Cycloalkanecarboxylic Esters Derived from Lysergol, Dihydrolysergol-I, and Elymoclavine as Partial Agonists and Antagonists at Rat 5-HT_{2A} Receptors: Pharmacological Evidence that the Indolo[4,3-*fg*]quinoline System of the Ergolines Is Responsible for High 5-HT_{2A} Receptor Affinity

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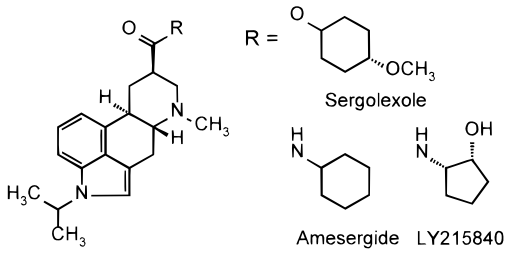
Three series of cycloalkanecarboxylic esters derived from the naturally occurring clavine alkaloids lysergol, dihydrolysergol-I, and elymoclavine were synthesized to study their interaction with 5-HT_{2A} receptors and α_1-adrenoceptors in rat tail artery and aorta, respectively. Especially cycloalkanecarboxylic esters derived from lysergol showed complex behavior as partial agonists and antagonists of the contractile effect of 5-HT. Within this group, partial 5-HT_{2A} receptor agonist activity was most potent for cyclopropanecarboxylic ester **6a** ($pK_P = 7.67$, $\alpha = 0.21$) and decreased as the volume requirement of the alicyclic ring increased. This tendency was echoed in experiments where the compounds were used as antagonists of the contractile effect of 5-HT. From the structure−activity study, the *N*-1-isopropyl homologue of **6a**, compound **6b**, emerged as the ligand with the highest affinity for rat 5-HT_{2A} receptors ($pA_2 = 8.74$). For cycloalkanecarboxylic esters derived from dihydrolysergol-I and elymoclavine, no clear structure−affinity relationship could be deduced, although those compounds that had smaller cycloalkyl rings in the acyl portion and an isopropyl substituent at N-1 showed the highest 5-HT_{2A} receptor affinity. On the other hand, cycloalkanecarboxylic esters derived from lysergol, dihydrolysergol-I, and elymoclavine displayed low or marginal affinity at α_1-adrenoceptors. A further aim of the study was to examine to what extent the complete removal of the acyl portion of the esters would affect 5-HT_{2A} receptor affinity. The parent alcohols of the three series of *N*-1-isopropyl homologues, 1-isopropyllysergol (**1b**), 1-isopropyldihydrolysergol-I (**2b**), and 1-isopropylelymoclavine (**3b**), displayed higher affinity for 5-HT_{2A} receptors ($pA_2 = 9.15$, 8.50, 9.14) than the corresponding esters. Compounds **1b**−**3b** had no contractile effects by themselves and displayed low affinity at guinea-pig 5-HT_{1B} receptors and rat α_1-adrenoceptors. The high affinity for rat 5-HT_{2A} receptors was retained when clavines even more simple in structure than **1b**−**3b**, compounds **4b** and **5b**, were examined as 5-HT_{2A} receptor antagonists. The nanomolar antagonist activity of simple clavines (**1b**−**5b**) in the rat suggests that the indolo-[4,3-*fg*]quinoline system of the ergolines is the molecular fragment that is responsible for 5-HT_{2A} receptor affinity, and not the substituent at position C-8.

Introduction

Despite their low selectivity, ergolines are important ligands for serotonin 5-HT_{2A} receptors, where they display complex behavior as partial agonists or antagonists.[1] For example, it has been suggested that the classical hallucinogenic agent lysergic acid diethylamide (LSD) may exert its psychotomimetic effect predominantly by acting as a partial agonist at 5-HT_{2A} receptors.[2,3] Furthermore, there is evidence that the powerful contractions produced by ergometrine and methylergometrine on uterine smooth muscle involve partial agonism of these agents at 5-HT_{2A} receptors, since the effects can be blocked by the 5-HT_{2A} receptor antagonists methysergide and ICI169,369, respectively.[4] On the other hand, ergoline-based 5-HT_{2A} receptor antagonists such as the widely used LY53857 and structurally related compounds such as sergolexole, amesergide, and LY215840 are known to inhibit 5-HT-induced platelet aggregation and to block the direct contractile effect of platelet-released 5-HT on vascular smooth muscle.[5−9] These agents may be useful in the treatment of ischemic heart disease and other vascular disorders.[10] From the chemical standpoint, sergolexole is a 6-methylergoline-8-carboxylic acid cycloalkyl ester, while amesergide and LY215840 are 6-methylergoline-8-carboxylic acid cycloalkylamides. The compounds share the structural property to be substituted with an isopropyl group at the indole nitrogen (Chart 1).

Chart 1



Recently, we have found that ester formation of the naturally occurring clavines lysergol (9,10-didehydro-

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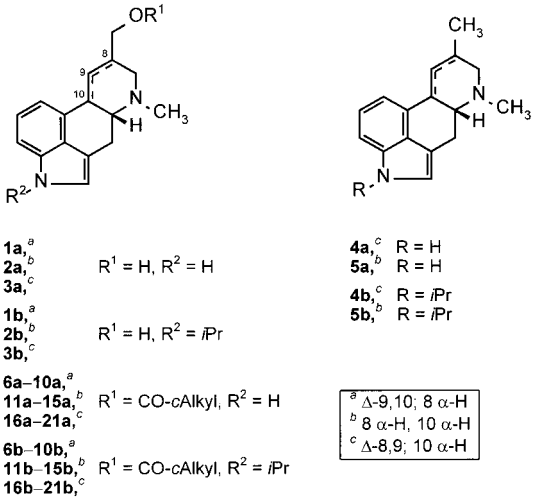
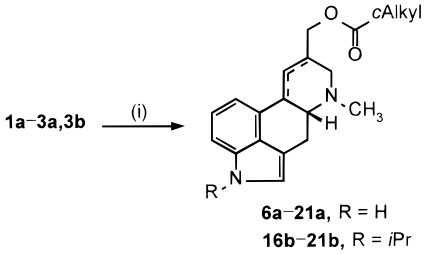
Chart 2



8β-hydroxymethyl-6-methylergoline) and dihydrolysergol-I (8β-hydroxymethyl-6-methylergoline) with suitable aliphatic branched-chain carboxylic acids and *N*-1-isopropyl substitution afforded compounds with enhanced antagonist activity at vascular 5-HT_{2A} receptors.[11] As an extension of our previous efforts, we now report the conversion of lysergol (**1a**), dihydrolysergol-I (**2a**), and elymoclavine (**3a**) into three series of cycloalkanecarboxylic esters (**6a,b**−**21a,b**) (Chart 2) and the interaction of the new ergolines with 5-HT_{2A} receptors in rat tail artery and α_1-adrenoceptors in rat aorta. It was hypothesized that alicyclic carboxylic esters derived from lysergol, dihydrolysergol-I, and elymoclavine could ultimately have a similar affinity profile at 5-HT_{2A} receptors as 6-methylergoline-8-carboxylic acid cycloalkyl esters such as sergolexole.

It has recently been demonstrated that N-1-unsubstituted ergolines have higher affinity for the human versus the rat 5-HT_{2A} receptor, whereas the corresponding ergolines with an alkyl (methyl, isopropyl) substituent at N-1 have higher affinity for the rat versus the human 5-HT_{2A} receptor.[12,13] A single amino acid difference, Ser242 in the human receptor and Ala242 in the rat receptor, is responsible for the differential affinities.[14] In addition, it has been shown that mutation of Ala242 → Ser242 in the rat 5-HT_{2A} receptor converts the operational characteristics of the rat receptor to that of the human receptor.[15] It has been speculated that serine in the human 5-HT_{2A} receptor serves as a hydrogen bond acceptor for the indole NH of N-1-unsubstituted ergolines, whereas alanine in the rat 5-HT_{2A} receptor allows aliphatic stacking with the alkyl group of N-1-alkylated ergolines.[15] Hence, a further aim of the present study was to investigate the pharmacological profile of the parent alcohols of the *N*-1-isopropyl homologues (1-isopropyllysergol (**1b**), 1-isopropyldihydrolysergol-I (**2b**), 1-isopropylelymoclavine (**3b**)) in order to estimate their possible contribution to 5-HT_{2A} receptor antagonist activity in the rat. Affinity estimates of even more simple ergolines such as 1-isopropylagroclavine (**4b**) and 1-isopropylfestuclavine (**5b**) should elucidate the structural requirements of the 5-HT_{2A} receptor with regard to the ergoline molecule. **4b** and **5b** have a methyl group instead of a hydroxymethyl group at C-8, and **5b** has a C-8-C-9 single bond (Chart 2). Since ergolines generally exert their pharmacological effects in the vasculature not only via interaction with 5-HT_{2A} receptors but also with 5-HT_{1B} (formerly 5-HT_{1D}-like) receptors[16−18] and α_1-adrenoceptors, it was of special interest to examine whether selected compounds would display selectivity for vascular 5-HT_{2A} receptors over these other sites. A preliminary report of some of these data has been published.[19]

Scheme 1[a]



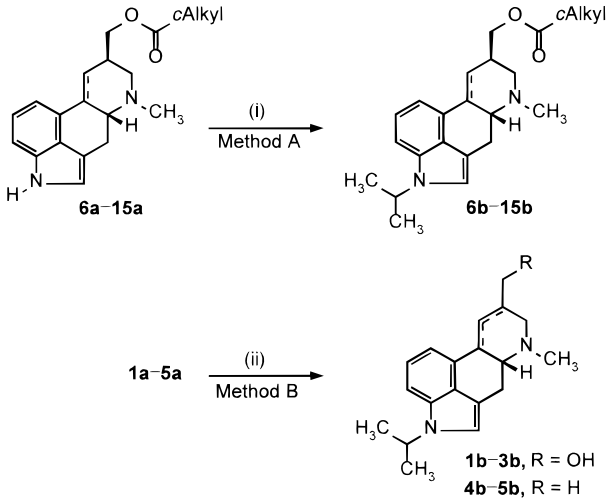
[a] Reagents and experimental conditions: (i) pyridine, 4-DMAP, addition of the respective acid chloride at 0 °C within 160 min, then 12 h stirring at rt. Chemical structures: for details, see Chart 2.

Chemistry

Sergolexole is the most prominent 6-methylergoline-8-carboxylic acid cycloalkyl ester that shows potent 5-HT_{2A} receptor antagonist activity (pA_2 of 9.05 in rat jugular vein) without appreciably binding to 5-HT_1, dopamine D_1, dopamine D_2, histamine H_1, muscarinergic, α_1-adrenergic, and β-adrenergic receptors.[6,7] We intended to synthesize structurally related cycloalkanecarboxylic esters derived from 9,10-didehydro-8β-hydroxymethyl-6-methylergoline (lysergol), 8β-hydroxymethyl-6-methylergoline (dihydrolysergol-I), and 8,9-didehydro-8-hydroxymethyl-6-methylergoline (elymoclavine), so-called ergoline "reverse esters" (reverse in comparison with lysergic acid esters), which have an alicyclic ring in the acyl portion and an isopropyl substituent at the indole nitrogen. Lysergol (**1a**), dihydrolysergol-I (**2a**), and elymoclavine (**3a**) served as starting points for the preparation of the esters. Elymoclavine (**3a**) was available from submerged cultures of *Claviceps* strain SD-58 which can be readily adapted to large-scale production.[20,21] Lysergol (**1a**) was prepared from elymoclavine (**3a**) by heating on activated Al_2O_3 in pyridine[22] and dihydrolysergol-I (**2**) from lysergol (**1a**) by catalytic hydrogenation with 10% Pd/C in DMF/pyridine (100:2, v/v) at 5 bar.[23] Agroclavine (**4a**) was isolated from *Claviceps* strain SD-58, while festuclavine (**5a**) was synthesized from **4a** by catalytic transfer hydrogenation using Raney nickel in EtOH.[24] O-Acylation of **1a**−**3a** was achieved by Einhorn reaction using the respective alicyclic acid chloride in pyridine in the presence of 4-DMAP (Scheme 1).

Introduction of the isopropyl substituent at the N-1 position of the esters derived from **1a** and **2a** was achieved by addition of isopropyl iodide to a solution of the respective O-acylated lysergol or dihydrolysergol-I derivative in THF using as base powdered KOH in the presence of 18-crown-6 (method A). Simple clavines such as **1a**−**5a** were alkylated by using isopropyl tosylate as alkylating agent in DMSO (method B) (Scheme 2).[25] O-Acylated derivatives of **3a** with an isopropyl substituent at the N-1 position were prepared from **3b** because

Scheme 2[a]



[a] Reagents and experimental conditions: (i) dry THF, 18-crown-6, powdered KOH, isopropyl iodide, 1 h stirring at rt (method A); (ii) DMSO, powdered KOH, isopropyl tosylate, 2 h stirring at rt (method B). Chemical structures: for details, see Chart 2.

the direct alkylation of the esters led to hydrolysis. After workup, all compounds were purified and separated by radial chromatography under an atmosphere of nitrogen or argon.

Biology

The interaction of the compounds with 5-HT_{2A} and 5-HT_{1B} receptors and α_1-adrenoceptors was studied in various functional in vitro assays. Partial agonism at 5-HT_{2A} receptors was studied in the absence and presence of ketanserin (3 nM) in cylindrical segments of the isolated rat tail artery. The effectiveness of the compounds in blocking 5-HT_{2A} receptor-mediated contractions by 5-HT was determined in the same tissue. The ability of the compounds to block α_1-adrenoceptor-mediated contractions by (*R*)-phenylephrine was studied in cylindrical segments of the isolated rat thoracic aorta. The interaction of selected compounds (**1b**−**3b**) with 5-HT_{1B} receptors was examined in cylindrical segments of the isolated guinea-pig iliac artery, moderately precontracted by $PGF_{2\alpha}$ (50−500 nM).[26]

Antagonism by cycloalkanecarboxylic esters derived from lysergol (**6a,b**−**10a,b**), dihydrolysergol-I (**11a,b**−**15a,b**), and elymoclavine (**16a,b**−**21a,b**) of the contractile effect of 5-HT via 5-HT_{2A} receptors and (*R*)-phenylephrine via α_1-adrenoceptors is summarized in Tables 1−3. The contractile effects of O-acylated lysergol derivatives **6a**−**10a** on 5-HT_{2A} receptors are summarized in Table 4. The interaction of selected clavine derivatives **1b**−**5b** with 5-HT_{2A} receptors, 5-HT_{1B} receptors, and α_1-adrenoceptors is summarized in Table 5. Partial agonists were characterized by estimation of the equilibrium dissociation constant K_P (given as pK_P).[27−29] Antagonist dissociation constants are given as apparent or full pA_2 values.[30−32] For details, see the Experimental Section.

Results and Discussion

Cycloalkanecarboxylic Esters Derived from Lysergol, Dihydrolysergol-I, and Elymoclavine as Antagonists at 5-HT_{2A} Receptors and α_1-Adrenoceptors. "Reverse esters" of lysergol, dihydrolysergol-I, and elymoclavine were tested for their ability to inhibit 5-HT-induced contractions of rat tail artery and (*R*)-phenylephrine-induced contractions of rat aorta. All compounds studied caused a rightward shift of the concentration−response curves to 5-HT and (*R*)-phenylephrine, respectively, with little or no effect on maximum responses (Tables 1−3). Cyclopropanecarboxylic acid ester **6a** (pK_P = 8.40, Figure 1A) and its *N*-1-isopropyl homologue **6b** (pA_2 = 8.74) showed the highest affinity for 5-HT_{2A} receptors. Within the series of O-acylated derivatives of lysergol (**6a,b**−**10a,b**) 5-HT_{2A} receptor affinity continuously decreased as the volume requirement of the alicyclic ring increased. Increasing the size of the alicyclic ring resulted in a decrease in antagonist activity for N-1-unsubstituted homologues (**6a**−**10a**) that paralleled the potency in experiments for 5-HT_{2A} receptor agonist activity (see below). It should be mentioned that the pK_P values for the partial agonist−5-HT_{2A} receptor complex calculated from antagonism by **6a**−**10a** of the contractile response to 5-HT were slightly higher than the pK_P values calculated from the contractile response to **6a**−**10a**. This suggests that

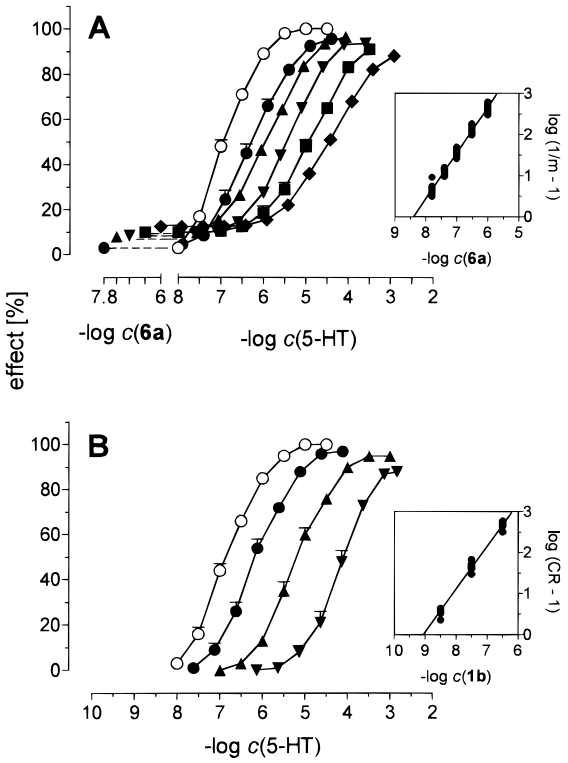
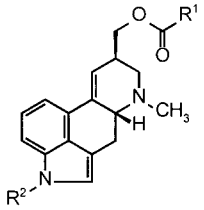


Figure 1. Antagonism of 5-HT-induced contractions by the partial agonist **6a** and the silent antagonist **1b** in rat tail artery. Shown are concentration−effect curves for 5-HT in the absence (○, 17−42) and presence of **6a** and **1b**, respectively. A. Compound **6a** at 15.8 nM (●, n = 8), 39.8 nM (▲, n = 8), 100 nM (▼, n = 8), 300 nM (■, n = 8), and 1000 nM (◆, n = 10). Contractions elicited by 15.8, 39.8, 100, 300, and 1000 nM **6a** were 3 ± 1, 9 ± 2, 9 ± 2, 11 ± 2, and 14 ± 3% (not shown) and partially faded to 3 ± 1, 8 ± 2, 9 ± 2, 10 ± 2, and 13 ± 2% within 30 min. Contractions of **6a** after 30 min are shown in the left segment of the abscissa. Inset: Kaumann−Marano plot for the calculation of partial agonist affinity (pK_P).[29] pK_P was 8.40 ± 0.04 (slope m = 1.11 ± 0.02, significantly different from unity (P < 0.05)). B. Compound **1b** at 3 nM (●, n = 10), 30 nM (▲, n = 9), and 300 nM (▼, n = 6). Inset: Schild analysis of antagonist-induced curve displacements. pA_2 was 9.15 ± 0.04 (slope m = 1.05 ± 0.03, not significantly different from unity (P < 0.05)). All values are means ± SEM.

Table 1. Physical Properties and Pharmacological Effects of Cycloalkanecarboxylic Esters Derived from Lysergol on 5-HT-Induced Contractions of Rat Tail Artery and (*R*)-Phenylephrine-Induced Contractions of Rat Aorta



compd	R^1	R^2	molecular formula[a]	% yield	mp, °C	5-HT_{2A} receptor affinity[b]	α_1-adrenoceptor affinity[b,c]	specificity 5-HT_{2A}/α_1[d]
6a		H	$C_{24}H_{26}N_2O_6$	79	178–180	8.40 ± 0.04[e,f] (42)	6.66 ± 0.11 (4)	55
6b		$CH(CH_3)_2$	$C_{27}H_{32}N_2O_6$	65	185–186	8.74 ± 0.11[g,h] (16)	5.08 ± 0.11 (4)	4571
7a		H	$C_{25}H_{28}N_2O_6$	73	198–200	7.76 ± 0.06[e,i] (16)	5.52 ± 0.04 (4)	174
7b		$CH(CH_3)_2$	$C_{28}H_{34}N_2O_6$	68	178–179	8.22 ± 0.09[g,j] (12)	4.75 ± 0.08 (4)	2951
8a		H	$C_{26}H_{30}N_2O_6$	66	217–219	7.40 ± 0.09[k] (8)	5.32 ± 0.07 (3)	120
8b		$CH(CH_3)_2$	$C_{29}H_{36}N_2O_6$	62	184–185	8.25 ± 0.04[c] (12)	4.57 ± 0.07 (4)	4786
9a		H	$C_{27}H_{32}N_2O_6$	68	218–219	6.88 ± 0.09[k] (8)	5.05 ± 0.04 (4)	68
9b		$CH(CH_3)_2$	$C_{30}H_{38}N_2O_6$	65	190–191	7.92 ± 0.05[c] (8)	4.77 ± 0.01 (4)	1413
10a		H	$C_{28}H_{34}N_2O_6$	56	211–213	6.71 ± 0.06[k] (12)	4.73 ± 0.09 (4)	95
10b		$CH(CH_3)_2$	$C_{31}H_{40}N_2O_6$	60	195–196	7.75 ± 0.05[c] (8)	4.93 ± 0.09 (4)	661

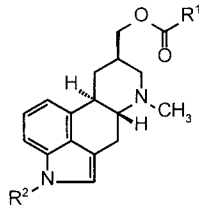
[a] All compounds exhibit ^{1}H NMR spectral data in agreement with assigned structures. All compounds were characterized and tested as their hydrogen maleate salts. Salt **6b** was hydrated with 0.5 mol of water. All salts were crystallized from a THF/Et_2O mixture. Elemental analyses were within ±0.4% of the theoretical values for C, H, N. [b] Values are expressed as mean ± SEM from *n* individual vascular segments of at least two animals (number *n* of independent experiments in parentheses). [c] These values are apparent pA_2 values which were estimated according to ref 30. [d] 5-HT_{2A}/α_1 is the ratio of K_B values. [e] This value is the pK_P value which was estimated from a Kaumann−Marano plot according to ref 29. [f] Slope 1.11 ± 0.02 of the Kaumann−Marano plot, significantly different from unity ($P < 0.05$). [g] This value is the full pA_2 value which was estimated from the Schild plot according to ref 31. [h] Slope 1.05 ± 0.06 of the Schild plot. [i] Slope 1.00 ± 0.12 of the Kaumann−Marano plot. [j] Slope 1.10 ± 0.07 of the Schild plot. [k] This value is the pK_P value which was estimated according to ref 28.

6a−**10a** and 5-HT may bind in two slightly different orientations at the 5-HT_{2A} receptor. In the series of O-acylated derivatives of dihydrolysergol-I (**11a,b**−**15a,b**), increasing the size of the alicyclic ring resulted in a decrease in 5-HT_{2A} receptor affinity only for *N*-1-isopropyl homologues. For unsubstituted homologues no clear structure−affinity relationship could be deduced. Moreover, 5-HT_{2A} receptor affinity was only slightly affected by the structure of the cycloalkyl ring in the acyl portion of the esters. This finding was echoed in the series of O-acylated derivatives of elymoclavine (**16a,b**−**21a,b**), where 5-HT_{2A} receptor affinities for unsubstituted and *N*-1-isopropyl homologues showed differences of only 0.86 and 0.36 log unit between the most potent compound and the weakest compound. It should be mentioned that the introduction of a methoxy group in the 4-position of the cyclohexyl ring (**19a,b** and **20a,b**) failed to enhance 5-HT_{2A} receptor affinity. This was in contrast to the observation of Garbrecht et al. who synthesized substituted 6-methylergoline-8-carboxylic acid cyclohexyl esters of which an oxygen in the 4-position of the ester cyclohexyl ring was of crucial importance for 5-HT_{2A} receptor affinity.[5] Moreover, the findings in our laboratory suggest that the stereochemical orientation of the substituent in the 4-position seems to be of minor importance for 5-HT_{2A} receptor affinity. Compounds **20a**,**b** with a cyclohexyl ring in the acyl portion characterized by a 4-methoxy group trans to the carboxyl group showed nearly equal antagonist activity compared to **19a**,**b** with a 4-methoxy-substituted cyclohexyl ring existing as a cis/trans diastereomeric mixture.

Comparing the 5-HT_{2A} receptor affinity of N-1-unsubstituted derivatives with that of compounds with an *N*-1-isopropyl group revealed the importance of a substituent at N-1. "Reverse esters" derived from lysergol and elymoclavine, respectively, with an *N*-1-isopropyl group showed higher affinity at rat 5-HT_{2A} receptors than their unsubstituted homologues with the exception of one case (compound **21b**). Similar findings have previously been reported with 8β-6-methylergoline-8-carboxylic acid cycloalkyl esters and amides.[12,13] With O-acylated derivatives of dihydrolysergol-I, no clear structure−affinity relationship could be deduced. N-1-Alkylation resulted in higher 5-HT_{2A} receptor affinity only for those derivatives that were characterized by smaller cycloalkyl rings (cyclopropyl derivative **11b**, cyclobutyl derivative **12b**).

The examination of the pharmacological activity of lysergol, dihydrolysergol-I, and elymoclavine "reverse esters" at α_1-adrenoceptors revealed low affinity at these sites for all compounds tested. This was especially true for ergolines with an *N*-1-isopropyl substituent. Ergolines with an isopropyl group at N-1 seem to be highly specific antagonists at 5-HT_{2A} receptors (see ratio 5-HT_{2A}/α_1 of Tables 1−3).

Table 2. Physical Properties and Pharmacological Effects of Cycloalkanecarboxylic Esters Derived from Dihydrolysergol-I on 5-HT-Induced Contractions of Rat Tail Artery and (*R*)-Phenylephrine-Induced Contractions of Rat Aorta



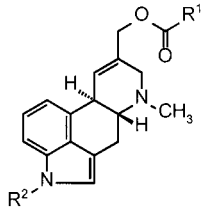
compd	R^1	R^2	molecular formula[a]	% yield	mp, °C	5-HT_{2A} receptor affinity[b]	α_1-adrenoceptor affinity[b,c]	specificity 5-HT_{2A}/α_1[d]
11a		H	$C_{24}H_{28}N_2O_6$	80	205–206	7.67 ± 0.05[e] (8)	7.20 ± 0.09 (4)	3
11b		$CH(CH_3)_2$	$C_{27}H_{34}N_2O_6$	54	190–191	8.22 ± 0.09[f,g] (16)	5.55 ± 0.02 (4)	468
12a		H	$C_{25}H_{30}N_2O_6$	75	225–226	7.30 ± 0.05[e] (6)	6.59 ± 0.02 (4)	5
12b		$CH(CH_3)_2$	$C_{28}H_{36}N_2O_6$	65	184–185	8.06 ± 0.14[f,h] (14)	4.59 ± 0.10 (4)	2951
13a		H	$C_{26}H_{32}N_2O_6$	72	228–229	7.50 ± 0.05[e] (6)	5.95 ± 0.13 (4)	35
13b		$CH(CH_3)_2$	$C_{29}H_{38}N_2O_6$	59	194–195	7.65 ± 0.07[c] (8)	4.68 ± 0.02 (4)	933
14a		H	$C_{27}H_{34}N_2O_6$	70	224–225	7.34 ± 0.05[e] (6)	5.62 ± 0.09 (4)	52
14b		$CH(CH_3)_2$	$C_{30}H_{40}N_2O_6$	63	213–214	7.14 ± 0.05[c] (10)	4.59 ± 0.06 (4)	355
15a		H	$C_{28}H_{36}N_2O_6$	71	216–218	7.10 ± 0.08[e] (6)	4.86 ± 0.09 (4)	174
15b		$CH(CH_3)_2$	$C_{31}H_{42}N_2O_6$	56	214–215	7.05 ± 0.10[c] (10)	4.78 ± 0.05 (4)	186

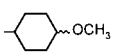
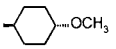
[a] All compounds exhibit ^{1}H NMR spectral data in agreement with assigned structures. All compounds were characterized and tested as their hydrogen maleate salts. All salts were crystallized from a THF/Et_2O mixture. Elemental analyses were within ±0.4% of the theoretical values for C, H, N. [b] Values are expressed as mean ± SEM from *n* individual vascular segments of at least two animals (number *n* of independent experiments in parentheses). [c] These values are apparent pA_2 values which were estimated according to ref 30. [d] 5-HT_{2A}/α_1 is the ratio of K_B values. [e] This value is the pK_P value which was estimated according to ref 28. [f] This value is the full pA_2 value which was estimated from the Schild plot according to ref 31. [g] Slope 0.92 ± 0.05 of the Schild plot. [h] Slope 0.84 ± 0.05 of the Schild plot, significantly different from unity ($P < 0.05$).

Cycloalkanecarboxylic Esters Derived from Lysergol as Partial Agonists at 5-HT_{2A} Receptors. Within the series of O-acylated lysergol derivatives, N-1-unsubstituted compounds (**6a**−**10a**) contracted rat tail arteries with pK_P values of 6.35−7.67 and intrinsic activities α of 0.11−0.21 with respect to 5-HT (Table 4). Partial agonist effects of the compounds were surmountably antagonized by ketanserin (3 nM). The similarity of blocking potency of ketanserin (pA_2 of 9.10−9.18) is consistent with an interaction of the compounds with the same receptor class (5-HT_{2A}) (Table 4). Compounds **6a**−**10a** are therefore further ergolines with 5-HT_{2A} receptor agonist activity. In agreement with the affinities obtained from antagonist experiments (see above), agonist activity was most potent for cyclopropanecarboxylic acid ester **6a** ($pK_P = 7.67$) and continuously decreased in this series of homologues to reveal the lowest activity for cycloheptanecarboxylic acid ester **10a** ($pK_P = 6.35$). Especially compounds **6a**, **7a**, and **8a** were more potent than 5-HT and lysergol (**1**), respectively (see relative potencies of Table 4). On the other hand, O-acylated dihydrolysergol-I derivatives produced only marginal contractile responses in rat tail artery (α of 0.02−0.05). Therefore pK_P values for the agonist effects of these compounds were not calculated. Compound **11a** was the only outlier in this series. The compound showed appreciable agonist potency and intrinsic activity ($pK_P = 6.10$, $\alpha = 0.35$), but the contractile effect was resistant to blockade by ketanserin (3 nM). It should be mentioned that there was no compound within the series of O-acylated elymoclavine derivatives that produced any measurable contractile effect by itself at vascular 5-HT_{2A} receptors. Furthermore, when O-acylated derivatives of lysergol and dihydrolysergol-I had an *N*-1-isopropyl substituent, no agonist activity was observed. The finding that partial 5-HT_{2A} receptor agonism of the ergolines examined is restricted to N-1-unsubstituted compounds with a double bond in the 9,10-position is consistent with previously reported findings on several ergolines which show the same structural pattern. The most prominent representative in this connection is LSD which has been reported to act as a partial agonist at 5-HT_{2A} receptors of rabbit aorta ($\alpha = 0.14$) and calf coronary artery ($\alpha \approx 0.2$).[34,35] In addition LSD has been reported to act as a partial agonist ($\alpha = 0.25$) at 5-HT_{2A} receptors coupled to the phosphatidylinositol (PI) second-messenger system.[36] Further ergoline-based compounds with partial agonist activity at 5-HT_{2A} receptors are lisuride, and ergometrine.[37,38,4] Although it has been found that N-1-unsubstituted ergolines such as LSD, lisuride and ergometrine display selectivity for human versus rat 5-HT_{2A} receptors (see above) and thus are reliable probes for unmasking species differences,[12,13,39] it should be emphasized that these ergolines have a different quality of action (partial agonism) compared to ergolines for which silent 5-HT_{2A} receptor antagonism can be demonstrated.

N-1-Substituted Clavines as Antagonists at 5-HT_{2A} Receptors. The observation that ergoline "re-

Table 3. Physical Properties and Pharmacological Effects of Cycloalkanecarboxylic Esters Derived from Elymoclavine on 5-HT-Induced Contractions of Rat Tail Artery and (*R*)-Phenylephrine-Induced Contractions of Rat Aorta



compd	R^1	R^2	molecular formula[a]	% yield	mp, °C	5-HT_{2A} receptor affinity[b,c]	α_1-adrenoceptor affinity[b,c]	specificity 5-HT_{2A}/α_1[d]
16a		H	$C_{25}H_{28}N_2O_6$	60	136–137	7.11 ± 0.06 (4)	6.42 ± 0.02 (4)	5
16b		$CH(CH_3)_2$	$C_{24}H_{31}ClN_2O_2$	55	117–118	8.03 ± 0.05 (14)	4.69 ± 0.12 (4)	2181
17a		H	$C_{26}H_{30}N_2O_6$	69	164–165	7.31 ± 0.11 (14)	5.99 ± 0.01 (4)	21
17b		$CH(CH_3)_2$	$C_{25}H_{33}ClN_2O_2$	70	120–121	8.05 ± 0.04 (15)	4.89 ± 0.05 (5)	1445
18a		H	$C_{27}H_{32}N_2O_6$	63	158–159	7.40 ± 0.15 (4)	5.89 ± 0.13 (4)	32
18b		$CH(CH_3)_2$	$C_{30}H_{38}N_2O_6$	62	165–166	7.80 ± 0.05 (14)	4.65 ± 0.06 (4)	1413
19a		H	$C_{28}H_{34}N_2O_7$	29	94 – 96	7.32 ± 0.07 (4)	6.32 ± 0.07 (4)	12
19b		$CH(CH_3)_2$	$C_{31}H_{40}N_2O_7$	53	147–149	7.94 ± 0.07 (15)	4.84 ± 0.05 (5)	1259
20a		H	$C_{28}H_{34}N_2O_7$	22	104–105	7.24 ± 0.02 (4)	6.00 ± 0.06 (4)	17
20b		$CH(CH_3)_2$	$C_{31}H_{40}N_2O_7$	52	152–154	8.13 ± 0.06 (12)	4.67 ± 0.03 (4)	2884
21a		H	$C_{28}H_{34}N_2O_6$	37	149–151	7.97 ± 0.08 (4)	5.05 ±0.17 (4)	832
21b		$CH(CH_3)_2$	$C_{31}H_{40}N_2O_6$	32	186–187	7.77± 0.06 (10)	<4.5 (4)	>1862

[a] All compounds exhibit ^1H NMR spectral data in agreement with assigned structures. All compounds were characterized and tested as their hydrogen maleate salts with the exception of compounds **16b** and **17b** which were characterized and tested as their hydrochloride salts. All salts with the exception of compounds **16b** and **17b** were crystallized from a THF/Et_2O mixture. Compounds **16b** and **17b** were crystallized from a MeOH/Et_2O mixture. The 4-methoxy-substituted cyclohexyl ring of **19a**,**b** exists as cis/trans diastereomeric mixture (1:3). Elemental analyses were within ±0.4% of the theoretical values for C, H, N. [b] Values are expressed as mean ± SEM from *n* individual vascular segments of at least two animals (number *n* of independent experiments in parentheses). [c] These values are apparent pA_2 values which were estimated according to ref 30. [d] 5-HT_{2A}/α_1 is the ratio of K_B values.

Table 4. Cycloalkanecarboxylic Esters Derived from Lysergol as Agonists at 5-HT_{2A} Receptors of Rat Tail Artery

	agonism[a]			Ketanserin antagonism[b]
compd	α ± SEM	rel pot (95% c.l.)	pK_P[c] ± SEM	pA_2[d] ± SEM
5-HT	1	100		9.55 ± 0.03[e]
1a[f]	0.18 ± 0.03	74 (52–105)	6.88 ± 0.07	9.10 ± 0.10
6a	0.21 ± 0.02	331 (245–447)	7.67 ± 0.05	9.13 ± 0.11
7a	0.13 ± 0.02	185 (126–272)	7.36 ± 0.06	9.13 ± 0.04
8a	0.11 ± 0.02	128 (98–167)	7.27 ± 0.07	9.13 ± 0.07
9a	0.18 ± 0.02	49 (32–75)	6.92 ± 0.06	9.18 ± 0.06
10a	0.18 ± 0.02	14 (9–21)	6.35 ± 0.09	9.11 ± 0.06

[a] Number *n* of independent experiments was 11−12. [b] The concentration of ketanserin was 3−10 nM ($n = 5-6$). [c] pK_P values were estimated according to ref 27. [d] Apparent pA_2 values were estimated according to ref 30. [e] Full pA_2 value from the Schild plot according to ref 31. [f] Data are from ref 33.

verse esters" with a small alicyclic ring in the acyl portion and an isopropyl substituent at the indole nitrogen were potent 5-HT_{2A} receptor antagonists led us to examine to what extent the complete removal of the acyl portion would affect 5-HT_{2A} receptor affinity. Most surprisingly, the parent alcohols of the isopropyl homologues (1-isopropyllysergol (**1b**), 1-isopropyldihydrolysergol-I (**2b**), and 1-isopropylelymoclavine (**3b**)) showed higher affinity for 5-HT_{2A} receptors of rat tail artery than the corresponding esters (Table 5). Compounds **1b**, **2b**, and **3b** produced potent antagonism of the effects of 5-HT with pA_2 values of 9.15 (Figure 1B), 8.50, and 9.14, respectively. In contrast, **1b**, **2b**, and **3b** showed lower affinity for vascular 5-HT_{1B} receptors and vascular α_1-adrenoceptors (Table 5). It is worth mentioning that **1b**, **2b**, and **3b** had no contractile effects by themselves at 5-HT_{2A} receptors, 5-HT_{1B} receptors, and α_1-adrenoceptors. The high affinity for rat 5-HT_{2A} receptors was retained when analogues even more simple in structure than **1b**, **2b**, and **3b** were examined as antagonists of 5-HT-induced contractions of rat tail artery. Simple clavines such as compounds **4b** and **5b**, in which a methyl group instead of a hydroxymethyl group is attached to C-8, produced competitive antagonism of the effects of 5-HT with pA_2 values of 8.84 and 8.50, respectively. Compared to the high antagonist activity of **4b** and **5b** at 5-HT_{2A} receptors, antagonist activity of the compounds at α_1-adrenoceptors of rat aorta was low (Table 5).

Perhaps most informative is the comparison between the antagonist activities of the N-1-alkylated esters and their corresponding alcohols **1b**−**3b**. O-Acylated derivatives of lysergol, dihydrolysergol-I, and elymoclavine which are characterized by an isopropyl substituent at N-1 have 2.5−25-, 2−28-, and 10−23-fold decreased

Table 5. Physical Properties and Pharmacological Effects of N-1-Substituted Clavine Alkaloids

compd	molecular formula[a]	% yield	mp, °C	5-HT_{2A} receptor $pA_2 \pm SEM$[b]	5-HT_{1B} receptor $pA_2 \pm SEM$[c]	α_1-adrenoceptor $pA_2 \pm SEM$[c]	Specificity 5-HT_{2A}/5-HT_{1B}[d]	Specificity 5-HT_{2A}/α_1[d]
1b	$C_{23}H_{28}N_2O_5$	38	204–205	9.15 ± 0.04[e] (25)	6.68 ± 0.06 (5)	6.05 ± 0.09 (4)	295	1259
2b	$C_{23}H_{30}N_2O_5$	40	185–186	8.50 ± 0.04[f] (26)	5.73 ± 0.11 (5)	6.12 ± 0.10 (4)	589	240
3b	$C_{23}H_{28}N_2O_5$	55	162–164	9.14 ± 0.11[g] (24)	6.07 ± 0.04 (5)	5.69 ± 0.07 (4)	1175	2818
4b	$C_{23}H_{28}N_2O_4$	75	152–153	8.84 ± 0.07[h] (14)	n. d.	6.34 ± 0.05 (6)	n. d.	316
5b	$C_{23}H_{30}N_2O_4$	70	222–223	8.50 ± 0.06[i] (12)	n. d.	6.70 ± 0.03 (4)	n.d.	63

[a] All compounds exhibit ^1H NMR spectral data in agreement with assigned structures. All compounds were characterized and tested as their hydrogen maleate salts. Salt **1b** was hydrated with 0.5 mol of water. All salts were crystallized from a THF/Et_2O mixture. Elemental analyses were within ±0.4% of the theoretical values for C, H, N. [b] Full pA_2 values were estimated from the Schild plot according to ref 31. [c] Apparent pA_2 values were estimated according to ref 30 (concentration of antagonists 3−30 μM). [d] 5-HT_{2A}/5-HT_{1B} and 5-HT_{2A}/α_1 are the ratios of K_B values. [e] Slope 1.05 ± 0.03 of the Schild plot. [f] Slope 1.03 ± 0.03 of the Schild plot. [g] Slope 0.89 ± 0.04 of the Schild plot, significantly different from unity ($P < 0.02$). [h] Slope 1.06 ± 0.08 of the Schild plot. [i] Slope 1.00 ± 0.07 of the Schild plot. Number *n* of independent experiments in parentheses.

antagonist activity at 5-HT_{2A} receptors compared to the corresponding parent alcohols **1b**−**3b**. It could be shown that a number of variations at the 8-position may be tolerated without a major effect on antagonist potency. Moreover, the nanomolar antagonist activity of the parent drugs demonstrates that the derivatization of the C-8 substituent is not crucial for 5-HT_{2A} receptor affinity. The observation is consistent with the nanomolar antagonist activity of compounds **4b** and **5b** of which the pharmacophore is structurally reduced to the crude 8,9-didehydro-6,8-dimethylergoline or 6,8-dimethylergoline skeleton due to the lack of the −OH group at C-17 and the double bond in the D-ring, respectively. Thus, the indolo[4,3-*fg*]quinoline system of the ergolines is, in fact, the molecular fragment that is somehow ultimately responsible for 5-HT_{2A} receptor affinity, and not the substituent at position C-8. The present study of structure−activity relationships shows that the importance of the tetracyclic structure of the ergolines can be demonstrated by means of simple ergolines, of which the indolo[4,3-*fg*]quinoline system represents more or less the complete molecule. The crucial role of aromatic groups of many 5-HT receptor ligands including ergolines for 5-HT receptor interaction has recently been demonstrated by molecular modeling studies.[40−42] In addition, site-directed mutagenesis techniques have shown that within the 5-HT_{2A} receptor protein it is the highly conserved aromatic residue phenylalanine at position 340 which is essential for ergoline binding. It has been suggested that the phenyl moiety of phenylalanine may allow a specific aromatic interaction (e.g., π or hydrophobic) with the aromatic ring of the ergoline nucleus of compounds such as LY53857 and amesergide.[42]

In conclusion, the present findings demonstrate that cycloalkanecarboxylic esters derived from lysergol, dihydrolysergol-I, and elymoclavine exhibit complex behavior as partial agonists and antagonists at rat 5-HT_{2A} receptors. Those compounds that have smaller cycoalkyl rings in the acyl portion and an isopropyl substituent at the indole nitrogen emerged as silent 5-HT_{2A} receptor antagonists of high potency. Partial 5-HT_{2A} receptor agonist activity is restricted to N-1-unsubstituted derivatives of lysergol (double bond in the 9,10-position). The complete removal of the acyl portion in the series of *N*-1-isopropyl homologues yields compounds which are even more potent 5-HT_{2A} receptor antagonists than their corresponding esters and display low affinity at 5-HT_{1B} receptors and α_1-adrenoceptors. Therefore, it is suggested that the indolo[4,3-*fg*]quinoline system of the ergolines is the structural fragment that is decisive for 5-HT_{2A} receptor affinity, and not the substituent at position C-8.

Experimental Section

Chemistry. ^1H NMR spectra were recorded on a Bruker AC 300 or AC 400 spectrometer. Chemical shifts are given in ppm (δ) downfield from TMS. EIMS were obtained using a MAT-711 spectrometer operating at 70 eV. Elemental analyses (C, H, N) for novel compounds were determined with a Perkin-Elmer 240C instrument. Melting points were taken on a Büchi 530 melting point apparatus and are uncorrected. Chemical purifications on a preparative scale were performed by radial centrifugal chromatography with a Chromatotron 7924 (Harrison Research, Palo Alto, CA) using glass rotors with 1-, 2-, or 4-mm layers of silica gel 60 PF_{254} containing gypsum (Merck art. no. 7749) and appropriate eluents. The Chromatotron chamber was continuously purged with dry nitrogen and protected from light. All experiments were monitored by thin-layer chromatography (TLC) using aluminum sheets coated with a 0.2-mm layer of silica gel 60 F_{254} (Merck art. no. 5554) and appropriate eluents. Detection of compounds on TLC was additionally achieved with van Urk's reagent. All reactions were carried out in the dark under an inert atmosphere of dry nitrogen or argon using glassware that had been carefully cleaned and dried overnight in a 120 °C oven.

General Procedure for the Preparation of Cycloalkanecarboxylic Esters Derived from Lysergol, Dihydrolysergol-I, and Elymoclavine. 9,10-Didehydro-6-methylergolin-8β-ylmethyl Cyclohexanecarboxylate (9a). To a cooled (0 °C) and stirred solution of **1a** (0.75 g, 2.96 mmol) in dry pyridine (45 mL) was added dropwise, and in the presence of catalytic amounts of 4-DMAP, a solution of freshly distilled cyclohexanecarbonyl chloride (0.86 g, 5.85 mmol) in $CHCl_3$ (12 mL) over 160 min. After the addition was finished, the reaction mixture was allowed to stand at room temperature overnight. The resulting solution was evaporated to dryness and the residue partitioned between $CHCl_3$ and a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4 and the solvent removed under vacuum. Radial centrifugal chromatography (Chromatotron instrument, eluent CH_2Cl_2/cyclohexane/MeOH, 100/10/2 (v/v/v)) of the residue afforded a yellow oil. The hydrogen maleate salt of **9a** was precipitated

from THF/Et_2O to give a white powder: yield 0.96 g (68%); mp 218−219 °C dec; ^{1}H NMR (pyridine-d_5) δ 1.10−1.20 (m, 3 H), 1.48−1.62 (m, 5 H), 1.94 (m, 2 H), 2.37 (m, 1 H), 2.56 (quasi t, J = 12.0 Hz, 1 H, H-7β), 2.67 (s, 3 H, NCH_3), 3.00 (quasi t, J = 14.5 Hz, 1 H, H-4α), 3.38−3.45 (m, 2 H, H-8, H-7α), 3.49 (m, 1 H, H-5), 3.67 (dd, J = 14.5, 5.5 Hz, 1 H, H-4β), 4.29 (d, J = 6.0 Hz, 2 H, H-17β, H-17α), 6.60 (br s, 1 H, H-9), 6.66 (s, 2 H, maleate CH=CH), 7.25 (s, 1 H, H-2), 7.33 (t, J = 7.5 Hz, 1 H, H-13), 7.41 (d, J = 7.5 Hz, 1 H, H-12 or H-14), 7.44 (d, J = 8.0 Hz, 1 H, H-12 or H-14), 11.77 (br s, 1 H, NH); MS (m/z) 364 ($M^{+\bullet}$, 100). Anal. ($C_{27}H_{32}N_2O_6$) Calcd: C, 67.5; H, 6.7; N, 5.8; Found: C, 67.3; H, 6.8; N, 5.8.

General Procedure for the Preparation of Cycloalkanecarboxylic Esters Derived from Lysergol and Dihydrolysergol-I with an Isopropyl Substituent at N-1 (Method A). 9,10-Didehydro-1-isopropyl-6-methyl-8β-ergolinylmethyl Cyclohexanecarboxylate (9b). To a stirred solution of **9a**-base (0.465 g, 1.28 mmol) in dry THF (20 mL) were added 18-crown-6 (0.24 g, 0.91 mmol), powdered KOH (0.965 g, 17.2 mmol), and 2-iodopropane (1.52 mL, 15.2 mmol). The mixture was stirred for 1 h, diluted with CH_2Cl_2, and filtered to remove solids. After the filtrate was evaporated to dryness, the residue was partitioned between CH_2Cl_2 and a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4 and the solvent removed under vacuum. Radial centrifugal chromatography (Chromatotron instrument, eluent CH_2Cl_2/cyclohexane/MeOH, 100/20/2 (v/v/v)) of the residue afforded a yellow oil. The hydrogen maleate salt of **9b** was precipitated from THF/Et_2O to give a white powder: yield 0.43 g (65%); mp 190−191 °C dec; ^{1}H NMR (pyridine-d_5) δ 1.09−1.16 (m, 3 H), 1.36 (2 d, J = 6.5 Hz, 6 H), 1.48−1.62 (m, 5 H), 1.94 (m, 2 H), 2.38 (m, 1 H), 2.44 (quasi t, J = 10.0 Hz, 1 H, H-7β), 2.60 (s, 3 H, NCH_3), 2.89 (quasi t, J = 14.5 Hz, 1 H, H-4α), 3.20−3.27 (m, 2 H, H-8, H-7α), 3.32 (m, 1 H, H-5), 3.60 (dd, J = 14.5, 5.5 Hz, 1 H, H-4β), 4.28 (d, J = 6.0 Hz, 2 H, H-17β, H-17α), 4.53 (sept, J = 6.5 Hz, 1 H), 6.57 (br s, 1 H, H-9), 6.66 (s, 2 H, maleate CH=CH), 7.04 (s, 1 H, H-2), 7.31−7.41 (m, 3 H, H-12, H-13, H-14); MS (m/z) 406 ($M^{+\bullet}$, 100). Anal. ($C_{30}H_{38}N_2O_6$) Calcd: C, 68.9; H, 7.3; N, 5.4. Found: C, 69.1; H, 7.3; N, 5.3.

General Procedure for the Preparation of *N*-1-Isopropyl Derivatives of Simple Clavines (Method B). 1-Isopropylelymoclavine (3b). To a stirred solution of **3a** (1.27 g, 5 mmol) in DMSO (10 mL) were added 18-crown-6 (1.32 g, 5 mmol) and powdered KOH (1.72 g, 30.66 mmol). A solution of isopropyl tosylate (1.6 g, 7.5 mmol) in DMSO (5 mL) was added dropwise over 45 min. The mixture was stirred for further 60 min and then poured into a saturated solution of $NaHCO_3$. The solution was extracted with Et_2O (4 × 100 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed under vacuum. Radial centrifugal chromatography (Chromatotron instrument, eluent CH_2Cl_2/MeOH, 95/5 (v/v)) of the residue afforded a white solid. The hydrogen maleate salt of **3b** was precipitated from THF/Et_2O to give a white powder: yield 1.13 g (55%); mp 162−164 °C; ^{1}H NMR (pyridine-d_5) δ 1,37 (2 d, J = 6.5 Hz, 6 H), 2,91 (s, 3 H, NCH_3), 3.17 (dd, J = 12.5, 11.5 Hz, 1 H, H-4α), 3.24 (ddd, J = 11.5, 9.5, 3.0 Hz, 1 H, H-5), 3.52 (dd, J = 13,5, 3,0 Hz, 1 H, H-4β), 3.83 (d, J = 16.5 Hz, 1 H, H-7β), 4.13 (d, J = 16.5 Hz, 1 H, H-7α), 4.21 (br s, 1 H, H-10), 4.44 (s, 2 H, H-17), 4.55 (sept, J = 6.5 Hz, 1 H), 6.63 (s, 2 H, maleate CH=CH), 6.81 (br s, 1 H, H-9), 7.03 (s, 1 H, H-2), 7.15 (d, J = 7.0 Hz, 1 H, H-12 or H-14), 7.27−7.37 (m, 2 H, H-13 and H-12 or H-14); MS (m/z) 296 ($M^{+\bullet}$, 100). Anal. ($C_{23}H_{28}N_2O_5$) Calcd: C, 67.0; H, 6.8; N, 6.8. Found: C, 66.8; H, 6.8; N, 6.9.

Pharmacology. Functional 5-HT$_{2A}$ Receptor Assay (rat tail artery). Male Wistar rats (280−350 g) were killed by cervical dislocation. The ventral caudal artery was quickly dissected and cleared of adhering connective tissue. A stainless steel wire (diameter 0.3 mm) was inserted into the artery to rub off the endothelium. Up to 24 cylindrical segments of 4−5-mm length were prepared from each artery and horizontally suspended between two L-shaped stainless steel hooks (diameter 0.15 mm) gently inserted into the lumen for the recording of contractile responses.[43] Each preparation was mounted in a 20-mL organ bath containing modified Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and D-glucose 10. The solution was continuously gassed with 95% O_2/5% CO_2 and warmed to a constant temperature of 37 °C. Preparations were connected to a force displacement transducer attached to a TSE 4711 transducer coupler and a Siemens C 1016 compensograph for the continuous recording of isometric changes in tension. Resting force was adjusted to 5 mN at the beginning of each experiment. During an equilibration period of 120 min, preparations were stimulated once (after 60 min) with 5-HT (1 μM).

In experiments with agonists two cumulative concentration−effect curves were determined on each arterial segment at an interval of 60 min: the first curve for 5-HT and the second for the respective agonist in the absence or presence of ketanserin (3 nM). Ketanserin was incubated 30 min before the second curve. The shift to the right observed in the presence of ketanserin was calculated by comparing with the shift measured for the respective control preparation in the absence of ketanserin. In additional experiments with antagonists (partial agonists) two cumulative concentration effect curves for 5-HT were determined at an interval of 60 min in the absence and presence of antagonists (partial agonists). Antagonists (partial agonists) were incubated 30−60 min before the second curve. Prazosin (30 nM) and cocaine (6 μM) were present throughout the experiments to block α_1-adrenoceptors and neuronal uptake of 5-HT.

Partial agonists were characterized by the equilibrium dissociation constant K_P. In experiments where the compounds were studied as agonists, K_P was estimated according to the method of Kenakin.[27] K_P was estimated from the slope m of a plot which related equieffective concentrations of 5-HT and the partial agonist P according to the equation: c(5-HT) = m·c(5-HT)/c(P) + b, where b is the ordinate intercept. pK_P = −log K_P was calculated from −log K_P = log m. In experiments where the compounds were studied as antagonists of the effects of 5-HT, K_P was estimated according to the method of Marano and Kaumann.[28] K_P was estimated from the slope m of a weighted plot which related equieffective concentrations of 5-HT in the absence (c(5-HT)) and presence (c(5-HT)*) of the partial agonist P: c(5-HT) = m·c(5-HT)* + b, where b is the ordinate intercept. pK_P = −log K_P was calculated from log(1/m − 1) = log c(P) − log K_P. Kaumann−Marano plots were constructed in those cases where antagonist concentrations were used over 2 log units.[29] After log(1/m − 1) versus log c(P) was plotted, a regression line was constructed and the intercept on the log concentration axis provided the estimate of pK_P. For the calculation of pK_P values, the slope of the Kaumann−Marano plot was constrained to unity unless it was significantly different from unity (P < 0.05). Antagonist dissociation constants given as apparent pA_2 values were calculated from the equation: pA_2 = −log c(B) + log(CR − 1).[30] Antagonist dissociation constants given as full pA_2 values were estimated using the method of Arunlakshana and Schild.[31] Schild plots were constructed to estimate the pA_2 value and the slope m of the regression line from each experimental series, which generally comprised at least three different antagonist concentrations over 2 log units. For the calculation of pA_2 values from Schild plot, the slope was constrained to unity unless it was significantly different from unity (P < 0.05).[32]

Functional 5-HT$_{1B}$ Receptor Assay (guinea-pig iliac artery). Guinea pigs of either sex, 300−450 g, were stunned by a blow on the neck and bled. The abdominal aorta and the right and left common iliac arteries were removed and cleared of adhering connective tissue. Two or three cylindrical segments of 1−2-mm length from each iliac artery were horizontally suspended between two L-shaped stainless steel hooks (diameter 0.15 mm) and isometrically mounted as described for rat tail artery experiments (see above). The bath fluid (modified Krebs-Henseleit solution with $CaCl_2$ (1.25 mM) and D-glucose (11.5 mM)) contained ketanserin (1 μM), mepyramine

(0.3 μM), cimetidine (30 μM), and cocaine (30 μM) to block 5-HT_{2A} receptors, α_1-adrenoceptors, histamine H_1 receptors, histamine H_2 receptors, and neuronal uptake of 5-HT. The applied resting force was 5 mN. During an equilibration period of 4.5 h, the organs were stimulated after 100 min with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 30 μM). Relaxation was achieved by subsequent addition of carbachol (10 μM). After 175 min the organs were moderately precontracted with an EC_{10}−EC_{20} (50−500 nM) of $PGF_{2\alpha}$ and subsequently stimulated with 5-HT (0.3 μM). Two cumulative concentration−effect curves for 5-HT were determined at an interval of 80 min in the absence and presence of **1b**−**3b**, on organs precontracted with an EC_{10}−EC_{20} of $PGF_{2\alpha}$ as above. Compounds **1b-3b** were incubated for 45 min.

Functional α_1-Adrenoceptor Assay (rat aorta). The thoracic aorta was removed from rats used for studies at 5-HT_{2A} receptors in rat tail artery (see above). When cleared of connective tissue the aorta was cut into 6−12 rings of 4−6-mm length. Each cylindrical segment was rolled with a pair of tweezers to destroy the endothelium. The segments were horizontally suspended between two L-shaped stainless steel holders (diameter 0.3 mm).[44] The organs were isometrically mounted as described for rat tail artery experiments (see above). The bath fluid (modified Krebs-Henseleit solution of the above composition at 37 °C, gassed with 95% O_2/5% CO_2) contained (*R,S*)-propranolol (1 μM) to block β-adrenoceptors. The applied resting force was 20 mN. During an equilibration period of 140 min the organs were stimulated twice with (*R*)-phenylephrine (100 nM). Two cumulative concentration−response curves for the contractile effect of (*R*)-phenylephrine were determined in the absence and presence of antagonist. Antagonists were incubated 30 min before the second curve.

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