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Dopamine Receptor Ligands. Part VII [1]: Novel 3-Substituted 5-Phenyl-1,2,3,4,5,6-hexahydro-azepino-[4,5-*b*]indoles as Ligands for the Dopamine Receptors

A number of 5-phenyl-1,2,3,4,5,6-hexahydro-azepino-[4,5-*b*]indoles 3 were synthesized with different substituents at the azepine-N position (methyl-, allyl-, 2-phenylethyl-, cyclopropylmethyl- and unsubstituted). Furthermore, the indole-N-methylated compound was generated and by using norephedrines and norpseudoephedrines as a chiral pool, 4-methyl-5-phenyl-1,2,3,4,5,6-hexahydro-azepino-[4,5-*b*]indoles were prepared which contained racemisation at the reacting C-atom. These compounds, as well as the ring-open amino-alcohols, were screened for their affinity to the hD_1-, hD_5-, hD_{2L}-, and hD_4-receptors (ş please check sentence). They had micromolar affinities for the receptors and showed the highest affinity to the D_1-subtype family. The cyclic compounds possessed the highest affinity, with the cyclopropylmethyl-(**3 c**) and methyl-substituents (**3 e**) being the most active of the tested compounds. Based on an intracellular cAMP-assay, the unsubstituted compound (at the azepine-N position) turned out to be an agonist for the D_1- and D_5-subtype family, whereas the substituted compounds showed (partial) agonistic, or even inverse agonistic activity.

Keywords: Indolo-azepines; Dopamine receptors; Chiral pool; Inverse agonists

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Introduction

A number of compounds are known to interact selectively with the hD_1-receptor including the well known hD_1-selective antagonist SCH 23390 **2 a** [2], the agonist SKF 38393 **2 b** [3] and the indoloazezine LE 300 **1**, a new lead structure containing both a tryptamine unit and a β-phenylethylamine structure [4, 5] (ş please check sentence). We were interested in synthesizing corresponding indoloazepines **3 a–i** with the goal of generating a new class of antipsychotics selective for the hD_1-receptor family but which lack the extrapyramidal side effects of classical antipsychotic drugs [6] (Figure 1).

The stereochemistry of a compound determines whether it will have a high affinity interaction with members of the dopamine receptor family. We wanted to develop a synthesis for dopamine receptor ligands, in which we could make use of the chiral pool of norephedrines **4** and ephedrines **5** (Scheme 1; the [1*R*, 2*S*]-enantiomers are shown) as synthones (ş please check sentence).

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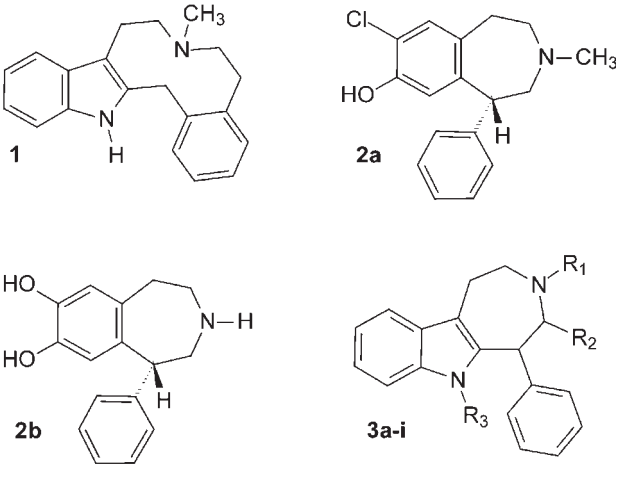
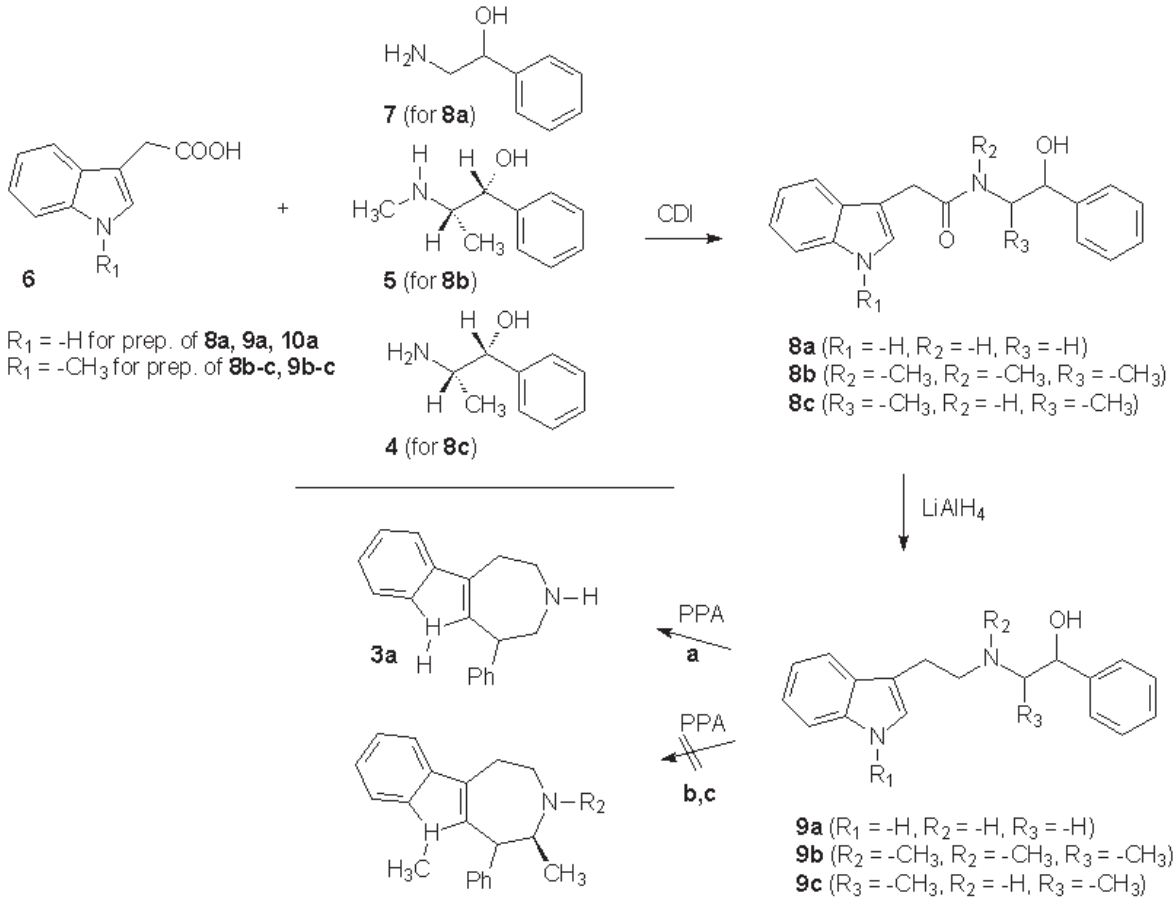


Figure 1. Lead structures (LE 300 **1**, SCH 23390 **2 a**, SKF 38393 **2 b**) and novel 5-phenyl-1,2,3,4,5,6-hexahydro-[4,5-*b*]indoles **3 a–i**.

Results

Chemistry

In order to use norephedrines as synthones, we made use of a modified synthesis procedure described by



Scheme 1. Synthesis of compounds **8a–c**, **9a–c** and **3a**.

Elliot et al. [7]. Amides (**8a–c**) were prepared by first activating 1*H*-indol-3-ylacetic acid with *N, N′*-carbonyldiimidazole and subsequently reacting it with 2-amino-1-phenyl-ethanol **7** or norephedrines **4** and ephedrines **5**, respectively. The amides were then reduced to the corresponding amines (**9a–c**) using lithium aluminium hydride. Subsequent cyclisation of the compounds to azepines was performed with polyphosphoric acid (PPA) (Scheme 1). The indoloazepines were directly alkylated to tertiary amines with the appropriate alkyl bromides using potassium carbonate in dimethylformamide (DMF) as described for the benzoazepines [8] (Scheme 2). Methylation at the azepine-*N* atom was achieved by reaction with ethyl chloroformate followed by reduction with lithium aluminium hydride [6]. Methylation at both nitrogen atoms was achieved by the use of methyl iodide with sodium hydride in tetrahydrofuran (THF) (Scheme 2). The use of ephedrine and norephedrine allowed the syntheses of a variety of ring-open compounds. By using (1-methyl-1*H*-indol-3-yl)acetic acid, prepared by a Fischer-indole reaction [9], the corresponding indole-*N*-methylated compounds could be prepared (see Table 2).

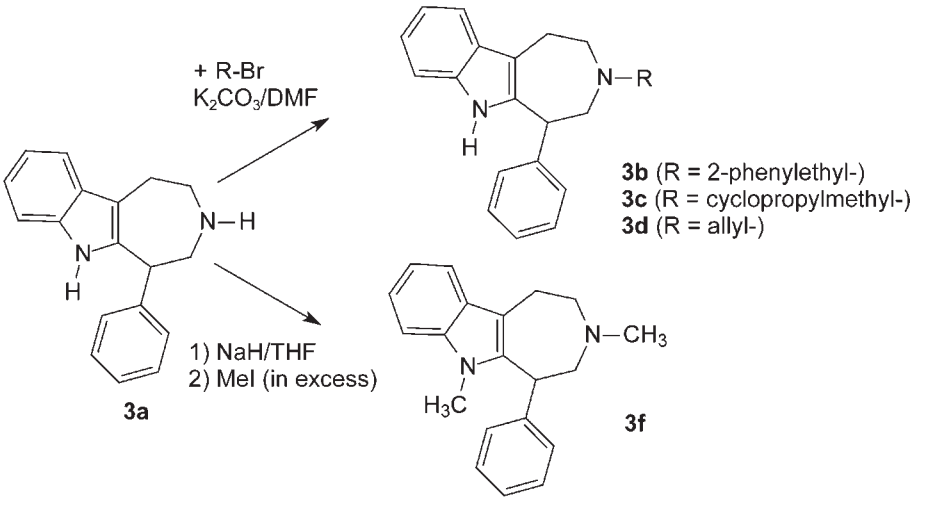
As previously described [10], racemisation occurs during cyclisation to the seven-membered ring at the chiral centre which contains a hydroxy-group (Scheme 3). Optical purity during the course of the reaction, and final racemisation, which resulted in the formation of diastereomers (**3g–i**), was determined using capillary electrophoresis (NMR spectra at 300 MHz were identical) with heptakis(2,3-*O*-diacetyl-6-sulfato)b-cyclodextrin as a chiral selector [10, 11]. The chemical and analytical data for the respective compounds has been previously described as were the detailed conditions for analytical separation using CE [10, 11].

Pharmacology

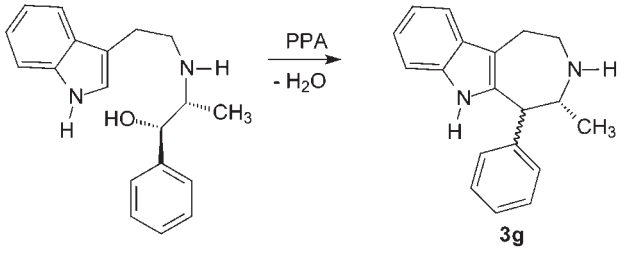
The compounds were tested in radioligand binding studies using Chinese hamster ovary (CHO)-cells stably expressing hD_1-, hD_{2L}-, hD_4- and hD_5-receptors. In an initial screen, the decrease in receptor-bound radioactivity, by a 10 µM solution of the test compound, was measured. When the decrease was greater then 70 %, the K_i-value

Table 1. Specifications of indoloazepines **3 a–i** and their radioligand binding results (see Figure 1 for the basic structure). A100 % decrease in radioactive binding was reached by using fluphenazine (10 μM) for the D_1 and D_5 receptors and haloperidol (1 μM) for the D_2 and D_4 receptors.

Compound	R_1	R_2	R_3	Decrease in receptor-bound radioactivity by a 10 μM solution	K_i-value ± SEM
3 a	H	H	H	**D_1: –82** **D_{2L}: –33** **D_4: –31** **D_5: –59**	**2112 ± 61 nM**
3 b	2-Phenylethyl-	H	H	**D_1: –73** **D_{2L}: –23** **D_4: –24** **D_5: –19**	**3901 ± 27 nM**
3 c	Cyclopropylmethyl-	H	H	**D_1: –99** **D_{2L}: –76** **D_4: –56** **D_5: –85**	**214 ± 19 nM** **1572 ± 26 nM** **1424 ± 52 nM**
3 d	Allyl-	H	H	**D_1: –75** **D_{2L}: –36** **D_4: –51** **D_5: –41**	**2781 ± 43 nM**
3 e	CH_3	H	H	**D_1: –95** **D_{2L}: –59** **D_4: –56** **D_5: –72**	**750 ± 34 nM** **2413 ± 31 nM**
3 f	CH_3	H	CH_3	**D_1: –66** **D_{2L}: –35** **D_4: –19** **D_5: –48**	
3 g	H	CH_3 (bound to *R*-configurated *C*-atom)	H	**D_1: –54** **D_{2L}: –39** **D_4: –22** **D_5: –46**	
3 h	H	CH_3 (bound to *S*-configurated *C*-atom)	H	**D_1: –45** **D_{2L}: –4** **D_4: –13** **D_5: –22**	
3 i	CH_3	CH_3 (bound to *R*-configurated *C*-atom)	H	**D_1: –66** **D_{2L}: –5** **D_4: –7** **D_5: –69**	



Scheme 2. Synthesis of compounds **3b–d** and **3f**.



Scheme 3. Synthesis and partial racemisation of norephedrinoid indoloazepines [10].

was also determined. The results are summarized in Tables 1 and 2. Curves for the most potent compounds at the hD_1 receptor are shown in Figure 2.

Three of the cyclic compounds were also tested in a cellular assay, in which the change in cAMP formation was measured after incubation with the test compounds. Specifically, Human embryonic kidney (HEK)-cells expressing the hD_1, hD_{2L} and hD_5 receptors were preincubated with forskolin, which unspecifically stimulates adenylate cyclase. Preincubation was necessary to produce adequate levels of cAMP for detection. Cells that expressed the hD_1 and hD_5 receptors, could then be

Table 2. Radioligand binding results for ring–open compounds **9a–e** (preparation and properties of **9d** and **e** have been previously described [10]). A 100 % decrease in radioactive binding was reached by using fluphenazine (10 µM) for the hD_1 and hD_5 receptors and haloperidol (1 µM) for the hD_2 and hD_4 receptors (ş please check sentence).

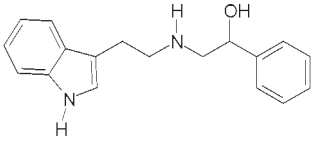
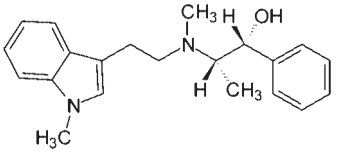
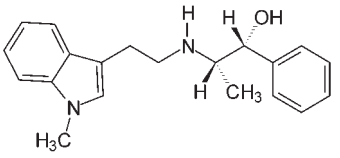
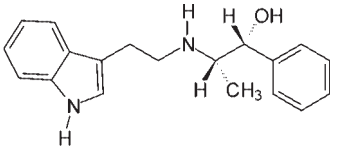
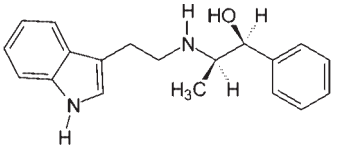
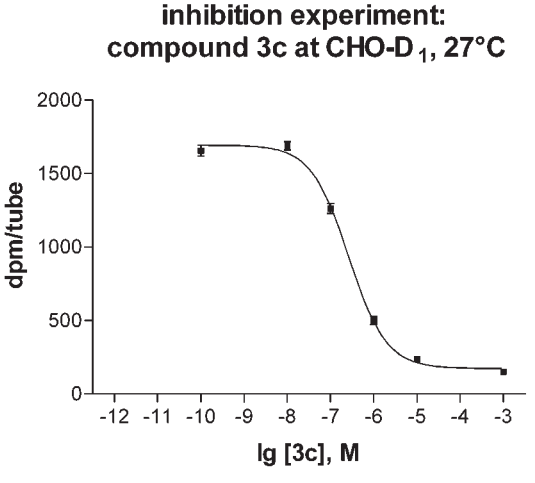
Starting materials	Compound	Decrease in receptor-bound radioactivity by a 10 µM solution
2-Amino-1-phenyl-ethanol and 1*H*-indol-3-ylacetic acid	 **9a**	**D_1: –45** **D_{2L}: –26** **D_4: –27** **D_5: –22**
(1*R*, 2*S*)-Ephedrine and (1-methyl-1*H*-indol-3-yl)acetic acid	 **9b**	**D_1: –55** **D_{2L}: –51** **D_4: –27** **D_5: –42**

Table 2. (continued).

Starting materials	Compound	Decrease in receptor-bound radioactivity by a 10 µM solution
(1*R*, 2*S*)-Norephedrine and (1-methyl-1*H*-indol-3-yl)acetic acid	 **9c**	**D_1: –69** **D_{2L}: –48** **D_4: –71°** **D_5: –46**
(1*R*, 2*S*)-Norephedrine and 1*H*-indol-3-ylacetic acid	 **9d**	**D_1: –42** **D_{2L}: –23** **D_4: –31** **D_5: –23**
(1*S*, 2*R*)-Norephedrine and 1*H*-indol-3-ylacetic acid	 **9e**	**D_1: –22** **D_{2L}: –54** **D_4: –23** **D_5: –9**

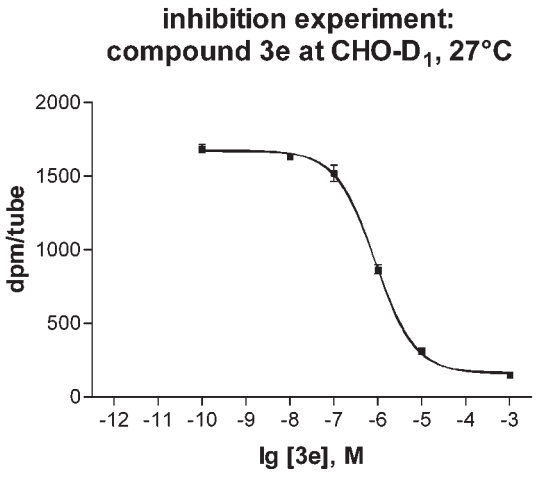
° (K_I = 492 ± 21 nM)



lg [3c], M	dpm		
	Y1	Y2	Y3
-10	1623	1732	1611
-8	1697	1734	1636
-7	1252	1326	1209
-6	553	459	483
-5	228	231	246
-3	151	176	127

Best-fit values	
BOTTOM	173.9
TOP	1694
LOGEC50	-6.576
EC50	2.6560e-007
KI	2.2320e-007
Ligand (Constant)	0.11
Kd (Constant)	0.58
Std. Error	
BOTTOM	21.95
TOP	22.90
LOGEC50	0.04476
95% Confidence Intervals	
BOTTOM	127.1 to 220.6
TOP	1645 to 1743
LOGEC50	-6.671 to -6.480
EC50	2.1320e-007 to 3.3080e-007
KI	1.7920e-007 to 2.7810e-007
Goodness of Fit	
Degrees of Freedom	15
R^2	0.9943
Absolute Sum of Squares	42700
Sy.x	53.35
Data	
Number of X values	6
Number of Y replicates	3
Total number of values	18
Number of missing values	0

Figure 2a.

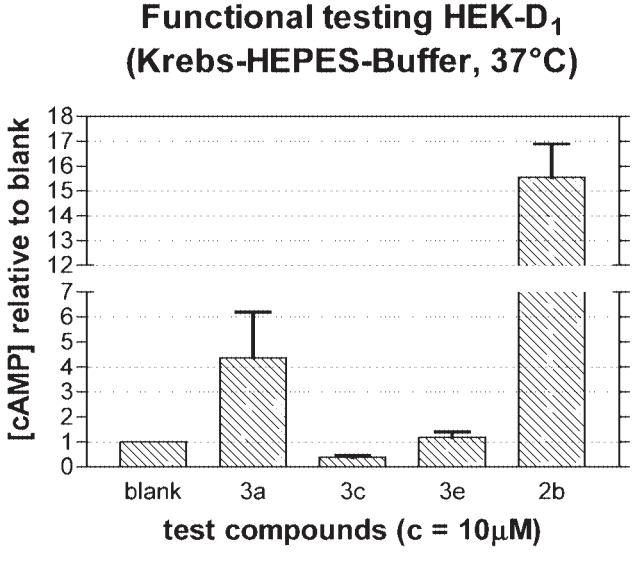


lg [3e], M	dpm		
	Y1	Y2	Y3
-10	1697	1734	1636
-8	1616	1643	1631
-7	1410	1595	1556
-6	868	814	920
-5	272	336	326
-3	151	176	126

Best-fit values	
BOTTOM	166.4
TOP	1669
LOGEC50	-6.050
EC50	8.9060e-007
KI	7.4860e-007
Ligand (Constant)	0.11
Kd (Constant)	0.58
Std. Error	
BOTTOM	23.68
TOP	19.00
LOGEC50	0.04001
95% Confidence Intervals	
BOTTOM	116.0 to 216.9
TOP	1628 to 1709
LOGEC50	-6.136 to -5.965
EC50	7.3180e-007 to 1.0840e-006
KI	6.1510e-007 to 9.1100e-007
Goodness of Fit	
Degrees of Freedom	15
R^2	0.9945
Absolute Sum of Squares	38710
Sy.x	50.80
Data	
Number of X values	6
Number of Y replicates	3
Total number of values	18
Number of missing values	0

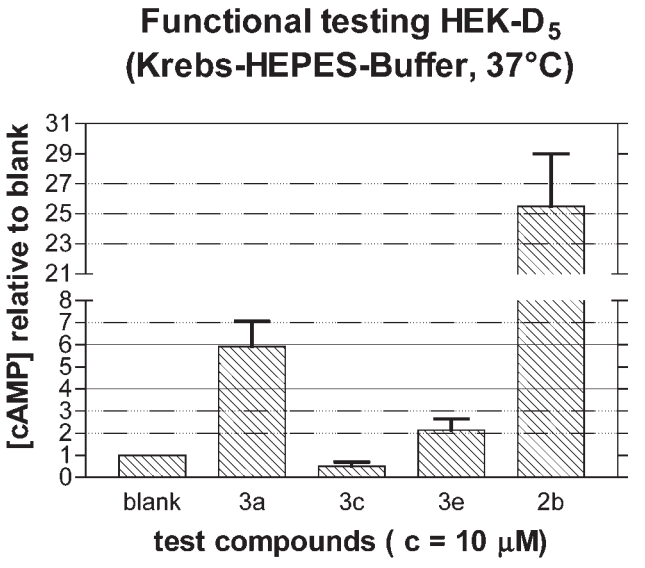
Figure 2b.

Figure 2. Heterologous competition experiments at the hD_1 receptor using [^{3}H]-SCH23390 and compounds **3 c** and **3 e**. Shown is a representative example of one of the three experiments performed.



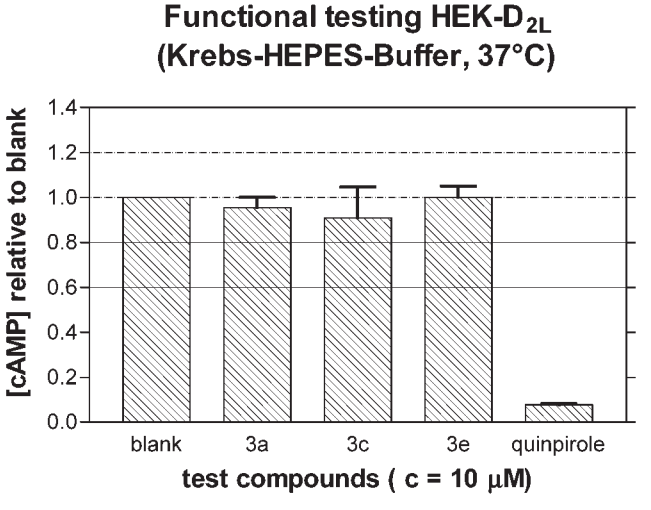
Compound	normalized [cAMP]	
	Y1	Y2
blank	1.000	1.000
3a	2.526	6.190
3c	0.280	0.472
3e	0.952	1.415
2b	16.902	14.280

Figure 3. Influence of test compounds **3 a**, **c**, **e** and the hD_1 receptor agonist SKF 38393 (**2 b**) on intracellular cAMP formation in HEK-D_1 cells (G_s coupled).



Compound	normalized [cAMP]	
	Y1	Y2
blank	1.000	1.000
3a	4.790	7.056
3c	0.680	0.344
3e	1.632	2.647
2b	22.096	27.830

Figure 4. Influence of the test compounds **3 a**, **c**, **e** and the hD_1 receptor agonist SKF 38393 (**2 b**) on intracellular cAMP formation in HEK-D_5 cells (G_s coupled).



Compound	normalized [cAMP]	
	Y1	Y2
blank	1.000	1.000
3a	0.910	1.123
3c	0.774	1.046
3e	0.950	1.002
quinpirole	0.082	0.074

Figure 5. Influence of the test compounds **3 a**, **c**, **e** and the hD_2 receptor agonist quinpirole on intracellular cAMP formation in HEK-D_{2L} cells (G_i coupled).

stimulated with agonists (Figures 3 and 4), and dose-response curves generated. An increase in cAMP formation indicates that the test compound possesses intrinsic affinity and acts therefore as an agonist (G_s coupling). With the D_{2L} receptor, which is G_i coupled, an agonist lowers cAMP formation. The results are summarized in Figures 3, 4 and 5.

Discussion

While structure-activity relationships cannot be deduced from the initial screen, general trends may become apparent from the data. Both ring-open as well as cyclic compounds showed affinity in the micromolar range to both dopamine receptor subtype families. The

cyclic compounds showed the highest affinity for the receptors and also appeared to have greater selectivity for the D_1-receptor family (**3 a**–**i**; **9 a**–**e**). A methyl-group at the azepine-*N* atom (**3 e**, Figure 2) resulted in a compound with higher affinity to the hD_1 receptor compared to the *N-H* compound (**3 a**). With the exception of the cyclopropylmethyl-group (**3 c**, Figure 2), sterically larger substituents gave lower affinities (**3 b**, **d**). **3 c** was the most potent of the tested compounds. A second methyl-group at the indole-*N* seemed to decrease the corresponding affinities, especially for the cyclic compounds (**3 f**). This also seemed to be the case for a methyl-group (originating from an ephedrine or norephedrine) bound to the aliphatic part of the molecules (**3 g**–**i**).

From the results of the cAMP testing at the hD_1 and hD_5 receptors (Figures 3 and 4), it could be shown that only the *N-H* compound (**3 a**) is an agonist, that stimulates adenylate cyclase *via* the corresponding receptor. This has already been shown for the benzo-azepines (**2 a** and **2 b**, the latter showed a stronger signal in our experiments due to its much higher affinity for the receptors) [2, 3]. The *N*-methyl compound (**3 e**) also showed some intrinsic activity at the hD_5 receptor. However, considering the fact that it generally showed a higher affinity at the dopamine receptor and there was only a moderate increase in cAMP formation, the compound seems to be a partial agonist with fairly small agonistic activity.

Another remarkable feature is the moderate decreases of cAMP formation (apart from compound **3 e** at the hD_1 receptor, all of the values determined are statistically significantly different from the blank) during incubation with the cyclopropylmethyl-compound **3 c**, which was also observed with many "antagonistic" compounds not described in this paper, including LE 300 [1]. Furthermore, this observation is consistent with recent findings by M. W. Martin et al., which showed that typical antipsychotics (including fluphenazine and thioridazine) showed inverse agonist activity at the rat D_1-like receptor [12]. For the hD_{2L} receptor, the standard agonist quinpirole lowered cAMP formation as expected, but none of the test compounds altered its formation at the concentrations tested (Figure 5).

In summary, cyclic compounds with micromolar affinities were synthesized (**3 a**–**e**), that showed some selectivity for the hD_1 receptor (**3 c** had ~eight times higher affinity to hD_1 than to hD_{2L} or hD_5). Interestingly, in contrast to the azepine *N-H* compound (**3 a**) which is an agonist, the data suggests that compounds **3 c** and **3 e** are a partial and an inverse agonist, respectively.

Experimental

Chemistry

Melting points were determined on a "Melting Point Apparatus" by Gallenkamp in open capillary tubes and are uncorrected. Elemental analyses were carried out on "Vario EL" by Elementar. ^{1}H-NMR spectral data were obtained on a "Varian XL 300" (300 MHz). Unless otherwise stated, the solvent was d_6-DMSO. IR-data were measured with a "1420" by Perkin Elmer.

General procedure for synthesis of 3-alkyl-5-phenyl-1,2,3,4,5,6-hexahydroazepino-[4,5-b]indoles **3 b**–**d**

5-Phenyl-1,2,3,4,5,6-hexahydroazepino-[4,5-*b*]indole (**3 a**, 2.3 mmol, 0.6 g) and anhydrous potassium carbonate (2.5 mmol, 0.35 g) were dissolved or suspended, respectively, in a mixture of 6 mL of DMF and 0.25 mL of water. A solution of 2.5 mmol of the appropriate alkylbromide in 3 mL of dichloromethane was added over a 30 min period and the mixture was stirring under nitrogen over night. The solution was poured into 50 mL of water, the organic phase separated, the aqueous phase extracted twice with 10 mL of dichloromethane, and the combined organic phases washed with water and brine. It was dried over anhydrous calcium chloride and the solvent removed under reduced pressure. The crude product was purified by column chromatography (200 g SiO_2^{60}, CH_2Cl_2/MeOH/NH_3conc. = 85/14/1).

3-Phenylethyl-5-phenyl-1,2,3,4,5,6-hexahydro-[4,5-b]indole **3 b**

Yield: 0.67 g (76 %), yellow crystals, mp 121 °C; ^{1}H-NMR: 2.6–3.19 (m, 10 H, aliphat. -CH_2-), 4.3 (s, 1 H, -CH-), 6.9–7.4 (m, 14 H, aromatic H), 10.3 (s, 1 H, indole-NH); IR (KBr, cm^{-1}): 3446, 2926, 1454, 1336, 1123, 750, 702; $C_{26}H_{26}N_2 \cdot H_2O$ (384.2) calc. (CHN): 81.2, 7.3, 7.3 found: 81.1, 6.9, 7.0.

3-Cyclopropylmethyl-5-phenyl-1,2,3,4,5,6-hexahydro-[4,5-b]indole **3 c**

Yield: 0.61 g (81 %), yellow-brown crystals, mp 45 °C; ^{1}H-NMR: 0.02 (m, 2 H, cyclopropane -C**H$_2$**-), 0.38 (m, 2 H, cyclopropane -C**H$_2$**-), 0.7 (m, 1 H, cyclopropane -CH-), 2.4 (dq, ^2J = 11 Hz, ^3J = 6.5 Hz, 2 H, cyclopropyl-C**H$_2$**-); 2.8–3.1 (m, 5 H, aliphat. H); 3.43 (dd, ^2J = 13 Hz, ^3J = 6 Hz, 1 H, Ph-CHC**H**-), 4.3 (dd, ^3J = 6 Hz, ^3J = 5.5 Hz, 1 H, Ph-CH-), 6.9–7.48 (m, 9 H, aromatic H), 10.3 (s, 1 H, indole-NH); IR (KBr, cm^{-1}): 3409, 2361, 1654, 1458, 742, 700; $C_{22}H_{20}N_2 \cdot ½\ H_2O$ (325.2) calc. (CHN): 81.2, 7.7, 8.6 found: 80.7, 7.5, 8.7.

3-Allyl-5-phenyl-1,2,3,4,5,6-hexahydro-[4,5-b]indole **3 d**

Yield: 0.52 g (73 %), yellow-brownish crystals, mp 77 °C; ^{1}H-NMR: 2.7–3.22 (m, 8 H, aliphatic -CH_2-), 4.31 (dd, ^3J = 3.5 Hz, ^3J = 3 Hz, 1 H, Ph-CH), 5.0–5.15 (m, 2 H, C=C**H$_2$**), 5.6–5.75 (m, 1 H, -CH=C**H$_2$**), 6.9–7.45 (m, 9 H, aromatic H), 10.3 (s, 1 H, indole-NH); IR (KBr, cm^{-1}): 3422, 2361, 1654, 1458, 668; $C_{21}H_{22}N_2 \cdot ¼\ H_2O$ (306.9) calc. (CHN): 82.2, 7.4, 9.1 found: 82.1, 7.3, 9.1.

3,6-Dimethyl-5-phenyl-1,2,3,4,5,6-hexahydro-azepino-[4,5-b]indole **3 f**

5-Phenyl-1,2,3,4,5,6-hexahydroazepino-[4,5-b]indole (**3 a**, 7.6 mmol, 1.98 g) was dissolved in 10 mL of dried THF. The solution was poured, under nitrogen and ice-cooling, into a suspension of 0.125 mol (**3 g**) sodium hydride in 150 mL of THF. Under cooling, a solution of 30 mmol (4.26 g) methyliodide in 15 mL of anhydrous THF was added dropwise. Stirring was continued overnight at room temperature. Sodium hydride was

destroyed with a 1:1 mixture of water/methanol, the precipitation filtered and then resuspended in THF. It was refiltered, the combined filtrates dried over anhydrous sodium sulphate and the solvent removed in vacuo. The crude product was purified by column chromatography (200 g SiO_2^{60}, CH_2Cl_2/MeOH/NH_3conc. = 85/14/1).

Yield: 0.24 g (10.3 %), yellow crystals, mp 135 °C; ^{1}H-NMR: 2.3 (s, 3 H, azepine-*N*-methyl), 2.75 (m, 1 H, Ph-CH-CH_2-), 3.05–3.1 (m, 4 H, aliphat. H), 3.4 (d, ^2J = 10 Hz, ^3J = 5 Hz, 1 H, Ph-CH-CH_2-), 3.45 (s, 3 H, indole-methyl); 4.43 (dd, ^3J = 5 Hz, ^3J = 5 Hz, 1 H, Ph-CH-), 7.1–7.6 (m, 9 H, aromatic H); IR (KBr, cm^{-1}): 3458, 2927, 2361, 1472, 1372, 1123, 735, 701; $C_{20}H_{22}N_2 \cdot H_2O$ (308.4) calc. (CHN): 77.9, 7.8, 9.1 found: 78.5, 7.7, 8.2. The NMR-data confirmed the structure. Satisfying elemental analysis of the hygroscopic compound could not be obtained.

5-Phenyl-1,2,3,4,5,6-hexahydroazepino-[4,5-b]indole **3 a**

2-{[2-(1*H*-Indol-3-yl)ethyl]amino}-1-phenylethanol (**9 a**, 0.025 mol, 6.98 g) was added to a stirred mixture of 500 mL of chloroform and 200 mL of PPA. The mixture was stirred and boiled under reflux (of chloroform) for 90 min. After cooling, the organic phase was decanted, and under ice-cooling and heavy stirring, 1 L of water was added until complete homogeneity was reached. The solution was then made basic (pH = 8), under continued ice-cooling, with 6N NaOH. The product was extracted twice with 500 mL of ethylacetate. The combined organic phases were washed twice with water, dried with anhydrous sodium sulphate and the solvent was removed under reduced pressure. Chromatography on silica (500 g SiO_2^{60}, CH_2Cl_2/MeOH/NH_3conc. = 85/14/1) gave 1.03 g of **3 a** (15 %).

yellowish crystals, mp 141 °C; ^{1}H-NMR (deuteriochloroform): 3.0–3.38 (m, 6 H, aliphat. H), 4.23 (dd, ^3J = 4 Hz, ^3J = 3.5 Hz, 1 H, Ph-CH-), 7.04–7.68 (m, 9 H, aromatic H), 10.23 (s, 1 H, indole-NH); IR (KBr, cm^{-1}): 3399, 2361, 1456, 743, 701; $C_{18}H_{18}N_2 \cdot ¾\ H_2O$ (275.5) calc. (CHN): 78.4, 7.4, 9.7 found: 78.7, 6.9, 9.8.

General procedure for the synthesis of **8 a**–**c**

1*H*-indol-3-ylacetic acid (0.05 mol, 8.76 g) or (1-methyl-1*H*-indol-3-yl)acetic acid (0.05 mol, 9.46 g), were dissolved in 50 mL of dried THF and 0.05 mol (8.11 g) of *N, N′*-carbonyldiimidazole (CDI) was added. Following formation of carbon dioxide (~15 min), the solution of activated acid was allowed to stand for three hours. It was then slowly added dropwise, during vigorous stirring, to a solution of 0.05 mol of the corresponding amino-alcohol (**7**, ephedrine **5**, or norephedrine **4**, respectively). The resulting mixture was stirred at room temperature over night. The solution was washed once with 25 mL of 1N H_2SO_4 and twice with water. During the course of the second wash with water the separation of phases does not take place and additional water has to be added for separation. This time, the organic phase is the lower one. The organic phase is dried over $MgSO_4$ and the solvent is removed under reduced pressure.

The synthesis of analogous compounds out of 1*H*-indol-3-ylacetic acid and norephedrines was previously described [10].

N-(2-Hydroxy-2-phenylethyl)-2-(1H-indol-3-yl)acetamide **8 a**

Starting materials: 1*H*-indol-3-ylacetic acid, 2-amino-1-phenylethanol **7**; yield: 12.8 g (86 %), white powder, mp 132 °C; ^{1}H-NMR: 3.1–3.6 (m, 4 H, -CH_2-groups), 4.57 (s, 1 H, Ph-CH-), 5.45 (s, 1 H, amide-NH), 6.95–7.6 (m, 10 H, aromatic H), 10.9 (s, 1 H, indole-NH); IR (KBr, cm^{-1}): 3348, 2369, 1643, 1558, 1054, 742, 668; $C_{18}H_{18}N_2O_2 \cdot ¼\ H_2O$ (299.9) calc. (CHN): 72.1, 6.5, 9.3 found: 72.4, 6.1, 9.5.

N-[(1S, 2R)-2-Hydroxy-1-methyl-2-phenylethyl]-N-methyl-2-(1-methyl-1H-indol-3-yl)acetamide **8 b**

Starting materials: (1-methyl-1*H*-indol-3-yl)acetic acid, (1*R*, 2*S*)-ephedrine **5**; yield: 14.2 g (82 %), yellowish powder, mp 51 °C; IR (KBr, cm^{-1}): 3368, 2934, 2361, 1616, 1474, 1330, 1117, 743, 701; $C_{21}H_{24}N_2O_2 \cdot ½\ H_2O$ (345.4) calc. (CHN): 73.0, 7.3, 8.1 found: 72.5, 7.3, 7.6 (satisfying elemental analysis was difficult to obtain due to the hygroscopy of the compound).

N-[(1S, 2R)-2-Hydroxy-1-methyl-2-phenylethyl]-2-(1-methyl-1H-indol-3-yl)acetamide **8 c**

Starting materials: (1-methyl-1*H*-indol-3-yl)acetic acid, (1*R*, 2*S*)-norephedrine **4**; yield: 13.4 g (82 %), light yellow powder, mp 128.5 °C; IR (KBr, cm^{-1}): 3285, 2361, 1654, 1543, 1253, 736; $C_{21}H_{24}N_2O_2 \cdot ¼\ H_2O$ (326.9) calc. (CHN): 73.5, 6.9, 8.6 found: 73.8, 6.9, 8.6.

General procedure for the synthesis of **9 a**–**c**

The corresponding amide (**8 a**–**c**, 0.05 mol) was dissolved in 80 mL of dried THF. The solution was slowly added to an ice-cold suspension of 2 mol (75.9 g) lithium aluminium hydride in 300 mL of dried diethylether. The suspension was boiled under reflux and stirred for 18 hours. After cooling, excess $LiAlH_4$ was destroyed with 0.5 N NaOH, which was carefully added dropwise under ice-cooling until the formation of hydrogen was finished.

The inorganics were filtered, washed intensively twice with ether and the filtrate was dried with $MgSO_4$ and evaporated in vacuo. The products were purified by column chromatography on silica (500 g SiO_2^{60}, CH_2Cl_2/MeOH/NH_3conc. = 85/14/1).

Synthesis of the analogous compounds **9 d**, **e** (see Table 2) out of 1*H*-indol-3-ylacetic acid and norephedrines was previously described [10].

2-[2-(1H-Indol-3-yl)ethylamino]-1-phenylethanol **9 a**

Yield: 8.4 g (58 %), yellow powder, mp 120 °C; ^{1}H-NMR: 2.75–2.9 (m, 6 H, -CH_2-groups), 4.65 (t, ^3J = 5 Hz, 1 H, -CH-), 6.9–7.5 (m, 10 H, aromatic H), 10.8 (s, 1 H, indole-NH); IR (KBr, cm^{-1}): 3295, 2894, 2361, 1451, 1421, 1066, 819, 734, 702; $C_{18}H_{20}N_2O \cdot ½\ H_2O$ (289.4) calc. (CHN): 75.0, 7.0, 9.7 found: 75.0, 7.2, 9.8.

(1R, 2S)-2-{Methyl[2-(1-methyl-1H-indol-3-yl)ethyl]amino}-1-phenylpropan-1-ol **9 b**

Yield: 5.3 g (32 %), yellow powder, mp 51 °C; ^{1}H-NMR: 0.96 (d, ^3J = 7 Hz, 3 H, aliphatic -CH_3), 2.36 (s, 3 H, N-CH_3), 2.48–2.9 (m, 5 H, aliphatic H), 3.65 (s, 3 H, indole-CH_3), 4.6 (s[b], 1 H, Ph-CH-), 5.02 (s[b], 1 H, -OH), 6.9–7.45 (m, 10 H, aromatic H); IR (KBr, cm^{-1}): 3422, 2361, 1473, 740, 668; $C_{21}H_{26}N_2O \cdot ½\ H_2O$ (331.5) calc. (CHN): 76.1, 8.2, 8.5 found: 76.5, 8.2, 8.3.

(1R, 2S)-2-{[2-(1-Methyl-1H-indol-3-yl)ethyl]amino}-1-phenylpropan-1-ol **9 c**

Yield: 5.6 g (36 %), yellow oil; ^{1}H-NMR: 0.91 (d, ^3J = 7 Hz, 3 H, N-CH_3), 2.74–2.9 (m, 5 H, aliphat. H), 3.78 (s, 3 H, indole-CH_3), 4.62 (d, ^3J = 5 Hz, 1 H, -CH-Ph), 7.0–7.5 (m, 10 H, aromatic H); IR (KBr, cm^{-1}): 3446, 2361, 1654, 1474, 736, 698, 668; $C_{20}H_{24}N_2O$ (308.4) calc. (CHN): 77.9, 7.8, 9.1 found: 77.5, 7.7, 8.8

Pharmacology

The methods used have been previously described in further detail with special regards to the pharmacological behaviour of compound **1** (LE 300) [5].

Materials

[^{3}H]-Spiperone and [^{3}H]-SCH 23390 were purchased from Amersham, UK, and had a specific activity of 97,0 Ci/mmol and 83,0 Ci/mmol, respectively.

Methods

Cell culture

Human D_1, D_{2L}, D_4, and D_5 receptors were stably expressed in Chinese hamster ovary (CHO) cells as previously described by Sunahara et al. [13]. The cDNAs for the transfection were provided by Dr. D.Grandy (Portland, OR, USA) (D_1, D_5) and Dr. Shine (Darlinghurst, AUS) (D_{2L}). The stably transfected CHO-$D_{4.4}$ cell line was kindly donated to us by Dr. H.H.M. Van Tol (Toronto, CA). The donations are gratefully acknowledged. The densities of receptors measured with [^{3}H]-SCH 23390 were 307.15 fmol/mg protein for the D_1 receptor and 679.44 fmol/mg protein for the D_5 receptor. The densities of receptors measured with [^{3}H]-Spiperone were 2020.92 fmol/mg protein for the D_{2L} receptor and 137.21 fmol/mg protein for the D_4 receptor. Cells were grown at 37 °C under a humidified atmosphere of 5 % CO_2: 95 % air in HAM/F12-medium (Sigma-Aldrich) supplemented with 10 % fetal bovine serum, 1mM *L*-glutamine, 20 U/ml penicillin G, 20 µg/ml streptomycin and 0,2 µg/ml G 418 (all by Sigma-Aldrich).

Preparation of whole-cell-suspensions

Human D_{2L}, D_4, D_5 and D_1 receptor cell lines were grown to 85 % confluency on T 175 culture dishes (Nunc). The medium was removed and the cells were incubated with 6 mL trypsine-EDTA-solution (Sigma-Aldrich) to remove the cells from the culture dish. The resulting suspension was centrifuged (1000 rot/min, 4 °C, 4 min), the pellet resuspended in 10 mL of PBS (ice-cooled, calcium- and magnesium-free), pelleted and the procedure repeated. The resulting pellet was resuspended in 12 mL of Tris-Mg^{2+}-buffer (5 mM magnesium chloride, 50 mM Tris-HCl, pH = 7,4) and used directly for the radioligand binding assay.

Radioligand binding assay

Binding assays, with whole-cell-suspensions, were carried out in triplicate according to the method described by Mierau et al. [14]. Specifically, assays were carried out in a volume of 1.1 mL containing Tris-Mg^{2+}-buffer (690 mL), [^{3}H]-ligand (100 µL), whole-cell-suspension (200 µL) and appropriate drugs (110 µL). Non-specific binding in the assays containing the D_2 or D_4 receptors was determined using fluphenazine (10 µM, final concentration). Haloperidol (1 µM, final concentration) was used to determine non-specific binding in the assays containing the D_1 or D_5 receptors. Drugs were used at a concentration of 100 µM in the initial screen and the percentage of removed radioligand was determined. They were first dissolved in 0.5 mL of DMSO, and then diluted with distilled water to the desired concentration. Concentrations of 100, 10, 1 and 0,1 µM were used to determine the K_I-value. The incubation was initiated by addition of the radioligand and carried out at 27 °C for 2 h. It was stopped by rapid filtration through a glass fiber filter (GF 6, Schleicher and Schüll, Germany) previously treated with 0.25 % polyethyleneimine solution (Sigma-Aldrich), and washed twice with ice-cold water. The radioactivity retained on the filters was counted in five mL of scintillation cocktail Ready-Protein® (Beckman, USA) using a Beckman LS 6000 SC scintillation counter. The competition binding data was analyzed with GraphPad Prism™ software using nonlinear least squares fit. Experiments for determining the K_I values were carried out three times using two different dilutions of drugs.

cAMP assay

A commercially available cAMP Assay Kit from Amersham, UK, was used to measure the formation of cAMP. Human D_1, D_{2L} and D_5 receptors were stably expressed in HEK 293 cells as previously described by Dal Toso et al.[15]. The densities of D_1 and D_5 receptors measured with [^{3}H]-SCH 23390 were 8183.72 fmol/mg protein and 7021.1 fmol/mg protein, respectively. The density of D_2 receptors measured with [^{3}H]-Spiperone was 2022.45 fmol/mg protein. The cells were grown at 37 °C under a humified atmosphere of 5 % CO_2: 95 % air in Dulbecco's modified Eagles minimal essential medium (MEM, Sigma-Aldrich) with 15 mM HEPES, pyridoxine and $NaHCO_3$, supplemented with 10 % fetal bovine serum, 1mM *L*-glutamine, 20 U/ml penicillin G, 20 µg/ml streptomycin and 0,2 µg/ml G 418 (all from Sigma-Aldrich). HEK cells were harvested as described above. The cells were washed with 10 mL of Krebs-HEPES buffer, pH = 7.4, and the resulting pellet resuspended in 3 mL of the same buffer.

Assays were performed in duplicate two independent times (means are given in Figures 3, 4 and 5). The incubation was carried out in a volume of 100 µL which contained the whole-cell-suspension (80 µL), forskolin solution (10 µL, final concentration 10 µM) and 10 µL distilled water (for determining the blank) or test substance solution (10 µM final concentration). Reaction tubes were incubated for 15 min at 37 °C and the reaction stopped by the addition of 100 µL of ethanolic HCl (1 mL 1N HCl/100 mL EtOH). The mixture was allowed to stand for 5 min and then centrifuged (14 000 rot/min, 4 °C, 5 min). Supernatants were stored at –24 °C without any change in cAMP content.

To measure the amount of cAMP produced, 80 µL of the supernatant was dried with a refrigerated vapour trap (Speed Vac SC 100®, Refrigerated Vapor Trap RVT 100®, Savant, UK) and then dissolved in 25 µL of Tris-EDTA-buffer (0.05 M tris, 4 mM EDTA), pH = 7.5.

Radio-immuno-assay

The radio-immuno-assay was performed as described by Amersham. Aliquots of the obtained radioactive solution (100 µL) were diluted in five mL of scintillation cocktail Ready-Protein® (Beckman, USA) and counted in a Beckman LS 6000 SC scintillation counter.

Data analysis (cAMP assay)

Measured radioactivity (in decays per minute, dpm) was converted to quantity of cAMP (pmol/tube) by comparison with a standard curve. The amount of cAMP produced was then related to the number of cells, as determined by a cell counter (0.1 mm depth, 0.0025 mm^2, Brand, D), so that the production of cAMP per cell was obtained. This value was related to cAMP produced in the absence of a test compound (blank = 1).

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