5100C, Wescor, Inc., Logan, UT). The dilution of the disk fluid below controls without peptide was taken as a measure of osmotic water flow from the mucosal bath into the serosal surface disk. For these studies AVP (Pitressin, Parke-Davis, Morris Plains, NJ) was used as the reference standard.

The Ringer's solution employed in these experiments was composed of the following solutes in mmol/L: NaCl, 110; KCl, 3.5; $CaCl_2$, 1; $MgCl_2$, 1; glucose, 5.5; tris(hydroxymethyl)aminomethane hydrochloride, 10; pH, 7.5; osmolality, 236 mOsm/kg $H_2O$.

**Toad Bladder Hydroosmotic Assay in the Presence of Avidin.** Toad bladders were preincubated for 15 min in Ringer's solution containing 4 mg/mL avidin (Sigma Chemical Co.). Analogue 1 and AVP were assayed in the continued presence of avidin.

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# Binary Drugs: Conjugates of Purines and a Peptide That Bind to Both Adenosine and Substance P Receptors

Kenneth A. Jacobson,*[†] Andrzej W. Lipkowski,[‡,§] Terry W. Moody,[‖] William Padgett,[⊥] Evelyn Pijl,[†] Kenneth L. Kirk,[†] and John W. Daly[⊥]

*Laboratory of Chemistry and Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, Department of Chemistry, Warsaw University, 02-093 Warsaw, Poland, and Department of Biochemistry, George Washington University, School of Medicine and Health Sciences, Washington, D.C. 20037.* *Received March 9, 1987*

A "functionalized congener" approach to adenosine receptor antagonists has provided a means to synthesize highly potent peptide conjugates of 1,3-dialkylxanthines. The antagonist XAC, such a functionalized xanthine amine congener, has been attached to a segment derived from the neurotransmitter peptide substance P (SP) to form a binary drug that binds to both receptors with $K_i$ values of 35 nM (central $A_1$-adenosine) and 300 nM (striatal SP). Coupling of the functionalized adenosine agonist $N^6$-[*p*-(carboxymethyl)phenyl]adenosine to an SP C-terminal peptide also resulted in a binary drug that binds to both receptors. The demonstration that the biochemical properties of two unrelated drugs, both of which act through binding at extracellular receptors, may be combined in the same molecule suggests a novel strategy for drug design. In principle, a combined effect of the two different substances that produce the same final effect (e.g., hypotension by adenosine agonists and by SP analogues) might occur in vivo. Adenosine analogues have analgesic properties, and the binary drug derived from substance P and adenosine agonists or antagonists might provide useful tools for probing interrelationships of SP pathways and sites for the antinociceptive action of adenosine.

A "functionalized congener" approach to adenosine receptor antagonists[1,2] has resulted in highly potent 1,3-dialkylxanthines having amino and carboxylic acid functionalized chains. These functional groups have been coupled covalently to amino acids and dipeptides. The resulting amino acid and oligopeptide conjugates have full, and in some cases enhanced, biological activity. In our previous work[2] we used nonhormonal peptide sequences to alter the physicochemical properties of the drugs. We now report the attachment of a xanthine to a segment derived from a putative neurotransmitter peptide, substance P (SP) (reviews, ref 3,4), to form a binary drug **1** (Figure 1). This drug binds both to adenosine receptors and to substance P receptors with affinity comparable to that of each component at the appropriate receptor. In addition, coupling of the adenosine agonist $N^6$-[*p*-(carboxymethyl)phenyl]adenosine,[5] **2a**, to substance P segment 7-11 produced the binary drug **3**, which also binds to both receptors.

We have demonstrated a potentially general strategy via "functionalized congeners" for the synthesis of binary drugs that combine pharmacological properties of both elements. The choice of combination of adenosine receptor ligands and substance P in target molecules stems from the peripheral hypotensive effect elicited by agonists for both receptors.[6,7] Moreover, since both receptors mediate central nociceptive effects,[8,9] the binary conjugates may prove to be useful tools in probing neuronal pathways

Table I.[a] Potencies of the Binary Drugs and Various Truncated Derivatives and Intermediates at $A_1$-Adenosine Receptors and at Substance P Receptors

| | $K_i$, nM | |
|---|---|---|
| | $A_1$-adenosine receptors | SP receptors |
| substance P analogues | | |
| H-Phe-Phe-Gly-Leu-Met-$NH_2$ (**8a**) | >30 000 | >10 000 |
| Ac-Phe-Phe-Gly-Leu-Met-$NH_2$ (**11**) | >30 000 | >10 000 |
| H-Gln-Phe-Phe-Gly-Leu-Met-$NH_2$ (**8b**) | | 450 |
| H-Gln-Gln-Phe-Phe-Gly-Leu-Met-$NH_2$ (**8c**) | | 180 |
| substance P | >30 000 | 1.1 |
| xanthine derivatives | | |
| XAC (**4a**) | 1.2 ± 0.3 | >10 000 |
| H-Gly-XAC (**4b**) | 2.1 ± 0.1 | >10 000 |
| H-Asn-XAC (**4c**) | 4.3 ± 0.2 | |
| H-Asp(XAC)-Phe-Phe-Gly-$NH_2$ (**10**) | 30 ± 6.0 | >10 000 |
| H-Asp(XAC)-Phe-Phe-Gly-Leu-Met-$NH_2$ (**1**) | 35 ± 3.4 | 300 |
| adenosine derivatives | | |
| $N^6$-$C_6H_4CH_2COOH$ (**2a**) | 210 ± 57 | |
| $N^6$-$C_6H_4CH_2CONHCH_3$ (**2b**) | 16 ± 10 | >10 000 |
| $N^6$-$C_6H_4CH_2CO$-Phe-Phe-Gly-Leu-Met-$NH_2$ (**3**) | 16 ± 0.9 | 2 000 |

[a] The complete sequence of substance P is Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-$NH_2$.

[†] Laboratory of Chemistry, NIDDK, NIH.
[‡] Warsaw University.
[§] While on leave at the University of Minnesota, Department of Medicinal Chemistry and Pharmacognosy, Minneapolis, MN.
[‖] George Washington University, School of Medicine and Health Sciences.
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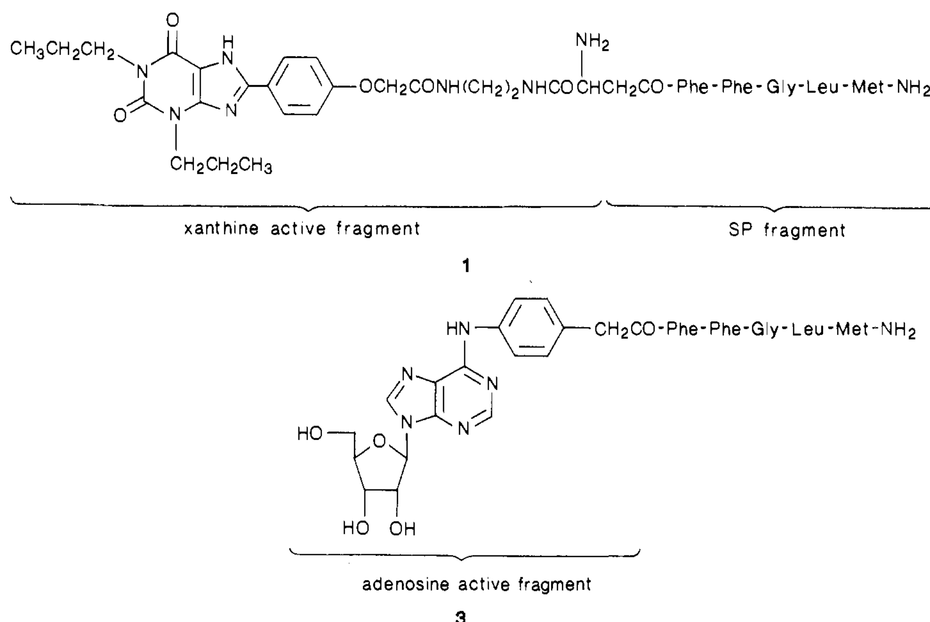


**Figure 1.**

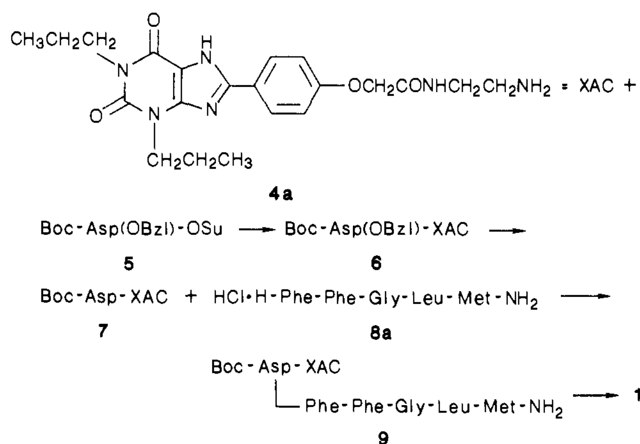


**Figure 2.**

involved in perception of pain.

## Results

The xanthine amine congener (XAC) has several features in its structure that lead to enhanced receptor affinities. Thus, we have found from a study of many xanthine derivatives that an amino group in the appended chain[1,2,10] enhances binding. The 1,3-dipropyl and 8-phenyl substituents present in the parent xanthine amine congener (XAC, 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine), compound **4a** (Figure 2), are also features that enhance receptor binding. Thus XAC was chosen as a promising adenosine ligand for the design of binary drugs.

Various amide derivatives of the substance P terminal pentapeptide amide, **8a** (SP 7–11, see Table I),[11] are highly active. The C-terminal hexapeptide segment of substance P, **8b**, administered intravenously to anesthetized rats has the same hypotensive activity as substance P.[7a] In addition, the pentapeptide has been coupled to other pharmacologically active substances,[13,14] including an enkephalin active fragment, with retention of substance P activity. The pentapeptide itself was reported to be 100- and 2000-fold less potent than substance P in receptor binding[12] and contraction of guinea pig ileum,[4] respectively. Thus, SP 7–11 seemed a promising choice for the SP portion of the binary drug.

XAC and SP 7–11, **8a**, were joined covalently through the $\alpha$- and $\beta$-carboxylate groups, respectively, of L-aspartic acid, thus preserving a free amino group on the chain, to form compound **1** in several steps (Figure 2). In a one-step reaction, $N^6$-[(carboxymethyl)phenyl]adenosine, **2a**, was coupled to the same peptide precursor, forming compound **3**.

The binary drugs **1** and **3** inhibited the binding of [$^3$H]-$N^6$-(phenylisopropyl)adenosine to rat cerebral cortex membranes, a measure of potency at $A_1$-adenosine receptors, with $K_i$ values in the range of $10^{-8}$ M (Table I). Potency at substance P receptors was measured by in-

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hibition of specific binding of [$^{125}$I]physalaemin[15] to the receptors in rat telencephalon slices. The results show that the ability of one molecule to bind to either receptor is achieved when both components are structurally complete, e.g., 1 and 3. The results from a truncated xanthine–peptide derivative, 10, demonstrate that the entire C-terminal SP sequence is required for binding to SP receptors, but not to adenosine receptors. It is curious that the affinity of 1 at SP receptors depends largely on the presence of the xanthine-bearing aspartyl residue bound at Phe$^7$. In contrast, compound 11 (*N*-acetyl SP 7–11), a truncated variation of 1, does not bind measurably to SP receptors.

The affinity of the adenosine–SP conjugate 3 at $A_1$-adenosine receptors is much greater than that of the precursor, **2a**. The relatively low potency of carboxylic acid congener **2a** is likely due to the presence of an anionic group (unpublished) on this analogue. In contrast, the simple *N*-methyl carboxamide derivative, **2b**, has 13-fold-greater affinity for $A_1$-adenosine receptors (Table I) than **2a**. Both compounds **2a** and **2b** were inactive at substance P receptors, while the adenosine–SP conjugate **3** was only 4-fold less potent than a substance P analogue containing a glutamine residue in place of the functionalized adenosine moiety.

## Discussion

The demonstration that the interactive properties of two unrelated drugs that act through binding at extracellular receptors may be combined in the same molecule suggests a novel and potentially general strategy for drug design. Adenosine agonists, including amide derivatives of $N^6$-[*p*-(carboxymethyl)phenyl]adenosine,[6] produce in vivo an intense vasodilatory response, related to the $A_2$-adenosine receptor. Substance P and its agonist analogues long have been known to lower blood pressure[7] when administered systemically. Thus the adenosine agonist–SP binary conjugate 3 might be expected to cause hypotension by two possible mechanisms, involving different receptors. The goal in the design of binary drugs is to achieve a desirable combination of final effects in vivo. A conjugate of two substances having an overlapping physiological action might produce such an additive effect.

The adenosine antagonist–SP binary conjugate 1, administered in combination with an adenosine agonist, also may lead to a combined antihypertensive effect, depending on the receptor subtype selectivity of the xanthine portion. The xanthine precursor of 1, XAC, has been shown to be a selective $A_1$-adenosine receptor antagonist (20-fold) in cardiovascular studies in vivo.[16] Other related xanthine derivatives,[2,17] including some amino acid conjugates, are also $A_1$ selective. In vivo, an $A_1$-selective adenosine antagonist, in combination with a potent and nonselective adenosine agonist, e.g., 5′-deoxy-5′-(ethylamino)-5′-oxoadenosine (NECA), contributes to a net effect of vasodilation ($A_2$) without cardiac depression ($A_1$).[16] In vivo studies with the binary conjugates are in progress.

In the case of the present binary drugs, antagonism of central adenosine receptors by the xanthine portion in combination with activation of substance P neuronal pathways by the peptide portion might lead to a profoundly nociceptive drug when administered centrally. Adenosine analogues do have analgesic properties,[18] and the binary drug containing an adenosine moiety also might provide a useful tool for probing interrelationships with substance P pathways.

Another goal of the binary drug approach is to take advantage of the proposed phenomenon of bridging between two receptors in proximity on the same membrane. Such bridging has been postulated for bivalent conjugates of opiate receptor ligands.[19] It is unknown whether this phenomenon occurs with these binary ligands for two different receptors, but it remains a possibility in light of the high affinity of conjugate 1. It is to be noted that SP receptors[15] and one subclass of $A_2$-adenosine receptors both occur in high density in the striatum relative to other brain regions.

Diminished potency of binding, resulting from aggregation of the two moieties, as was observed for dimeric enkephalins,[20] was not apparent in the case of these binary drugs.

## Experimental Section

**Synthesis.** Compounds **2a**, **b**, **4a–c**, and **8a** were prepared as described.[1,5,11] 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDAC) and 1-hydroxybenzotriazole (HOBt) were from Sigma (St. Louis, MO). Reagent grade dimethylformamide (Aldrich, gold label) was stored over 3 Å molecular sieves. Thin-layer chromatography was carried out on Analtech silica gel GF plates. Reverse-phase high-pressure liquid chromatography (HPLC) was carried out on an Altex Ultrasphere ODS 5 μm column. NMR specrta (300 MHz; Varian) of all compounds reported here were consistent with the assigned structures. Elemental analyses were carried out by Atlantic Microlabs (Atlanta, GA). Larger molecular weight species were analyzed by using californium plasma desorption mass spectroscopy[21] by H. Fales and L. Pannell of NIH.

***N*$^\alpha$-[*N*$^6$-[(Carboxymethyl)phenyl]adenosinyl]-L-phenylalanyl-L-phenylalanylglycyl-L-leucyl-L-methionine Amide (3).** $N^6$-[(Carboxymethyl)phenyl]adenosine, **2a** (67 mg, 0.17 mmol), was coupled, by using EDAC (77 mg, 0.39 mmol) and HOBt (40 mg, 0.30 mmol) in DMF (3 mL), to the substance P segment 7–11 (**8a**, 92 mg, 0.14 mmol) in 92% yield. The solid product, **3** (128 mg), recrystallized from DMF/water, melted at 230–235 °C, and was homogeneous by TLC, anal. ($C_{49}H_{61}N_{11}O_{10}S.H_2O$) C,H,N. NMR ($(CD_3)_2SO$): δ 8.53, 8.38 (each s, 1 H, adenine), 8.2 (2 H, NH's), 8.0 (3 H, NH's), 7.76 (d, 2 H, C-2 of NHAr, $J$ = 9 Hz), 7.2 (m, 13 H, aromatic), 7.0–7.1 (3 H, NH's), 5.95 (d, 1 H, ribose $C_1$, $J$ = 6.6 Hz), 5.50 (d, 1 H, OH), 5.31 (t, 1 H, OH), 5.22 (d, 1 H, OH), 4.64 (m, 1 H, ribose CHO), 4.51 (m, 2 H, $C_\alpha$Phe), 4.2–4.3 (2 H, $C_\alpha$Leu, Met), 4.17, 3.98 (each m, 1 H, ribose CHO), 3.73 (d, $CH_2$Gly), 3.68 (m, 1 H, ribose $CH_{5'}$), 3.6 (3 H, Ar$CH_2$CO + ribose $CH_{5'}$), 2.7–3.0 (4 H, $C_\beta$Phe), 2.4 (m, 2 H, $C_\gamma$Cys), 2.01 (s, 3 H, $CH_3$Met), 1.8–2.0 (4 H, $C_\beta$, Leu, Met), 1.71 (s, 3 H, Ac), 1.48 (m, 1 H, $C_\gamma$Leu), 0.86 (dd, 6 H, $C_\delta$Leu).


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**$N^{\alpha}$-(*tert*-Butyloxycarbonyl)-α-L-aspartyl-8-[4-[[[[(aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine β-Benzyl Ester (6).** The xanthine amine congener, **4a** (0.12 g, 0.28 mmol), was added in small portions to a solution of Boc-β-benzyl-L-aspartic acid succinimido ester (Sigma, 0.16 g, 0.38 mmol) in DMF (10 mL). The reaction mixture was worked up as described,[2] to give the amino acid conjugate, **6** (0.21 g, 100% yield): mp 199–201 °C; $[\alpha]^{22}_{D}$ −3.7° (*c* 1.2, $Me_2SO$). Anal. ($C_{37}H_{47}N_7O_9{\cdot}H_2O$) C, H, N.

**[$N^{\alpha}$-(*tert*-Butyloxycarbonyl)-α-aspartyl[8-[4-[[[[(aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthin-4.2-yl]]-L-phenylalanyl-L-phenylalanylglycyl-L-leucyl-L-methionine Amide (9).** The benzyl ester of **6** (80 mg, 0.11 mmol) was removed by hydrogenolysis in 5 mL of DMF over 10% Pd/C to give the resulting carboxylic acid, **7**, which was homogeneous by TLC. The catalyst was removed by filtration through Celite, and **7** was coupled in situ. The substance P derived peptide, L-phenylalanyl-L-phenylalanylglycyl-L-leucyl-L-methionine carboxamide hydrochloride, **8a** (120 mg, 0.18 mmol), excess EDAC (200 mg, 0.90 mmol), and HOBt (120 mg, 0.90 mmol) were added. After 15 min, the peptide amine was liberated upon addition of diisopropylethylamine (0.15 mL). After 1 h, water (12 mL) was added, and a white precipitate formed. The product, **9** (55% yield), mp 253–255 °C (recrystallized twice from DMF/ethyl acetate/ether), was homogeneous by TLC: silica, $CHCl_3$/MeOH/HOAc, 85:10:5, $R_f$ 0.64. Anal. ($C_{61}H_{83}N_{13}O_{13}S{\cdot}0.7DMF{\cdot}0.3H_2O$) C, H, N. NMR ($(CD_3)_2SO$): δ 8.23 (d, 1 H, NH, $J$ = 7.8 Hz), 8.16 (t, 1 H, NHGly), 8.08 (d, 2 H, 2-position of 8-aryl, $J$ = 8.8 Hz), 7.9–8.1 (m, NH's, 6 H), 7.1–7.3 (14 H, aromatic + $NH_2$), 6.84 (d, 1 H, NH Boc, $J$ = 7.7 Hz), 4.54 (s, 2 H, $CH_2OAr$), 4.4 (m, 2 H, $C_{\alpha}$Phe), 4.1–4.3 (3 H, $C_{\alpha}$'s), 4.02, 3.87 (each d, 2 H, $C_{\alpha}$ *n*-propyl), 3.71 (d, $CH_2$Gly, $J$ = 5.4 Hz), 3.17 (s, 4 H, NH($CH_2$)$_2$NH), 2.6–3.1 (4 H, $C_{\beta}$Phe), 2.5 (m, $C_{\beta}$Asp), 2.4 (m, $C_{\gamma}$Cys), 2.01 (s, 3 H, $CH_3$Met), 1.8–2.0 (4 H, $C_{\beta}$, Leu, Met), 1.74, 1.58 (each m, 2 H, $C_{\beta}$ *n*-propyl), 1.48 (m, 1 H, $C_{\gamma}$Leu), 1.39 (s, 9 H, *t*-Bu), 0.9 (12 H, $C_{\delta}$Leu + $C_{\gamma}$ *n*-propyl).

**[α-Aspartyl[8-[4-[[[[(aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthin-4.2-yl]]-L-phenylalanyl-L-phenylalanylglycyl-L-leucyl-L-methionine Amide Trifluoroacetate (1).** The BOC group of **9** was removed in neat trifluoroacetic acid containing 2% thiophenol. Evaporation of the solution and trituration of the residue with ether gave the binary drug **1**, mp 160–163 °C. Californium plasma desorption mass spectroscopy[21] showed intense peaks at 1140 and 1161 (+ ions) corresponding to M + 1 and M + Na + 1. By HPLC (C-18, mobile phase: 65–75% MeOH/0.05 M triethylammonium trifluoroacetate, 1.0 mL/min) compound **1** was >95% pure, and biologically active xanthine precursors were undetectable (<1%).

**[α-Aspartyl[8-[4-[[[[(aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthin-4.2-yl]]-L-phenylalanyl-L-phenylalanylglycine Amide Trifluoroacetate (10).** Compound **7** was coupled to HCl·H-$Phe_2$-Gly-$NH_2$ to give Boc-Asp-α-XAC[β-($Phe_2$-Gly-$NH_2$)] in 58% yield, mp 288–290 °C, and the Boc group was deprotected as above to give compound **10**, melting at 216–220 °C dec.

**Acetyl-L-phenylalanyl-L-phenylalanylglycyl-L-leucyl-L-methionine Amide (11).** Compound **7** (80 mg) was treated with acetic anhydride in 10% pyridine/THF to give Ac-$Phe_2$-Gly-Leu-Met-$NH_2$ (58 mg, 73% yield), **11**, mp 254–257 °C. Anal. ($C_{33}H_{46}N_6O_6S$) C, H, N. NMR ($(CD_3)_2SO$): δ 8.18 (t, 1 H, NHGly, $J$ = 5.6 Hz), 8.11, 8.03, 7.98, 7.94 (each d, NH, $J$ = 8 Hz), 7.2 (m, 11 H, aromatic), 7.06 (s, 1 H, $CONH_2$), 4.5 (7, 2 H, $C_{\alpha}$Phe), 4.30 (m, 1 H, $C_{\alpha}$), 4.23 (m, 1 H, $C_{\alpha}$), 3.73 (d, $CH_2$Gly), 3.05, 2.94, 2.82, 2.66 (each dd, 1 H, $C_{\beta}$Phe), 2.42 (7 2 H, $C_{\gamma}$Cys), 2.02 (s, 3 H, $CH_3$Met), 1.8–2.0 (4 H, $C_{\beta}$, Leu, Met), 1.71 (s, 3 H, Ac), 1.48 (m, 1 H, $C_{\gamma}$Leu), 0.87 (dd, 6 H, $C_{\gamma}$Leu).

**Receptor Binding.** Competitive binding assays for adenosine receptor affinity were carried out as described[1,10] by using [$^3$H](phenylisopropyl)adenosine. Each value respresents the average of three determinations, each run in triplicate. Protease inhibitors[22] (leupeptin, 4 μg/mL, and bacitracin, 20 μg/mL) were present during incubation of the membranes in the presence of the drug derivatives.

The substance P competitive binding assay was carried out as described.[15] [$^{125}$I]Physalaemin, present during the assay at a concentration of $4 \times 10^{-11}$ M, was the radioligand.

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