(51) International Patent Classification 6 : A61K 51/04, C07D 451/02	A1	(11) International Publication Number: WO 97/40859
		(43) International Publication Date: 6 November 1997 (06.11.97)

(54) Title: BRIDGE-SUBSTITUTED TROPANES AND USES

(57) Abstract

    Disclosed are bridge-substituted analogs of tropanes and benztropines as well as methods for, imaging of cocaine receptors, treatment of cocaine abuse, and imaging and treatment of Parkinson's disease.

BRIDGE-SUBSTITUTED TROPANES AND USES
Cross-reference to related application

This application is a continuation-in-part of our
5 earlier commonly owned co-pending application U.S.S.N.
08/548,271, filed October 25, 1995, which was a
divisional of USSN 08/111,141, filed August 24, 1993 (now
U.S. 5,506,359), which in turn was a continuation-in-part
of USSN 07/934,362, filed August 24, 1992. Each of the
10 above applications and any patents issuing on them are
hereby incorporated by reference.

Background of the Invention

The invention relates to cocaine analogues.

The abuse of cocaine is a health problem of
national significance. Despite intensive study, there is
5 still inadequate information about the neurochemical
mechanisms mediating cocaine's detrimental effects and
abuse liability or about drug therapies for cocaine
abuse.

Recent studies have identified biologically
10 relevant binding sites for cocaine in brain tissue of
rodents (Reith et al., Biochem. Pharmacol. 35:1123-1129,
1986; Kennedy and Hanbauer, 41:172-178, 1983; Calligaro
and Eldefrawi, J. Pharmacol. Exp. Ther. 243:61-68, 1987;
Calligaro and Eldefrawi, Membrane Biochem. 7:87-106,
15 1988), humans (Schoemaker et al., Naunyn-Schmiedeberg's
Arch. Pharmacol. 329:227-235, 1985), and nonhuman
primates (Madras et al., FASEB J. 2:A1137, 1988; Madras
et al., ASP/ASPET Abstr. A197, 1988; Madras et al., J.
Pharmacol. Exp. Ther. 251:131-141, 1989). These sites,
20 which are associated with monoamine uptake systems, can
be labeled with [^{3}H]cocaine and have properties
characteristic of a pharmacological receptor. First, the
sites bind [^{3}H]cocaine saturably with affinities in the
10^{-8} to 10^{-6} M range, concentrations comparable to those

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achieved in brain or plasma after peripheral
administration of cocaine to animals or humans (Van Dyke
et al., Science 191:859-861, 1976; Misra et al., Drug
Alcohol Depend. 2:261-272, 1977; Javaid et al., Science

5   200:227-228, 1978). Second, the sites display
stereoselectivity for (-)-cocaine over (+)-cocaine or its
C-2 epimer pseudococaine (Madras et al., APS/ASPET Abstr.
A197, 1988; Madras et al., J. Pharmacol. Exp. Ther.
251:131-141, 1989). Third, and perhaps most importantly,

10   there is a high degree of correspondence between the
relative potencies of various cocaine analogs for
producing cocaine-like effects in vivo and relative
binding affinities of the drugs for [^{3}H]cocaine binding
sites in vitro. Specifically, the ED_{50} values of cocaine

15   and several cocaine analogs for producing behavioral
stimulation (Spealman et al., J. Pharmacol. Exp. Ther.
251:142-149, 1989) or for maintaining self-administration
in non-human primates (Bergman et al., J. Pharmacol. Exp.
Ther. 251:150-155, 1989) parallel their IC_{50} values for

20   displacing [^{3}H]cocaine from binding sites in monkey brain
(Madras et al., FASEB J. 2:A1137, 1988; Madras et al., J.
Pharmacol. Exp. Ther. 251:131-141, 1989).

Although cocaine inhibits the uptake of dopamine,
serotonin, and norepinephrine with similar potencies,

25   [^{3}H]cocaine recognition sites on the dopamine transporter
are particularly relevant to the behavioral effects of
cocaine (Reith et al., Biochem. Pharmacol. 35:1123-1129,
1986; Madras et al., FASEB J. 2:A1137, 1988; Madras et
al., J. Pharmacol. Exp. Ther. 251:131-141, 1989, Bergman

30   et al., J. Pharmacol. Exp. Ther. 251:150-155, 1989;
Spealman et al., J. Pharmacol. Exp. Ther. 251:142-149,
1989). Cocaine congeners and other drugs block
behaviorally relevant [^{3}H]cocaine binding in monkey
caudate-putamen with a rank order of potency that

35   corresponds closely to their reported potencies for

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inhibiting uptake of dopamine but not norepinephrine or
serotonin (Madras et al., J. Pharmacol. Exp. Ther.
251:131-141, 1989). Furthermore, selective
norepinephrine or serotonin inhibitors do not produce the
5   characteristic stimulant, reinforcing and interoceptive
effects elicited by cocaine (Spealman et al., J.
Pharmacol. Exp. Ther. 251:142-149, 1989; Spealman et al.,
J. Pharmacol. Exp. Ther. 258:945-953, 1991; Bergman et
al., J. Pharmacol. Exp. Ther. 251:150-155, 1989).
10 Together, these studies support the view that cocaine
recognition sites associated with the dopamine
transporter are important mediators of the behavioral
effects of cocaine.

Further clarification of the mechanisms by which
15 cocaine and related drugs alter behavior and maintain
abuse will likely emerge from molecular characterization
of cocaine receptor sites and imaging these sites in the
brain. These studies have been hampered, however, by the
relative inefficiency of [^{3}H]cocaine as a radioligand and
20 the lack of versatile probes to elucidate the molecular
properties of the receptor complex. In particular,
[^{3}H]cocaine binds to the receptor with modest affinity in
all brain regions studied ($K_{0.50} \approx 300$ nM) and dissociates
rapidly. This modest affinity of [^{3}H]cocaine (25-30
25 Ci/mmole) make it a relatively poor tag for imaging
cocaine receptors, either in vitro or in vivo.

Drug therapies for cocaine abuse also are needed,
and therapeutic agents based on cocaine congeners are
proposed.
30   There is also a need for suitable agents and
procedures to diagnose neurodegenerative disorders, such
as Parkinson's disease. In particular, exclusion of
Parkinson's disease as the cause of symptoms at an early
stage may be useful information in diagnosing other
35 conditions such as Alzheimer's disease. In addition,

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early diagnosis of Parkinson's disease facilitates the
introduction of prophylactic drug therapy (e.g., deprenyl
administration) prior to the onset of symptoms.

　　　　Finally, there is a need for agents that treat
5　other disorders by targeting brain monamine
neurotransporters.

　　　　　　　　　　　Summary of the Invention
　　　　It has been generally assumed that nitrogen should
be incorporated into drugs used to target brain monoamine
10　neurotransporters, e.g., agents for diagnosing or
treating cocaine addiction, Parkinson' disease, Attention
Deficit Hyperactivity Disorder, depression, and other
conditions. We have determined that compounds lacking
nitrogen can be effective for these purposes. In
15　particular, we have discovered bridge-substituted
tropanes and uses for those compounds described below.

　　　　One aspect of the invention features a compound
having one of the following general formulas A., B., C.,
or D.:

20

A.

B.

C.

D.
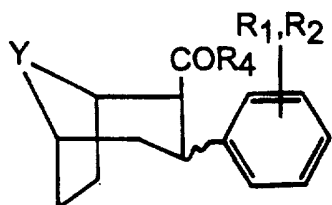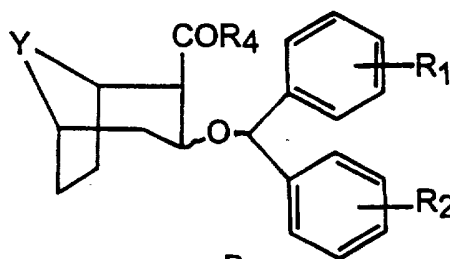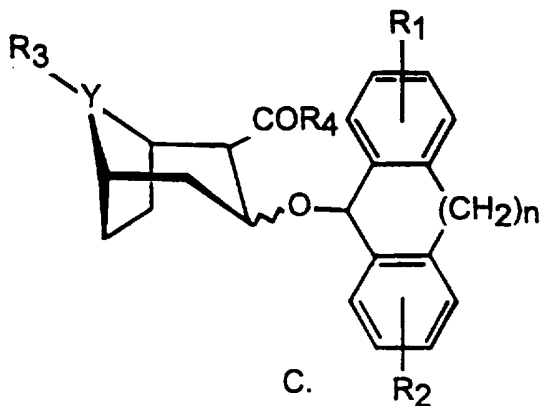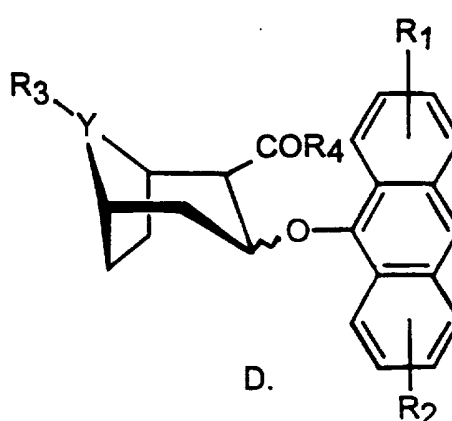
- 5 -

In those formulas:

Y is C, O, S, SO, or SO_2; and

R_1 and R_2 are both 4-F, 4-Cl, 3,4-diCl, 4-I, H, 3,4-diOH, 3,4-diOAc, or 3,4-diOCH$_3$; or

R_1 is H and R_2 is 4-F, 4-Cl, 4-I, 2-F, 2-Cl, 2-I, 3-F, 3-Cl, 3-I, 3,4-diCl, 3,4-diOH, 3,4-diOAc, or 3,4-diOCH$_3$; or

R_1 is H and R_2 is 3-OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH, or 3-F-4-OH; or

R_1 is the same as R_2 and both are chosen from 3-OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH, or 3-F-4-OH; or

R_1 is not the same as R_2 and both are chosen from 3-OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH, or 3-F-4-OH; and

wherein R_4 is CH_3, CH_3CH_2, $(CH_3)_2CH$, $CH_3(CH_2)_n$, $(CH_2)_nC_6H_4X$, C_6H_4X, C_6H_5, OCH_3, OCH_3CH_2, $OCH(CH_3)_2$, OC_6H_5, OC_6H_4X, $O(CH_2)_nC_6H_4X$, or $O(CH_2)_nCH_3$,

wherein X is Br, Cl, I, F, OH, OCH_3, CF_3, NO_2, NH_2, CN, $NHCOCH_3$, $N(CH_3)_2$, $(CH_2)_nCH_3$, $COCH_3$ or $C(CH_3)_3$, wherein n is between 0 and 6 inclusive.

Those formulas cover compounds in which the linkage at the 2- and 3- position can be (independently) α or β. They also cover compositions enriched in either the R enantiomer or in the S enantiomer, as well as racemic mixtures of those enantiomers.

In more detailed embodiments, Y is O; the linkage at the 3-position is α; and each R_1 and R_2, independently, is Cl, I, or F. In another instance, R_4 is OCH_3; and Y is C; and R_1 and R_2 are both F or both I.

In a preferred embodiment, the compound is labelled with a radioactive or fluorescent label; preferable labels include ^{3}H, [11]C (e.g., on the aromatic ring substituent(s)) [123]I or ^{125}I or [18]F (e.g., on the aromatic ring(s) of the C-3 substituent.

These compounds have several uses based on the finding that cocaine recognition sites are localized on

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the dopamine transporter, which itself is localized on
dopamine nerve terminals. Uses for drugs that bind to
these sites include (i) imaging probes for dopamine
transporter/cocaine binding sites, (ii) imaging probes
5   for neurodegenerative disorders, (iii) drug therapies for
cocaine abuse, and (iv) drug therapies for
neurodegenerative disorders such as Parkinson's disease.
        The compounds may therefore be used as cocaine
substitutes to treat cocaine addiction and, when
10   detectably labelled, may be used as tags to assay the
number of cocaine receptors in the brain. Because such
receptors are present in decreased number in the brains
of patient's afflicted with Parkinson's disease, such
synthetic cocaine analogs facilitate a method of _in vivo_
15   imaging for diagnosing this disease. In addition, such
analogs, by virtue of their ability to inhibit dopamine
transport and increase synaptic dopamine levels may serve
as therapeutics for Parkinson's disease.
        Additionally, because of the unique anatomical
20   location of the cocaine recognition sites, a high
affinity probe for imaging of these sites _in vivo_ in the
brain may be carried out using, e.g., PET or SPECT
imaging. Such imaging is useful for the purposes of (i)
assaying cocaine receptors in chronic cocaine users and
25   in individuals exposed to cocaine prenatally, (ii)
assaying the receptor occupancy of potential cocaine
therapeutics, and (iii) assaying cocaine receptors in
individuals that abuse other drugs. Such imaging is also
useful for monitoring the occupancy of the dopamine
30   transporter by established and novel drugs that are
targeted to and/or bind to these sites; these drugs
include, but are not limited to, antidepressants (e.g.,
bupropion), attention deficit disorder/hyperactivity
syndrome therapies (e.g., methylphenidate or pemoline),
35   and dopamine uptake inhibitors useful for treating

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Parkinson's disease (e.g., benztropine, also termed
cogentin). Finally, such imaging agents are useful for
diagnosing or monitoring Parkinson's disease, a
neurological disorder characterized by the degeneration
of dopamine nerve terminals.

Apart from the uses described above, cocaine
analogs may also provide alternatives to cocaine in the
pharmacological management of cocaine abuse. The
development of cocaine drug therapies which can be
readily administered on an out-patient basis under
controlled conditions may diminish the illicit drug
trade, reduce the probability of AIDS transmission by
frequent intravenous dose administration of cocaine, and
enable abusers greater access to treatment programs. In
a related aspect, the compounds of the invention may be
formulated as therapeutic compositions, essentially
including the compound in a pharmaceutically acceptable
carrier.

Another object of the invention was to develop
compounds useful for cocaine drug therapy. Such
compounds include cocaine-like drugs preferably having a
longer onset time, a longer duration of action, and a
diminished abuse liability relative to cocaine; compounds
described herein are expected to have such properties.
The need also exists for cocaine antagonists which
preferably bind sites normally bound by cocaine but
without inhibiting dopamine transport. The compounds of
the invention which lack a ring nitrogen or possess a
catechol moiety likely provide such useful cocaine
antagonists. A number of the compounds described herein
are useful for the treatment of Parkinson's disease and
similar neurodegenerative disorders.

Thus, in another aspect, the invention features a
method of treating cocaine addiction in a mammal,

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involving administering a therapeutic composition of the
invention to the mammal.

In still another aspect, the invention features a
method of selectively imaging cocaine binding regions of
the central nervous system of a human patient, involving
administering to the central nervous system a detectably
labelled compound of the invention or CFT and detecting
the binding of that compound to CNS tissue (e.g., by
position emission tomography (PET) or single-photon
emission computed tomography (SPECT)).

In a fifth aspect, the invention features a
method of detecting parkinsonism in a human patient,
involving administration to the patient of a detectably
labelled compound of the invention or CFT.

In a sixth aspect, the invention features a method
of treating a neurodegenerative disorder (e.g.,
Parkinson's disease) characterized by monoamine nerve
terminal degeneration in a human patient involving
administration to the patient of a therapeutic compound
of the invention.

Detailed Description of the Drawings

FIGs. 1A and 1B are graphs showing the
relationship between drug potencies at [^{3}H]CFT binding
sites and drug potencies (FIG 1A) at [^{3}H]cocaine binding
sites; (FIG1B) at the dopamine transporter in the
caudate-putamen; and (FIG1C) at the dopamine transporter
in the nucleus accumbens.

FIGs. 2A and 2B are graphs showing the
relationship (FIG 2A) between [^{3}H]CFT binding and
psychomotor stimulation and (FIG 2B) between [^{3}H]CFT
binding and maintenance of drug self-administration.

FIG. 3 is a bar graph showing a comparison between
the amount of [^{3}H]CFT bound in normal and Parkinson's-
diseased human putamen and the relationship to dopamine
levels.

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FIG. 4 shows the structure of (-)cocaine and benztropine to illustrate the stereospecificity of those compounds at the 3 position.

FIG. 5 shows R and S enantiomers of various
5   compounds.

FIG. 6 shows one synthetic scheme for the preparation of analogs of the invention which lack a nitrogen in the tropane ring.

FIG. 7 shows another synthetic scheme for the
10 preparation of analogs of the invention which lack a nitrogen in the tropane ring.

FIG. 8 depicts various benzotropine analogs.

FIGS. 9 and 10 depict the results of competitive binding experiments for four compounds.

15   Description of the Preferred Embodiment
I.   NOVEL COCAINE ANALOGS

General formulas for compounds of the invention are given above and in Fig. 5. Preferred compounds are those in which Y=C, O, S; and SO, SO_2, linkage at 2 is α-
20 or β-, linkage at 3 is α- or β-. The molecules may be racemic, and/or R-enantiomers, and/or S-enantiomers, n=0,1,2, R_1 and R_2 are both 4-F, 4-Cl, 3, 4-diCl, 4-I, H, 3, 4-diOH, 3, 4-diOAc, 3, 4-$diOCH_3$, or R_1=H and R_2=4-F, 4-Cl, 4-I, 2-F, 2-Cl, 2-I, 3-F, 3-Cl, 3-I, 3, 4-diCl, 3, 4-
25 diOh, 3, 4-diOAc, 3, 4-$diOCH_3$, or R_1=H and R_2= 3-OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH, 3-F-4-OH, or R_1=R_2=3-OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH, 3-F-4-OH, or R_1 not = R_2; both = 3-OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH, 3-F-4-OH where X=Br, Cl, I, F, OH, OCH_3, CF_3, NO_2, NH_2, CN, $NHCOCH_3$, $N(CH_3)_2$, $(CH_2)nCH_3$, $COCH_3$,
30 $C(CH_3)_3$ where n=0-6.

R_4=CH_3, CH_3CH_2, $CH_3(CH_2)_n(CH_2)_nC_6H_4X$, C_6H_4X, C_6H_5, OCH_3, OCH_3CH_2, OC_6H_5, OC_6H_4X, OC_6H_3XY, $OCH(CH_3)_2$, $CHCH_2$.

Those compounds can be evaluated to optimize activity according to the techniques described below.
35 Desirable properties included high affinity and high

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selectivity for the dopamine transporter (preferably in
the picomolar-low nanomolar range). The affinity and
selectivity of the congener is preferably at least
comparable to that of cocaine so that the full spectrum
5   of biologically relevant cocaine recognition sites
throughout the brain may be monitored. The compounds of
the invention are examples of such improved imaging
probes.

II. Techniques for evaluating and using cocaine mimics.

10   In reviewing the claimed compounds and their uses,
it is helpful to refer to experiments using two known
compounds which have been studied for their ability to
mimic cocaine binding in the brain, and to mimic the in
vivo effects of cocaine. These compounds are: a) 2β-
15   carbomethoxy-3β-(4-fluorophenyl)tropane or "CFT"; it is
also designated "WIN 35,428"; and b) 2β-carbomethoxy-3β-
(4-iodophenyl)tropane or "CIT".

        The cocaine receptor-binding assays, and the other
protocols for testing the ability of CFT to mimic
20   cocaine, can also be used for evaluating the other
cocaine analogs described below. Those skilled in the
art will understand that the procedures detailed below
for CFT are generally applicable to other cocaine analogs
discussed below.

25   A. Binding Studies

        Specifically, in early studies, the binding
properties of the cocaine congener [³H]CFT were determined
in tissue homogenates and compared with those of
[³H]cocaine (Madras et al., Mol. Pharmacol. 36:518-524,
30   1989). All studies were conducted in monkey brain
caudate-putamen for two reasons. First, this dopamine-
rich brain region has the highest density of [³H]cocaine
receptors in the brain, and dopamine has been implicated
in mediating the effects of cocaine. Second, primates
35   are consistently used to test diagnostic imaging agents

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and to test for cocaine-like drugs and drug therapies for Parkinson's disease. These studies indicated that [³H]CFT and [³H]cocaine binding in monkey caudate-putamen were similar in the following respects:

5   (1) The density of [³H]CFT binding sites (Bmax: 388 pmol/g) was similar to the density of [³H]cocaine binding sites (Bmax: 471 pmol/g).

       (2) Both [³H]cocaine and [³H]CFT labeled high- and low-affinity binding components, with the high-affinity

10   component constituting approximately 10% of the total number of sites.

       (3) NaCl was necessary for specific binding of [³H]CFT and [³H]cocaine.

       (4) Both [³H]CFT and [³H]cocaine were inhibited

15   stereoselectively by the enantiomers of cocaine and pseudococaine and by the diastereoisomers of WIN 35,065-2.

       (5) A high positive correlation between the potencies of drugs for inhibiting specifically bound

20   [³H]CFT and [³H]cocaine was clearly demonstrated (0.99; $p < 0.001$; Fig. 1A).

       (6) A high positive correlation was found between the relative potencies of drugs for inhibiting [³H]CFT or [³H]cocaine binding and for inhibiting dopamine uptake

25   (Fig. 1B).

       (7) The relative potencies of cocaine analogs and other monoamine uptake inhibitors for inhibiting [³H]CFT binding in monkey caudate-putamen corresponded closely to their relative potencies for producing behavioral

30   stimulation (Fig. 2A) and for maintaining intravenous drug self-administration (Fig. 2B). This high degree of correspondence supports the view that in caudate-putamen, the pharmacological profiles of [³H]CFT and [³H]cocaine are virtually identical and that [³H]CFT labels

35   behaviorally relevant cocaine receptors.

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Depletion of [³H]cocaine binding sites in post-mortem Parkinson's diseased putamen has been attributed to the pre-synaptic location of cocaine binding sites associated with the dopamine transporters (Schoemaker et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 329:227-235, 1985). [³H]CFT binding sites are severely depleted in post-mortem human Parkinson's diseased caudate-putamen using either tissue homogenates (Fig. 3) or tissue sections to monitor [³H]CFT binding. These results suggest that CFT is a suitable marker for Parkinson's disease (Madras et al., Soc. Neurosci. Abst. 16:14, 1991; Kaufman and Madras, Synapse 9:43-49, 1991).

Moreover, the affinity of [³H]CFT was higher and its dissociation rate slower than that of [³H]cocaine. Improvements in both parameters were essential for detailed mapping and characterization of cocaine binding sites in the brain. The affinity of [³H]CFT ($K_{0.50}$: 16.0 nM) was 18 times higher than that of [³H]cocaine ($K_{0.80}$: 283 nM) and the dissociation rate considerably slower. Although unanticipated, [³H]CFT offered other advantages over [³H]cocaine. A higher degree of reproducibility using [³H]CFT enabled previously unrecognized differences in the binding of drugs to become apparent. For example, competition curves for certain cocaine congeners and other drugs (e.g., CFT, cocaine, and (-)-norcocaine) were shallow (0.61-0.89), reached full displacement of [³H]CFT and were characterized by at least two binding components. In contrast, competition curves for monoamine uptake inhibitors structurally distinct from cocaine (e.g., Lu 19-005, GBR 12909 and bupropion) were steeper (0.94-1.1), plateaued at levels corresponding to about 90-93% and were characterized by a single binding component. Furthermore, the level of non-specific binding of [³H]CFT was lower than that of [³H]cocaine. The low level of non-specific binding suggested that

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[³H]CFT and other radiolabeled forms of CFT would be useful probes for imaging cocaine recognition sites and dopamine terminals and dopamine terminals *in vitro* and *in vivo*.

5   B. Behavioral Effects

To determine whether CFT also mimicked cocaine's behavioral effects *in vivo* (specifically the reinforcing and interoceptive effects), squirrel monkeys were trained to respond under a second order schedule of i.v. drug

10   self-administration (see detailed description below). These studies demonstrated that CFT was approximately six times more potent than cocaine in maintaining responding. The potency relationships were similar to the potency relationships for cocaine and CFT for inhibiting [³H]CFT

15   or [³H]cocaine binding and for inhibiting dopamine uptake. The results were consistent with the view that the reinforcing effects of cocaine are mediated by cocaine recognition sites associated with the dopamine uptake system.

20   The subjective (interoceptive) effects of cocaine were studied in monkeys through the use of a drug discrimination procedure. This procedure was used to determine whether CFT produced a stimulus cue comparable to that of cocaine. Squirrel monkeys were trained to

25   discriminate cocaine (0.3-0.56 mg/kg) from saline using a two-level choice procedure. CFT fully generalized to the cocaine cue, and their potency relationships relative to cocaine corresponded to the potency relationships for inhibiting [³H]CFT binding sites *in vitro*. These results

30   indicated that the subjective effects of CFT and cocaine were similar again suggesting that [³H]CFT represented a useful probe for characterizing behaviorally relevant cocaine binding sites.

C. Distribution in Brain

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To identify the distribution of cocaine recognition sites in the brain, autoradiographic techniques were utilized. Such techniques were particularly appropriate for this purpose because they are 100-1,000 times more sensitive than radioreceptor assays conducted in tissue homogenates. The moderately high affinity, slow dissociation, and low level of non-specific binding of [^{3}H]CFT made this congener particularly well suited for autoradiography and for imaging. Three sequential procedures: parametric studies, in vitro autoradiographic techniques, and ex vivo autoradiographic techniques were conducted in tissue sections of squirrel monkey brains. It should be noted that the pharmacological specificity of [^{3}H]CFT binding sites in squirrel monkey and cynomolgus striatum are virtually identical.

In order to characterize binding sites for [^{3}H]CFT in tissue sections, competition experiments were conducted using a fixed concentration of [^{3}H]CFT (3 nM) and a range of concentrations of (-)-cocaine, (+)-cocaine, CFT, Lu 19-005, GBR 12909, bupropion and citalopram. The IC_{50} values for the drugs in tissue sections corresponded closely with their reported IC_{50} values in monkey caudate-putamen membranes (r: 0.99; p < 0.001), suggesting that [^{3}H]CFT binding is similar in the two preparations. The pharmacological specificity of [^{3}H]CFT binding sites in substantia nigra and zona incerta were similar.

After establishing suitable conditions for autoradiography, systematic mapping of cocaine recognition sites were conducted in squirrel monkey brain at nine anterior - posterior levels. Tissue sections were incubated alone, or in the presence of excess (-)-cocaine to determine total and nonspecific binding of [^{3}H]CFT, respectively. High densities of [^{3}H]CFT binding

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were present in the dopamine-rich brain regions,
including the caudate nucleus, putamen, nucleus
accumbens, and olfactory tubercle. In each of these
brain regions, specific binding was >90% of total
5   binding. Intermediate densities of [³H]CFT binding were
detected in substania nigra, zona incerta, amygdala, and
the hypothalamus. Low, though measurable, levels of
[³H]CFT binding were observed in the bed nucleus of the
stria terminalis, the ventral tegmental area, the medial
10   preoptic area, the pineal, the hippocampus, and the
thalamic central nuclei. Near background levels were
found in the white matter, cerebellum, globus pallidus,
and cortical regions See Figs. 4a-A through 4a-F of the
'359 patent referenced above. The distribution and
15   density of [³H]CFT binding sites closely paralleled the
distribution of D_1 and D_2 dopamine receptors and the
concentration of dopamine in primate brain, mediating the
behavioral effects of cocaine (Kaufman et al., Synapse,
supra, 1991).
20   From the in vitro autoradiographic studies, two
conclusions were drawn: (a) cocaine receptors labelled
by [³H]CFT distributed primarily to dopamine-rich brain
regions and (b) the highly circumscribed distribution and
low level of non-specific binding of [³H]CFT further
25   supported the development of CFT as a PET imaging ligand
for cocaine receptors in vivo. However, as the
distribution pattern of a probe in vitro may not
necessarily reflect its distribution in vivo, it was
necessary to determine whether similar conclusions could
30   be drawn using ex vivo autoradiographic techniques. The
ex vivo autoradiographic distribution of [³H]CFT was
determined following intravenous administration of [³H]CFT
(1.0 or 2.5 nmol/kg) to squirrel monkeys. See Figs. 4b-
through 4bL of the '359 patent referenced above. Brain
35   sections from several A-P levels were exposed to [³H]-

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sensitive film. The resulting autoradiograms revealed
binding of [³H] CFT primarily in dopamine-rich brain
regions: caudate, putaman > nucleus accumbens/olfactory
tubercla, stria terminalis > substantia nigra >
5   hypothalamus. Several regions such as cortex and
thalamus displayed more prominent binding in vivo than in
vitro. Nevertheless, the ex vivo labeling of brain
regions by [³H]CFT corresponded closely to the in vitro
autoradiographic pattern.

10   At the time these studies were completed, a higher
affinity iodinated analog of [³H]CFT, [¹²⁵I]RTI-55 (i.e.,
CIT, 2β-carbomethoxy-3β-(4-iodophenyl)tropane; Dupont-
NEN), became available. [¹²⁵I] RTI-55 labels sites in the
caudate putamen with an approximately 10 fold higher
15   potency than [³H]CFT. This ligand was also tested by ex
vivo autoradiography as an imaging probe. Experiments
carried out as described above revealed that highest
densities of [¹²⁵I]RTI-55 were detected in the caudate-
putamen, and moderately high densities also were found in
20   the thalamus, cortex and brain stem nuclei. The results
indicated that although both probes labeled dopamine-rich
brain areas, [¹²⁵I]RTI-55 labeled sites in addition to
those recognized by [³H]CFT. The additional sites labeled
by [¹²⁵I]RTI-55 are associated with the serotonin
25   transporter (Kaufman et al., Soc. Neurosci. Abst., 1991).

          In a collaborative study with the PET imaging
group at the Massachusetts General Hospital, a PCR-I
camera was used to conduct preliminary PET imaging
studies with [¹¹C]CFT in cynomolgus and squirrel monkeys
30   in order to determine the distribution and kinetic
properties of the ligand. The monkeys were injected with
[¹¹C]CFT (Sp. act.: 400-700 Ci/mmol, 3-9 μg, 0.5-1 ml),
arterial blood samples were collected, and dynamic
imaging was carried out for 90 minutes. Images showed
35   high uptake of [¹¹C]CFT in the caudate-putamen with a

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resulting striatal-to-cerebellar ratio of > 4.0 at 58
min. Mazindol and other dopamine uptake inhibitors
displaced bound [^{11}C]CFT from the striatum. [^{11}C]CFT
binding was reduced in MPTP-treated Parkinsonian monkeys
5   and depletion was found in asymptomatic monkeys. These
results further suggest the use of [^{11}C]CFT to monitor
Parkinson's disease (Hantraye et al., Neuro. Reports
3:265-268, 1992).

        Results from the PET imaging studies were
10   sufficiently encouraging to support further evaluation of
CFT as a potential imaging probe for human brain. In
order to determine whether [^{3}H]CFT labels sites associated
with the dopamine transporter, the binding of [^{3}H]CFT to
homogenates of human caudate nucleus and putamen, was
15   characterized. [^{3}H]CFT binding was saturable,
stereoselective, and inhibited by drugs with a rank order
of potency: Lu 19-005 > CFT, mazindol, GBR 12909 > (-)-
cocaine > bupropion > (+)-cocaine, that was identical
with that of cocaine receptors associated with the
20   dopamine transporter in monkey caudate-putamen.

II. Synthesis

        To prepare the compounds of the invention which
lack a ring nitrogen, the following procedures may be
utilized.

25   **Synthesis of 2β-Carboxymethyl-3β-
        arylbicyclo[3.2.1]octanes.**

        To prepare the oxa, thia, and carbo analogs of
CFT, the following synthetic procedures may be used.

        Initially, compound **22'** is prepared, preferably as
30   shown in Fig. 6 or 7 (where it is given the number **3**).
From that point, alternative synthesis routes are shown.

        In Fig. 7, one synthesis of the 2-carbomethoxy-3-
arylbicyclo[3.2.1]octanes utilizes the following route
(see separate scheme provided). The essential feature of
35   this alternative route is the reaction of a suitably

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substituted boronic acid with the intermediate triflate
formed from **22'** (Fig. 6). Thus, **22'** is formed as
described by Brownridge and Chan (Brownridge and Chan,
Tetrahedron Letters, 46:4437, 1979)(the appropriate thia

5 or carbon analogs are used as precursors for the
synthesis of the thia and carbobicyclo[3.2.1]octanes).
The enol triflate, **4**, (See Fig. 7) is then formed by
reaction with N-phenyltrifluoromethansulfonimide and
sodium bistrimethylsilylamide. Coupling of the

10 appropriate preformed aryl boronic acid is accomplished
in the presence of
tris(dibenzylideneacetone)dipalladium(0) to provide **5**.
Reduction of 5 with samarium iodide them provides the
mixture of final products, **6** and **7**. These are obtained

15 as 2α and 2β and 3α and 3β substituted isomers and can be
separated by careful column chromatography.
        Separation of enantiomers can be accomplished as
described earlier, or alternatively by formation of the
camphor enolate ester of **3** and column chromatography of

20 the diastereoisomers thus obtained. Hydrolysis of
theseenantiomerically pure esters then provides
enantiopure **3**. Each enantiomer is then taken through the
sequence described earlier to obtain the enantiopure
products **6** and **7**.

25   In Fig. 6, an alternative synthesis is shown,
involving the reaction of a suitable aromatic Grignard
reagent with the oxabicyclo[3.2.1]octene methyl ester
24'. This methyl ester is obtained by dehydration of the
3-hydroxy compound, **23'**, which is, in turn, obtained by

30 reduction of the ketone, **22'** with sodium borohydride.
The ketone **22'** is prepared as described by Brownridge and
Chan (Tetrahedron Letters, 46:4437, 1979). Resolution is
carried out on the alcohol, **23'** (or **27'**), by
crystallization of a chiral ester. Thus, 2,5-

35 dimethozytetrahydrofuran, **20'**, (the appropriate thia or

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carbon analogs are used as precursors for the synthesis
of the thia and carbbicyclo[3.2.1]octanes) is reacted
with 1,3-bis(trimethylsiloxy)-1-methoxybuta-1,3-diene,
21', in methylene chloride in the presence of titanium
5   tetrachloride to give **22'** in 79% yield. Reduction of the
ketone, **22'**, with sodium borohydride then provides the
alcohol as a mixture of stereoisomers, with **23'** as a
major stereoisomer present and **27'** as the minor. The
desired syn stereoisomer is separated and the enantiomers
10 are separated by fractional crystallization of chiral
esters such as the tartrate ester. The enantiomerically
pure alcohol is dehydrated and reesterified with
phosphorus exychloride and methanol to provide the
anhydromethyl ester, **24'**, which is reacted with a
15 suitably chosen Grignard reagent (described above) to
provide the desired products, (for example, **25'**).

    **Synthesis of 2β-Carboxymethyl-3α-diphenylmethoxy
[3.2.1]octanes.** To prepare the oxa analogs of the 3α-
diphenylmethoxytropanes, the synthetic procedure shown in
20 Fig. 6 is followed. The 3α-diphenylmethoxy[3.2.1]octanes
are made from the ketone **22'**. Either reduction of **22'**
with sodium borohydride will provide **27'** as the minor
isomer, or reduction with sodium will provide **27'**. As
before, **27'** is then reacted with a suitably chosen
25 diphenyl diazomethane or dibenzhydrol to provide the
desired compounds, **28'**.

    **Synthesis of 2β-Carboxymethyl-3β-diphenylmethoxy
[3.2.1]octanes.** To prepare the oxa analogs of the 3β-
diphenylmethoxytropanes, the synthetic procedure shown in
30 Fig. xx is followed. Compound **23'** is prepared and
resolved, and purified, and the enantiomerically pure
alcohol, **23'**, is then reacted with a suitably selected
diphenyldiazomethane to provide the desired target
compounds, **26'**.

35   Reaction of **25'**, **26'** or **28'** with suitably chosen
lithium reagents will provide the 2β-ketones.

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Experimental Details for Synthesis of the 8-oxabicyclo[3.2.1] octanes

Methyl 3-trimethylsiloxybut-2-enoate (1)

5   A mixture of freshly fused ZnCl2 (1.1 g) and triethylamine (86 mL, 0.62 mol) was stirred for 1 h at room temperature. Methyl acetoacetate (40 mL, 0.37 mol) in benzene (80 mL) was added dropwise, followed by chlorotrimethylsilane (90 mL, 0.72 mol). The mixture was stirred overnight at 40°C. After cooling, ether (500 mL)

10   was added. The mixture was stirred for 1 h and filtered through celite. Volatiles were removed in vacuo. Hexanes (200 mL) were added to the residue which was filtered through celite once again. The filtrate was concentrated to dryness and the residue was distilled

15   through a vigreux column at 79-82° C (1.2 mm) to afford 57.6 g (83 %) of 1 as a clear oil.

1,3-Bis(trimethylsiloxy)-1-methoxybuta-1,3-diene (2)

       To dry diisopropylamine (52 mL, 0.39 mol) in THF (400 mL) at 0° C under nitrogen was added n-BuLi (146 mL,

20   2.5 M in hexanes, 0.365 mol) followed by TMEDA (48.5 mL, 0.32 mol). The solution was stirred at 0° C for 30 min then cooled to -78° C. Methyl 3-trimethylsiloxybut-2-enoate, 1, (57.6 g, 0.3 mol) was added to the solution. After stirring for 10 min, chlorotrimethylsilane (61 mL,

25   0.48 mol) was added. The mixture was allowed to warm up to 0° C and was then concentrated to dryness on a rotary evaporator at 0° C (the compound is heat sensitive). Hexanes (dried over molecular sieves; 1 L) were added. The insolubles were removed by filtration and washed with

30   hexanes. The combined filtrate was concentrated to dryness on a rotary evaporator at 0° C and dried overnight, under high vacuum, at room temperature, to afford 82 g of crude product, 2, which was used in the next step without further purification.

35   ### 2-Carbomethoxy-8-oxabicyclo[3.2.1] octan-3-one (3)

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To 2,5-dimethoxytetrahydrofuran (39.6 g, 0.3 mol)
in CH_2Cl2 (anhydrous, 200 mL) at -78° C under nitrogen was
added $TiCl_4$ (66 mL, 0.6 mol). After stirring for 30 min,
1,3-bis(trimethylsiloxy)-1-methoxybuta-1,3-diene, 2, (78

5   g, 0.3 mol) in CH_2Cl_2 (anhydrous, 400 mL) was added at a
rate such that the internal temperature was maintained
below -55° C. The mixture was stirred for 3 h.
Saturated $NaHCO_3$ was added until the mixture was neutral
to pH paper. The aqueous layer was extracted with ether

10 (3 x 1 L). The dried ($MgSO_4$) combined organic layers were
concentrated to dryness. The residue was purified by
flash chromatography (20% EtOAc/hexanes) to afford 20.5 g
(37 %) of 3 as a light brown oil.

2-Carbomethoxy-3-{[(trifluoromethyl)sulfonyl]oxy}-8-
15 **oxabicyclo[3.2.1]-2-octene (4)**

Sodium bistrimethylsilylamide (1.0 M solution in
THF, 45 mL) was added dropwise to 2-carbomethoxy-8-
oxabicyclo[3.2.1] octanone, 3, (7.12 g, 38.65 mmol) in
THF (100 mL) at -70° C under nitrogen. After stirring

20 for 30 min, N-phenyltrifluoromethanesulfonimide (15.19 g,
42.52 mmol) was added as a solid at -70° C. The reaction
was allowed to warm to room temperature and was then
stirred overnight. The volatiles were removed on a
rotary evaporator. The residue was dissolved in CH_2Cl_2

25 (200 mL) and washed with H_2O (100 mL) and brine (100 mL).
The dried ($MgSO_4$) CH_2Cl_2 layer was concentrated to
dryness. The residue was purified by flash
chromatography (10% EtOAc/hexanes) to afford 9.62 g (79
%) of 4 as a pale yellow oil.

30 ^{1}H NMR ($CDCL_3$, 100MHz): δ 5.05 (bm, 1H),4.70 (t, 1H), 3.83
(s, 3H), 3.0 (dd, 1H), 2.0-2.35 (m, 5H)

4-Fluorophenyl boronic acid

To a stirred solution of triisopropyl borate (4.6
mL, 20 mmol) in THF (1 mL) at -78° C under nitrogen was
35 added 4-fluorophenylmagnesium bromide (2 M solution in

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diethylether, 10 mL) dropwise. The mixture was allowed
to warm to room temperature. Ether (50 mL) was added,
followed by 10% HCl (50 mL). The ether layer was then
washed with H_2O (25 mL) and brine (25 mL). The dried
5   ($MgSO_4$) ether layer was concentrated to dryness. The
residue was triturated with hexanes to provide an off-
white solid which was filtered and dried under vacuum to
afford 1.38 g of 4-fluorophenyl boronic acid.

2-Carbomethoxy-3-(4-fluorophenyl)-8-oxabicyclo[3.2.1]-2-
10   **octene (5a)**
          2-Carbomethoxy-3-{[(trifluoromethyl)sulfonyl]oxy}-
8-oxabicyclo[3.2.1]-2-octene, **4**, (1.87 g, 5.9 mmol), 4-
fluorophenyl boronic acid (1.09 g, 7.8 mmol),
diethoxymethane (20 mL), LiCl (535 mg, 12.6 mmol),
15   tris(dibenzylideneacetone)dipalladium(0) (230 mg, 0.25
mmol) and Na_2CO_3 (2 M solution, 5.7 mL) were combined and
heated at reflux for 1 h. The mixture was cooled to room
temperature, filtered through celite and washed with
ether (100 mL). The mixture was basified with NH_4OH and
20   washed with brine. The dried ($MgSO_4$) ether layer was
concentrated to dryness. The residue was purified by
flash chromatography (10 % EtOAc/hexanes) to afford 1.36
g (88 %) of **5a** as a light brown viscous oil.
^1H NMR (CDCl$_3$, 100MHz): δ 6.99-7.12 (m, 4H), 5.00 (bm,
25   2H), 4.64 (t, 1H), 3.52 (s, 3H), 2.95 (dd, 1H), 1.71-2.19
(m, 5H)

2-Carbomethoxy-3-phenyl-8-oxabicyclo[3.2.1]-2-octene (5b)
          Reaction of 2-carbomethoxy-3-
{[(trifluoromethyl)sulfonyl]oxy}-8-oxabicyclo[3.2.1]-2-
30   octene, **4**, (2.0 g, 6.32 mmol), phenyl boronic acid (1.02
g, 8.36 mmol), diethoxymethane (20 mL), LiCl (578 mg,
13.6 mmol), tris(dibenzylideneacetone)dipalladium(0) (247
mg, 0.25 mmol) and Na_2CO_3 (2M solution, 6.1 mL), as
described above, gave 1.28 g (82 %) of **5b** as a light
35   brown viscous oil.

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^{1}H NMR (CDCl$_3$, 100MHz): δ 7.1-7.5 (m, 5H), 5.00 (bm, 1H), 4.64 (t, 1H), 3.52 (s, 3H), 2.95 (dd, 1H), 1.71-2.19 (m, 5H)

2-Carbomethoxy-3-(4-chlorophenyl)-8-oxabicyclo[3.2.1]-2-octene (5c)

Reaction of 2-carbomethoxy-3-{[(trifluoromethyl)sulfonyl]oxy}-8-oxabicyclo[3.2.1]-2-octene, **4**, (1.0 g, 3.16 mmol), 4-chlorophenyl boronic acid (653 mg, 4.17 mmol), diethoxymethane (10 mL), LiCl (286 mg, 6.75 mmol), tris(dibenzylideneacetone)dipalladium(0) (123 mg, 0.13 mmol) and Na$_2$CO$_3$ (2M solution, 3.0 mL), as described above, gave 0.81 g (92 %) of **5c** as a light brown viscous oil. ^{1}H NMR (CDCl$_3$, 100MHz): δ 7.0-7.35 (q, 4H), 5.00 (bm, 1H), 4.64 (t, 1H), 3.52 (s, 3H), 2.95 (dd, 1H), 1.71-2.19 (m, 5H)

2-Carbomethoxy-3-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]-2-octene (5d)

Reaction of 2-carbomethoxy-3-{[(trifluoromethyl)sulfonyl]oxy}-8-oxabicyclo[3.2.1]-2-octene, **4**, (1.0 g, 3.16 mmol), 3,4-dichlorophenyl boronic acid (796 mg, 4.17 mmol), diethoxymethane (10 mL), LiCl (286 mg, 6.75 mmol), tris(dibenzylideneacetone)dipalladium(0) (123 mg, 0.13 mmol) and Na$_2$CO$_3$ (2 M solution, 3.0 mL), as described above, gave 0.96 g (97 %) of **5d** as a light brown viscous oil. ^{1}H NMR (CDCl$_3$, 100MHz): δ 6.9-76.5 (m, 4H), 5.00 (bm, 1H), 4.64 (t, 1H), 3.52 (s, 3H), 2.95 (dd, 1H), 1.71-2.19 (m, 5H)

2β-Carbomethoxy-3α-(4-fluorophenyl)-8-oxabicyclo[3.2.1]octane (6a) and 2β-Carbomethoxy-3β-(4-fluorophenyl)-8-oxabicyclo[3.2.1]octane (7a)

To 2-carbomethoxy-3-(4-fluorophenyl)-8-oxabicyclo[3.2.1]-2-octene, **5a**, in THF (10 mL) at -70° C under N$_2$ was added SmI$_2$ (0.1 M in THF, 230 mL, 23.0 mmol). After the mixture was stirred for 30 min, MeOH (anhydrous, 25 mL) was added. The mixture was stirred

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at -70° C for a further 2 h. The mixture was quenched
with TFA (5 mL) and H$_2$O (100 mL). After warming to 0° C,
NH$_4$OH was added to attain a pH 11 and the mixture was then
stirred for 30 min. The mixture was filtered through

5 celite and washed with ether (400 mL) and then saturated
with Na$_2$S$_2$O$_3$. The ether layer was washed with brine. The
dried (MgSO$_4$) ether layer was concentrated to dryness.
The isomers were separated by gravity column
chromatography (10 % EtOAc/hexanes) to afford 834 mg (62

10 %) of **6a** as a white solid, mp. 58-60° C and 300 mg (22 %)
of **7a** as a white solid, mp.118.5-120.5° C. ^{1}H NMR (CDCl$_3$,
400 MHz) (**6a**): δ 6.92-7.17(m, 4H), 4.48 (bd, 2H), 3.58
(s, 3H), 3.20 (dt, 1H), 2.44 (dd, 1H),2.38 (m, 1H), 2.12
(m, 1H), 2.00 (1H), 1.75 (m, 1H),1.63 (m, 1H), 1.32 (ddd,

15 1H)

^{1}H NMR (CDCl$_3$, 400 MHz) (**7a**): δ 6.93-7.20 (m, 4H), 4.65
(m, 2H),3.48 (s, 3H), 3.17 (dt, 1H), 2.78 (d, 1H), 2.73
(dt, 1H), 2.13 (1H), 2.05 (m, 1H), 1.90 (m, 1H), 1.78 (m,
1H), 1.59 (m, 1H)

20 **2β-Carbomethoxy-3α-phenyl-8-oxabicyclo[3.2.1]octane (6b)
and 2β-Carbomethoxy-3β-phenyl-8-oxabicyclo[3.2.1]octane
(7b)**

Reaction of 2-carbomethoxy-3-phenyl-8-
oxabicyclo[3.2.1]-2-octene, **5b**, (1.17 g, 4.8 mmol) in THF

25 (10 mL) and SmI$_2$ (0.1 M in THF, 215 mL, 21.5 mmol), as
described above, gave 789 mg (67 %) of **6b** as a white
solid, mp. 96.5-98° C and 270 mg (23 %) of **7b** as a white
solid, mp. 102.5-104° C. ^{1}H NMR (CDCl$_3$, 100 MHz) (**6b**): δ
7.25(bs, 5H), 4.51 (bd, 2H), 3.58 (s, 3H), 3.25 (dt, 1H),

30 2.51 (dd, 1H),2.38 (m, 1H), 1.6-2.2 (m, 4H), 1.41 (ddd,
1H)

^{1}H NMR (CDCl$_3$, 100 MHz) (**7b**): δ 7.25 (bs, 5H), 4.65 (m,
2H),3.48 (s, 3H), 3.25 (dt, 1H), 2.78 (d, 1H), 2.73 (dt,
1H), 1.5-2.3 (m, 5H)

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2β-Carbomethoxy-3α-(4-chlorophenyl)-8-oxabicyclo[3.2.1]octane (6c) and 2β-Carbomethoxy-3β-(4-chlorophenyl)-8-oxabicyclo[3.2.1]octane (7c)

Reaction of 2-carbomethoxy-3-(4-chlorophenyl)-8-oxabicyclo[3.2.1]-2-octene, **5c**, (808 mg, 2.9 mmol) in THF (8 mL) and SmI$_2$ (0.1 M in THF, 130 mL, 13.0 mmol), as described above, gave 418 mg (51 %) of (**6c**) as a white solid, mp. 89-90° C and 152 mg (19 %) of **7c** as a white solid, mp. 116-117° C.

^{1}H NMR (CDCl$_3$, 100 MHz) (**6c**): δ 7.1-7.3(m, 4H), 4.51 (bd, 2H), 3.58 (s, 3H), 3.25 (dt, 1H), 2.51 (dd, 1H),2.38 (m, 1H), 1.6-2.2 (m, 4H), 1.35 (ddd, 1H)

^{11}H NMR (CDCl$_3$, 100 MHz) (**7c**): δ 7.1-7.4 (m, 4H), 4.65 (m, 2H),3.48 (s, 3H), 3.20 (dt, 1H), 2.6-2.9(m, 2H), 1.5-2.3 (m, 5H)

2β-Carbomethoxy-3α-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]octane (6d) and 2β-Carbomethoxy-3β-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]octane (7d)

Reaction of 2-carbomethoxy-3-(3,4-dichlorophenyl)-8-oxabicyclo[3.2.1]-2-octene, **5d**, (829 mg, 2.65 mmol) in THF (5 mL) and SmI$_2$ (0.1 M in THF, 119 mL, 11.9 mmol), as described above, gave 455 mg (55 %) of **6d** as a white solid, mp. 88.5-90° C and 115 mg (14 %) of **7d** as a white solid, mp. 132-133.5° C.

^{1}H NMR (CDCl$_3$, 100 MHz) (**6d**): δ 7.0-7.3 (m, 3H), 4.51 (bd, 2H), 3.61 (s, 3H), 3.25 (dt, 1H), 2.55 (dd, 1H), 1.6-2.4 (m, 5H), 1.35 (ddd, 1H)

^{1}H NMR (CDCl$_3$, 100 MHz) (**7d**): δ 7.0-7.4 (m, 3H), 4.65 (m, 2H), 3.48 (s, 3H), 3.20 (dt, 1H), 2.6-2.9(m, 2H), 1.5-2.3 (m, 5H)

2-Carbomethoxy-3-camphanate-8-oxabicyclo[3.2.1]oct-2-ene

The ketone, **3** was dissolved in anhydrous THF and cooled to -78° C under nitrogen. Butyl lithium (1.1 eq.) was added slowly to this cold solution. The resulting

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solution was allowed to stir for 15 min. The (1S) or
(1R) camphanic chloride (1.1 eq.) was added in one
portion and the cooling bath was removed. As the
solution reached room temperature (ca. 15 min) TLC
5   indicated (30% ethyl acetate in hexane: Rf 0.25)
indicated completion of reaction. The reaction was
quenched with saturated NaHCO₃ and extracted with ether.
The layers were separated and the ether further washed
with brine. Drying, (MgSO₄) filtration and evaporation
10   gave a yellow solid which was purified by flash
chromatography (30% ethyl acetate/hexanes) to yield (86%)
a white solid, mp. 122-123.5° C .

        The single diastereomers were separated by
repeated recrystallization from CH₂Cl₂/hexanes.

15   ¹H NMR (CDCl₃, 100 MHz): δ_5.02 (br s, 1H), 4.65 (br t,
1H), 3.72 (s, 3H), 1.6-3.1 (m, 10H), 1.14 (s, 3H), 1.11,
1.13 (2s, 3H), 1.04, 1.06 (2s, 3H).

Hydrolysis of 2-Carbomethoxy-3-camphanate-8-oxabicyclo[3.2.1]oct-2-ene

20   The camphanate ester was dissolved in a 3:1:1
mixture of THF:H₂O:MeOH and LiOH hydrate (1.6 eq.) was
added to this in one portion. TLC showed completion of
hydrolysis within 1 min. The solution was brought to pH
7 with 1M HCl and organic solvents were evaporated. To
25   the residue was added brine and CH₂Cl₂ and the layers were
separated. The aqueous fraction was extracted with
CH₂Cl₂. Organic extracts were combined and dried (MgSO₄),
filtered and evaporated. The residue was chromatographed
(2% Et₂O/CH₂Cl₂) to give the product in 57% yield.
30   Compound Characterization

        Characterization of the compounds is carried out
using standard methods of high field NMR spectra as well
as IR, MS and optical rotation. Elemental analysis, TLC
and/or HPLC is used as a measure of purity. A purity of

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>98% is preferred before biological evaluation of these compounds is undertaken. These standards have been easily achieved in prior preparations of CFT itself as well as in the preparation of a number of CFT analogs.

5   IV. Binding Assays for Candidate Compounds

        To evaluate derivatives as to selectivity for the dopamine transporter, compounds are screened in radioreceptor assays using both [³H]CFT and [³H]citalopram as probes for the dopamine and serotonin receptors,
10 respectively. The relative affinities of a compound for either site establishes whether it binds selectively to the dopamine transporter or non-selectively to the serotonin transporter as well. Assays are carried out as follows.

15   Competition studies to determine the potency of a compound for inhibiting specifically bound [³H]CFT in monkey caudate-putamen membranes are conducted using 0.3-10nM [³H]CFT and the brain tissue of drug- and disease-free cynomolgus monkeys (Macaca fascicularis), which is
20 stored at -85°C. The caudate-putamen is dissected from coronal sections and suspended in 10 vol Tris-HCl (50 mH, pH 7.4 at 4°C). The tissue is homogenized in a Potter-Elvehjem (glass-teflon) homogenizer with 8 up-and-down strokes using a Tri-R electronic control stirrer motor
25 (2000 rpm approx.). The homogenate is centrifuged twice for 30 min at 38,000 x g and resuspended in buffer to yield a final concentration of 4 mg original wet tissue weight/ml of assay buffer. The assay medium contains, in order of addition, buffer (50 mM, Tris-HCl, 120mM NaCl)
30 or buffer plus test compound (0.2 ml); [³H]CFT (80 Ci/mMol, 0.2ml, 1nM); and membrane suspension (0.2ml) to a final volume of 0.6 ml. Unlabeled (-)-cocaine (30 μM) serves as the baseline drug to detect non-specific binding. Incubation proceeds at 4°C for 60 min, and the
35 assay is terminated by vacuum filtration over glass fiber

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filters (Whatman GF/B) pre-soaked with 0.1% bovine serum
albumin. The filters are washed with two 5-ml rinses of
ice-cold buffer, incubated overnight in fluor (Beckman
ready-solve, EP grade), and total radioactivity
5   determined by liquid scintillation spectrometry at 50%
counting efficiency. All assays are performed in
triplicate, and each experiment is repeated at least
twice using tissue from different brains.

To assay binding of the analog to the serotonin
10   transporter, analogs are tested for their ability to
compete with labeled [^{3}H]citalopram, a high affinity and
selective ligand for serotonin transporter sites (D'Amato
et al., J. Pharmacol. Exp. Ther. 242:364-371, 1987).
Radioreceptor assays are conducted using tissues prepared
15   as described above. Analogs are incubated with buffer
(50 mM Tris HCl; 100mM NaCl), [^{3}H]citalopram (1 nM), and
tissue (1 mg/ml wet tissue weight), incubated 2 h at 4°C,
and the incubation terminated by rapid filtration. Non-
specific binding is monitored with fluoxetine (1 μM).
20   The sites may first be characterized using other
serotonin uptake inhibitors (sertraline, citalopram,
fluoxetine, paroxetine, imipramine), other monoamine
uptake inhibitors (cocaine, mazindol, GBR 12909,
talsupram), and neurotransmitters (dopamine, serotonin,
25   norepinephrine). Subsequently, the potency of CFT or the
analog is determined. Parallel studies are conducted
with [^{3}H]CFT in order to determine the relative potencies
of the analogs at each transporter.

Oxy analogs of potent phenyltropanes were
30   synthesized and their potencies were determined at the
dopamine, norepinephrine and serotonin transporters.
Several compounds in this series retained the full
spectrum of biological activity as the parent
nitrogen-containing drugs, particularly high affinity
35   binding to the dopamine, norepinephrine and serotonin

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transporters in nonhuman primate striatum and to the
dopamine transporter in human striatum.

The drugs were assessed in brain tissue of adult
cynomolgus monkeys (Macaca fascicularis), a species used
5   in our program for brain imaging of the dopamine
transporter Madras et al., *FASEB J.* 2:A1137 (1988),
Madras et al., J. Pharmacol. Exp. Ther. 251:131-141,
(1989a); Madras et al., Mol. Pharmacol. 36:518-524,
(1989b); Madras et al. *NIDA Res. Monograph* 138:57-69
10   (1994), Madras et al. *Synapse* 22:239-246 (1996)).
Caudate-putamen was the source of the dopamine and
serotonin transporters and thalamus was used to assay the
norepinephrine transporter. Within this series of
non-nitrogen compounds, several exhibited affinities for
15   monoamine transporters in the low nanomolar range. Each
of the drugs inhibited radioligand binding to monoamine
transporters in a concentration dependent and saturable
manner (Figs. 9 (monkey) and Fig. 10 (human)). Most of
the drugs, however, did not fully displace [^{3}H]WIN 35,428
20   binding to the dopamine transporter, an observation that
we have noted in the past for non-phenyltropane drugs
(Madras et al., 1989a,b,). The high affinity of
tropoxane (0-914) for the dopamine transporter was
similar to its affinity for the serotonin transporter and
25   lower for the norepinephrine transporter (Table 1).
These values compare favorably with affinities of
antidepressant drugs for the norepinephrine and serotonin
transporters. They are considerably higher than the
affinity of cocaine for the dopamine transporter but
30   within the range of cocaine congeners. The a-epimer of
0914, 0-913, had similar affinity for the dopamine
transporter. The halogen substituent on the aromatic
ring was critical for high affinity binding as the
nonhalogenated compounds bound in the micromolar range.
35   Based on aromatic ring substitutions, the resulting rank

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order of potency for binding was Cl2 > Cl > F > H. The
rank order corresponds closely to that observed with the
parent nitrogen-containing phenyltropanes, although the
effects of halogen substitution on affinity are more
5   pronounced with this series.

In striatum of human post-mortem brain, the drugs
bound with similar affinities (Table 2). This series of
drugs demonstrates that a nitrogen is not needed for
dopamine transporter binding. They furthermore indicate
10   that nitrogen-containing drugs may not be necessary for
blockade of the serotonin or norepinephrine transporters.
Accordingly, they reveal the possibility of developing a
new generation of drugs for the treatment of depression,
ADHD, and cocaine abuse. This unique series of compounds
15   indicate new avenues for drug discovery and drug
development, significantly modify conventional thinking
about transporter-drug interactions, revise
structure-activity relationships of the cocaine series,
highlight significant differences between the human and
20   monkey dopamine transporter, differences in binding and
transport blockade.

Table 1 shows data for binding of novel drugs to
the dopamine (A), serotonin (B) and norepinephrine (C)
transporters in cynomolgus monkey (Macaca fascicularis)
25   brain. Competition studies were conducted with various
concentrations of the novel compounds and a fixed
concentration of radioligand to label the three monoamine
transporters. (A) Inhibition of [^{3}H]WIN 35,428 (1 nM)
binding to the dopamine transporter in cynomolgus monkey
30   caudate-putamen. The competition curve for WIN 35,428 is
used as a reference standard for each brain preparation;
(B) Inhibition of [^{3}H]citalopram (1 nM) binding to the
serotonin transporter in monkey caudate-putamen. The
competition curve for citalopram is used as a reference
35   standard for each brain preparation; (C) Inhibition of

[^{3}H]nisoxetine (0.6 nM) binding to the norepinephrine
transporter in monkey thalamus. The competition curve
for nisoxetine is used as a reference standard for each
brain preparation. Each curve is representative of
several experiments, each conducted in triplicate.

Table 1. Affinities and monoamine transporter
selectivities of tropoxanes in monkey (Macaca
fasciculans) brain. The affinity of each compound for
the dopamine and serotonin transporter was determined in
striatum with [^{3}H]WIN 35,428 and [^{3}H]citalopram,
respectively, or the norepinephrine transporter, labeled
with [3H]nisoxetine in the thalamus, by radioreceptor
assay procedures reported previously (Madras et al.,
1996).

Compound	Dopamine Transporter [^{3}H]WIN 35,428 IC$_{50}$ (nM)	Serotonin Transporter [^{3}H]Citalpram IC$_{50}$ (nM)	Norepineohrine Transporter [^{3}H]Nisoxetine IC$_{50}$ (nM)
O–905	1,900 ± 247	11,440 ± 447	
O–895	546 ± 67	2,580 ± 404	
O–913	3.06 ± 0.12	64.5 ± 10.3	62.6 ± 4.7
O–914	3.35 ± 0.39	6.52 ± 2.05	27.0 ± 6.7
O–915	34.0 ± 2.4	816 ± 79	
O–916	9.99 ± 2.26	107 ± 17.9	

Note that O-895 is compound 25' in Fig. 6, in which R$_1$ is
-H and R$_2$ is -F; the 3- substituent is in the β
configuration. Compound O-905 is the same as O-895
except that R$_2$ is -F. In each of O-913 through O-916, R$_2$
is -Cl. For O-913 and O-914, R$_1$ is also -Cl, the former
having the 3- substituent in the α position, and the
latter having that substituent in the β position. For O-
915 and O-916, R$_1$ is -H, again, the former having the 3-
substituent in the α position, and the latter having that
substituent in the β position.

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Table 2. Affinities of tropoxanes for the dopamine
transporter in human putamen. The affinity of each
compound for the dopamine transporter, labeled with
[3H1WIN 35,428 (1 nM), was measured by radioreceptor
5   assays using standard procedures (Madras et al., 1996),
with a striatal tissue concentration of 1 mg/ml.

Compound	Dopamine Transporter Affinity [^{3}H]WIN 35,428 OC$_{50}$ (nM)
WIN 35,428	9.95 ± 1.09
(-)-Cocaine	57.7 ± 0.8
O-913 (3)	4.53 ± 1.58
O-914 (2)	5.01 ± 1.74
O-915 (2)	28.5 ± 10.8
O-916 (2)	32.3 ± 0.35

10

Assays

15   The caudate-putamen was homogenized in 10 volumes
(w/v) of ice-cold Tris.HCI buffer (50 mM, pH 7.4 at 4°C)
and centrifuged at 38,700 x g for 20 min in the cold.
The resulting pellet was suspended in 40 volumes of
buffer and the wash procedure was repeated twice. The
20   membrane suspension (25 mg original wet weight of
tissue/ml) was diluted in assay buffer to 12 mg/ml for
[^{3}H]WIN 35,428 or [3Hlcitalopram. The affinity of the
novel compounds was The dopamine transporter was labeled
with [^{3}H]WIN 35,428 ([^{3}H]CFT,
25   2,B-carbomethoxy-3,B-(4-fluorophenyl)-
N-[^{3}H]methyltropane, DuPont-NEN). The affinity of novel
compounds for the dopamine transporter was determined in
experiments by incubating tissue with a fixed
concentration of [^{3}H1WIN 35,428 and a range of
30   concentrations of the compound as previously described
(Madras et al., 1989b). The stock solutions were diluted
serially in the assay buffer and added (0.2 ml) to the
assay medium. The assay tubes received, in Tris.HCl

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buffer (50 mM, pH 7.4 at 0 - 4°C; NaCl 100 mM), the
following constituents at a final assay concentration:
drug (0.2 ml; 1 pM - 300 yM, depending on affinity),
[³H]WIN 35,428 (0.2 ml; 0.3 or 1 nM); membrane preparation
5   (0.2 ml; 1 or 4 mg original wet weight of tissue/ml).
The 2 h incubation (0 - 4°C) was initiated by addition of
membranes and terminated by rapid filtration over Whatman
GF/B glass fiber filters pre-soaked in 0.1% bovine serum
albumin (Sigma Chem. Co.). The filters were washed twice
10   with 5 ml Tris.HCl buffer (50 mM), incubated overnight at
0 - 4°C in scintillation fluor (Beckman Ready-Value, 5
ml) and radioactivity (dpm) was measured by liquid
scintillation spectrometry (Beckman 1801). Total binding
was defined as [³H]WIN 35,428 bound in the presence of
15   ineffective concentrations of the drug. Non-specific
binding was defined as [³H]WIN 35,428 bound in the
presence of an excess (30 μM) of (-)-cocaine. Specific
binding was the difference between the two values.
[³H]Citalopram binding- Serotonin transporter assay

20   The serotonin transporter was assayed in
caudate-putamen membranes using conditions similar to
those for the dopamine transporter. The affinity of
drugs for the serotonin transporter labeled by
[³H]citalopram (spec. act.: 81.86 Ci/mmol, DuPontNEN) was
25   determined by incubating tissue with a fixed
concentration of [³H]citalopram and a range of
concentrations of the test compounds. The assay tubes
received, in Tris.HCl buffer (50 mM, pH 7.4 at 0 - 4°C;
NaCl 100 mM), the following constituents at a final assay
30   concentration: drug (0.2 ml of various concentrations);
[³H]citalopram (0.2 ml; 1 nM); membrane preparation (0.2
ml; 4 mg original wet weight of tissue/ml). The 2 h
incubation (0 - 4°C) was initiated by addition of
membranes and terminated by rapid filtration over Whatman
35   GF/B glass fiber filters pre-soaked in 0.1 %

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polyethyleneimine. The filters were washed twice with 5
ml Tris.HCl buffer (50 mM), and the remaining steps were
carried out as described above. Total binding was
defined as [³H]citalopram bound in the presence of

5   ineffective concentrations of unlabeled citalopram (1 pM)
or the test compounds. Non-specific binding was defined
as [³H]citalopram bound in the presence of an excess (10
yM) of fluoxetine. Specific binding was the difference
between the two values. After conducting preliminary

10 studies with [³H]WIN 35,428 to determine the affinity of
altropane for the transporters, [¹²⁵I]altropane was used
for subsequent studies.

[³H]Nisoxetine binding: Norepinephrine transporter assay

The norepinephrine transporter was assayed in

15 thalamus membranes using conditions similar to those for
the serotonin transporter and adapted from whole rat
brain (Gehlert et al., 1995). The affinity of
[³H]nisoxetine (spec. act.: 74 Ci/mmol, DuPont-NEN) for
the norepinephrine transporter was determined in

20 experiments by incubating tissue with a fixed
concentration of [³H] nisoxetine and a range of
concentrations of unlabeled nisoxetine. The assay tubes
received the following constituents at a final assay
concentration: nisoxetine or drug (0.2 ml; 1 pM - 300

25 yM), [³H]nisoxetine (0.2 ml; 0.6 nM); membrane preparation
(0.2 ml; 4 mg original wet weight of tissue/ml). The
buffer in the assay medium was Tris-HCl: 50 mM, pH 7.4 at
0 - 4°C; NaCl 300 mM. The 16 h incubation at 0 - 4°C was
initiated by addition of membranes and terminated by

30 rapid filtration over Whatman GF/B glass fiber filters
presoaked in 0.3% polyethyleneimine for 1 hr. The
remaining steps are described above. Total binding was
defined as [³H]nisoxetine bound in the presence of
ineffective concentrations of drug. Non-specific binding

35 was defined as [³H]nisoxetine bound in the presence of an

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excess (10 yM) of desipramine. Specific binding was the
difference between the two values.

V.   Behavioral Effects of Candidate Compounds

    To the degree possible, candidate compounds for
molecular probes should have a full range of cocaine-like
biological activity. Candidate compounds found above to
have cocaine-like properties in binding studies are
assessed further for cocaine-like discriminative stimulus
(subjective) effects and their capacity to maintain
intravenous self-administration.

    For drug discrimination and self administration
studies, experiments are conducted with rodents or
squirrel monkeys (Saimiri sciureus) seated in standard
primate chairs enclosed in ventilated, sound-attenuating
chambers. The chairs are equipped with response levers,
stimulus lights, food pellet dispensers, and pumps for
injecting drugs intravenously. Four animals are studied
in each experiment, with each subject receiving all drug
treatments whenever possible. Response rates and
temporal patterns of responding are monitored
continuously during daily experimental sessions. Effects
of drugs are analyzed in individual subjects by comparing
performances during drug sessions with those during
control sessions. In experiments involving drug self-
administration, i.v. injections of drug solutions (0.2
ml, infused over 200 msec) are scheduled intermittently
(as described below). Monkeys in these studies are
prepared with indwelling venous catheters according to
the general procedures described by Herd et al., (Am. J.
Physiol. 27:24-29, 1969). Surgery is conducted in
aseptic conditions under halothane/oxygen anesthesia.
Catheters are passed by way of a jugular or femoral vein
to the level of the right atrium. The distal end of the
catheters is passed subcutaneously to a midscapular exit
point. Catheters are flushed with 0.9% saline solution

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and sealed with stainless steel obturators when not in
use. Monkeys wear nylon mesh jackets at all times to
protect the catheters.

VI. Drug Discrimination

5   Drug discrimination experiments are conducted
using cumulative-dose procedures similar to those
described in Madras et al. (Soc. Neurosci. Abst. $\underline{16}$:14,
1990; Pharmacol. Biochem. Behav. $\underline{35}$:949-953, 1990) and
Spealman et al. (J. Pharmacol. Exp. Ther. $\underline{258}$:945-953,
10   1991). Drugs acting in the central nervous system can
induce changes in interoceptive states that serve as
discriminative stimuli in animals, and there is high
pharmacological specificity in the classification of
drugs based on their generalized discriminative-stimulus
15   effects. Cumulative-dose procedures have a major
advantage over single-dose procedures in that they permit
determination of a full dose-effect curve during a single
experimental session. Preliminary experiments are first
conducted to determine the effective dose ranges and the
20   onsets and durations of action of the drugs. Good
agreement has been found between the effects of drugs
determined by cumulative-dose procedures and by single-
dose procedures when appropriate parameters are selected.
Effective doses are studied using single-dose procedures
25   to directly assess the comparability of effects
determined by the two techniques.

          Drug-discrimination assays are used to
characterize the effects of drugs in monkeys trained to
discriminate cocaine from vehicle. During experimental
30   sessions, monkeys are seated in chairs equipped with two
levers, and a food pellet dispenser mounted between the
levers. Each monkey is trained under a 10-response
fixed-rate (FR) schedule of food presentation to respond
differentially on the left and right levers depending on
35   whether cocaine or vehicle is injected. After i.m.

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injection of cocaine, 10 consecutive responses on one lever (left for half the monkeys in each group, right for the reminder) will produce food; after i.m. injection of vehicle, 10 consecutive responses on the other lever will
5   produce food. Responses on the inappropriate lever resets the FR requirement. Daily sessions consist of a variable number (n=1-4) of components of the FR schedule. Each component ends after the completion of 10 FRs or after 10 min, whichever occurs first, and each is
10   preceded by an extended timeout period. During most sessions, vehicle is injected during the timeout period preceding the first n-1 components; drug is injected during the timeout period preceding the nth component of the session. During some sessions, however, vehicle is
15   injected during all timeout periods to prevent an invariant association between the fourth component and injection of drug. The number of components per session is varied randomly from day to day. Training continues until a criterion of > 90% of responses are made on the
20   injection-appropriate lever. The training dose of cocaine is 0.3 - 1.0 mg/kg, depending on the performance of individual subjects.

         Drug testing begins after criterion-level performances are maintained consistently. Thereafter,
25   test sessions are conducted once per week, always preceded by training sessions with criterion performances. Test sessions consist of four FR components, with each component preceded by an extended timeout period. In each FR component, every tenth
30   response, regardless of lever, produces food. Drugs are studied using the cumulative dosing procedure described previously. Incremental doses are injected i.m. during the timeout periods preceding sequential components of the FR schedule, permitting determination of a four-point
35   dose-effect curve in a single session. In most

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experiments, five or six different doses of a test drug
are studied by determining the effects of overlapping
ranges of doses in separate sessions. A test drug is
considered to substitute full for cocaine if it engenders
5   > 90% of responses on the drug-associated lever.
VII. Drug Self-administration
        Self-administration of cocaine and other
psychomotor-stimulant drugs is studied under second-order
schedules of i.v. drug injection (Bergman et al., J.
10   Pharmacol. Exp. The. 251:150-155, 1989, Spealman et al.,
Pharmacol. Biochem. Behav. 31:1011-1013, 1991). Under
these schedules, high rates of responding can be
maintained for extended periods of time by only one or a
few injections of drug per session. This feature is
15   particularly useful for comparing drugs with different
time courses, which may complicate interpretation of
results if injections are scheduled more frequently.
Squirrel monkeys are implanted with venous catheters as
described previously and trained to respond under a
20   second-order schedule of i.v. cocaine injection similar
to the schedule described by Bergman et al. (1989,
supra). In the presence of a red light, every 30th
response (FR unit) produces a 1-sec change in
illumination from red to amber (brief stimulus), and the
25   first FR unit completed after elapse of a 10-min fixed-
interval (FI) produces both the brief stimulus and an
i.v. injection of cocaine. Following a timeout period,
the entire cycle is repeated for a total of ten
injections of drug per session.
30   Initially, responding is maintained using an
injection dose of 100 μg/kg cocaine, which usually is
optimal for developing i.v. self-administration. After
consistent rates and patterns of responding are
maintained by cocaine, the effects of a full range of
35   doses of cocaine and of selected candidate drugs is

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determined. Drugs are studied in different order in different subjects, and experiments with one drug are completed before another drug is studied. Each dose is studied for a block of at least 5 sessions and until no

5   systematic trends in responding are observed for at least 3 consecutive sessions. Blocks of sessions in which vehicle is substituted for drug and responding is extinguished separates experiments with different drugs.

VIII. PET IMAGING

10   A. PET Imaging Probes

The cocaine analogs described herein provide useful PET imaging probes. PET imaging has at least two applications: to evaluate the time course of accumulation of a candidate cocaine substitute in the brain as well as

15   the duration of receptor occupancy and to monitor cocaine receptors and dopamine nerve terminals in competitor studies (see above).

Cocaine analogs of high affinity are most useful as PET imaging probes because the dose of radioactivity

20   needed to image a target decreases along with decreases in organ dosimetry. In addition, CFT displays a markedly low level of non-specific binding. High affinity analogs are also preferable because dopamine may compete effectively with trace doses of low affinity analogs in

25   vivo, thereby reducing the apparent accumulation in striatum. Affinity of a compound is determined using the in vitro binding assay, and distribution is determined by in vivo and ex vivo receptor autoradiography (described above).

30   Compounds may also be analyzed for possible in vivo breakdown. Arterial blood samples are withdrawn at pre-determined intervals, plasma separated by centrifugation, basified, extracted with CH_2Cl_2, the solvent evaporated, and compound metabolites analyzed by

35   HPLC. The percent of water soluble metabolites in the

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plasma residue is determined by radioactive monitoring and analyzed by HPLC. After appropriate corrections for decay, sensitivity, uniformity and attenuation, the collected image data is reconstructed for tissue
5   concentration maps using a Hanning windowed filtered backprojection. The tissue concentration maps are combined with arterial plasma data to calculate receptor uptake using the model developed by Patlak et al., (Blood Flow Metab. $\underline{5}$:584, 1986). This model relies on the
10 hypotheses (1) that the specific binding of [^{11}C]radioligand to striatal membranes can be considered as irreversible for the duration of the PET study; and (2) that non-specific ligand binding is distributed evenly through brain and can be determined from the
15 activity in the cerebellum.

        Arterial blood sampling of the monkey will be taken for the same period of time to determine the arterial blood activity curve. The brain distribution, striatum:cerebellar ratio as a function of time and the
20 blood levels of the compound are performed in three monkeys. The following steps are used to evaluate model parameters. 1) Time course of plasma radioactivity is determined. 2) Plasma curves are corrected for metabolism of the tracer and a biexponential function
25 (Gauss-Newton algorithm/non-linear curve-fitting) is fit to the data. 3) From dynamic PET images, time activity curves for striatum, occipital cortex and cerebellum are obtained. 4) The plasma activity of the tracer at the time of PET measurements is determined. 5) The corrected
30 plasma curve at the time of the PET measurements is integrated. 6) The tissue activity ratio (t) [Striatum/Cerebellum] = f[integrated plasma activity (t)/plasma activity (t)] is determined. Initially striatum:cerebellar ratios are calculated as a function
35 of time. The compounds with the highest striatal

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concentration, fastest blood clearance, least metabolism
and least non-specific binding are most preferable in the
invention.

Compounds to be used as imaging probes are
5   labelled as follows. This procedure is performed each
time before the labeling procedure is done. [^{11}C]methyl
iodide is prepared as follows: nitrogen gas is bombarded
for 15 minutes with 50 μA. The process of converting
gaseous COffi to methyl iodide is automated and uses
10 between 1.32-1.5Ci ^{11}CO$_2$. A freshly prepared (under
argon) LAH solution is used for the reduction of the CO$_2$.
The methyl iodide specific activity is generally 1.32-1.5
Ci. The carboxylic acid (C$_2$) precursor is methylated with
[^{11}C]methyl iodide in DMF. The mixture is heated at 80°C
15 for a period of 5 min. The mixture is then flushed with
helium or argon into an HPLC system for separation and
analysis. The specific activity of the final product
should be between 1.- 2. Ci/mmol. The column for
separation of precursor-product is an Altech C18 prep-
20 column. The solvent system is 40% acetonitrile, 60%
water and 1% ammonia formate. The extract is evaporated
and prepared for i.v. administration.

Prior to PET imaging studies, test animals are
subjected to one magnetic resonance imaging (MRI) to
25 determine stereotaxic coordinates of regions of interest
using a stereotaxic head holder designed for these
studies. The anaesthetized animal (ketamine 10 - 20
mg/kg/; xylazine 1 - 2 mg/kg) is placed on the computer
controlled imaging table face down. The head is placed
30 into a stereotaxic head holder and the table is moved
until the plane of interest is positioned in the imaging
field. Two planes of interest are selected, the caudate-
putamen and the cerebellum and the selected brain levels
are imaged sequentially. Generally, the compound (for
35 example, [^{11}C]CFT (400-2,000 Ci/mmol; approximately 1-5

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nmoles) is administered to control monkeys and the
regional brain distribution association and dissociation
rates determined. Following rapid intravenous injection
of the compound, dynamic imaging in the selected brain
level is started; 30 s scans for 5 min, 1 min scans for
next 10 min, 2 min scans for 45 min and 5 min scans
thereafter for 30 min. Arterial blood samples of 0.25-
0.5 ml for determination of radioactivity and 1.2 ml (*)
for determination of lipophilic metabolites by H.P.L.C.
are collected according the following time table; 0, 15"
30", 45", 60" (*), 75", 90", 105", 120" (*),5'(*), 10'(*),
15', 20', 30'(*), 45', 60'(*), 75', 90'(*). Plasma and
total blood radioactivity are counted in a well counter
that is cross calibrated with the tomograph.

        PET imaging may be carried but using any
appropriate apparatus but is preferably carried out using
coded single ring positron tomograph (Brownell et al.,
Intl. J. Imaging Syst. Tech. $\underline{1}$:207-217, 1989). The
analog ring design offers a number of advantages for
positron tomography. First, high resolution tomographs
can be obtained without sacrificing sensitivity. The
analog ring design does not use absorbing masks or
intercrystal shielding and is not limited by rejecting
photons scattered into adjacent detector elements.
Second, high resolution can be obtained without
mechanical motion. This results from the use of thin,
closely packed detector elements and permits fast dynamic
imaging in an easily assembled system (Burnham et al.,
IEEE Trans. Nucl. Sci. NS $\underline{32}$:889-893, 1991). A single
ring analog coded position tomograph (PCR-I) may be used.
PCR-I provides adequate resolution (spatial resolution of
4.5 mm) and sensitivity (46 kHz for the slice thickness
of 1 cm and activity concentration of 1 μCi/ml) for
quantitative biological studies. A moving computer
controlled table connected to the camera enables imaging

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of sequential slices, so images can be acquired in three
dimensions. Based on PCR-I, an analog coded cylindrical
tomograph, PCR-II, capable of volumetric reconstruction
of three-dimensional sources at 3mm resolution has been
5   designed (Brownell et al., Intl. J. Imaging Syst. Tech.
1:207-217, 1989) and may be used for further analysis.

        PET imaging is carried out on conscious human
subjects using the techniques outlined above or any other
equivalent techniques. SPECT imaging may also be used on
10   human subjects (see, e.g., *Medicine*, Scientific American,
Inc., ed. Rubenstein and Federman, 1988; Jaszczak and
Coleman, Invest. Radiol. 20:897, 1985; Coleman et al.,
Invest. Radiol. 21:1, 1986); preferably SPECT imaging
employes gamma-imitting derivatives of the analogs
15   described herein (e.g., analogs labelled with ^{123}I or
^{99}Tc).

XI.   Analytical Considerations

        All target compounds are characterized and their
purity analyzed prior to any biological evaluation. High
20   field NMR spectra are measured as well as IR, MS and
optical rotation for all test compounds. Elemental
analysis, TLC and/or HPLC are used as a measure of
purity. A purity of >98% is required before any
biological evaluation of these compounds is undertaken.

25   X.   Therapy

        The cocaine analogs described herein are useful as
cocaine substitutes for treatment of cocaine addiction.
The cocaine analogs, may also be useful for the treatment
of neurodegenerative diseases, such as Parkinson's
30   disease. For either application, the analogs may be
administered, e.g., orally or intravenously in an
appropriate dosage, generally 0.01-10mg/kg.

        What is claimed is:

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1.   A compound of formula:
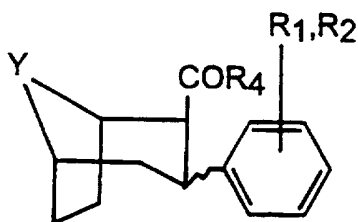
        wherein the linkage at each of the 2- and 3-
positions independently may be α or β;

        wherein Y is C, O, S, SO; or SO_2;

        wherein R_1 and R_2 are each chosen independently
from OH, Br, Cl, F, I, OCH_3, CF_3, NO_2, NH_2, CN, NCS, OAc,
H, or N_3; and

        wherein R_4 is CH_3, CH_3CH_2, $(CH)_2CH$, $CH_3(CH_2)_n$,
$(CH_2)_nC_6H_4X$, C_6H_4X, C_6H_5, OCH_3,
$OCH(CH_3)_2$, OC_6H_5, OC_6H_4X, $O(CH_2)_nC_6H_4X$, $O(CH_2)_nCH_3$, or OCH_3CH_2,

        wherein X is Br, Cl, I, F, OH, OCH_3, CF_3, NO_2, NH_2,
CN, $NHCOCH_3$, $N(CH_3)_2$, $(CH_2)_nCH_3$, $COCH_3$, or $C(CH_3)_3$, wherein n
is between 0 and 6 inclusive.

        2.   The compound of claim 1, wherein R_4 is OCH_3;
and   Y is C, O or S; and
        wherein R_1 is H and R_2 is F, I, or Cl; or
        R_1 and R_2 are both Cl or OH.

        3.   A compound of formula A., B. or C.:

A.

B.

C.

SUBSTITUTE SHEET (RULE 26)
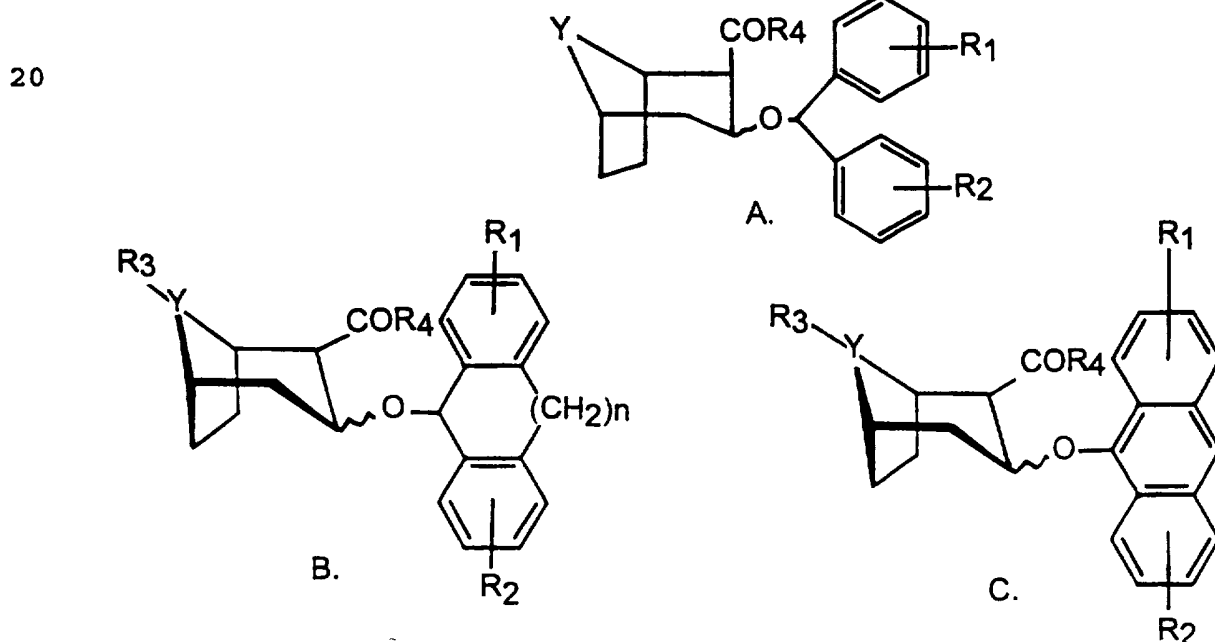
- 45 -

wherein Y is C, O, S, SO, or SO_2; and

wherein the linkage at each of the 2- and 3-positions independently may be α or β; and

wherein R_1 and R_2 are both 4-F, 4-Cl, 3,4-diCl, 4-
5   I, H, 3,4-diOH, 3,4-diOAc, or 3,4-diOCH$_3$; or

R_1 is H and R_2 is 4-F, 4-Cl, 4-I, 2-F, 2-Cl, 2-I,
3-F, 3-Cl, 3-I, 3,4-diCl, 3,4-diOH, 3,4-diOAc, or 3,4-
diOCH$_3$; or

R_1 is H and R_2 is 3-OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH,
10   or 3-F-4-OH; or

R_1 is the same as R_2 and both are chosen from 3-
OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH, or 3-F-4-OH; or

R_1 is not the same as R_2 and both are chosen from
3-OH-4-Cl, 3-OH-4-F, 3-Cl-4-OH, or 3-F-4-OH; and

15   wherein R_4 is CH_3, CH_3CH_2, $(CH_3)_2CH$, $CH_3(CH_2)_n$,
$(CH_2)_nC_6H_4X$, C_6H_4X, C_6H_5, OCH_3, OCH_3CH_2, $OCH(CH_3)_2$, OC_6H_5,
OC_6H_4X, $O(CH_2)_nC_6H_4X$, or $O(CH_2)_nCH_3$,

wherein X is Br, Cl, I, F, OH, OCH_3, CF_3, NO_2, NH_2,
CN, $NHCOCH_3$, $N(CH_3)_2$, $(CH_2)_nCH_3$, $COCH_3$ or $C(CH_3)_3$, wherein n
20   is between 0 and 6 inclusive.

4.   The compound of claim 3, wherein R_4 is OCH_3;
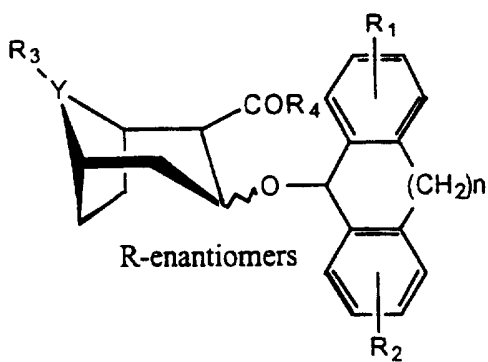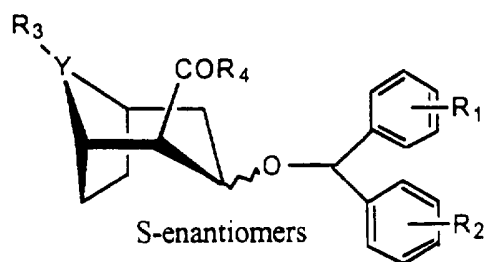and   wherein Y is C; and R_1 and R_2 are both F or both
I; or

wherein Y is O; the linkage at the 3-position is
25   α; and each R_1 and R_2, independently, is Cl, I, or F.

5.   A compound of claim 1, said compound being
labelled with a radioactive or fluorescent label.

6.   A therapeutic composition comprising a
compound of claim 1, said compound being formulated in a
30   pharmaceutically-acceptable carrier.

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7. A method of treating cocaine addiction in a mammal, comprising administering a therapeutic composition of claim 20 to said mammal.

8. A method of selectively imaging cocaine binding regions of the central nervous system of a human patient comprising administering to the central nervous system a detectably labelled compound according to claim 1 and detecting the binding of that compound to CNS tissue.

9. A method of detecting parkinsonism in a human patient comprising administration to said patient of a detectably labelled compound selected from the group consisting of a) compounds according to claim 1 and detecting the binding of said compound to CNS tissue.

10. The method of claim 1 in which detecting is by position emission tomography (PET).

11. The method of claims 9 or 10 in which said detection is by single-photon emission computed tomography (SPECT).

12. A method of treating neurodegenerative disorders characterized by monoamine nerve terminal degeneration in a human patient comprising administration to said patient of a compound according to claims 1-5.

13. The method of claim 12, wherein said neurodegenerative disorder is Parkinson's disease.
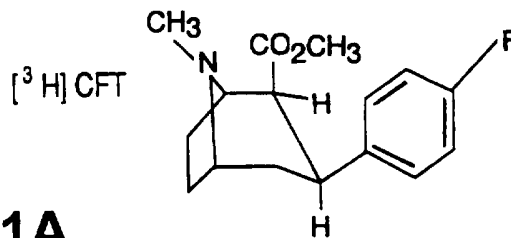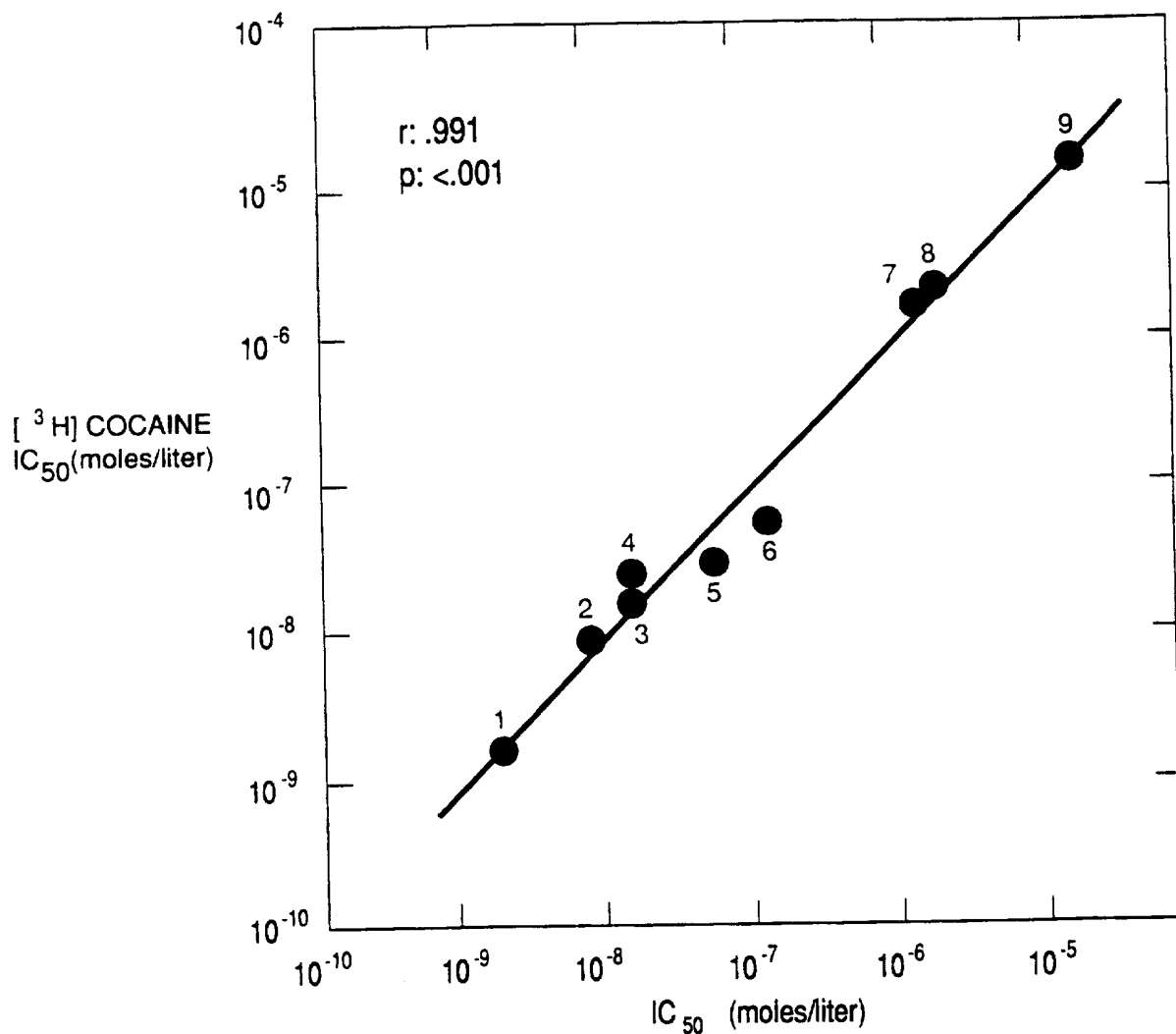
FIG. 1A
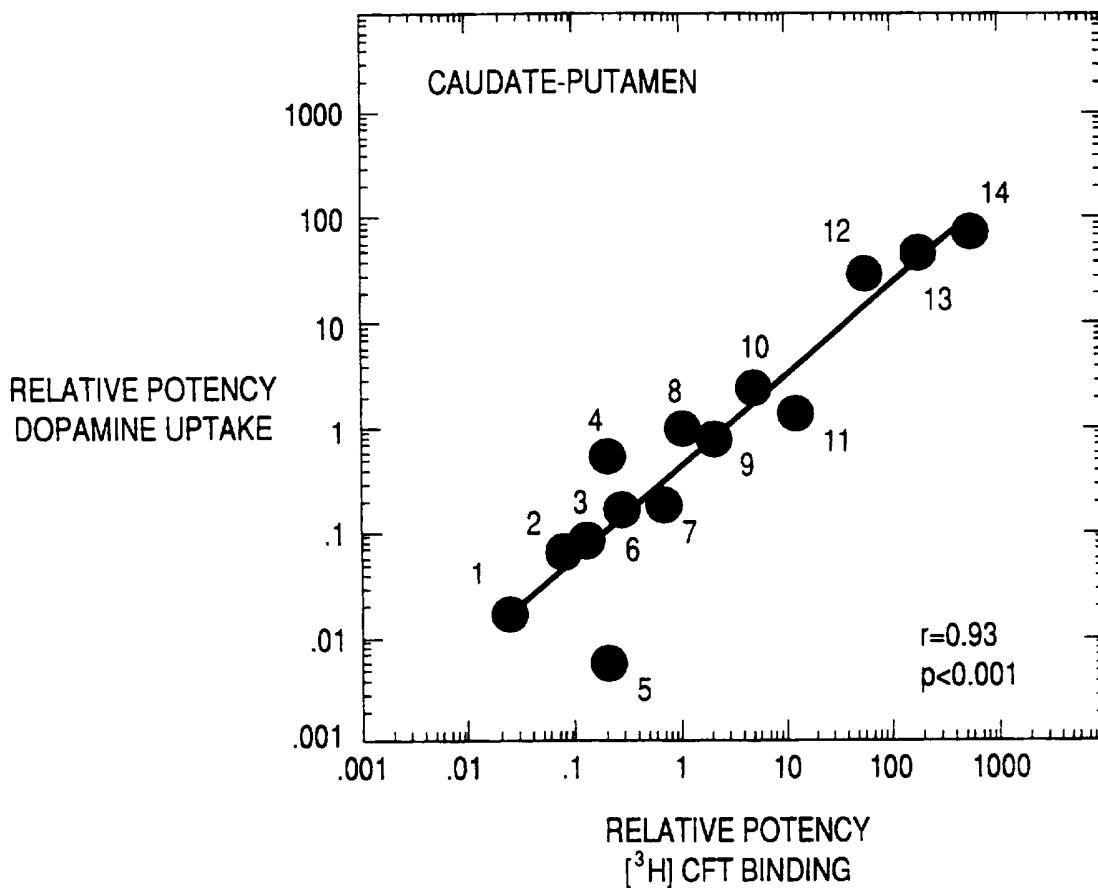
FIG. 1B

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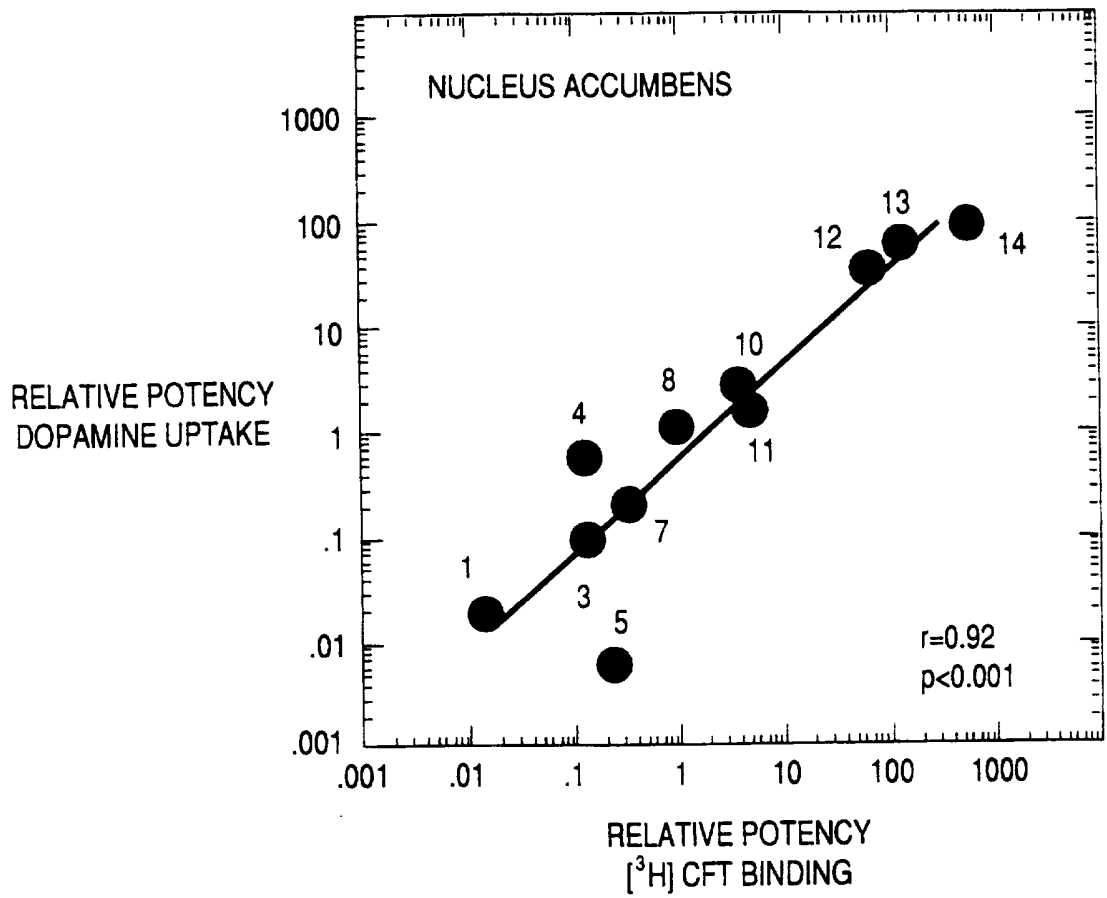
FIG. 1C
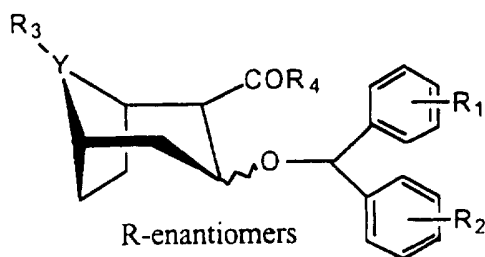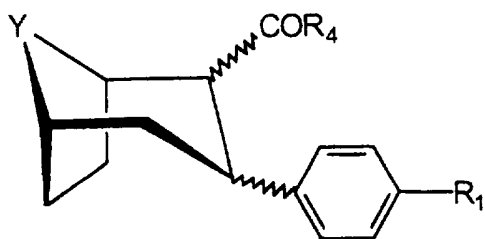
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FIG. 2A

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FIG. 2B

FIG. 3

(-) COCAINE

BENZTROPINE
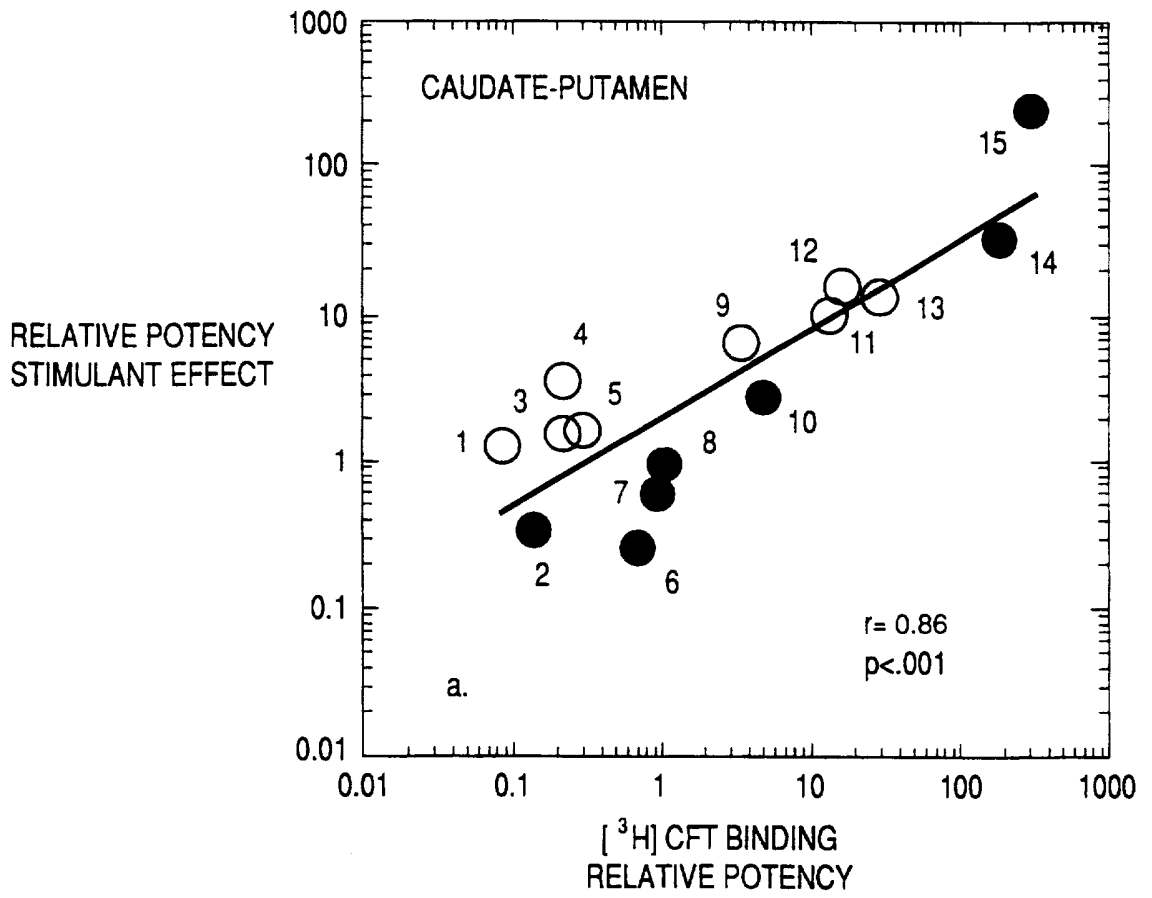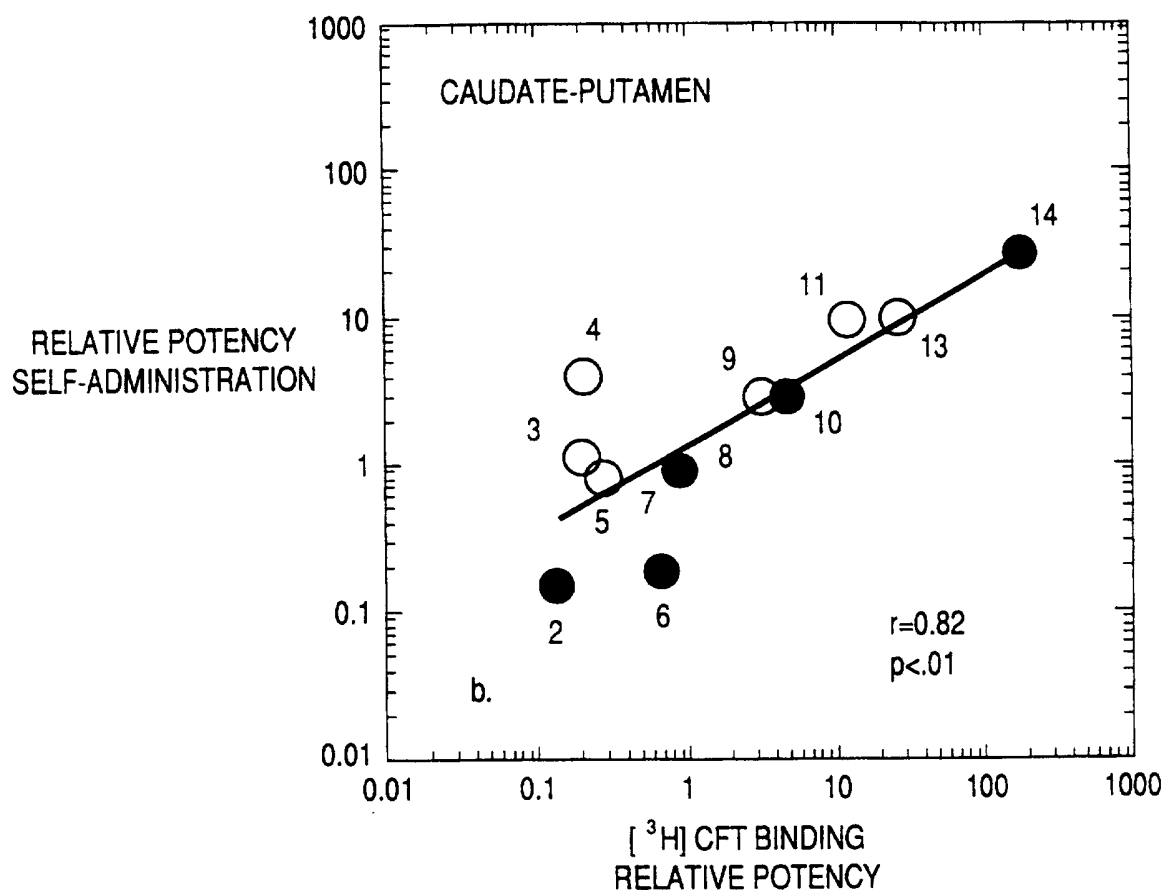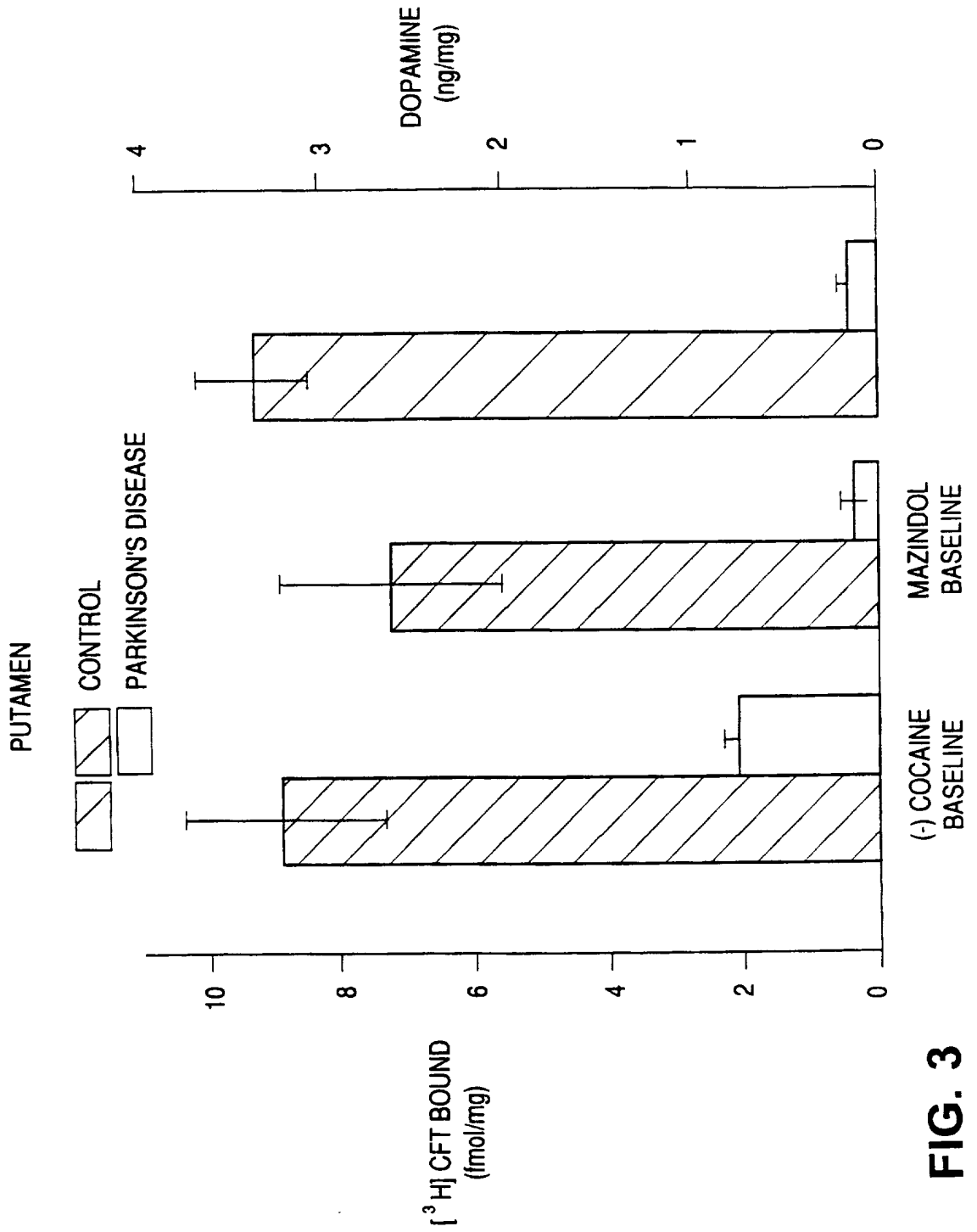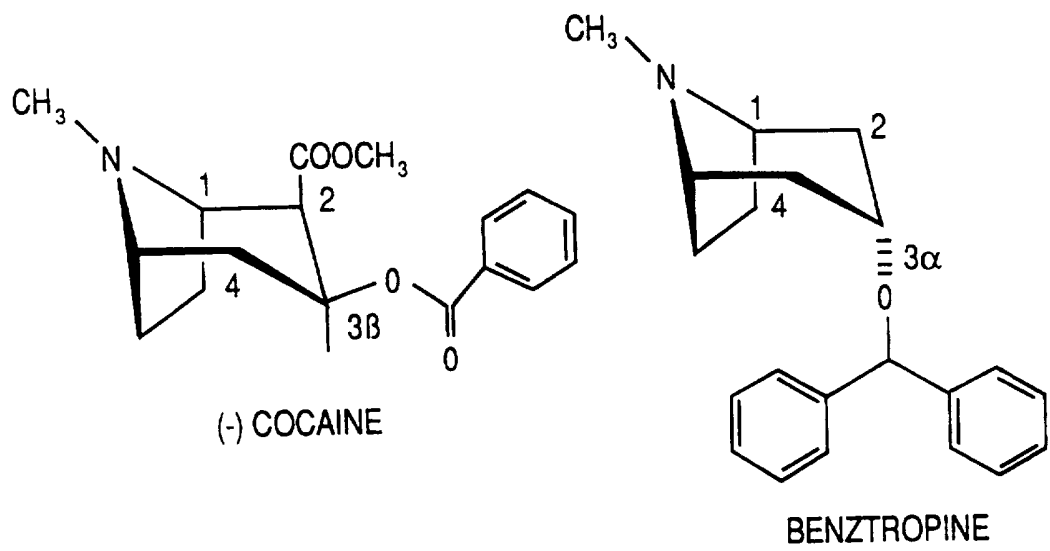
FIG. 4

FIG. 5

SUBSTITUTE SHEET (RULE 26)

a. $R_1=R_2=Cl$
b. $R_1=R_2=OH$
c. $R_1=R_2=OCH_3$
d. $R_1=R_2=OAc$
e. CFT: $R_1=H$, $R_2=F$
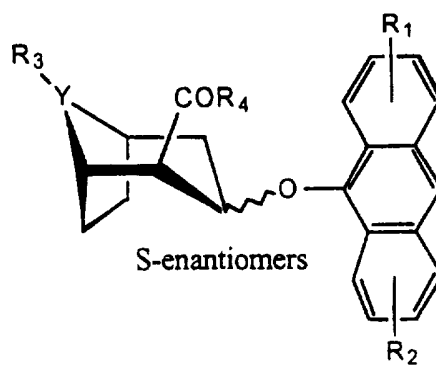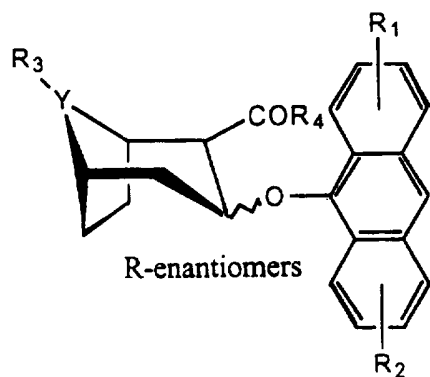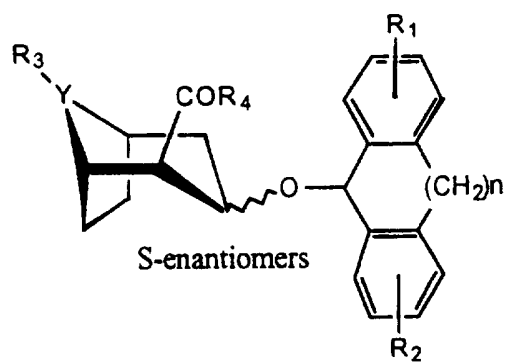
a. $R_1=R_2=H$
b. $R_1=R_2=4-Cl$
c. $R_1=R_2=4-F$
d. $R_1=H$; $R_2=4-I$
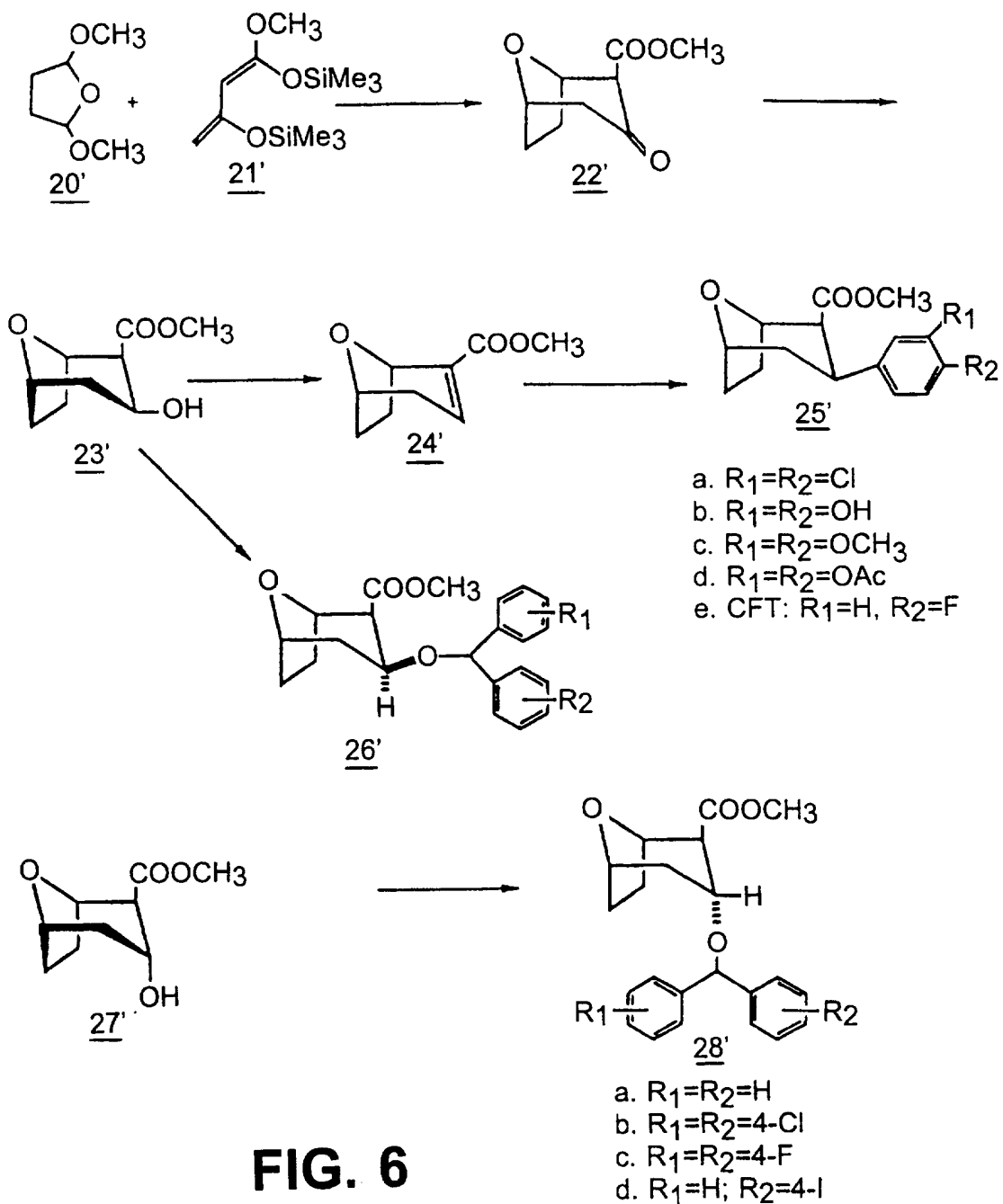
FIG. 6
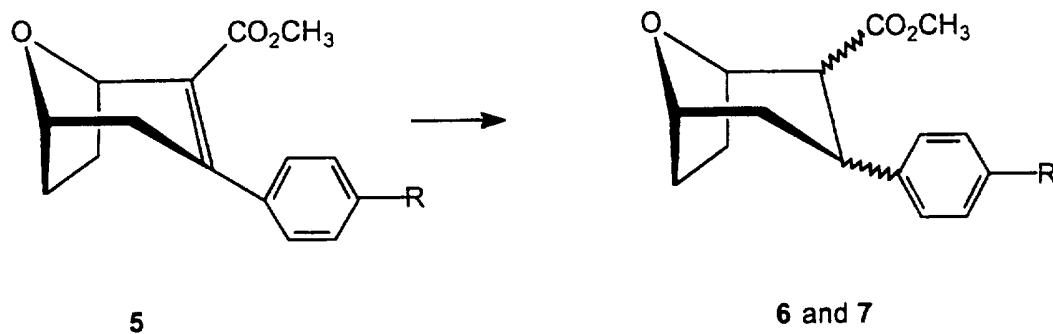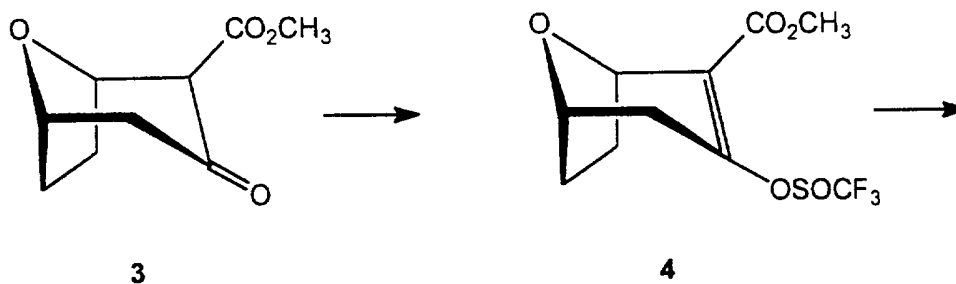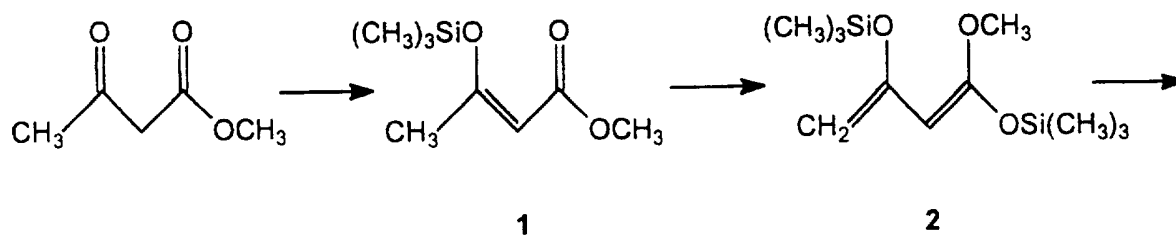
a.	R = 4-F
b.	R = 4-H
c.	R = 4-Cl
d.	R = 3,4-Cl$_2$

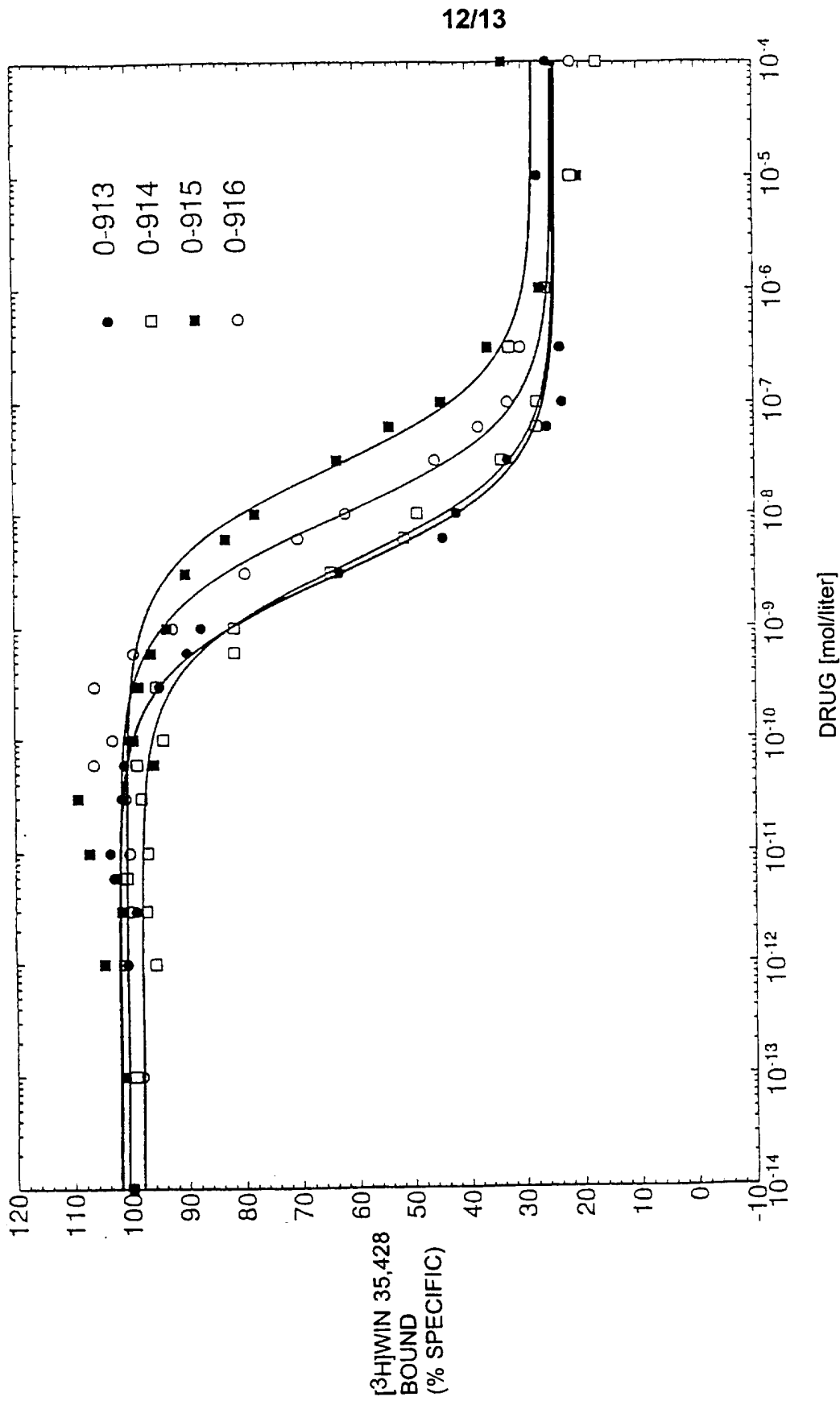# FIG. 7

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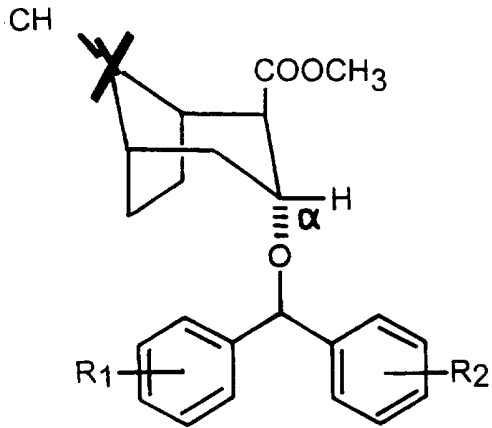
a. R₁=R₂=H
b. R₁=R₂=4-Cl
c. R₁=R₂=4-F
d. R₁=H; R₂=4-I
e. R₁=H; R₂=3.4diOX
where X=H, Acetyl, Alkyl
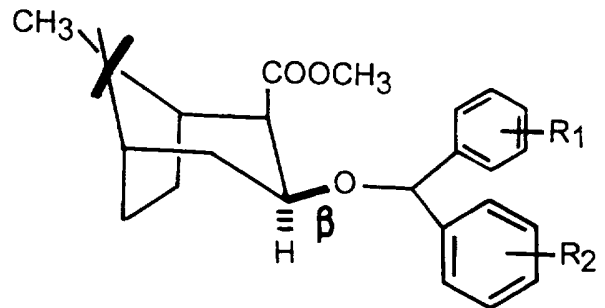
a. R₁=R₂==H
b. R₁=R₂=4-Cl
c. R₁=R₂=4-F
d. R₁=H; R₂=4-I
e. R₁=H; R₂=3,4diOX
where X=H, Acetyl, Alkyl

FIG. 8
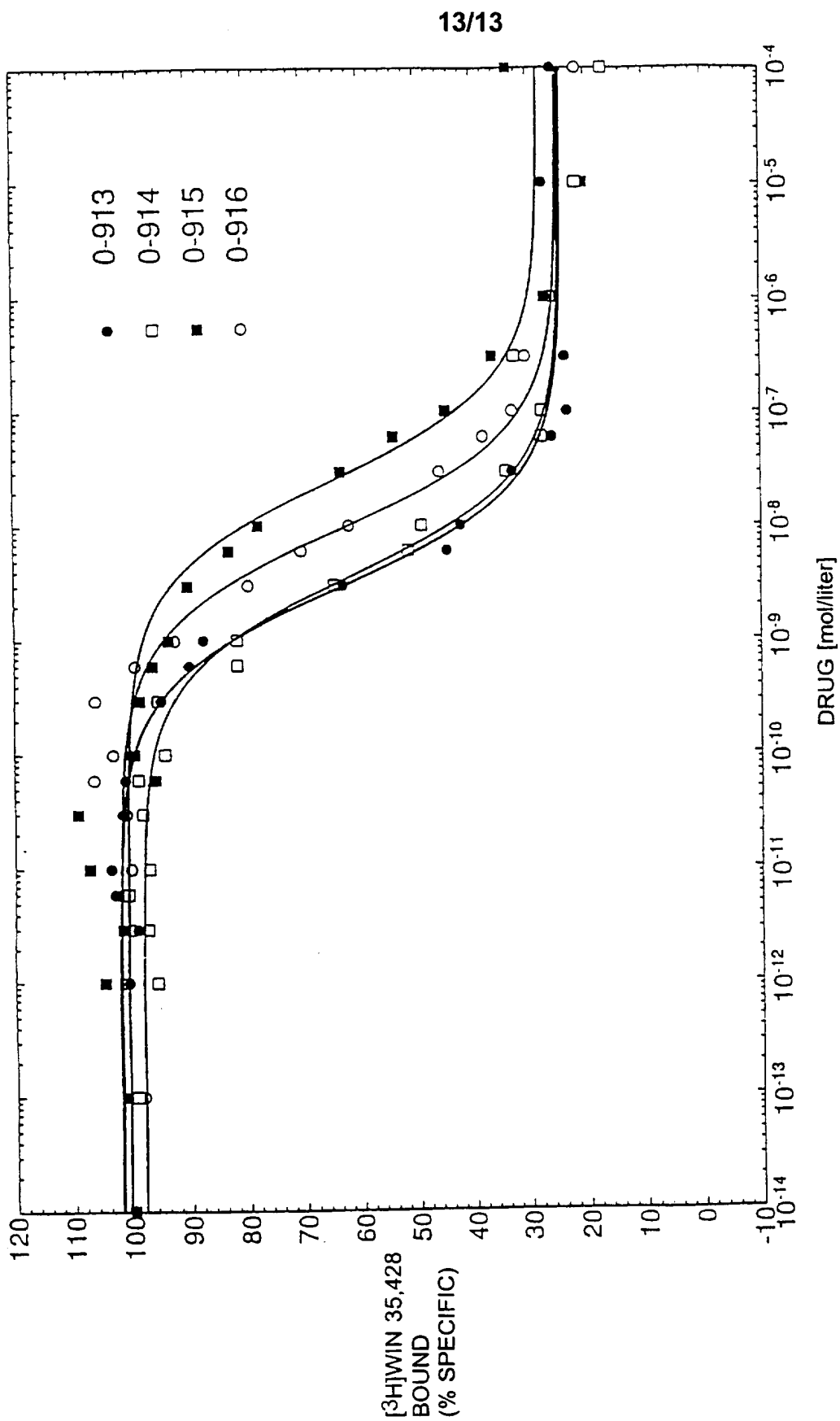
FIG. 9

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FIG. 10

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :A61K 51/04; C07D 451/02
US CL   :424/1.85; 546/129, 130, 132; 514/304
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

    U.S. :   424/1.85; 546/129, 130, 132; 514/304

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

    REGISTRY STRUCTURE SEARCH, CAPLUS, MEDLINE, APS
    search terms: tropane, benztropane, cocaine, dopamine, serotonin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 5,413,779 A (KUHAR ET AL.) 09 May 1995, see column 2.	1-2 ---- 1-13
X ---- Y	US 5,439,666 A (NEUMEYER ET AL.) 08 August 1995, see column 3, lines 14+.	1-2 ---- 1-13
X	US 5,506,359 A (MADRAS ET AL.) 09 April 1996, see columns 3-6.	1-13

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 JUNE 1997	2 0 AUG 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Michael G. Hartley
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*