ferens with the prostatic and epididymal portions of this tissue following a protocol already described in detail.[4] In order to allow comparison of the results, we used the same techniques and statistical evaluation of the bioassays as for other benzodioxan-related compounds.[4]

The results of 4–6 are shown in Table I together with pA_2 values of parent compounds 1 and 3. It can be seen that insertion of a phenyl ring at position 3 of 1, affording 4 and 5 (Phendioxan) alters markedly both activity and selectivity toward α-adrenoreceptor subtypes. It is also evident that the stereochemical relation between the 2-side chain and the 3-phenyl ring plays a crucial role in drug–receptor interaction. In fact, both isomers 4 and 5 were very weak α_2-adrenoreceptor antagonists while trans isomer 5 (Phendioxan) was more than 3 orders of magnitude more potent than cis isomer 4 at α_1-adrenoreceptors. This finding clearly indicates that the insertion of a phenyl ring at position 3 of 1 is highly detrimental toward α_2-adrenoreceptors either in a trans or in a cis relation with respect to the 2-chain whereas the activity for α_1-adrenoreceptors is negatively affected, compared to that of 1, only for a cis relation as in 4. In fact, 4 is more than 10000 times less active than 1 at α_1-adrenoreceptors whereas the trans isomer 5 is only five times less potent than the parent compound. Furthermore, isomers 4 and 5 not only displayed toward α_1-adrenoreceptors a markedly different activity but also a different type of antagonism, owing to the observation that 5 was a competitive antagonist over a wide range of concentrations (3–3000 μM) whereas 4 behaved as a noncompetitive antagonist.[19] However, the most stricking result of the present investigation is the selectivity toward α_1-adrenoreceptors displayed by 5 which resulted in markedly increased selectivity compared to that of the prototype 1. The high selectivity of 5 could be the result of an unfavorable binding of the 3-phenyl group with α_2-adrenoreceptors, presumably by way of a steric hindrance, while that moiety is still tolerated at the α_1-site where it produces only a slight decrease in affinity (Table I).

We demonstrated that replacement of the oxygen at position 1 of 1 with a carbonyl function, affording 3, does not alter the biological profile of the molecule.[4] Again, the insertion of a phenyl ring at position 2 of 3, affording 6, resulted in an effect which is similar to that observed above for the same structural manipulation performed on 1.

In conclusion, to our knowledge, 5 (Phendioxan) represents, until now, the most selective α_1-adrenoreceptor antagonist in in vitro experiments and it might be not only a useful tool in the characterization of α-adrenoreceptor subtypes but also a lead compound for the design of more selective and more potent antagonists.[23]

(19) This compound behaves as a noncompetitive α_1-adrenoreceptor antagonist since it caused a depression of the maximum response to norepinephrine. Thus, its activity for α_1-adrenoreceptors was expressed as the IC_{50} value that represents the concentration which produces 50% inhibition of the agonist maximal response (Table I).

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(23) Detailed pharmacological characterization of 5 shall appear elsewhere together with the results of ongoing relevant research.

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(1*R*,3*S*)-1-(Aminomethyl)-3,4-dihydro-5,6-dihydroxy-3-phenyl-1*H*-2-benzopyran: A Potent and Selective D1 Agonist

It is now generally accepted that there are two major subtypes of the dopamine receptor in the central nervous system (CNS), designated as D1 and D2.[1] Both receptors are located in postsynaptic neuronal membranes and are linked to the enzyme adenylate cyclase. The receptors differ since activation of the D1 receptor enhances the production of cyclic adenosine monophosphate (cAMP) while stimulation of the D2 receptor subtype inhibits cAMP production. A presynaptic dopamine receptor has also been characterized and is believed to be of the D2 type.[2] The D1 dopamine receptor was once thought to have no important function in the CNS. More recently, however, with the aid of selective agonists and antagonists, significant roles of the central dopamine D1 receptor have been documented.[3] Conversely, the actions of the peripheral DA1 receptor have been understood for some time.[4]

Defects in the dopaminergic neuronal systems in the brain have been implicated in a number of disease states. Parkinson's disease in particular has been widely studied and is characterized by the degeneration of the dopamine-producing neurons in the substantia nigra.[5] We believe that a selective D1 agonist could have therapeutic potential in the treatment of Parkinson's disease. A D1 agonist incapable of CNS penetration, such as fenoldopam, may also have application as a novel antihypertensive. Out of a program aimed at the development of dopaminergic compounds, A68930 [1, (1*R*,3*S*)-1-(aminomethyl)-3,4-dihydro-5,6-dihydroxy-3-phenyl-1*H*-2-benzopyran] was identified as a potent and selective D1 agonist.

Compound 1 was synthesized as shown in Scheme I. The protected catechol derivative 2[6] was lithiated with *n*-BuLi in THF and condensed with styrene oxide to afford the alcohol 3 in 50% yield. The key step was a stereo-

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Table I. In Vitro Pharmacology[a]

compound	D1 K_i, nM	D1 EC_{50}, nM	D1 %IA	D2 K_i, nM	D2 EC_{50}, nM	D2 %IA
1	3.0 (2.1–4.3)	2.1 (1.7–2.64)	72 ± 6.5	776 (501–1200)	3910 (3110–4930)	101 ± 1.9
9	1.6 (1.07–2.4)	1.95 1.5–2.5)	60 ± 3.4	807 (486–1340)	10200 (5920–17700)	98 ± 6.1
10	7200 (6580–7880)	8577 (7330–10000)	99 ± 8.6	>10000	>10000	
11	1030 (681–1570)	490 (370–649)	71 ± 7	4610 (2290–9280)	2850 (2290–3550)	96 ± 2.5
12 (SKF38393)	64.1 (49–84)	386 (244–612)	61 ± 3	6870 (5350–8800)	>10000[b]	

[a] Values represent the mean with the range of the SEM in parentheses. Binding tissue/ligand preparations were as follows. D1: homogenized rat caudate/[^{125}I]SCH23982.[13] D2: homogenized rat caudate/[^{125}I]-*N*-(*p*-aminophenethyl)spiroperidol.[14] Functional assay tissues were as follows. D1: cell free homogenate of carp retina. D2: cell free homogenate of rat intermediate lobe.[15] [b] Data extracted from ref 16.

specific cyclization to the isochroman system using bromoacetaldehyde dimethyl acetal under BF_3–OEt_2 catalysis. From this reaction, only the 1,3-cis-substituted product **4** could be isolated. Conversion of the bromide to an amine (LiN_3, DMF; LAH, Et_2O; 70%) followed by deprotection (HCl, EtOH) gave catechol amine **1** as its HCl salt.

Since the chiral center formed at C1 is controlled by the center at C3, a straightforward enantioselective synthesis was envisioned and carried out as follows. Oxidation of alcohol **3** with PCC afforded ketone **6** in high yield. Reduction of **6** with the (+)- or (−)-*B*-chlorodiisopinocampheylborane reagent developed by H. C. Brown[7] furnished the (*R*)- and (*S*)-alcohols, respectively, in 75% yield and 98% ee.[8] The synthesis was completed as described above, and the enantiomeric purity of compounds **9** and **10** was determined to be ≥98% ee.[8]

The in vitro activity upon the D1 receptor of the synthesized compounds was assessed in two assays.[9] The first test is a binding assay determining the ability of a compound to displace a radiolabeled ligand ([^{125}I]SCH23982).[10] The binding K_i for **1** resulting from this analysis was 3.0 nM. The second assay is a functional test determining the formation of cAMP on D1 receptor stimulation. From this assay, an EC_{50} is obtained as well as an intrinsic activity (IA) based upon the maximal response to dopamine. Isochroman **1** exhibited an EC_{50} of 2.1 nM with an IA of 72% (Table I). Our data for benzazepine **12** (SKF38393),[10] the most widely studied D1 agonist, is shown for comparison.

To determine the selectivity of the agonists, they are screened in similar assays for the D2 receptor. The result for **1** was a D2 receptor binding K_i of 776 nM and an EC_{50} of 3910 nM for inhibition of cAMP release, indicating a D1/D2 selectivity in the functional assay of greater than 1500:1.

The pharmacology of the enantiomers proved to be quite remarkable. The 1*R**,3*S** isomer **9** has a D1 binding K_i of 1.6 nM and an EC_{50} of 1.95 nM. At the same receptor, the 1*S**,3*R** isomer **10** exhibited a K_i of 7200 nM and an EC_{50} value of 8580 nM, indicating that all of the dopaminergic activity resides in one enantiomer.[11] Furthermore the importance of the 3-phenyl substituent was demonstrated by the comparison of **1** with desphenyl analogue **11**, which was 200–400-fold less potent.[12]

Scheme I

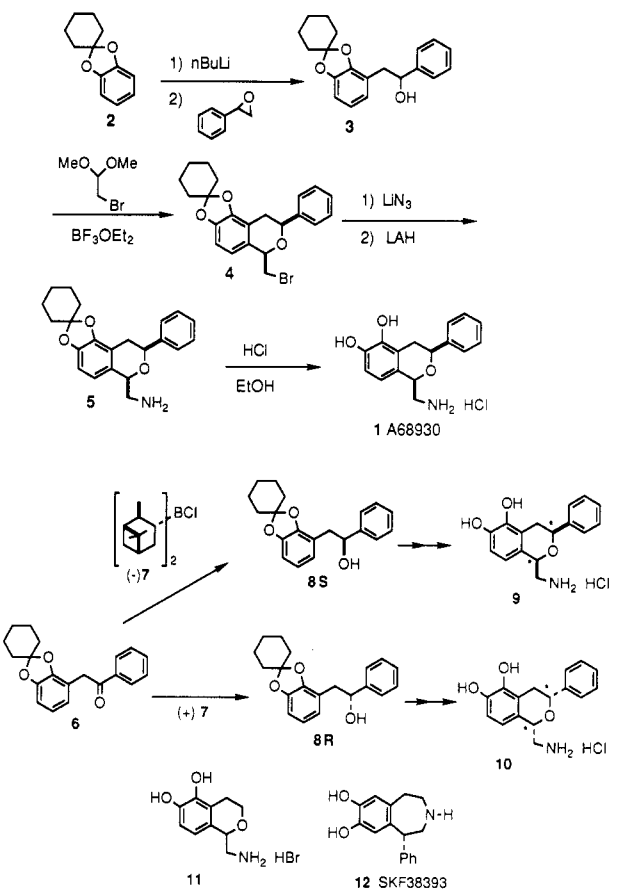

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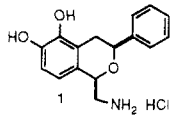
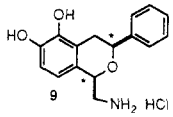
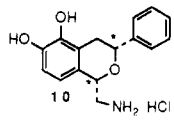
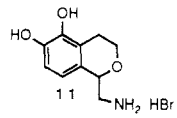
Table II. In Vivo Behavioral Data

structure	dose, μmol/kg (sc)	contralateral rotations/2 h
 1	0.41	192 ± 83
	1.2	895 ± 185
	4.1	1554 ± 194
	20.3[a]	326 ± 117
	40.6[a]	1066 ± 308
 9	0.20	348 ± 210
	1.02	1113 ± 227
	2.03	1525 ± 384
 10	2.03	-22 ± 13
	20.3	-24 ± 10
 11	65.0	93 ± 66
	130	286 ± 119
	260	584 ± 161

[a] Oral dose.

A standard behavioral method to access central dopaminergic receptor stimulation is the rat rotation model.[17] Rats with unilateral 6OHDA lesions of the nigro-striatal bundle rotate contralaterally to the lesion in response to exposure to a direct D1 or D2 agonist. The results from this assay using the isochromans are shown in Table II. Compound 1 was shown to be a very potent agonist in vivo by the subcutaneous (sc) route of administration. The compound was also demonstrated to be orally active, although the data suggests it has a low bioavailability. The activity of the enantiomers follow as predicted from the in vitro data. Compound **9** was potent in the whole animal while **10** was inactive in doses as high as 20 μmol/kg (sc).

In conclusion, A68930 (1) has been demonstrated to be a potent and selective D1 agonist. It is potent in vitro and is orally active in vivo. It has also been shown that all of the dopaminergic activity residues in the 1*R**,3*S** enantiomer. In addition to their therapeutic potential, these compounds represent useful probes of the D1 receptor. Further work on the SAR of this class of dopaminergic compounds will be disclosed in future publications.

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Development of CCK-Tetrapeptide Analogues as Potent and Selective CCK-A Receptor Agonists

Cholecystokinin (CCK) is a member of the brain-gut family of peptides that is found in a number of mammalian species including man. Various biological functions are attributed to this peptide including gall bladder contraction and release of pancreatic enzymes.[1,2] In addition, CCK may be involved with mechanisms linked to analgesia, appetite regulation, and dopamine modulation.[3,4] The receptors for CCK are classified into two sub-types: CCK-A (alimentary), found predominantly in peripheral tissues such as the pancreas and gall bladder, and CCK-B (brain), localized in the central nervous system and shown to have a similar ligand-binding profile to the gastrin receptor.[5,6] These receptors can be differentiated by their relative affinity for CCK and its fragments. While CCK and CCK-8 bind potently (low nanomolar) to both the peripheral and brain type receptors, BOC-CCK-4, the C-terminal tetrapeptide, exhibits high affinity only for the CCK-B receptor (Table I). The desulfated octapeptide (CCK-8-DS) is significantly less potent for the pancreatic receptor. The CCK-A receptor in the pancreas is coupled to phosphatidylinositol turnover (PI) while no such second messenger system has been definitely linked to the CCK-B receptor.

Recently, much attention has focused on the development of potent antagonists for the CCK-A receptor. A number of structurally diverse compounds is now available that exhibit high potency and selectivity for the CCK-A receptor and are being used to investigate the physiological roles of these receptors. Examples of these antagonists include the benzodiazepine MK-329, developed by Merck,[7] and the glutamic acid derivative A-65186, disclosed by Abbott.[8] Efforts to determine what structural relationships exist between these two series of compounds are underway. Recently, the Merck group has further modified the benzodiazepine structure to produce a derivative, L-365,260, with high potency and selectivity for the gastrin and CCK-B receptors.[9]

Although much progress has been made in developing non-peptide CCK-A antagonists, potent CCK-A agonists reported to date have remained sizeable peptides consisting of seven or eight amino acids.[10-12] In addition, an acidic function such as a sulfated hydroxyl on tyrosine was found to be necessary for high potency at the CCK-A receptor, the desulfated analogues being roughly 2-3 orders of magnitude less potent. Thus, the potent peptide agonists described in the literature do not differ significantly in structure from CCK-8 itself. Many of these peptides share another common feature to CCK-8 by binding with similar

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