Improvement in the Selectivity and Metabolic Stability of the Serotonin 5-HT$_{1A}$ Ligand, S 15535: A Series of *cis*- and *trans*-2-(Arylcycloalkylamine) 1-Indanols

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S 15535 (**1**) displays a distinctive profile of agonist and antagonist (weak partial agonist) activity at pre- and postsynaptic 5-HT$_{1A}$ receptors, respectively. It has proven to be active in several models predictive of anxiolytic, antidepressant, and procognitive properties. In an attempt to increase its selectivity and metabolic stability, and guided by the results of human metabolic studies, we prepared a series of *cis*- and *trans*-2-(arylcycloalkylamine) 1-indanols. Irrespective of the nature of the arylcycloalkylamine moiety or the presence of substituents on the indanol ring, trans isomers invariably showed the highest affinity for human, recombinant h5-HT$_{1A}$ receptors. Among them, compounds **39**, **42**, **45**, **49**, **52**, **53**, **54**, **57**, **61**, **64**, **67**, and **70** displayed similar or higher affinity than the parent compound **1** (p$K_i \geq 9.1$). Lack of selectivity toward α_1-adrenoceptors has been frequently encountered with 5-HT$_{1A}$ ligands. While S 15535 itself presents reasonable selectivity (158-fold) in this respect, trans piperazine derivatives **4-trans**, **35**, **39**, **41**, **47**, **64**, **68**, **69**, **70**, **71** displayed even more pronounced selectivity vs α_1-adrenoceptors, with the nitro derivative **70** being highly selective (1259-fold). However, among the set of trans piperidines prepared, only **64**, which also bears a nitro on the indanol ring, displayed selectivity greater than the parent compound **1.** All trans derivatives behaved as partial agonists at h5-HT$_{1A}$ receptors, as determined by their submaximal stimulation of [^{35}S]GTPγS binding to a level comparable to that observed with S 15535. In metabolic stability studies in vitro using human microsomes and hepatocytes, only trans piperazines and, in particular, **35**, **39**, **41**, **68**, **69**, and **70**, showed an improvement relative to **1**, whereas trans piperidines did not. Compounds **35**, **39**, **41**, and **70**, which combined both improved selectivity and metabolic stability, and which retained the distinctive pharmacological characteristics of S 15535, were evaluated in animal models of anxiety. Of these, **35**, which showed the highest oral bioavailability in vivo in rats, was resolved into its two isomers **36** and **37**. The eutomer **37** displayed 47% oral bioavailability in the rat and was potently active (0.1−0.5 mg/kg, s.c.) in the rat ultrasonic vocalization and social interaction models, predictive of anxiolytic activity. In conclusion, 2-(arylcycloalkylamine) 1-indanols represent a novel class of potent 5-HT$_{1A}$ ligands in which the presence of the hydroxyl group in the benzylic position enhances selectivity, while substituents on the phenyl ring of the indanol moiety improve both selectivity and metabolic stability.

Introduction

S 15535 (**1**), a benzodioxopiperazine derivative,[1−3] is active in diverse, experimental models predictive of antidepressant,[4] anxiolytic,[5,6] and procognitive[7] properties, and displays a distinctive profile of agonist and antagonist (weak partial agonist) properties at pre- and postsynaptic serotonin 5-HT$_{1A}$ receptors, respectively.[8] In analogy to the selective and pure 5-HT$_{1A}$ antagonist,[9] WAY 100635 (**2**), S 15535 shows high affinity for human 5-HT$_{1A}$ receptors (p$K_i = 9.1$) and reasonable selectivity over dopamine D$_2$ (195-fold) and α_1-adrenoceptors (α_1-AR) (158-fold), a characteristic seldom encountered in 5-HT$_{1A}$ receptors antagonists.[10a,b] In a previous article,[1] we provided full details concerning the structure−activity relationships which led to the selection of **1** for further development and clinical evaluation.Unfortunately, during the course of clinical trials, it was established that S 15535 is extensively metabolized. Several circulating hydroxylated metabolites were identified: the 5-hydroxyindane **3** was the most abundant, whereas the 1-hydroxyindane **4** was present only in small amounts. However, in a radioreceptor assay from plasma of treated patients, only **4** appeared to have marked affinity for 5-HT1A receptors.To study their pharmacology and metabolic stability in detail, we prepared both diastereoisomers of **4**. The cis isomer of **4** displayed weak affinity for human serotonin h5-HT$_{1A}$ receptors (p$K_i = 7.2$), while **4-trans** retained almost the same affinity as the parent compound **1**. Moreover, in vitro, metabolic stability studies using human hepatic microsomes and hepatocytes revealed an improvement

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Chart 1

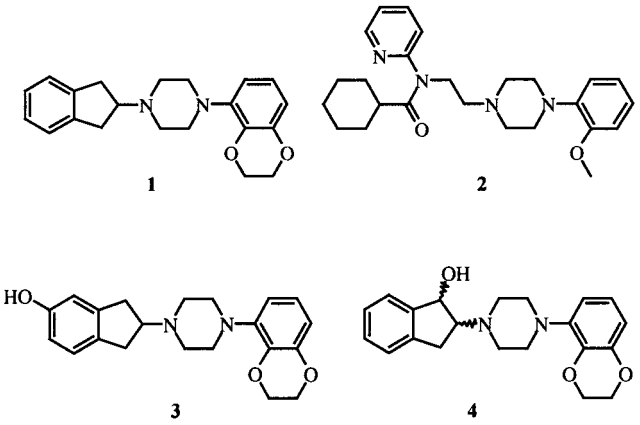
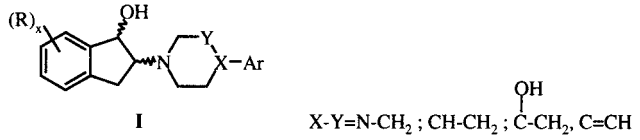


Chart 2



in the stability of **4-trans** relative to **1**. Encouraged by the observation that the indanol backbone could also lead to potent and more stable 5-HT_{1A} ligands, we hypothesized that blockade of the probable metabolic (hydroxylation) position of the phenyl ring of the indanol moiety of **4** might generate novel ligands of superior metabolic stability while retaining the original pharmacological profile of S 15535. Surprisingly, few 1-indanols substituted by an arylpiperazine in position 2 are described in the literature.[11] This prompted us to investigate a series of cis and trans compounds with the general structure **I** shown in Chart 2, where the arylpiperazine group **IIA** could be extended to closed analogues **IIB**−**IIL** such as shown in Chart 3.

In the present paper, we describe the synthetic methodologies used for the preparation of compounds of structure **I** and examine structure−activity relationships based on the results of binding studies at human 5-HT_{1A} receptors. We also assessed selectivity versus native, rat α_1-adrenoceptors and, for the most selective ligands, efficacy at human 5-HT_{1A} receptors, as well as antagonist properties in the rat in vivo. In parallel, we evaluated oral bioavailability prediction from in vitro metabolic stability (human hepatic microsomes) and intestinal permeability (Caco-2 cell line). Because of possible primary metabolic pathways activated by phase II conjugation enzymes, the evaluation was completed for several key compounds, by assessing metabolic stability using human hepatocytes and by determination of oral bioavailability in the rat.

Chemistry

By introducing an hydroxyl function on the benzylic position of S 15535, we created geometrical and optical isomers and were confronted with the problem of either designing synthetic routes able to produce regiospecifically cis or trans isomers, or finding appropriate experimental conditions generating an equilibrated mixture of both isomers.

Piperazines **4-cis**, **4-trans**, **34**−**41**, **46**, **47**, and piperidines **44**, **45** listed in Table 2 were prepared as illustrated in Scheme 1 by a classical set of reactions. 4-Piperidinol **50** was prepared by an alternative route depicted in Scheme 2. Because of their relative instability, indanone intermediates **V** (Table 1) had to be rapidly reduced to indanols by sodium borohydride, affording a mixture of cis and trans isomers where the cis isomer was always present in a much larger proportion than the trans isomer (about 85/15), and the workup procedure did not allow isolation of the trans isomer in sufficient amounts to permit pharmacological evaluation. Consequently, we sought a strategy where the ratio of trans isomer could be significantly improved. We developed, thus, a simple and quick method starting from 2-bromo 1-indanones **IV**, which is characterized by the ready generation of stable pyridinium ions **VI** as key intermediates (Table 1). Reduction of **VI** by sodium borohydride in methanol afforded a 50/50 mixture of *cis*- and *trans*-1,2,5,6-tetrahydropyridine derivatives **I**, which could be either separated by flash chromatography to lead to the above-mentioned compounds, or further reduced by catalytic hydrogenation to give piperidine derivatives **52**, **53**, **55**−**59**, **61**−**63** (Scheme 3).

4-Arylpyridines **VIII A**−**E** used in the first step of this synthesis were obtained through a Suzuki reaction as depicted in Scheme 4, starting from the corresponding aryl triflate or aryl bromide **VII**.

However, this synthetic pathway applied only to piperidines and tetrahydropyridines. Moreover, we presumed that, in comparison to **4-cis** and **4-trans**, trans derivatives would be more potent. Consequently, we attempted to develop a diastereospecific and general method (Scheme 5) for exclusively obtaining the trans isomer of piperidines and piperazines. When epoxides **IX** were treated by aryl cyclic amines **IIA**−**IIL**, a regiospecific ring opening occurred. This led to trans 2-indanols **X** (Table 1), which were isomerized into the ester of trans 1-indanols **XI** by a Mitsunobu reaction at −25 °C in the presence of *p*-nitrobenzoic acid under conditions described in the literature,[12a,b] providing a diastereomeric excess greater than 99%. Saponification of the resulting *p*-nitrobenzoate **XI** led to compounds **42**, **43**, **51**, **64**−**71** (Table 2). The mechanism of this isomerization reaction could involve an aziridinium ion, as suggested by Freedman[13] and Solà.[12b] In regard to the hindered nature of the secondary alcohols **X**, rather unusual Mitsunobu conditions had to be employed.[14]

Assignment to either cis or trans configuration was made by means of IR spectra as described by Rimek.[15] In addition, X-ray crystallography was performed on compound **4-cis** and **4-trans** to verify attributions made by the IR method.

Biology

The biological characterization of compounds prepared in the present work was based partly upon experimental strategies discussed previously.[1,2] Affinities at h5-HT_{1A} receptors and α_1-adrenoceptors were assessed by inhibition of [^{3}H]-8-OH-DPAT binding in Chinese hamster ovary cells line stably expressing recombinant h5-HT_{1A} receptors[16] and inhibition of [^{3}H]-prazosin binding to rat cortex, respectively. Efficacy of compounds at h5-HT_{1A} sites were determined by measuring 5-HT_{1A} receptor-linked G-protein activation by

Chart 3

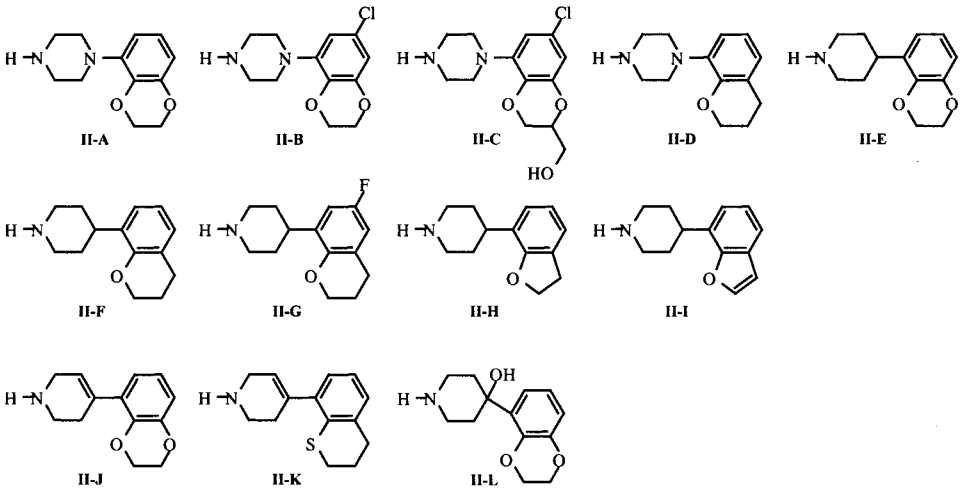
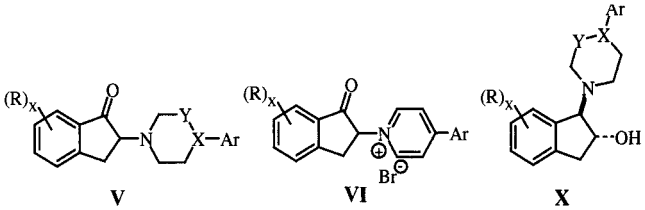


Table 1. Key Intermediates. Indanones **V** and **VI**, 2-Indanols **X** (Preparation and Physical Properties)



Compounds	$(R)_X$	—N(Y)X—Ar	Method	mp (°C)[a]
5	H	II-A	A	oil
6	5-OMe	II-A	A	foam
7	6-OMe	II-A	A	oil
8	5-F	II-A	A	110-115
9	5-F	II-D	A	foam
10	5-F	II-E	A	oil
11	H	VIII-A	B	251-254
12	5-F	VIII-A	B	190-195
13	6-F	VIII-A	B	218-222
14	5-CH_3	VIII-A	B	242-244
15	6-CH_3	VIII-A	B	200-202
16	5,6-diF	VIII-A	B	240-242
17	5,6-OCH_2O	VIII-A	B	243-246
18	5,6-diOMe	VIII-A	B	258-261
19	5-F	VIII-B	B	215-217
20	5-F	VIII-C	B	199-202
21	5-F	VIII-D	B	211-214
22	5-F	VIII-E	B	208-212
23 (+)	5-F	II-A	C	148-150
24 (–)	5-F	II-A	C	146-149
25	5,6-diF	II-A	C	foam
26	6-F	II-A	C	foam
27	6-NO_2	II-A	C	202-205
28	5-F	II-B	C	foam
29	5-F	II-F	C	193 (decomp.)
30	5-F	II-C	C	foam
31	6-OMe	II-E	C	oil
32	6-NO_2	II-E	C	141-143
33	5-F	II-L	C	166-168

[a] All melting points were determined on a Reichert Thermovar apparatus and are uncorrected.

[^{35}S]GTPγS binding.[17a,b] The ability of compounds to inhibit hypothermia elicited by s.c. administration of 8-OH-DPAT in rats was employed as a measure of antagonist properties at postsynaptic 5-HT$_{1A}$ receptors.

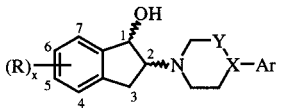
Results

Structure−Activity Relationships. Concerning affinities at h5-HT$_{1A}$ receptors, the tendency observed with compounds **4-cis** and **4-trans** was confirmed with the other couples of cis/trans isomers (Tables 2 and 3), irrespective of the nature of the cyclic amine moiety or the presence of substituents on the indanol ring. That is, trans isomers invariably showed the highest affinity.

With respect to the influence of the heterocyclic aromatic moiety upon affinity at 5-HT$_{1A}$ receptors (Table 4), there were only minor variations in affinity when we compared benzodioxan derivatives **41** and **45** to their respective benzopyran analogues **47** and **67**, and when a chlorine atom was introduced into the benzene ring of the benzodioxan part of **41** to give **71.** However, a marked decrease was seen when a fluorine atom was introduced on the benzopyran ring of **67** leading to **59**. A reduction of affinity was also obtained when a dihydrobenzofuran ring, as in **62**, replaced the benzopyran ring of **67**. Finally, the most marked loss of affinity was observed in the piperazine series upon transformation of the benzodioxan moiety of **41** to **66**, as undertaken by Hartog and colleagues[18] with flesinoxan. As for compound **1**, the benzodioxan ring conferred maximal affinity at h5-HT$_{1A}$ sites.

On the other hand, the affinities of benzodioxan derivatives at h5-HT$_{1A}$ receptors (Table 5) were only slightly affected by variation of the cyclic amine component inasmuch as piperazines **4-trans** and **41** displayed almost similar affinity to tetrahydropiperidines **49** and **54,** and piperidine analogues **53** and **45** showed only a slight increase in affinity. However, a piperidinol was deleterious for affinity since compound **51** exhibited a pK_i < 6. Major differences were seen with respect to the nature of the substituent on the indanol moiety (Table 6). In the piperazine series, a methoxy in position 6, as for compound **39,** conferred maximal activity (pK_i = 9.35) and a 5,6-difluoro lower activity (pK_i = 8.67 for compound **68**). In the piperidine series, a 6-nitro (compound **64)** afforded the highest affinity among the compounds prepared in this study, in contrast to the 5,6-dimethoxy analogue **63** (pK_i = 7.1), which showed the lowest affinity of the series.

Table 2. Preparation, Physical Properties, and Affinities at h5-HT_{1A} Receptors of Compounds **I**



I

Compd	isomer	R(x)	-N(Y)-X-Ar	key interm.	method	recryst. solvent	mp (°C)[b]	formula[c]	pK_i[f] h5-HT_{1A}
4	cis	H	II-A	**5**	A	CH_3CN	196-198	$C_{21}H_{24}N_2O_3$	7.17 ± 0.09
4	trans	H	II-A	**5**	A	CH_2Cl_2	211-214	$C_{21}H_{24}N_2O_3$	8.79 ± 0.09
34	cis	5-OMe	II-A	**6**	A	CH_3CN	214-217	$C_{22}H_{26}N_2O_4$	7.63 ± 0.03
35	(±) trans	5-OMe	II-A	**6**	A	CH_3CN	137-140	$C_{22}H_{26}N_2O_4$	8.90 ± 0.26
36	(+) trans	5-OMe	II-A	**6**	A[a]	CH_3CN	149-152	$C_{22}H_{26}N_2O_4$	8.18 ± 0.03
37	(–) trans	5-OMe	II-A	**6**	A[a]	CH_3CN	152-154	$C_{22}H_{26}N_2O_4$	8.99 ± 0.05
38	cis	6-OMe	II-A	**7**	A	CH_3CN	207-210	$C_{22}H_{26}N_2O_4$	7.76 ± 0.06
39	trans	6-OMe	II-A	**7**	A	CH_3CN	145-148	$C_{22}H_{26}N_2O_4$	9.35 ± 0.13
40	cis	5-F	II-A	**8**	A	CH_3CN	210-213	$C_{21}H_{23}FN_2O_3$	7.94 ± 0.05
41	(±) trans	5-F	II-A	**8**	A	CH_3CN	220-224	$C_{21}H_{23}FN_2O_3$[d]	8.90 ± 0.01
42	(+) trans	5-F	II-A	**23**	C	CH_3CN	157-161	$C_{21}H_{23}FN_2O_3$	9.20 ± 0.19
43	(–) trans	5-F	II-A	**24**	C	CH_3CN	158-161	$C_{21}H_{23}FN_2O_3$	8.69 ± 0.03
44	cis	5-F	II-E	**10**	A	CH_3CN	197-201	$C_{22}H_{24}FNO_3$	7.57 ± 0.12
45	trans	5-F	II-E	**10**	A	CH_3CN	204-209	$C_{22}H_{24}FNO_3$	9.36 ± 0.31
46	cis	5-F	II-D	**9**	A	CH_3CN	209-210	$C_{22}H_{25}FN_2O_2$	6.50 ± 0.10
47	trans	5-F	II-D	**9**	A	CH_3CN	148-150	$C_{22}H_{25}FN_2O_2$	8.89 ± 0.10
48	cis	H	II-J	**11**	B	C_2H_5OH	109-112	$C_{22}H_{23}NO_3$	7.64 ± 0.04
49	trans	H	II-J	**11**	B	CH_3CN	182-184	$C_{22}H_{23}NO_3$	9.08 ± 0.17
50	cis	5-F	II-L	-	A'	CH_3CN	189-191	$C_{22}H_{24}FNO_4$	< 6
51	trans	5-F	II-L	**33**	C	CH_3CN	174-175	$C_{22}H_{24}FNO_4$	< 6
52	trans	6-F	II-E	**13**	B	CH_3CN	159-163	$C_{22}H_{24}FNO_3$	9.11 ± 0.01
53	trans	H	II-E	**11**	B	CH_3CN	173-174	$C_{22}H_{25}NO_3$	9.15 ± 0.16
54	trans	5-F	II-J	**12**	B	CH_3CN	198-202	$C_{22}H_{22}FNO_3$	9.07 ± 0.05
55	trans	5-F	II-I	**20**	B	CH_3CN	170-172	$C_{22}H_{22}FNO_2$	N.T.[g]
56	trans	5-CH_3	II-E	**14**	B	CH_3CN	191-192	$C_{23}H_{27}NO_3$	8.93 ± 0.01
57	trans	6-CH_3	II-E	**15**	B	C_2H_5OH	225-226	$C_{23}H_{27}NO_3$	9.26 ± 0.04
58	trans	5,6-diF	II-E	**16**	B	CH_3CN	167-169	$C_{22}H_{23}F_2NO_3$	8.66 ± 0.11
59	trans	5-F	II-G	**19**	B	CH_3CN	160-162	$C_{23}H_{25}NO_2F_2$	8.31 ± 0.10
60	trans	5-F	II-K	**22**	B	CH_3CN	143-147	$C_{23}H_{24}NOSF$	7.93 ± 0.15
61	trans	5,6-OCH_2O	II-E	**17**	B	CH_3CN	212-215	$C_{23}H_{25}NO_5$	9.28 ± 0.05
62	trans	5-F	II-H	**21**	B	CH_3CN	215-217	$C_{22}H_{24}NO_2F$	8.82 ± 0.19
63	trans	5,6-diOMe	II-E	**18**	B	CH_3CN	180-183	$C_{24}H_{29}NO_5$	7.10 ± 0.14
64	trans	6-NO_2	II-E	**32**	C	CH_3CN	181-184	$C_{22}H_{24}N_2O_5$	9.54 ± 0.17
65	trans	6-OMe	II-E	**31**	C	CH_3CN	204-205	$C_{23}H_{27}NO_4$	8.96 ± 0.31
66	trans	5-F	II-C	**30**	C	CH_3CN	195-198	$C_{22}H_{24}N_2O_4ClF$[e]	7.98 ± 0.19
67	trans	5-F	II-F	**29**	C	CH_3CN	163-167	$C_{23}H_{26}NO_2F$	9.52 ± 0.09
68	trans	5,6-diF	II-A	**25**	C	CH_3CN	169-171	$C_{21}H_{22}N_2O_3F_2$	8.67 ± 0.13
69	trans	6-F	II-A	**26**	C	CH_3CN	185-186	$C_{21}H_{23}N_2O_3F$	8.88 ± 0.25
70	trans	6-NO_2	II-A	**27**	C	CH_3CN	194-196	$C_{21}H_{23}N_2O_5$	9.13 ± 0.29
71	trans	5-F	II-B	**28**	C	CH_3CN	190-193	$C_{21}H_{22}N_2O_3ClF$	8.88 ± 0.31
1									9.10 ± 0.15

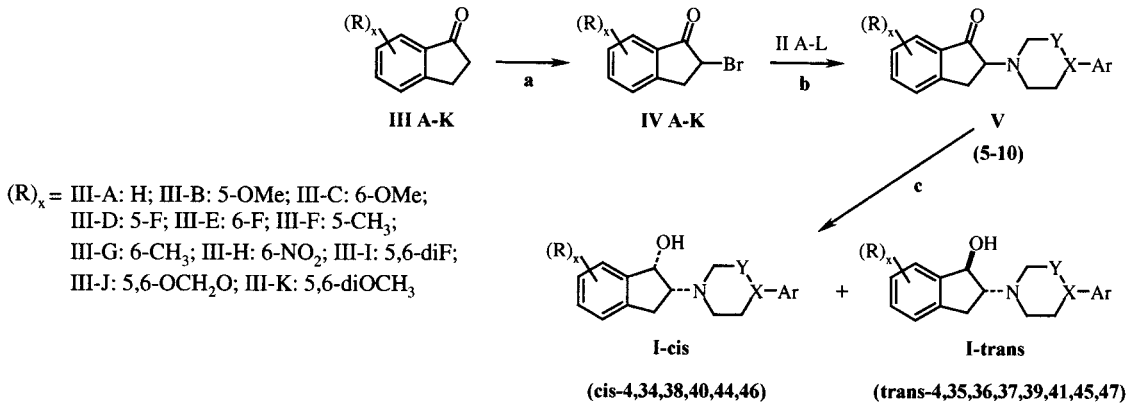
[a] Resolution of **35** by HPLC on chiral column. [b] See corresponding footnote Table 1. [c] Compounds were purified by column chromatography; C, H, and N analyses were within 0.4% of theoretical values for the formulas given, unless otherwise stated. All compounds exhibited NMR consistent with assigned structures. [d] C: % calculated = 68.09, % found = 67.39. [e] N: % calculated = 6.44, % found = 7.06. [f] pK_i ± SEM values are from two to five independent experiments. [g] N. T. = not tested.

It is of importance to note that compounds **39**, **42**, **45**, **49**, **52**, **53**, **54**, **57**, **61**, **64**, **67**, and **70** displayed similar or higher affinity than the parent compound **1** (Table 2).

Concerning selectivity toward α_1-adrenoceptors, the unsubstituted or monosubstituted benzodioxan seemed to confer the highest selectivity, as illustrated by compounds **41** and **71** (Table 4), but disubstitution led to a decrease of selectivity mainly due to a decrease in affinity at h5-HT_{1A} receptors, since in **66** affinity at α_1-adrenoceptors was not affected. On the other hand, in the benzopyran analogue **47,** a rise in affinity at α_1 sites was responsible for decreased selectivity since affinity at h5-HT_{1A} receptors was not modified.

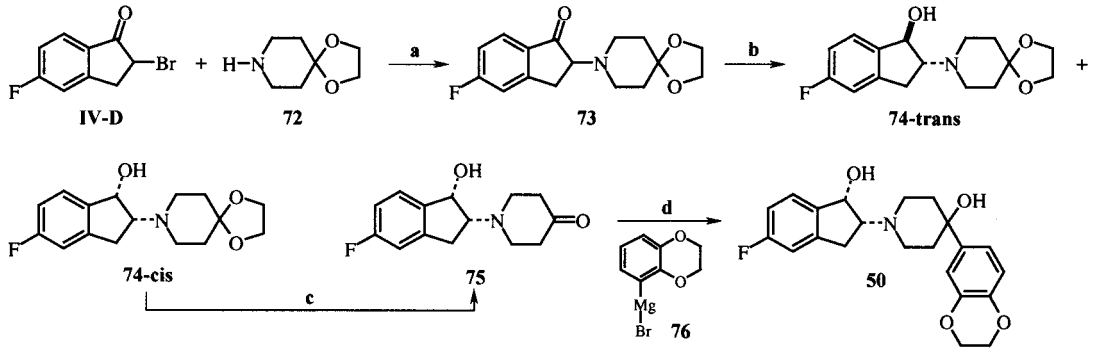
The high selectivity shown by **41** and **71** was also due to the presence of the piperazine ring since the 1,2,5,6-tetrahydropyridine ring, as in **54**, or the piperidine ring, as in **45**, led to a decrease of selectivity due to an increase in α_1-AR affinity. Similar observations was made upon comparing the piperazine derivative **4-trans** with its 1,2,5,6-tetrahydropyridine analogue **49** and its piperidine analogue **53**, respectively (Table 5), or piperazine **47**, with its piperidine homologue **67** (Table 4). Substitution on the phenyl ring of the indanol moiety

Scheme 1. Synthesis of Piperazine and Piperidine Derivatives I (Method A)[a]



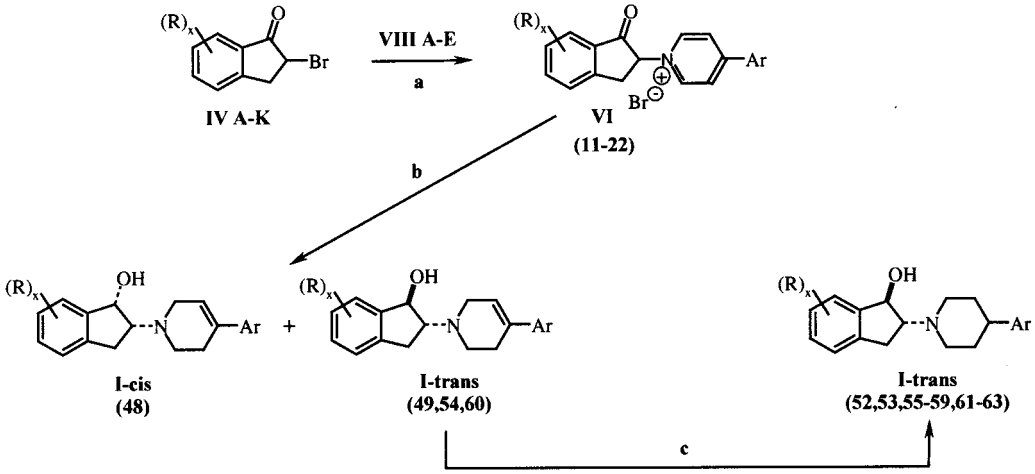
[a] Reagents and conditions: (a) $^{n}Bu_4NBr_3$, CH_2Cl_2, MeOH, R.T.; (b) K_2CO_3, DMF, R.T.; (c) $NaBH_4$, THF, R.T.

Scheme 2. Synthesis of **50** (Method A′)[a]



[a] Reagents and conditions: (a) K_2CO_3, DMF, R.T.; (b) $NaBH_4$, THF, R.T.; (c) HCl concentrated, AcOH, 50 °C, 24 h; (d) THF, R.T., then reflux 1 h.

Scheme 3. Synthesis of 1,2,5,6-Tetrahydropyridine and Piperidine Derivatives **I** (Method B)[a]

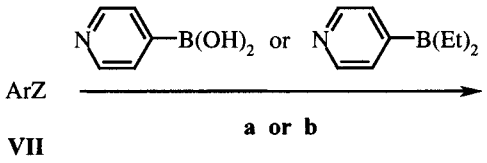


[a] Reagents and conditions: (a) methylethyl ketone or acetone, reflux; (b) 1. $NaBH_4$, methanol, 2. AcOH; (c) H_2/PtO_2, MeOH, THF.

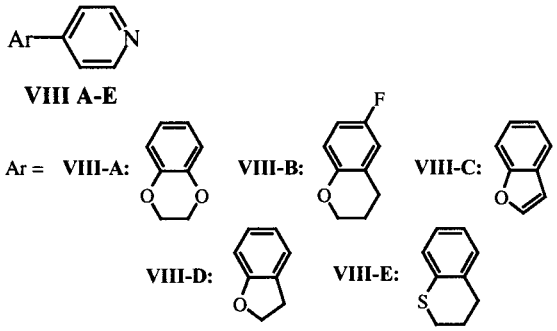
apparently played a limited role with regard to selectivity (Table 6), except in the case of a nitro substitution in position 6 which appears crucial since piperazine **70** and piperidine **64** displayed the highest selectivity among piperazine and piperidine series, respectively, more than double those seen with their unsubstituted analogues **4-trans** and **53**, their 5-fluoro analogues **41** and **45,** or their 6-fluoro analogues **69** and **52.** Finally, all piperazines listed in Table 6 and piperidine **64** showed a higher affinity than the parent compound **1**.

Biological Results. In analogy to the parent compound **1**, all ligands examined (Table 7) behaved as partial agonists at h5-HT$_{1A}$ receptors expressed in CHO cells, as determined by their submaximal stimulation of [^{35}S]GTPγS binding to a level comparable to that observed with S 15535, and markedly inferior to 5-HT itself (defined as a full agonist, 100% stimulation). No systematic differences in this regard were noted between trans piperazines and trans piperidines. This modest level of efficacy is sufficient to activate highly sensitive 5-HT$_{1A}$ receptors but not low-sensitivity postsynaptic 5-HT$_{1A}$ receptors.[19−21] Correspondingly, in analogy to S 15535, both trans piperazine and trans piperidine derivatives displayed pronounced and com-

Scheme 4. Synthesis of Arylpyridines **VIII**[a]

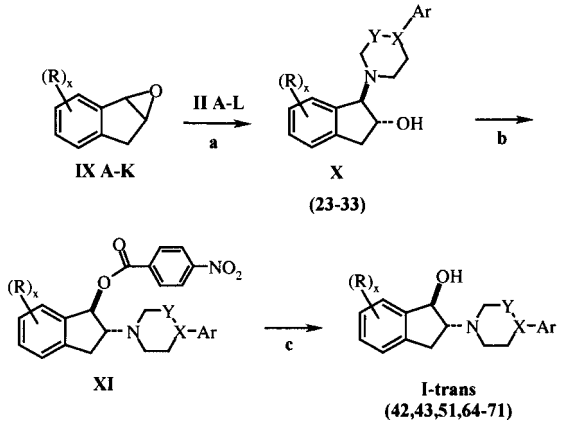


Z = OTf, Br



[a] Reagents and conditions: (a) Na_2CO_3, $Pd(PPh_3)_4$, toluene, ethanol, reflux; (b) $^nBu_4NBr$, KOH, $Pd(PPh_3)_4$, THF.

Scheme 5. Synthesis of Trans Piperazine and Piperidine Derivatives **I** (Method C)[a]



[a] Reagents and conditions: (a) CH_3CN, R.T.; (b) DEAD, PPh_3, *p*-nitrobenzoic acid, toluene, THF, −25 °C; (c) KOH, CH_3OH, H_2O, R.T.

parable activity in vivo in blocking the induction of hypothermia by 8-OH-DPAT, with the exception of compounds of **52**, **64**, and **68.** This paradigm similarly revealed antagonist properties of the trans 1,2,4,5-tetrahydropyridines at postsynaptic 5-HT_{1A} receptors. Although trans piperazines and trans piperidines displayed, thus, very similar profiles as concerns their affinities and functional activities at 5-HT_{1A} receptors and showed high Caco-2 permeability corresponding to total oral absorption prediction (Table 7), they differed markedly as concerns their metabolic stability. Thus, the former ligands, with the exception of **4-trans**, showed relatively substantial stability upon incubation with human microsomes (60 to 80% of metabolic stability prediction), whereas the latter revealed little improvement relative to S 15535.

As all these compounds include an hydroxyl group that may be metabolized by phase II conjugation enzymes (UDP-glucuronyl transferases and/or sulfotransferases), and inasmuch hepatic microsomes are deprived of functional phase II enzymes under the incubation conditions used, the apparent improvement in metabolic stability was confirmed using human hepatocytes that present both functional microsomal enzymes and phase II conjugation enzymes. Metabolic bioavailability predictions using human hepatocytes were confirmed to be very much in line with the results of human hepatic microsomes for compounds **35**, **39**, and **41** (74, 62, and 58% vs 80, 62, and 65%). Such comparison also confirmed the low metabolic stability seen with **45**, **52**, **53**, and **64** (15, 9, 10, and 22% vs 7, 8, 7 and 22% for human hepatocytes and human hepatic microsomes, respectively).

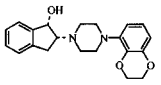
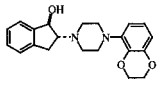
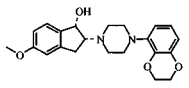
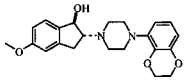
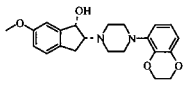
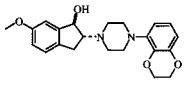
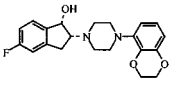
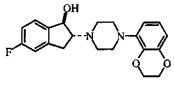
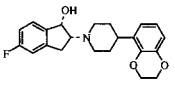
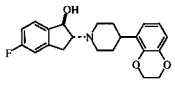
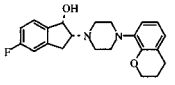
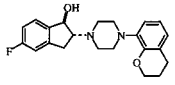
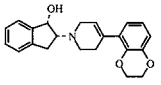
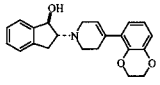
Discussion and Conclusion

It is of interest to note that **I-trans** compounds led not only to the discovery of a novel family of antagonists/partial agonists at postsynaptic 5-HT_{1A} receptors but also to the identification, with regard to piperidines **64** and **67**, of compounds with affinities among the highest ever obtained at these sites. In contrast, **cis-I** isomers displayed much lower affinities than their trans analogues. This result can lead to a further comprehension of the structural requirements for optimal recognition of 5-HT_{1A} receptors. Molecular modeling studies are currently underway to define which interactions are decisive for high recognition and to explain the poor affinity of **I-cis** isomers in comparison to **I-trans** analogues.

Among the numerous points that may be considered regarding the structure−activity study, one particularly intriguing aspect concerns the comparison between the behavior of benzopyran derivatives **59** and **67**, on one hand, and benzodioxan derivatives **71** and **41**, on the other. In the former case, the presence of a fluorine led to a decrease in affinity of 16-fold, but in the latter case, a chlorine had no effect (Table 4). Interestingly, Kuipers[22] made a similar observation: incorporation of a fluorine atom into the 5-position of the benzofuran cycle of 1-(7-benzofuranyl)piperazine led to a decrease in affinity of 10-fold, whereas a bromine atom at the same position caused only a 2.5-fold decrease. This observation led the authors to speculate that steric effects play only a minor role at this 5-position. In extension to their analysis, we may hypothesize that an electronic effect on the para-oxygen atom explains the influence of the fluorine atom, in **59** and in Kuiper's derivative. In general, this oxygen atom is frequently found to confer high 5-HT_{1A} affinities in arylpiperazine derivatives (cf. compounds **1** and **2**). The strong electronegative effect of a fluorine atom may sufficiently modify the electronic environment of a para-oxygen atom to induce a marked decrease in affinity. In contrast, a chlorine (or a bromine) atom, with its electronegative effect less pronounced than the fluorine, does not reduce the electronic density on the oxygen atom enough to cause a noticeable change in affinity.

Lack of selectivity toward α_1-adrenoceptors is a major consideration in the design of 5-HT_{1A} ligands,[23] and in the resolution of this difficulty, diverse strategies have been adopted. For example, Hibert,[23] on the basis of graphical computer-generated maps of 5-HT_{1A} receptors and α_1-adrenoceptors, increased the size of the aromatic moiety of the 20-fold selective compound MDL 72832

Table 3. Affinities at h5-HT$_{1A}$ Receptors: Comparison of Cis and Trans Compounds **I**

cis compounds		pK_i[a]	trans compounds		pK_i[a]
4-cis		7.17 ± 0.09	**4-trans**		8.79 ± 0.09
34		7.63 ± 0.03	**35**		8.90 ± 0.26
38		7.76 ± 0.06	**39**		9.35 ± 0.13
40		7.94 ± 0.05	**41**		8.90 ± 0.01
44		7.57 ± 0.12	**45**		9.36 ± 0.31
46		6.50 ± 0.10	**47**		8.89 ± 0.10
48		7.64 ± 0.04	**49**		9.08 ± 0.17

[a] See corresponding footnote of Table 2.

Table 4. Influence of the Nature of the Heterocyclic Moiety upon Affinity at h5-HT$_{1A}$ Receptors and Selectivity vs α_1-Adrenoceptors for Trans Piperazines and Trans Piperidines **I**

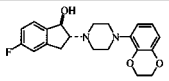
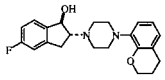
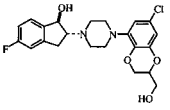
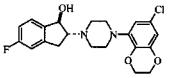
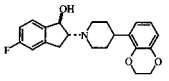
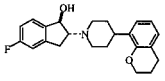
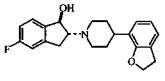
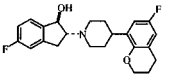
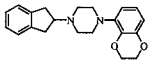
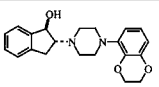
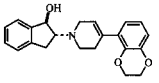
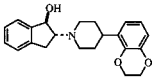
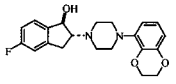
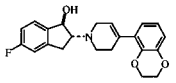
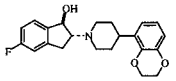
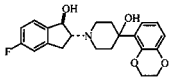
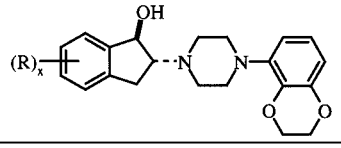
Compd	Structure	pK_i h5-HT$_{1A}$	pK_i α_1	selectivity, K_i ratio α_1 / h5-HT$_{1A}$
41		8.90	6.15	562
47		8.89	6.67	166
66		7.98	6.17	64
71		8.88	6.07	645
45		9.36	7.19	148
67		9.52	7.64	76
62		8.82	7.34	30
59		8.31	7.26	11
1		9.10	6.90	158

Table 5. Influence of the Nature of the Central Amino Ring upon Affinity at h5-HT$_{1A}$ Receptors and Selectivity vs α_1-Adrenoceptors for Trans Isomers **I**

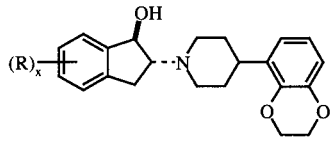
compd	Structure	pK_i h5-HT$_{1A}$	pK_i α_1	selectivity, K_i ratio α_1 / h5-HT$_{1A}$
4-trans		8.79	6.03	575
49		9.08	7.00	120
53		9.15	7.10	112
41		8.90	6.15	562
54		9.07	6.96	128
45		9.36	7.19	148
51		<6	6.31	<1

to obtain a 140-fold selective derivative, which poorly recognized α_1-adrenoceptors. Bolognesi,[24] in introducing a bulky tether into the mixed 5-HT$_{1A}$/α_1-AR ligand, WB 4101, was able to prepare a selective (468-fold) 5-HT$_{1A}$ ligand. Lopez-Rodriguez,[25] working with a series of arylpiperazines bearing a 3-(diphenylmethylene or a 9*H*-fluoren-9-ylidene) 2,5-pyrrolidinedione-1-yl group in the N-4 position, demonstrated the preponderant role for selectivity played by the length of the tether between this group and the piperazine ring. In previous work,[1] we showed that, when the substituent in the N-4 position of arylpiperazines is an indan-2-yl, reasonable selectivity could be attained. In the present work, we exploited this 4-(indan-2-yl) 1-arylpiperazine backbone in structure **I** to further increase selectivity. Three

Table 6. Influence of the Nature of $(R)_x$ Substituents upon Affinity at h5-HT_{1A} Receptors and Selectivity vs α_1-Adrenoceptors for Trans Piperazines and Trans Piperidines **I**



		pK_i		selectivity, K_i ratio
compd	$(R)_x$	h5-HT_{1A}	α_1	α_1 / h5-HT_{1A}
4-trans	H	8.79	6.03	575
35	5-OMe	8.90	6.55	224
39	6-OMe	9.35	6.57	603
41	5-F	8.90	6.15	562
68	5,6-diF	8.67	6.15	332
69	6-F	8.88	6.13	561
70	6-NO_2	9.13	6.03	1259



45	5-F	9.36	7.19	148
52	6-F	9.11	7.10	102
53	H	9.15	7.10	112
56	5-Me	8.93	7.77	14.5
57	6-Me	9.26	7.44	66
58	5,6-diF	8.66	7.08	38
61	5,6-OCH_2O	9.28	7.53	56
63	5,6-diOMe	7.10	6.90	1.5
64	6-NO_2	9.54	6.95	389
65	6-OMe	8.96	7.22	55

regions of molecules **I** were of significance concerning selectivity for 5-HT_{1A} receptors versus α_1-adrenoceptors. First, the heteroaryl region had a greater influence on selectivity than affinity, even if affinity at 5-HT_{1A} receptors was somewhat dependent on the presence of substituents on the ring, since the fluorine atom of **59** and the chlorine atom plus the hydroxymethyl group of **66** were responsible for the decreased 5-HT_{1A} affinity of their respective analogues **67** and **41**. However, as far as selectivity is concerned, marked differences were noted even for minor structural variations, for example, in comparing the benzodioxan **41** (selectivity 562) to its benzopyran analogue **47** (selectivity 166), or **71** versus **66** (selectivity of 645 vs 64). Second, the cyclic amino region also played a major role in determining selectivity, mainly reflecting its influence upon α_1-AR affinity. Piperazines **4-trans** and **41** were far more selective than their tetrahydropyridine analogues **49** and **54** and their piperidine analogues **53** and **45** (Table 5). The presence of a nitrogen atom linked to the phenyl ring of the heteroaromatic part was of great importance for selectivity, since it markedly suppressed α_1-AR affinity, but only lowered 5-HT_{1A} receptor affinity to a lesser extent. Third, substitution of the phenyl component of theindanol ring may play a major role, in certain cases, in influencing both affinity and selectivity. Thus, for both piperazines and piperidines, a 6-nitro substitution simultaneously conferred high affinity at 5-HT_{1A} sites and low affinity at α_1-AR sites, a property not shared by any other substituent and leading, in the case of **70,** to a pronounced improvement of selectivity in comparison to **1**. Compounds **4-trans**, **35**, **39**, **41**, **64**, **68**, **69** also showed high selectivity, although less marked than **70**, underlining the key role of the hydroxyl function as the main structural feature underlying this increased selectivity in comparison to **1** (Table 6). The choice of the 4-(indan-2-yl) 1-arylpiperazine backbone seemed judicious since appropriate substitution on the indan ring generated selective 5-HT_{1A} ligands.

In addition, the present series of derivatives retained the distinctive pharmacological characteristics of **1** inasmuch as they behaved as partial agonists at cloned h5-HT_{1A} receptors (Table 7), thereby preserving agonist properties at 5-HT_{1A} autoreceptors (unpublished observations) and antagonist actions at postsynaptic sites (Table 7).

Concerning metabolic stability in human microsomes, the hydroxyl function in the benzylic position appeared insufficient to afford in itself a superior pharmacokinetic profile than **1**, since **4-trans** presented only a modest improvement in metabolic stability. Further, replacement of the piperazine ring of **4-trans** by a 1,2,5,6-tetrahydropyridine ring as in **49** or by piperidine ring as in **53** did not result in an improvement in stability (Table 7). Even heteroaryl groups different to benzodioxan were not able to confer improved metabolic stability (data not shown). In the **trans-I** piperidine series, mono or disubstitutions on the aromatic part of the indanol ring did not stabilize these compounds toward metabolic attack, in contrast to the clear improvement shown by substituted piperazines **35**, **39**, **41**, and **70** and, to a lesser extent by **68** and **69** (Table 7). These observations were confirmed with hepatocytes in which possible hydroxyl conjugation could be taken into account. Together with the satisfactory absorption predicted by high permeability in the in vitro Caco-2 model, the data suggest that these compounds should display good oral bioavailability in man.

To summarize, the presence of an hydroxyl group in the benzylic position of S 15535 increased its selectivity, and substituents on the phenyl ring of the indanol moiety enhanced both selectivity and metabolic stability. Compounds **35**, **39**, **41**, and **70**, which combined both improvements, were evaluated in animal models of anxiety and depression.[6] Of these, **35**, which showed a marked increase in oral bioavailability relative to **1** in in vivo metabolic studies in the rat, was resolved into its two isomers **36** and **37**. **37** showed oral bioavailability in the rat of 47% and was potently active (0.1−0.5 mg/kg, s.c.) in rat the ultrasonic vocalization and social interaction models predictive of anxiolytic activity.[6] **41** and **70**, which also demonstrated robust activity in the ultrasonic vocalisation test, are currently being resolved.

In conclusion, this study demonstrates that structural modification of S 15535 results in compounds retaining its distinctive profile of interaction with 5-HT_{1A} receptors and pronounced anxiolytic properties, yet present-

Table 7. Receptor Binding Affinities, Efficacies, Antagonist Properties at Postsynaptic 5-HT$_{1A}$ Receptors and in Vitro Oral Bioavailability Predictions of Trans Piperazines, Trans Piperidines, and Trans 1,2,5,6-Tetrahydropyridines **I**

compd	pK_i h5-HT$_{1A}$	[^{35}S] GTPγS[a] (n =)	8-OH-DPAT[b] hypothermia ID$_{50}$ mg/kg s.c. (95% C. L.)	MF %[e] human microsomes	Abs %[f] Caco-2 cells
			trans piperazines		
4	8.79 ± 0.09	52 ± 2% (3)	0.96 (0.26−3.52)	21	100
35	8.90 ± 0.26	21 ± 2% (3)	0.72 (0.21−2.51)	80	100
39	9.35 ± 0.13	31 ± 7% (2)	1.25 (0.37−4.27)	62	100
41	8.90 ± 0.01	60 ± 5% (3)	1.42 (0.45−4.51)	65	100
68	8.67 ± 0.13	54 ± 1% (2)	∼2.50 (N.C.)[c]	51	100
69	8.88 ± 0.25	41% (1)	1.00 (0.40−2.70)	46	100
70	9.13 ± 0.29	49 ± 2% (2)	0.90 (0.10−6.20)	71	100
			trans piperidines		
45	9.36 ± 0.31	50% (1)	N. T.[d]	7	100
52	9.11 ± 0.01	45% (1)	2.10 (0.80−5.80)	8	100
53	9.15 ± 0.16	47% (1)	0.50 (0.20−1.60)	7	100
58	8.66 ± 0.11	45% (1)	5.00 (1.50−16.5)	5	100
61	9.28 ± 0.05	24% (1)	1.00 (0.60−1.60)	8	100
64	9.54 ± 0.17	54% (1)	8.10 (2.20−30.5)	22	100
65	8.96 ± 0.31	N. T.	0.60 (0.20−1.80)	N. T.[d]	N. T.[d]
			trans 1,2,5,6-tetrahydropyridines		
49	9.08 ± 0.19	N. T.	1.30 (0.40−4.00)	4	100
54	9.07 ± 0.05	N. T.	1.70 (0.50−5.30)	8	100
					100
					100
1	9.10 ± 0.09	39 ± 2% (2)	1.44 (0.72−2.87)	4	100
2	9.30 ± 0.21	0% (3)	0.01 (0.003−0.03)	1	100

[a] Agonist efficacies (compared to 5-HT at 5-HT$_{1A}$ receptors) ± SEM values are from two to three independent experiments, except when n = 1. [b] ID$_{50}$ = inhibitory dose$_{50}$; $n \geq 5$ per dose tested. [c] N. C. = not computable. [d] N. T. = not tested. [e] MF % = metabolic bioavailability prediction; experiment were performed once (100% = no metabolism). [f] Abs % = oral absorption prediction; experiments were performed in duplicate and led to the same prediction (100% = total intestinal absorption)

ing a marked improvement in selectivity and metabolic stability. Of these, the optical isomers of **35** are under more intensive pharmacological evaluation.

Experimental Section

Chemistry. Reactions performed in nonaqueous solvents were carried out under an atmosphere of nitrogen. Column chromatography was carried out with Merck Kieselgel 60 (230−400 mesh) under a nitrogen pressure of 0.5 atm. Microanalyses were performed on solid samples only, with a Carlo-Erba autoanalyzer. IR spectra were recorded on a Bruker IFS 28 infrared spectrometer. ^{1}H NMR spectra were recorded on either a Bruker AC 200 or Bruker AM 300 spectrometer at 200 and 300 MHz, respectively. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (δ, 0.00) used as internal standard. Assignment to either cis or trans configuration was made by means of IR spectra as described by Rimek.[15] Cis compounds, which form intramolecular hydrogen bonds, showed in solution, a broad hydroxyl band around 3400 cm^{-1}, whereas trans compounds where the hydroxyl function is free, showed a weak band around 3600 cm^{-1}. Optical activity was measured at 20 °C with a Perkin-Elmer 241 polarimeter.

General Method A. 2-[4-(2,3-Dihydrobenzo [1,4] dioxin-5-yl) piperazin-1-yl] 1-indanone (5). A suspension of 2-bromo 1-indanone (15.9 g, 75 mmol), compound **II-A** (19.3 g, 75 mmol), and potassium carbonate (10.4 g, 75 mmol) in dimethylformamide (100 mL) was stirred at room temperature for 48 h. The mixture was poured in water (750 mL) and extracted by AcOEt (2 × 200 mL). After the samples were washed, the combined extracts were dried ($MgSO_4$) and concentrated under reduce pressure to yield 26.1 g of a violet oil (100%).

***cis*-2-[4-(2,3-Dihydrobenzo [1,4] dioxin-5-yl) piperazin-1-yl] indan-1-ol (4-cis) and *trans* 2-[4-(2,3-Dihydrobenzo [1,4] dioxin-5-yl) piperazin-1-yl] indan-1-ol (4-trans).** Sodium borohydride (2.2 g, 4.3 mmol) was added by portion, at room temperature, to a solution of compound **5** (20 g, 57 mmol) in tetrahydrofuran (170 mL). At the end of the addition, the mixture was stirred overnight and evaporated to dryness, and the residue was taken up in methylene chloride (200 mL), washed with water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue obtained (14 g) was chromatographed (CH_2Cl_2/MeOH, 97/3). The first compound eluted (9 g) was recrystallized from CH_3CN: yield 7.4 g (35.5%) mp 196−198 °C and identified to be **4-cis** by its IR spectrum. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3380 (br, OH). ^{1}H NMR (300 MHz, DMSO, δ): 2.65 (m, 2H, CH*H* pip), 2.8 (m, 3H, C*H*H pip + C*H*N indan), 2.93 (m, 2H, CH_2 indan), 3.04 (m, 4H, CH_2 pip), 4.23 (m, 4H, OCH_2CH_2O), 4.52 (d, 1H, OH) 4.87 (t, 1H, C*H*OH), 6.48 (d, 1H, Bzd H-6), 6.51 (d, 1H, Bzd H-8), 6.73 (t, 1H, Bzd H-7), 7.21 (m, 1H, arom), 7.26 (d, 2H, arom), 7.39 (d, 1H, arom). Confirmation of the cis configuration was done by X-ray crystallography.

The second compound eluted (1.3 g) was washed with hot acetonitrile: yield 1.1 g (5.4%); mp 211−214 °C and identified to be **4-trans**. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3580 (w, OH). ^{1}H NMR (300 MHz, DMSO, δ): 2.8−3.25 (cluster of 11H, 8H pip + 1H, C*H*N indan, + 2H, CH_2 indan), 4.25−4.35 (m, 4H, OCH_2CH_2O), 5.22 (d, 1H, C*H*OH), 6.55 (d, 1H, Bzd H-8), 6.6 (d, 1H, Bzd H-6), 6.8 (t, 1H, Bzd H-7), 7.22 (m, 3H, arom), 7.4 (m, 1H, arom). Confirmation of the trans configuration was done by X-ray crystallography.

***cis*-2-[4-(2,3-Dihydrobenzo [1,4] dioxin-5-yl) piperazin-1-yl] 5-methoxyindan-1-ol (34) and *trans*-2-[4-(2,3-Dihydrobenzo [1,4] dioxin-5-yl) piperazin-1-yl] 5-methoxyindan-1-ol (35).** Prepared as described for **4-cis** and **4-trans** starting from compound **6**.

(+)-*trans*-2-[4-(2,3-Dihydrobenzo [1,4] dioxin-5-yl) piperazin-1-yl] 5-methoxyindan-1-ol (36) and (−)-*trans* 2-[4-(2,3-Dihydrobenzo [1,4] dioxin-5-yl) piperazin-1-yl] 5-methoxyindan-1-ol (37). Compound **35** (3 g) was resolved in its enantiomers on a Chiralpak AD column by HPLC (eluting system: EtOH/diethylamine, 1000/1). The first enantiomer eluted (880 mg) was recrystallized from CH_3CN: yield 0.61 g (20%) mp 149−152 °C; [α_D] +12.5° (*c* 0.5, MeOH) and identified to be **(+)-36**. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3500 (w, OH). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2 (br m, 1H, OH), 2.75−3.1 (cluster of 7H, 4H pip + 1H, C*H*N indan, + 2H, CH_2 indan), 3.15 (m, 4H, 4H pip), 3.8 (s, 3H, OMe), 4.3 (m, 4H, OCH_2CH_2O), 5.2 (d, 1H, C*H*OH), 6.55 (m, 2H, Bzd H-6 and H-8), 6.75 (m, 3H, 1H Bzd H-7 + 2H arom), 7.25 (d, 1H, arom).

The second compound eluted (870 mg) was recrystallized from CH_3CN: yield 0.62 g (21%) mp 151−153 °C; $[\alpha_D]$ −12.1° (*c* 0.5, MeOH) and identified to be **(−)-37**. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3500 (w, OH). ^1H NMR (300 MHz, $CDCl_3$, δ): 2 (br m, 1H, OH), 2.75−3.1 (cluster of 7H, 4H pip + 1H, C*H*N indan, + 2H, CH_2 indan), 3.15 (m, 4H, 4H pip), 3.8 (s, 3H, OMe), 4.3 (m, 4H, OCH_2CH_2O), 5.2 (d, 1H, C*H*OH), 6.55 (m, 2H, Bzd H-6 and H-8), 6.75 (m, 3H, 1H Bzd H-7 + 2H arom), 7.25 (d, 1H, arom).

***cis*-2-[4-(2,3-Dihydrobenzo [1,4] dioxin-5-yl) 4-hydroxy piperid-1-yl] 5-fluoroindan-1-ol (50). (Method A′).** *cis*-2-(1,4-Dioxo 8-azaspiro [4.5] dec-8-yl) 5-fluoroindan-1-ol **74-cis** (7.04 g, 24 mmol), obtained as described for compound **4-cis** in reacting 2-bromo 5-fluoro indan-1-one **IV-D** with 1,4-dioxo 8-azaspiro [4.5] decane **72** and then reducing the ketone obtained (**73**) by $NaBH_4$, was deprotected in 1-(5-fluoro 1-hydroxy indan-2-yl) piperid-4-one **75** (mp 178−181 °C) by HCl concentrated (24 mL) and AcOH (48 mL). Compound **75** (0.85 g, 3.41 mmol) in THF (34 mL) was introduced at 0 °C in 22 mL of a solution prepared by reacting magnesium (485 mg) on 5-bromo 2,3-dihydrobenzo[1,4]dioxine (4.3 g, 20 mmol) in THF (50 mL). At the end of the addition, the reaction mixture was stirred 1 h at room temperature then 1 h at reflux. After hydrolysis by a saturated solution of NH_4Cl (50 mL) and extraction by ether (3 × 50 mL), evaporation to dryness led to **50**: yield 1.52 g, mp 189−191 °C. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3366 (br, OH). ^1H NMR (300 MHz, DMSO, δ): 2.03−2.25 (m, 4H, CH_2 pip), 2.7−3.18 (cluster, 7H, 2H, CH_2NCH_2 + 3H, CH_2CH indan), 3.68 (s, 1H, OH), 4.25 (m, 1H, OH), 4.32 (m, 4H, OCH_2-CH_2O), 4.9 (d, 1H, C*H*OH), 6.8−7 (cluster, 5H, 3H, Bzd + 2H, arom), 7.42 (dd, 1H, arom).

General Method B. 4-(2,3-Dihydrobenzo[1,4]dioxin-5-yl) pyridine (VIII-A). 4-Pyridineboronic acid (9.85 g, 80 mmol) in EtOH (120 mL) and sodium carbonate (59.4 g, 560 mmol) in water (240 mL) were successively added to a solution of 5-bromo 2,3-dihydrobenzo[1,4]dioxine (22.4 g, 104 mmol), tetrakis(triphenylphosphine) palladium (3.7 g, 3.2 mmol), and toluene (380 mL), and the resulting mixture was heated at reflux for 28 h and then poured in water (1 L). After decantation, the aqueous phase was extracted by AcOEt (2 × 250 mL), and the joint organic phases were extracted by HCl 1 N (2 × 200 mL). Acidic phases were basified by concentrated NaOH and extracted by CH_2Cl_2. After the usual work up, 13.4 g of **VIII-A** were isolated: yield 78%; mp < 60 °C. ^1H NMR (200 MHz, $CDCl_3$, δ): 4.3 (s, 4H, OCH_2CH_2), 6.95 (s, 3H, Bzd), 7.5 (d, 2H, CHCCH pyr), 8.65 (d, 2H, CHNCH).

Compounds **VIII-C**, **VIII-D**, **VIII-E** were prepared according to the same procedure:

4-Benzofuran-7-yl pyridine (VIII-C): yield 71%; ^1H NMR (200 MHz, $CDCl_3$, δ): 6.82 (d, 1H, OCHC*H*), 7.34 (t, 1H, Bzd), 7.54 (d, 1H, Bzd), 7.65 (m, 2H, 1H, OCH + 1H, Bzd), 7.78 (dd, 2H, CHCCH pyr), 8.68 (dd, 2H, CHNCH).

4-(2,3-Dihydrobenzofuran-7-yl) pyridine (VIII-D): yield 73%.

4-Thiochroman-8-yl pyridine (VIII-E): using the triflate derivative instead of the bromo: yield 36%. 3.24 (t, 2H, OCH_2CH_2), 4.61 (t, 2H, OCH_2), 6.92 (t, 1H, Bzd), 7.23 and 7.32 (d, 2H, Bzd), 7.65 (d, 2H, CHCCH pyr), 8.61 (d, 2H, CHNCH).

4-(6-Fluorochroman-8-yl) pyridine (VIII-B). A mixture of diethyl 4-pyridinyl borane (0.45 g, 3 mmol), 8-bromo 6-fluorochroman (1 g, 4.5 mmol), KOH (0.5 g, 9 mmol), tetrabutylammonium bromide (0.48 g, 1.5 mmol), and tetrakis-(triphenylphosphine) palladium in THF (15 mL) was heated at reflux for 3 days. At the end of the reaction, AcOEt (75 mL) was added. The resulting mixture was washed with brine, dried over $MgSO_4$, and concentrated. After purification by chromatography (CH_2Cl_2 then CH_2Cl_2/AcOEt, 90/10), 0.6 g of **VIII-B** was isolated; yield 59%; mp 76−78 °C. ^1H NMR (200 MHz, $CDCl_3$, δ): 2 (m, 2H, $OCH_2CH_2CH_2$), 2.82 (m, 2H, OCH_2-CH_2CH_2), 4.14 (m, 2H, OCH_2), 6.7−6.9 (m, 2H, chroman), 7.44 (d, 2H, CHCCH pyr), 8.58 (d, 2H, CHNCH).

4-(2,3-Dihydrobenzo[1,4]dioxin-5-yl) 1-(1-oxoindan-2-yl) pyridinium, bromide (11). A solution of 2-bromo 1-indanone (2.45 g, 11.6 mmol) and compound **VIII-A** (2.41 g, 11.3 mmol) in acetone (35 mL) was heated under reflux for 24 h. On cooling (ice), a precipitate appeared that was filtered off, washed with cold acetone, and dried on vacuum under P_2O_5: yield 3.1 g (64%); mp 251−254 °C.

***cis*-2-[4-(2,3-Dihydrobenzo[1,4]dioxin-5-yl) 3,6-dihydro-2*H*-pyridin-1-yl] indan-1-ol (48) and *trans*-2-[4-(2,3-Dihydrobenzo[1,4]dioxin-5-yl) 3,6-dihydro-2*H*-pyridin-1-yl] indan-1-ol (49).** Sodium borohydride (1.18 g, 31 mmol) was added by portion, at room temperature, to a suspension of compound **11** (2.64 g, 6.2 mmol) in methanol (50 mL). At the end of the addition, the mixture was stirred 1 h at room temperature, and acetic acid (2.8 mL, 49.6 mmol) was added. After evaporation to dryness, the residue was taken up in NaOH 1 N (50 mL) and extracted by CH_2Cl_2 (2 × 50 mL). The combined organic layers were concentrated under reduce pressure, and the residue was chromatographed (CH_2Cl_2/AcOEt, 90/10). The first compound eluted was recrystallized from C_2H_5OH: yield 0.57 g (26%); mp 109−112 °C and identified to be of cis configuration by IR spectrum. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3400 (br, OH). ^1H NMR (300 MHz, $CDCl_3$, δ): 2.5−3.25 (cluster of 6H, 4H NCH_2CH_2 pip + 2H, CH_2 indan), 3.40 (m, 2H, $NCH_2CH{=}C$), 4.30 (m, 5H, 4H, OCH_2CH_2O + 1H, C*H*N indan), 5.0 (d, 1H, C*H*OH), 5.90 (m, 1H, $NCH_2CH{=}C$), 6.8 (m, 3H, Bzd), 7.25−7.5 (m, 4H, arom).

The second compound eluted was recrystallized from CH_3CN: yield 0.67 g (28%); mp 182−184 °C and identified to be trans. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3600 (w,OH). ^1H NMR (200 MHz, $CDCl_3$, δ): 2.6 (m, 2H, NCH_2CH_2), 2.8−3.2 (cluster of 5H, 2H NCH_2CH_2 + 1H, C*H*N indan + 2H, CH_2 indan), 3.95 (m, 2H, $NCH_2CH{=}C$), 4.25 (s, 4H, OCH_2CH_2O), 5.25 (d, 1H, C*H*OH), 5.85 (m, 1H, $NCH_2CH{=}C$), 6.8 (m, 3H, Bzd), 7.1−7.5 (m, 4H, arom).

***trans*-2-[4-(2,3-Dihydrobenzo[1,4]dioxin-5-yl) piperid-1-yl] indan-1-ol (53).** Compound **49** (0.4 g, 1.15 mmol) in methanol (20 mL) was hydrogenated over PtO_2 during 19 h, at room temperature and under atmospheric pressure. After filtration and concentration of the solvent, the solid residue was recrystallized from CH_3CN: yield 0.21 g (52%); mp 173−174 °C. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3600 (w,OH). ^1H NMR (300 MHz, $CDCl_3$, δ): 1.84 (m, 4H, CH_2CHCH_2), 2.34 (m, 2H, NCH_2-CH_2), 2.85−3.42 (cluster of 6H, 2H, NCH_2CH_2 + 1H, CH_2CHCH_2 + 1H, C*H*N indan + 2H, CH_2 indan), 4.28 (s, 4H, OCH_2CH_2O), 5.25 (d, 1H, C*H*OH), 6.75 (m, 3H, Bzd), 7.1−7.45 (m, 4H, arom).

General Method C. *trans*-1-[4-(2,3-Dihydrobenzo[1,4]-dioxin-5-yl) piperid-1-yl] 6-nitroindan-2-ol (27). A solution of 6-nitroindene oxide **IX-H** (6.2 g, 35 mmol) and compound **II-A** in CH_3CN (30 mL) was stirred at room temperature for 20 h and at reflux for 4 h. After concentration under vacuum, the residue was taken up in CH_2Cl_2, washed with water, dried, and concentrated. The residue was purified by flash-chromatography (CH_2Cl_2/EtOH, 98/2): yield 5.9 g (52%); mp 202−205 °C. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3600 (w,OH). ^1H NMR (200 MHz, $CDCl_3$, δ): 1.9 (br m, 1H, OH), 2.85 (m, 4H, CH_2NCH_2 pip), 2.9 (dd, 1H, CH*H* indan), 3.15 (cluster, 5H, 4H, CH_2NCH_2 pip + 1H, C*H*N indan), 3.35 (dd, 1H, CH*H* indan), 4.3 (m, 4H, OCH_2CH_2O), 4.85 (d, 1H, C*H*OH), 6.6 (m, 2H, Bzd-6 + Bzd-8), 6.8 (t, 1H, Bzd-7), 7.35 (d, 1H, arom-4), 8.15 (dd, 1H, arom-5), 8.25 (d, 1H, arom-7).

***trans*-2-[4-(2,3-Dihydrobenzo[1,4]dioxin-5-yl) piperid-1-yl] 6-nitroindan-1-ol (70).** Diethylazodicarboxylate (9.5 mL, 60.3 mmol) was added dropwise, in 15 min, to a suspension of compound **27** (4.9 g, 12.3 mmol), *p*-nitrobenzoic acid (9.0 g, 54.1 mmol), triphenylphosphine (15.8 g, 60.3 mmol), in THF (125 mL) and toluene (125 mL) cooled at −20 °C. The mixture was kept at this temperature for 1 h and overnight at room temperature. After the sample was evaporated to dryness, the residue was filtered off on silica (CH_2Cl_2/AcOEt, 98/2) to yield 16 g of a residue which was taken up in ether and HCl 1 N to lead, after filtration, to 3.7 g (48%) of the dihydrochloride salt of *p*-nitrobenzoic acid 2-[4-(2,3-dihydrobenzo[1,4]dioxin-5-yl) piperazin-1-yl] 6-nitro indan-1-yl ester. The dihydrochloride salt was suspended in a solution of KOH (1.63 g, 29 mmol), water (16 mL), and methanol (160 mL) and

heated at reflux for 1 h. The solution was concentrated, and the residue was taken up in water (100 mL) and extracted three times by CH_2Cl_2 (70 mL) and after usual workup led to 2.4 g of a light dark solid which was recrystallized from CH_3-CN: yield 1.7 g (74%); mp 194−196 °C. IR ($CHCl_3$) γ_{max} (cm^{-1}): 3600 (w, OH). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.2 (br m, 1H, OH), 3.20 (cluster, 6H, 4H, C*H*$_2$NC*H*$_2$ pip + 1H, C*H*N indan + 1H, CH*H* indan), 3.9 (cluster, 5H, 4H, C*H*$_2$NC*H*$_2$ pip + 1H, C*H*H indan), 4.3 (m, 4H, OCH$_2$CH$_2$O), 5.25 (m, 1H, C*H*OH), 6.6 (m, 2H, Bzd-6 + Bzd-8), 6.8 (t, 1H, Bzd-7) 7.33 (d, 1H, arom-4), 8.13 (dd, 1H, arom-5), 8.23 (s, 1H, arom-7).

Biology. 1. Binding Studies at α_1 and at Human 5-HT$_{1A}$ Receptors. (a) Determination of Affinity for Rat Cortex α_1-Receptors. Binding affinity at rat α_1-adrenoceptors was determined by competition with [^{3}H]-prazosin (Amersham, Les Ulis, France) as described by Millan.[26] Briefly, membranes were prepared from rat frontal cortex and incubated in triplicate with 0.2 nM [^{3}H]-prazosin and competing ligand in a final volume of 0.5 mL, for 1 h at 22 °C. The incubation buffer contained: 50 mM TRIS−HCl, pH 7.4, 4 mM $CaCl_2$, 0.1% w/v ascorbic acid. Nonspecific binding was defined with 10 μM phentolamine.

(b) Determination of Affinity for Recombinant Human 5-HT$_{1A}$ Receptors. Binding affinity at human 5-hydroxytryptamine 5-HT$_{1A}$ receptors was determined by competition with [^{3}H]-8-OH-DPAT (Amersham, Les Ulis, France) as described by Newman-Tancredi.[27] Briefly, membranes were incubated with [^{3}H]-8-OH-DPAT at 22 °C for 2.5 h. Nonspecific binding was defined with 5-HT (10 μM).

(c) Data Analysis. At the end of the incubation period, membranes were filtered through Whatman GF/B filters pretreated with 0.1% de polyethylenimine. Radioactivity retained on the filters was determined by scintillation counting. Binding isotherms were analyzed by nonlinear regression using the program Prism (GraphPad Software Inc., San Diego, USA) to determine IC_{50} values. These were converted to inhibition constants (K_i) by the use of the Cheng-Prusoff equation $K_i = IC_{50}/\{(L/K_D) - 1\}$ where L is the concentration of [^{3}H]-ligand and K_D is its dissociation constant. The K_D values were 0.1 nM for [^{3}H]-prazosin, 0.6 nM for [^{3}H]-8-OH-DPAT.

2. Determination of Agonist Efficacy at Recombinant Human 5-HT$_{1A}$ Receptors. Efficacy was determined by measuring agonist stimulation of [^{35}S]GTPγS binding, as described previously by Newman-Tancredi.[27] Briefly, CHO-h5-HT$_{1A}$ membranes (50 μg of protein) were incubated (20 min, 22 °C) in triplicate in a buffer containing 20 mM HEPES (pH 7.4), 3 μM GDP, 3 mM $MgSO_4$, NaCl 100 mM, and 0.1 nM [^{35}S]GTPγS (1300 Ci/mmol, NEN). Nonspecific binding was defined with 10 μM GTPγS. Agonist efficacy is expressed relative to that of 5-HT (= 100%), which was tested at a maximally effective concentration (10 μM) in each experiment.

3. Inhibition of Hypothermia Induced by 8-OH-DPAT. Male Wistar rats of 200−220 g, housed singly, were removed from home cages, and core (rectal) temperature was determined by use of a digital thermistoprobe. Then the rats were treated with either vehicle or the putative antagonist and returned to their home cages. Thirty minutes later, they were reinjected with either vehicle or 8-OH-DPAT (0.16 mg/kg) and returned to their cages for a further 30 min, and then the core temperature was redetermined. The difference between basal and posttreatment values was calculated. The mean decrease in core temperature elicited by 8-OH-DPAT in the presence of vehicle was, typically, -2.2 ± 0.2 °C ($n = 10$). The ID_{50} (95% confidence limits) was calculated according to the method of Finney, 1964. All drugs were dissolved in distilled water and given s.c. in a volume of 1.0 mL/kg.

4. Determination of Metabolic Bioavailability.[28] Metabolic bioavailability predictions (MF%) were based on in vitro metabolic stability measurements with hepatic microsomes assuming total absorption. Briefly, unchanged drugs were quantified by LC-MS-MS following incubation (10^{-7} M) with rat and human hepatic microsomes (0.33 mg of protein/mL) after 0, 5, 15, 30, and 60 min of incubation. Hepatic microsomes were obtained from a pool of five different subjects. The in vitro intrinsic clearances (vitroClint) were the slope (after LN linearization) of the curve of the unchanged drug remaining concentration versus incubation time. In vitro Clint were then scaled up to in vivo whole body (vivoClint) using 0.045 mg of protein/kg of liver and liver weight of 11 g for the rat and 1.2 kg for man. In vivo Clint were then transformed into hepatic clearances (HepCl) using the well-stirred model (HepCl = vivoClint*HBF/(vivoClint + HBF) where HBF (hepatic blood flow) were taken as 22 mL/min for the rat (250 g) and 1500 mL/min for man. The MF% were then deducted from the extraction ratio with the following equation (MF% = 1 − HepCl/HBF). This method was shown internally to give a good in vitro/in vivo correlation on about 40 chemically nonrelated compounds. When human hepatocytes were required to confirm the metabolic stability measurements the same method was employed using in that case an hepatocytes suspension in a classical culture media (0.2×10^6 cells/mL), and kinetic time points were 10 min, 30 min, 1 h, 2 h, and 4 h.

5. Determination of Intestinal Absorption.[29] Assuming no possible limitation due to solubility, absorbed fractions (Abs%) were estimated using permeability measurements through Caco-2 cell monolayers. Briefly, unchanged drugs were incubated with Caco-2 monolayers (apical compartment) at 20 μM and concentration versus incubation time appearance in the basal compartment were quantified by LC-MS-MS after 0, 15, 30, 60, and 120 min of incubation. Apparent permeabilities (Papp) were calculated from the slope of the appearance line (permeability rate) with the following equation (Papp = permeability rate/initial apical concentration/surface area of the monolayer). The absorbed fraction prediction (Abs%) were then obtained from the plot against an internal calibration curve performed from Papp of six reference compounds for which oral absorption in man was described (mannitol with 16%, atenolol with 50%, cimetidine with 70%, propranolol with 90%, and antipyrine and testosterone with 100%). This method was shown internally and by others to give a good in vitro/in vivo correlation for human intestinal absorption.

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