High-Efficacy 5-HT$_{1A}$ Agonists for Antidepressant Treatment: A Renewed Opportunity

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We report the discovery of novel 5-HT$_{1A}$ receptor agonists and describe the process that led to the antidepressant candidate **9** (F 15599). **9** has nanomolar affinity for 5-HT$_{1A}$ binding sites and is over 1000-fold selective with respect to the other 5-HT$_1$ receptor subtypes, 5-HT$_{2-7}$ receptor families, and also numerous GPCRs, transporters, ion channels, and enzymes. In a cellular model of signal transduction, **9** activates h5-HT$_{1A}$ receptors with an efficacy superior to that of the prototypical 5-HT$_{1A}$ agonist (±)-8-OH-DPAT and comparators undergoing clinical trials. After acute oral administration in rats, **9** totally reverses immobility in the forced swimming test and produces behaviors characteristic of 5-HT$_{1A}$ receptor activation. However, these effects occurred at widely separated doses, suggesting that **9** discriminates between distinct populations of 5-HT$_{1A}$ receptors. While the clinical relevance of these observations is still unknown, this opens new perspectives for the treatment of depressive disorders.

Introduction

Previous reports from this laboratory[1] described a novel series of 5-HT$_{1A}$[a] agonists exhibiting unprecedented selectivity and efficacy at the 5-HT$_{1A}$ receptor subtype. This research effort culminated in the discovery of **1** (F 13640, Figure 1), which is currently undergoing phase II clinical trials for the treatment of severe, chronic pains.[2] In preclinical models, the analgesia produced by **1** excels that of available therapies in many respects: (1) the magnitude of the analgesic effect equals that of opioids with nociceptive pain and surpasses that of opioids, antiepileptic, NMDA-antagonist, and antidepressant agents with neuropathic pain;[3] (2) its spectrum of activity is much broader, encompassing acute and chronic, central and peripheral pains of different origins;[4] and (3) the time-course of the effect is exceptional; the analgesia grows rather than decays upon chronic administration (inverse-tolerance),[5] and pain relief persisted for up to 2 months after discontinuation of the treatment (curative-like action).[6] Clearly, compound **1** opens novel therapeutic and research avenues in the field of analgesia.[7]

Despite a compelling pharmacological rationale gained over more than two decades of intense research,[8,9] only two poorly selective, 5-HT$_{1A}$ partial agonists are marketed as anxiolytics, that is, buspirone[10] and tandospirone.[11] Still, the results accumulated on **1** suggested that 5-HT$_{1A}$ agonists have indeed therapeutic potential in domains other than anxiety. The enticing prospect that the story of **1** in pain states might repeat itself in other CNS disorders in which 5-HT$_{1A}$ transmission has been implicated, such as depression[12] and neuroprotection,[13] prompted us to the search for novel high-efficacy 5-HT$_{1A}$ agonists. Given that compound **1** survived the evermore-demanding toxicity and tolerance requirements, we focused our medicinal chemistry effort on close structural analogues of **1**. In practice, therefore, we sought after compounds endowed with similar pharmacodynamics but having altered pharmacokinetics relative to **1**.[14]

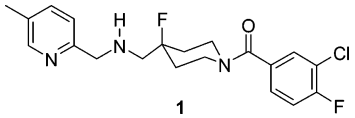

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Figure 1. Structure of F 13640.

Here, we describe the process through which we discovered compound **9** (F 15599) and highlight some of the pharmacological features that distinguish **9** from the parent molecule **1**. We also touch upon the early experimental cues that nurtured the hypothesis that **9** interacts preferentially with certain populations of 5-HT$_{1A}$ receptors rather than others. Such a profile, we believe, renders **9** particularly adapted for exploring its antidepressant potential in human, a domain in which there is a significant unmet need.[15]

Chemistry

Target compounds **2**−**21** were all prepared by reductive amination either between an arylmethylamine of the type **XII** or **XIII** and the cyanohydrine **XIVa** (Scheme 2), or between an arylcarboxaldehyde of the type **XVIII** or **XIX** and the amine **XIVb** (Scheme 3). The synthesis of intermediates **XIVa** and **XIVb** has been described previously.[16]

Scheme 1 summarized the routes used to access the pyrimidylmethylamines of the type **XII**. From the commercially available 2,4-dichloro-5-methylpyrimidine **I**, successive introduction of a cyano group (**II**), then of a methylamino group, led to **III** in which R$_1$ = CH$_3$ and R = NHCH$_3$. On the other hand, displacement of the 2-methylsulfonyl group in the known substrate **IV**[17] by cyanide anion[18] yielded **III** in which R$_1$ = CO$_2$CH$_3$ and R = H. Alternatively, the 2-chlorine on the Boc-carbamate derived from **V**[19] was first converted into a methylsulfonyl group, and then the cyano group was installed as above to provide **III** in which R$_1$ = H and R = NBocCH$_3$. Reduction of the cyano function in **III** by catalytic hydrogenation furnished the corresponding amines **XII**. This reaction was, however, more conveniently carried out in two steps: trapping

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[a] Abbreviations: HT, hydroxytryptamine; GPCRs, G-protein coupled receptors; NMDA, *N*-methyl-D-aspartic acid; D, dopamine; E_{max}, maximum effect; FPT, forepaw treading; FBP, flat body posture; FST, forced swimming test; RGS, regulator of G-protein signaling; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin and noradrenaline reuptake inhibitors; TCAs, tricyclic antidepressants; MAOIs, monoamine oxidase inhibitors; pEC$_{50}$, negative logarithm of the dose that is effective in 50% of test subject; MSD, minimum significant dose; Boc, *tert*-butoxycarbonyl; DAST, (diethylamino)sulfur trifluoride.

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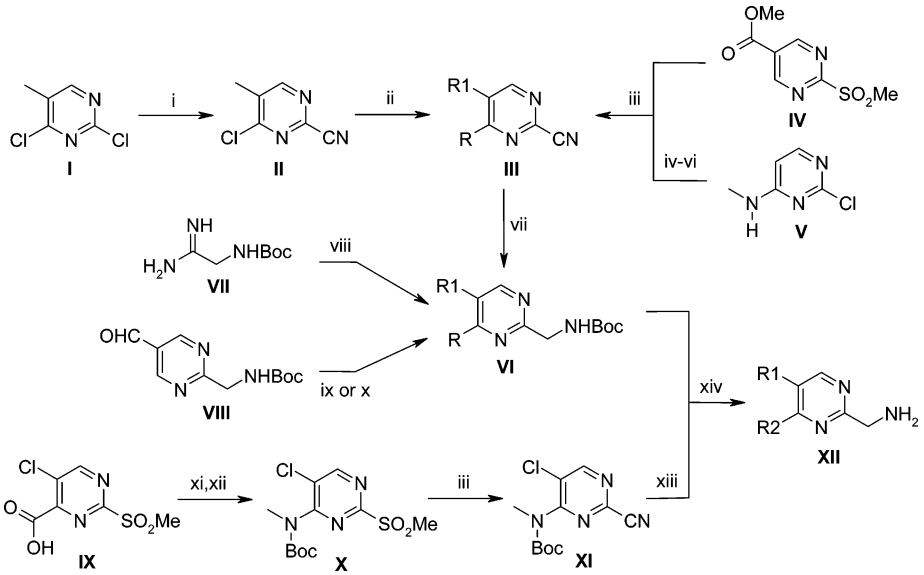
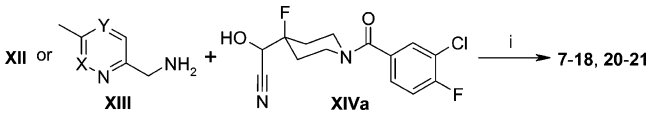
Scheme 1. Synthesis of Pyrimidine Intermediates of the Type **XII**[a]



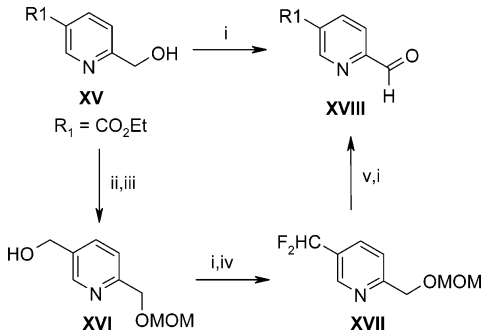
[a] Reagents and conditions: (i) NaCN, H_2O, $(CH_3)_3N$; (ii) CH_3NH_2, EtOH; (iii) NaCN, DMSO; (iv) $(Boc)_2O$, DMAP, THF; (v) CH_3SNa, DMF; (vi) mCPBA, CH_2Cl_2; (vii) H_2 Pd/C, $(Boc)_2O$, EtOAc; (viii) CH_3OH, CH_3ONa; 1,3-di-CO equivalent; (ix) $NaBH_4$, EtOH; (x) DAST, CH_2Cl_2; (xi) DPPA, NEt_3, tBuOH; (xii) NaH, $(CH_3)_2SO_4$; (xiii) H_2 Pd/C, CH_3OH; (xiv) HCl, iPrOH, EtOAc.

Scheme 2. Preparation of Ligands **7**−**18**, **20**, and **21**[a]



[a] Reagents and conditions: (i) $NaBH_3CN$, CH_3OH, DABCO.

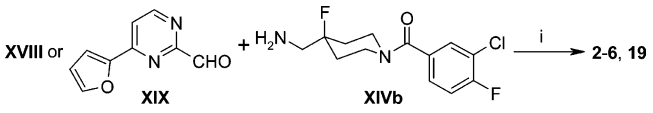
Scheme 3. Synthesis of Pyridine Intermediates **XVIII**[a]



[a] Reagents and conditions: (i) MnO_2, $CHCl_3$; (ii) NaH, MOMCl, THF; (iii) $LiAlH_4$, THF; (iv) DAST, $CHCl_3$; (v) HCl, EtOH.

first the primary amine as a Boc-carbamate (**VI**), which can be purified if needed, then releasing the amine just before the reductive amination step. The synthesis of the intermediate **XI** was achieved from the known 5-chloro-2-(methylsulfonyl)-4-pyrimidinecarboxylic acid **IX**.[20] Thus, a Curtius rearrangement[21] from **IX** afforded the corresponding Boc-protected amine, which was then *N*-methylated under basic conditions to give **X**. The cyano function was incorporated by applying the same method as that used for the conversion of **IV** into **III**. Some intermediates of the type **VI** (R = H and R_1 = CH_3, CH_2CH_3, and CHO) were more conveniently accessed by constructing the pyrimidine ring. To this end, the amidine **VII**[22] was reacted with the appropriate 1,3-dicarbonyl equivalent[23] to deliver either **VI** (R = H and R_1 = CH_3 or CH_2CH_3) or **VIII** (R = H and R_1 = CHO). The latter (**VIII**) served also as precursor for the 5-difluoro derivative **VI** in which R = H and R_1 = CHF_2. Cleavage of the Boc-protecting group in **VI** produced the amines **XII**, which were engaged in the reductive amination step to give **9**−**18**, **20**, and **21** (Scheme 2).

Scheme 4. Preparation of Ligands **2**−**6**, **19**[a]



[a] Reagents and conditions: (i) $NaBH(AcO)_3$, NEt_3, $C_2H_4Cl_2$.

The diazine-type intermediates **XIII** were obtained from the known 2-cyano-5-methylpyrazine[24] and 3-cyano-6-methylpyridazine.[25] Thus, hydrogenation of 2-cyano-5-methylpyrazine and 3-cyano-6-methylpyridazine on deactivated Pd^0 catalyst provided the 5-aminomethyl-2-methylpyrazine[26] and the 6-aminomethyl-3-methylpyridazine **XIII**, respectively (see Supporting Information). Reductive amination between **XIII** and **XIVa** then supplied compounds **7** and **8** (Scheme 2).

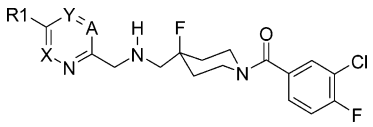
The aldehydes of the type **XVIII** were synthesized according to Scheme 3. Among compounds **XVIII**, a few were known (R_1 = Cl, CH_2OH, and CH_2F),[27] and the others were prepared from the ester **XV**.[28] Thus, the alcohol function in **XV** was protected as a MOM-ether, and then the C-5 ester function was reduced to the alcohol **XVI**. Adjusting the oxidation state in **XVI** to the aldehyde level followed by *gem*-difluoration with an excess of DAST led to the derivative **XVII**.[29] Cleavage of the MOM-protecting group and then oxidation of the resulting alcohols with Mn(IV) oxide completed the synthesis of aldehydes **XVIII**. The pyrimidine carboxaldehyde **XIX** was prepared in three steps and modest overall yield from the known compound 4-(fur-2-yl)-2-methyl-pyrimidine[30] (see Supporting Information).

Reductive amination between aldehydes **XVIII** or **XIX** and amine **XIVb** led to the final products **3**−**6**. Compound **2** required an extra step (i.e., hydrolysis of the 5-ester group) to secure the corresponding 5-carboxylic acid (Scheme 4).

Results and Discussion

In humans and animals (rat and dog), the carboxylic acid **2** (Figure 2) constitutes the major metabolite of compound **1**.[31] Following the research direction defined above, we set out to disrupt the metabolism of **1** by acting on the pathway(s) responsible for the oxidation at the 5-methyl group.[32] For this

Table 1. Activities of Compounds **1**−**9** and Reference at 5-HT_{1A} Receptors in Vitro



compd	X	Y	A	R_1	5-HT_{1A} affinity pK_i[a]	5-HT_{1A} efficacy pEC_{50}[b]	5-HT_{1A} efficacy E_{max}[c]
1	CH	CH	CH	CH_3	9.07 ± 0.05	7.63 ± 0.13	74.7 ± 1.5
2	CH	CH	CH	CO_2H	<5		
3	CH	CH	CH	CH_2OH	8.13 ± 0.01	5.69 ± 0.03	80.0 ± 5.7
4	CH	CH	CH	CH_2F	8.92 ± 0.01	6.64 ± 0.09	61.0 ± 5.2
5	CH	CH	CH	CHF_2	8.42 ± 0.05	6.62 ± 0.02	96.4 ± 4.6
6	CH	CH	CH	Cl	9.16 ± 0.06	7.16 ± 0.18	80.4 ± 10.1
7	N	CH	CH	CH_3	8.25 ± 0.07	5.10 ± 0.34	89.7 ± 0.7
8	CH	N	CH	CH_3	7.18 ± 0.01		
9	CH	CH	N	CH_3	8.65 ± 0.11	6.41 ± 0.06	70.3 ± 1.3
8-OH-DPAT					8.85 ± 0.07	7.49 ± 0.02	35.9 ± 0.7
PRX-00023					7.52 ± 0.13[d]	7.03 ± 0.26	6.6 ± 1.6
flibanserin					7.93 ± 0.02	6.20 ± 0.03	72.0 ± 2
5-HT					8.73 ± 0.04	6.64 ± 0.14	

[a] Binding affinity values are expressed as means ± SEM of separate experiments, each performed in triplicate. [b] Concentration of agonist for 50% of [^{35}S]GTPγS binding in C6-glial cells expressing human 5-HT_{1A} receptors; −log EC_{50} (pEC_{50}) values were estimated using the mean values of three separate experiments. [c] E_{max} refers to maximal agonist effect; results expressed as % relative to 5-HT (100%). [d] Affinity reported in ref 35b: 17 nM.

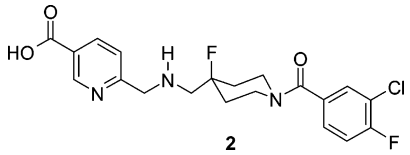


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Figure 2. The major metabolite of F 13640.

purpose, we relied on two strategies: (1) the oxidation potential at the 5-methyl group was altered either by substituting hydrogen atom(s) for fluorine (cf., **4** and **5**, Table 1) or by lowering the electron density in the aromatic ring (cf., diazines **7**−**9**); or (2) the 5-methyl group was replaced by a non-oxidizable, isosteric group (e.g., **6**).[33] Each one of these approaches provided ligands that exhibited a varying degree of agonism at the 5-HT_{1A} receptor (e.g., **4**, **5**, **6**, and **9**). Of these, however, the pyrimidine motif proved interesting enough to be investigated further. Throughout this work, three compounds are used as references: (±)-8-OH-DPAT, as it is the prototypic 5-HT_{1A} agonist;[34] *N*-{3-(4-(4-cyclohexylmethanesulfonylaminobutyl)piperazin-1-yl)phenyl}acetamide (PRX-00023), which is claimed to be a potent, full 5-HT_{1A} agonist;[35] and flibanserin, which is reported as a preferential postsynaptic 5-HT_{1A} agonist,[36] although it interacts also with 5-HT_2 and dopaminergic D_4 receptors.

All of the ligands examined in Table 1 had nanomolar affinity for 5-HT_{1A} binding sites, with the exception, however, of compounds **2** and **8**. Of interest, **2**, the major metabolite of **1**, does not recognize the 5-HT_{1A} receptor (nor any other monoamine receptors) and, therefore, cannot contribute to the 5-HT_{1A} profile of **1**. In C6-glial cells transfected with h5-HT_{1A} receptors, all of the derivatives listed in Table 1 exerted partial agonist activities as evidenced by stimulation of [^{35}S]GTPγS binding. The magnitude of the responses (E_{max}) in this recombinant system was similar for compounds **3**, **5**−**7**, and **9** and in the range of that of **1**. Because the potency of **3** and **7** was weak as compared to that of **1**, they were not investigated further. In fact, the experimental conditions used in this cell-based functional assay have been optimized to discriminate between ligands with high intrinsic activity.[37] Hence, 8-OH-DPAT was found to act in this system as a weak agonist with a maximal effect of only 35% of that of 5-HT. In these conditions, PRX-00023 hardly stimulated the 5-HT_{1A} receptor (6% relative to 5-HT), whereas flibanserin had a modest affinity but achieved marked efficacy at the 5-HT_{1A} receptor. Compounds **4**, **5**, **6**, and **9**, which displayed in vitro characteristics similar to that of **1**, were examined in vivo.

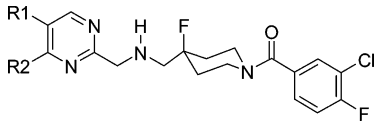
Table 2. Induction of Elements of the Behavioral 5-HT Syndrome in Rats by Selected Ligands

compd	FPT[a] ED_{50}[c]	FBP[b] ED_{50}[c]	ratio FBP/FPT
1	0.38 (0.16−0.88)	1.40 (0.59−3.20)	3.7
4	6.10 (3.40−11.00)	16.00 (9.00−28.00)	2.6
5	2.50 (0.75−8.50)	4.20 (1.80−9.90)	1.7
6	3.80 (2.0−7.3)	4.90 (2.80−8.60)	1.3
9	1.50 (0.81−2.90)	8.30 (2.00−34.00)	5.5
8-OH-DPAT	40	>40	
PRX-00023	>40	>40	
flibanserin[d]	>40	20 (10−40)	

[a] Forepaw treading induction scored at 60 min after treatment po. [b] Flat body posture scored at 60 min after treatment po. [c] ED_{50} effective dose values are mg/kg (95% confidence limit). [d] Observed 60 min after ip administration.

Direct and indirect 5-HT receptor agonists produce postural movements in naïve rats such as forepaw treading (FPT) and flat body posture (FBP).[38] These paradigms were used here[38b] as the first-line screen for a central 5-HT_{1A} agonistic activity. Thus, upon acute, oral administration in rats, compounds **1**, **4**−**6**, and **9** (Table 2) produced dose-dependent FPT and FBP, indicating that they exerted 5-HT_{1A} agonist action in vivo. There was no simple correlation between affinity, functional activity, and occurrence of these behavioral signs in rats. The results shown in Table 2 revealed that the potency order of the ligands was the same for both signs, except for compound **9**. The latter caused FPT in all animals from 10 mg/kg upward; in contrast, FBP occurred in less than 60% of the animals, and the dose−response curve was markedly shifted rightward. Thus, even though **9** induces both stereotypic behaviors (FPT and FBP), the responses were sign-dependent and the separation between those was larger with **9** than with the other substrates (**4**−**6**). In comparison, 8-OH-DPAT and flibanserin (even given ip) performed poorly on these markers; flibanserin was also the only agent for which FBP preceded FPT. Further, PRX-00023 evoked none of the elements of the 5-HT syndrome in rats despite its alleged oral bioavailability.[35b]

Table 3. Activities of Compounds **10**−**21** at 5-HT$_{1A}$ Receptors in Vitro



				5-HT$_{1A}$ efficacy	
compd	R_1	R_2	5-HT$_{1A}$ affinity pK_i[a]	pEC$_{50}$[b]	E_{max}[c]
10	H	H	7.02 ± 0.03		
11	CO_2CH_3	H	6.66 ± 0.03		
12	CH_2OH	H	6.93 ± 0.16		
13	CH_2CH_3	H	7.25 ± 0.02		
14	CHF_2	H	7.60 ± 0.10	5.44 ± 0.45	82.5 ± 4.4
15	Cl	H	8.47 ± 0.06	6.81 ± 0.04	76.0 ± 2.7
16	H	CH_3O	8.19 ± 0.01	6.35 ± 0.01	75.5 ± 2.5
17	H	CH_3NH	8.32 ± 0.01	6.51 ± 0.03	58.8 ± 2.6
18	H	$(CH_3)_2N$	8.64 ± 0.03	7.05 ± 0.05	63.4 ± 3.3
19	H	2-furyl	9.37 ± 0.12	8.00 ± 0.03	79.0 ± 0.1
20	CH_3	CH_3NH	8.76 ± 0.10	7.07 ± 0.02	64.1 ± 4.1
21	Cl	CH_3NH	9.13 ± 0.08	7.37 ± 0.03	71.8 ± 2.2

[a] Binding affinity values are expressed as means ± SEM of separate experiments, each performed in triplicate. [b] Concentration of agonist for 50% of [^{35}S]GTPγS binding in C6-glial cells expressing human 5-HT$_{1A}$ receptors; −log EC$_{50}$ (pEC$_{50}$) values were estimated using the mean values of three separate experiments. [c] E_{max} refers to maximal agonist effect; results expressed as % relative to 5-HT (100%).

Table 4. Antidepressant-like Activity and Elements of the 5-HT Syndrome in Rats of Selected Ligands

	forced swimming test[a]				
	potency		efficacy		
compd	ED$_{50}$[c] (mg/kg)	MSD[d]	MED[e]	max effet[f]	FPT[b] ED$_{50}$ (mg/kg)
1	0.06 (0.02−0.10)	0.08	5	100	0.38[g]
4	0.17 (0.08−0.36)	0.31	10	95	6.10[g]
9	0.05 (0.02−0.09)	0.08	5	100	1.50[g]
15	0.03 (0.01−0.11)	0.04	40	99	2.8 (0.72−11)
19	>2.5				5.0 (2.5−10.0)
21	0.32 (0.16−0.61)	0.63	2.5	100	5.0 (2.5−10.0)
8-OH-DPAT	0.75 (0.08−6.8)	2.5	10	80	
PRX-00023	>40				
flibanserin	>320	NS[h]	160	32	

[a] All of the compounds were administered po 60 min before test. [b] Forepaw treading induction scored at 60 min after treatment po. [c] Based on the number of animals affected. [d] Minimum significant dose (mg/kg). [e] The first dose at which the effects are maximal. [f] Percentage of animals affected. [g] See Table 2. [h] NS: not significant.

It is clear that the ability to trigger FPT and FBP differs substantially between 5-HT$_{1A}$ agonists, even between close structural analogues. This aspect will be revisited in the context of the forced swimming test (vide infra).

Compound **9** displayed marked binding affinity, high selectivity,[39] and functional activity at 5-HT$_{1A}$ receptor subtype. It behaves as an agonist in vivo and was orally bioavailable in rats.[40] To gain further insight into the pharmacological consequences of the change from a pyridine to a pyrimidine motif, we initiated a limited SAR program around **9**, guided by the knowledge garnered in previous studies.[1] The results obtained are summarized in Table 3.

No doubt, the presence of a methyl group at C-5 on the pyrimidine ring is beneficial (**9** vs **10**). In addition, any substituent larger than a methyl (e.g., **9**) or a chlorine (e.g., **15**) at the 5-position ruined the binding at the 5-HT$_{1A}$ sites (e.g., **12**−**14**). Such a trend, although reminiscent of that seen in the pyridine series, was even clear-cut in the case of a ligand containing a pyrimidine pharmacophore (e.g., **3**, **5**, Table 1 and **12**, **14**, Table 3), possibly underlying subtle differences in the way both types of molecules dock in the active site of the receptor. In contrast to the tight constraints imposed at C-5 on the pyrimidine, the 6-position was quite tolerant to chemical modulations, and groups of various sizes and electronic properties were all accommodated (e.g., **16**−**19** vs **10**). Of these, the 2-furyl (**19**) was optimal. Combining a 5-methyl or -chlorine with a group at C-6 improved affinity (**20** and **21** vs **17**). Subsequently, however, such combinations were shown to have a negative impact in the forced swimming test (vide infra). We assumed that the contribution of the 6-substituent was mostly lipophilic and, therefore, not productive in a drug-like sense.

Overall, the 5-HT$_{1A}$ receptor appeared to be less permissive toward ligands containing a pyrimidine than toward those having a pyridine pharmacophore.

With a set of promising compounds in hand, we probed their antidepressant potential by means of the forced swimming test (FST). The latter is the most widely used and the best-established model of antidepressant-like efficacy.[41] Further, activity in this procedure is not restricted to a particular pharmacological mechanism.[42] FST data obtained on selected ligands are summarized in Table 4. For comparative purposes, FPT data are also included as it is the most sensitive component of the 5-HT syndrome in rats.

Upon single, oral administration, compounds **1**, **4**, **9**, **15**, and **21** dose-dependently (0.01−40 mg/kg) and completely decrease immobility time relative to vehicle.[43] Remarkably, the activity of **9** and **15** in the FST was superimposable to that of **1** despite their significant affinity and potency differences in vitro (Tables 1 and 3) and in vivo as regards syndrome-induction (Tables 2 and 4). Thus, **9** and **15** significantly shortened the latency to

swim at doses as low as 0.05 and 0.03 mg/kg, respectively, whereas locomotor activation (FPT) only appeared at doses 30- to 70-fold higher. Incidentally, it can be appreciated that, under the experimental conditions used,[42c] motor effects did not interfere with the response measured in the FST. What began as a "routine" effort, merely directed toward finding a back-up for compound **1**, took an unforeseen and exciting turn.

Obviously, processes beyond G-protein signaling take part in the control of in vivo outcomes. For instance, antidepressant-like activity was favored over other behavioral effects with compounds **9** and **15** but not with relatives (e.g., **19**). Also, the ED_{50} in FPT test increases with **9** and **15**, whereas that in FST does not (Table 4). This undermines any pharmacokinetic contribution to this "in vivo selectivity" because it is unlikely that these compounds distributed differently into the brain so as to activate neural pathways unevenly. In contrast, the erosion of all in vivo responses seen with **4** would fit in with a decline in brain exposure as compared to that of **1**.

In agreement with published data, flibanserin (even given ip) was found inactive in the FST.[44] Likewise, PRX-00023 exhibited no antidepressant-like activity in that procedure, a result consistent with the in vitro data we generated (cf., Table 1). It thus would seem that the agonist properties of PRX-00023 at the 5-HT_{1A} receptor subtype have been largely overestimated.[35]

The finding that the profiles of **9** and **15** in the FST do not match those observed in other behavioral tests has several implications: (1) the separation between antidepressant-like and other 5-HT_{1A}-mediated effects may (in theory) still be enhanced; (2) the 5-HT syndrome can no longer be taken as a "quantitative" index of 5-HT_{1A} agonist activity in rats; and (3) further mechanistic studies on compound **9** are warranted. In any case, the heterogeneity in the in vivo responses must reflect heterogeneous activation of the 5-HT_{1A} receptors regulating these processes.[45] It should be taken into account that, for each compound, the data were all compared at 60 min after po dosing to minimize pharmacokinetic influences. Hence, the picture that begins to emerge is that FPT, FBP, and FST patterns are controlled by distinct population(s) of 5-HT_{1A} receptors, which, themselves, are differentially regulated by these ligands.

Although we do not understand the molecular mechanism(s) involved in the discrepancies highlighted above, this cannot be explained by the conventional partition between pre- and postsynaptic 5-HT_{1A} receptors inasmuch as FPT, FBP, and anti-immobility (FST) are predominantly linked to postsynaptic actions of the agonist.[46] We are currently exploring features known to modulate signal transduction in GPCRs (e.g., coupling with different G-proteins, RGS accessory proteins...) and possibly involved in the original profile of **9**.[47,48]

In a separate set of experiments, the anti-immobility effect of **9** was blocked by pretreatment of the animals with the selective 5-HT_{1A} antagonist WAY-100635 (0.63 mg/kg sc), confirming that the anti-immobility effect is attributable to 5-HT_{1A} activation. Importantly, the antidepressant-like profile of **9** was maintained upon repeated administrations, demonstrating that no tachyphilaxis developed to the antidepressant-like response.

Conclusions

Structural modifications of compound **1** with the objective of altering its pharmacokinetic properties provided a novel series of 5-HT_{1A} agonists. SAR study then led us to the selection of compound **9** as a candidate for development in depression. The latter exhibited the desired in vitro 5-HT_{1A} characteristics: marked affinity, specificity, and high efficacy for the 5-HT_{1A} receptor subtype. In vivo profiling of **9** revealed pronounced differences in the 5-HT_{1A}-mediated responses. Thus, **9** was extremely potent in the FST, whereas its ability to produce stereotypic behaviors (FPT and FBP) was much weaker. The molecular mechanism(s) at the origin of this "FST-selectivity" has not been elucidated yet, but it would appear that **9** stimulates preferentially certain (postsynaptic) populations of 5-HT_{1A} receptors.

Conceptually, all anti-depressant medication that has been (at least partly) successful addressed multiple targets, generally through increased levels of endogenous neurotransmitters (e.g., SSRIs, SNRIs, TCAs, MAOIs).[49] In sharp contrast, the present work reinforces our previous finding[12] that, at least in animals, 5-HT_{1A} activation can produce maximally effective antidepressant-like activity, provided the intrinsic activity of the agonist is sufficient. In addition, we disclose preliminary evidence that 5-HT_{1A} activation can be channeled toward the receptor population(s) controlling the desired pharmacological effects, promoting thus the idea that 5-HT_{1A} agonist-directed trafficking may be exploited.[50] Further research is clearly needed to progress on this topic.

Indeed, we hope that the benefits seen in animals with compound **9** will translate into clinical advantages. From FST data, it is expected that **9** will be more effective[51a] and faster acting[51b] than available antidepressant drugs. From syndrome data, it is expected that **9** will be better tolerated insofar as symptoms mediated by 5-HT_{1A} receptors not implicated in the antidepressant action might be spared at antidepressant doses.

Experimental Section

Chemistry. Melting points were determined on a Büchi 530 melting point apparatus and were not corrected. ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in δ value (ppm) relative to an internal standard of tetramethylsilane. Infrared (IR) spectra were obtained on a Nicolet FT 510 P spectra photometer. Microanalyses were obtained on a Fison EA 1108/CHN analyzer. Mass spectra (TSQ 7000 Finnigan, Thermoelectron Corp.) were determined by electron spay ionization (ESI); only 100% relative intensity peaks are given. Analytical thin-layer chromatography was carried out on pre-coated plates (silicagel, 60 F 254 Merck).

General Method for the Reductive Amination between Amines (XII, XIII) and XIVa. Preparation of 3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(heteroarylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone. To a solution of the chlorhydrate of the amine **XII** or **XIII** (1 equiv) in methanol (7 mL per mmol) were added successively **XIVa** (1.1 equiv), 1,4-diazabicyclo[2.2.2]-octane (3 equiv), $NaBH_3CN$ (1.15 equiv), and molecular sieves 4 Å. The mixture was stirred at 50 °C for 6 h, and then the solid was filtered out and the filtrate was concentrated under vacuum. The residue was taken up in ethyl acetate, washed with water, brine, dried over Na_2SO_4, filtered, and concentrated in vacuo.

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(6-methylpyridazin-3-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (7). Reductive amination between **XIIIa** (7.3 g, 59 mmol) and **XIVa** (15.5 g, 49 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol/ammonia, 97.5:2.25:0.25) afforded 3.0 g (16%) of **7** as a yellow solid. Crystallization of **7** as a chlorhydrate salt gave a white powder, mp = 205 °C; HPLC purity 98.3% (eluent, acetonitrile−water−KH_2PO_4, 250:750:6.8 g, pH 4); IR (KBr) ν 3426, 1630, 1444 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.71−2.02 (m, 4H), 2.69 (s, 3H), 2.96−3.19 (m, 1H), 3.21−3.62 (m, 2H), 3.38 (d, J = 19.70 Hz, 2H), 4.36−4.45 (m, 1H), 4.53 (s, 2H), 5.41 (s, 1H), 7.43−7.49 (m, 1H), 7.52 (t, J = 8.60 Hz, 1H), 7.67 (d, J = 6.57 Hz, 1H), 8.75 (d, J = 8.59 Hz, 1H), 7.92 (d, J = 8.59 Hz, 1H), 10.29 (s, 2H); ^{13}C NMR (DMSO-d_6) δ

20.3, 31.9, 32.2, 37.2, 42.7, 49.4, 52.8 (d, J = 22.0 Hz), 92.5 (d, J = 176.0 Hz), 117.2, 119.8, 127.8, 129.2, 130.8, 131.5, 133.7, 153.7, 157.7 (d, J = 249.1 Hz), 159.89, 166.19; MS (ESI) m/z = 494 [M$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methylpyrazin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (8). Reductive amination between **XIIIb** (1.0 g, 8.1 mmol) and **XIVa** (2.51 g, 8.0 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol/ammonia, 95:4.5:0.5) afforded 0.47 g (15%) of **8**. Crystallization of **8** as a chlorhydrate salt gave a white powder, mp = 155 °C; HPLC purity 100% (eluent, acetonitrile−water−KH_2PO_4, 250:750:6.8 g, pH 4); IR (KBr) ν 3426, 1637, 1442 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.69−2.10 (m, 4H), 2.53 (s, 3H), 2.93−3.17 (m, 1H), 3.24 (d, J = 20.0 Hz, 2H), 3.32 (s, 3H), 3.32−3.50 (m, 1H), 4.33 (s, 2H), 7.43−7.49 (m, 1H), 7.52 (t, J = 8.8 Hz, 1H), 7.66 (d, J = 6.8 Hz, 1H), 8.61 (s, 1H), 8.68 (s, 1H), 10.00 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 20.9, 31.7, 32.2, 37.2, 42.7, 48.9, 52.6 (d, J = 22.0 Hz), 92.5 (d, J = 174.9 Hz), 117.2, 119.8, 127.8, 129.2, 133.7, 143.5, 144.4, 144.5, 153.6, 157.7 (d, J = 249.2 Hz), 166.7; MS (ESI) m/z = 394 [M$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methylpyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (9). Reductive amination between **XIIe** (8.21 g, 42.4 mmol) and **XIVa** (13.29 g, 43.2 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol/ammonia, 95:4.5:0.5) afforded 11.9 g (71%) of **9**. Crystallization of **9** as a tosylate salt gave a white powder, mp = 195 °C; HPLC purity 99.8% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 5.5); IR (KBr) ν 3500−3300, 1622 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.75−2.25 (m, 4H), 2.33 (s, 3H), 2.34 (s, 3H), 3.23−3.35 (m, 2H), 3.60−4.52 (m, 2H), 3.52 (d, J = 19.50 Hz, 2H), 4.51 (s, 2H), 4.84 (s, 2H), 7.19 (d, J = 7.95 Hz, 2H), 7.34 (dd, J = 8.60 Hz, 1H), 7.43 (ddd, J = 8.60, 4.60, 2.05 Hz, 1H), 7.61 (dd, J = 7.02, 2.05 Hz, 2H), 7.64 (d, J = 7.95 Hz, 2H), 8.65 (s, 2H); ^{13}C NMR (CD_3OD) δ 15.4, 21.3, 33.3, 33.5, 38.9, 44.3, 52.4, 55.2 (J = 20.8 Hz), 93 (J = 175.8 Hz), 118.3 (J = 21.8 Hz), 122.4 (J = 18.2 Hz), 127, 128.8 (J = 7.9 Hz), 129.8, 130.9, 132.3, 134.2 (J = 4.2 Hz), 141.7, 143.6, 159.0, 159.4, 160.1 (J = 251 Hz), 170.0; MS (ESI) m/z = 395 [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(pyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (10). Reductive amination between **XIIk**[52] (0.77 g, 5.29 mmol) and **XIVa** (1.82 g, 5.79 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 98:2) afforded 0.88 g (44%) of **10**. Crystallization of **10** as a fumarate salt gave an off-white powder, mp = 157 °C; HPLC purity 99.8% (eluent, acetonitrile−water−KH_2PO_4, 200:800:6.8 g, pH 4); IR (KBr) ν 3500, 1702, 1625, 1287 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.64−1.93 (m, 4H), 2.85 (d, J = 21 Hz, 2H), 3.01−3.41 (m, 3H), 3.99 (s, 2H), 4.25 (s, 1H), 6.62 (s, 2H), 7.41 (t, J = 4.8 Hz, 1H), 7.45−7.51 (m, 2H), 7.68 (d, J = 6.4 Hz, 1H), 8.78 (d, J = 4.8 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 31.8, 32.2, 38.8, 43.1, 55.1, 55.5 (d, J = 21.6 Hz), 94.5 (d, J = 170 Hz), 116.9, 119.6, 119.7, 127.6, 129.1, 133.7, 133.8, 157.1 (2C), 157.5 (d, J = 247 Hz), 166.1, 166.6, 167.8; MS (ESI): m/z = 381 [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-carbomethoxypyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (11). Reductive amination between **XIIb** (1.12 g, 5.5 mmol) and **XIVa** (1.74 g, 5.51 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 97:3) afforded 0.24 g (10%) of **11**. Crystallization of **11** as a fumarate salt gave an off-white powder, mp = 134 °C; HPLC purity 86.8% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 4); IR (KBr) ν 2987, 1716, 1685, 1436, 1277 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.67−1.90 (m, 4H), 2.79 (d, J = 22 Hz, 2H), 3.10−3.58 (m, 3H), 3.91 (s, 3H), 4.08 (s, 2H), 4.25 (s, 1H), 6.62 (s, 2H), 7.31−7.49 (m, 2H), 7.61−7.67 (m, 1H), 9.21 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 30.6, 32.4, 37.5, 42.4, 52.5, 55.2, 55.5 (d, J = 21.6 Hz), 95.1 (d, J = 167 Hz), 116.9, 119.6, 121.9, 127.6, 129.0, 133.8, 134.0, 157.5 (2C), 157.5 (d, J = 247.4 Hz), 163.8, 166.0, 166.6, 171.8; MS (ESI) m/z = 439 [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-hydroxymethylpyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (12). Reductive amination between **XIIg** (0.6 g, 3.4 mmol) and **XIVa** (1.18 g, 3.7 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 97:3) afforded 0.44 g (31%) of **12**. Crystallization of **12** as a fumarate salt gave an off-white powder, mp = 166 °C; HPLC purity 99.8% (eluent, acetonitrile−water−KH_2PO_4, 200:800:6.8 g, pH 4); IR (KBr) ν 3083, 1683, 1425, 1274 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.65−1.91 (m, 4H), 2.84 (d, J = 21 Hz, 2H), 3.07−3.51 (m, 3H), 3.99 (s, 2H), 4.25 (s, 1H), 4.54 (s, 2H), 6.62 (s, 2H), 7.43−7.52 (m, 2H), 7.67 (d, J = 6.9 Hz, 1H), 8.72 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 31.7, 32.6, 37.8, 43.0, 54.5, 55.5 (d, J = 21.8 Hz), 58.7, 94.4 (d, J = 171 Hz), 117.4, 120.0, 128.1, 129.6, 133.6, 134.2, 134.4, 156.1 (2C), 157.9 (d, J = 247.5 Hz), 165.6, 166.4, 167.0; MS (ESI) m/z = 411 [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-ethylpyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (13). Reductive amination between **XIIf** (0.59 g, 3.4 mmol) and **XIVa** (1.18 g, 3.7 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 97:3) afforded 0.88 g (63%) of **13**. Crystallization of **13** as a fumarate salt gave a white powder, mp = 172 °C; HPLC purity 99.7% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 4); IR (KBr) ν 2961, 2927, 1629, 1444, 1402 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.6 Hz, 3H), 1.65−1.92 (m, 4H), 2.61 (q, J = 7.6 Hz, 2H), 2.83 (d, J = 21 Hz, 2H), 3.01−3.41 (m, 3H), 3.95 (s, 2H), 4.25 (s, 1H), 6.62 (s, 2H), 7.43−7.51 (m, 2H), 7.67 (d, J = 6.6 Hz, 1H), 8.66 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 14.8, 22.4, 31.9, 32.6, 37.5, 43.0, 54.7, 55.6 (d, J = 21.5 Hz), 94.9 (d, J = 170.3 Hz), 116.9, 119.6, 127.7, 129.1, 133.8, 134.0, 134.3, 156.3 (2C), 157.5 (d, J = 247.5 Hz), 165.4, 166.1, 166.6; MS (ESI) m/z = 409 [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-difluoromethylpyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (14). Reductive amination between **XIIh** (0.38 g, 1.6 mmol) and **XIVa** (0.54 g, 1.7 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 98:2) afforded 0.26 g (37%) of **14**. Crystallization of **14** as a fumarate salt gave a pale yellow powder, mp = 142 °C; HPLC purity 98.8% (eluent, acetonitrile−water−KH_2PO_4, 250:750:6.8 g, pH 4); IR (KBr) ν 2964, 1620, 1438, 1405 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.65−1.91 (m, 4H), 2.78 (d, J = 21 Hz, 2H), 2.99−3.51 (m, 6H), 4.03 (s, 2H), 4.25 (s, 1H), 6.62 (s, 2H), 7.20 (t, J = 55 Hz, 1H), 7.44−7.51 (m, 2H), 7.67 (d, J = 7 Hz, 1H), 9.01 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 32.0, 32.4, 37.7, 43.3, 55.1, 55.5 (d, J = 21.8 Hz), 95.1 (d, J = 170 Hz), 112.9 (t, J = 235 Hz), 116.9, 119.6, 125.7, 127.7 (2C), 129.1, 133.8, 133.9, 155 (t, J = 6 Hz), 157.5 (d, J = 247.7 Hz), 166.0, 166.6, 170.5; MS (ESI) m/z = 431 [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-chloropyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (15). Reductive amination between **XIIm**[53] (1.62 g, 8.0 mmol) and **XIVa** (2.65 g, 8.4 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 98:2) afforded 1.8 g (54%) of **15**. Crystallization of **15** as a fumarate salt gave an off-white powder, mp = 152 °C; HPLC purity 99.4% (eluent, acetonitrile−water−KH_2PO_4, 250:750:6.8 g, pH 4); IR (KBr) ν 3123, 1696, 1623, 1430 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.64−1.91 (m, 4H), 2.65 (d, J = 20.8 Hz, 2H), 3.09−3.50 (m, 3H), 4.01 (s, 2H), 4.25 (s, 1H), 6.63 (s, 2H), 7.42−7.52 (m, 2H), 7.67 (d, J = 7.2 Hz, 1H), 8.92 (s, 2H), 9.20 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 31.8, 32.4, 39.3, 43.0, 54.4, 55.4 (d, J = 21.6 Hz), 94.4 (d, J = 170.3 Hz), 116.9, 119.6, 127.6, 127.7, 128.8, 129.1, 133.8, 156.2 (2C), 157.5 (d, J = 247.3 Hz), 165.9, 166.2, 166.6; MS (ESI) m/z = 415 [M$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(4-methoxypyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (16). Reductive amination between **XIIn**[54] (0.73 g, 5.21 mmol) and **XIVa** (2.0 g, 6.3 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 95:5) afforded 1.1 g (51%) of **16.** Crystallization of **16** as a fumarate salt gave a white powder, mp = 149 °C; HPLC purity 98.6% (eluent, acetonitrile−water−KH_2PO_4, 250:750:6.8 g, pH 4); IR (KBr) ν 3054, 2077, 1710, 1629, 1583 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.68−1.99 (m,

5H), 2.87 (d, $J = 20.8$ Hz, 2H), 3.08−3.91 (m, 3H), 3.88 (s, 2H), 3.92 (s, 3H), 4.27 (s, 1H), 6.62 (s, 2H), 6.82 (d, $J = 5.6$ Hz, 1H), 7.45−7.52 (m, 2H), 7.67 (d, $J = 6.7$ Hz, 1H), 8.48 (d, $J = 5.6$ Hz, 1H), 9.30 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 32.4, 32.9, 38.0, 43.5, 53.9, 55.1, 56.0 (d, $J = 21.6$ Hz), 94.8 (d, $J = 170.4$ Hz), 106.4, 117.4, 120.0, 128.1, 129.6, 134.2, 134.5, 157.9 (d, $J = 247.5$ Hz), 157.9, 166.6, 167.0, 168.2, 169.4; MS (ESI) $m/z = 411$ [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(4-methylaminopyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (17). Reductive amination between **XIIc** (0.55 g, 3.98 mmol) and **XIVa** (1.5 g, 4.8 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol/ammonia, 90:9:1) afforded 0.16 g (10%) of **17**. Crystallization of **17** as an oxalate salt gave a white powder, mp = 180 °C; HPLC purity 98.8% (eluent, acetonitrile−water−KH_2PO_4, 150:850:6.8 g, pH 2.5); IR (KBr) ν 3023, 1653, 1603, 1464 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.52−2.03 (m, 4H), 2.85 (s, 3H), 2.98−3.45 (m, 3H), 3.28 (d, $J =$ 20 Hz, 2H), 4.11 (s, 2H), 4.30 (s, 1H), 6.45 (d, $J = 6$ Hz, 1H), 7.32−7.54 (m, 2H), 7.68 (d, $J = 7$ Hz, 1H), 7.82 (s, 1H), 8.02 (s, 1H), 9.70 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 31.3, 32.0, 36.1, 38.8, 42.7, 51.3, 53.6 (d, $J = 21.5$ Hz), 92.7 (d, $J = 175$ Hz), 103.6, 117.1, 119.6, 127.7, 129.1, 133.6, 156.3, 159.0 (d, $J = 249$ Hz), 162.9, 163.6, 166.6; MS (ESI) $m/z = 410$ [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(4-dimethylaminopyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (18). Reductive amination between **XIId** (0.74 g, 4.8 mmol) and **XIVa** (1.8 g, 5.7 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 90:10) afforded 0.66 g (33%) of **18**. Crystallization of **18** as a fumarate salt gave a white powder, mp = 160 °C; HPLC purity 99.4% (eluent, acetonitrile−water−KH_2PO_4, 250:750:6.8 g, pH 4); IR (KBr) ν 3013, 1623, 1599, 1259 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.71−1.94 (m, 4H), 2.87 (d, $J = 20.8$ Hz, 2H), 3.06 (s, 6H), 3.00−3.42 (m, 3H), 3.77 (s, 2H), 4.26 (s, 1H), 6.54 (d, $J = 5.2$ Hz, 1H), 6.60 (s, 2H), 7.46−7.50 (m, 3H), 7.67, (d, $J = 5.6$ Hz, 1H), 8.13 (d, $J = 5.2$ Hz, 1H), 8.96 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 32.3, 33.0, 36.8 (2C), 37.9, 43.4, 55.0, 55.9 (d, $J = 21.6$ Hz), 94.7 (d, J = 170.6 Hz), 101.4, 117.4, 120.1, 128.1, 129.6, 134.2, 134.6, 155.1, 157.9 (d, $J = 247.6$ Hz), 159.2, 161.8, 166.2, 166.7, 167.0; MS (ESI) $m/z = 424$ [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(4-methylamino-5-methylpyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (20). Reductive amination between **XIIa** (0.63 g, 4.1 mmol) and **XIVa** (1.45 g, 4.6 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 90:10) afforded 1.15 g (63%) of **20**. Crystallization of **20** as a fumarate salt gave a white powder, mp = 157 °C; HPLC purity 99.1% (eluent, acetonitrile−water−KH_2PO_4, 250:750:6.8 g, pH 4); ^{1}H NMR (DMSO-d_6) δ 1.53−1.81 (m, 4H), 1.98 (s, 3H), 2.85 (d, $J =$ 20.5 Hz, 2H), 2.86 (d, $J = 4.4$ Hz, 3H), 3.02−3.41 (m, 4H), 3.73 (s, 2H), 4.25 (s, 1H), 6.61 (s, 4H), 6.87 (m, 1H), 7.42−7.51 (m, 2H), 7.67 (d, $J = 6.6$ Hz, 1H), 7.84 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 13.3, 27.3, 32.0, 33.0, 37.2, 42.9, 54.5, 55.7 (d, $J = 21$ Hz), 94.1 (d, $J = 171$ Hz), 111.7, 116.9, 117.1, 127.7, 129.1, 133.8, 134.1, 151.8, 156.3, 158.8, 162.5 (d, $J = 270$ Hz), 166.1, 166.6; MS (ESI) $m/z = 424$ [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(4-methylamino-5-chloropyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (21). Reductive amination between **XIIj** (0.8 g, 4.6 mmol) and **XIVa** (1.54 g, 4.9 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 95:5) afforded 0.30 g (15%) of **21**. Crystallization of **21** as a di-fumarate salt gave a white powder, mp = 92 °C; HPLC purity 90.0% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 4); IR (KBr) ν 3084, 1600, 1430, 1277 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.67−1.91 (m, 4H), 2.90 (d, $J = 20$ Hz, 2H), 2.91 (s, 3H), 3.07−3.41 (m, 3H), 3.81 (s, 2H), 4.26 (s, 1H), 6.61 (s, 4H), 7.43−7.51 (m, 3H), 7.67 (d, $J = 7$ Hz 1H), 8.15 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 27.4, 32.4, 32.5, 38.6, 42.9, 53.9, 55.2 (d, $J = 21.3$ Hz), 94.2 (d, $J =$ 170.5 Hz), 111.9, 117.0, 119.6, 127.6, 129.1, 133.8, 134.0, 151.6, 157.8 (d, $J = 248$ Hz), 157.9, 164.5, 166.1, 166.6; MS (ESI) m/z = 444 [M − H$^+$].

General Method for the Reductive Amination between Aldehydes (XVIII, XIX) and XIVb. To a solution of **XIVb** (1 equiv) and **XVIII** or **XIX** (1 equiv) in dichloroethane (14 mL per mmol of XIVb) was added sodium triacetoxyborohydride (1.5 equiv), and the mixture was stirred at room temperature. Water was added, the aqueous was extracted with dichloromethane, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo.

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(4-fur-2-yl-pyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (19). Reductive amination between **XIX** (0.8 g, 4.6 mmol) and **XIVb** (1.33 g, 4.6 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 95:5) afforded 1.98 g (96%) of **19**. Crystallization of **19** as a hemi-fumarate salt gave a white powder, mp = 122 °C; HPLC purity 98.9% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 4); IR (KBr) ν 1631, 1445 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.70−1.93 (m, 4H), 2.85 (d, $J = 21.2$ Hz, 2H), 3.08−3.51 (m, 3H), 3.97 (s, 2H), 4.25 (s, 1H), 6.61 (s, 1H), 6.75 (m, 1H), 7.42−7.51 (m, 3H), 7.62 (d, $J = 5.2$ Hz, 1H), 7.67 (dd, $J = 7.2$ Hz, 1H), 7.99 (s, 1H), 8.79 (d, $J = 5.3$ Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 32.4, 32.9, 39.2; 45.0, 55.5, 56.0 (d, $J = 21.5$ Hz), 95.4 (d, $J = 170.1$), 113.0, 113.4, 117.3, 117.5, 120.2, 128.0, 129.6, 134.2, 134.5, 146.7, 151.3, 154.8, 157.9 (d, $J = 248$ Hz), 158.4, 166.6, 167.0, 168.6; MS (ESI) $m/z = 447$ [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-carboxy-pyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (2). Reductive amination between **XVIIIa** (0.9 g, 5.0 mmol) and **XIVb** (1.45 g, 5.0 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 95:5) afforded 2.1 g (95%) of the adduct, mp = 177 °C. The ester was dissolved in a solution of lithium hydroxide in water (25 mL) and ethanol (30 mL) and stirred for 3 h at room temperature. Compound **2** was purified by flash column chromatography (silica gel, dichloromethane/methanol, 75:25) to afford 0.5 g (24%) as a white powder, mp = 184 °C; HPLC purity 100% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 4); IR (KBr) ν 3665, 3350, 3201, 1616 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.65−1.91 (m, 4H), 2.74 (d, $J = 21.2$ Hz, 2H), 3.06−3.51 (m, 5H), 3.92 (s, 2H), 4.25 (s, 1H), 7.40−7.51 (m, 2H), 7.58 (dd, $J = 8$ Hz, 1H), 7.66 (dd, $J = 6.4$ Hz, 1H), 8.23 (d, $J = 6$ Hz, 1H), 8.98 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 32.1, 32.5, 37.6, 43.1, 54.7, 55.8 (d, $J = 21.8$ Hz), 94.5 (d, $J = 170.1$ Hz), 116.9, 119.7, 120.9, 127.7, 128.7, 129.2, 133.8, 137.0, 149.7, 157.6 (d, $J = 247.5$ Hz), 162.6, 166.7, 167.2; MS (ESI) $m/z = 424$ [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-hydroxymethylpyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (3). Reductive amination between **XVIIIc** (0.28 g, 2.0 mmol) and **XIVb** (0.59 g, 2.0 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 95:5) afforded 0.47 g (57%) of **3**. Crystallization of **3** as a fumarate salt gave a white powder, mp = 167 °C; HPLC purity 99.7% (eluent, acetonitrile−water−KH_2PO_4, 200:800:6.8 g, pH 4); IR (KBr) ν 3350, 1697, 1616, 1263 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.66−1.99 (m, 4H), 2.79 (d, $J = 20.8$ Hz, 2H), 3.06−3.47 (m, 3H), 3.91 (s, 2H), 4.27 (s, 1H), 4.52 (s, 1H), 6.61 (s, 2H), 7.40−7.51 (m, 3H), 7.67 (d, $J =$ 7 Hz, 1H), 7.70 (d, $J = 8$ Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 32.4, 32.8, 39.2, 44.0, 54.4, 55.7 (d, $J = 22$ Hz), 60.8, 95.1 (d, $J = 170.6$ Hz), 117.3, 120.1, 121.9, 128.1, 129.6, 134.2, 134.3, 135.4, 136.3, 147.7, 157.7, 157.9 (d, $J = 247.5$ Hz), 166.7, 167.0; MS (ESI) $m/z = 410$ [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-fluoromethylpyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (4). Reductive amination between **XVIIId** (0.39 g, 2.8 mmol) and **XIVb** (0.82 g, 2.8 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 95:5) afforded 0.82 g (71%) of **4**. Crystallization of **4** as a fumarate salt gave a white powder, mp = 160 °C; HPLC purity 99.9% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 4); IR (KBr) ν 2964, 1622, 1432 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.56−2.04 (m, 4H), 2.75 (d, $J =$ 20.8 Hz, 2H), 3.06−3.26 (m, 3H), 3.90 (s, 2H), 4.26 (s, 1H), 5.46

(d, J = 48 Hz, 2H), 6.62, (s, 2H), 7.42−7.52 (m, 3H), 7.66 (dd, J = 8.8 Hz, 1H), 7.85 (d, J = 8 Hz, 1H), 8.57 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 32.3, 32.9, 37.9, 43.5, 54.6, 55.5 (d, J = 21.7 Hz), 81.9 (J = 160 Hz), 94.8 (d, J = 170.4 Hz), 116.9, 119.6, 121.6, 127.7, 129.7, 133.7, 133.8, 136.5, 148.4, 157.5 (d, J = 247 Hz), 160.1, 166.1, 166.6; MS (ESI) m/z = 411 [M$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-difluoromethylpyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (5). Reductive amination between **XVIIIb** (0.69 g, 4.4 mmol) and **XIVb** (1.28 g, 4.4 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/acetonitrile, 70:30) afforded 1.67 g (85%) of **5**. Crystallization of **5** as a fumarate salt gave a white powder, mp = 164 °C; HPLC purity 99.9% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 4); IR (KBr) ν 3050, 2964, 1629, 1434 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.66−1.95 (m, 4H), 2.77 (d, J = 20.8 Hz), 2.81−3.42 (m, 3H), 3.96 (s, 2H), 4.28 (s, 1H), 6.62 (s, 2H), 7.14 (t, J = 55.2 Hz), 7.43−7.51 (m, 2H), 7.62 (d, J = 8 Hz, 1H), 7.67 (d, J = 7 Hz, 1H), 7.99 (d, J = 8 Hz, 1H), 8.72 (s, 1H), 9.30 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 32.4, 32.8, 37.9, 43.5, 54.7, 55.9 (d, J = 21.7 Hz), 95.2 (d, J = 170.3 Hz), 114.4 (t, J = 234.3 Hz), 117.4, 120.1, 122.2, 128.1, 128.4, 128.6, 129.6, 134.2, 134.4, 146.6, 157.9 (d, J = 247.4 Hz), 163.0, 166.5, 167.0; MS (ESI) m/z = 430 [MH$^+$].

3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-chloropyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone (6). Reductive amination between **XVIIIe** (0.2 g, 1.4 mmol) and **XIVb** (0.4 g, 1.4 mmol) and then purification by flash column chromatography (silica gel, dichloromethane/methanol, 95:5) afforded 0.30 g (51%) of **6**. Crystallization of **6** as a fumarate salt gave a white powder, mp = 175 °C; HPLC purity 99.8% (eluent, acetonitrile−water−KH_2PO_4, 300:700:6.8 g, pH 4); IR (KBr) ν 3052, 1621, 1431 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.65−1.92 (m, 4H), 2.60 (d, J = 21 Hz, 2H), 2.75−3.39 (m, 4H), 3.85 (s, 2H), 4.25 (s, 1H), 6.62 (s, 2H), 7.43−7.52 (m, 3H), 7.66 (d, J = 7 Hz, 1H), 7.89 (dd, J = 8.4, 2.4 Hz, 1H), 8.54 (s, 1H), 9.58 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 32.1, 33.3, 38.0, 42.8, 53.9, 55.5 (d, J = 21.6 Hz), 94.5 (d, J = 170 Hz), 116.9, 120.2, 123.1, 127.6, 129.0, 129.1, 133.8, 133.9, 136.2, 147.1, 157.3 (d, J = 250 Hz), 158.7, 166.0, 166.6; MS (ESI) m/z = 414 [M$^+$].

Radioligand Binding at the 5-HT$_{1A}$ Receptor. The membrane preparations (rat cortex 10 mg/mL) and binding assays were performed as described previously.[55] At the end of the incubation period (30 min at 23 °C, Tris-HCl buffer), the tube contents were filtered under vacuum through GF/B filters with two 5 mL washes of Tris-HCl buffer (50 mM, pH 7.4 at 25 °C). The radioactivity retained on the filters was measured by scintillation spectroscopy in 4 mL of scintillation fluid (Emulsifier safe, Packard). The results of displacement experiments were analyzed using the nonlinear regression program KELL RADLIG version 6 (Biosoft, Cambridge, UK),[56] and pK_i values are given as means ± SEM of three experiments, each comprising 6−7 concentrations differing by one log unit interval. The −log of K_i values (pK_i) were calculated from the Cheng−Prusoff equation $K_i = IC_{50}/(1 + [\text{radioligand}]/K_d)$. The K_d value of 8-OH-DPAT is 3.1 nM. [^{3}H]8-OH-DPAT (0.2 nM, TRK.850: 160−240 Ci/mmol) was purchased from Amersham; nonspecific binding was determined with 5-HT (10 μM) purchased from Sigma.

[^{35}S]GTPγS Binding in C6 Glial Cells. This was adapted from the procedure described in detail by Koek.[12] C6-glial cells were collected in phosphate-buffered-saline (pH 7.4) and centrifuged for 20 min at 48 000g. The pellet was homogenized with a Polytron in 20 mM HEPES containing 10 mM EDTA (pH 7.4) and centrifuged for 10 min. The resulting pellet was washed twice in 10 mM HEPES (pH 7.4) containing 0.1 mM EDTA. The pellet was stored at −80 °C in fractions of 600−750 μg of protein. The pellet was thawed, diluted 20 times in 20 mM HEPES supplemented with the 30 μM GDP, 100 mM NaC1, 3 mM $MgC1_2$, and 0.2 mM ascorbic acid. Incubation mixtures were prepared in glass tubes and consisted of 0.4 mL of membrane preparation (16−38 μg of protein) and 0.05 mL of compound. After an incubation period of 30 min at 25 °C, 0.05 mL of [^{35}S]GTPγS (500 pM) was added for an additional period of 30 min. The reactions were stopped by adding 3 mL of ice-cold 20 mM HEPES containing 3 mM $MgCl_2$ and rapid filtration over Whatman GF/B glass fiber filters using a Brandel harvester. The filters were rinsed three additional times with 3 mL of HEPES buffer, placed in scintillation vials, and the radioactivity was extracted in 4 mL of Emulsifier-Safe. Nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. Maximal stimulation of [^{35}S]GTPγS binding was defined in the presence of 10 μM 5-HT. Bovine serum albumin was used as a standard. Each compound was tested at six concentrations, and [^{35}S]-GTPγS binding values were expressed as a percentage of the maximal response obtained with 10 μM 5-HT. Data were estimated by means of nonlinear regression (sigmoidal model with unit slope; Graphpad Prism). E_{max} value differences were analyzed statistically by a one-way analysis of variance followed by paired comparisons by Newman-Keuls tests; P values <0.05 were considered statistically significant.

Elements of the 5-HT Syndrome. The methods used were similar to those described previously.[38b,57] Behavioral observations were made at two time points, from 10 to 20 min and from 55 to 65 min post-administration po. Four animals were observed individually during each 10 min period; the rats were successively observed during a 10 s period (i.e., one animal every 15 s) for the presence (1) or absence (0) of forepaw treading (FPT). The behavior was considered present if the animal showed uninterrupted sign for at least 3 s. This cycle was repeated 10 times during a 10 min period; thus, the incidence of a particular behavior could vary from 0 to 10 for any observation period. Flat body posture (FBP) was scored present (1) if it occurred during the entire observation period, otherwise, the score was 0. Compounds were dissolved in H_2O and administered in a volume of 10 mL/kg and tested in five animals per dose. Doses refer to the weight of the free base and the order of treatment with doses was unsystematic. Dose−response functions were determined from the percentage of rats showing FBP, and the percentage of rats showing FPT scores of 1 or more. These criteria were based upon the incidence of each particular behavior observed in control animals treated with saline. ED_{50} values and their 95% confidence limits were calculated with the Litchfield and Wilcoxon probit analysis.[58]

Forced Swimming Test. This was adapted from the procedure described by Porsolt.[42b] A rat was placed in a Plexiglas cylinder (height 45 cm, diameter 20 cm) containing 17 cm of water (at 25 °C) for 15 min on the first day of the experiment, and placed again 24 h later for 5 min. The duration of immobility during the 5 min period was measured by an observer who was unaware of the treatment conditions. Each animal was treated po (administration volume: 10 mL/kg) with vehicle immediately after the first session, and with a dose of the compound (n = 5−12 per dose), or its vehicle control, 1 h before the second session. Drug effects on the median immobility time were analyzed statistically by means of a Kruskal−Wallis nonparametric analysis of variance, followed, where appropriate, by comparisons using Dunns test. Dose−response functions were determined from the percentage of rats with immobility times less than 138 s (a criterion based on the immobility times obtained in 1500 controls over more than 10 years), and ED_{50} values and their 95% confidence limits were calculated with the Litchfield and Wilcoxon probit analysis.[58]

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Supporting Information Available: Experimental and analytical data for intermediates **II**, **III**, **VI**, **VIII**, **X**−**XIII**, and **XVI**−**XIX**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Vacher, B.; Bonnaud, B.; Funes, P.; Jubault, N.; Koek, W.; Assié, M.-B.; Cosi, C. Design and synthesis of a series of 6-substituted-2-pyridinylmethylamine derivatives as novel, high-affinity, selective agonists at 5-HT_{1A} receptors. *J. Med. Chem.* **1998**, *41*, 5070−5083. (b) Vacher, B.; Bonnaud, B.; Funes, P.; Jubault, N.; Koek, W.; Assié, M.-B.; Cosi, C.; Kleven, M. Novel derivatives of 2-pyridinemethylamine as selective, potent, and orally active agonists at 5-HT_{1A} receptors. *J. Med. Chem.* **1999**, *42*, 1648−1660.

(2) Colpaert, F. 5-HT_{1A} receptor activation: New molecular and neuroadaptative mechanisms of pain relief. *Curr. Opin. Invest. Drugs* **2006**, *7*, 40−47.

(3) Colpaert, F. C.; Tarayre, J. P.; Koek, W.; Pauwels, P. J.; Bardin, L.; Xu, X. J.; Wiesenfeld-Hallin, Z.; Cosi, C.; Carilla-Durand, E.; Assié, M. B.; Vacher, B. Large-amplitude 5-HT_{1A} receptor activation: A new mechanism of profound central analgesia. *Neuropharmacology* **2002**, *43*, 945−958. Bardin, L.; Tarayre, J. P.; Malfetes, N.; Koek, W.; Colpaert, F. C. Profound, non-opioid analgesia produced by the high efficacy 5-HT_{1A} agonist F 13640 in the formalin model of tonic nociceptive pain. *Pharmacology* **2003**, *67*, 182−194.

(4) Deseure, K. R.; Koek, W.; Colpaert, F. C.; Adriaensen, H. The 5-HT_{1A} receptor agonist F 13640 attenuates mechanical allodynia in a rat model of trigeminal neuropathic pain. *Eur. J. Pharmacol.* **2002**, *456*, 51−57. Kiss, I.; Degryse, A. D.; Bardin, L.; Alvarez Gomez de Segura, I.; Colpaert, F. C. The novel analgesic, F 13640, produces intra- and postoperative analgesia in a rat model of surgical pain. *Eur. J. Pharmacol.* **2005**, *523*, 29−39.

(5) Deseure, K. R.; Koek, W.; Adriaensen, H.; Colpaert, F. C. Continuous administration of the 5-HT_{1A} agonist (3-chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-amino]-methyl}piperidin-1-yl]-methanone (F 13640) attenuates allodynia-like behavior in a rat model of trigeminal neuropathic pain. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 505−514.

(6) Colpaert, F. C.; Wu, W. P.; Hao, J. X.; Royer, I.; Sautel, F.; Wiesenfeld-Hallin, Z.; Xu, X. J. High-efficacy 5-HT_{1A} receptor activation causes a curative-like action on allodynia in rats with spinal cord injury. *Eur. J. Pharmacol.* **2004**, *497*, 29−33.

(7) Butera, J. A. Current and emerging targets to treat neuropathic pain. *J. Med. Chem.* **2007**, *50*, 2543−46. Rice, A. S. C.; Hill, R. G. New treatments for neuropathic pain. *Annu. Rev. Med.* **2006**, *57*, 535−551. Mico, J. A.; Ardid, D.; Berrocso, E.; Eschalier, A. Antidepressants and pain. *Trends Pharmacol. Sci.* **2006**, *27*, 348−354. Davies, S. L. Rising to the pain challenge. *Drug News Perspect.* **2006**, *19*, 653−658.

(8) Pedigo, N. W.; Yamamura, H. I.; Nelson, D. L. Discrimination of multiple [3H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* **1981**, *36*, 220−226.

(9) Examples of 5-HT_{1A} agonists that underwent clinical trials: zalospirone [114298-18-9]; umespirone [107736-98-1]; S-15535 [146998-34-7]; repinotan [144980-29-0]; Org-13011 [142494-12-0]; lesopitron [132449-46-8]; sunepitron [148408-65-5]; ipsapirone [95847-70-4]; flesinoxan [98206-10-1]; eptapirone [179756-85-5]; enilospirone [59798-73-1]; AP-521 [151227-08-6]; alnespirone [138298-79-0]; adatanserin [127266-56-2]; VML-670 (Mealy, N. E. *Drugs Future* **2004**, *29*, 662); U-93385 [147145-16-2]; EMD-56551 [133109-87-2]; CGP-50281 [100746-36-9]; Ebalzotan [149494-37-1].

(10) Buspirone: 8-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-8-azaspiro-(4.5)decane-7,9-dione, [36505-84-7].

(11) Tandospirone: 4,7-methano-1*H*-isoindole-1,3(2*H*)-dione, hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-, (3aα,4*β*,7*β*,7aα)-, 2-hydroxy-1,2,3-propanetricarboxylate, [112457-95-1].

(12) Koek, W.; Vacher, B.; Cosi, C.; Assié, M. B.; Patoiseau, J.-F.; Pauwels, P. J.; Colpaert, F. C. 5-HT_{1A} receptor activation and antidepressant-like effects: F 13714 has high efficacy and marked antidepressant potential. *Eur. J. Pharmacol.* **2001**, *420*, 103−112.

(13) Appert-Collin, A.; Duong, F. H. T.; Passilly Degrace, P.; Warter, J. M.; Poindron, P.; Gies, J. P. MAPK activation via 5-hydroxytryptamine 1A receptor is involved in the neuroprotective effects of xaliproden. *Int. J. Immunopathol. Pharmacol.* **2005**, *18*, 21−31. Kline, A. E.; Massucci, J. L.; Dixon, C. E.; Zafonte, R. D.; Bolinger, B. D. The therapeutic efficacy conferred by the 5-HT(1A) receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) after experimental traumatic brain injury is not mediated by concomitant hypothermia. *J. Neurotrauma* **2004**, *21*, 175−185. Kline, A. E.; Yu, J.; Horvath, E.; Marion, D. W.; Dixon, C. E. The selective 5-HT-(1A) receptor agonist repinotan HCl attenuates histopathology and spatial learning deficits following traumatic brain injury in rats. *Neuroscience* **2001**, *106*, 547−555.

(14) In rat and dog, **1** (F 13640) has a bioavailability greater than 70%. In human, its suitable pharmacokinetic properties were confirmed (e.g., mean half-life elimination from plasma was 35 h). The aim was not to improve over any particular PK parameter relative to **1** but rather to obtain a profile somewhat different. Indeed, extending the patent life of these series was also a major incentive.

(15) Murray, C. J. L.; Lopez, A. D. Alternative projections of mortality and disability by cause 1990−2020: Global Burden of Disease Study. *Lancet* **1997**, *349*, 1498−1504. Andlin-Sobocki, P.; Jonsson, B.; Wittchen, H.-U.; Olesen, J. Cost of disorders of the brain in Europe. *Eur. J. Neurol.* **2005**, *12* (supp. 1), 1−27.

(16) See ref 1b. Also: Vacher, B.; Bonnaud, B.; Koek, W. Pyridin-2-yl-methylamine derivatives, method of preparing and application as medicine. Pierre Fabre Medicament WO/9822459, 1998. Maurel, J. L.; Bonnaud, B.; Ribet, J. P.; Vacher, B. Synthesis method and intermediates of pyridin-2-yl-methylamine. Pierre Fabre Medicament WO 02/064585, 2002. Vacher, B.; Bonnaud, B.; Maurel, J. L.; Colpaert, F. Novel aryl-{4-halo-4-[heteroarylmethylamino)-methyl]-piperidin-1-yl}-methanone derivatives, methods for production and use thereof as medicaments. WO 03/106449, 2003.

(17) Methyl 2-(methylsulfonyl)pyrimidine-5-carboxylate, [38275-49-9].

(18) Ohta, K.; Kawachi, E.; Inoue, N.; Fukasawa, H.; Hashimoto, Y.; Itai, A.; Kagechika, H. Retinoidal pyrimidinecarboxylic acids. Unexpected diaza-substituent effects in retinobenzoic acids. *Chem. Pharm. Bull.* **2000**, *48*, 1504−1513. Liang, Y.-M.; Luo, S.-J.; Zhang, Z.-X.; Ma, Y.-X. Efficient synthesis of a new pyrimidine derivative. *Synth. Commun.* **2002**, *32*, 153−157.

(19) 2-Chloro-4-(methylamino)pyrimidine, [66131-68-8].

(20) 5-Chloro-2-(methylsulfonyl)-4-pyrimidinecarboxylic acid, [38275-34-2].

(21) Pie, P. A.; Green, T. P.; Hennequin, L. F.; Curwen, J.; Fennell, M.; Allen, J.; Lambert-van der Brempt, C.; Costello, G. Discovery of a new class of anilinoquinazoline inhibitors with high affinity and specificity for the tyrosine kinase domain of c-Src. *J. Med. Chem.* **2004**, *47*, 871−887.

(22) Lange, U. E. W.; Schäfer, B.; Baucke, D.; Buschmann, E.; Mack, H. A new mild method for the synthesis of amidines. *Tetrahedron Lett.* **1999**, *40*, 7067−7071. Bose, A. K.; Greer, F.; Gots, J. S.; Price, C. C. Some derivatives of glycineamidine. *J. Org. Chem.* **1959**, *24*, 1309−1313.

(23) Stolle, W. A. W.; Frissen, A. E.; Marcelis, A. T. M.; van der Plas, H. C. Intramolecular Diels-Alder reactions of pyrimidines and a computational study toward their structure and reactivity. *J. Org. Chem.* **1992**, *57*, 3000−3007. Gupton, J. T.; Gall, J. E.; Riesinger, S. W.; Smith, S. Q.; Bevirt, K. M.; Sikorski, J. A.; Dahl, M. L.; Arnold, Z. An alternative preparation of the 2-dimethylaminomethylene-1,3-bis(dimethylimmonio)propane salt from phosphonoacetic acids and some applications in heterocyclic synthesis. *J. Heterocycl. Chem.* **1991**, *28*, 1281−1285. Isoda, S.; Yamaguchi, H.; Satoh, Y.; Miki, T.; Hirata, M. Medicinal chemical studies on antiplasmin drugs. VI. Aza analogs of 4-aminomethylbenzoic acid. *Chem. Pharm. Bull.* **1980**, *28*, 1408−1414.

(24) 2-Cyano-5-methylpyrazine, [98006-91-8].

(25) 3-Cyano-6-methylpyridazine, [49840-90-6].

(26) 5-Aminomethyl-2-methylpyrazine, [132664-85-8].

(27) (a) 5-Chloro-2-pyridinecarboxaldehyde, [31181-89-2]. (b) 5-(Hydroxymethyl)-2-pyridinecarboxaldehyde, [40749-33-5]. (c) 5-Fluoromethylpyridine-2-carboxaldehyde, [635323-74-9].

(28) Ethyl 6-(hydroxymethyl)nicotinate, [35005-81-3].

(29) Liu, Y.; Lien, I.-F. F.; Ruttgaizer, S.; Dove, P.; Taylor, S. D. Synthesis and protection of aryl sulfates using the 2,2,2-trichloroethyl moiety. *Org. Lett.* **2004**, *6*, 209−212.

(30) 4-(Fur-2-yl)-2-methyl-pyrimidine, [28565-17-5].

(31) Unpublished data.

(32) The bio-transformation of **1** into **2** is likely to proceed through the alcohol **3**. However, **3** could not be detected in vivo (neither any phase 2 metabolite).

(33) The extra nitrogen in the diazines decreased the lipophilicity of the compound (e.g., **9**: log $D = 1.5$, p$K_a = 6.7$) as compared to **1**: log $D = 2.6$, p$K_a = 6.4$. This, in turn, influenced ADM parameters.

(34) 8-OH-DPAT: 8-hydroxy-2-dipropylaminotetralin, [76135-31-4].

(35) (a) de Paulis, T. Drug evaluation: PRX-00023, a selective 5-HT_{1A} receptor agonist for depression. *Curr. Opin. Invest. Drugs* **2007**, *8*, 78−86. Iyer, G. R.; Reinhard, J. F.; Oshana, S.; Kauffman, M; Donahue, S. Tolerability, pharmacokinetics, and neuroendocrine effects of PRX-00023, a novel 5-HT1A agonist, in healthy subjects. *J. Clin. Pharmacol.* **2007**, *47*, 817−824. (b) Becker, O. M.; Dhanoa, D. S.; Marantz, Y.; Chen, D.; Shacham, S.; Cheruku, S.; Heifetz, A.; Mohanty, P.; Fichman, M.; Sharadendu, A.; Nudelman, R.; Kauffman, M.; Noiman, S. An integrated in silico 3D model-driven discovery of a novel, potent, and selective amidosulfonamide 5-HT_{1A} agonist (PRX-00023) for the treatment of anxiety and depression. *J. Med. Chem.* **2006**, *49*, 3116−3135. This compound is in Phase IIb for major depressive disorder.

(36) Flibanserin: 1,3-dihydro-1-[2-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]ethyl]-2*H*-benzimidazol-2-one, [167933-07-5]. Invernizzi, R. W.; Sacchetti, G.; Parini, S.; Acconcia, S.; Samanin, R. Flibanserin, a potential antidepressant drug, lowers 5-HT and raises dopamine and noradrenaline in the rat prefrontal cortex dialysate: Role of 5-HT$_{1A}$ receptors. *Br. J. Pharmacol.* **2003**, *139*, 1281−1288. Borsini, F.; Evans, K.; Jason, K.; Rohde, F.; Alexander, B.; Pollentier, S. Pharmacology of flibanserin. *CNS Drug Rev.* **2002**, *8*, 117−142. Initially, this compound underwent clinical trial in depression but was then switched to sexual dysfunction in women (Phase III).

(37) See ref 8. See also: Pauwels, P. J.; Tardif, S.; Wurch, T.; Colpaert, F. C. Stimulated [^{35}S]GTPγS binding by 5-HT$_{1A}$ receptor agonists in recombinant cell lines: modulation of apparent efficacy by G-protein activation state. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *356*, 551−561.

(38) (a) Tricklebank, M. D.; Forler, C.; Fozard, J. R. The involvement of subtypes of the 5-HT1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino)tetraline in the rat. *Eur. J. Pharmacol.* **1984**, *106*, 271−282. (b) Colpaert, F. C.; Koek, W.; Lehmann, J.; Rivet, J. M.; Lejeune, F.; Canton, H.; Bervoets, K.; Millan, M. J.; Laubie, M.; Lavielle, G. S 14506: A novel, potent, high-efficacy 5-HT$_{1A}$ agonist and potential anxiolytic agent. *Drug Dev. Res.* **1992**, *26*, 21−48.

(39) Compound **9** selectivity was over 1000-fold with respect to the other serotonin subtypes, but also toward an extensive panel of other receptors (e.g., dopaminergic, adrenergic...), transporters, ion channels, and enzymes.

(40) Initially, we estimated the bioavailability of these compounds from the ratios of the doses inducing FPT at fixed intervals after ip and po administrations. Subsequently, the absolute bioavailability in rat of compound **9** was measured (F > 80%, unpublished data).

(41) See ref 8 and references therein.

(42) (a) Belozertseva, I. V.; Kos, T.; Popik, P.; Danysz, W.; Bespalov, A. Y. Antidepressant-like effects of mGluR1 and mGluR5 antagonists in the rat forced swim and the mouse tail suspension tests. *Eur. Neuropsychopharmacol.* **2007**, *17*, 172−179. Cryan, J. F.; Valentino, R. J.; Lucki, I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci. Biobehav. Rev.* **2005**, *29*, 547−569. Lucki, I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav. Pharmacol.* **1997**, *8*, 523−532. Detke, M. J.; Lucki, I. Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. *Behav. Brain Res.* **1996**, *73*, 43−46. Borsini, F.; Meli, A. Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology* **1988**, *94*, 147−160. Porsolt, R. D.; Bertin, A.; Blavet, N.; Deniel, M.; Jalfre, M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. *Eur. J. Pharmacol.* **1979**, *57*, 201−210. (b) Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. Behavioral despair in rats: a new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.* **1978**, *47*, 379−391. (c) An important experimental parameter in the FST that varies between laboratories is the depth of the water in the cylinder.

(43) A previous study in this laboratory established that the tricyclic antidepressant imipramine and the SSRI paroxetine reversed immobility only weakly and partially upon repeated administration. Koek, W.; Patoiseau, J. F.; Assié, M.-B.; Cosi, C.; Kleven, M. S.; Dupont-Passelaigue, E.; Carilla-Durand, E.; Palmier, C.; Valentin, J. P.; John, G. W.; Pauwels, P. J.; Tarayre, J. P.; Colpaert, F. C. F11440, a potent, selective, high efficacy 5-HT$_{1A}$ receptor agonist with marked anxiolytic and antidepressant potential. *J. Pharmacol. Exp. Ther.* **1998**, *287*, 266−283.

(44) Borsini, F.; Cesana, R. Further characterization of potential antidepressant action of flibanserin. *Psychopharmacology* **2001**, *159*, 64−69.

(45) The progress made in understanding the mechanism of action of **9** will be reported in due course. For abstracts, see: Newman-Tancredi, A.; Assié, M. B.; Cosi, C.; Bruins-Slot, L.; Cussac, D.; Martel, J. C. F15599 a highly selective serotonin 5-HT$_{1A}$ receptor agonist: in vitro affinity and efficacy profile at native rat and recombinant human 5-HT$_{1A}$ receptors. *Eur. Neuropsychopharmacol.* **2006**, *16*, S232. Assié, M. B.; Bardin, L.; Depoortere, R.; Carilla-Durand, E.; Newman-Tancredi, A. F15599 a highly selective serotonin 5-HT$_{1A}$ receptor agonist: in vivo profile in neurochemical and behavioural rat models of serotoninergic activity. *Eur. Neuropsychopharmacol.* **2006**, *16*, S233.

(46) Hjorth, S.; Magnusson, T. The 5-HT$_{1A}$ receptor agonist 8-OH-DPAT preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1988**, *338*, 463−471. Yocca, F. D.; Iben, L.; Meller, E. Lack of apparent receptor reserve at postsynaptic 5-hydroxytryptamine1A receptors negatively coupled to adenyl cyclase activity in rat hippocampal membranes. *Mol. Pharmacol.* **1992**, *41*, 1066−1072. Lohse, M. J. G protein-coupled receptors: too many dimers? *Nat. Methods* **2006**, *3*, 972−973.

(47) Sullivan, N. R.; Crane, J. W.; Damjanoska, K. J.; Carrasco, G. A.; D'Souza, D. N.; Garcia, F.; Van de Kar, L. D. Tandospirone activates neuroendocrine and ERK (MAP kinase) signaling pathways specifically through 5-HT$_{1A}$ receptor mechanisms in vivo. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2005**, *371*, 18−26. Newman-Tancredi, A.; Cussac, D.; Marini, L.; Millan, M. J. Antibody capture assay reveals bell-shaped concentration-response isotherms for h5-HT$_{1A}$ receptor-mediated Gαi3 activation: Conformational selection by high-efficacy agonists, and relationship to trafficking of receptor signaling. *Mol. Pharmacol.* **2002**, *62*, 590−601. Martinez, D.; Hwang, D.-R.; Mawlawi, O.; Slifstein, M.; Kent, J.; Simpson, N.; Parsey, R. V.; Hashimoto, T.; Huang, Y.; Shinn, A.; Van Heertum, R.; Abi-Dargham, A.; Caltabiano, S.; Malizia, A.; Cowley, H.; Mann, J. J.; Laruelle, M. Differential occupancy of somatodendritic and postsynaptic 5HT(1A) receptors by pindolol: a dose occupancy study with [^{11}C]WAY 100635 and positron emission tomography in humans. *Neuropsychopharmacology* **2001**, *24*, 209−229. Clarke, W. P.; Yocca, F. D.; Maayani, S. Lack of 5-hydroxytryptamine1A-mediated inhibition of adenyl cyclase in dorsal raphe of male and female rats. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1259−1266.

(48) Mannoury la Cour, C.; El Mestikawy, S.; Hanoun, N.; Hamon, M.; Lanfumey, L. Regional differences in the coupling of 5-hydroxytryptamine-1A receptors to G proteins in the rat brain. *Mol. Pharmacol.* **2006**, *70*, 1013−1021. Marazziti, D.; Palego, L.; Giromella, A.; Mazzoni, M. R.; Borsini, F.; Mayer, N.; Naccarato, A. G.; Lucacchini, A.; Cassano, G. B. Region-dependent effects of flibanserin and buspirone on adenylyl cyclase activity in the human brain. *Int. J. Neuropsychopharmacol.* **2002**, *5*, 131−140. Evans, K. L. J.; Cropper, J. D.; Berg, K. A.; Clarke, W. P. Mechanisms of regulation of agonist efficacy at the 5-HT(1A) receptor by phospholipid-derived signaling components. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 1025−1035. Rabiner, E. A.; Gunn, R. N.; Wilkins, M. R.; Sargent, P. A.; Mocaer, E.; Sedman, E.; Cowen, P. J.; Grasby, P. M. Drug action at the 5-HT(1A) receptor in vivo: autoreceptor and postsynaptic receptor occupancy examined with PET and [carbonyl-^{11}C]WAY-100635. *Nucl. Med. Biol.* **2000**, *27*, 509−513.

(49) Schildkraut, J. J. The catecholamine hypothesis of affective disorders: A review of supporting evidence. *Am. J. Psychiatry* **1965**, *122*, 509−522.

(50) Gettys, T. W.; Fields, T. A.; Raymond, J. R. Selective activation of inhibitory G-protein alpha-subunits by partial agonists of the human 5-HT1A receptor. *Biochemistry* **1994**, *33*, 4283−4290. Kenakin, T. Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB J.* **2001**, *15*, 598−611.

(51) (a) Dremencov, E.; El Mansari, M.; Blier, P. Noradrenergic augmentation of escitalopram response by risperidone: Electrophysiologic studies in the rat brain. *Biol. Psychiatry* **2007**, *61*, 671−678. (b) Blier, P. The pharmacology of putative early-onset antidepressant strategies. *Eur. Neuropsychopharmacol.* **2003**, *13*, 57−66. Artigas, F.; Celada, P.; Laruelle, M.; Adell, A. How does pindolol improve antidepressant action? *Trends Pharmacol. Sci.* **2001**, *22*, 224−228. Blier, P.; Bergeron, R.; De Montigny, C. Selective activation of postsynaptic 5-HT-1A receptors induces rapid antidepressant response. *Neuropsychopharmacology* **1997**, *16*, 333−338.

(52) 2-Pyrimidinemethanamine, [75985-45-4].

(53) 5-Chloro-2-pyrimidinemethanamine, [426266-77-5].

(54) 4-Methoxy-2-pyrimidinemethanamine, [909563-18-4].

(55) Newman-Tancredi, A.; Assié, M. B.; Leduc, N.; Ormière, A.-M.; Danty, N.; Cosi, C. Novel antipsychotics activate recombinant human and native rat serotonin 5-HT$_{1A}$ receptors: affinity, efficacy and potential implications for treatment of schizophrenia. *Int. J. Neuropsychopharmacol.* **2005**, *8*, 341−356.

(56) McPherson, G. A. Analysis of radioligand binding experiments. A collection of computer programs for the IBM PC. *J. Pharmacol. Methods* **1985**, *14*, 213−228.

(57) Kleven, M.; Ybema, C.; Carilla, E.; Hamon, M.; Koek, W. Modification of behavioral effects of 8-hydroxy-2-(di-n-propylamino)tetralin following chronic ethanol consumption in the rat: evidence for the involvement of 5-HT$_{1A}$ receptors in ethanol dependence. *Eur. J. Pharmacol.* **1995**, *281*, 219−228.

(58) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations with Computer Programs*, 2nd ed.; Springer-Verlag: New York, 1987.

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