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Perspective

Cocaine Receptor: Biochemical Characterization and Structure–Activity Relationships of Cocaine Analogues at the Dopamine Transporter

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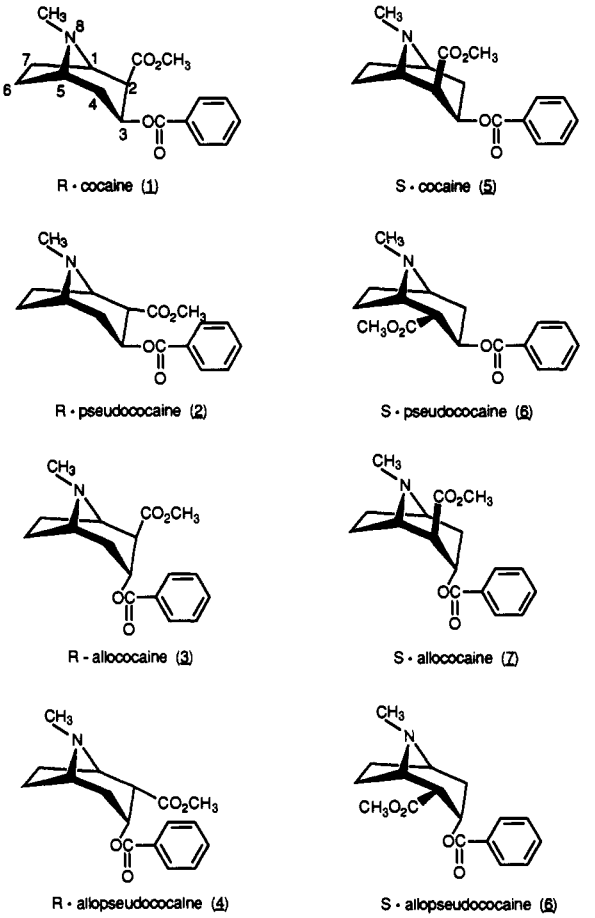
Introduction

(*R*)-Cocaine (1) is a plant alkaloid purified from the leaves of *Erythroxylon coca* and has been a subject of scientific investigation since the late 1800s. It is one of the eight possible stereoisomeric forms of methyl 3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octan-2-carboxylate.[1] The other three natural diastereomers are designated as (*R*)-pseudococaine (2), (*R*)-allococaine (3), and (*R*)-allopseudococaine (4), while the four isomers deriving from the enantiomer of natural cocaine are (*S*)-cocaine (5), (*S*)-pseudococaine (6), (*S*)-allococaine (7), and (*S*)-allopseudococaine (8).[2]

It is well known that Sigmund Freud was one of the first investigators who studied the effects of cocaine and who in fact proposed its use in the treatment of alcohol and opiate abuse! Even though this error was soon recognized, cocaine has continued to be abused, and we have experienced a serious cocaine abuse epidemic in this country over the past decade or so.[3] In both animals and humans, cocaine is one of the most reinforcing drugs known,[4,5] which presumably relates to its great abuse potential.

Cocaine has many physiological effects. It is a local anesthetic and this property is responsible for its early legitimate use in medicine. However, many newer compounds have been developed that are superior to cocaine for this purpose. Cocaine is also a powerful vasoconstrictant and as such has some current use in medicine during nasal or throat surgery where control of bleeding is desired. Cocaine also has very potent effects on the sympathetic nervous system, and it is well known to increase heart rate and blood pressure. From the point of view of drug abuse, the most relevant effects of the drug include its ability to produce euphoria and its reinforcing properties. The latter are readily demonstrated in animal models and the euphorigenic effects of cocaine are amply



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documented in human subjects.[5,6] In addition to being a powerful reinforcer, cocaine also has properties common to other drugs of abuse. For example, tolerance occurs to some of its effects, and its psychological withdrawal syndrome takes place over a long time period, which includes periods of craving during which relapse to drug use may often occur.[5,7]

Over the past 10 years, there have been significant advances in understanding the mechanism of action of cocaine. The development of drug self-administration[6] as a useful animal model for reinforcing properties has led to exploration of many of the physiological, neurochemical, neuroanatomical, and pharmacological correlates.

Cocaine has several sites of action in the central nervous system. It has been shown to block the reuptake of norepinephrine (NE), serotonin (5-HT), and dopamine (DA)[8-13] as well as to exert effects on the cholinergic muscarinic, and σ receptors.[14,15] Any one or a combination of these could mediate effects related to the abuse of cocaine. At present, however, it is mainly the dopaminergic pathway that has been implicated in the reinforcing properties of cocaine.[16]

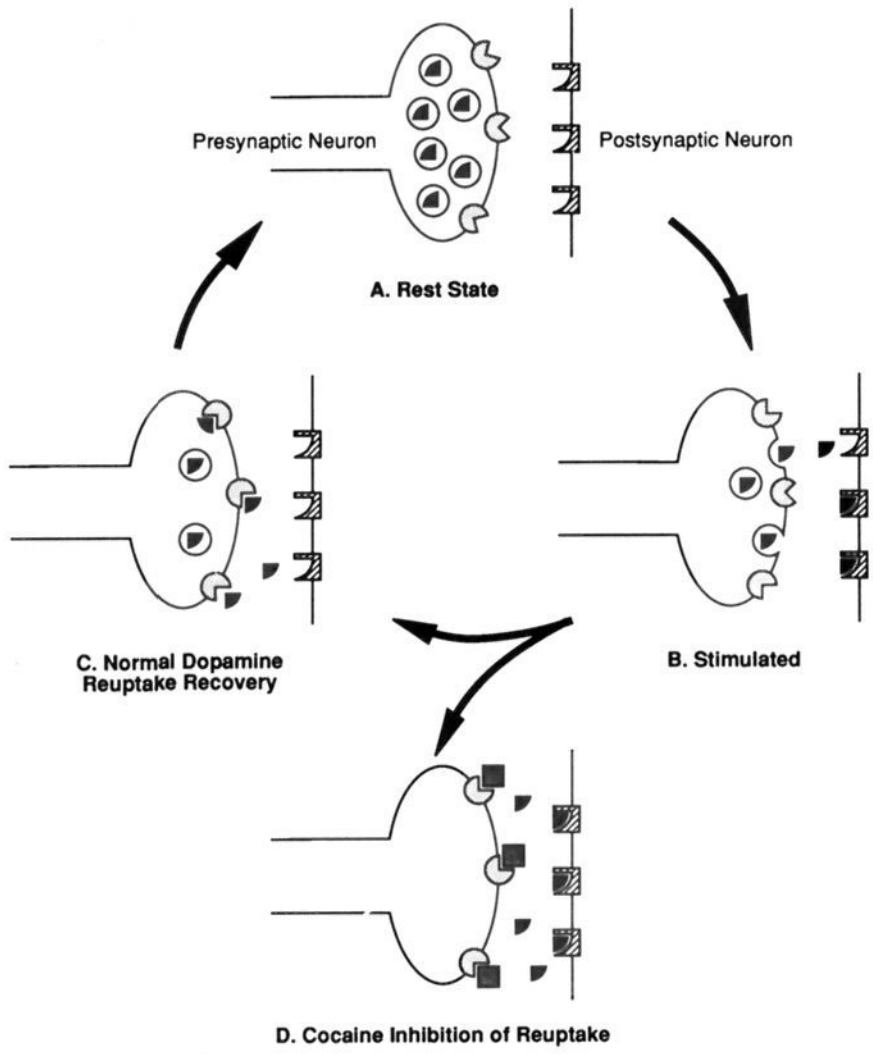
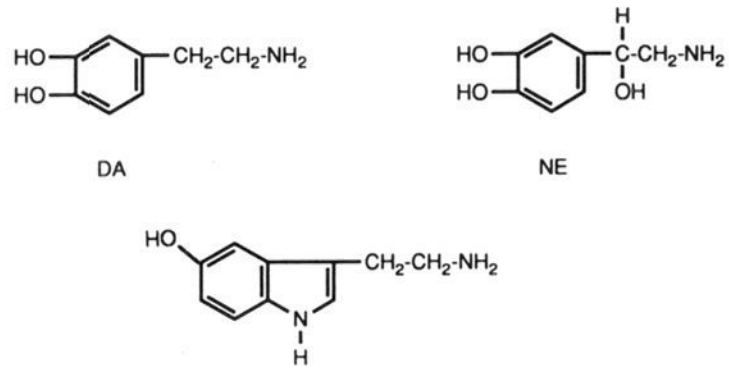


Figure 1. A hypothesis of cocaine's reinforcing action. When a nerve terminal in the resting state (A) is stimulated (B), dopamine (black triangles) is released and diffuses across the synaptic gap. The stimulating action of dopamine ends with its reuptake, by dopamine transporters, into the presynaptic neuron (C). Cocaine (squares) binds to and blocks the transporter function, flooding the synapse with excess dopamine (D). Other inhibitors of dopamine uptake would act in similar fashion.



In fact, the predominant theoretical base for the reinforcing properties of cocaine is the so called "dopamine hypothesis"[16-18] (cf. Figure 1 for a pictorial representation of the hypothesis). This hypothesis assumes that cocaine binds to the dopamine transporter site in a way which inhibits dopamine transport. Since the dopamine transporter is a unique constituent of dopaminergic nerve terminals serving as the primary mechanism to remove dopamine from the synaptic cleft after its release,[19] inhibition

(1) The *Chemical Abstracts* name for natural (−)-cocaine is [1*R*-(*exo*,*exo*)]-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester. The 1*R* designation refers to the configuration of the natural cocaine ring structure following the Cahn, Ingold, and Prelog (1966) convention. For simplicity, the more familiar traditional names are used in this perspective. However, all structures derivable from the natural and unnatural cocaine ring system will be designated by an *R* and *S* prefix, respectively. Thus, natural and unnatural cocaine are designated (*R*)-cocaine (**1**) and (*S*)-cocaine (**2**), respectively.

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of dopamine uptake results in a buildup of dopamine in the synaptic cleft, leading to significant potentiation of dopaminergic transmission. This potentiation, which presumably does not occur under natural circumstances, is responsible for the reinforcing properties of cocaine and perhaps for some of its euphorigenic effects as well. The mesolimbocortical pathway is the circuit that has been related to reinforcing effects of cocaine, particularly dopaminergic nerve terminals in the nucleus accumbens and prefrontal cortex.[20-22] Thus, dopamine interaction (binding) with the dopamine receptors alters the functional state of limbic areas leading to the observed behavioral effects of cocaine.

Because of the important role of the dopamine transporter in the action of cocaine, a very large effort has been focused on the transporter and its characteristics and properties have been the subject of many studies.

1. The Dopamine Transporter

A. Early Studies. Dopamine transport into dopaminergic nerve terminals was demonstrated many years ago (see Horn[23] for references). The process was shown to be saturable and to depend on both sodium and choride ions.[19,23,24] Also, it was shown quite early that a variety of drugs inhibited dopamine transport. These included cocaine, amphetamine, benztropine and some of the tricyclic drugs, and closely related compounds.[23] The fruits of this early work were significant in that it became clear that the dopamine transporter was an important component of a functioning dopaminergic nerve terminal. These experiments also suggested that the transporter was pharmacologically significant since it was affected by a number of drugs the actions of which were, at least in part, attributed to interaction with this transporter.

B. The Dopamine Transporter as a Cocaine Receptor. As mentioned briefly above, binding data have indicated that the dopamine transporter, or at least the cocaine binding site associated with the transporter, has the properties associated with drug self-administration. These studies showed that a highly significant correlation exists between the potencies of a variety of cocaine-like compounds in binding at the dopamine transporter with the potencies of these same compounds in eliciting self-administration behavior.[25,26] Because of this close correlation between the action of drugs at the transporter with their action in an animal model of the abuse liability of cocaine, it was hypothesized that the dopamine transporter is the cocaine "receptor", or the initial site of action that ultimately causes the reinforcing properties of the drug. This is the cornerstone of the dopamine hypothesis. The extensive evidence supporting this hypothesis has been summarized[16] and includes the following:

(a) The potency of various cocaine-related compounds in maintaining self-administration can be predicted by each compound's affinity for the dopamine transporter but cannot be predicted by the compound's affinity for the norepinephrine or serotonin transporters.[25]

(b) Lesion of the dopaminergic innervation of the nucleus accumbens disrupts self-administration of cocaine,[27-29] whereas lesions of the noradrenergic system or dopaminergic terminal in the striatum are without similar effect.[27,30]

(c) Cocaine craving in humans is reduced by indirect agonists of dopamine (compounds which block DA uptake such as mazindol and methylphenidate).[31,32]

(d) The increase of extracellular dopamine levels in the brain following administration of cocaine has a time course that is in approximate agreement with the time course of the euphoria reported by humans taking cocaine.[33-36]

C. Development of Binding Agents. The development of receptor binding techniques in general[37] has led to the identification of many drug related sites. This approach has been enormously successful in pharmacology and has led to a new level of understanding of drug receptors in the brain and other tissues. While neurotransmitter receptors have been a major focus of binding studies, other drug receptors have been studied as well. Indeed, many important biological macromolecules can be

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Table I. Potentials for Inhibition of Radioligand Binding and Dopamine Uptake at the Dopamine Transporter for Selected Dopamine Inhibitors

		IC_{50}, μM, for inhibition of							
		binding							uptake:
entry no.	compd	[^{3}H]cocaine	[^{3}H]WIN-35428	[^{3}H]WIN-35065-2	[^{125}I]RTI-55	[^{3}H]-BTCP	[^{3}H]-mazindol	[^{3}H]GBR-12935	[^{3}H]dopamine
1	(*R*)-cocaine	0.139[a]	0.15[b]	1.81[c]	0.066[d]			1.45[e]	0.843[a]
2		0.065[f]	0.102[g]				0.387[g]		5.02[g]
3		0.068[h]	0.1[i]						2.75[i]
4		0.138[j]	0.208[k]			0.161[k]		0.687[k]	0.87[j]
5		0.36[l]					0.64[n]	5.2[o]	2.42[l]
6		0.068[m]						2.4[q]	0.815[q]
7		0.032[p]						0.525[r]	0.69[r]
8						0.384[s]			0.317[s]
9							0.178[t]		2.48[t]
10									0.8[u]
11									1.4[v]
12									2[w]
13	mazindol	0.009[h]	0.014[k]	0.29[c]	0.007[d]	0.013[k]		0.077[k]	0.085[v]
14			0.008[h]					0.093[r]	0.029[r]
15								0.276[q]	0.252[q]
16								0.072[e]	
17								0.33[o]	
18	GBR 12909	0.036[h]	0.033[k]	0.024[d]	0.001[c]	0.004[k]		0.003[k]	
19								0.004[r]	0.001[r]
20								0.009[q]	0.035[q]
21								0.0047[e]	
22								0.092[o]	
23	BTCP	0.042[h]	0.002[k]			0.005[4]k		0.02[k]	
24						0.008[s]			0.014[s]
25	Nomifensine	0.12[l]	0.046[k]	0.43[c]		0.034[k]		0.115[k]	0.24[l]
26		0.02[h]				0.14[s]		0.25[e]	0.077[s]
27									0.22[v]

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identified and characterized by binding techniques.[38]

[^{3}H](*R*)-Cocaine binding was first reported in 1980 by Reith and co-workers[39] who identified a sodium-independent binding site in brain that was later shown to have the characteristics of the serotonin transporter. Kennedy and Hanbauer[40] showed that [^{3}H](*R*)-cocaine could bind to the dopamine transporter in rat striatal tissue; this, of course, is the site that later proved to be the cocaine "receptor" associated with drug self-administration.[25,26]

Several binding ligands for the dopamine transporter have been identified and developed. These include nomifensine,[41] methylphenidate,[42] 1-[2-(diphenylmethoxy)-ethyl]-4-(3-phenylpropyl)piperazine (GBR 12935),[43,44] mazindol,[13] *N*-[1-(2-benzo(*b*)thiophenyl)cyclohexyl]-piperidine (BTCP),[45] and cocaine and its analogues[39,40,46–52]

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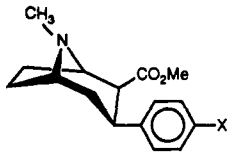
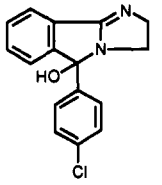
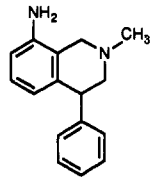
(Table I). Of these, [^{3}H]cocaine has appeal as a probe for the effects of cocaine-like compounds at the dopamine transporter. However, in addition to being nonselective,[25,39] it suffers from relatively low affinity associated with a high dissociation rate.[39,48,49] The other radioligands, mazindol, GBR 12935, BTCP, 3β-(4-fluorophenyl)tropan-2β-carboxylic acid methyl ester (WIN 35,428), 3β-phenyltropan-2β-carboxylic acid methyl ester (WIN 35,065-2), and 3β-(4-iodophenyl)tropan-2β-carboxylic acid methyl ester (RTI-55), all have higher affinity and are, therefore, expected to be experimentally more useful. However, the observation that mazindol and GBR bind to only one site while [^{3}H](*R*)-cocaine labels two sites suggests that the former two ligands may not reveal all the important information regarding cocaine binding.



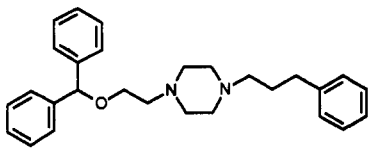
WIN 35,065-2, X = H
WIN 35,428, X = F
RTI-55, X = I



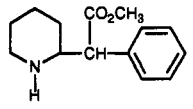
Mazindol



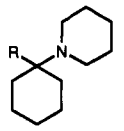
Nomifensine

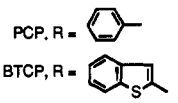


GBR-12935



Methylphenidate





Four ligands, [^{3}H]WIN 35,428, [^{3}H]WIN 35,065-2, [^{3}H]BTCP, and [^{125}I]RTI-55, exhibit the presence of both high- and low-affinity binding sites, just like cocaine. For binding assays, [^{3}H]WIN 35,428 and [^{3}H]WIN 35,065-2 are both useful. The ratios of their binding affinities at the two sites are 14 and 29,[50,51] respectively, in reasonable agreement with the ratio of 58, observed for [^{3}H](*R*)-cocaine.[47] The B_{max} ratios of the low- to high-affinity binding sites are 13 and 26, respectively; the ratio observed for (*R*)-cocaine is 15. Both ligands exhibit reasonable selectivity for the dopamine transporter, and both are likely to be more resistant than cocaine to metabolic or chemical degradation due to the replacement of the benzoyl group by an aromatic ring. This is manifested in the greater percentage of specific binding observed in in vivo binding studies with these ligands,[53] although the higher affinities of these compounds may be an important factor as well. The higher specific to nonspecific binding ratios observed for [^{3}H]WIN 35,428 and [^{3}H]WIN 35,065-2 compared to cocaine recommend these ligands for reducing methodological difficulties and uncertainties.[50,51] Another promising ligand is [^{3}H]BTCP, which, in spite of its structural similarity to phencyclidine (PCP), has been found to be highly selective for the dopamine transporter.[45] The relatively high affinity, low dissociation rate and low nonspecific binding displayed by this ligand highly recommend it for binding assays. A possible concern is that although the affinity ratio for the two binding sites of [^{3}H]BTCP resembles that of cocaine (22 and 58, respectively), the B_{max} ratio is only 2. A similar situation obtains for the iodinated ligand [^{125}I]RTI-55 which has a similar ratio of binding affinities (23) but exhibits a B_{max} ratio of 3 for the low- to high-affinity sites. This is apparently due to larger B_{max} for the high-affinity site.[52] The high affinity of [^{125}I]RTI-55 for the dopamine transporter and the possibility of labeling it with iodine-123 or with carbon-11 suggest that compounds of this basic structure will be useful in imaging studies such as autoradiography, positron emission tomography (PET), and single photon emission computed tomography (SPECT) scanning[52] (see section 2.A).

D. Cocaine Binding Site. Competitive or Allosteric. Cocaine, methylphenidate, and dopamine have been shown to competitively inhibit [^{3}H]GBR 12935 binding.[54,55] In addition, competitive inhibition of dopamine uptake in striatal tissue by 1-[2-[bis(4-fluorophenyl)methoxy]-ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909), cocaine, methylphenidate, benztropine, and nomifensine has been demonstrated.[54,55] These reports are consistent with the hypothesis that [^{3}H]GBR 12935 labels the dopamine binding site on the transporter. However, other studies with cocaine and mazindol in rat striatum, and with mazindol and dopamine in human caudate, suggest that cocaine binds to an allosterically-linked site.[56] In these studies, cocaine, but not mazindol, was found to decrease the B_{max} of [^{3}H]GBR 12935 binding rather than decreasing affinity, suggesting allosteric interactions between cocaine and the GBR 12935 site.

Several investigators have reported evidence which supports the premise that cocaine inhibition of the dopamine transporter occurs by its binding to a site on the transporter that is different from the dopamine recognition

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site on the transporter.[13,39,40] In addition, the fact that dopamine is more potent than cocaine in inhibiting [^{3}H]dopamine accumulation in vitro but is much less potent than cocaine in displacing specifically bound [^{3}H]-(*R*)-cocaine is consistent with different recognition sites for dopamine and cocaine.[49,57] A different explanation for this observation was suggested in a detailed study of the effect of cations on [^{3}H]mazindol binding and on [^{3}H]-dopamine binding and uptake.[58] In this study, the lower potency of dopamine to displace [^{3}H]mazindol relative to its potency to inhibit [^{3}H]dopamine uptake was explained based on a model involving initial binding, translocation and a rate-determining reorientation step. Nevertheless, the authors concluded that the binding of dopamine and mazindol took place at overlapping sites.

One Site or Multisite. Two early studies suggested a single site for [^{3}H](*R*)-cocaine binding to mammalian brain.[39,40] In addition, mazindol and GBR 12935 have been shown to bind to only one site on the dopamine transporter.[13] However, present evidence has demonstrated that there are at least two binding sites for (*R*)-cocaine associated with the dopamine transporter, one with high and another with low affinity. Thus, [^{3}H](*R*)-cocaine,[46-49] [^{3}H]WIN 35,428,[50,59] [^{3}H]WIN 35,065-2,[51] [^{3}H]BTCP,[45] and [^{125}I]RTI-55[52] all label two binding sites. The two sites have kd = 0.1–210 nM and 2.57 nM to 26.4 μM, depending on the radioligand, tissue source, species, and buffer used. Both WIN 35,428 and (*R*)-cocaine are reported to bind with higher affinity to the less abundant site. Since mazindol and GBR 12909 inhibited only approximately 90% of total [^{3}H]WIN 35,428 binding,[50] it may be that the site labeled by the GBR compounds and mazindol corresponds to a subset of sites labeled by [^{3}H]WIN 35,428, but it is not clear how these sites relate to the high- and low-affinity sites for (*R*)-cocaine. A key unanswered question is whether the two cocaine binding sites are related to distinct proteins, distinct sites on the same protein, or to two different affinity states of one protein.[48]

The fact that the clinical effects of cocaine are produced by plasma concentrations of less than 1 μM[60] suggests that the high-affinity site may be the most physiologically relevant receptor site, although significant numbers of low-affinity sites would be occupied at this concentration as well. Whether the high-affinity site alone is relevant to cocaine abuse or whether both high- and low-affinity sites play a role in the reinforcing properties of cocaine is unknown.

E. Isolation and Characterization Studies. Despite efforts in several laboratories, the dopamine transporter protein(s) responsible for cocaine's reinforcing properties remain largely uncharacterized. In part, this is due to difficulties in solubilization, purification, and low abundance.

Nevertheless, several studies have appeared in which photoaffinity and irreversible binding ligands of GBR-type compounds were utilized in labeling the transporter protein.[61-63] The bulk of the evidence indicates that the dopamine transporter is a glycoprotein of molecular weight between 58 000 and 80 000 Da.[62-64] The carbohydrate moiety contains sialic acid residues and is N-linked to the protein. Interestingly, recent studies suggest that there is a heterogeneity of dopamine transporters and that the mesolimbic transporters may not be the same as those in the nigrostriatal neurons.[65,66]

In addition to the dopamine (DA) transporter, several other sodium-dependent transporters have been identified including transporters for serotonin, norepinephrine (NE), glucose, γ-aminobutyric acid (GABA), glycine, L-glutamate, and choline. A GABA,[67] a glucose,[67-70] and more recently a norepinephrine[71] transporter have been characterized and cloned. While no significant sequence similarity was found between sodium-dependent NE transporter and the sodium-dependent glucose transporter, 46% identity and 68% homology with the human GABA transporter was observed. This homology suggests domains responsible for common mechanisms, while regions of divergence could account for specificity in substrate/ligand interactions. Considering the extensive overlap in biochemical properties between the DA and NE transporters, similar sequence homology may exist between them. Thus, some of the information about the NE transporter protein could be significant for characterization of the DA transporter. In this regard, the NE transporter was characterized to possess 12–13 highly hydrophobic regions compatible with transmembrane domains. Similarly to other transporters,

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sites for N-linked glycosylation and charged residues for binding the substrate are present.

With the research that is underway at several laboratories, the real structure of the DA transporter should be forthcoming shortly. The availability of protein sequence data, and ultimately an X-ray structure of the cocaine binding site at the dopamine transporter would enhance the process of rational drug design and development.

2. Chemical and Medicinal Chemical Studies

The chemistry of cocaine and its analogues has been described in several reviews.[72-77] In addition, an annotated bibliography of cocaine has also been published.[78] In this perspective, only cocaine analogues prepared as biochemical probes for the cocaine receptor are addressed.

A. Cocaine Biochemical Probes. At present biochemical probes are used in conjunction with imaging and for receptor isolation and characterization. Imaging techniques provide information about locus and density of binding sites, while site-directed probes aid in characterization at the molecular level. The information obtained from these probes is essential for understanding of the biochemical mechanism of cocaine abuse and for the design of analogues with potential for therapeutic use. Both techniques require high-affinity ligands with high selectivity for and low dissociation rate from the receptor. In addition, a radioactive label is frequently required or desirable. Several of these approaches have been applied to investigations of the cocaine binding site.

PET is an imaging method that has been widely used for the measurements of blood flow, glucose metabolism, as well as drug binding sites, and other processes. PET studies utilizing [^{11}C-*N-methyl*](*R*)-cocaine have shown that in humans the highest uptake is in the striatum[79] and that the time course of [^{11}C-*N-methyl*](*R*)-cocaine in human striatum[80] was similar to the time courses of the "high" after intravenous or smoked cocaine.[60] In addition, [^{11}C-*N-methyl*](*R*)-cocaine binding in human striatum was reduced by (*R*)-cocaine and nomifensine but not by desipramine, suggesting that cocaine binding occurs predominantly at the dopamine reuptake site.[79,81]

SPECT is another imaging technique with certain advantages over the PET method. The SPECT method uses gamma emitting radioisotopes such as iodine-123. No cocaine ligands suitable for SPECT are presently available. However, [^{125}I]2′-iodococaine and [^{125}I]RTI-55 have been prepared; studies with these compounds suggest that the iodine-123 labeled analogues may be useful SPECT probes for the cocaine receptor.[52,82]

The concept of site-directed receptor probes[83-85] has been useful in studies of several enzyme and receptor systems. Receptor "labeling" by means of irreversible bonding to the binding site allows for "tracking" of the receptor protein by monitoring radioactivity or by detection of fluorescence. This allows for the determination of receptor subtypes, the molecular weight of the receptor protein, the number of protein subunits, their stoichiometry and biochemical properties. In some cases the duration of pharmacological effects can be monitored as well. The irreversible bond can be established either by photoactivation of a photoactive ligand at the receptor site or by chemical reaction of a bionucleophile in the vicinity of the binding site with a reactive substituent in the ligand. Typical photoactive moieties are azido groups; while isothiocyanato, bromoacetamido, and maleimido are some of the chemically reactive substrates used. Photoaffinity labeling has been used successfully in the identification and characterization of the dopamine D_2 receptor,[86,87] and the two photoaffinity analogues of GBR 12935 (discussed in section 1.E) have provided valuable information that suggests some properties of a receptor protein associated with the GBR site. However, other photoaffinity ligands are desirable. Since (*R*)-cocaine and WIN 35,428 bind at two sites while GBR 12935 binds at only one site, photoaffinity probes with structures similar to cocaine may label other parts of the receptor protein. Potent photoaffinity probes for the cocaine receptor that are analogues of WIN 35,065-2 have been developed.[88] The azido ligand **9** was shown to significantly inhibit [^{125}I]1-[2-(diphenylmethoxy)ethyl-4-[2-(azido-3-iodophenyl)ethyl]-piperazine binding to the dopamine transporter.[88] Furthermore, **9** and two iodoazido compounds, **10** and **11**, displace specifically bound [^{3}H]WIN 35,428 with high affinity[88] and show irreversible covalent attachment to a

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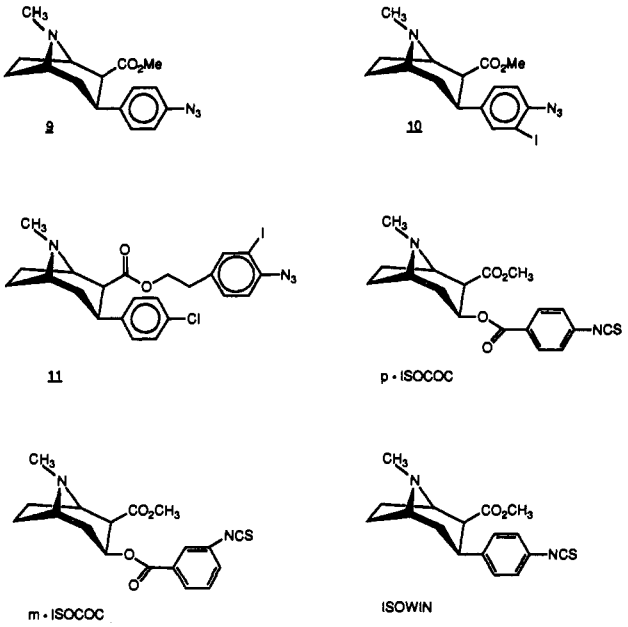
protein located within the dopamine transporter on exposure to UV light. These photoaffinity probes in radiolabeled form may be useful in the purification and characterization of the receptor proteins.



The first acylating ligand used to study the cocaine receptor was metaphit, the *m*-isothiocyanate derivative of phencyclidine. Metaphit was reported to bind irreversibly to the dopamine transporter.[61,89-91] However, metaphit also binds irreversibly to the PCP binding site on the NMDA receptor complex.

(*R*)-*p*-Isothiocyanatobenzoylecgonine methyl ester (*p*-isococ) and (*R*)-*m*-isothiocyanatobenzoylecgonine methyl ester (*m*-isococ), compounds more structurally similar to (*R*)-cocaine, were found to be somewhat more specific than metaphit as irreversible binding inhibitors at the cocaine receptor.[59] In contrast, (*R*)-3β-(4-isothiocyanatophenyl)-tropan-2β-carboxylic acid methyl ester (isowin), an analogue of WIN 35,065-2, was ineffective as a site-directed acylator of the cocaine receptor. *p*-Isococ also irreversibly blocked dopamine uptake by the transporter. Most significantly, it blocked the high-affinity cocaine site in preference to the low-affinity site, suggesting that it may be a powerful probe for studying the role of the high versus low affinity site.

B. Structure–Activity Studies. Important insights into the interactions of cocaine with the dopamine transporter which appear to be significantly implicated to the CNS effects of cocaine are provided by an understanding of the structure–activity correlation of cocaine. Such correlations can be carried out by comparing effects of structure variation on the IC_{50} values for inhibition of binding and of dopamine uptake at the dopamine transporter. A listing of these values for selected cocaine analogues and derivatives is shown in Table II.

Several structure–activity studies have been reported.[2,92-96] These studies have concluded that requirements for cocaine binding at the dopamine transporter, which are associated with the reinforcing effects of cocaine, include: (a) *R* configuration of the cocaine structure,[2,92,93] (b) a β substituent[92,93] at C-2, preferably a carbomethoxy group,[93] and (c) a benzene ring at C-3;[93] an ester link at C-3 was not required.[92] The effects of stereochemistry,[2] substitution at C-2,[96] C-3,[94] and N-8 position, as well as the effect of the position of the nitrogen[95] have been further elucidated.

In order to analyze the effect of a single structural parameter on the activity of cocaine-like compounds at the dopamine transporter, the ratios obtained by dividing the IC_{50} value for (*R*)-cocaine (or for an (*R*)-cocaine analogue) by the IC_{50} value for an analogue different in a single parameter were calculated. Table III lists the ratios of the IC_{50} values for inhibition of binding by [^{3}H](*R*)-cocaine, [^{3}H]WIN 35,428, and [^{3}H]mazindol as well as for inhibition of [^{3}H]dopamine uptake. In order to eliminate variations due to experimental parameters, the ratios were obtained for IC_{50} values which had been reported by the same investigator, usually in the same publication.

This analysis led to the following observations:

1. The largest factor in the activity of cocaine-like compounds is the configuration. Inversion of configuration decreases the activity of cocaine by a factor in the range of 140–330 (entries 12 and 13) and this range increases to 1000 (entry 17) for the WIN analogue. This effect is not carried over into the cocaine isomers. Thus, the activities of (*S*)-pseudococaine (entry 14), (*S*)-allococaine (entry 15) and (*S*)-allopseudococaine (entry 16) are only 1.2–7.4 times smaller than the activities of the *R* analogues; however, they are all much weaker than cocaine.

2. The second largest factor is the substituent at C-2. Replacement of the carbomethoxy group by hydrogen (entry 24) or by a carboxy group (entry 28) or by an *N*-methylcarboxamido group (entry 27) decreases the activity by 25–2000. Replacement by acetoxymethyl or hydroxymethyl groups (entries 26 and 25) decreases the activity by factors of only 2–5. Considering the ester group it appears as though a variety of ester groups can be accommodated without substantially altering the activity (entries 18–23).

3. Stereochemistry at C-2 has the third largest effect on activity. Epimerization from β to α at C-2 results in

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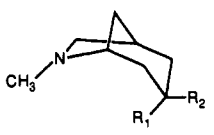
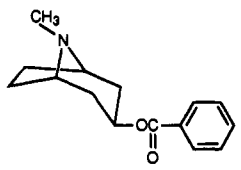
30–200 times lower activity (entries 1, 2, 7, and 8). This effect is not carried over into compounds with C-3 hydroxy substituent (entry 3) or C-3α substituents (entry 4), but these latter compounds have reduced potency.

4. Substitution at nitrogen can have a fairly large effect, in particular when it changes the electron density at nitrogen. Thus, replacement of the 8-methyl group by a propyl (entry 40) has almost no effect, and replacement by allyl (entries 38 and 39) or benzyl substituent (entry 41) reduces the activity by a factor of 7 or less. However, replacement by an acetyl group to create an amide (entry 42) or addition of a methyl group to create a quaternary salt (entry 37) reduces the activity by factors of 33 and 111, respectively.

5. Significant and important effects on activity are obtained by substitution at C-3. Thus, although substitution of the aromatic ring of the benzoyl group reduces the activity by a factor of 5–15 (entries 44–47), replacement of the benzoyl group by an aryl group (entries 48–54, WIN compounds) mostly leads to enhanced activity by factors of 3–51. Within the WIN series (entries 55–66), the effect of aromatic substitution on reactivity appears to be well correlated and predicted by Comparative Molecular Field Analysis (CoMFA).[94] Stereochemical change at C-3 from β to α decreases the activity by a factor of 63 in the *R* configuration (entry 10) but slightly enhances the activity in the *S* configuration (entry 11). In 6-methyl-6-azabicyclo[3.2.1]octan-3β-ol and -3α-ol benzoates (**12a** and **12b**, respectively), the ratio of IC_{50} values for inhibition of [^{3}H]WIN 35,428 binding for the β (4.95 μM) and α (19.8 μM) benzoates is 0.25; that is, there is a 4-fold decrease in activity for the α epimer.[95]



12a. R_1 = H, R_2 = $CO_2C_6H_5$
b. R_1 = $CO_2C_6H_5$, R_2 = H



Tropacocaine

Substitution at C-3 has had profound effects but is probably the least understood, in spite of the CoMFA correlation.[94] It appears that rigid β configuration and electron density are required for high activity.

6. The ratio of the IC_{50} values for inhibition of [^{3}H]WIN 35,428 binding of tropacocaine (5.18 μM) to that of 6-methyl-6-azabicyclo[3.2.1]octan-3β-ol benzoate (**12a**) (4.95 μM) is only 1.05.[95] This suggests that the nitrogen of (*R*)-cocaine can be moved from the 8- to the 6- and/or 7-position of the azabicyclo[3.2.1]octane ring system without loss in binding potency.

Several of these observations suggest that compounds with C-3α stereochemistry may not, in fact, bind in the exact same manner or to the exact same site as the C-3β epimers. Thus, for example, inversion of configuration of (*R*)-allo- (entry 15) and (*R*)-allopseudococaine (entry 16) does not have the same effect on activity as that observed for inversion of configuration of (*R*)-cocaine (entries 12 and 13) and (*R*)-WIN (entry 17). Similarly, the effect of C-2 stereochemistry is much smaller for C-3α substituted compounds than for C-3β epimers (compare entries 1, 2, and 4), but all α compounds are weak compared to (*R*)-cocaine.

Systematic variation of the O-substituent at C-2 appears to allow for fairly large changes in bulk and lipophilicity without substantially affecting the activity. The main advantage of the relative tolerance of the ester moiety to substitution lies in the potential for introduction of groups suitable for use as irreversible ligands, affinity ligands, photoaffinity labels, and fluorescent probes.

The effects of nitrogen substitution suggest that decreased electron density at nitrogen is detrimental to activity. Thus, the methiodide salt (entry 37), with full positive charge at nitrogen, is 111 times less active than cocaine, the zwitterion (entry 34), with a high percentage of positive charge at nitrogen is about 100 times less active, while the amide (entry 42), with partial positive charge at nitrogen, is 33 times less active. Consistently, replacement of the methyl group, which is inductively an electron donor, by groups such as benzyl (entry 41) or allyl (entry 38), which are electron withdrawers, reduces the activity by a factor of 7, while replacement by a hydrogen reduces the activity by 2–7.

With the exception of the GBR series, other inhibitors of dopamine uptake do not lend themselves to structure–activity analysis, because not enough structural variants are known. Within the GBR series, literally hundreds of compounds have been prepared. However, data relating to the inhibition of [^{3}H](*R*)-cocaine or [^{3}H]WIN 35,428 binding are insufficient for structure–activity analysis.

3. Conclusions and Perspectives for Future Research

Present evidence strongly suggests that inhibition of the dopamine transporter is necessary for the reinforcing properties of cocaine. The dopamine hypothesis stipulates that the reinforcing effects of cocaine are produced by initial blockage of dopamine reuptake, which potentiates dopaminergic transmission. Thus, the cocaine binding site at the dopamine transporter is, in fact, the cocaine receptor that is somehow ultimately responsible for the reinforcing properties.

It has been pointed out that not all dopamine uptake inhibitors exert reinforcing properties similar to those of cocaine.[97] Do these compounds bind at different sites on the transporter, or do they have other properties that make them different from cocaine? It has been suggested that the abuse liability of reinforcing drugs is greater for those drugs that enter the brain and occupy receptors rapidly.[98] Since it is possible to quantify the relative rate of occupancy of the cocaine receptor after injection of cocaine and other uptake inhibitors such as mazindol and GBR 12909 using in vivo binding studies, this question can be examined directly.[99]

Little is known about the molecular mechanism by which the binding of cocaine to the sodium-dependent dopamine transporter leads to inhibition of reuptake. Key unanswered questions are: How does cocaine block reuptake? Does it block a carrier channel that dopamine is transported through, or does it operate by some different mechanism? Whereas it is known that there are two cocaine binding sites in tissues, a high- and a low-affinity site, additional studies are needed to determine if the two sites are related to distinct proteins or to two affinity states of a single protein. More work will also be required to de-

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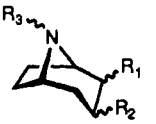
Table II. Potencies for Inhibition of Radioligand Binding and Dopamine Uptake at the Dopamine Transporter for Selected Cocaine Analogues



entry no.	compd.[a]	structure[b] configuration	R1	R2	R3	$IC_{50}(\mu M)$ for inhibition of binding [^{3}H]cocaine	[^{3}H]WIN-35428	[^{3}H]-mazindol	uptake: [^{3}H]dopamine
1		*R*	β-$CO_2CH_2CH_3$	β-OC(O)Ph	CH_3		0.13[c]		
2		*R*	β-CO_2Ph	β-OC(O)Ph	CH_3		0.112[c]		
3		*R*	β-CO_2CH_2Ph	β-OC(O)Ph	CH_3		0.257[c]		
4		*R*	β-CO_2CH_2-CH_2Ph	β-OC(O)Ph	CH_3		0.248[c]		
5		*R*	β-CO_2CH_2-CH_2Php-NO_2	β-OC(O)Ph	CH_3		0.601[c]		
6		*R*	β-CO_2CH_2-CH_2Php-NH_2	β-OC(O)Ph	CH_3		0.071[c]		
7		*R*	β-CO_2CH_2-CH_2Php-NCS	β-OC(O)Ph	CH_3		0.196[c]		
8		*R*	β-CO_2CH_2-CH_2Php-N_3	β-OC(O)Ph	CH_3		0.227[c]		
9	tropacocaine	*R*	H	β-OC(O)Ph	CH_3	3.3[d]	5.18[c]	15[e]	186[d]
10		*R*	β-CH_2OH	β-OC(O)Ph	CH_3		0.561[c]		
11		*R*	β-$CH_2OC(O)CH_3$	β-OC(O)Ph	CH_3		0.272[c]		
12		*R*	β-$C(O)NHCH_3$	β-OC(O)Ph	CH_3		3.18[c]		
13		*R*	β-CO_2CH_3	β-OC(O)Php-NH_2	CH_3		1.23[f]	3.04[e]	
14		*R*	β-CO_2CH_3	β-OC(O)Php-N_3	CH_3		0.748[g]		
15	*p*-isococ	*R*	β-CO_2CH_3	β-OC(O)Php-NCS	CH_3		1.05[h]		2.78[h]
16	*m*-isococ	*R*	β-CO_2CH_3	β-OC(O)Phm-NCS	CH_3		1.46[h]		
17		*R*	β-CO_2CH_3	β-OC(O)NHPhm-NO_2	CH_3	0.037[i]			0.178[i]
18	(*R*)-ecgonine methyl ester	*R*	β-CO_2CH_3	β-OH	CH_3			62[e]	
19	(*R*)-benzoyl-ecgonine	*R*	β-CO_2H	β-OC(O)Ph	CH_3	62[j]	195[c]	392[e]	
20		*R*	β-CO_2H	β-OC(O)Php-NH_2	CH_3		8.25[f]	4.51[e]	
21	(*R*)-ecgonine	*R*	β-CO_2H	β-OH	CH_3			>1000[e]	
22	(*R*)-norcocaine	*R*	β-CO_2CH_3	β-OC(O)Ph	H	0.414[j]	0.303[k]	1.21[e]	
23		*R*	β-CO_2CH_3	β-OC(O)Ph	H	0.27[d]			2.5[d]
24		*R*	β-CO_2CH_3	β-OC(O)Ph	$(CH_3)_2$		10.7[k]	20[e]	
25		*R*	β-CO_2CH_3	β-OC(O)Ph	$CH_2CH{=}CH_2$	1.024d			2.5[d]
26		*R*	β-CO_2CH_3	β-OC(O)Ph	CH_2Ph		0.668[k]		
27		*R*	β-CO_2CH_3	β-OC(O)Ph	$C(O)CH_3$		3.37[k]		
28		*R*	β-CO_2H	β-OC(O)Ph	H	78.2[j]		342[e]	
29	(*R*)-pseudococaine	*R*	α-CO_2CH_3	β-OC(O)Ph	CH_3	14.2[j]	15.8[l]	97[e]	
30		*R*	α-CO_2CH_3	β-OC(O)Ph	CH_3	8.54d			36[d]
31	(*R*)-pseudo-ecgonine methyl ester	*R*	α-CO_2CH_3	β-OH	CH_3			87[e]	
32	(*R*)-allococaine	*R*	β-CO_2CH_3	α-OC(O)PH	CH_3		6.2[l]		
33	benztropine	*R*	H	α-OC(O)PH	CH_3	23[d]	21.2[k]		110[d]
34		*R*	H	α-OC(O)PH	CH_3	0.2[m]	0.157[n]		0.7[m]
35		*R*	H	α-OC(O)PH	CH_3				0.175[o]
36		*R*	H	α-OC(O)PH	CH_3				0.24[p]
37		*R*	H	α-OC(O)PH	CH_3				0.094[q]
38		*R*	H	α-OC(O)PH	CH_3				1[r]
39		*R*	H	α-OC(O)PH	CH_3				0.175[s]
40	(*R*)-allopseudo-cocaine	*R*	α-CO_2CH_3	α-OC(O)PH	CH_3		28.5[l]		
41		*R*	α-CO_2CH_3	α-OC(O)PH	CH_3		5[d]		18[d]
42	(*S*)-cocaine	*S*	β-CO_2CH_3	β-OC(O)Ph	CH_3	13.1[j]	15.8[l]	136[e]	
43		*S*	β-CO_2CH_3	β-OC(O)Ph	CH_3	10.5[t]	30[u]		
44	(*S*)-pseudococaine	*S*	α-CO_2CH_3	β-OC(O)Ph	CH_3	109[j]	22.5[l]	116[e]	
45	(*S*)-allococaine	*S*	β-CO_2CH_3	α-OC(O)Ph	CH_3		9.82[l]		
46	(*S*)-allopseudo-cocaine	*S*	α-CO_2CH_3	β-OC(O)Ph	CH_3		67.7[l]		
47	WIN 35065-2	*R*	β-CO_2CH_3	β-Ph	CH_3	0.044[j]	0.023[v]	0.119[v]	0.96[v]
48		*R*	β-CO_2CH_3	β-Ph	CH_3	0.041[d]	0.065[u]	0.26[e]	0.19[d]
49	*RS*	*RS*	β-CO_2CH_3	β-Ph	CH_3	0.022[w]			1.33[w]
50	WIN 35065-3	*S*	β-CO_2CH_3	β-Ph	CH_3	32[d]			200[d]
51	WIN 35428	*R*	β-CO_2CH_3	β-Php-F	CH_3	0.027[d]			0.08[d]
52		*R*	β-CO_2CH_3	β-Php-F	CH_3	0.018[j]	0.016[v]	0.056[v]	0.51[v]
53		*R*	β-CO_2CH_3	β-Php-F	CH_3		0.03[n]	0.17[e]	
54		*R*	β-CO_2CH_3	β-Php-NH_2	CH_3	0.192[w]	0.025[x]		0.557[w]

Table II (Continued)

entry no.	compd.[a]	structure[b] configuration	R1	R2	R3	$IC_{50}(\mu M)$ for inhibition of binding [^{3}H]cocaine	[^{3}H]WIN-35428	[^{3}H]-mazindol	uptake: [^{3}H]dopamine
55		*R*	β-CO_2CH_3	β-Php-NO_2	CH_3	0.137[w]	0.010[x]		0.616[w]
56		*RS*	β-CO_2CH_3	β-Php-NO_2	CH_3	0.213[w]			0.903[w]
57		*R*	β-CO_2CH_3	β-Php-Me	CH_3		0.002[v]	0.005[v]	0.11[v]
58		*R*	β-CO_2CH_3	β-Php-CF_3	CH_3		0.013[v]		
59		*R*	β-CO_2CH_3	β-Php-OMe	CH_3		0.008[x]		
60		*R*	β-CO_2CH_3	β-Php-I	CH_3		0.001[x,y]		
61		*R*	β-CO_2CH_3	β-Php-Cl	CH_3		0.001[v]	0.002[v]	0.03[v]
62		*R*	β-CO_2CH_3	β-Php-N_3	CH_3		0.002[x]		0.2[z]
63		*R*	β-CO_2CH_3	β-Php-NHAc	CH_3		0.064[x]		
64	WIN 35981	*R*	β-CO_2CH_3	β-Ph	H	0.086[j]		0.36[e]	
65		*R*	β-CO_2CH_3	β-Php-F	H	0.036[aa]			
66		*R*	β-CO_2CH_3	β-Php-F	$CH_2CH_2CH_3$	0.043[aa]			
67		*R*	β-CO_2CH_3	β-Php-F	$CH_2CH{=}CH_2$	0.023[aa]			
68	WIN 35140	*R*	α-CO_2CH_3	β-Ph	CH_3	2.6[j]	2.9[u]	385[e]	
69		*R*	α-CO_2CH_3	β-Ph	CH_3	3.3[d]			5.5[d]
70		*RS*	α-CO_2CH_3	β-Ph	CH_3	1.2[w]			2.1[w]

[a] Only common names are given. [b] See structure. [c] See ref 96. [d] See ref 92. [e] See ref 93. [f] Unpublished results. [g] See ref 88. [h] See ref 59. [i] Kline, R. H., Jr.; Wright, J.; Eshleman, A. J.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3-carbamoylecgonine methyl ester analogues as inhibitors of cocaine binding and dopamine uptake. *J. Med. Chem.* **1991**, *34*, 702–705. [j] See ref 49. [k] See ref 95. [l] See ref 2. [m] See ref 46. [n] Rothman, R. Private communication. [o] Sharif, N. A.; Nunes, J. L.; Michel, J. L.; Whiting, R. L. Comparative properties of the dopamine transport complex in dog and rodent brain: striatal [^{3}H]GBR 12935 binding and [^{3}H]dopamine uptake. *Neurochem. Int.* **1989**, *15*, 325–332. [p] Bonnet, J.-J.; Lemasson, M.-H.; Costentin, J. Simultaneous evaluation by a double labeling method of drug-induced uptake inhibition and release of dopamine in synaptosomal preparation of rat striatum. *Biochem. Pharmacology* **1984**, *33*, 2129–2135. [q] See ref 45. [r] Ross, S. B; Renyi, A. L. Inhibition of the uptake of ^{3}H-dopamine and ^{14}C-5-hydroxytryptamine in mouse striatum slices. *Acta Pharmacol. Toxicol.* **1975**, *36*, 56–66. [s] See ref 44. [t] See ref 48. [u] See ref 50. [v] Boja, J. W.; Carroll, F. I.; Rahman, M. A.; Philip, A.; Lewin, A. H.; Kuhar, M. J. New, potent cocaine analogs: Ligand binding and transport studies in rat striatum. *Eur. J. Pharmacol.* **1990**, *184*, 329–332. [w] Kline, R. H., Jr.; Wright, J.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3-arylecgonine analogues as inhibitors of cocaine binding and dopamine uptake. *J. Med. Chem.* **1990**, *33*, 2024–2027. [x] See ref 94. [y] See ref 52. [z] Boja, J. W.; Kuhar, M. J. [^{3}H]Cocaine binding and inhibition of [^{3}H]dopamine uptake is similar in both the rat striatum and nucleus accumbens. *Eur. J. Pharmacol.* **1989**, *173*, 215–217. [aa] Madras, B. K.; Kamien, J. B.; Fahey, M. A.; Canfield, D. R.; Milius, R. A.; Saha, J. K.; Neumeyer, J. L.; Spealman, R. D. N-Modified fluorophenyltropane analogs of cocaine with high affinity for cocaine receptors. *Pharmacol. Biochem. Behav.* **1990**, *35*, 949.

termine if both the high- and low-affinity sites are involved in the reinforcing properties of cocaine. Despite these gaps in knowledge, it is reasonable to speculate that a compound capable of competing with cocaine for its binding site(s) on the dopamine transporter could modulate dopamine reuptake. Consequently, cocaine analogues that act as partial agonists[97] or antagonists, if they exist, could have important clinical applications.

The neuronal dopamine transporter has not been purified and/or cloned. Once the primary structure of the dopamine transporter is known, information concerning the location of binding site(s) might be inferred from sequence homology with receptor(s) of known three-dimensional structure. Only after the tertiary structure is known will information at the molecular level be available. Such information will greatly enhance SAR studies and the ability to predict binding properties of untested analogues. Recent successes with the characterization and closing of other transporter proteins such as the GABA and norepinephrine transporters suggest that the dopamine transporter protein will be characterized and cloned in the near future. Purification of the protein associated with the dopamine transporter has now become a possibility due to the availability of the newly developed, more potent and selective radiolabeled photoaffinity and irreversible probes described in this perspective.

The SAR data combined with some preliminary molecular modeling studies[94] allow speculation on a preliminary pharmacophoric model for the cocaine receptor (Figure 2). All high-affinity cocaine ligands require the presence of a basic amino group which is involved in an electrostatic or hydrogen bonding interaction with the receptor protein. The SAR data concerning substituents at the 2-position of cocaine suggest the presence of at least one and probably two additional hydrogen bond acceptor

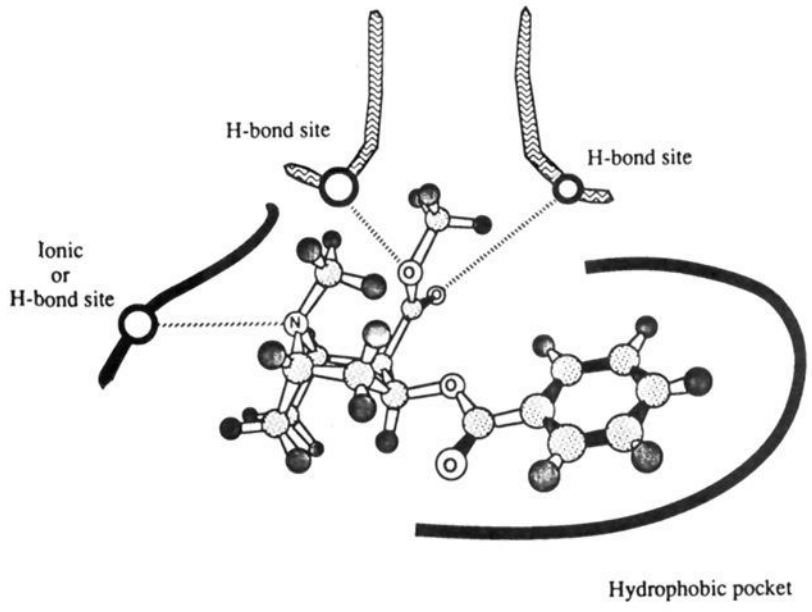
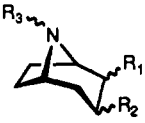


Figure 2. Schematic representation of putative interactions of cocaine with its receptor at the dopamine transporter. It is not known whether the conformation of the *N*-methyl group has to be as shown, nor whether the H-bonding sites are discrete. It is known that the hydrophobic pocket accommodates benzoyl as well as phenyl groups.

sites localized in the vicinity of the two oxygens of the 2β-carbomethoxy group of cocaine. Potency was reduced when the 2-position ester group was missing (entry 9, Table II) or possessed α orientation (entries 29 and 30, Table II). Affinity for the receptor was also reduced when the ester group of (*R*)-cocaine was replaced by substituents containing only one oxygen. The low potency of the *N*-methyl amide (entry 12, Table II) suggests that the hydrogen bond(s) are highly specific. Since electron-donating groups would be expected to potentiate the hydrogen-bonding capability of the two oxygens of the ester group, studies directed toward correlating the negative electrostatic po-

Table III. Effect of Structural Variation on Potencies of Cocaine Analogues To Inhibit Radioligand Binding and Dopamine Uptake at the Dopamine Transporter



	structure A[a]				structure B[a]				IC_{50} A/IC_{50} B[b] for inhibition of			
									binding			uptake:
entry no.	configuration	R1	R2	R3	configuration	R1	R2	R3	[^{3}H]cocaine	[^{3}H]WIN 35428	[^{3}H]mazindol	[^{3}H]-dopamine
1	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	α-CO_2CH_3	β-OC(O)Ph	CH_3	0.005[c]	0.007[d]	0.007[e]	
2	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	α-CO_2CH_3	β-OC(O)Ph	CH_3	0.016[f]			0.024[f]
3	*R*	β-CO_2CH_3	β-OH	CH_3	*R*	α-CO_2CH_3	β-OH	CH_3			0.712[e]	
4	*R*	β-CO_2CH_3	α-OC(O)Ph	CH_3	*R*	α-CO_2CH_3	α-OC(O)Ph	CH_3		0.218[d]		
5	*S*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*S*	α-CO_2CH_3	β-OC(O)Ph	CH_3	0.12[c]	0.702[d]	1.17[e]	
6	*S*	β-CO_2CH_3	α-OC(O)Ph	CH_3	*S*	α-CO_2CH_3	α-OC(O)PH	CH_3		0.145[d]		
7	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	α-CO_2CH_3	β-Ph	CH_3	0.017[c]	0.022[g]	0.001[e]	
8	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	α-CO_2CH_3	β-Ph	CH_3	0.014[f]			0.035[f]
9	*RS*	β-CO_2CH_3	β-Ph	CH_3	*RS*	α-CO_2CH_3	β-Ph	CH_3	0.08[h]			0.062[h]
10	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	α-OC(O)Ph	CH_3		0.017[d]		
11	*S*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*S*	β-CO_2CH_3	α-OC(O)Ph	CH_3		1.61[d]		
12	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*S*	β-CO_2CH_3	β-OC(O)Ph	CH_3	0.005[c]	0.007[d]	0.005[e]	
13	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*S*	β-CO_2CH_3	β-OC(O)Ph	CH_3	0.003[i]			
14	*R*	α-CO_2CH_3	β-OC(O)Ph	CH_3	*S*	α-CO_2CH_3	β-OC(O)Ph	CH_3	0.130[c]	0.702[d]	0.836[e]	
15	*R*	β-CO_2CH_3	α-OC(O)Ph	CH_3	*S*	β-CO_2CH_3	α-OC(O)Ph	CH_3		0.631[d]		
16	*R*	α-CO_2CH_3	α-OC(O)Ph	CH_3	*S*	α-CO_2CH_3	α-OC(O)Ph	CH_3		0.421[d]		
17	*R*	β-CO_2CH_3	β-Ph	CH_3	*S*	β-CO_2CH_3	β-Ph	CH_3	0.001[f]			0.001[f]
18	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-$CO_2CH_2CH_3$	β-OC(O)Ph	CH_3		0.785[j]		
19	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2Ph	β-OC(O)Ph	CH_3		0.911[j]		
20	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_2Ph	β-OC(O)Ph	CH_3		0.397[j]		
21	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-$CO_2CH_2CH_2$-Ph	β-OC(O)Ph	CH_3		0.411[j]		
22	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-$CO_2CH_2CH_2$-Php-NO_2	β-OC(O)Ph	CH_3		0.170[j]		
23	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-$CO_2CH_2CH_2$-Php-NH_2	β-OC(O)Ph	CH_3		1.437[j]		
24	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	H	β-OC(O)Ph	CH_3	0.041[f]	0.02[j]	0.043[e]	0.005[f]
25	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CH_2OH	β-OC(O)Ph	CH_3		0.182[j]		
26	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-$CH_2OC(O)CH_3$	β-OC(O)Ph	CH_3		0.376[j]		
27	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-$C(O)NHCH_3$	β-OC(O)Ph	CH_3		0.032[j]		
28	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2H	β-OC(O)Ph	CH_3	0.001[c]	0.0005[j]	0.002[e]	
29	*R*	β-CO_2CH_3	β-OC(O)PHp-NH_2	CH_3	*R*	β-CO_2H	β-OC(O)Php-NH_2	CH_3		0.15[k]	0.674[e]	
30	*R*	β-CO_2CH_3	β-OH	CH_3	*R*	β-CO_2H	β-OH	CH_3			0.062[e]	
31	*R*	β-CO_2CH_3	β-OC(O)Ph	H	*R*	β-CO_2H	β-OC(O)Ph	H	0.005[c]		0.004[e]	
32	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Ph	H	0.164[c]	0.337[l]	0.529[e]	
33	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Ph	H	0.5[f]			0.348[f]
34	*R*	β-CO_2H	β-OC(O)Ph	CH_3	*R*	β-CO_2H	β-OC(O)Ph	H	0.793[c]		1.146[e]	
35	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Ph	H	0.512[c]		0.722[e]	
36	*R*	β-CO_2CH_3	β-Php-F	CH_3	*R*	β-CO_2CH_3	β-Php-F	H	1.2[m]			
37	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Ph	$(CH_3)_2$		0.001[l]	0.032[e]	
38	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Ph	$CH_2CH{=}CH_2$	0.132[f]			0.348[f]
39	*R*	β-CO_2CH_3	β-Php-F	CH_3	*R*	β-CO_2CH_3	β-Php-F	$CH_2CH{=}CH_2$	0.782[n]			
40	*R*	β-CO_2CH_3	β-Php-F	CH_3	*R*	β-CO_2CH_3	β-Php-F	$CH_2CH_2CH_3$	0.419[n]			
41	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_2Ph		0.153[l]		
42	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Ph	$C(O)CH_3$		0.030[l]		
43	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OH	CH_3			0.103[e]	
44	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Php-NH_2	CH_3		0.082[k]	0.211[e]	
45	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Php-N_3	CH_3		0.136[o]		
46	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Php-NCS	CH_3		0.097[p]		1.81[p]
47	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-OC(O)Phm-NCS	CH_3		0.070[p]		
48	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-Ph	CH_3	1.545[c]		2.46[e]	
49	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-Ph	CH_3	3.000[f]			4.579[f]
50	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-Ph	CH_3		4.435[o]	3.252[q]	5.229[q]
51	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-Php-NH_2	CH_3	0.724[h]	4.080[o]		1.514[h]
52	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-Php-N_3	CH_3		51[o]		
53	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-Php-F	CH_3	5.00[f]	6.375[q]	6.91[q]	4.579[f]
54	*R*	β-CO_2CH_3	β-OC(O)Ph	CH_3	*R*	β-CO_2CH_3	β-Php-F	CH_3	3.78[c]	6.93[r]	3.764[e]	9.84[q]
55	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-F	CH_3	1.519[f]		2.125[q]	2.375[f]
56	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-F	CH_3	2.44[c]			
57	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-NH_2	CH_3		0.92[o]		
58	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-NO_2	CH_3		2.3[o]		
59	*RS*	β-CO_2CH_3	β-Ph	CH_3	*RS*	β-CO_2CH_3	β-Php-NO_2	CH_3	0.103[h]			1.44[h]
60	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-CH_3	CH_3		11.5[q]	23.8[q]	8.73[q]
61	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-CF_3	CH_3		1.769[o]		
62	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-OCH_3	CH_3		2.875[o]		
63	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-I	CH_3		23[o]		
64	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-Cl	CH		23[q]	28[q]	17[q]
65	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-N_3	CH_3		11.5[o]		
66	*R*	β-CO_2CH_3	β-Ph	CH_3	*R*	β-CO_2CH_3	β-Php-$NHC(O)CH_3$	CH_3		0.359[o]		

[a] See structure. [b] The actual IC_{50} values are in Tables I and II. [c] See ref 49. [d] See ref 2. [e] See ref 93. [f] See ref 92. [g] See ref 50. [h] Kline, R. H., Jr.; Wright, J.; Eshleman, A. J.; Fox, K. M.; Eldefrawi, M. E. Synthesis of 3-carbamoylecgonine methyl ester analogues as inhibitors of cocaine binding and dopamine uptake. *J. Med. Chem.* **1991**, *34*, 702–705. [i] See ref 48. [j] See ref 96. [k] Unpublished results. [l] See ref 95. [m] Boja, J. W.; Kuhar, M. J. [^{3}H]Cocaine binding and inhibition of [^{3}H]dopamine uptake is similar in both the rat striatum and nucleus accumbens. *Eur. J. Pharmacol.* **1989**, *173*, 215–217. [n] Madras, B. K.; Kamien, J. B.; Fahey, M. A.; Canfield, D. R.; Milius, R. A.; Saha, J. K.; Neumeyer, J. L.; Spealman, R. D. N-Modified fluorophenyltropane analogs of cocaine with high affinity for cocaine receptors. *Pharmacol. Biochem. Behav.* **1990**, *35*, 949. [o] See ref 94. [p] See ref 59. [q] Boja, J. W.; Carroll, F. I.; Rahman, M. A.; Philip, A.; Lewin, A. H.; Kuhar, M. J. New, potent cocaine analogs: Ligand binding and transport studies in rat striatum. *Eur. J. Pharmacol.* **1990**, *184*, 329–332. [r] Rothman, R. Private communication.

tential exerted by the ester oxygens should provide information on binding requirements at the 2-position of the tropane ring.

An aromatic ring connected directly or indirectly to the 3β-position of the tropane ring is required for good affinity to the receptor. The optimum location and properties of this binding site have not been defined. Present data indicate that hydrophobicity, charge, and size are all important. The evaluation of additional 3β-(substituted phenyl)tropan-2β-carboxylic acid methyl esters and aromatic ring-substituted ring analogues of cocaine, as well as new analogues with the aromatic rings linked to the 3-position in chemically different ways will help define the binding requirements for the aromatic ring site.

From a steric standpoint, the receptor can accommodate only small increases in size at the nitrogen position or on the aromatic ring at C-3. In contrast, large groups can replace the methyl of the carbomethoxy group at C-2 with very little loss in affinity for the receptor. The solid lines in Figure 2 roughly represent the sterically disallowed area of the receptor. Additional studies will be required to more accurately define the steric requirements of the receptor.

Much additional research is needed before the biochemical, pharmacological, and behavioral roles of the cocaine receptor(s) are understood. An important issue is the feasibility of developing a clinically useful competitive cocaine antagonist. If, as some studies suggest, cocaine interacts competitively with the dopamine binding site, a compound capable of blocking cocaine binding without blocking dopamine uptake would be a suitable antagonist. At this point, no such compound is known. On the other hand, knowledge of the receptor protein is limited as is the cascade of molecular events that lead to the inhibition of dopamine uptake and the reinforcing properties of cocaine. Present SAR data have provided a working pharmacophore model for the cocaine binding site. However, additional new and novel compounds are needed to more precisely define the structural requirements for potent and selective binding to the cocaine receptor.

Articles

A Novel Class of Calcium-Entry Blockers: The 1-[[4-(Aminoalkoxy)phenyl]sulfonyl]indolizines

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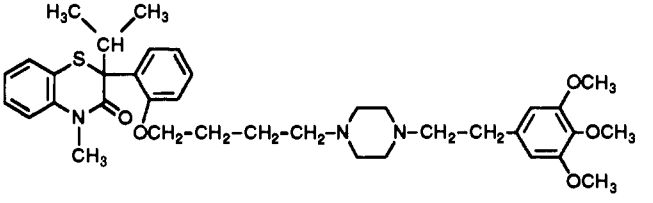
The synthesis and initial biological evaluation of a series of 1-sulfonylindolizines is described. These compounds have been shown to be representatives of a novel class of potent, slow-channel calcium antagonists. All compounds were found to be at least as active as the reference calcium antagonists verapamil and *cis*-(+)-diltiazem. Structure–activity relationship studies have shown that all compounds possessing an aralkyl group in the amine moiety and an isopropyl or cyclopropyl group at the 2 position of the indolizine are among the most potent calcium antagonists known outside the 1,4-dihydropyridine series. The IC_{50} values for the inhibition of [^{3}H]nitrendipine binding vary between 0.19 and 4.5 nM whereas the IC_{50} value for nifedipine is 2.5 nM. One of the compounds in this group (**9ab**) has now been selected for clinical development.

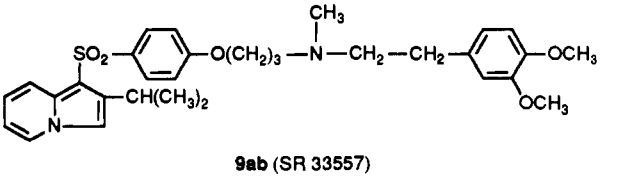
Despite the enormous growth in interest in calcium antagonism during the last 2 decades and its recognition as a principle with a great potential impact on the treatment of ischemic heart disease and hypertension, there are comparatively few calcium channel blocking agents currently in clinical use. These drugs are characterized by the fact that they belong to only three classes of compounds which are chemically unrelated: the phenylalkylamines, the 1,4-dihydropyridines, and the benzothiazepines. The three well-known prototypes of three chemical classes are shown in Chart I.

More recently several novel classes of calcium blockers have emerged: diphenylbutylpiperidines (fluspirilene),[1,2] 1,3-diphosphonates (belfosdil),[3] and, in addition, benzothiazinone (HOE 166),[4,5] the chemical structure of which bears some resemblance to diltiazem.

Previous studies by us have led to the discovery of 1-sulfonylindolizines as a new class of potent calcium antagonists.[6-8] The biochemical studies carried out to date



HOE 166



9ab (SR 33557)

implicate a new binding site associated with the L-type calcium channel for the 1-sulfonylindolizines in addition

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