related to hit **12a** (Table 4) were also synthesized, taking into account the SAR around the indazole-3-carboxamide series. Due to their cumbersome syntheses, substitution on the benzene of the pyrroloquinoline ring was not considered in this study. Contrary to that observed in the previous series, the methyl derivative **12c** showed 5-HT_4R binding affinity 2 orders of magnitude higher than that of the isopropyl counterpart **12d** (Table 4, pK_i = 8.7 and 6.9, respectively). This could be due to the steric clash caused by the isopropyl group, on the rigid tricyclic pyrroloquinoline ring, with the receptor. This negative interaction may not occur for the more conformationally free indazolecarboxamide moiety. Thus, the methyl group was selected for the remainder of the SAR studies.

Finally, a few derivatives of **12a** were designed, in which the butyl group on the piperidine ring was removed or substituted with the groups that gave the most potent and selective ligands within the indazolecarboxamide series (see the sections "Selectivity for 5-HT4R vs 5-HT2AR" and "Effect on the hERG Ion Channel"). These included the morpholinylethyl or (un)substituted phenylethyl moieties.

Four substituted phenylethyl derivatives (**12h−k**) were synthesized to further explore the chemical space around the phenyl ring that seemed to be relevant for both affinity and selectivity in the previous series.

Substitution on the nitrogen of the piperidine ring gave similar results in both series. The phenylethyl derivative **12c** showed the highest affinity (pK_i = 8.7), and an increase in affinity was also observed for the 4-carboxylic derivative **12g** and the amide **12k**. Removal of the acetyl group of **12k** gave amine **12f**, which showed a slight decrease in affinity (pK_i = 8.3) compared to the parent compound. Replacement of the carboxylic group of **12g** with a hydroxymethyl or a methoxymethyl moiety gave compounds that showed a moderate decrease in activity (**12i** and **12j**, pK_i = 8.0 and 7.7, respectively), while moving the carboxylic group from the 4- to the 2-position of the phenyl ring gave compound **12h**, which showed a 2 orders of magnitude drop in binding affinity. Unsubstituted piperidine derivative **12a** was 200 times less active than phenylethyl derivative **12c**, while the butyl analogue **12b** had pK_i = 8.1. Finally, the morpholine derivative **12e** and

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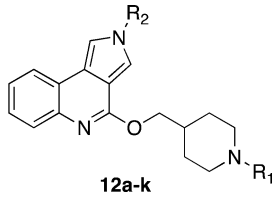
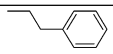
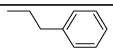
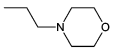
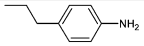
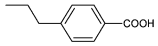
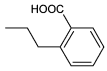
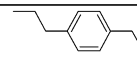
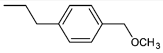
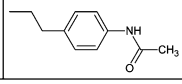
Table 4. Binding Properties of Derivatives 12a–k for the Human 5-HT$_4$ and 5-HT$_{2A}$ Receptors, Functional Inhibition of the hERG Potassium Ion Channel, and Calculated log *D* at pH 7.4



12a-k

compd	R_1	R_2	5-HT$_4$[a] p*K*i	5-HT$_{2A}$[b] p*K*i	hERG[c] IC_{50} (μM)	log$D_{(7.4)}$[d]
12a	H	CH_3	6.4			0.98
12b	$(CH_2)_3CH_3$	CH_3	8.1	< 6		3.88
12c		CH_3	8.7	7.1	[91][e]	4.71
12d[f]		$CH(CH_3)_2$	6.9	7.0		5.58
12e[f]		CH_3	8.6	< 5	1.22	2.27
12f[f]		CH_3	8.3	6.6	0.05	3.36
12g		CH_3	8.7	< 5	9.96	2.85
12h		CH_3	6.7	6.1		3.01
12i		CH_3	8.0	7.5	0.01	3.59
12j		CH_3	7.7	6.7	0.02	4.37
12k		CH_3	8.7	5.5	0.02	3.65
4			9.8		67	
Methysergide				8.8		

[a]Binding affinity for the human recombinant 5-HT$_4$ receptor, displacement of the [^{3}H]**5** ligand expressed as pK_i, mean values of duplicate measurements. Confidence intervals of 95% for K_i are not greater than 10% of K_i. [b]Binding affinity for the human recombinant 5-HT$_{2A}$ receptor, displacement of the [^{3}H]ketanserin ligand expressed as pK_i, mean values of duplicate measurements. Confidence intervals of 95% for K_i are not greater than 10% of K_i. [c]See ref 37. [d]log $D_{(7.4)}$ values calculated by ACDLabs 12.0. [e]Percent inhibition at a concentration of 30 μM. [f]Tested as hydrochloride salt.

the phenyl derivative **12c** showed comparable binding activities (pK_i = 8.6 and 8.7, respectively).

Selectivity over 5-HT$_4$R vs 5-HT$_{2A}$R. Throughout the SAR study directed toward obtaining potent 5-HT$_4$R ligands, attention was paid to the selectivity of our compounds versus some off-target proteins. Our concern for a potential lack of selectivity arose from (i) literature data showing that 5-HT$_4$R has structural features partially overlapping those of 5-HT$_{2A}$R[41] and (ii) preliminary binding data of a few initial hits (**11b**, **11d**, and **11m**) for all the 5-HT subtype receptors and transporter (see the Supporting Information), which confirmed the presence of significant activity for the off-target 5-HT$_{2A}$R. We therefore decided to screen the most interesting compounds against 5-HT$_{2A}$R (Tables 1–4).

Low selectivity between 5-HT$_4$ and 5-HT$_{2A}$ receptors was confirmed for a number of compounds within the indazole-carboxamide series, which show pK_i values for 5-HT$_{2A}$R between 7 and 8 (**11m,r,y,aa**). However, small chemical

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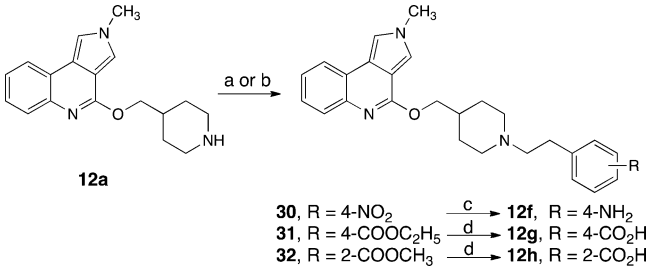
Scheme 6. Synthetic Route to Compounds 12f–h[a]



[a]Reagents and conditions: (a) ethyl 4-(2-chloroethyl)benzoate[36] or methyl 2-(2-bromoethyl)benzoate (**35**), NaI, Et_3N, 2-butanone, reflux; (b) 4-(2-bromoethyl)-1-nitrobenzene, K_2CO_3, DMF, 70 °C; (c) H_2, 10% Pd/C, EtOAc (d) 1 N NaOH, THF, EtOH.

modifications led to significant changes in activity. Among the most potent 5-HT_4 ligands, low or very low 5-HT_{2A}R binding affinity (pK_i < 6) was obtained with derivatives having polar terminal moieties (**11u–w**) or electron-withdrawing substituents on the phenyl moiety (**11z** and **11ab**) (Table 3). These results gave us useful information about the electronic and structural requirements needed to obtain selectivity toward the 5-HT_{2A}R, particularly in the region "beyond" the basic site of the molecules, where the 5-HT_4R shows more tolerance.

Interestingly, the 4-(4-piperidinylmethoxy)-2*H*-pyrrolo[3,4-*c*]quinolines display lower affinity for the 5-HT_4 and 5-HT_{2A} receptors compared to their indazolecarboxamide counterparts. Fortunately, the positive effect on 5-HT_4 selectivity shown by the morpholinylethyl and (4-carboxyphenyl)ethyl moieties in the indazolecarboxamide series was retained in the pyrroloquinolines, with compounds **12e** and **12g** having pK_i < 5 on 5-HT_{2A}R. The 4-acetamido derivative **12k** also showed high selectivity (5-HT_{2A}, pK_i = 5.5).

Effect on the hERG Ion Channel. Our ligands are lipophilic molecules with a basic site, and these structural features are often associated with hERG inhibition.[42] We therefore carried out a functional hERG/Kv11.1 cellular assay on selected compounds using **1** as a reference compound (100% at 10 μM).[38] The hERG inhibitions shown as percent inhibition at 1 μM or IC_{50} values are reported in Tables 1–4, together with the calculated values of log *D* (pH 7.4) (Tables 3 and 4). log $D_{(7.4)}$ (octanol/water) was selected as a measure of hydrophobicity since it considers the predominance of protonated species or zwitterions at pH 7.4. The results show that in general there is a correlation between the lipophilicity and the hERG activity.[42] Within the indazole series, neither the Me_2N– or $MeSO_2NH$– groups on the alkyl chain terminal moiety nor the –OH and $-NO_2$ groups on the 4-position of the phenyl ring led to a significant reduction of hERG inhibition activity (see compounds **11v,w,y,z**). On the other hand, the morpholine and aniline derivatives **11u** and **11aa** (IC_{50} = 32.5 and 22.6 μM) showed a reduction in inhibition. The best result was obtained with carboxylic acid derivative **11ab**, which was inactive in the hERG assay at the highest concentration tested (100 μM). The use of carboxylic acid groups to mitigate hERG has been previously reported.[43,44]

Unfortunately, the newly synthesized 2*H*-pyrrolo[3,4-*c*]-quinoline derivatives generally exhibited high affinity for the hERG channel. All tested compounds showed IC_{50} values ranging from 0.01 to 1.22 μM, including morpholine and aniline derivatives **12e,f**. One notable exception was carboxylic acid derivative **12g** (IC_{50} = 9.96 μM). The high hERG affinity found in this chemical series could be attributed to the higher lipophilicity of these compounds, which have log $D_{(7.4)}$ values almost 1 unit higher than those of their *N*-(4-piperidinylmethyl)-1-isopropyl-1*H*-indazole-3-carboxamide counterparts (compare log $D_{(7.4)}$ values of **12c,e–g** with those of **11m,u,aa,ab**, respectively). Only the introduction of the carboxylic acid group (**12g**) significantly decreased the affinity for the hERG ion channel.

Taking into account the interesting in vitro properties of the carboxylates **11ab** and **12g** (high affinity for 5-HT_4R and good selectivity toward the 5-HT_{2A}R and hERG channel), we decided to further investigate their pharmacological profile with several biologically relevant receptors and enzymes.[45] Compound **11ab** did not show any significant interaction (<50% inhibition at 100 μM) for all the biological targets assayed, whereas compound **12g** shows only very weak binding affinity for σ1 and σ2 receptors, with 58% and 68% inhibition at 10 μM, respectively (Table 2 in the Supporting Information).

5-HT_4R Antagonism. The 5-HT_4R antagonism was tested for a few of the most interesting derivatives. Compounds **11b,v,x,y,aa** lacked any intrinsic activity on intracellular basal Ca^{2+} levels in the HEK293 cellular functional assay, whereas they antagonized (1 μM) 5-HT-induced intracellular ion levels (Supporting Information). The same result was also obtained for **11y** and **11aa** in an intracellular cAMP level functional assay for human 5-HT_4R (Supporting Information). The most interesting compounds **11ab** and **12g** showed full receptor antagonism in the cAMP-related functional assay on human recombinant 5-$HT_{4(e)}$ receptor (Chinese hamster ovarian, CHO; **5** as reference antagonist compound), with IC_{50} values of 9.8 and 2.8 nM and K_b values of 1.4 and 0.41 nM, respectively (**5**, IC_{50} = 0.21 nM, K_b = 0.03 nM) (Supporting Information).

Pharmacokinetic Studies. **11ab** and **12g** were tested in an in vitro liver microsome assay from five different species (rat, dog, miniature pig, monkey, and human) (Table 5). Intrinsic clearance was also calculated for rat and human microsomes (Table 6). Both compounds showed high metabolic stability in all the tested species, with the exception of **12g** in cynomolgus monkey liver microsomes, where it showed low and moderate stability at 1 and 10 μM, respectively. This metabolic behavior could possibly be attributed to the presence of a specific cytochrome P450 in monkey (CYP2C76), where compound **12g** could be metabolized, as observed for other compounds such as tolbutamide and testoterone.[46] The low values of intrinsic clearance for **11ab** (3.9 and 2.5 mL/min/kg, respectively) and **12g** (8.32 and 0.65 mL/min/kg, respectively) supported the selection of these compounds for further in vivo evaluation.[47]

Antinociceptive Effect. The results reported in the literature for 5-HT_4R antagonists in animal models of analgesia[16–21] prompted us to assay **11ab** and **12g** in two standard antinociceptive assays in rats: the hot plate test and the formalin test.[48,49] The maximal dose for *in vivo* studies (10 mg/kg) was selected according to preliminary behavioral and toxicological results obtained in the Irwin test on male rats after a single oral dose administration of compound (data not shown).[50,51]

Oral administration of **11ab** in the hot plate test produced a weak, but significant nociceptive response at the highest dose tested (20% increase of latency at 10 mg/kg, p < 0.05 vs vehicle group). Rats treated with **12g** in the same experiment showed a significant latency increase of antinociceptive response at 5 and

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Table 5. Metabolic Stability of 11ab and 12g in Pooled Liver Microsomes from Different Species

	percentage of compd remaining			
	11ab[a]		**12g**[a]	
conc (μM)	30 min	60 min	30 min	60 min
	Human			
1	101	103	104	99
10	99	99	106	99
	Mini-Pig			
1	110	109	101	96
10	106	99	102	102
	Cynomolgus Monkey			
1	91	86	9	0.4
10	93	87	80	61
	Beagle Dog			
1	97	93	115	107
10	101	97	98	94
	Rat			
1	86	85	87	79
10	87	91	93	89
	Mouse			
1	92	97	85	79
10	100	104	96	97

[a]Mean values from three experiments. Warfarin, propranolol, and testosterone incubated as a cocktail were used as a positive control.

Table 6. Evaluation of the in Vitro Cross-Species Intrinsic Clearance with Rat and Human Liver Microsomes for 11ab and 12g

	CL_{int} (μL/min/mg)[a]		CL_{int} (μL/min/kg)[b]	
compd	rat	human	rat	human
11ab	2.0	2.3	3.9	2.5
12g	4.2	0.6	8.3	0.7

[a]Mean values from three experiments. Midazolam and propranolol were used as a positive control. [b]Scale-up factors for microsomes used are 45 mg of liver/g of body mass and 44 and 24 g of liver/kg of body mass for rat and human, respectively.

10 mg/kg ($p < 0.05$ vs vehicle group) with an improvement of 39% and 26%, respectively. Morphine, which was used as a reference drug, produced a significant antinociceptive response ($p < 0.05$ vs vehicle group) at 6 mg/kg with a 67% latency increase (Table 7).

The results obtained in the formalin test are reported in Figure 4. Compounds **11ab** and **12g** orally administered at 10 mg/kg did not inhibit the paw licking in mice during the first phase of the model (0−10 min), when there is a direct effect on nociceptors, whereas a significant analgesic effect was observed in the second phase of the experiment (panel A, 10−40 min, $p < 0.05$ vs vehicle group), when an inflammatory response is present. Analysis of the area under the curve ($AUC_{0-40'}$) confirmed a statistically significant analgesic activity following administration of **12g** (Figure 4B, $p < 0.05$ vs vehicle group). As expected, morphine at 3 mg/kg significantly inhibited the licking time in both the early and late phases (data not shown).

CONCLUSIONS

A VS computational approach performed on our in-house library led us to identify hit compounds **11b** and **12a** as new 5-HT_4R ligands. The activity of these hits was confirmed in in vitro assays on human 5-HT_4R. A number of derivatives of both **11b** and **12a** were designed, synthesized, and tested to define the SAR in these new classes of ligands. Potent ligands were found in both series. Selectivity over undesirable biological targets such as 5-HT_{2A}R and hERG was required, so screening on these off-target proteins was performed to find compounds with the highest 5-HT_4R binding affinity and the lowest interaction with the 5-HT_{2A}R and hERG potassium ion channel. Introduction of large lipophilic or weak polar terminal groups on the basic site of the hit compounds of both series led to ligands with high 5-HT_4R affinities. However, among these, only derivatives with a carboxylate function on the phenethyl moiety (compounds **11ab** and **12g**) showed good selectivity over 5-HT_{2A} and the hERG potassium ion channel. Therefore, **11ab** and **12g** were further studied in vitro (i) in a functional test of 5-HT_4 antagonism, (ii) for their selectivity toward a large panel of biological targets, and (iii) to assess their metabolic stability in several species. Both compounds were potent antagonists with a good selectivity profile and metabolic stability. Finally, **12g** showed promising pharmacological properties in in vivo models of analgesia (hot plate and formalin tests), demonstrating significant antinociceptive activity after oral administration. Therefore, we considered **12g** as a candidate for preclinical studies as a potent and selective 5-HT_4 antagonist with excellent analgesic properties.

Table 7. Antinociceptive Activity of 11ab, 12g, and Morphine in the Hot Plate Test after Oral Administration in Rats

	dose (mg/kg, po)	latency[a] (s)
vehicle		3.3 ± 0.15
11ab	0.5	3.8 ± 0.13
	1	3.7 ± 0.15
	5	3.7 ± 0.20
	10	4.1 ± 0.21[b]
12g	0.5	3.3 ± 0.15
	1	3.6 ± 0.28
	5	4.4 ± 0.22[b]
	10	4.0 ± 0.22[b]
morphine	6	5.3 ± 0.34[b]

[a]Latency was measured 1 h postdosing. [b]$p < 0.05$ vs vehicle group by analysis of variance (ANOVA) followed by Dunnet's test for multiple comparisons.

EXPERIMENTAL SECTION

Computational Studies. The computational study was performed using the Schrödinger suite (www.schrodinger.com). Active compounds were extracted from the literature[9,14,15] and used as a basic set for pharmacophore modeling. 3D structures of the ligands were first generated by means of Macromodel (version 9.1, 2006, Schrödinger LLC) and then minimized using the OPLS-2005 force field (version 2.0, 2006, Schrödinger LLC) in a continuum dielectric model with a dielectric constant of 1.0. Molecules were kept in their neutral form throughout the process. LigPrep (version 2.0, 2006, Schrödinger LLC) was then used to generate stereoisomers and the most probable ionization states at pH 7 ± 2. Conformers were generated using a maximum of 1000 steps of rapid ConfGen sampling, followed by up to 5000 iterations of truncated Newton conjugate gradient minimization. The OPLS-2005 force field with distance-dependent dielectric solvation treatment was employed. Each minimized conformer was filtered through a relative energy window of 10 kcal/mol and a redundancy check of 1 Å in the heavy atom positions. Pharmacophore development was carried out using PHASE,[52] where each ligand structure is represented by a series of chemical features (points) in the

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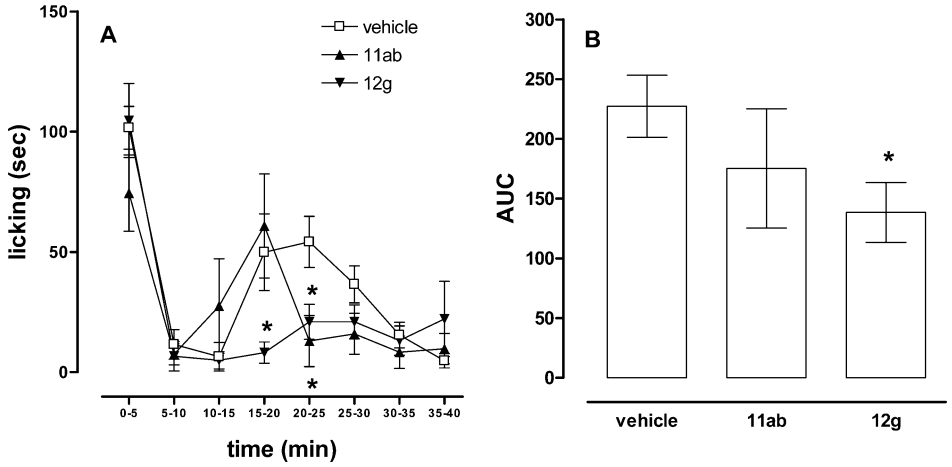

Figure 4. Effect of **11ab** and **12g** oral administration in the formalin test in mice: (A) time-course curve, (B) area under time-course curve (0−40 min). The asterisk indicates $p < 0.05$ vs vehicle group by ANOVA followed by Dunnet's test for multiple comparisons.

3D space, which set the noncovalent binding properties between the ligand and its target receptor. After applying default feature definitions to each ligand, we performed the common pharmacophore search using a terminal box size of 1 Å, and we required that all the actives should be matched. These pharmacophore sites are characterized by type, location, and, if applicable, directionality. PHASE provides six built-in types of pharmacophore features: hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic (H), negative ionizable (N), positive ionizable (P), and aromatic ring (R).

Pharmacophores with features common to all training set active compounds were identified and scored according to superposition of pharmacophore site points, alignment of vector characteristics, overlap of molecular volumes, and penalization of matches to the set of inactive compounds. Different 3D queries were generated and then used to discriminate true positives. The model was validated by means of the search of 5-HT_4 active compounds in a database composed of known actives (not used in the development of the model) and decoy ligands from Schrödinger.[53] The validated pharmacophoric model was then used to identify hits from the proprietary database. The proprietary database was first prepared using LigPrep to generate 3D structures, stereoisomers, tautomers, and ionization states. Then the structures were used to create a PHASE database, which entails the following steps: expanding structures into conformational ensembles and mapping pharmacophore features to each molecule. The phase database was the starting point for the ligand-based VS. When the pharmacophore hypothesis was used to filter the phase database, all features of the pharmacophore hypothesis were required to match. The distance tolerance was set to its default value of 2.0 Å in all pharmacophore searches. The pharmacophore matching score of PHASE was used in the VS procedure. The identified virtual hits were then submitted to the in vitro assay.

Chemistry. *General Procedures.* Reagents were purchased from Sigma-Aldrich and were used as received. Reaction progress was monitored by TLC using Merck silica gel 60 F_{254} (0.04−0.063 mm) with detection by UV (214 or 254 nm). Merck silica gel 60 or aluminum oxide 90 (active neutral) was used for column chromatography. Melting points (uncorrected) were determined in open Pyrex capillary tubes using a Buchi 510 melting point apparatus. The compounds' purities were always ≥95% determined by high-pressure liquid chromatography (HPLC). HPLC analysis was carried out with a pump/autosampler from Waters (2695-Alliance model), a UV photodiode array detector from Waters (2996 model), and a data management system from Waters (Empower 2). The column used was generally Suplex pkb-100 (250 × 4.6 mm, 5 μm). Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Bruker Avance system, operating at 300 or 400 MHz. All resonance bands were referenced to tetramethylsilane (internal standard). UV−vis spectra were recorded using a Perkin-Elmer (UV/vis) Lambda 25 spectrophotometer. The UV measurement was carried out using matched 1 cm quartz cells and substance dissolved in 95° ethanol. Vibration infrared (IR) spectroscopy was performed using a Perkin-Elmer model FT-2000 infrared spectrophotometer equipped with a universal attenuated total reflectance (UATR) accessory. Elemental analysis was conducted by means of a CHNS-O EA1108 elemental analyzer, Carlo Erba Instruments, and the results were within ±0.4% of the theoretical values, unless otherwise noted. Ultraperformance liquid chromatography/quadrupole time-of-flight (UPLC/QToF) exact mass data were obtained by means of a SYNAPT MS−ACQUITY UPLC system, Waters. The system was operated in positive ion mode in the "V-Optics" configuration. Leucine enkephalin (200 pg/μL) was employed as the lock mass to provide authenticated exact mass measurement in MS and MS/MS modes within 5 ppm rms mass accuracy. The column was an Acquity BEH C18 (2.1 × 50 mm, 1.7 μm). Differential scanning calorimetry data were obtained on a Perkin-Elmer DSC7 differential scanning calorimeter.

1-[1-(Phenylmethyl)-4-piperidinyl]methylamine, 1-methyl-1*H*-indazole-3-carbonyl chloride (**13**), 1,5-dimethyl-1*H*-indazole-3-carboxylic acid (**17**), 2-methyl-2*H*-indazole-3-carboxylic acid (**20**), methyl 5-chloro-1*H*-indazole-3-carboxylate, 1-(1-butyl-4-piperidinyl)-methylamine, 1-[(2-phenylethyl)-4-piperidinyl]methylamine, 1*H*-indazole-3-carbonyl chloride, (2-bromoethyl)cyclohexane, *N*-(2-bromoethyl)methansulfonamide, 2-(4-hydroxyphenyl)ethyl bromide, ethyl 3-(2-nitrophenyl)propenoate, 1-butyl-4-piperidinemethanol, 1-(2-phenylethyl)-4-piperidinemethanol, 4-piperidinemethanol, 1-(phenylmethyl)-4-piperidinemethanol, 1-(2-bromoethyl)-4-nitrobenzene, *N*-[4-(2-bromoethyl)phenyl]acetamide, and 2-(4-chlorophenyl)ethyl bromide are commercially available. 1-Isopropyl-1*H*-indazole-3-carbonyl chloride (**14**),[31] ethyl 4-(2-bromoethyl)benzoate,[34] ethyl 4-(2-chloroethyl)benzoate,[36] 5-methoxy-1-methyl-1*H*-indazole-3-carboxylic acid (**18**),[32] and ethyl 3-(2-nitrophenyl)propanoate (**22**)[35] were prepared as previously described.

Syntheses. Specific examples presented below illustrate general synthetic procedures.

*1-Isopropyl-N-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11q**).* 1-Isopropyl-1*H*-indazole-3-carbonyl chloride (**14**;[31] 9.3 g, 42 mmol) was added in portions into a well-stirred suspension of 1-[1-(phenylmethyl)-4-piperidinyl]methylamine (8.6 g, 42 mmol) in toluene (100 mL) at room temperature. After being stirred for 12 h, the mixture was filtered, and the crude product obtained was recrystallized from isopropyl alcohol/water (50:1) to achieve **11q**. This compound was treated with 2.5 N HCl in ethanol to furnish the corresponding hydrochloride, which was recrystallized from *n*-hexane/ethyl acetate (7:3; 31%, 109−111 °C). IR (KBr): ν 3498.24, 2931.81, 2538.38, 1630.07, 1543.92, 1455.10, 1203.76, 947.24, 763.94 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.55 (d, J = 7 Hz, 6H), 1.10−2.25 (m, 5H), 2.80−3.80 (m, 10H), 4.0−4.6 (m, 2H), 5.08

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(sept, J = 7 Hz, 1H), 7.0−8.0 (m, 8H), 8.05−8.70 (m, 2H), 11.04 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.84, 26.61, 33.98, 43.21, 50.20, 51.13, 58.88, 110.23, 121.77, 122.18, 126.22, 128.58, 129.22, 129.89, 131.37, 136.67, 139.49, 162.21. Anal. ($C_{24}H_{30}N_4O$·HCl·2H_2O) C, H, N, Cl.

*N-[[1-(2-Phenylethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11l**).* **11l** was prepared according to the procedure used for compound **11q** starting from commercially available 1*H*-indazole-3-carbonyl chloride using 1-[(2-phenylethyl)-4-piperidinyl]methylamine as reactant. **11l**·HCl was recrystallized from ethyl acetate/ethanol (33%, 192−194 °C). IR (KBr): ν 2927.12, 2552.32, 1650.83, 1549.71, 1469.81, 1232.18, 1154.07, 954.45, 763.04 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 13.83 (s, 1H), 10.93 (br s, 1H), 8.60 (t, J = 5.72 Hz, 1H), 8.21 (d, J = 7.63 Hz, 1H), 7.65 (d, J = 8.00 Hz, 1H), 7.02−7.54 (m, 7H), 2.64−4.04 (m, 11H), 1.26−2.29 (m, 5H). ^{13}C NMR (DMSO-d_6): δ 26.87, 29.31, 33.94, 43.13, 51.42, 56.58, 110.64, 121.50, 121.88, 126.36, 126.66, 128.56, 137.25, 141.05, 162.50. Anal. ($C_{22}H_{26}N_4O$·HCl·$^1/_3H_2O$) C, H, N, Cl.

*N-[[1-(Phenylmethyl)-4-piperidinyl]methyl]-1-methyl-1H-indazole-3-carboxamide (**15**).* **15** was prepared according to the procedure used for compound **11q** starting from commercially available 1-methyl-1*H*-indazole-3-carbonyl chloride (**13**) using 1-[1-(phenylmethyl)-4-piperidinyl]methylamine as reactant. **15**·HCl was obtained (71%, 247−248 °C). IR (KBr): ν 3398.6, 2918.29, 2488.17, 1658.98, 1540.34, 1495.21, 1432.32, 1227.29, 1168.33, 747.70, 700.79 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.20−2.20 (m, 5H), 2.63−3.68 (m, 6H), 4.13 (s, 3H), 4.12−4.36 (m, 2H), 7.26−7.81 (m, 8H), 8.15−8.25 (m, 1H), 8.40−8.75 (m, 1H), 11.10 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 23.01, 26.56, 33.89, 35.79, 43.13, 47.37, 51.13, 58.90, 110.24, 121.68, 122.17, 126.46, 128.60, 129.23, 129.87, 131.35, 136.77, 140.84, 162.00. Anal. ($C_{22}H_{26}N_4O$·HCl) C, H, N, Cl.

*N-[(1-Butyl-4-piperidinyl)methyl]-1,5-dimethyl-1H-indazole-3-carboxamide (**11h**).* Thionyl chloride (4 mL, 54 mmol) was added to a stirred solution of the commercially available **17** (5.1 g, 29.6 mmol) in toluene, and the mixture was stirred under reflux for 2 h. After removal of the solvent under vacuum, the residue was recrystallized from *n*-hexane to give 3.5 g of 1,5-dimethyl-1*H*-indazol-3-carbonyl chloride. 1-(1-Butyl-4-piperidinyl)methylamine (2.4 g, 14 mmol) in toluene (30 mL) was added dropwise to a suspension in toluene (30 mL) of 2.9 g of the intermediate (14 mmol). The mixture was stirred for 3 h at room temperature. The solid was filtered and dissolved in H_2O. The solution was treated with 6 N NaOH solution until pH 8 and extracted with CH_2Cl_2 (2 × 200 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH (95:5) as eluent) and then treated with 2.5 N HCl in ethanol to give the corresponding hydrochloride. **11h**·HCl was recrystallized from isopropyl ether/isopropyl alcohol (4 g, 75%, 212−213 °C). IR (KBr): ν 3421.06, 2957.55, 2496.59, 1659.87, 1538.80, 1500.34, 1459.45, 1181.06, 1163.97, 804.76 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 10.54 (br s, 1H), 8.43 (t, J = 6.11 Hz, 1H), 7.89−7.99 (m, 1H), 7.62 (d, J = 8.59 Hz, 1H), 7.30 (dd, J = 1.49, 8.75 Hz, 1H), 4.10 (s, 3H), 3.29−3.52 (m, 2H), 3.21 (t, J = 6.28 Hz, 2H), 2.70−3.11 (m, 4H), 2.44 (s, 3H), 1.83 (d, J = 12.88 Hz, 3H), 1.48−1.76 (m, 4H), 1.30 (m, J = 7.40 Hz, 2H), 0.90 (t, J = 7.20 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 13.42, 19.48, 20.97, 23.17, 25.01, 26.78, 31.18, 33.97, 35.82, 43.10, 47.72, 51.33, 55.58, 109.94, 120.58, 122.49, 128.46, 131.26, 136.13, 139.64, 162.10. Anal. ($C_{20}H_{30}N_4O$·HCl) C, H, N, Cl.

*N-[(1-Butyl-4-piperidinyl)methyl]-5-methoxy-1-methyl-1H-indazole-3-carboxamide (**11i**).* **11i** was prepared according to the procedure described for **11h** starting from **18**.[32] Crude **11i** was then converted to the corresponding oxalic salt by treatment with oxalic acid in ethanol (29%, 191−192 °C). IR (KBr): ν 3410.07, 2944.72, 2677.99, 1720.71, 1655.28, 1541.25, 1497.68, 1271.15, 1206.59, 814.91, 707.69 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 9.21 (br s, 2H), 8.41 (t, J = 6.14 Hz, 1H), 7.65 (d, J = 9.35 Hz, 1H), 7.56 (d, J = 2.05 Hz, 1H), 7.11 (dd, J = 2.48, 9.21 Hz, 1H), 4.10 (s, 3H), 3.81 (s, 3H), 3.41 (d, J = 11.98 Hz, 2H), 3.23 (t, J = 6.28 Hz, 2H), 2.91−3.03 (m, 2H), 2.84 (t, J = 11.55 Hz, 2H), 1.84 (d, J = 12.57 Hz, 3H), 1.38−1.70 (m, 4H), 1.30 (m, J = 7.50 Hz, 2H), 0.90 (t, J = 7.20 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 13.43, 19.45, 25.31, 26.64, 33.63, 35.99, 42.77, 51.09, 55.27, 100.42, 111.46, 118.68, 122.82, 135.85, 136.82, 155.34, 162.26, 164.51. Anal. ($C_{20}H_{30}N_4O_2$·$C_2H_2O_4$·$^1/_2H_2O$) C, H, N, Cl.

*N-[(1-Butyl-4-piperidinyl)methyl]-5-chloro-1-methyl-1H-indazole-3-carboxamide (**11j**).* **11j** was prepared according to the procedure described for **11h** starting from **19**. Crude **11j** was then treated with 2.5 N HCl in ethanol to obtain the corresponding hydrochloride (22%, 250−251 °C). IR (KBr): ν 3418.18, 2933.70, 2496.57, 1656.26, 1536.17, 1480.92, 1213.02, 806.84 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 10.35 (br s, 1H), 8.58 (t, J = 5.94 Hz, 1H), 8.15 (d, J = 1.98 Hz, 1H), 7.82 (d, J = 8.92 Hz, 1H), 7.50 (dd, J = 1.98, 8.92 Hz, 1H), 4.15 (s, 3H), 3.44 (d, J = 11.56 Hz, 2H), 3.21 (t, J = 6.28 Hz, 2H), 2.71−3.12 (m, 4H), 1.76−2.05 (m, 3H), 1.47−1.75 (m, 4H), 1.31 (m, J = 7.30 Hz, 2H), 0.90 (t, J = 7.30 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 13.42, 19.48, 23.14, 25.01, 26.76, 31.15, 33.87, 36.14, 43.18, 47.72, 51.31, 55.58, 112.36, 120.48, 122.88, 126.84, 136.30, 139.47, 161.59. Anal. ($C_{19}H_{27}N_4OCl$·HCl) C, H, N, Cl.

*N-[(1-Butyl-4-piperidinyl)methyl]-2-methyl-2H-indazole-3-carboxamide (**11k**).* **11k** was prepared according to the procedure described for **11h** starting from **20**. **11k** (5.8 g, 17.6 mmol) was converted to the maleate salt by treatment with maleic acid (2.1 g, 17.6 mmol) in ethanol (15 mL), which was recrystallized from ethyl acetate (40%, 136−137 °C). IR (KBr): ν 3318.37, 2958.21, 2687.75, 1654.57, 1533.95, 1362.15, 1227.22, 1047.89, 872.64, 760.12 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 19.99 (br s, 1H), 9.17 (br s, 1H), 8.67 (t, J = 5.44 Hz, 1H), 7.77 (dd, J = 7.42, 13.69 Hz, 2H), 7.00−7.49 (m, 2H), 6.06 (s, 2H), 4.31 (s, 3H), 2.67−3.72 (m, 8H), 1.06−2.22 (m, 9H), 0.91 (t, J = 7.00 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 13.42, 19.37, 25.36, 27.13, 33.62, 39.92, 43.74, 51.62, 55.70, 117.22, 119.97, 120.32, 122.73, 125.76, 128.79, 136.00, 146.40, 159.86, 167.19. Anal. ($C_{19}H_{28}N_4O$·$C_4H_4O_4$) C, H, N, Cl.

*1-Methyl-N-(4-piperidinylmethyl)-1H-indazole-3-carboxamide (**11a**).* Compound **15** (6 g, 16.6 mmol) was dissolved in 312 mL of ethanol/acetic acid (30:1.2), and the resulting solution was hydrogenated by a Parr apparatus (28 psi) in the presence of 10% Pd/C (2.4 g) for 24 h at room temperature. The catalyst was filtered off, the solvent was evaporated, and the residue was portioned between 2 N NaOH (100 mL) and CH_2Cl_2 (100 mL). The organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuum to obtain the crude product **11a**, which was directly converted to maleic salt by treatment with 0.66 g of maleic acid (5.7 mmol) dissolved in 25 mL of absolute ethanol, which was recrystallized from ethyl acetate/ethanol (1:1) to provide 1.0 g of salt (16%, 153−154 °C). IR (KBr): ν 3389.42, 2929.19, 1659.66, 1538.79, 1362.74, 1214.19, 1164.27, 867.19, 752.18 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.1−1.65 (m, 5H), 1.65−2.15 (m, 6H), 4.14 (s, 3H) 6.08 (s, 2H), 7.1−8.7 (m, 7H), 20.12 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 26.30, 33.66, 35.81, 42.98, 110.27, 121.68, 122.20, 126.49, 136.09, 136.77, 140.86, 162.06, 167.19. Anal. ($C_{15}H_{20}N_4O$·$C_4H_4O_4$) C, H, N, Cl.

*1-Isopropyl-N-(4-piperidinylmethyl)-1H-indazole-3-carboxamide (**16**).* **16** was prepared according to the procedure described for **11a**, starting from **11q**·HCl salt, 35 psi. Compound **16** was treated with 2.5 N HCl in ethanol solution to afford the corresponding hydrochloride, which was recrystallized from ethanol/ethyl acetate (42%, 211−214 °C). IR (KBr): ν 3315.32, 2912.86, 2784.55, 1658.70, 1541.40, 1279.82, 1203.35, 750.09, 691.72, 619.88 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.55 (d, J = 7 Hz, 6H), 1.31−2.18 (m, 5H), 2.58−3.64 (m, 6H), 5.09 (m, J = 7 Hz, 1H), 7.12−7.60 (m, 2H), 7.80 (d, J = 8 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 8.41 (t, J = 6 Hz, 1H), 8.82−9.60 (2 br s, 2H). Anal. ($C_{17}H_{24}N_4O$·HCl) C, H, N, Cl.

*2-Methyl-4-(4-piperidinylmethoxy)-2H-pyrrolo[3,4-c]quinoline (**12a**).* Pd/C (10%, 200 mg) was added into a solution of **29** (3.5 mmol, 1.34 g) in methanol (150 mL). The mixture was stirred for 4 days under a H_2 atmosphere at room temperature and pressure. A H_2 stream was passed through every 3 h, and Pd/C (10%, 200 mg) was added every 24 h. After the palladium was filtered off, the solvent was removed under reduced pressure to obtain a residue, which was purified by column chromatography on aluminum oxide (chloroform/methanol (2:1) as eluent) to obtain 410 mg of pure **12a** as an oil (40%). ^{1}H NMR ($CDCl_3$): δ 1.47−1.57 (m, 2H), 1.91−2.29 (m, 5H),

2.76 (m, 1H), 3.12 (m, 1H), 3.26 (m, 1H), 4.03 (s, 3H), 4.49 (d, 2H), 7.30−7.47 (m, 4H), 7.78 (m, 1H), 7.94 (m, 1H). Anal. ($C_{18}H_{21}N_3O$) C, H, N.

*N-[(1-Butyl-4-piperidinyl)methyl]-1-methyl-1H-indazole-3-carboxamide (**11b**).* 1-Bromobutane (3.75 g, 27 mmol) and K_2CO_3 (3.76 g, 27 mmol) were added to a solution of **11a** (3.6 g, 13 mmol) in 15 mL of ethanol. The reaction mixture was stirred under reflux for 2 h. The solid was filtered off, and the solution was concentrated under vacuum. The residue was diluted with ethyl acetate and treated with 1 N HCl solution. The aqueous layer was neutralized with 1 N NaOH solution until pH 8 was reached and then extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was filtered through a silica gel pad that was washed with $CHCl_3$. The crude product was converted to the corresponding hydrochloride in the presence of 2.5 N HCl in ethanol. The salt that was obtained was recrystallized from isopropyl alcohol (4.3 g, 91%, 194−195 °C). IR (KBr): ν 3343.52, 2931.41, 2486.99, 1650.42, 1546.64, 1433.36, 1227.18, 1173.28, 953.87, 754.06 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.94 (t, J = 7 Hz, 3H), 1.2−2.2 (m, 9H), 2.7−3.6 (m, 8H), 4.08 (s, 3H), 7.2−7.5 (m, 4H), 8.3 (d, J = 7 Hz, 1H), 10.5 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 13.42, 19.48, 25.00, 26.78, 33.92, 35.81, 43.17, 51.31, 55.58, 110.26, 121.68, 122.17, 126.46, 136.79, 140.84, 162.01. Anal. ($C_{19}H_{28}N_4O$·HCl) C, H, N, Cl.

*N-[[1-(2-Cyclohexylethyl)-4-piperidinyl]methyl]-1-methyl-1H-indazole-3-carboxamide (**11c**).* **11c** was prepared according to the procedure described for **11b** using (2-bromoethyl)cyclohexane as reactant. The crude product was treated with 2.5 N HCl in ethanol to furnish the corresponding hydrochloride, which was recrystallized from ethyl acetate/ethanol (8:2; 78%, 227−230 °C). IR (KBr): ν 3370.73, 2927.48, 2850.47, 2497.79, 1650.41, 1536.21, 1493.85, 1217.49, 1164.54, 945.18, 753.33 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.66 (br s, 1H), 8.57 (t, J = 5.50 Hz, 1H), 8.19 (d, J = 7.57 Hz, 1H), 7.75 (d, J = 8.00 Hz, 1H), 7.37−7.59 (m, 1H), 7.16−7.36 (m, 1H), 4.15 (s, 3H), 2.67−3.66 (m, 8H), 0.59−2.16 (m, 18H). ^{13}C NMR (DMSO-d_6): δ 23.15, 25.44, 25.83, 26.81, 30.14, 32.34, 33.92, 35.02, 35.81, 43.15, 47.72, 51.33, 54.15, 110.26, 121.68, 122.17, 126.46, 136.79, 140.84, 162.00. Anal. ($C_{23}H_{34}N_4O$·HCl) C, H, N, Cl.

*1-Methyl-N-[[1-(2-phenylethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11d**).* **11d** was prepared according to the procedure used for compound **11b** using (2-bromoethyl)benzene as reactant. The crude product was treated with 2.5 N HCl in ethanol to obtain the corresponding hydrochloride, which was recrystallized from ethyl acetate/ethanol (8:2; 82%, 219−220 °C). IR (KBr): ν 3412.96, 2938.59, 2511.29, 1676.74, 1540.82, 1496.40, 1545.13, 1219.78, 1170.78, 949.11, 746.37, 698.47 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.5−2.1 (m, 5H), 2.8−3.7 (m, 10H), 4.1 (s, 3H), 7.2−7.9 (m, 9H), 8.2 (d, J = 7 Hz, 1H), 8.6 (t, 1H). ^{13}C NMR (DMSO-d_6): δ 23.21, 26.85, 29.27, 33.91, 35.81, 43.15, 51.41, 56.55, 110.26, 121.70, 122.18, 126.48, 126.68, 128.56, 136.79, 137.23, 140.84, 162.03. Anal. ($C_{23}H_{28}N_4O$·HCl) C, H, N, Cl.

*N-[[1-[2-(4-Chlorophenyl)ethyl]-4-piperidinyl]methyl]-1-methyl-1H-indazole-3-carboxamide (**11f**).* **11f** was prepared according to the procedure used for compound **11b** using 2-(4-chlorophenyl)ethyl bromide as reactant. The crude product was treated with 2.5 N HCl in ethanol to furnish the corresponding hydrochloride, which was recrystallized from ethanol/water (58%, 245−246 °C). IR (KBr): ν 3402.56, 2945.21, 2332.05, 1661.10, 1534.41, 1493.17, 1215.08, 858.67, 771.02 cm^{-1}. ^1H NMR (DMSO-d_6): δ 11.08 (br s, 1H), 8.59 (t, J = 5.69 Hz, 1H), 8.20 (d, J = 7.66 Hz, 1H), 7.75 (d, J = 8.00 Hz, 1H), 7.14−7.59 (m, J = 3.00 Hz, 6H), 4.15 (s, 3H), 2.63−3.74 (m, 10H), 1.33−2.23 (m, 5H). ^{13}C NMR (DMSO-d_6): δ 26.94, 28.68, 33.98, 35.91, 38.79, 39.06, 39.34, 39.61, 39.89, 40.18, 40.45, 43.24, 51.54, 56.34, 110.36, 121.78, 122.29, 126.58, 128.61, 130.60, 131.45, 136.37, 136.89, 140.94, 162.13. Anal. ($C_{23}H_{27}N_4ClO$·HCl) C, H, N, Cl.

*1-Methyl-N-[[1-(4-phenylbutyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11g**).* **11g** was prepared according to the procedure used for compound **11b** using 4-phenylbutyl bromide as reactant. The obtained crude product was reacted with oxalic acid in ethanol to give the corresponding oxalic salt, which was recrystallized from ethyl acetate/ethanol (18%, 170−171 °C). IR (KBr): ν 3407.39, 2926.15, 1653.92, 1542.02, 1494.36, 1404.81, 1222.82, 1166.49, 753.52, 720.00 cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.36 (br s, 2H), 8.54 (t, J = 5.62 Hz, 1H), 8.19 (d, J = 7.81 Hz, 1H), 7.74 (d, J = 8.00 Hz, 1H), 7.47 (dt, J = 1.22, J = 7.57 Hz, 1H), 7.04−7.35 (m, 6H), 4.13 (s, 3H), 2.33−3.63 (m, 10H), 1.07−2.07 (m, 9H). ^{13}C NMR (DMSO-d_6): δ 22.98, 26.59, 28.01, 33.55, 34.45, 35.79, 42.77, 51.09, 55.40, 110.26, 121.68, 122.17, 125.73, 126.46, 128.21, 136.79, 140.84, 141.54, 162.01, 164.46. Anal. ($C_{25}H_{32}N_4O·C_2H_2O_4$) C, H, N, Cl.

*1-Isopropyl-N-[[1-(2-phenylethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11m**).* **11m** was prepared according to the procedure described for **11b** starting from **16** using 1-[1-(2-phenylethyl)-4-piperidinyl]methylamine as reactant. **11m** was purified by flash chromatography on silica gel ($CHCl_3$/MeOH (95:5) as eluent) and then treated with 2.5 N HCl in EtOH solution to afford the corresponding hydrochloride (72%, 211−212 °C). IR (KBr): ν 3331.53, 2945.48, 2558.39, 1652.94, 1546.02, 1206.58, 942.45, 743.17 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.56 (d, J = 7 Hz, 6H), 1.50−2.30 (m, 5H), 2.70−3.90 (m, 10H), 5.10 (m, J = 7 Hz, 1H), 7.05−7.63 (m, 7H), 7.81 (d, J = 8 Hz, 1H), 8.21 (d, J = 8 Hz, 1 H), 8.47 (t, J = 6 Hz, 1H), 11.05 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.86, 26.90, 29.27, 33.98, 43.24, 50.22, 51.41, 56.57, 110.26, 121.79, 122.20, 126.23, 126.68, 128.56, 136.67, 137.26, 139.52, 162.22. Anal. ($C_{25}H_{32}N_4O$·HCl) C, H, N, Cl.

*N-[[1-(2-Cyclohexylethyl)-4-piperidinyl]methyl]-1-isopropyl-1H-indazole-3-carboxamide (**11t**).* **11t** was prepared according to the procedure described for **11b** starting from **16** using (2-bromoethyl)-cyclohexane as reactant. The crude **11t** was treated with 2.5 N HCl in ethanol to afford the corresponding hydrochloride, which was recrystallized from ethyl acetate/ethanol (36%, 244−246 °C dec). IR (KBr): ν 3329.19, 2922.70, 2552.33, 1655.91, 1544.81, 1441.61, 1207.91, 942.82, 738.08 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.55 (d, J = 7 Hz, 6H), 0.68−2.18 (m, 17H), 2.63−3.70 (m, 10H), 5.09 (m, J = 7 Hz, 1H), 7.12−7.60 (m, 2H), 7.80 (d, J = 8 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 8.41 (t, J = 6 Hz, 1H), 10.70 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.83, 25.42, 25.82, 26.82, 30.11, 32.32, 33.98, 35.00, 43.23, 50.19, 51.31, 54.13, 110.23, 121.76, 122.15, 126.20, 136.65, 139.49, 162.18. Anal. ($C_{25}H_{38}N_4O$·HCl·$^1/_2H_2O$) C, H, N, Cl.

*1-Isopropyl-N-[[1-(2-morpholin-4-ylethyl]-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11u**).* **11u** was prepared according to the procedure described for **11b** starting from **16** using 4-(2-chloroethyl)morpholine as reactant. The crude **11u** was treated with 2.5 N HCl in ethanol to give the corresponding dihydrochloride, which was recrystallized from ethanol (63%, 266−267 °C dec). IR (KBr): ν 3375.90, 2934.70, 2448.48, 1652.35, 1539.86, 1455.66, 1203.01, 1133.42, 1100.85, 754.59 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.55 (d, J = 7 Hz, 6H), 1.30−2.25 (m, 5H), 2.75−4.30 (m, 19H), 5.09 (m, J = 7 Hz, 1H), 7.12−7.60 (m, 2H), 7.81 (d, J = 8 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 8.45 (t, J = 6 Hz, 1H), 10.80 (br s, 1H), 10.60 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.86, 26.84, 33.68, 43.17, 49.17, 49.65, 50.20, 51.33, 51.95, 63.13, 110.26, 121.77, 122.20, 126.23, 136.64, 139.50, 162.22. Anal. ($C_{23}H_{35}N_5O_2$·2HCl·$^1/_2H_2O$) C, H, N, Cl.

*N-[[1-[3-(Dimethylamino)propyl]-4-piperidinyl]methyl]-1-isopropyl-1H-indazole-3-carboxamide (**11v**).* **11v** was prepared according to the procedure described for **11b** starting from **16** (480 mg, 1.6 mmol) using *N*-(3-chloropropyl)-*N*,*N*-dimethylamine hydrochloride (580 mg, 3.7 mmol) as reactant. Compound **11v** was treated with maleic acid in ethanol to afford the corresponding dimaleate, which was recrystallized from ethanol (840 mg, 84%, 155−156 °C). IR (KBr): ν 3433.88, 2934.55, 2697.84, 1572.28, 1385.77, 1200.30, 1005.05, 875.99, 864.97, 751.10 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.55 (d, J = 7 Hz, 6H), 1.20−1.60 (m, 2H), 1.68−2.28 (m, 5H), 2.81 (s, 6H), 2.75−3.75 (m, 13H), 5.09 (sept, J = 7 Hz, 1H), 6.09 (s, 4H), 7.12−7.60 (m, 2H), 7.81 (d, J = 8 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 8.45 (t, J = 6 Hz, 1H), 19 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 19.13, 21.86, 26.82, 33.54, 42.36, 42.71, 50.20, 51.45, 52.67, 53.92, 110.26, 121.74, 122.21, 126.26, 135.75, 136.64, 139.52, 162.27, 167.17. Anal. ($C_{22}H_{35}N_5O·C_8H_8O_8·^1/_2H_2O$) C, H, N.

*1-Isopropyl-N-[[1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11w**).* **11w** was

prepared according to the procedure described for **11b** starting from **16** (4.8 g, 16 mmol) using *N*-(2-bromoethyl)methanesulfonamide (3.4 g, 16.8 mmol) as reactant. The compound was treated with 2.5 N HCl in ethanol solution to furnish the corresponding hydrochloride, which was recrystallized with ethyl acetate/ethanol (1.5 g, 20%, 186−187 °C dec). IR (KBr): ν 3341.98, 2938.43, 2543.75, 1651.59, 1542.67, 1325.86, 1208.22, 1157.42, 967.42, 750.30 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.55 (d, J = 7 Hz, 6H), 1.40−2.30 (m, 5H), 3.00 (s, 3H), 2.75−3.80 (m, 10H), 5.09 (m, J = 7 Hz, 1H), 7.12−7.70 (m, 3H), 7.80 (d, J = 8 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 8.45 (t, J = 6 Hz, 1H), 10.73 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.86, 26.78, 33.75, 36.98, 39.30, 43.20, 50.20, 51.79, 55.53, 110.24, 121.77, 122.18, 122.24, 126.23, 136.65, 139.50, 162.21. Anal. ($C_{20}H_{31}N_5O_3S$·HCl) C, H, N, Cl, S.

*1-Isopropyl-N-[[1-[2-(4-hydroxyphenyl)ethyl]-4-piperidinyl]-methyl]-1H-indazole-3-carboxamide (**11y**).* **11y** was prepared according to the procedure described for **11b** starting from **16** using 2-(4-hydroxyphenyl)ethyl bromide as reactant. Crude **11y** was treated with 2.5 N HCl in ethanol to give the corresponding hydrochloride, which was crystallized from absolute ethanol (29%, 218−220 °C). IR (KBr): ν 3167.62, 2511.31, 1632.00, 1557.96, 1515.87, 1457.54, 1206.14, 833.57, 746.65 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1. 55 (d, J = 7 Hz, 6H), 1. 63−2.15 (m, 5H), 2.70−3.75 (m, 10H), 5.09 (m, J = 7 Hz, 1H), 6.75 (d, J = 8 Hz, 2H), 7.06 (d, J = 8 Hz, 2H), 7.21−7. Thirty (m, 1 H), 7.40−7.50 (m, 1 H), 7.8 (d, J = 8 Hz, 1H), 8.21 (d, J = 8 Hz, 1H), 8.46 (m, 1H), 9.40 (s, 1H), 10.80 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.86, 26.91, 28.50, 33.97, 43.21, 50.22, 51.44, 57.00, 110.24, 115.36, 121.77, 122.20, 126.23, 126.98, 129.48, 136.67, 139.50, 156.15, 162.22. Anal. ($C_{25}H_{32}N_4O_2$·HCl) C, H, N, Cl.

*1-Isopropyl-N-[[1-[2-(4-nitrophenyl)ethyl]-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11z**).* **11z** was prepared according to the procedure described for **11b** starting from **16** using 1-(2-bromoethyl)-4-nitrobenzene as reactant. The product was purified by flash chromatography on silica gel (ethyl acetate as eluent). Pure **11z** was treated with a stoichiometric amount of oxalic acid in ethyl acetate to generate the corresponding oxalate, which was recrystallized twice from ethyl acetate/ethanol (9:1; 39%, 98 °C dec). IR (diffuse reflectance with KBr): ν 3325.5, 2981.0, 2937.6, 1654.7, 1520.1, 1347.6, 1203.8, 855.8, 751.0, 707. 0 cm^{-1}. ^1H NMR (DMSO-d_6 + D_2O): δ 1.55 (d, J = 7 Hz, 6H), 1.44−1.66 (m, 2H), 1.83−2.02 (m, 3H), 2.98 (t, J = 12 Hz, 2H), 3.10−3. 40 (m, 6H), 3.55 (d, J = 12 Hz, 2H), 5.07 (m, J = 7 Hz, 1H), 7.28 (t, J = 8 Hz, 1H), 7.46 (t, J = 7 Hz, 1H), 7.59 (d, J = 9 Hz, 2H), 7.79 (d, J = 8 Hz, 1H), 8.11−8.26 (m, 3H), 8.42 (t, J = 6 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 21.86, 26.81, 29.58, 33.78, 42.92, 50.22, 51.25, 55.73, 110.24, 121.80, 122.20, 122.26, 123.58, 126.25, 130.06, 136.68, 139.52, 145.84, 146.38, 162.22, 164.60. Anal. ($C_{25}H_{31}N_5O_3$·$C_2H_2O_4$·$^1/_2H_2O$) C, H, N.

*1-Isopropyl-N-[[1-[3-(methylamino)-3-oxo-1-propyl]-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11x**).* A mixture of **16** (6.9 g, 23 mmol), 3-chloro-*N*-methylpropanamide (4.8 g, 35.4 mmol), and K_2CO_3 (10 g, 72 mmol) in DMF (100 mL) was stirred at 80 °C overnight. The cooled solution was poured into water and extracted with ethyl acetate. The organic layer was washed with 0.5 N NaOH, dried over Na_2SO_4, filtered, and concentrated under vacuum. Crude **11x** was treated with Et_2O/HCl to afford the corresponding hydrochloride, which was recrystallized from isopropyl alcohol/diisopropyl ether (5.2 g, 52%, 190−191.5 °C). IR (diffuse reflectance with KBr): ν 3474, 3352, 3203, 1638, 1541, 1383, 1263, 1207, 948, 832, 760, 642 cm^{-1}. ^1H NMR (DMSO-d_6): δ 10.31 (br s, 1H), 8.36 (t, J = 6.19 Hz, 1H), 8.17 (m, J = 0.93, 8.22 Hz, 1H), 8.10 (q, J = 4.30 Hz, 1H), 7.79 (d, J = 8.59 Hz, 1H), 7.43 (m, J = 1.16 Hz, J = 6.98 Hz, J = 8.38 Hz, 1H), 7.25 (m, J = 0.66 Hz, J = 6.98 Hz, J = 8.05 Hz, 1H), 5.08 (m, J = 6.61 Hz, 1H), 3.12−3.58 (m, 8H), 2.77−2.98 (m, 2H), 2.65 (t, J = 8.17 Hz, 2H), 2.59 (d, J = 4.46 Hz, 3H), 1.78−2.12 (m, 3H), 1.46−1.74 (m, 2H), 1.55 (d, J = 6.61 Hz, 6H). ^{13}C NMR (DMSO-d_6): δ 21.84, 25.47, 26.88, 29.29, 33.84, 43.17, 50.20, 51.50, 52.08, 110.24, 121.77, 122.18, 126.23, 136.65, 139.50, 162.21, 169.05. Anal. ($C_{21}H_{31}N_5O_2$·HCl·$^3/_4H_2O$) C, H, N, Cl.

*2-Methyl-4-[[1-[2-(4-nitrophenyl)ethyl]-4-piperidinyl]methoxy]-2H-pyrrolo[3,4-c]quinoline (**30**).* 2-(4-Nitrophenyl)ethyl bromide (2.6 mmol, 0.61 g) and K_2CO_3 (6.6 mmol, 0.91 g) were added into a solution of **12a** (2.2 mmol, 0.65 g) in DMF (5 mL). The mixture was stirred at 70 °C for 2 h and 45 min. After cooling, the mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (3 × 50 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give a crude product, which was purified by column chromatography on silica gel (chloroform/methanol (10:1) as eluent) to give 0.62 g of pure product (62%, 157−159 °C, recrystallized from toluene/cyclohexane). ^1H NMR ($CDCl_3$): δ 1.65 (m, 2H), 2.00 (m, 3H), 2.19 (m, 2H), 2.72 (t, J = 8.2 Hz, 2H), 3.02 (t, J = 8.2 Hz, 2H), 3.12 (m, 2H), 4.03 (s, 3H), 4.53 (d, 2H), 7.32−7.47 (m, 5H), 7.78 (m, 1H), 7.93 (m, J = 8.1 Hz, J = 1.1 Hz, 2H), 8.20 (d, J = 8.7 Hz, 2H). Anal. ($C_{26}H_{28}N_4O_3$) C, H, N.

*1-Methyl-N-[[1-(2-pyridin-2-ylethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11e**).* 2-Vinylpyridine (14.8 mmol, 1.6 mL) was added to a solution of **11a** (4 g, 14.7 mmol) in 1.8 mL of water/glacial acetic acid (4:5). The mixture was stirred under reflux for 5 h and then at room temperature overnight. The solution was treated with water and then with solid NaOH until pH 8 was reached. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was filtered through a silica gel pad that was washed with $CHCl_3$/MeOH (95:5). The crude **11e** was recrystallized from *n*-hexane/ethyl acetate to give 3.8 g of the title product. The dihydrochloride salt was prepared by treating **11e** with 0.1 N HCl in diethyl ether (3.3 mL) and was recrystallized from ethyl acetate/ethanol (95:5; 2.5 g, 35.5%, 214−215 °C). IR (KBr): ν 3386.36, 2598.25, 1655.28, 1619.82, 1538.55, 1232.88, 939.72, 777.15, 751.34 cm^{-1}. ^1H NMR (DMSO-d_6): δ 11.36 (br s, 1H), 8.82 (d, J = 4.64 Hz, 1H), 8.32−8.70 (m, 2H), 7.67−8.26 (m, 4H), 7.48 (dt, J = 1.22, 7.57 Hz, 1H), 7.17−7.36 (m, 1H), 4.15 (s, 3H), 2.70−3.95 (m, 11H), 1.30−2.45 (m, 5H). ^{13}C NMR (DMSO-d_6): δ 26.83, 28.19, 33.67, 35.91, 38.77, 39.05, 39.34, 39.61, 39.89, 40.16, 40.43, 43.15, 51.58, 54.16, 110.36, 121.78, 122.29, 124.72, 126.57, 136.88, 140.94, 143.55, 143.85, 153.48, 162.13. Anal. ($C_{22}H_{27}N_5O$·2HCl) C, H, N, Cl.

*1-Isopropyl-N-[[1-(2-pyridin-2-ylethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11r**).* **11r** was prepared according to the procedure described for **11e** starting from **16**. The residue was purified by flash chromatography on silica gel ($CHCl_3$/MeOH (97:3) as eluent) to yield **11r**, which was treated with 2.5 N HCl in ethanol solution to give the hydrochloride, which was recrystallized from ethyl acetate/ethanol (33%, 122−123 °C). IR (KBr): ν 3322.07, 2934.88, 2635.47, 1643.93, 1548.14, 1439.24, 1206.50, 941.32, 753.85 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.55 (d, J = 7 Hz, 6H), 1.68−2.30 (m, 5H), 2.80−3.78 (m, 12H), 5.10 (m, J = 7 Hz, 1H), 7.12−7.60 (m, 4H), 7.68−8.00 (m, 2H), 8.21 (d, J = 7 Hz, 1H), 8.33−8.70 (m, 2H), 11.05 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.86, 31.16, 50.20, 110.24, 121.77, 122.00, 122.18, 123.34, 126.23, 136.87, 139.50, 149.02, 157.00, 162.22. Anal. ($C_{24}H_{31}N_5O$·HCl·H_2O) C, H, N Cl.

*4-[[[(1-Isopropyl-1H-indazol-3-yl)carbonyl]amino]methyl]-1-methyl-1-(2-phenylethyl)piperidinium Iodide (**11s**).* Methyl iodide (0.6 mL, 10 mmol) was slowly added into a solution of **11m** (4 g, 9.9 mmol) in acetone (40 mL). The mixture was stirred at room temperature for 3 h and then filtered, and the solid that was obtained was recrystallized from ethyl acetate/ethanol (1:1; 1.4 g, 26%, 209−210 °C). ^1H NMR (DMSO-d_6): δ 8.48 (t, J = 5.67 Hz, 1H), 8.20 (d, J = 7.43 Hz, 1H), 7.82 (d, J = 8.20 Hz, 1H), 7.12−7.58 (m, 7H), 5.11 (m, J = 6.68 Hz, 1H), 2.87−3.93 (m, 13H), 1.36−2.23 (m, 5H), 1.56 (d, J = 6.65 Hz, 6H). ^{13}C NMR (DMSO-d_6): δ 21.86, 23.56,27.51, 33.46, 42.63, 43.93, 50.23, 59.51, 66.91, 110.26, 121.73, 122.20, 126.25, 126.87, 128.61, 128.96, 136.39, 136.67, 139.50, 162.29. IR (KBr): ν 3314.16, 2976.00, 1646.96, 1550.21, 1487.41, 1204.03, 1130.61, 930.35, 753.43, 710.40, 640.40 cm^{-1}. Anal. ($C_{26}H_{35}IN_4O$) C, H, N, I.

*2-Methyl-4-[[1-(2-morpholin-4-ylethyl)-4-piperidinyl]methoxy]-2H-pyrrolo[3,4-c]quinoline (**12e**).* 4-(2-Chloroethyl)morpholine hydrochloride (1.3 mmol, 240 mg) and sodium hydrogen carbonate (3.7 mmol, 310 mg) were added into a solution of **12a** (1.3 mmol, 380 mg) in absolute ethanol (12 mL). The mixture was stirred at reflux for 3 h and 15 min. After cooling, the solvent was removed under reduced pressure and the residue was taken up in water (50 mL) and extracted

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with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give 480 mg of pure **12e** (90%, oil). ^{1}H NMR ($CDCl_3$): δ 1.61 (m, 2H), 1.89−1.97 (m, 3H), 2.13 (m, 2H), 2.56 (m, 4H), 2.63 (m, 4H), 3.09 (m, 2H), 3.78 (m, 4H), 4.03 (s, 3H), 4.49 (d, 2H), 7.32−7.46 (m, 4H), 7.78 (m, 1H), 7.93 (m, 1H). Anal. ($C_{24}H_{32}N_4O_2$) C, H, N. The product was converted to the hydrochloride as described for the compound **12d** (41%, 190−192 °C, from isopropyl ether/isopropyl alcohol). ^{1}H NMR (DMSO-d_6): δ 1.71−2.01 (m, 2H), 2.16−2.48 (m, 3H), 2.94−3.14 (m, 4H), 3.19−3.39 (m, 4H), 3.57 (t, J = 7.31 Hz, 2H), 3.81 (d, J = 12.57 Hz, 2H), 3.97 (t, J = 4.38 Hz, 3H), 4.01 (s, 3H), 4.42 (d, J = 5.70 Hz, 2H), 7.42 (d, J = 1.75 Hz, 1H), 7.45−7.58 (m, 4H), 7.61 (s, 1H), 7.78 (d, J = 7.75 Hz, 1H).

*4-[2-[4-[[(2-Methyl-2H-pyrrolo[3,4-c]-4-quinolinyl)oxy]methyl]-1-piperidinyl]ethyl]benzenemethanol (**12i**).* Compound **33** (800 mg, 3.7 mmol) was added into a solution of **12a** (3.3 mmol, 990 mg) in 2-butanone (33.5 mL). The mixture was stirred at reflux for 30 min, and then triethylamine (130 mg, 1.29 mmol, 0.2 mL) was added. After the mixture was stirred for 2 h at the same temperature, a further portion of triethylamine (3.3 mmol, 0.33 g) was added. After 2 h the mixture was cooled at room temperature, diluted with water, and extracted with 2-butanone. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to give crude **12i**, which was purified by column chromatography on aluminum oxide (ethyl acetate as eluent) to obtain 0.67 g of pure **12i** (48.5%, 145−146 °C, from benzene/cyclohexane). ^{1}H NMR ($CDCl_3$): δ 1.31 (s, 1H), 1.64 (m, 2H), 1.84 (s, 1H), 1.99 (m, 3H), 2.11 (m, 1H), 2.64−2.68 (m, 2H), 2.87−2.92 (m, 2H), 3.14 (d, 2H), 4.03 (s, 3H), 4.53 (d, 2H), 4.72 (d, 2H), 7.26−7.28 (m, 2H), 7.32−7.38 (m, 5H), 7.44 (m, 1H), 7.78−7.80 (dd, 1H), 7.93 (dd, 1H). Anal. ($C_{27}H_{31}N_3O_2$) C, H, N.

*4-[[1-[2-[4-(Methoxymethyl)phenyl]ethyl]-4-piperidinyl]-methoxy]-2-methyl-2H-pyrrolo[3,4-c]quinoline (**12j**).* **12j** was prepared according to the procedure described for **12i** using **34** as alkylating agent (48.5%, 102−105 °C, from 2-propanol/*n*-hexane). ^{1}H NMR ($CDCl_3$: δ 1.61−1.68 (m, 2H), 1.96−1.99 (m, 3H), 2.11 (m, 2H), 2.66 (m, 2H), 2.89 (m, 2H), 3.13 (m, 2H), 3.44 (s, 3H), 4.03 (s, 3H), 4.48 (s, 2H), 4.51−4.54 (d, 2H), 7.25 (m, 2H), 7.32−7.34 (m, 3H), 7.36−7.39 (m, 2H), 7.42−7.45 (dd, 1H), 7.78−7.80 (dd, 1H), 7.92−7.94 (dd, 1H). Anal. ($C_{28}H_{33}N_3O_2$) C, H, N.

*4-[2-[4-[[(2-Methyl-2H-pyrrolo[3,4-c]-4-quinolinyl)oxy]methyl]-1-piperidinyl]ethyl]phenylacetamide (**12k**).* A mixture of **12a** (0.5 g, 1.7 mmol), 4-(2-bromoethyl)phenylacetamide (2.2 g, 9.0 mmol), NaI (9.0 mmol, 1.34 g), and triethylamine (0.9 g, 9.0 mmol) in 2-butanone (22 mL) was stirred at reflux for 12 h. After cooling, the mixture was poured into water (200 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were extracted with 1 N HCl (3 × 50 mL) to extract the final amine as a hydrochloride. The solid that formed and the acidic phases were combined and treated with sodium carbonate until pH 8 and then extracted again with ethyl acetate (3 × 50 mL). The organic layers were collected, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on aluminum oxide (ethyl acetate as eluent) to give 330 mg of pure **12k** (42.5%, 145−146 °C, from ethanol/hexane). ^{1}H NMR ($CDCl_3$): δ 1.64−1.76 (m, 6H), 1.99−2.02 (m, 2H), 2.23−2.24 (s, 3H), 2.93−2.95 (m, 2H), 2.97 (m, 2H), 3.26 (m, 2H), 4.04 (s, 3H), 4.53−4.54 (d, 2H), 7.18 (m, 1H), 7.22−7.24 (m, 2H), 7.35 (m, 1H), 7.39 (m, 4H), 7.77−7.79 (m, 1H), 7.93 (dd, 1H). Anal. ($C_{28}H_{32}N_4O_2$) C, H, N.

*Ethyl 4-[2-[4-[[(2-Methyl-2H-pyrrolo[3,4-c]-4-quinolinyl)oxy]-methyl]-1-piperidinyl]ethyl]benzoate (**31**).* **31** was obtained according to the procedure described for **12k** using ethyl 4-(2-chloroethyl)-benzoate[36] as reactant. The crude product was purified by column chromatography on aluminum oxide (chloroform as eluent) to give pure **31** (51%, brown oil). ^{1}H NMR ($CDCl_3$): δ 1.44 (t, 3H), 1.65 (m, 2H), 1.98 (m, 3H), 2.18 (m, 2H), 2.70 (t, 2H), 2.97 (t, 2H), 3.14 (m, 2H), 4.01 (s, 3H), 4.42 (q, 2H), 4.52 (d, 2H), 7.32−7.46 (m, 6H), 7.78 (dd, J = 8.1 Hz, J = 1.1 Hz, 1H), 7.92 (dd, J = 8.1 Hz, J = 1.1 Hz, 1H), 8.02 (d, J = 8.4 Hz, 2H). Anal. ($C_{29}H_{33}N_3O_3$) C, H, N.

*Methyl 2-[2-[4-[[(2-Methyl-2H-pyrrolo[3,4-c]-4-quinolinyl)oxy]-methyl]-1-piperidinyl]ethyl]benzoate (**32**).* **32** was obtained according to the procedure described for **12k** using **35** as alkylating agent. Crude **32** thus obtained was purified by column chromatography on silica gel (ethyl acetate as eluent) to give pure **32** (30%, yellow oil). ^{1}H NMR ($CDCl_3$): δ 1.28 (m, 1H), 1.65−1.68 (m, 1H), 1.96−1.98 (m, 3H), 2.26 (m, 2H), 2.70−2.74 (m, 2H), 3.17−3.29 (m, 4H), 3.92 (s, 3H), 3.99 (s, 3H), 4.50 (dd, 2H), 7.27−7.36 (m, 5H), 7.39−7.48 (m, 2H), 7.75 (dd, 1H), 7.88−7.92 (m, 2H). Anal. ($C_{28}H_{31}N_3O_3$) C, H, N.

*Ethyl 4-[2-[4-[[[(1-Isopropyl-1H-indazol-3-yl)carbonyl]amino]-methyl]-1-piperidinyl]ethyl]benzoate (**21**).* A solution of **16** (5.3 g, 17.6 mmol), ethyl 4-(2-bromoethyl)benzoate[34] (19 g, 74 mmol), potassium iodide (15.6 g, 94 mmol), and triethylamine (13.1 mL, 94 mmol) in 2-butanone (200 mL) was stirred under reflux for 48 h. The mixture was cooled to room temperature, poured into water (1.7 L), and extracted with ethyl acetate (3 × 350 mL). The organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude product was purified by column chromatography on aluminum oxide (Et_2O/*n*-hexane (1:1 → 7:3) as eluent) to obtain 3.5 g (42%) of the title compound, which was used for the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 1.32−1.51 (m, 2H), 1.38 (t, J = 7.16 Hz, 3H), 1.61 (d, J = 6.72 Hz, 6H), 1.64−1.91 (m 3H), 2.05 (m, J = 11.55 Hz, J = 2.34 Hz, 2H), 2.54−2.66 (m, 2H), 2.81−2.91 (m, 2H), 3.02 (d, J = 11.69 Hz, 2H), 3.42 (t, J = 6.43 Hz, 2H), 4.36 (q, J = 7.16 Hz, 2H), 4.87 (m, J = 6.72 Hz, 1H), 7.15 (br t, J = 6.28 Hz, 1H), 7.22−7.31 (m, 3H), 7.35−7.49 (m, 2H), 7.96 (d, J = 7.92 Hz, 2H), 8.39 (m, J = 8.18 Hz, J = 1.02 Hz, 1H). Anal. ($C_{28}H_{36}N_4O_3$) C, H, N.

*1-sec-Butyl-N-[[1-(2-phenylethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11n**).* **11l** (15.8 g, 44 mmol) was added into a stirred suspension of NaH (1.76 g, 60% suspension in mineral oil) in DMF (100 mL) cooled at 0 °C. The mixture was stirred at room temperature for 1 h, and then 2-bromobutane (6 mL, 44 mmol) was added. After 24 h at the same temperature, water (200 mL) was added, the solution was filtered, and the solid was dissolved in ethyl acetate. The residual inorganic material was filtered off, and the solution was concentrated under vacuum and treated with Et_2O/HCl. The hydrochloride that formed was recrystallized from ethanol/ethyl acetate (11.6 g, 65%, 235−237 °C). IR (KBr): ν 3327.21, 2930.72, 2545.81, 1650.95, 1545.41, 1489.88, 1200.85, 941.16, 748.85, 702.42 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 12.27 (br s, 1H), 8.33 (m, J = 0.98 Hz, J = 1.10 Hz, J = 7.93 Hz, 1H), 6.98−7.61 (m, 9H), 4.37−4.82 (m, 1H), 1.69−3.94 (m, 18H), 1.58 (d, J = 6.84 Hz, 3H), 0.79 (t, J = 7.30 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 10.68, 20.18, 26.90, 29.03, 29.27, 33.98, 43.27, 51.39, 55.91, 56.57, 110.23, 121.77, 122.00, 122.14, 126.25, 126.66, 128.56, 137.26, 140.43, 162.26. Anal. ($C_{26}H_{34}N_4O\cdot HCl\cdot{}^1/_2H_2O$) C, H, N, Cl.

*1-Isopentyl-N-[[1-(2-phenylethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11o**).* **11o** was prepared according to the procedure described for **11n** using isopentyl bromide as reactant. Crude **11o** was treated with 2.5 N HCl in ethanol solution to give the corresponding hydrochloride, which was recrystallized from isopropyl alcohol (25%, 217−218 °C). IR (KBr): ν 3369.1, 2924.6, 2553.2, 1655.4, 1542.4, 1440.0, 1183.1, 943.3, 754.9, 708.8 cm^{-1}. ^{1}H NMR (100 MHz, DMSO-d_6): δ 11.03 (br s, 1H), 8.51 (t, J = 5.66 Hz, 1H), 8.21 (d, J = 7.68 Hz, 1H), 7.77 (d, J = 8.20 Hz, 1H), 7.08−7.60 (m, 7H), 4.50 (t, J = 7.31 Hz, 2H), 2.63−4.04 (m, 10H), 1.26−2.27 (m, 8H), 0.94 (d, J = 6.21 Hz, 6H). ^{13}C NMR (DMSO-d_6): δ 22.19, 25.24, 26.85, 29.26, 33.91, 38.02, 43.21, 47.09, 51.36, 56.55, 110.17, 121.80, 122.15, 126.43, 126.65, 128.55, 136.88, 137.25, 140.25, 162.09. Anal. ($C_{27}H_{36}N_4O\cdot HCl$) C, H, N, Cl.

*2-Methyl-2H-pyrrolo[3,4-c]quinolin-4(5H)-one (**25**).* Methyl iodide (1.5 g, 11 mmol) and anhydrous potassium carbonate (1.5 g, 11 mmol) were added into a solution of **24** (2.0 g, 11 mmol) in anhydrous DMF (10 mL). The mixture was stirred at 90 °C for 15 h. After cooling, the reaction mixture was treated with water (30 mL) and filtered. The solid obtained was purified by column chromatography on silica gel (chloroform/methanol mixture (10:1) as eluent) to give 1.0 g of **25** (46%, sublimes at 225 °C, from toluene). ^{1}H NMR

(DMSO-d_6): δ 3.91 (s, 3H), 7.09−7.25 (m, 3H), 7.58−7.81 (m, 3H), 10.73 (s, 1H). Anal. ($C_{12}H_{10}N_2O$) C, H, N.

*2-Isopropyl-2H-pyrrolo[3,4-c]quinolin-4(5H)-one (**26**).* To a solution of **24** (3.0 g, 16 mmol) in dioxane (150 mL) brought to reflux was added potassium metal (580 mg, 15 mmol), and the mixture was stirred at reflux until total disappearance of the metal was observed (2 h). After cooling, 2-iodopropane (2.8 g, 16 mmol) and 18-crown-6 ether (3.9 g, 15 mmol) were added, and the mixture was refluxed for 5.5 h. A further portion of 2-iodopropane (1.4 g, 8.1 mmol) was then added, and the reaction mixture was stirred at reflux for a further 15 h. After cooling, the dioxane was removed under reduced pressure, and the residue was taken up in ethyl acetate (100 mL) and washed with brine (3 × 50 mL). The organic solution was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to obtain a crude product, which was purified by column chromatography of aluminum oxide (ethyl acetate as eluent, 0.9 g, 25%, 189−190 °C, from toluene). ^{1}H NMR (DMSO-d_6): δ 1.48 (d, 6H), 4.52 (m, 1H), 7.02−7.20 (m, 3H), 7.63−7.70 (m, 2H), 7.77 (m, 1H), 10.62 (s, 1H). Anal. ($C_{14}H_{14}N_2O$) C, H, N.

*1-Acetyl-N-[[1-(2-phenylethyl)-4-piperidinyl]methyl]-1H-indazole-3-carboxamide (**11p**).* Acetic anhydride (0.26 mL, 2.75 mmol) was added into a suspension of **11l** (500 mg, 1.4 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at room temperature overnight. The solution was treated with 1 N NaOH until pH 8 and extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was treated with Et_2O/HCl, and the hydrochloride that formed was recrystallized from *n*-hexane/ethyl acetate (280 mg, 42%, 246−247 °C). IR (KBr): ν 3431.82, 2937.44, 2547.28, 1727.70, 1676.48, 1536.32, 1375.93, 1325.32, 1375.93, 1325.45, 1197.07, 1147.76, 953.51, 767.44 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 11.13 (br s, 1H), 8.96 (t, J = 5.82 Hz, 1H), 8.31 (t, J = 7.35 Hz, 2H), 7.06−7.87 (m, 7H), 2.68−3.90 (m, 13H), 1.37−2.31 (m, 5H). ^{13}C NMR (DMSO-d_6): δ 22.59, 26.82, 29.26, 33.80, 43.45, 51.34, 56.54, 114.75, 122.40, 124.00, 125.34, 126.66, 128.55, 129.86, 137.23, 139.44, 161.01, 171.09. Anal. ($C_{24}H_{28}N_4O_2$·HCl) C, H, N, Cl.

*N-[[1-[2-(4-Aminophenyl)ethyl]-4-piperidinyl]methyl]-1-isopropyl-1H-indazole-3-carboxamide (**11aa**).* A solution of **11z** (2.7 g, 6 mmol) in ethanol (30 mL) was hydrogenated on 10% Pd/C (270 mg) by a Parr apparatus at 40 psi for 5 h at room temperature. The mixture was then filtered, and the filtrate was concentrated at reduced pressure. The crude **11aa** was treated with 2.5 N HCl in ethanol to afford the corresponding hydrochloride, which was recrystallized from ethyl acetate/ethanol (8:2; 1.4 g, 45%, 278 °C dec). IR (diffuse reflectance with KBr): ν 3504, 3413, 3237, 2634, 2053, 1630, 1547, 1514, 1287, 1210, 941, 762, 642 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.55 (d, J = 7 Hz, 6H), 1.45−2.13 (m, 5H), 2.80−3.84 (m, 12H), 5.08 (sept, J = 7 Hz, 1H), 7.20−7.49 (m, 6H), 7.79 (d, J = 9 Hz, 1H), 8.18 (d, J = 9 Hz, 1H), 8.39 (t, J = 6 Hz, 1H), 9.15−11.18 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 21.86, 26.90, 28.74, 33.95, 43.21, 50.22, 51.44, 56.32, 110.26, 121.77, 122.20, 122.82, 126.23, 129.77, 131.66, 136.26, 136.65, 139.50, 162.22. Anal. ($C_{25}H_{33}N_5O$·2HCl·H_2O) C, H, N, Cl.

*4-[[1-[2-(4-Aminophenyl)ethyl]-4-piperidinyl]methoxy]-2-methyl-2H-pyrrolo[3,4-c]quinoline (**12f**).* To a solution of the product **30** (1.4 mmol, 0.61 g) in ethyl acetate (100 mL) was added 10% Pd/C (200 mg). The mixture was stirred under a H_2 atmosphere at room temperature and pressure for 4 h. A further portion of 10% Pd/C (100 mg) was then added, and the mixture was left under a H_2 atmosphere at room temperature and pressure for 19 h. A stream of H_2 was passed through every 3 h. Then the mixture was filtered under vacuum on a Merck RP18 cartridge to remove the palladium, and the solvent was removed under reduced pressure to give 0.57 g of pure **12f** (99%, 150−152 °C, from toluene/cyclohexane). ^{1}H NMR ($CDCl_3$): δ 1.69 (m, 2H), 1.98 (m, 3H), 2.21 (m, 2H), 2.69 (m, 2H), 2.87 (m, 2H), 3.20 (m, 2H), 3.64 (br s, 2H), 4.04 (s, 3H), 4.53 (d, 2H), 6.69 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 7.32−7.47 (m, 4H), 7.79 (dd, J = 7.7 Hz, J = 1.2 Hz, 1H), 7.93 (m, J = 7.7 Hz, J = 1.2 Hz, 1H). Anal. ($C_{26}H_{30}N_4O$) C, H, N. The product was converted to the hydrochloride as described for the compound **12e** (95%, 165−167 °C, from diisopropyl ether/isopropyl alcohol). ^{1}H NMR (DMSO-d_6): δ 1.66−1.94 (m, 2H), 1.96−2.29 (m, 3H), 2.86−3.89 (m, 8H), 4.00 (s, 3H), 4.49 (d, J = 6.04 Hz, 2H), 7.22−7.46 (m, 6H), 7.63−7.76 (m, 2H), 7.80 (d, J = 1.83 Hz, 1H), 8.01 (dd, J = 7.50, J = 1.65 Hz, 1H), 10.00 (br s, 3H), 10.68 (br s, 1H).

*4-[2-[4-[[[(1-Isopropyl-1H-indazol-3-yl)carbonyl]amino]methyl]-1-piperidinyl]ethyl]benzoic Acid (**11ab**).* **21** (3.5 g, 7.3 mmol) was dissolved in THF/EtOH (1:1; 34 mL), and 1 N NaOH (15.8 mL) was added. The reaction was stirred at room temperature overnight. HCl (1 N; 15.8 mL) was added until precipitation, the mixture was cooled in an ice bath, and the resulting solid was filtered and recrystallized twice from ethanol/ethyl acetate (35:25; 1.9 g, 58%, 180−182 °C). IR (UATR): ν 3314, 2980, 2934, 2862, 1659, 1535, 1487, 1463, 1357, 1206, 753, 650 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 15−9 (very br s, 1H), 8.11−8.24 (m, 2H), 7.85 (d, J = 8.26 Hz, 2H), 7.77 (d, J = 8.59 Hz, 1H), 7.43 (m, J = 0.99, 7.10, 8.42 Hz, 1H), 7.33 (d, J = 8.26 Hz, 2H), 7.21−7.29 (m, 1H), 5.07 (m, J = 6.61 Hz, 1H), 3.21 (t, J = 6.28 Hz, 2H), 2.95 (d, J = 11.23 Hz, 2H), 2.74−2.86 (m, 2H), 2.53−2.61 (m, 2H), 1.99 (t, J = 10.73 Hz, 2H), 1.58−1.75 (m, 3H), 1.54 (d, J = 6.61 Hz, 6H), 1.08−1.32 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 21.84, 29.26, 32.18, 35.60, 43.71, 50.19, 52.50, 58.88, 110.20, 121.85, 122.12, 122.26, 126.20, 128.60, 129.25, 129.69, 136.80, 139.52, 144.89, 162.06, 167.66. Anal. ($C_{26}H_{32}N_4O_3$) C, H, N.

*4-[2-[4-[[(2-Methyl-2H-pyrrolo[3,4-c]-4-quinolinyl)oxy]methyl]-1-piperidinyl]ethyl]benzoic Acid (**12g**).* **12g** was prepared according to the procedure described for **11ab** using ethanol as solvent (27%, 155−166 °C, from ethanol). ^{1}H NMR (DMF-d_7): δ 1.53 (m, 2H), 1.93 (m, 3H), 2.23 (m, 2H), 2.74 (m, 2H), 2.97 (m, 2H), 3.17 (m, 2H), 3.25 (m, 2H), 4.13 (s, 3H), 4.48 (d, 2H), 7.36 (m, 1H), 7.43 (m, 1H), 7.49 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 1.9 Hz, 1H), 7.83 (d, J = 1.9 Hz, 1H), 7.70 (d, 1 H), 8.01 (d, J = 8.1 Hz, 2H), 8.08 (m, 1H), 14−16 (br s, 1H). Anal. ($C_{27}H_{29}N_3O_3$) C, H, N.

*2-[2-[4-[[(2-Methyl-2H-pyrrolo[3,4-c]-4-quinolinyl)oxy]methyl]-1-piperidinyl]ethyl]benzoic Acid (**12h**).* **12h** was prepared according to the procedure described for **11ab** using ethanol as solvent (50%, 165 °C, from ethyl acetate). ^{1}H NMR (DMF-d_7): δ 1.30 (m, 2H), 1.51 (m, 2H), 1.79−1.82 (m, 1 H), 2.51 (m, 5H), 2.87−2.92 (m, 2H), 3.07−3.10 (m, 2H), 3.98 (s, 3H), 4.35 (d, 2H), 7.23−7.37 (m, 5H), 7.57−7.62 (m, 3H), 7.72 (d, 1 H), 7.96 (dd, 1 H). Anal. ($C_{27}H_{29}N_3O_3$) C, H, N.

*Ethyl 4-(2-Nitrophenyl)-1H-pyrrole-3-carboxylate (**23**).* A solution of ethyl 3-(2-nitrophenyl)propanoate (**22**)[35] (26.6 g, 120 mmol) and TosMIC (25.4 g, 130 mmol) in anhydrous dimethyl sulfoxide/ethyl ether (1:2; 450 mL) was added dropwise to a well-stirred suspension of 60% sodium hydride in paraffin (10.4 g, 260 mmol) in anhydrous ethyl ether (300 mL) under a stream of argon. After the addition, the mixture was stirred at room temperature for 25 min, water (500 mL) was then added, and the resulting solution was extracted with ethyl acetate (3 × 600 mL). The combined organic phases were washed with brine (3 × 300 mL), dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The crude product obtained was purified by column chromatography on aluminum oxide (chloroform/ethyl acetate (1:1) as eluent) to give 12.8 g of **23** (41%, 159−161 °C, from ethanol). ^{1}H NMR ($CDCl_3$): δ 2.51 (m, 3H), 4.01 (q, 2H), 6.51 (m, 1H), 7.31−7.33 (m, 1H), 7.39−7.41 (m, 2H), 7.49−7.53 (m, 1H), 7.92−7.94 (m, 1H), 12.0 (s, 1H). Anal. ($C_{13}H_{12}N_2O_4$) C, H, N.

*2H-Pyrrolo[3,4-c]quinolin-4(5H)-one (**24**).* Iron powder (6.7 g, 120 mmol) was added over 15 min into a solution of **23** (2.0 g, 7.7 mmol) in glacial acetic acid (100 mL) mechanically stirred at 85 °C. The mixture was stirring at the same temperature for 45 min. After cooling, the iron was removed by filtration and washed several times with tetrahydrofuran, and the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography on aluminum oxide (ethyl acetate as eluent) to give 1.1 g of **24** (77%, sublimes at 280 °C from ethanol). ^{1}H NMR (DMSO-d_6): δ 7.05−7.28 (m, 3H), 7.57−7.63 (m, 2H), 7.84−7.88 (m, 1H), 10.7 (s, 1H), 12.1 (s, 1H). Anal. ($C_{11}H_8N_2O$) C, H, N.

*4-Chloro-2-methyl-2H-pyrrolo[3,4-c]quinoline (**27**).* A mixture of product **25** (1.0 g, 5.0 mmol), phosphorus oxychloride (16.2 mL), and triethylamine (1.2 mL) was stirred at 120 °C for 6 h. After cooling, the reaction mixture was poured cautiously into ice and extracted with

ethyl acetate (3 × 100 mL). The combined organic phases were washed with brine (1 × 50 mL), with saturated sodium bicarbonate solution (3 × 50 mL), and then again with brine (3 × 50 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product obtained was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate (1:1) as eluent) to give 1.0 g of **27** (92%, 123−124 °C, from benzene). ^{1}H NMR (DMSO-d_6): δ 4.09 (s, 3H), 7.51−7.58 (m, 2H), 7.76 (m, 1H), 7.82−7.85 (m, 1H), 7.97 (m, 1H), 8.15−8.17 (m, 1H). Anal. ($C_{12}H_9N_2Cl$) C, H, N, Cl.

*4-Chloro-2-isopropyl-2H-pyrrolo[3,4-c]quinoline (**28**).* **28** was obtained according to the procedure used for the preparation of compound **27** starting from **26**. The crude product obtained was purified by column chromatography on silica gel (chloroform as eluent) to give pure **28** (91%, 70−72 °C, from cyclohexane). ^{1}H NMR ($CDCl_3$): δ 1.64 (d, 6H), 4.59 (m, 1H), 7.46−7.49 (m, 3H); 7.55 (m, 1H), 7.93−7.98 (m, 2H). Anal. ($C_{14}H_{13}N_2Cl$) C, H, N, Cl.

*4-[(1-Butyl-4-piperidinyl)methoxy]-2-methyl-2H-pyrrolo[3,4-c]-quinoline (**12b**).* A solution of 1-butyl-4-piperidinemethanol (1.14 g, 6.72 mmol) in anhydrous DMF (14 mL) was added dropwise into a suspension of 60% NaH in paraffin (270 mg, 6.72 mmol) in the same solvent (14 mL). The reaction mixture was stirred at room temperature for 10 min, and then the alkoxide that formed was added into a solution of **27** (470 mg, 2.17 mmol) in DMF (14 mL) preheated to 146 °C. The reaction mixture was stirred at 146 °C for 1 h. After cooling, it was poured onto ice and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine (3 × 20 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on aluminum oxide (chloroform as eluent) to give 520 mg of pure **12b** (68%, 83−85 °C, from benzene/cyclohexane). ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H), 1.35−1.59 (m, 6H), 1.90−2.07 (m, 5H), 2.37 (m, 2H), 3.03 (m, 2H), 3.99 (s, 3H), 4.49 (d, 2H), 7.28-7.49 (m, 4H), 7.75 (m, 1 H), 7.88 (m, 1 H). Anal. ($C_{22}H_{29}N_3O$) C, H, N.

*2-Methyl-4-[[1-(2-phenylethyl)-4-piperidinyl]methoxy]-2H-pyrrolo[3,4-c]quinoline (**12c**).* **12c** was prepared according to the procedure described for **12b** starting from **27** (0.50 g, 2.3 mmol) using 1-(2-phenylethyl)-4-piperidinemethanol (1.77 g, 8.05 mmol) as reactant. The crude product was purified by column chromatography on aluminum oxide (*n*-hexane/ethyl acetate (4:1) as eluent) to give 620 mg of pure **12c** (67%, 90−92 °C, recrystallized from benzene/cyclohexane). ^{1}H NMR ($CDCl_3$): δ 1.67 (m, 2H), 1.94−1.97 (m, 3H), 2.17 (m, 2H), 2.69 (m, 2H), 2.90 (m, 2H), 3.15 (m, 2H), 3.98 (s, 3H), 4.49 (d, 2H), 7.20−7.42 (m, 9H), 7.74 (m, 1H), 7.88 (m, 1H). Anal. ($C_{26}H_{29}N_3O$) C, H, N.

*2-Isopropyl-4-[[1-(2-phenylethyl)-4-piperidinyl]methoxy]-2H-pyrrolo[3,4-c]quinoline (**12d**).* Compound **12d** was prepared according to the procedure described for **12b** starting from **28** using 1-(2-phenylethyl)-4-piperidinemethanol as reactant. The residue was purified by column chromatography on aluminum oxide (*n*-hexane/ethyl acetate (4:1) as eluent) to give pure **12d** (73%, 90−93 °C, from *n*-hexane). ^{1}H NMR ($CDCl_3$): δ 1.61−1.66 (m, 8H), 1.94 (m, 3H), 2.12 (m, 2H), 2.65 (m, 2H), 2.86 (m, 2H), 3.12 (m, 2H), 4.49 (d, 2H), 4.54 (m, 1H), 7.21−7.45 (m, 9H), 7.74 (m, 1H), 7.90 (m, 1H). Anal. ($C_{28}H_{33}N_3O$) C, H, N. A solution of HCl in methanol was prepared by addition dropwise of acetyl chloride (2.67 mmol, 200 mg) to 10 mL of methanol cooled at 0 °C. The solution was gently stirred for a few minutes, followed by addition dropwise of a solution of **12d** (1.0 g, 2.34 mmol) in methanol (5.0 mL). When the addition was complete, the mixture was stirred at 0 °C for 45 min, followed by addition of anhydrous ethyl ether (about 200 mL) until precipitation of the salt was observed. The obtained salt was filtered, washed with anhydrous ethyl ether (3 × 2 mL), and dried under vacuum at 45 °C for 6 h (630 mg, 63%, 138−140 °C, from diisopropyl ether/isopropyl alcohol). ^{1}H NMR (DMSO-d_6): δ 1.57 (d, J = 6.59 Hz, 6H), 1.79−2.29 (m, 5H), 2.86−3.47 (m, 6H), 3.63 (d, J = 11.71 Hz, 2H), 4.55 (d, J = 4.03 Hz, 2H), 4.68 (m, J = 6.59 Hz, 1H), 7.20−7.48 (m, 7H), 7.80 (br s, 1H), 7.95−8.16 (m, 3H), 10.93 (br s, 1H).

*4-[[1-(Phenylmethyl)-4-piperidyl]methoxy]-2-methyl-2H-pyrrolo-[3,4-c]quinoline (**29**).* Compound **29** was prepared according to the procedure described for **12b** using 1-(phenylmethyl)-4-piperidinemethanol as reactant. The residue was purified by column chromatography on aluminum oxide (chloroform/petroleum ether (1:1) as eluent) to give pure **29** as an oil (81%). ^{1}H NMR ($CDCl_3$): δ 1.58−1.59 (m, 2H), 1.91−2.11 (m, 5H), 2.94−3.02 (m, 2H), 3.59 (s, 2H), 4.02 (s, 3H), 4.50 (d, 2H), 7.31−7.39 (m, 8H), 7.44 (m, 1H), 7.77 (m, 1H), 7.92 (m, 1H). Anal. ($C_{25}H_{27}N_3O$) C, H, N.

Biological Assays. *In Vitro Binding Assay for 5-HT$_4$R.* The 5-HT$_4$R binding assay was performed on homogenates from human embryonic kidney (HEK-293) cells transfected with the human 5-HT$_4$R, using 0.2 nM [^{3}H]**5** as radioligand. Nonspecific binding was measured in the presence of 10 μM **4**·HCl. Assays were conducted in 25 mM Tris−HCl (pH 7.4), 0.5 mM EDTA, and 10 mM $MgSO_4$. Binding was initiated by addition of 200 μL of membrane homogenates (43−61 μg of protein/mL); after 60 min of incubation at 27 °C, the membranes were harvested onto glass fiber (GF/B) filters (Unifilter, Packard) (treated with 0.3% poly(ethylenimine)) using a Filtermate cell harvester (Packard). Subsequently, the filters were washed with 2.5 mL of ice-cold buffer and dried for 30 min in an oven at 45 °C, and 30−35 μL of Microscint 20 (Packard) was added to each well. At least 10 h later the radioactivity was measured using a TopCount (Packard) for 1 min. The compounds were tested in duplicate at eight concentrations ranging from 10^{-12} to 10^{-5} M in duplicate competition curves. The compounds were dissolved in dimethyl sulfoxide (DMSO), serially diluted 1:10 in DMSO, and further diluted in incubation buffer (1% vehicle DMSO final concentration).

The protein concentration was determined by the bicinchoninic acid (BCA) method (Pierce) using bovine serum albumin (BSA) as the standard.

Results at a single concentration are expressed as percent inhibition vs a positive control. The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) with Hill coefficients (n_H) were determined by computer-assisted nonlinear regression analysis of eight concentrations in duplicate competition curves. The inhibition constants pK_i (defined as the negative log of K_i) were calculated from the Cheng−Prusoff equation.

In Vitro Binding Assay for 5-HT$_{2A}$R. The 5-HT$_{2A}$R binding assay was performed on cell homogenates obtained from a stable recombinant CHO-K1 cell line expressing the human 5-HT$_{2A}$R, using 0.7 nM [^{3}H]ketanserin as ligand. Nonspecific binding was measured in the presence of 20 μM mianserin. Assays were conducted in buffer containing 50 mM Tris (pH 7.4), 5 mM $CaCl_2$, 0.1% ascorbic acid, and 10 μg/mL saponin. Binding was initiated by addition of 20 μL of membrane homogenates (750 μg/mL protein); after 60 min of incubation at 25 °C, the membranes were harvested onto glass fiber (GF/B) filters (Unifilter, Packard) (treated with 0.3% poly-(ethylenimine)) using a Filtermate cell harvester (Packard). Subsequently, the filters were washed with 2.5 mL of ice-cold buffer and dried for 30 min in an oven at 45 °C, and 30−35 μL of Microscint 20 (Packard) was added to each well. At least 10 h later the radioactivity was measured using a TopCount (Packard) for 1 min. The compounds were tested in duplicate at eight concentrations ranging from 10^{-12} to 10^{-5} M. The compounds were dissolved in DMSO and serially diluted 1:10 in DMSO, and each dilution was further diluted in incubation buffer (final DMSO concentration 1%).

The protein concentration was determined by the BCA method (Pierce) using BSA as the standard.

Results at a single concentration are expressed as percent inhibition vs a positive control. The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) with Hill coefficients (n_H) were determined by computer-assisted nonlinear regression analysis of eight concentrations in duplicate competition curves. The inhibition constants pK_i (defined as the negative log of K_i) were calculated from the Cheng−Prusoff equation.

In Vitro Cross-Species Intrinsic Clearance with Rat and Human Liver Microsomes. Compounds were incubated at 37 °C at a concentration of 1 μM in Dulbecco's buffer at pH 7.4 with rat and

human liver microsomes for up to 60 min to determine their metabolic stability and intrinsic clearance. Samples (25 μL) were taken at time 0 and after 5, 10, 20, 30, and 60 min and added to 50 μL of cold acetonitrile to stop the reaction and to 20 μL of cold acetonitrile containing the internal standard warfarin. The samples were then centrifuged, and the supernatant was analyzed immediately. Midazolam and propranolol were used as positive controls and incubated under the same conditions as the test compounds. Control tests were performed by incubating the compounds with rat and human liver microsomes in Dulbecco's buffer in the absence of NADPH for 60 min. The single time point metabolic stability of the test compounds was evaluated by calculating the percentage of parent compound remaining after 60 min of incubation. The intrinsic clearance (CL_{int}) of the test compounds was calculated using the half-life approach. The half-life and CL_{int} were determined from the concentration remaining at the different sampling points using the LC−MS/MS method.

Cross-Species Metabolic Stability in Liver Microsomes from Mouse, Rat, Dog, Miniature Pig, Monkey, and Human. The samples consisted of the test articles at 1 and 10 μM, pooled microsomes at 0.5 mg/mL final protein concentration, and NADPH regenerating system in a final volume of 200 μL. DMSO was the test article solvent, and the final DMSO concentration was 0.5%. The assay was standardized for both phosphate buffer (75 mM, pH 7.4) and the NADPH regenerating system ($MgCl_2$, 3.3 mM; G6P, 3.3 mM; G6PD, 0.4 U/mL; $NADP^+$, 1.3 mM). Positive controls (warfarin, propranolol, and testosterone, incubated as a cocktail) were treated as the test articles. The samples were incubated at 37 °C in a 96-well plate in a humidified incubator. At t = 0, 30, and 60 min, 100 μL of acetonitrile containing the internal standards (0.2 μM metoprolol and 0.4 μM diclofenac) for LC−MS analysis was added to stop the reaction. The samples were diluted 10-fold to bring them within the linear range of the instrumental measures. The samples were centrifuged prior to analyses by LC−MS/MS using positive electronspray ionization (ESI) and selected reaction monitoring (SRM) using a gradient LC method. The LC conditions included a 5−91% acetonitrile gradient in water containing 0.1% formic acid with a total run time of 6.5 min. The LC was isocratic for 0.5 min followed by a linear gradient of acetonitrile from 5% to 91% over 1 min, with a 2.5 min hold at 91% acetonitrile. The eluent flow rate was 0.5 mL/min, and the column was an XDBC18 (2.1 × 50 mm, Agilent). The re-equilibration period was 1.5 min at 1.4 mL/min followed by 0.5 min at 0.5 mL/min. The internal standard for positive mode ESI was metoprolol.

Hot Plate Test. The method originally described by Woolfe and Mac Donald was used with some modifications.[54] The hot plate consists of an electrically heated surface with the temperature fixed at 56 ± 0.2 °C. The animals are placed on the hot plate, and the time until a nocifensive response (i.e., licking, flinching, jumping) occurs is recorded by a stopwatch. During the test, latency for nocifensive response was recorded 1 h after drug administration. The maximum latency time was 30 s to minimize tissue damage. The effect on latency was also calculated as a percent response increase with respect to vehicle-treated rats.

Formalin Test. The formalin test in mice is a valid and reliable model of nociception. The noxious stimulus is a subcutaneous injection of 20 μL of a 1% solution of formalin in saline into the dorsal surface of the right hind paw of the mouse.[55] The formalin injection produces a distinct biphasic response consisting of licking or biting the paw. The early phase, occurring from 0 to 10 min after the formalin injection, is due to a direct effect on nociceptors, while the late phase from 15 to 40 min seems to be an inflammatory response where pain is responsive to anti-inflammatory drugs. Mice were placed in a Plexiglas cage, utilized as an observation chamber, 1 h before administration of the formalin injections. The tested drugs were orally given 30 min before formalin. The total amount of time (s) that the animal spent licking or biting the paw after the formalin injection was recorded for a period of 40 min within 5 min intervals.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental procedures regarding the pharmacophore approach, synthetic methods for compounds **29** and **33**−**35**, analyses, 5-HT_4R functional assay, and in vitro pharmacology profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

5-HT, 5-hydroxytryptamine or serotonin; 5-HTR, 5-HT receptor; 5-HT_4R, serotonin 4 receptor; 5-HT_{2A}R, serotonin 2A receptor; VS, virtual screening; EF, enrichment factor; TosMIC, (4-tolylsulfonyl)methyl isocyanide; CHO, Chinese hamster ovarian

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