Novel Ergopeptides as Dual Ligands for Adenosine and Dopamine Receptors

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Multivalent ligands are promising pharmacological tools that may be more efficacious for several diseases than highly selective single-target drugs. A combined therapy using dopaminergic agonists and adenosinergic antagonists is currently being evaluated for the treatment of Parkinson's disease. [(a) Kanda, T.; et al. *Exp. Neurol.* **2000**, *162*, 321−327. (b) Jenner, P. *Expert Opin. Invest. Drugs* **2005**, *14*, 729−738. (c) Kase, H.; et al. *Neurology* **2003**, *61* (Suppl 6), S97−S100.] Here we prepared dual ligands acting on adenosine and dopamine receptors by applying a combinatorial approach based on the ergolene privileged structure. The potency and efficacy of these novel compounds were determined by radioligand binding studies and intracellular cAMP production assays in cells expressing adenosine and dopamine receptors. Selected compounds displayed dual dopamine agonist and adenosine antagonist activity. Molecules with this pharmacological profile are potentially useful for studying dopamine−adenosine cross-talk in the central nervous system and for testing the therapeutic potential of multivalent drugs for Parkinson's disease.

Introduction

Recent studies indicate that highly selective drugs alone may not be sufficient to modulate complex in vivo systems, in spite of binding to a single given target.[2] This observation may help to explain the scarcity of successful new single-target drugs during the past decade, despite extensive drug-development efforts. Network models support the notion that partial inhibition of a minimal number of targets is more efficient than the complete inhibition of a single target.[3] The application of a multitarget ligand approach is a new trend in medicinal chemistry, and in some cases has superseded the traditional "one target−one disease" philosophy. This is especially true for disorders in which the alteration of a single receptor is therapeutically insufficient.[4] For these cases, balanced modulation of a small number of targets has been shown to have superior efficacy and fewer side effects than single-target treatments.[5]

The growth of this trend in pharmacological research is reflected in the numerous relevant compounds that are currently in late stages of clinical development. For instance, in the field of antipsychotic drugs, the first selective dopamine antagonists described, such as haloperidol,[6] have been gradually replaced by the so-called atypical antipsychotics, which have a broader receptor affinity profile. Recent research has focused on the synthesis of ligands with an optimum pK_i for 5-HT_{2A}/D_2 receptors, as this multireceptor affinity profile provides broader efficacy against both the negative and positive symptoms of schizophrenia.[7−9]

Multiple pharmacological targets can be affected through the use of drug cocktails, multicomponent drugs, or multivalent ligands.[10] However, the latter have become increasingly popular as they do not have drawbacks such as patient compliance, as in the first case, or complex pharmacokinetics and pharmacodynamics, as in the second.

The potential of multivalent ligands to relieve the symptoms and halt the progress of complex neurodegenerative diseases has been partially explored. The limited efficacy of some single-target drugs for certain disorders—mainly those related to cognition, memory, and movement—has been reported.[11] Thus, although patients suffering from neurodegenerative diseases such as Parkinson's disease (PD) or Alzheimer's disease (AD) are currently benefiting from single-target drugs, current pharmacological approaches are limited in their capacity to significantly alter the course of these diseases, and they offer partial or transient benefits at best.[12] This is particularly relevant to PD, where patients receiving traditional treatment, based on dopamine replacement therapy using L-DOPA or other dopamine agonists, experience response fluctuation, often accompanied by dyskinesias.[13,14] Recently several nondopaminergic receptors expressed in the basal ganglia have been reported as alternative targets for PD treatment.[15] For instance, a combination of $A_{2A}R$[a] antagonists with selective D_1R or D_2R agonists has exhibited antiparkinsonian activity in rodent as well as nonhuman primate models of the disease.[1a] Functional interactions between adenosine and dopamine receptors occur at both molecular and functional levels.[16,17] Molecular cross-talk between A_1R and D_1R has been reported, which demonstrates the adenosinergic negative modulation of dopaminergic activation of striatonigral−striatopenduncular neurons.[18,19] $A_{2A}R$ and D_2R form heteromeric complexes.[20−23] These receptors are colocalized in striatal medium spiny neurons, and it has been proposed that adenosine binding to $A_{2A}R$ lowers the affinity of dopamine for D_2R, thus modulating the function of the latter receptor.[24] $A_{2A}R$ selective antagonists have antiparkinsonian activities in animal models of PD,[25] and in addition, some have exhibited neuroprotective properties, a benefit rarely observed in most currently used antiparkinsonian drugs.[26] Thus, the synthesis of multivalent

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[a] Abbreviations: A_1R, adenosine A_1 receptor; $A_{2A}R$, adenosine A_{2A} receptor; cAMP, cyclic adenosine monophosphate; D_1R, dopamine D_1 receptor; D_2R, dopamine D_2 receptor. Abbreviations used for amino acids follow the IUPAC−IUB Comission of Biochemical Nomenclature in Jones, J.H. *J. Pept. Sci.* **2003**, *9*, 1−8.

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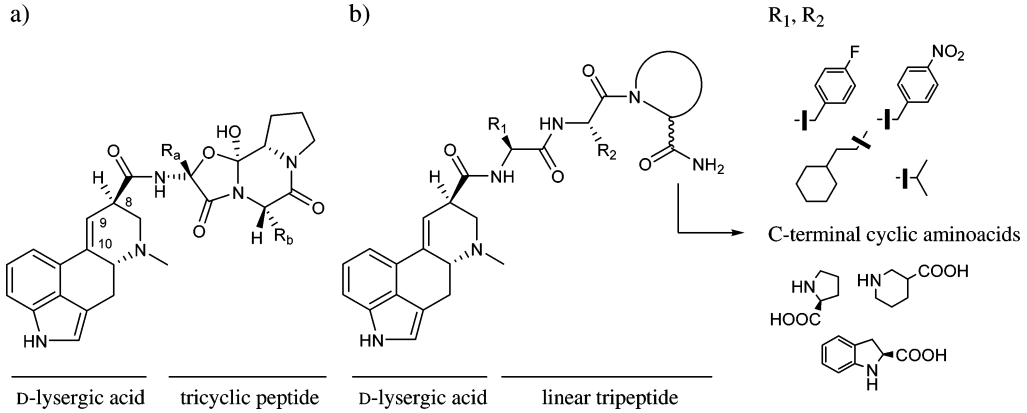


Figure 1. (a) General structure of natural ergopeptines; (b) scheme of the library of ergot linear tripeptide alkaloids synthesized.

ligands that combine D_1 and D_2 dopamine agonism to A_1 and A_{2A} adenosine antagonism in a single molecule may lead to novel treatments for PD.

Privileged structures,[27] with their inherent capacity to interact with diverse biological receptors, are an ideal source of scaffolds for the design and synthesis of combinatorial libraries targeted at multiple receptors.[28,29] Ergot alkaloids contain the tetracyclic ergolene or ergoline system and are privileged structures because of their interactions at more than one receptor.[30] Here we synthesized a 36-member library of ergolene analogs containing distinct linear peptidic moieties in order to study the interaction and pharmacology of novel ergopeptides at dopamine and adenosine receptors.

Results

Library Design and Synthesis. A number of ergolene derivatives interact selectively with some GPCRs. The affinity of these interactions varies with alkaloid structure. Selective modification of the ergolene system may therefore provide molecules that interact with a desired set of receptors. Among the many families of natural products containing the ergolene system, the ergot peptide alkaloids family, also known as ergopeptines, displays a broad spectrum of pharmacological activity. Ergopeptines comprise a D-lysergic acid skeleton linked to a tricyclic peptide moiety via an amide bond. Naturally occurring ergopeptines have a double bond at position 9,10 (Figure 1a). In this figure, R_a and R_b correspond to side chains of hydrophobic amino acids such as alanine, β-alanine, leucine, valine, isoleucine, or phenylalanine[30,31] (Figure 1a).

In addition to natural ergopeptines, the biosyntheses of several of these compounds have revealed the presence of intermediates in which the peptidic moiety is partially or completely linear.[32] Linear intermediates can be synthesized more easily than the corresponding cyclic compounds, thus accelerating the evaluation of the effects that peptide substitution has on the pharmacological properties of novel ergopeptides. On the basis of this premise, we synthesized a set of novel ergopeptides by attaching 36 distinct linear tripeptides to D-lysergic acid (Figure 1b).

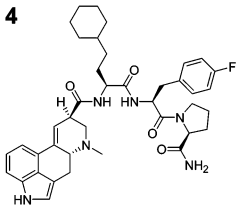
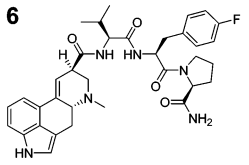
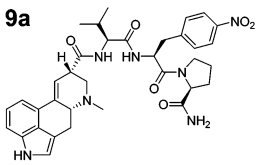
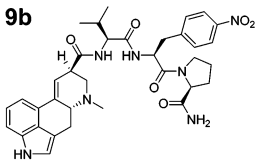
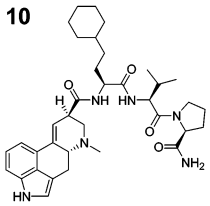
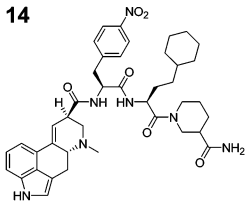
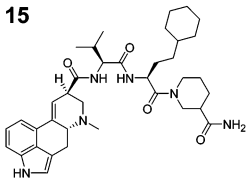
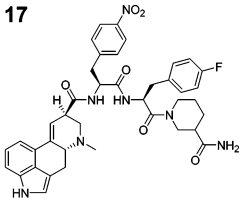
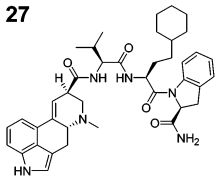
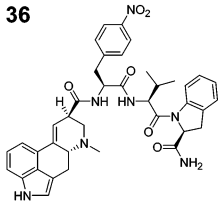
The library of ergopeptides was designed so that the ergolene system was attached to the N-terminal position of distinct linear tripeptides with a carboxamide group at the C-terminus. Peptidic moieties of natural ergopeptines always consist of an L-proline plus two other hydrophobic amino acids. Therefore, to mimic the diversity space defined by the natural products, we introduced three proline-like amino acids at the C-terminal position of the tripeptides while four different L-amino acids were introduced at the other two positions. In addition to L-proline, nipecotic acid and L-indoline-2-carboxylic acid were selected as proline-like amino acids to determine the role of the size of the C-terminal cyclic amino acid. In the other two positions, non-natural aromatic amino acids and hydrophobic amino acids, such as valine or homocyclohexylalanine, were used. The 36-compound library, resulting from the combination of the three C-terminal amino acids with 12 possible combinations of R_1 and R_2 (in Figure 1b, R_1 and R_2 were always distinct amino acids), was synthesized on solid-phase using Rink-PS-resin as a polymeric support. Tripeptides were synthesized using standard peptide coupling conditions (DIPCDI and HOBt as an additive). D-Lysergic acid was attached via an amide bond to the tripeptides using DIPCDI as the coupling reagent, HOAt as an additive, and *N*-methylpyrrolidinone (NMP) as solvent, because D-lysergic acid has low solubility in the other common solvents used for peptide synthesis (DMF, DCM, toluene, THF). Finally, cleavage with TFA−H_2O (95:5) released the final ergopeptides [**1**−**36**, Table 1 in the Supporting Information (SI)], which were then analyzed by RP-HPLC−MS. To check the binding properties of the ergolene scaffold itself, the corresponding primary amide of D-lysergic acid was also synthesized. In this case, direct coupling of D-lysergic acid to the Rink-PS resin (DIPCDI, HOAt in NMP) and subsequent cleavage with TFA−H_2O (95:5) yielded D-lysergilamide (**37**) (Table 1 in SI).

Biological Assays: Binding Properties of Ergopeptides. The binding properties of **1**−**37** were assayed via competitive radioligand binding experiments at 50 μM in D_1R, D_2R, A_1R, and $A_{2A}R$ (Figure 1 in SI)

For all the receptors except $A_{2A}R$, some ergopeptides were more potent than D-lysergilamide (**37**). Further screening at lower ligand concentrations was performed for compounds with the capacity to displace more than 50% of the specific radioligand binding (Figure 2 in SI). After this second screening, nine ergopeptides (**4**, **6**, **9**, **10**, **14**, **15**, **17**, **27**, and **36**), with relatively high affinity for either dopamine or adenosine receptors, were selected, resynthesized, and purified to confirm their binding properties and to characterize their pharmacological profiles. It should be noted that, to date, these molecules are the first derivatives of the ergolene system described to interact with adenosine receptors.

Resyntheses of Compounds with Higher Affinity (4, 6, 9, 10, 14, 15, 17, 27, and 36). Nine ergopeptides were resynthesized as previously described with the exception that D-lysergic acid was coupled in solution. The tripeptides were thus cleaved from the resin using TFA−H_2O (95:5) prior to coupling and D-lysergic acid was attached using DIPCDI, HOAt, and DIEA. Coupling of the acid in solution instead of on solid-phase afforded higher global yields and required a much smaller

Table 1. Chemical Structures of the Selected Ergopeptides and Their Corresponding K_D Values[a]

4  K_D (A_{2A}) = 33±22μM	**6**  K_D (D_1) = 0.6±0.1 μM K_D (D_2) = 16±4 μM	**9a**  K_D (D_1) = 0.7±0.4 μM **9b**  K_D (D_1) = 7.8±0.1 μM
10  K_D (D_1) = 1.2±0.3 μM	**14**  K_{Dh} (D_2) = 0.3±0.2 μM	**15**  K_D (A_{2A}) = 50±20 μM
17  K_D (D_1) = 0.7±0.3 μM K_{Dh} (D_2) = 0.02±0.01μM	**27**  K_D (A_{2A}) = 25±11 μM	**36**  K_D (A_1) = 0.10±0.02 μM K_{Dh} (D_2) = 2±1 μM

[a] K_{Dh} correspond to K_D in the receptor high-affinity state.

amount of the costly D-lysergic acid. However, coupling between tripeptides and D-lysergic acid proceeded with moderate epimerization (around 20% in most cases) at the C_8 carbon of the ergolene scaffold (Figure 1a), thereby leading to diastereoisomeric mixtures. As the chromatographic separation of both diastereoisomers was complex and there was no evidence of a preferred C_8 configuration to interact with dopamine and adenosine receptors, the crude mixtures were purified by semipreparative RP-HPLC, thereby collecting the two diastereoisomers for each sample. However, to evaluate the effects of the C_8 configuration on ergopeptide pharmacology, **9** was further purified so that the two corresponding diastereoisomers (**9a** and **9b**) were isolated. The chemical structures of **9a** and **9b** were determined by ^{1}H and ^{13}C NMR spectroscopy (Experimental Section), which indicate that **9b** is a D-lysergic acid derivative, whereas **9a** is an L-lysergic acid derivative. In addition, the corresponding tripeptides of the nine selected ergopeptides were synthesized using the same procedure described above but including an N^{α}-acetylation step at the N^{α}-terminus prior to cleavage. Purification by semipreparative RP-HPLC afforded the final tripeptides (**38**−**46**) (Table 2 in SI).

Biological Assays: K_D Determination. To determine the binding affinity of selected compounds to adenosine and dopamine receptors, the corresponding K_D values were determined from competition experiments (see Table 1).

The results shown in Table 1 indicate that incorporation of some linear peptidic moieties onto the ergolene structure leads to improved affinities for several subtypes of adenosine and dopamine receptors when compared to the ergolene scaffold alone. This improvement is dependent on the sequence of the peptidic moiety, with some amino acids exhibiting moderate selectivity at certain receptors. Regarding the contribution of the C-terminal amino acid, ergopeptides containing L-proline or nipecotic acid (**6**, **9**, **10**, **14**, and **17**) showed higher affinities for dopamine receptors, while those containing L-indolin-2-carboxylic acid (**27** and **36**) showed greater affinities for adenosine receptors. Concerning the amino acid in the intermediate position, stronger interactions at adenosine receptors were obtained with homocyclohexylalanine or valine (**15**, **27**, and **36**), while this specificity was not detected in the case of ergopeptides binding to dopamine receptors. Finally, in terms of the amino acid directly attached to the ergolene scaffold, *p*-nitrophenylalanine and valine conferred more affinity for adenosine and dopamine binding (**6**, **9**, **14**, **15**, **17**, **27**, and **36**). In addition, not only the amino acids but their relative position in the global structure is relevant to ergopeptide binding properties. Consequently, it is not surprising that the binding

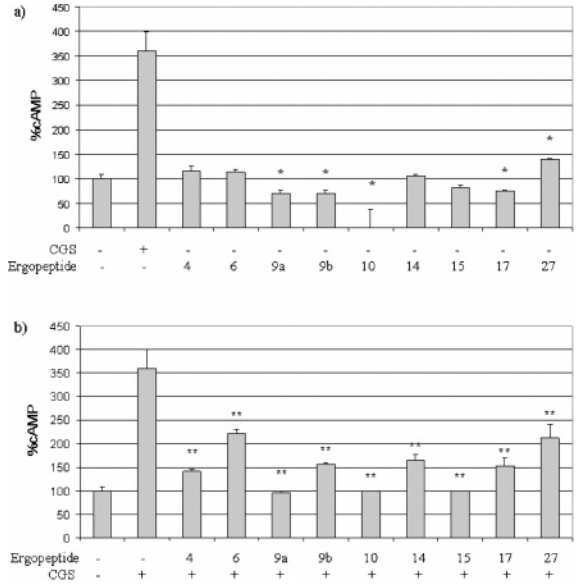


Figure 2. Effects of ergopeptides (50 μM) on intracellular cAMP levels in SH-SY5Y neuroblastoma cells. CGS (150 nM) is a selective $A_{2A}R$ agonist. *Significantly different ($p < 0.05$) compared to basal. **Significantly different ($p < 0.05$) compared to CGS-treated cells.

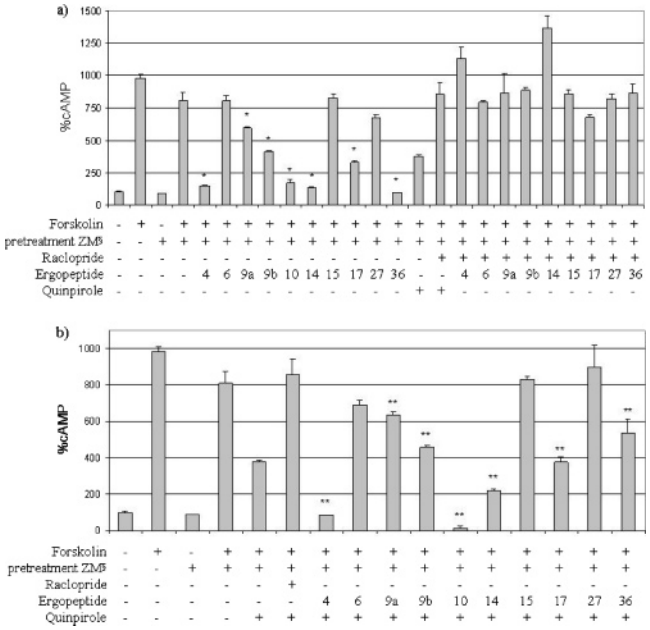


Figure 3. Effects of ergopeptides (50 μM) on intracellular cAMP levels in SH-SY5Y neuroblastoma cells. Raclopride (50 μM) is a selective D_2R antagonist and quinpirole (150 nM) is a selective D_2R agonist. §Cells were preincubated (5−10 min, 22 °C) with ZM (5 μM) to block binding of endogenous adenosine to $A_{2A}R$. *Significantly different ($p < 0.05$) compared to cells activated with Fk and pretreated with ZM. **Significantly different ($p < 0.05$) compared to cells activated with Fk, pretreated with ZM and after addition of raclopride and quinpirole.

properties of ergopeptides with the same composition but with amino acids placed in a different order vary greatly. For instance, matching pairs of ergopeptides (**6** and **11**, **9** and **12**, **36** and **33**, and **4** and **1**) exhibited very different affinities in D_1R, D_2R, A_1R, and $A_{2A}R$, respectively. Thus, whereas **6**, **9**, **36**, and **4** were selected from the nine ergopeptides with higher affinity, **11**, **12**, **33**, and **1** were discarded after the competition experiments.

To determine the contribution of the peptidic moiety to the affinity of ergopeptides for the receptors under study, radioligand binding competition studies were performed using **38**−**46** for D_1R, D_2R, A_1R, and $A_{2A}R$ under the same experimental conditions as those used for the ergopeptide library. None of the tripeptides at 50 μM significantly (more than 40%) displaced the specific radioligand binding at any receptor (see Figure 3 in SI); however, as previously mentioned, the peptidic moiety appeared to be crucial in determining the binding and selectivity properties of ergopeptides. This phenomenon may be explained by the fact that the ergolene scaffold interacts at the binding site itself, whereas tripeptides interact with adjacent amino acids. Therefore, the peptidic interaction would determine a better or a worse accommodation of the ergolene scaffolds in the binding site and thus establishes several selectivity criteria.

Biological Assays: Identification of Agonist/Antagonist Behavior. To characterize the pharmacological profile of **4**, **6**, **9a, 9b**, **10**, **14**, **15**, **17**, **27**, and **36** at D_1R, D_2R, A_1R, and $A_{2A}R$, the ligands were tested by evaluating intracellular cAMP levels derived from the activation or inhibition of adenylate cyclase in two cell lines: SH-SY5Y neuroblastoma cells, which endogenously express $A_{2A}R$ and were transfected with dopamine $D_{2L}R$, and fibroblasts cotransfected with A_1R and D_1R.

We first evaluated the effects of the compounds on $A_{2A}R$ activation by analyzing cAMP levels in SH-SY5Y cells separately treated with each of the nine ergopeptides or the $A_{2A}R$ agonist CGS [2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethyl-carboxamido adenosine hydrochloride] (Figure 2a). Activated $A_{2A}R$ couples to Gαs-protein, which leads to the activation of adenylate cyclase and therefore to an increase in cAMP levels.[33,34] As none of the ergopeptides increased basal cAMP levels, they were not considered to be $A_{2A}R$ agonists. To test whether they acted as $A_{2A}R$ antagonists, cAMP levels were determined in SH-SY5Y cells pretreated with the $A_{2A}R$ selective agonist CGS[35] (Figure 2b). All compounds antagonized CGS-induced cAMP production and therefore were A_{2A} antagonists, taking into consideration that **9a**, **9b**, **10**, **15**, and **17** may behave as inverse agonists.

A similar procedure was used to distinguish between agonism and antagonism in ergopeptides at D_2R. D_2R activation results in coupling to Gαi-proteins, which leads to the inhibition of adenylate cyclase and therefore to a decrease in cAMP levels.[36,37] Thus, D_2R agonism causes a decrease in the increased cAMP levels induced by forskolin. Given that activation of adenosine-binding $A_{2A}R$ decreases the affinity of dopamine for D_2R,[24a,38] SH-SY5Y cells were preincubated with the selective $A_{2A}R$ antagonist ZM [4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo-[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol], thereby preventing D_2R binding caused by endogenous adenosine. Experiments were performed in the presence of 10 μM forskolin to detect decreases in cAMP levels. Compounds **4**, **9a**, **9b**, **10**, **14**, **17**, and **36** provoked a significant decrease in the cAMP levels of forskolin-activated cells (Figure 3a). This effect was antagonized by the selective D_2R antagonist raclopride (3,5-dichloro-*N*-(1-ethylpyrrolidin-2-ylmethyl)-2-hydroxy-6-methoxybenzamide), thereby indicating that compounds **4**, **9a**, **9b**, **10**, **14**, **17**, and **36** behaved as D_2R agonists. Concerning the effect of C_8 configuration in D_2R pharmacology, the diastereoisomer **9b** was a slightly more potent agonist than its corresponding isomer **9a**. In contrast, ligands **6**, **15**, and **27** acted as D_2R antagonists, since they reverted the decrease in the cAMP levels of cells activated by the selective D_2R agonist quinpirole [(4a*R*,8a*R*)-5-propyl-4,4a,5,6,7,8,8a,9-octahydro-1*H*-pyrazolo[3,4-*g*]quinoline] (Figure 3b).

Analogously to $A_{2A}R$, D_1R couples to Gαs-protein; thus, activation of the receptors also causes an increase in cAMP levels.[36,37] The nine ergopeptides tested proved to be D_1R

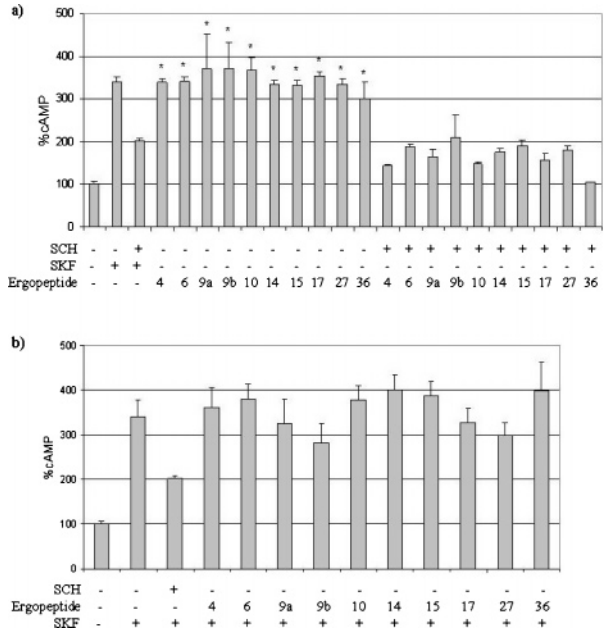


Figure 4. Effects of ergopeptides (50 μM) on intracellular cAMP levels in A_1R and D_1R transfected fibroblasts. SCH (50 μM) is a selective D_1R antagonist and SKF (20 nM) is a selective D_1R agonist. *Significantly different ($p < 0.05$) compared to basal.

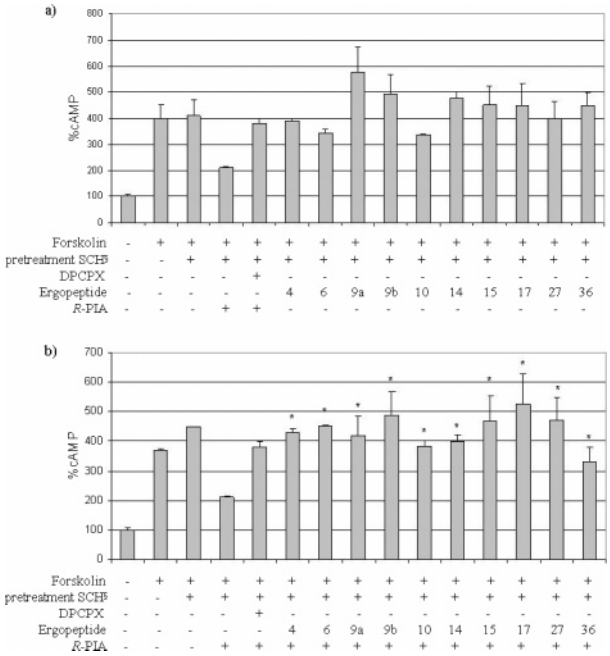


Figure 5. Effects of ergopeptides (50 μM) on intracellular cAMP levels in A_1R and D_1R transfected fibroblasts. DPCPX (50 μM) is a selective A_1R antagonist and *R*-PIA (20 nM) is a selective A_1R agonist. §Cells were preincubated (5−10 min, 22 °C) with SCH (50 μM) to block binding of ergopeptides as D_1R agonists. *Significantly different ($p < 0.05$) compared to cells activated with Fk, pretreated with SCH and after addition of *R*-PIA.

agonists since they increased the cAMP levels in A_1R-D_1R transfected fibroblasts to a similar extent as the selective D_1R agonist SKF [(±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol]. This increase was also antagonized by the selective D_1R antagonist SCH [(*R*)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine] (Figure 4a), thus confirming D_1R agonism. None of the compounds decreased the cAMP levels in SKF-activated fibroblasts (Figure 4b), thereby indicating that they were not D_1R antagonists.

Activated A_1R couples to Gαi-protein, which leads to the inhibition of adenylate cyclase. This observation implies that A_1R agonists decrease cAMP levels.[33,34] A_1R-D_1R transfected fibroblasts were preincubated with SCH (50 μM) to prevent any agonist interaction of the ergopeptides with D_1R during pharmacological evaluation of A_1R. Experiments were performed in the presence of 10 μM forskolin to detect decreases in cAMP levels. In contrast to the effect of the selective A_1R agonist *R*-PIA [N^6-(*R*)-(2-phenylisopropyl)adenosine], none of the ligands caused a decrease in the forskolin-induced cAMP levels (Figure 5a). Furthermore, all compounds acted as A_1R antagonists as they reverted the cAMP decrease induced by *R*-PIA, similarly to DPCPX (1,3-dipropyl-8-cyclopentylxanthine), a selective A_1R antagonist (Figure 5b).

Conclusions

The incorporation of linear peptidic moieties into the ergolene privileged structure has led to molecules with improved affinity for dopamine and adenosine receptors compared to the ergolene scaffold alone. This improvement is dependent on the sequence of the peptidic moiety, which therefore determines the binding and selectivity properties of ergopeptides. However, none of the tripeptides alone interacted significantly in the binding site of any receptor. A possible explanation for this binding mode considers the interaction of the ergolene scaffold at the binding site while tripeptides interact with adjacent amino acids. In addition, our pharmacological assays revealed that the agonist/antagonist behavior of ergopeptides relies on the ergolene system. This observation is also consistent with our hypothesis of ergopeptides binding mode, which assumes that the peptidic moiety does not directly interact at receptor binding sites. Concerning the effect of ergolene system chirality, the C_8 configuration is not relevant in ergopeptides pharmacology, as the diastereoisomers **9a** and **9b** showed similar behavior in the four receptors evaluated, observing only slight differences in their agonist potency at D_2R. All the selected ergopeptides behaved as A_1R antagonists, $A_{2A}R$ inverse agonists or antagonists, and D_1R agonists. Regarding D_2R, **4**, **9a**, **9b**, **10**, **14**, **17**, and **36** acted as agonists, whereas **6**, **15**, and **27** were antagonists. Therefore, these novel ergopeptides can be described as multifunctional compounds, as they induce distinct pharmacological responses for receptors. Given their affinities in the low micromolar range and complementary pharmacological profiles (D_1-D_2 agonism and A_1-A_{2A} antagonism), these ergopeptides provide a good starting point to generate new ligands with dual activity in dopamine and adenosine receptors. In addition, these molecules are useful tools to evaluate adenosine−dopamine cross-talk mechanisms and to test the potential of multivalent ligands in the treatment of PD and other neurological disorders. The incorporation of novel amino acids with several functional groups may lead to more potent and selective compounds. Using the above-mentioned ergopeptides, we are currently working on the design and synthesis of new molecules that show improved affinity for a number of subtypes of dopamine and adenosine receptors.

Experimental Section

Materials and Equipment. All Fmoc-amino acids were purchased from Neosystem (Strasbourg, France), and Fmoc-Rink-PS resin was supplied by Calbiochem-Novabiochem AG. DIPCDI was obtained from Fluka Chemika (Buchs, Switzerland) and HOBt from Albatross Chem, Inc. (Montreal, Canada). Solvents for peptide synthesis and RP-HPLC equipment were obtained from Scharlau (Barcelona, Spain). Trifluoroacetic acid was supplied by KaliChemie (Bad Wimpfen, Germany). Other chemicals were purchased

from Aldrich (Milwaukee, WI) and were of the highest commercially available purity. All commercial reagents and solvents were used as received.

Adenosine deaminase (EC 3.5.4.4) was purchased from Roche (Basel, Switzerland). Geneticine G-418, and [^{3}H]-*R*-PIA were supplied by Amersham Biosciences (Buckinghamshire, UK). Forskolin, quinpirole, raclopride, dopamine, $MgCl_2$, CGS 21680, *R*-PIA, and DPCPX were purchased from Sigma (St. Louis, MO). ZM 241385 and SCH 23390 were supplied by Tocris Biosciences (Avonmouth, UK). SKF 38393 was purchased from Research Biochemicals (Natick, MA) and [^{3}H]SCH 23390, [^{3}H]YM 09151-2, [^{3}H]ZM 241385, and [^{3}H]CGS 21680 were supplied by Perkin-Elmer (Boston, MA). Ecoscint H scintillation cocktail was purchased from National Diagnostics (Atlanta, GA) and cAMP Biotrak Enzyme Immunoassay (EIA) kits from Amersham Biosciences (Buckinghamshire, UK). Penicilin, streptomycin, hygromycine, and all other supplements were purchased from Invitrogen (Paisley, UK).

Analytical RP-HPLC−MS were performed using a 2795 Waters (Milford, MA) Alliance with a Micromass ZQ Mass Spectrometer and 996 PDA detector. Semipreparative RP-HPLC was performed on a 2767 Waters chromatography system with a Micromass ZQ mass spectrometer. Multiple sample evaporation was carried out in a Discovery SpeedVac ThermoSavant (Waltham, MA). ^{1}H and ^{13}C NMR spectra were recorded on a Mercury 400 spectrometer (Unitat de RMN, Serveis Cientifico-Tècnics, University of Barcelona); chemical shifts (δ scale) are reported in parts per million (ppm) and coupling constants are given in hertz (Hz). Radioligand binding experiments were performed using a Brandel (Gaithersburg, MD) cell harvester and a Packard 1600 TRI-CARB scintillation counter. Absorbance measures were carried out in a Merck Elisa System Mios plate reader.

Synthesis. Solid-Phase General Procedure. Peptide syntheses were performed manually in a polypropylene syringe fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. Washings between deprotection, coupling, and subsequent deprotection steps were carried out with DMF (5 × 1 min) and DCM (5 × 1 min) using 10 mL of solvent/g of resin each time. Peptide syntheses were carried out with Fmoc-Rink-PS resin using an Fmoc/tBu solid-phase strategy. Fmoc-AA-OH (3 equiv) was coupled using DIPCDI (3 equiv) and HOBt (3 equiv) in DCM−DMF (1:1) for 2−4 h at room temperature. After each coupling, the resin was washed with DMF (5 × 1 min) and DCM (5 × 1 min). Reaction completion was checked by the Kaiser or chloranil tests.

Fmoc group removal involved the following sequence**:** (i) DMF (5 × 1 min), (ii) piperidine−DMF (2:8) (1 × 1 min + 2 × 15 min), (iii) DMF (5 × 1 min).

Synthesis of Ergopeptides Library (1−36). Each compound was synthesized starting from Fmoc-Rink-PS resin (85 mg, loading 0.7 mmol/g). Upon synthesis of the tripeptides as described above, D-lysergic acid (47 mg, 3 equiv) was activated in NMP by HOAt (25 mg, 3 equiv). The resulting solution and DIPCDI (28 μL, 3 equiv) were added to the resin, which had previously been swollen in NMP. The coupling was carried out overnight at room temperature. After coupling, the resins were washed with NMP, DMF, DCM, and MeOH (5 × 1 min with every solvent). Cleavages were performed at room temperature with a solution of TFA−H_2O (95:5, v/v). The products were evaporated under pressure and lyophilized before biological assays. All compounds were characterized by analytical RP-HPLC−MS using a reverse-phase Symmetry C_{18} (3.9 × 150 mm^2) 5 μm column, flow 1 mL/min (HPLC data included in Table 1 in SI).

Synthesis of D-Lysergilamide (37). Fmoc-Rink-PS resin (50 mg) was swollen in DMF and the Fmoc group was removed as abovementioned. D-Lysergic acid (28 mg, 3 equiv) and HOAt (15 mg, 3 equiv) were dissolved in NMP. The solution obtained and DIPCDI (17 μL, 3 equiv) were added to the resin, and the coupling was carried out overnight at room temperature. Afterward, the resin was washed with NMP, DMF, DCM, and MeOH (5 × 1 min with every solvent). Cleavage at room temperature with a solution of TFA−H_2O (95:5, v/v) in 1 h afforded 3.0 mg of d-lysergilamide (yield: 32%), which was dried under vacuum and lyophilized before biological assays. The product was characterized using the same conditions as those used for the ergopeptide library (HPLC data included in Table 1 in SI).

Resyntheses of Compounds 4, 6, 9a, 9b, 10, 14, 15, 17, 27, and 36 and the Corresponding N$^\alpha$-Acetylated Tripeptides (38−46). Each synthesis was carried out using Fmoc-Rink-PS resin (400 mg). Upon synthesis of the free N$^\alpha$-terminus tripeptides, the resins were split equally into two syringes to eventually provide both the ergopeptides and the corresponding N$^\alpha$-acetylated tripeptides.

N$^\alpha$-Acetylated Tripeptides. The resins were treated with Ac_2O (135 μL, 10 equiv) and DIEA (240 μL, 10 equiv) in DCM (2 × 15 min). Reaction completion was checked by the Kaiser test. Cleavage for 1 h with a solution of TFA−H_2O (95:5, v/v) led to the crude N$^\alpha$-acetylated tripeptides.

Ergopeptides. Free N$^\alpha$-terminus tripeptides were cleaved from the resin with a solution of TFA−H_2O (95:5, v/v). After evaporation, the crude products were solved in DMF and treated with DIEA (24 μL, 1 equiv). D-Lysergic acid (38 mg, 1 equiv) was activated in DMF by HOAt (19 mg, 1 equiv). The resulting suspension and DIPCDI (22 μL, 1 equiv) were added to each crude peptide. Couplings were performed overnight at room temperature and the crude products were evaporated under vacuum. The compounds were purified by semipreparative RP-HPLC using a reverse-phase Symmetry C_{18} (3 × 10 cm^2) 5 μm column with elution systems (A) H_2O−TFA 99.9:0.1 and (B) ACN−TFA 99.9:0.1 at 25 mL/min flow rate and a purification gradient of 0% B to 100% B in 30 min.

Sample Characterization. The purified compounds were characterized by analytical RP-HPLC−MS with a reverse-phase Symmetry C_{18} (3.9 × 150 mm^2) 5 μm column and flow rate of 1 mL/min in two separate elution systems. The purities obtained using each of the two elution systems are specified in Table 3 (SI).

N$^\alpha$-Acetylated Tripeptides. HPLC data and yields are included in Table 2 (SI).

Ergopeptides. 4 (yield 31%): 37.6 mg, M_{exp} 697.4 (M_{calc} 696.4), 93%.

6 (yield 1%): 3.2 mg, M_{exp} 629.3 (M_{calc} 628.3), 94%.

9a (yield 5%): 4.4 mg, M_{exp} 656.3 (M_{calc} 655.3), 98%; ^{1}H NMR (400 MHz, DMSO-d_6) 11.03 (s, 1H), 10.37 (bs, 2H), 8.52 (d, 1H, J = 8.9 Hz), 8.41 (d, 1H, J = 7.9 Hz), 8,14 (d, 2H, J = 8.5 Hz), 7,67 (d, 2H, J = 8.6 Hz), 7.35 (d, 1H, J = 8.0 Hz), 7.30 (bs, 1H), 7.20 (t, 1H, J = 7.7 Hz), 7.10 (d, 1H, J = 7.3 Hz), 6.50 (bs, 1H), 4.92 (m, 1H), 4.38 (m, 1H), 4.33 (dd, 1H, J = 3.8−8.4 Hz), 4.27 (m, 1H), 3.98 (m, 1H), 3.72 (m, 1H), 3.70 (m, 1H), 3.60 (m, 2H), 3.50 (m, 1H), 3.35 (m, 1H), 3.19 (s, 3H), 3.05 (d, 1H, J = 9.3 Hz), 2.94 (m, 1H), 2.11 (m, 2H), 1.99 (m, 1H), 1.91 (m, 2H), 0.89 (d, 3H, J = 6.9 Hz), 0.87 (d, 3H, J = 6.9 Hz); ^{13}C NMR (400 MHz, DMSO-d_6) 174.1, 171.3, 169.6, 169.4, 146.7, 134.4, 131.4, 125.8, 125.4, 125.1, 123.4, 121.0, 120.1, 116.1, 112.4, 111.6, 110.0, 106.3, 61.9, 60.4, 58.6, 53.4, 52.0, 47.5, 41.9, 40.2, 37.1, 31.0, 30.0, 25.1, 24.5, 19.9, 18.9.

9b (yield: 3%): 2.9 mg, M_{exp} 656.3 (M_{calc} 655.3), 99%; ^{1}H NMR (400 MHz, DMSO-d_6) 10.94 (s, 1H), 9.15 (bs, 2H), 8.46 (d, 1H, J = 8.8 Hz), 8.43 (d, 1H, J = 7.9 Hz), 8,09 (d, 2H, J = 8.4 Hz), 7.56 (d, 2H, J = 8.4 Hz), 7.21 (bs, 1H), 7.16 (bs, 1H), 7.11 (bs, 1H), 7.10 (bs, 1H), 6.68 (d, 1H, J = 5.4 Hz), 4.76 (m, 1H), 4.30 (m, 1H), 4.18 (dd, 1H, J = 4.0−8.4 Hz), 4.14 (dd, 1H, J = 1.5−7.0 Hz), 3.72 (m, 1H), 3.62 (m, 4H), 3.51 (m, 1H), 3.22 (dd, 1H, J = 4.0−14.0 Hz), 3.11 (dd, 1H, J = 4.1−13.9 Hz), 3.10 (s, 3H), 2.01 (m, 1H), 1.90 (m, 1H), 1.89 (m, 2H), 1.80 (m, 1H), 0.73 (d, 3H, J = 6.4 Hz), 0.72 (d, 3H, J = 6.4 Hz); ^{13}C NMR (400 MHz, DMSO-d_6) 174.0, 171.2, 171.0, 169.6, 146.9, 134.5, 131.5, 125.9, 125.4, 123.9, 123.8, 123.7, 123.4, 121.2, 118.6, 112.1, 111.7, 106.3, 62.1, 60.4, 58.4, 53.4, 52.0, 47.5, 43.1, 39.3, 37.0, 31.4, 30.0, 25.1, 24.8, 19.9, 18.9.

10 (yield 27%): 30.0 mg, M_{exp} 631.4 (M_{calc} 630.4), 99%.

14 (yield 9%): 23.9 mg, M_{exp} 738.2 (M_{calc} 737.5), 87%.

15 (yield 6%): 10.6 mg, M_{exp} 645.4 (M_{calc} 644.5), 97%.

17 (yield 20%): 20 mg, M_{exp} 736.3 (M_{calc} 735.3), 99%.

27 (yield 13%): 22.7 mg, M_{exp} 679.4 (M_{calc} 678.4), 94%.

36 (yield 4%): 10.7 mg, M_{exp} 704.4 (M_{calc} 703.3), 97%.

Biological Assays. Radioligand Binding Experiments. General Procedure. Membrane suspensions of lamb striatum were obtained following the method described in refs 39 and 40. Radioligand binding assays of membrane suspensions (0.5 mg of protein/mL determined with bicinchoninic acid kits) were carried out at 22 °C in 50 mM Tris-HCl buffer, pH 7.4 (see conditions below used for each receptor). After radioligand incubation, free and membrane-bound ligand were separated by rapid filtration of 500 mL aliquots in a cell harvester through Whatman GF/C filters embedded in polyethylenimine (except for [^{3}H]CGS). Nonspecific binding was determined with unlabeled ligands at the concentration indicated below. In all cases, the filters were incubated with 10 mL of Ecoscint H scintillation cocktail overnight at room temperature. Radioactivity counts in the vials were determined in the counter with an efficiency of 60%.

Screening of Ergopeptides Library. Binding experiments of the whole library were performed at a concentration of 50 μM ergopeptide.

Dopamine D_1 Receptor. Membranes were incubated with 1.2 nM [^{3}H]SCH 23390 (85 Ci/mmol) in 50 mM Tris-HCl buffer (pH 7.4) for 1 h. Nonspecific binding was measured in the presence of 1 mM dopamine.

Dopamine D_2 Receptor. Membranes were incubated with 0.9 nM [^{3}H]YM 09151-2 (83 Ci/mmol) in 50 mM Tris-HCl buffer (pH 7.4) for 2 h. Nonspecific binding was measured in the presence of 100 μM raclopride.

Adenosine A_1 Receptor. Membranes were incubated with 2.2 nM [^{3}H]-*R*-PIA (46 Ci/mmol) in 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 0.2 U/mL ADA for 1 h. Nonspecific binding was measured in the presence of 50 μM *R*-PIA.

Adenosine A_{2A} Receptor. Membranes were incubated with 20.1 nM [^{3}H]CGS 21680 (40.5 Ci/mmol) in 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 2 U/mL ADA for 2 h. Nonspecific binding was measured in the presence of 1 mM CGS 21680.

Compounds with the capacity to displace more than 50% of the specific radioligand binding were then tested at lower concentrations using the same procedure. Each ergopeptide concentration is indicated in the corresponding legend of Figure 2a−d (SI).

Competition Experiments and K_D Determination. Competition experiments were performed by incubating membranes under the same conditions as described above, in the absence or presence of increasing concentrations of ergopeptides. Nonspecific binding was determined as previously outlined. Radioligand displacement curves were analyzed by nonlinear regression using the commercial program GRAFIT (Erithacus Software, Surrey, UK) by fitting the total binding data to the displacement models with one or two affinity sites.[41,42] Data are means ± SD of three to six determinations, and the differences with respect to controls were tested for significance (two-tailed; $p < 0.05$) using the Student's *t* test for unpaired samples. Goodness of fit was tested following the reduced χ^2 value given by the nonlinear regression program GRAFIT. A modified *F* test was used to analyze whether the fit to the two-site model significantly improved upon the fit to the one-site model, and $p < 0.05$ was taken as a criterion of significance; when no significant improvement over the one-site model was detected, the *p* values were >0.30.[35a]

Cell Cultures. A previously characterized[38] human neuroblastoma cell line SH-SY5Y cotransfected with human D_{2L}R cDNA (NBt cells) was used. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM L-glutamine, 100 UI/mL penicilin/streptomycin, 1% sodium pyruvate, 600 μg/mL geneticine G-418, and 10% (v/v) heat inactivated fetal bovine serum (FBS). For the experiments on A_1R and D_1R, the previously characterized mouse fibroblast Ltk$^-$ cells transfected with both human A_1R and human D_1R cDNAs (A_1D_1 cells) were used.[43] The A_1D_1 cells were grown under the same conditions as NBt cells but were supplemented with 300 μg/mL hygromicine B and 200 μg/mL geneticine G-418. Both cell lines were grown at 37 °C in saturation humidity in a 5% CO_2−95% air atmosphere.

cAMP Determination. The accumulation of cAMP was measured with a competitive enzyme immunoassay of the cAMP Biotrak enzyme immunoassay system. Cells showing 80% confluence (NBt or A_1D_1 cells) were serum-starved for 12−16 h, and the medium was replaced by fresh HBSS. The phosphodiesterase inhibitor zardaverine (50 μM) was added, and after 10 min of incubation, the ligands were added at the concentrations indicated in the legends of Figures 2−5. After 35 min overall incubation, the reactions were stopped by treatment with the lysis buffer provided by the kit and samples were processed following the manufacturer's instructions. Data are means ± SD of three to six determinations, and the differences with respect to controls were tested for significance (two-tailed; $p < 0.05$) using the Student's *t* test for unpaired samples.

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Supporting Information Available: Tables containing chemical structures, HPLC data, and yields for the whole library of ergopeptides, N^{α}-acetylated tripeptides, and resynthesized ergopeptides as well as results for preliminary radioligand binding experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kanda, T.; Jackson, M. J.; Smith, L. A.; Pearce, R. K. B.; Nakamura, J.; Kase, H.; Kuwana, Y.; Jenner, P. Combined use of the adenosine A_{2A} antagonist KW-6002 with L-DOPA or with selective D_1 or D_2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. *Exp. Neurol.* **2000**, *162*, 321−327. (b) Jenner, P. Istradefylline, a novel adenosine A_{2A} receptor antagonist, for the treatment of Parkinson's disease. *Expert Opin. Invest. Drugs* **2005**, *14*, 729−738. (c) Kase, H.; Aoyama, S.; Ichimura, M.; Ikeda, K.; Ishii, A.; Kanda, T.; Koga, K.; Koike, N.; Kurokawa, M.; Kuwana, Y.; Mori, A.; Nakamura, J.; Nonaka, H.; Ochi, M.; Saki, M.; Shimada, J.; Shindou, T.; Shiozaki, S.; Suzuki, F.; Takeda, M.; Yanagawa, K.; Richardson, P. J.; Jenner, P.; Bedard, P.; Borrelli, E.; Hauser, R. A.; Chase, T. N. Progress in pursuit of therapeutic A_{2A} antagonists: The adenosine A_{2A} receptor selective antagonist KW6002: Research and development toward a novel nondopaminergic therapy for Parkinson's disease. *Neurology* **2003**, *61* (Suppl 6), S97−S100.

(2) Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. Magic shotguns versus magic bullets: Selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discovery* **2004**, *3*, 353−359.

(3) Cmeserly, P.; Ágoston, V.; Pongor, S. The efficiency of multi-target drugs: The network approach might help drug design. *Trends Pharmacol. Sci.* **2005**, *26*, 178−182.

(4) Barbey, S.; Goosens, L.; Taverne, T.; Cornet, J.; Choesmel, V.; Rouaud, C.; Gimeno, G.; Yannic-Arnoult, S.; Michaux, C.; Charlier, C.; Houssin, R.; Henichard, J. P. Synthesis and activity of a new methoxytetrahydropyran derivative as dual cyclooxygenase-2/5-lipoxygenase inhibitor. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 779−782.

(5) Morphy, R.; Kay, C.; Rankovic, Z. From magic bullets to designed multiple ligands. *Drug Discovery Today* **2004**, *9*, 641−651.

(6) Carlsson, A. The current status of the dopamine hyphotesis of schizophrenia. *Neuropsychopharmacology* **1988**, *1*, 179−186.

(7) Campiani, G.; Butini, S.; Fattorusso, C.; Catalanotti, B.; Gemma, S.; Nacci, V.; Fiorini, I.; Morelli, E.; Cagnotto, A.; Mereghetti, I.; Mennini, T.; Carli, M.; Minetti, P.; Di Cesare, M. A.; Mastroianni, D.; Scafetta, N.; Galletti, B.; Stasi, M. A.; Castorina, M.; Pacifici, L.; Vertechy, M.; Di Serio, S.; Ghirardi, O.; Tinti, O.; Carminati, P. Pyrrolo[1,3]benzothiazepine-based serotonin and dopamine receptor antagonists. Molecular modeling, further structure-activity relationship studies, and identification of novel atypical antipsychotic agents. *J. Med. Chem.* **2004**, *47*, 143−157.

(8) Campiani, G.; Butini, S.; Fattorusso, C.; Trotta, F.; Gemma, S.; Catalanotti, B.; Nacci, V.; Fiorini, I.; Cagnotto, A.; Mereghetti, I.; Mennini, T.; Minetti, P.; Di Cesare, M. A.; Stasi, M. A.; Di Serio, S.; Ghirardi, O.; Tinti, O.; Carminati, P. Novel atypical antyphsicotic agents: Rational design, an efficient palladium-catalyzed route, and pharmacological studies. *J. Med. Chem.* **2005**, *48*, 1705−1708.

(9) Jones, H. M.; Pilowsky, L. S. New targets for antiphysotics. *Expert Rev. Neurother*. **2002**, *2*, 61−68.
(10) Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* **2005**, *48*, 1−21 and references therein.
(11) Buccafusco, J. J.; Terry, A. V., Jr. Multiple CNS targets for eliciting beneficial effects on memory and cognition. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 438−446.
(12) Youdim, M. B. H.; Buccafusco, J. J. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends Pharmacol. Sci.* **2005**, *26*, 27−35.
(13) Mercuri, N. B.; Bernardi, G. The 'magic' of L-dopa: Why is it the gold standard Parkinson's disease therapy? *Trends Pharmacol. Sci.* **2005**, *26*, 341−344.
(14) (a) Kurlan, R. Practical therapy of Parkinson's disease. *Semin. Neurol.* **1987**, *7*, 160−166. (b) Mouradian, M. M.; Heuser, I. J. E.; Baronti, F.; Fabbrini, G.; Juncos, J. L.; Chase, T. N. Pathogenesis of dyskinesias in Parkinson's disease. *Ann. Neurol.* **1989**, *25*, 523−526.
(15) Ferré, S.; Fredholm, B. B.; Morelli, M.; Popoli, P.; Fuxe, K. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. *Trends Neurosci.* **1997**, *10*, 482−487.
(16) Agnati, L. F.; Ferré, S.; Lluis, C.; Franco, R.; Fuxe, K. Mechanisms and therapeutical implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. *Pharmacol. Rev.* **2003**, *55*, 509−550.
(17) Franco, R.; Casadó, V.; Mallol, J.; Ferré, S.; Fuxe, K.; Cortés, A.; Ciruela, F.; Lluis, C.; Canela, E. I. Dimer-based model for heptaspanning membrane receptors. *Trends Biochem. Sci.* **2005**, *300*, 360−366.
(18) Ginés, S.; Hillion, J.; Torvinen, M.; Le Crom, S.; Casadó, V.; Canela, E. I.; Rondin, S.; Lew, J. Y.; Watson, S.; Zoli, M.; Agnati, L.; Vernier, P.; Lluis, C.; Ferré, S.; Fuxe, K.; Franco, R. Dopamine D_1 and adenosine A_1 receptors form functionally interacting heteromeric complexes. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8606−8611.
(19) Torvinen, M.; Ginés, S.; Hillion, J.; Latini, S.; Canals, M.; Ciruela, F.; Bordoni, F.; Staines, W.; Pedata, F.; Agnati, L.; Lluis, C.; Franco, R.; Ferré, S.; Fuxe, K. Interactions among adenosine deaminase, adenosine A_1 receptors and dopamine D_1 receptors in stably cotransfected fibroblast cells and neurons. *Neuroscience* **2002**, *113*, 709−719.
(20) Torvinen, M.; Kozell, L. B.; Neve, K. A.; Agnati, L. F.; Fuxe, K. Biochemical identification of the dopamine D_2 receptor domains interacting with the adenosine A_{2A} receptor. *J. Mol. Neurosci.* **2004**, *25*, 191−200.
(21) (a) Hillion, J.; Canals, M.; Torvinen, M.; Casadó, V.; Scott, R.; Terasmaa, A.; Hansonn, A.; Watson, S.; Olah, M. E.; Mallol, J.; Canela, E. I.; Zoli, M.; Agnati, L. F.; Ibáñez, C. F.; Lluis, C.; Franco, R.; Ferré, S.; Fuxe, K. Coaggregation, cointernalization and codesensitization of adenosine A_{2A} receptors and dopamine D_2 receptors. *J. Biol. Chem.* **2002**, *277*, 18091−18097. (b) Canals, M.; Marcellino, D.; Fanelli, F.; Ciruela, F.; de Benedetti, P.; Goldberg, S. R.; Neve, K.; Fuxe, K.; Agnati, L. F.; Woods, A. S.; Ferré, S.; Lluis, C.; Bouvier, M.; Franco, R. Adenosine A_{2A}−dopamine D_2 receptor−receptor heteromerization. *J. Biol. Chem.* **2003**, *278*, 46741−46749.
(22) Kamiya, T.; Saitoh, O.; Yoshioka, K.; Nakata, H. Oligomerization of adenosine A_{2A} and dopamine D_2 receptors in living cells. *Biochem. Biophys. Res. Commun.* **2003**, *306*, 544−549.
(23) Ciruela, F.; Burgueño, J.; Casadó, V.; Canals, M.; Marcellino, D.; Goldberg, S. R.; Bader, M.; Fuxe, K.; Agnati, L. F.; Lluis, C.; Franco, R.; Ferré, S.; Woods, A. S. Combining mass spectrometry and pull-down techniques for the study of receptor heteromerization. Direct epitope-epitope electrostatic interactions between adenosine A_{2A} and dopamine D_2 receptors. *Anal. Chem.* **2004**, *76*, 5354−5363.
(24) (a) Ferré, S.; von Euler, G.; Johansonn, B.; Fredholm, B. B.; Fuxe, K. Stimulation of high-affinity adenosine A_{2A} receptors decreases the affinity of dopamine D_2 receptors in rat striatal membranes. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7238−7241. (b) Popoli, P.; Pezzola, A.; de Carolis, A. S. Modulation of striatal adenosine A_1 and A_2 receptors induces rotational behaviour in response to dopaminergic stimulation in intact rats. *Eur. J. Pharmacology* **1994**, *257*, 21−25.
(25) Aoyama, S.; Kase, H.; Borrelli, E. Rescue of locomotor impairment in dopamine D_2 receptor-deficient mice by an adenosine A_{2A} receptor antagonist. *J. Neuroscience* **2000**, *20*, 5848−5852.
(26) Hockemeyer, J.; Burbiel, J. C.; Müller, C. E. Multigram-scale syntheses, stability and photoreactions of A_{2A} adenosine receptor antagonists with 8-styrylxanthine structure: Potential drugs for Parkinson's disease. *J. Org. Chem.* **2004**, *69*, 3308−3318.
(27) Evans, B. E.; Rittle, K. E.; Bock, M. G.; Dipardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. Methods for drug discovery−Development of potent, selective, orally effective cholecystokinin antagonists. *J. Med. Chem.* **1988**, *31*, 2235−2246.
(28) Guo, T.; Hobbs, D. W. Privileged structure-based combinatorial libraries targeting G protein-coupled receptors. *Assay Drug Dev. Technol.* **2003**, *1*, 579−592.
(29) Messer, W. S. Bivalent ligands for G-protein coupled receptors. *Curr. Pharm. Des.* **2004**, *10*, 2015−2020.
(30) Berde, B.; Stürmer, E. Introduction to the pharmacology of ergot alkaloids and related compounds as a basis of their therapeutic application. In *Ergot Alkaloids and Related Compounds*; Berde, B.; Schild, H. O. Eds.; Springer-Verlag: Berlin, 1978; pp 1−28.
(31) Stadler, P. A.; Stürmer, E. Comparative studies on the pharmacological properties of stereoisomerers of ergotamine and dihydroergotamine. *Naunyn-Schmiedeberg's Arch. Pharmacol*. **1970**, *266*, 457.
(32) Walzel, B.; Riederer, B.; Keller, U. Mechanism of alkaloid cyclopeptide synthesis in the ergot fungus *Claviceps purpurea*. *Chem. Biol.* **1997**, *4*, 223−230.
(33) Jacobson, K. A.; Gao, Z.-G. Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discovery* **2006**, *5*, 247−264.
(34) Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev*. **2001**, *53*, 527−552.
(35) (a) For CGS binding at A_{2A}R, see: Ciruela, F.; Casadó, V.; Rodrigues, R. J.; Luján, R.; Burgueño, J.; Canals, M.; Borycz, J.; Rebola, N.; Goldberg, S. R.; Mallol, J.; Cortés, A.; Canela, E. I.; López-Giménez, J. F.; Milligan, G.; Lluis, C.; Cunha, R. A.; Ferré, S.; Franco, R. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A_1−A_{2A} receptor heteromers. *J. Neurosci.* **2006**, *26*, 2080−2087. (b) For CGS binding at A_{2B}R, see: Casadó, V.; Casillas, T.; Mallol, J.; Canela, E. I.; Lluis, C.; Franco, R. The adenosine receptors present on the plasma membrane of chromaffin cells are of the A2b subtype. *J. Neurochem.* **1992**, *59*, 425−431.
(36) Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. Dopamine receptors: From structure to function. *Physiol. Rev.* **1998**, *78*, 189−225.
(37) Neve, K. A.; Seamans, J. K.; Trantham-Davidson, H. Dopamine receptor signalling. *J. Recept. Signal Transduction* **2004**, *24*, 165−205.
(38) Salim, H.; Ferré, S.; Dalal, A.; Peterfreund, R. A.; Fuxe, K.; Vincent, J.-D.; Lledo, P.-M. Activation of adenosine A_1 and A_{2A} receptors modulates dopamine D_2 receptor-induced responses in stably transfected human neuroblastoma cells. *J. Neurochem.* **2000**, *74*, 432−43.
(39) Casadó, V.; Cantí, C.; Mallol, J.; Canela, E. I.; Lluis, C.; Franco, R. Solubilization of A_1 adenosine receptor from pig brain: Characterization and evidence of the role of the cell membrane on the coexistence of high and low affinity states. *J. Neurosci. Res*. **1990**, *26*, 467−473.
(40) Sarrió, S.; Casadó, V.; Escriche, M.; Ciruela, F.; Mallol, J.; Canela, E. I.; Lluis, C.; Franco, R. The heat shock cognate protein hsc73 assembles with A_1 adenosine receptors to form functional modules in the cell membrane. *Mol. Cell. Biol.* **2000**, *20*, 5164−5174.
(41) Cheng, Y.-C.; Prusoff, W. H. Relation between the inhibition constant (K1) and the concentration of inhibitor which causes fifty per cent inhibition (I50) of an enzymic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(42) Casadó, V.; Casillas, T.; Mallol, J.; Canela, E. I.; Lluis, C.; Franco, R. The adenosine receptors present on the plasma membrane of chromaffin cells are of the A2b subtype. *J. Neurochem.* **2000**, *59*, 425−431.
(43) Ferré, S.; Torvinen, M.; Antoniou, K.; Irenius, E.; Civelli, O.; Arenas, E.; Fredholm, B. B.; Fuxe, K. Adenosine A_1 receptor-mediated modulation of dopamine D_1 receptors in stably cotransfected fibroblast cells. *J. Biol. Chem*. **1998**, *273*, 4718−4724.

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