

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
4 November 2004 (04.11.2004)

PCT

(10) International Publication Number
WO 2004/093789 A2

(51) International Patent Classification⁷: **A61K**

(21) International Application Number:
PCT/US2004/011698

(22) International Filing Date: 15 April 2004 (15.04.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/414,660 16 April 2003 (16.04.2003) US

(71) Applicant: **SYNAPTIC PHARMACEUTICAL CORPORATION** [US/US]; 215 College Road, Paramus, NJ 07652 (US).

(72) Inventors: **KONKEL, Michael**; 9-24 12th Street, Fair Lawn, NJ 07410 (US). **WETZEL, John, M.**; 16-08 Well Drive, Fairlawn, NJ 07410 (US). **TALISMAN, Jamie**; 526 West 113th Street #71, New York, NY 10025 (US).

(74) Agent: **WHITE, John, P.**; Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF GALR3 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF DEPRESSION AND/OR ANXIETY AND COMPOUNDS USEFUL IN SUCH METHODS

(57) Abstract: This invention is directed to indolone derivatives which are antagonists for the GALR3 receptor. The invention provides a pharmaceutical composition comprising a therapeutically effective amount of a compound of the invention and a pharmaceutically acceptable carrier. This invention also provides a pharmaceutical composition made by combining a therapeutically effective amount of a compound of the invention and a pharmaceutically acceptable carrier. This invention further provides a process for making a pharmaceutical composition comprising combining a therapeutically effective amount of a compound of the invention and a pharmaceutically acceptable carrier.



WO 2004/093789 A2

Use Of GALR3 Receptor Antagonists For The Treatment Of
Depression And/Or Anxiety And Compounds Useful in Such
Methods

5

Background of the Invention

This application claims priority of U.S. Serial No.
10/414,660, filed April 16, 2003.

10

Throughout this application, various publications are
referenced in parentheses by author and year. Full
citations for these references may be found at the end of
the specification immediately preceding the claims. The
disclosures of these publications in their entireties are
hereby incorporated by reference into this application to
describe more fully the art to which this invention
pertains.

Depression is the most common of mental disorders and yet
is often underdiagnosed and undertreated, inflicting
substantial morbidity and psychosocial impairment on its
sufferers. Depression is mainly characterized by sadness,
flatness, loss of feeling, anhedonia (lack of pleasure),
tearfulness, agitation or retardation, thoughts of guilt,
and worthlessness; in severe cases, suicide,
hallucinations and delusions.

Depression can be mainly categorized into bipolar
disorders, identifying wide swings of mood; major
depressive illness, marked by severe depressive symptoms
but without manic swings; and less defined milder forms
of bipolar and major depression that fall short of the

specific diagnostic criteria e.g. dysthymic disorder (formerly called depressive neurosis). The symptomatology and diagnostic criteria for depression are set out in the DSMIV guidelines (American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders). Although many patients have single episodes of major depressive illness, the condition also can be repetitive, and this recurrent condition is frequently called unipolar depressive illness.

10

The key features of depressive illness are a markedly gloomy mood in which there is a loss of interest in life, and general feelings of hopelessness and worthlessness. Depressive symptoms range in severity from mild mood swings to severe delusions about self-worth, accomplishments, and the future.

The "blackness" of the presentation in the depressed patient is most often accompanied by severe motor retardation with profound sleep and appetite disturbance and suicidal ideation. Furthermore, depressive illness can also present in a highly anxious or agitated state.

The degree to which the underlying brain mechanisms in anxiety and depression differ or overlap remains unknown. The fact, however, that to some extent the same neurotransmitter systems are involved in depression and anxiety does not mean that the mechanisms are identical. However, the majority of people in an episode of either depression or anxiety also meet criteria for at least one other psychiatric disorder. But by far the strongest comorbidities in both cases are between depression and

anxiety disorders. Therefore, it is now becoming common clinical practice to treat both indications with antidepressants such as SSRIs.

5 The key clinical features of anxiety disorders relate to various combinations of psychological and physical manifestations of anxiety, not attributable to real danger and occurring either in attacks (panic disorder - PD) or as a persisting state (generalized anxiety disorder - GAD). Other neurotic features may be present
10 (obsessional or hysterical symptoms) but do not dominate the clinical picture.

The Pathophysiology of Depression

15 Theories underlying the pathophysiology of depression have developed from several lines of evidence including:
1) changes in neurotransmitter monoamine levels;
2) endocrine imbalance; and 3) electrophysiological studies on sleep functions.

20 Evidence implicating the role of neurotransmitters in depression, in particular the monoamines serotonin, noradrenaline and dopamine, include the success of pharmacological agents in treating depressive disorders.
25 Many of the tricyclic antidepressants (TCAs), selective serotonin re-uptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs) effective in the treatment of depression increase the availability of the catecholamines (noradrenaline and dopamine) and
30 indolamines (serotonin) in the central nervous system (CNS). The clinical efficacy of these agents has given rise to the catecholamine-indolamine hypothesis of

depression. This theory postulates that a certain level of amines and/or receptor sensitivity to catecholamines functions to generate a normal mood. A receptor insensitivity, a depletion of monoamines, or a decrease
5 in their release, synthesis or storage have been postulated to lead to depression.

Current Treatments for Depression

A variety of pharmacological agents have been employed to
10 treat depression based on the catecholamine-indolamine hypothesis of depression. Drugs used to treat depression include MAOIs, atypical antipsychotics, lithium, TCAs, and SSRIs. In addition, a number of off-label agents such as antiepileptics are used to treat depression in
15 treatment-resistant patients.

Tricyclic antidepressants are about equal to SSRIs in effectiveness against depression thus providing supporting evidence for the catecholamine-indolamine
20 hypothesis of depression. However, SSRIs have largely displaced TCAs because of side effects associated with TCAs and the need to monitor EKG and plasma drug concentration. Although the SSRIs are viewed as an improvement over other antidepressants, they are not
25 without their clinical problems. Adverse effects on sexual function, primarily anorgasmia and delayed ejaculation, have been consistently reported. Other, common side-effects include sleep disorders, yawning, weight changes, suicidal ideation and extrapyramidal-like
30 side-effects such as dystonic reactions. Thus, there clearly remains a medical need for new treatments of depression, without the adverse side-effect profile of

existing agents and with improved efficacy.

Current treatments for anxiety

5 There is now considerable direct evidence for the efficacy of the SSRIs both in depression and in anxiety disorders.

Of the current SSRIs approved for marketing in the USA
10 all have shown sufficient efficacy to be further approved for the treatment of at least one anxiety disorder, for example; obsessive compulsive disorder (OCD) and generalized anxiety disorder (GAD). Compounds such as paroxetine and sertraline are also indicated for the
15 treatment of panic disorder (PD).

However, it is clear from the issues raised earlier relating to the efficacy and side-effect profile of SSRIs and for that matter the more widely prescribed
20 benzodiazapines, there still exists a real medical need for novel approaches for the treatment of anxiety and depression.

Discovery Of GALR3 Receptor Subtype And Its Role In
25 Depression and Anxiety

The investigations leading to the present invention arose from the discovery that mRNA for the GALR3 receptor is localized to areas of the rat brain associated with mood and emotion (see PCT International Publication No. WO
30 98/15570, published April 16, 1998), thus supporting the expression of GALR3 in those regions. Protein for the GALR3 receptor is also shown to localize to areas of the

rat brain associated with mood and emotion (see Table 11 and discussion herein).

This discovery led to the hypothesis that the GALR3
5 receptor may play a role in controlling the activity of catecholamine and indolamine neurons in the CNS. Galanin is known to hyperpolarize neurons, including monoaminergic neurons (Seutin, et al., 1989) and to have inhibitory effects on 5-HT neurons (Xu, et al., 1998),
10 and dopamine neurons (Gopalan, et al., 1993; De Weille, et al., 1989; Jansson, et al., 1989; Nordstrom, et al., 1987; Weiss, et al., 1998). In light of these reports, a series of in vivo behavioral experiments were carried out to evaluate the antidepressant properties of a selective
15 GALR3 receptor antagonist. The rat Forced Swim Test and the rat Social Interaction Test were employed to evaluate the use of selective GALR3 receptor antagonists to treat depression and anxiety. These models are considered by experts in the field to reflect the potential of agents
20 to treat depression and anxiety.

Rat Forced Swim Test (FST)

The rat Forced Swim Test (FST) is a behavioral test that is used to screen compounds for antidepressant efficacy
15 (Porsolt et al., 1977, 1978; Porsolt, 1981). This test is widely used as it is reliable across laboratories, relatively easy to perform and is sensitive to the effects of some of the major classes of antidepressant drugs, including TCAs and MAOIs, and various atypical
0 antidepressants. Furthermore, this test is relatively selective for antidepressant drugs, as few psychoactive drugs produce similar behavioral actions in the FST.

In the rat FST, animals are placed in a cylinder of water, from which there is no escape, for an extended period of time. Typically, animals will display a range of behaviors such as immobility, climbing, swimming, and diving, with immobility being predominant after several minutes of immersion in the water. Consequently, many past studies have only measured or scored immobility after the administration of the test agent. Unfortunately, this method does not score any other active behaviors that may be produced by potential antidepressants. Thus, if a particular class of antidepressant were to have very little effect on immobility, yet produce characteristic behaviors during the FST, these behaviors would not be scored and the conclusion would be that the compound in question does not possess antidepressant action.

Recently, however, a sampling technique was developed to score active behaviors in the FST, such as swimming, climbing and diving, in addition to immobility (Detke, et al., 1995; Lucki, 1997; Page, et al., 1999; Renner and Lucki, 1998). This modified sampling technique has indicated that SSRIs, such as fluoxetine, paroxetine and sertraline, significantly decrease immobility and increase swimming time (Detke, et al., 1995; Page, et al., 1999). In contrast, selective reuptake inhibitors of norepinephrine (NE) increase climbing behavior but do not alter swimming time (Detke, et al., 1995; Page, et al., 1999).

Rat Social Interaction Test (SIT)

There are a number of paradigms that have been used to determine whether a compound possesses anxiolytic action. A number of these tests involve food or water deprivation, punishment or measurement of consummatory behavior (see File, et al., 1980; File, 1985; Rodgers, et al., 1997; and Treit, 1985, for review). In addition, in these models, prior conditioning reduces the uncertainty or anxiety. In general, these tests lack ethological validity.

One model that is based upon an unconditioned response that does not involve punishment or deprivation is the Social Interaction Test (SIT) (File and Hyde, 1978, 1979). In this model, rats previously housed singly are placed in a familiar, dimly lit, test arena with weight-matched, novel partners. The principal anxiogenic stimulus under these conditions is the partner novelty, which involves an unconditioned response to a potential threat. After pharmacological treatments, the following behaviors are scored as active social interaction: grooming, sniffing, biting, boxing, wrestling, following, crawling over and crawling under. A wide range of psychoactive drugs have been examined in this paradigm and it has been shown that the social interaction test can distinguish anxiolytics from antidepressants, antipsychotics, analeptics and sedative agents (File, 1985; Guy and Gardner, 1985). This test can detect anxiolytic agents such as the benzodiazepines (File and Hyde, 1978; File and Hyde, 1979; File, 1980), in addition to non-benzodiazepines, including paroxetine and other SSRIs (Lightowler, et al., 1994). Finally, the social

interaction test can detect anxiogenic agents, including the inverse benzodiazepine receptor agonists (File, et al., 1982; File and Pellow, 1983; File and Pellow, 1984; File, 1985).

5

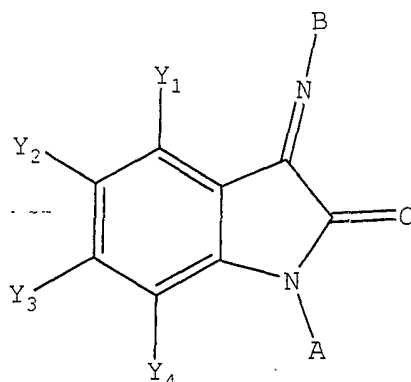
In an embodiment of the present invention the synthesis of indolones which bind selectively to the cloned human GALR3 receptor, compared to other cloned human G-protein coupled receptors, as measured in *in vitro* assays, is disclosed. The *in vitro* receptor assays described hereinafter were performed using various cultured cell lines, each transfected with and expressing only a single galanin-type receptor. Thus, we demonstrate that the GALR3 receptor antagonists, which may be classified as neutral antagonists, inverse agonists or allosteric modulators, provide a novel method to treat depressive disorders and/or anxiety.

10

15

Summary Of The Invention

The invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a
 5 compound having the structure:



wherein each of Y_1 , Y_2 , Y_3 , and Y_4 is independently -H; straight chained or branched C_1 - C_7 alkyl, monofluoroalkyl or polyfluoroalkyl; straight chained or branched C_2 - C_7 alkenyl or alkynyl; C_3 - C_7 cycloalkyl, or C_5 - C_7 cycloalkenyl; -F, -Cl, -Br, or -I; - NO_2 ; - N_3 ; -CN; - OR_4 , -
 10 $OCOR_4$, - COR_4 , - $NCOR_4$, - $N(R_4)_2$, - $CON(R_4)_2$, or - $COOR_4$; aryl or heteroaryl; or any two of Y_1 , Y_2 , Y_3 and Y_4 present on adjacent carbon atoms can constitute a methylenedioxy group;
 15 group;

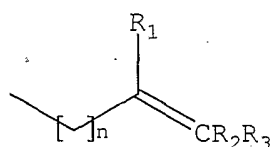
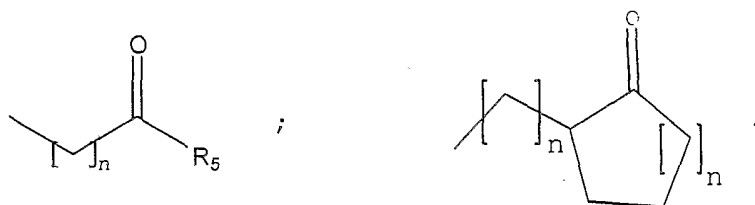
wherein each R_4 is independently -H; straight chained or branched C_1 - C_7 alkyl, monofluoroalkyl or polyfluoroalkyl; straight chained or branched C_2 - C_7 alkenyl or alkynyl; C_3 -
 20 C_7 cycloalkyl, C_5 - C_7 cycloalkenyl, aryl or aryl(C_1 - C_6)alkyl;

wherein A is A' , straight chained or branched C_1 - C_7 alkyl, aryl, heteroaryl, aryl(C_1 - C_6)alkyl or heteroaryl(C_1 -

11

C₆) alkyl;

wherein A' is



; or



5

wherein R₁ and R₂ are each independently H, straight chained or branched C₁-C₇ alkyl, -F, -Cl, -Br, -I, -NO₂, or -CN;

10 wherein R₃ is H, straight chained or branched C₁-C₇ alkyl, -F, -Cl, -Br, -I, -NO₂, -CN, -OR₆, aryl or heteroaryl;

wherein R₅ is straight chained or branched C₁-C₇ alkyl, -N(R₄)₂, -OR₄ or aryl;

15

wherein R₆ is straight chained or branched C₁-C₇ alkyl or aryl;

wherein B is C₃-C₇ cycloalkyl, C₅-C₇ cycloalkenyl,
 20 adamantyl, aryl, pyridyl, pyridazinyl, pyrimidinyl,
 pyrazinyl, triazinyl, indolizinyl, indol-4-yl, indol-5-yl,
 indol-6-yl, indol-7-yl, isoindolyl, benzo[b]furan-4-yl,
 benzo[b]furan-5-yl, benzo[b]furan-6-yl,
 benzo[b]furan-7-yl, benzo[b]thiophen-4-yl,
 25 benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl,

benzo[b]thiophen-7-yl, indazolyl, benzimidazolyl,
benzo[b]thiazolyl, purinyl, imidazo[2,1-b]thiazolyl,
quinolinyl, isoquinolinyl, quinazolinyl, 2,1,3-
benzothiazolyl, furanyl, thienyl, pyrrolyl, oxazolyl,
5 thiazolyl, imidazolyl, pyrazolyl, isoxazolyl,
isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl,
benzoxazolyl, benzisoxazolyl, cinnolinyl, quinoxalinyl,
1,8-naphthridinyl, pteridinyl, or phthalimidyl; provided
however, if B is aryl, pyridyl, pyridazinyl, pyrimidinyl,
10 pyrazinyl, triazinyl, indolizinyl, indol-4-yl, indol-5-
yl, indol-6-yl, indol-7-yl, isoindolyl, benzo[b]furan-4-
yl, benzo[b]furan-5-yl, benzo[b]furan-6-yl,
benzo[b]furan-7-yl, benzo[b]thiophen-4-yl,
benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl,
15 benzo[b]thiophen-7-yl, indazolyl, benzimidazolyl,
benzo[b]thiazolyl, purinyl, imidazo[2,1-b]thiazolyl,
quinolinyl, isoquinolinyl, quinazolinyl, 2,1,3-
benzothiazolyl, furanyl, thienyl, pyrrolyl, oxazolyl,
thiazolyl, imidazolyl, pyrazolyl, isoxazolyl,
20 isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl,
benzoxazolyl, benzisoxazolyl, cinnolinyl, quinoxalinyl,
1,8-naphthyridinyl, pteridinyl, or phthalimidyl the carbon
atom or carbon atoms ortho to the nitrogen atom of the
imine bond may only be substituted with one or more of
25 the following -F, -Cl, -Br, -I, -CN, methyl, ethyl or
methoxy;

wherein n is an integer from 1 to 4 inclusive.

30 In a further embodiment of the aforementioned
pharmaceutical composition, the compound has the
structure:

In a further embodiment, A is aryl, heteroaryl, heteroaryl(C₁-C₆)alkyl or -(CH₂)_n-CC-R₄; wherein the aryl is substituted with -OH;

5

In a further embodiment, A is aryl, heteroaryl, or heteroaryl(C₁-C₆)alkyl; and

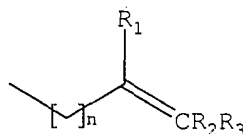
In a further embodiment, each of Y₁, Y₂, Y₃, and Y₄ is independently -H; straight chained or branched C₁-C₇ alkyl, -CF₃, -F, -Cl, -Br, -I, -OR₄, -N(R₄)₂, or -CON(R₄)₂;

wherein each R₄ is independently -H; straight chained or branched C₁-C₇ alkyl, -CF₃, or phenyl;

wherein A is A', straight chained or branched C₁-C₇ alkyl, aryl, heteroaryl, aryl(C₁-C₆)alkyl or heteroaryl(C₁-C₆)alkyl; and

20

wherein A' is



In a further embodiment, B is C₃-C₇ cycloalkyl or adamantyl.

25

In a further embodiment, B is pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolizinyl, indol-4-yl, indol-5-yl, indol-6-yl, indol-7-yl, isoindolyl, benzo[b]furan-4-yl, benzo[b]furan-5-yl, benzo[b]furan-6-

yl, benzo[b]furan-7-yl, benzo[b]thiophen-4-yl,
 benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl,
 benzo[b]thiophen-7-yl, indazolyl, benzimidazolyl,
 benzo[b]thiazolyl, purinyl, imidazo[2,1-b]thiazolyl,
 5 quinolinyl, isoquinolinyl, quinazolinyl, 2,1,3-
 benzothiazolyl, furanyl, thienyl, pyrrolyl, oxazolyl,
 thiazolyl, imidazolyl, pyrazolyl, isoxazolyl,
 isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl,
 benzoxazolyl, benzisoxazolyl, cinnolinyl, quinoxalinyl,
 10 1,8-naphthyridinyl, pteridinyl, or phthalimidyl.

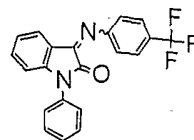
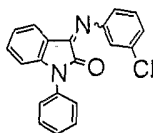
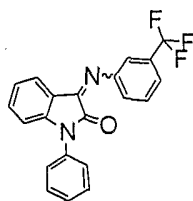
In a further embodiment, B is aryl.

In a further embodiment, B is phenyl and the phenyl is
 15 optionally substituted with one or more of the following:
 -F, -Cl, -Br, -CF₃, straight chained or branched C₁-C₇
 alkyl, -N(R₄)₂, -OR₄, -COR₄, -NCOR₄, -CO₂R₄, or -CON(R₄)₂.

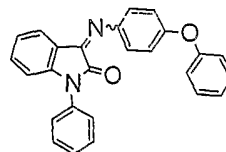
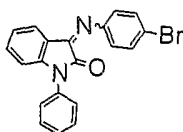
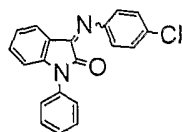
In a further embodiment, A is aryl.

20

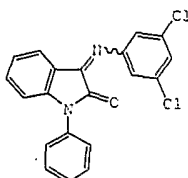
In a further embodiment, the compound is selected from
 the group consisting of:



25

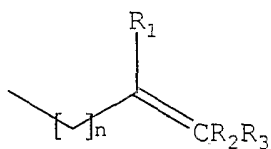


and



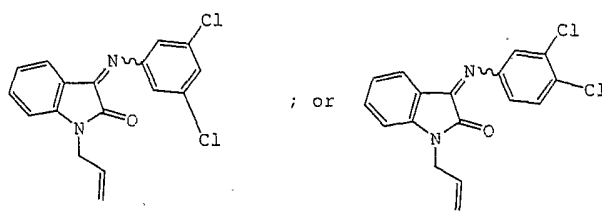
15

In a further embodiment, A is A' and A' is

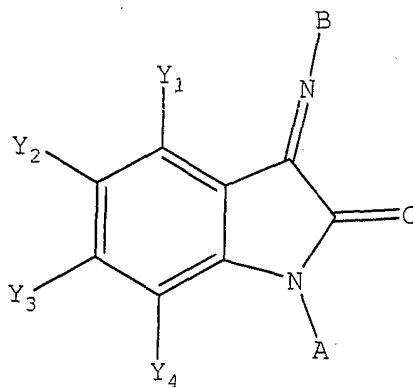


5

In a further embodiment, the compound is selected from the group consisting of:



10 The present invention also provides a compound having the structure:



wherein each of Y₁, Y₂, Y₃, and Y₄ is independently -H;
 straight chained or branched C₁-C₇ alkyl, monofluoroalkyl
 15 or polyfluoroalkyl; straight chained or branched C₂-C₇
 alkenyl or alkynyl; C₃-C₇ cycloalkyl or C₅-C₇ cycloalkenyl;

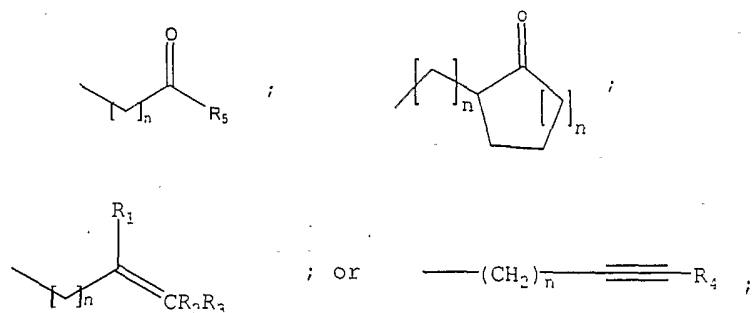
16

-F, -Cl, -Br, or -I; -NO₂; -N₃; -CN; -OR₄, -SR₄, -OCOR₄, -COR₄, -NCOR₄, -N(R₄)₂, -CON(R₄)₂, or -COOR₄; aryl or heteroaryl; or any two of Y₁, Y₂, Y₃ and Y₄ present on adjacent carbon atoms can constitute a methylenedioxy group;

wherein each R₄ is independently -H; straight chained or branched C₁-C₇ alkyl, monofluoroalkyl or polyfluoroalkyl; straight chained or branched C₂-C₇ alkenyl or alkynyl; C₃-C₇ cycloalkyl, C₅-C₇ cycloalkenyl, aryl or aryl(C₁-C₆)alkyl;

wherein A is A', straight chained or branched C₁-C₇ alkyl, aryl, heteroaryl, aryl(C₁-C₆)alkyl or heteroaryl(C₁-C₆)alkyl;

wherein A' is



wherein R₁ and R₂ are each independently H, straight chained or branched C₁-C₇ alkyl, -F, -Cl, -Br, -I, -NO₂, or -CN;

wherein R₃ is H, straight chained or branched C₁-C₇ alkyl,

-F, -Cl, -Br, -I, -NO₂, -CN, -OR₆, aryl or heteroaryl;

wherein R₅ is straight chained or branched C₁-C₇ alkyl, -N(R₄)₂, -OR₄ or aryl;

5

wherein R₆ is straight chained or branched C₁-C₇ alkyl or aryl;

wherein B is pyridyl, pyridazinyl, pyrimidinyl,
10 pyrazinyl, triazinyl, indoliziny, indol-4-yl, indol-5-yl, indol-6-yl, indol-7-yl, isoindolyl, benzo[b]furan-4-yl, benzo[b]furan-5-yl, benzo[b]furan-6-yl, benzo[b]furan-7-yl, benzo[b]thiophen-4-yl, benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl, benzo[b]thiophen-7-yl, indazolyl, benzimidazolyl,
15 benzo[b]thiazolyl, purinyl, imidazo[2,1-b]thiazolyl, quinolinyl, isoquinolinyl, quinazolinyl, 2,1,3-benzothiazolyl, furanyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl,
20 isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, benzoxazolyl, benzisoxazolyl, cinnolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl, or phthalimidyl; provided however, that the carbon atom or carbon atoms ortho to the nitrogen atom of the imine bond may only be
25 substituted with one or more of the following -F, -Cl, -Br, -I, -CN, methyl, ethyl or methoxy;

wherein n is an integer from 1 to 4 inclusive;

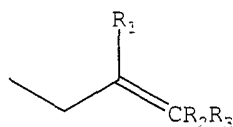
30 or a pharmaceutically acceptable salt thereof.

In a further embodiment,

In a further embodiment, A is A', straight chained or branched C₁-C₇ alkyl, aryl, heteroaryl, aryl(C₁-C₆)alkyl or heteroaryl(C₁-C₆)alkyl; and

5

A' is



10

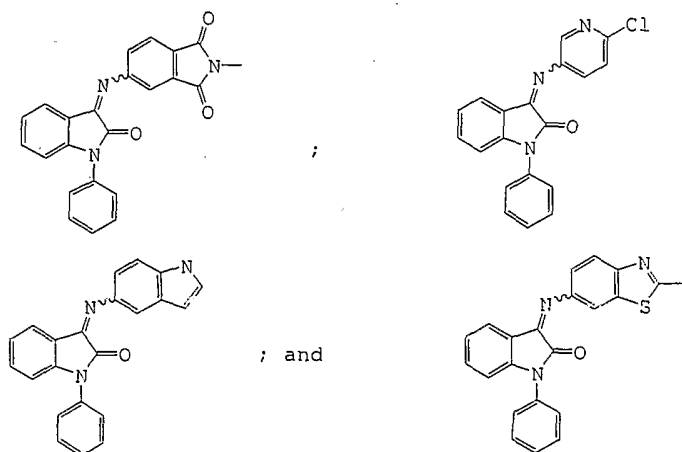
In an embodiment each of Y₁, Y₂, Y₃, and Y₄ is independently -H; straight chained or branched C₁-C₇ alkyl, -CF₃, -F, -Cl, -Br, -I, -OR₄, -N(R₄)₂, or -CON(R₄)₂.

15

In a further embodiment, A is aryl or aryl(C₁-C₆)alkyl.

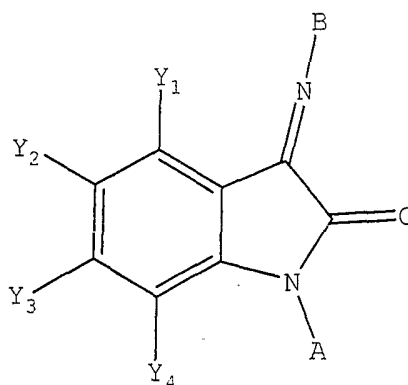
In a further embodiment, the compound is selected from the group consisting of:

20



Detailed Description Of The Invention

This invention further provides for a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound having the structure:



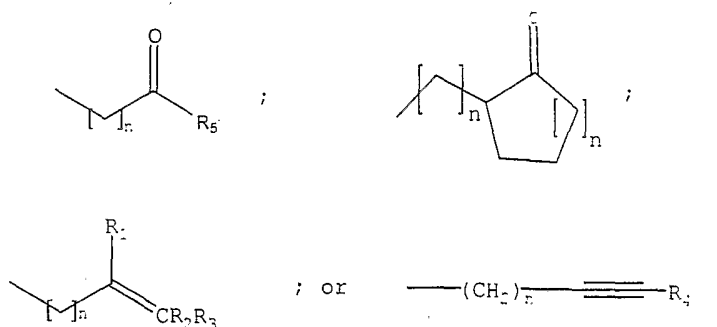
wherein each of Y_1 , Y_2 , Y_3 , and Y_4 is independently -H; straight chained or branched C_1 - C_7 alkyl, monofluoroalkyl or polyfluoroalkyl; straight chained or branched C_2 - C_7 alkenyl or alkynyl; C_3 - C_7 cycloalkyl, or C_5 - C_7 cycloalkenyl; -F, -Cl, -Br, or -I; - NO_2 ; - N_3 ; -CN; - OR_4 , - $OCOR_4$, - COR_4 , - $NCOR_4$, - $N(R_4)_2$, - $CON(R_4)_2$, or - $COOR_4$; aryl or heteroaryl; or any two of Y_1 , Y_2 , Y_3 and Y_4 present on adjacent carbon atoms can constitute a methylenedioxy group;

wherein each R_4 is independently -H; straight chained or branched C_1 - C_7 alkyl, monofluoroalkyl or polyfluoroalkyl; straight chained or branched C_2 - C_7 alkenyl or alkynyl; C_3 - C_7 cycloalkyl, C_5 - C_7 cycloalkenyl, aryl or aryl(C_1 - C_6)alkyl;

wherein A is A' , straight chained or branched C_1 - C_7 alkyl, aryl, heteroaryl, aryl(C_1 - C_6)alkyl or heteroaryl(C_1 -

C₆)alkyl;

wherein A' is



5

wherein R₁ and R₂ are each independently H, straight chained or branched C₁-C₇ alkyl, -F, -Cl, -Br, -I, -NO₂, or -CN;

10 wherein R₃ is H, straight chained or branched C₁-C₇ alkyl, -F, -Cl, -Br, -I, -NO₂, -CN, -OR₆, aryl or heteroaryl;

wherein R₅ is straight chained or branched C₁-C₇ alkyl, -N(R₄)₂, -OR₄ or aryl;

15

wherein R₆ is straight chained or branched C₁-C₇ alkyl or aryl;

wherein B is C₃-C₇ cycloalkyl, C₅-C₇ cycloalkenyl, adamantyl, aryl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolizinyl, indol-4-yl, indol-5-yl, indol-6-yl, indol-7-yl, isoindolyl, benzo[b]furan-4-yl, benzo[b]furan-5-yl, benzo[b]furan-6-yl, benzo[b]furan-7-yl, benzo[b]thiophen-4-yl, benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl,

20

25

benzo[b]thiophen-7-yl, indazolyl, benzimidazolyl,
benzo[b]thiazolyl, purinyl, imidazo[2,1-b]thiazolyl,
quinolinyl, isoquinolinyl, quinazolinyl, 2,1,3-
benzothiazolyl, furanyl, thienyl, pyrrolyl, oxazolyl,
5 thiazolyl, imidazolyl, pyrazolyl, isoxazolyl,
isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl,
benzoxazolyl, benzisoxazolyl, cinnolinyl, quinoxalyl,
1,8-naphthridinyl, pteridinyl, or phthalimidyl; provided
however, if B is aryl, pyridyl, pyridazinyl, pyrimidinyl,
10 pyrazinyl, triazinyl, indolizinyl, indol-4-yl, indol-5-
yl, indol-6-yl, indol-7-yl, isoindolyl, benzo[b]furan-4-
yl, benzo[b]furan-5-yl, benzo[b]furan-6-yl,
benzo[b]furan-7-yl, benzo[b]thiophen-4-yl,
benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl,
15 benzo[b]thiophen-7-yl, indazolyl, benzimidazolyl,
benzo[b]thiazolyl, purinyl, imidazo[2,1-b]thiazolyl,
quinolinyl, isoquinolinyl, quinazolinyl, 2,1,3-
benzothiazolyl, furanyl, thienyl, pyrrolyl, oxazolyl,
thiazolyl, imidazolyl, pyrazolyl, isoxazolyl,
20 isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl,
benzoxazolyl, benzisoxazolyl, cinnolinyl, quinoxalyl,
1,8-naphthridinyl, pteridinyl, or phthalimidyl the carbon
atom or carbon atoms ortho to the nitrogen atom of the
imine bond may only be substituted with one or more of
25 the following -F, -Cl, -Br, -I, -CN, methyl, ethyl or
methoxy;

wherein m is an integer from 0 to 4 inclusive; and

30 wherein n is an integer from 1 to 4 inclusive.

In a further embodiment, A is aryl, heteroaryl,

heteroaryl(C₁-C₆)alkyl or -(CH₂)_n-CC-R₄; wherein the aryl is substituted with -OH;

In a further embodiment, A is aryl, heteroaryl, or
5 heteroaryl(C₁-C₆)alkyl; and

As used in the present invention, the term "cycloalkyl" includes C₃-C₇ cycloalkyl moieties which may be
10 substituted with one or more of the following: -F, -NO₂, -CN, straight chained or branched C₁-C₇ alkyl, straight chained or branched C₁-C₇ monofluoroalkyl, straight chained or branched C₁-C₇ polyfluoroalkyl, straight chained or branched C₂-C₇ alkenyl, straight chained or
15 branched C₂-C₇ alkynyl, C₃-C₇ cycloalkyl, C₃-C₇ monofluorocycloalkyl, C₃-C₇ polyfluorocycloalkyl, C₅-C₇ cycloalkenyl, -N(R₄)₂, -OR₄, -COR₄, -NCOR₄, -CO₂R₄, -CON(R₄)₂ or (CH₂)_n-O-(CH₂)_m-CH₃.

20 As used in the present invention, the term "cycloalkenyl" includes C₅-C₇ cycloalkenyl moieties which may be substituted with one or more of the following: -F, -Cl, -Br, -I, -NO₂, -CN, straight chained or branched C₁-C₇ alkyl, straight chained or branched C₁-C₇ monofluoroalkyl,
25 straight chained or branched C₁-C₇ polyfluoroalkyl, straight chained or branched C₂-C₇ alkenyl, straight chained or branched C₂-C₇ alkynyl, C₃-C₇ cycloalkyl, C₃-C₇ monofluorocycloalkyl, C₃-C₇ polyfluorocycloalkyl, C₅-C₇ cycloalkenyl, -N(R₄)₂, -OR₄, -COR₄, -NCOR₄, -CO₂R₄, -
30 CON(R₄)₂ or (CH₂)_n-O-(CH₂)_m-CH₃.

In the present invention, the term "heteroaryl" is used

to include five and six membered unsaturated rings that may contain one or more oxygen, sulfur, or nitrogen atoms. Examples of heteroaryl groups include, but are not limited to, furanyl, thienyl, pyrrolyl, oxazolyl, 5 thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, and triazinyl.

10 In addition the term "heteroaryl" is used to include fused bicyclic ring systems that may contain one or more heteroatoms such as oxygen, sulfur and nitrogen. Examples of such heteroaryl groups include, but are not limited to, indolizinyll, indolyl, isoindolyl, 15 benzo[b]furanyl, benzo[b]thiophenyl, indazolyl, benzimidazolyl, purinyl, benzoxazolyl, benzisoxazolyl, benzo[b]thiazolyl, imidazo[2,1-b]thiazolyl, cinnolinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, phthalimidyl and 20 2,1,3-benzothiazolyl.

The term "heteroaryl" also includes those chemical moieties recited above which may be substituted with one or more of the following: -F, -Cl, -Br, -I, -NO₂, -CN, 25 straight chained or branched C₁-C₇ alkyl, straight chained or branched C₁-C₇ monofluoroalkyl, straight chained or branched C₁-C₇ polyfluoroalkyl, straight chained or branched C₂-C₇ alkenyl, straight chained or branched C₂-C₇ alkynyl, C₃-C₇ cycloalkyl, C₃-C₇ monofluorocycloalkyl, C₃-C₇ 30 polyfluorocycloalkyl, C₅-C₇ cycloalkenyl, -N(R₄)₂, -OR₄, -COR₄, -NCOR₄, -CO₂R₄, -CON(R₄)₂ or (CH₂)_n-O-(CH₂)_m-CH₃.

The term "heteroaryl" further includes the N-oxides of those chemical moieties recited above which include at least one nitrogen atom.

5 In the present invention the term "aryl" is phenyl or naphthyl. The term "aryl" also includes phenyl and naphthyl which may be substituted with one or more of the following: -F, -Cl, -Br, -I, -NO₂, -CN, straight chained or branched C₁-C₇ alkyl, straight chained or branched C₁-C₇
10 monofluoroalkyl, straight chained or branched C₁-C₇ polyfluoroalkyl, straight chained or branched C₂-C₇ alkenyl, straight chained or branched C₂-C₇ alkynyl, C₃-C₇ cycloalkyl, C₃-C₇ monofluorocycloalkyl, C₃-C₇ polyfluorocycloalkyl, C₅-C₇ cycloalkenyl, -N(R₄)₂, -OR₄, -
15 SR₄, -OCOR₄, -COR₄, -NCOR₄, -CO₂R₄, -CON(R₄)₂ or (CH₂)_n-O-(CH₂)_m-CH₃.

The present invention also provides for an enantiomerically and diastereomerically pure compound
20 administered in the aforementioned method. The present invention further provides for an enantiomerically or diastereomerically pure compound administered in the aforementioned method. The present invention further provides for a pure Z imine isomer or a pure Z alkene
25 isomer of the compound administered in the aforementioned method. The present invention also provides for a pure E imine isomer or a pure E alkene isomer of the compound administered in the aforementioned method.

30 In a further embodiment of the aforementioned pharmaceutical composition, each of Y₁, Y₂, Y₃, and Y₄ is independently -H; straight chained or branched C₁-C₇

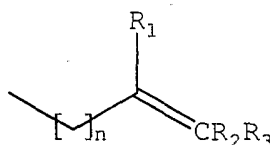
alkyl, $-\text{CF}_3$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{OR}_4$, $-\text{N}(\text{R}_4)_2$, or $-\text{CON}(\text{R}_4)_2$;

wherein each R_4 is independently $-\text{H}$; straight chained or
5 branched $\text{C}_1\text{-C}_7$ alkyl, $-\text{CF}_3$, or phenyl;

wherein A is A', straight chained or branched $\text{C}_1\text{-C}_7$ alkyl, aryl, heteroaryl, aryl($\text{C}_1\text{-C}_6$)alkyl or heteroaryl($\text{C}_1\text{-C}_6$)alkyl; and

10

wherein A' is

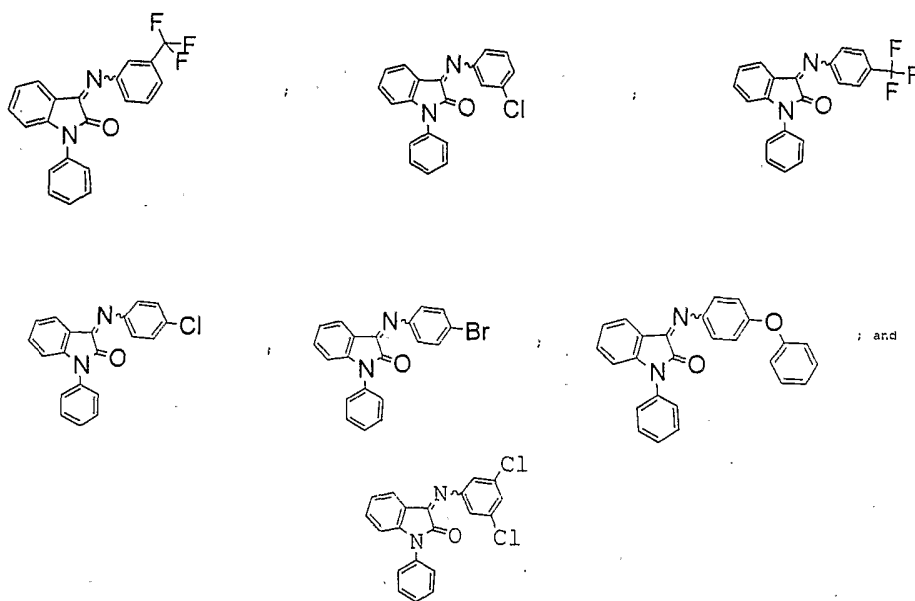


In another embodiment of the pharmaceutical composition, B is $\text{C}_3\text{-C}_7$ cycloalkyl or adamantyl. In yet another
15 embodiment, B is pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolizinyl, indol-4-yl, indol-5-yl, indol-6-yl, indol-7-yl, isoindolyl, benzo[b]furan-4-yl, benzo[b]furan-5-yl, benzo[b]furan-6-yl, benzo[b]furan-7-yl, benzo[b]thiophen-4-yl, benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl, benzo[b]thiophen-7-yl, indazolyl, benzimidazolyl, benzo[b]thiazolyl, purinyl, imidazo[2,1-b]thiazolyl, quinolinyl, isoquinolinyl, quinazolinyl, 2,1,3-benzothiazolyl, furanyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, benzoxazolyl, benzisoxazolyl, cinnolinyl, quinoxalinyl, 1,8-naphthridinyl, pteridinyl, or phthalimidyl. In a
25

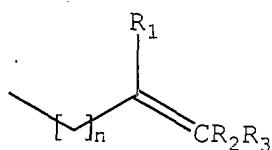
further embodiment of the pharmaceutical composition, B is aryl. In an additional embodiment of the pharmaceutical composition, B is phenyl and the phenyl is optionally substituted with one or more of the following:

5 -F, -Cl, -Br, -CF₃, straight chained or branched C₁-C₇ alkyl, -N(R₄)₂, -OR₄, -COR₄, -NCOR₄, -CO₂R₄, -CON(R₄)₂. In other embodiments, A is aryl.

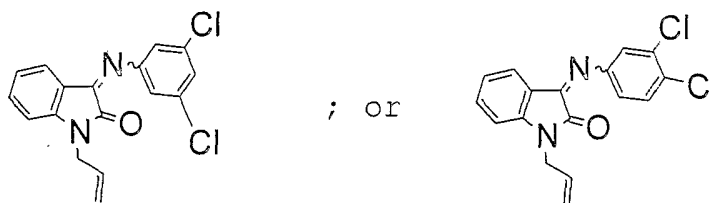
10. In an embodiment of the aforementioned pharmaceutical composition, the compound is selected from the group consisting of:



15 In a further embodiment of the pharmaceutical composition, A is A' and A' is

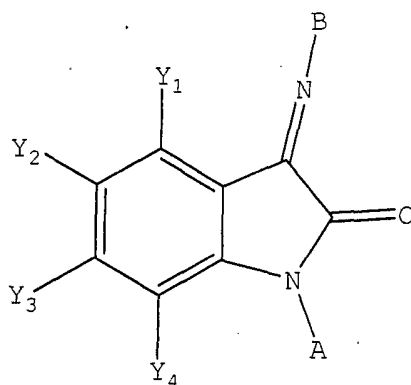


In still another embodiment of the pharmaceutical



composition described above, the compound is:

5. The claimed invention also provides for a compound having



the structure:

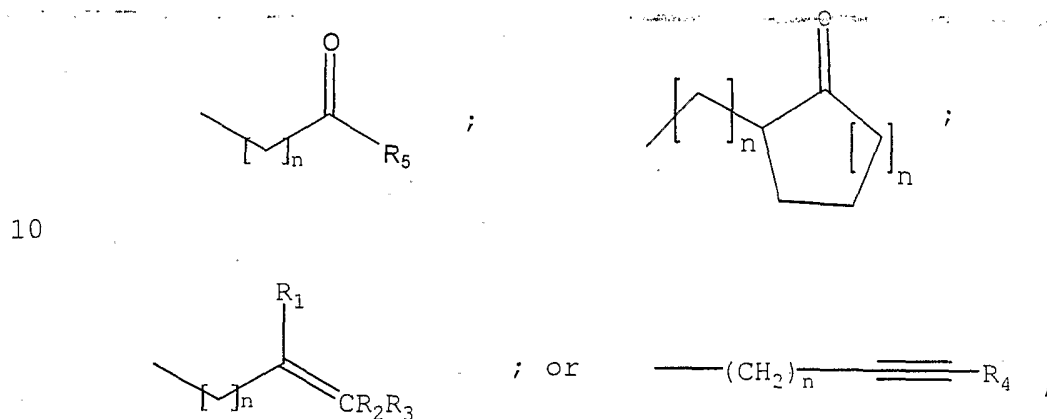
wherein each of Y_1 , Y_2 , Y_3 , and Y_4 is independently -H;
 straight chained or branched C_1 - C_7 alkyl, monofluoroalkyl
 10 or polyfluoroalkyl; straight chained or branched C_2 - C_7
 alkenyl or alkynyl; C_3 - C_7 cycloalkyl or C_5 - C_7 cycloalkenyl;
 -F, -Cl, -Br, or -I; - NO_2 ; - N_3 ; -CN; - OR_4 , - SR_4 , - $OCOR_4$, -
 COR_4 , - $NCOR_4$, - $N(R_4)_2$, - $CON(R_4)_2$, or - $COOR_4$; aryl or
 heteroaryl; or any two of Y_1 , Y_2 , Y_3 and Y_4 present on
 15 adjacent carbon atoms can constitute a methylenedioxy
 group;

wherein each R_4 is independently -H; straight chained or
 branched C_1 - C_7 alkyl, monofluoroalkyl or polyfluoroalkyl;

straight chained or branched C_1 - C_7 alkenyl or alkynyl; C_3 - C_7 cycloalkyl, C_3 - C_7 cycloalkenyl, aryl or aryl(C_1 - C_6)alkyl;

- 5 wherein A is A', straight chained or branched C_1 - C_7 alkyl, aryl, heteroaryl, aryl(C_1 - C_6)alkyl or heteroaryl(C_1 - C_6)alkyl;

wherein A' is



wherein R_1 and R_2 are each independently H, straight chained or branched C_1 - C_7 alkyl, -F, -Cl, -Br, -I, - NO_2 , or -CN;

15

wherein R_3 is H, straight chained or branched C_1 - C_7 alkyl, -F, -Cl, -Br, -I, - NO_2 , -CN, - OR_6 , aryl or heteroaryl;

20 wherein R_5 is straight chained or branched C_1 - C_7 alkyl, - $\text{N(R}_4\text{)}_2$, - OR_4 or aryl;

wherein R_6 is straight chained or branched C_1 - C_7 alkyl or aryl;

wherein B is pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolizinyl, indol-4-yl, indol-5-yl, indol-6-yl, indol-7-yl, isoindolyl, benzo[b]furan-4-yl, benzo[b]furan-5-yl, benzo[b]furan-6-yl, benzo[b]furan-7-yl, benzo[b]thiophen-4-yl, benzo[b]thiophen-5-yl, benzo[b]thiophen-6-yl, benzo[b]thiophen-7-yl, indazolyl, benzimidazolyl, benzo[b]thiazolyl, purinyl, imidazo[2,1-b]thiazolyl, quinolinyl, isoquinolinyl, quinazolinyl, 2,1,3-benzothiazolyl, furanyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, benzoxazolyl, benzisoxazolyl, cinnolinyl, quinoxalinyl, 1,8-naphthridinyl, pteridinyl, or phthalimidy; provided however, that the carbon atom or carbon atoms ortho to the nitrogen atom of the imine bond may only be substituted with one or more of the following -F, -Cl, -Br, -I, -CN, methyl, ethyl or methoxy;

wherein m is an integer from 0 to 4 inclusive;

wherein n is an integer from 1 to 4 inclusive;

or a pharmaceutically acceptable salt thereof.

25

In a further embodiment, A is aryl, heteroaryl, heteroaryl(C₁-C₆)alkyl or -(CH₂)_n-CC-R₄; wherein the aryl is substituted with -OH;

30 In a further embodiment, A is aryl, heteroaryl, or heteroaryl(C₁-C₆)alkyl; and

In a further embodiment, the above described compound is an enantiomerically and diastereomerically pure compound. In another embodiment, the above described compound is an enantiomerically or diastereomerically pure compound. In still another embodiment, the aforementioned compound is a pure Z imine isomer or a pure Z alkene isomer of the compound. In still another embodiment, the aforementioned compound is a pure E imine isomer or a pure E alkene isomer of the compound.

As used in the present invention, the term "cycloalkyl" includes C₃-C₇ cycloalkyl moieties which may be substituted with one or more of the following: -F, -NO₂, -CN, straight chained or branched C₁-C₇ alkyl, straight chained or branched C₁-C₇ monofluoroalkyl, straight chained or branched C₁-C₇ polyfluoroalkyl, straight chained or branched C₂-C₇ alkenyl, straight chained or branched C₂-C₇ alkynyl, C₃-C₇ cycloalkyl, C₃-C₇ monofluorocycloalkyl, C₃-C₇ polyfluorocycloalkyl, C₅-C₇ cycloalkenyl, -N(R₄)₂, -OR₄, -COR₄, -NCOR₄, -CO₂R₄, -CON(R₄)₂ or (CH₂)_n-O-(CH₂)_m-CH₃.

As used in the present invention, the term "cycloalkenyl" includes C₅-C₇ cycloalkenyl moieties which may be substituted with one or more of the following: -F, -Cl, -Br, -I, -NO₂, -CN, straight chained or branched C₁-C₇ alkyl, straight chained or branched C₁-C₇ monofluoroalkyl, straight chained or branched C₁-C₇ polyfluoroalkyl, straight chained or branched C₂-C₇ alkenyl, straight chained or branched C₂-C₇ alkynyl, C₃-C₇ cycloalkyl, C₃-C₇ monofluorocycloalkyl, C₃-C₇ polyfluorocycloalkyl, C₅-C₇ cycloalkenyl, -N(R₄)₂, -OR₄, -COR₄, -NCOR₄, -CO₂R₄, -

$\text{CON}(\text{R}_4)_2$ or $(\text{CH}_2)_n\text{-O-}(\text{CH}_2)_m\text{-CH}_3$.

In the present invention, the term "heteroaryl" is used to include five and six membered unsaturated rings that may contain one or more oxygen, sulfur, or nitrogen atoms. Examples of heteroaryl groups include, but are not limited to, furanyl, thienyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, and triazinyl.

In addition the term "heteroaryl" is used to include fused bicyclic ring systems that may contain one or more heteroatoms such as oxygen, sulfur and nitrogen. Examples of such heteroaryl groups include, but are not limited to, indolizinyll, indolyl, isoindolyl, benzo[b]furanyl, benzo[b]thiophenyl, indazolyl, benzimidazolyl, purinyl, benzoxazolyl, benzisoxazolyl, benzo[b]thiazolyl, imidazo[2,1-b]thiazolyl, cinnolinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, phthalimidyl and 2,1,3-benzothiazolyl.

The term "heteroaryl" also includes those chemical moieties recited above which may be substituted with one or more of the following: -F, -Cl, -Br, -I, -NO₂, -CN, straight chained or branched C₁-C₇ alkyl, straight chained or branched C₁-C₇ monofluoroalkyl, straight chained or branched C₁-C₇ polyfluoroalkyl, straight chained or branched C₂-C₇ alkenyl, straight chained or branched C₂-C₇ alkynyl, C₃-C₇ cycloalkyl, C₃-C₇ monofluorocycloalkyl, C₃-C₇

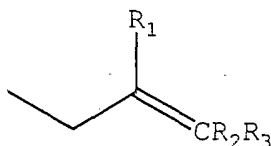
polyfluorocycloalkyl, C₅-C₇ cycloalkenyl, -N(R₄)₂, -OR₄, -COR₄, -NCOR₄, -CO₂R₄, -CON(R₄)₂ or (CH₂)_n-O-(CH₂)_m-CH₃.

The term "heteroaryl" further includes the N-oxides of those chemical moieties recited above which include at least one nitrogen atom.

In the present invention the term "aryl" is phenyl or naphthyl. The term "aryl" also includes phenyl and naphthyl which may be substituted with one or more of the following: -F, -Cl, -Br, -I, -NO₂, -CN, straight chained or branched C₁-C₇ alkyl, straight chained or branched C₁-C₇ monofluoroalkyl, straight chained or branched C₁-C₇ polyfluoroalkyl, straight chained or branched C₂-C₇ alkenyl, straight chained or branched C₂-C₇ alkynyl, C₃-C₇ cycloalkyl, C₃-C₇ monofluorocycloalkyl, C₃-C₇ polyfluorocycloalkyl, C₅-C₇ cycloalkenyl, -N(R₄)₂, -OR₄, -SR₄, -OCOR₄, -COR₄, -NCOR₄, -CO₂R₄, -CON(R₄)₂ or (CH₂)_n-O-(CH₂)_m-CH₃.

The claimed invention further provides for a compound, wherein A is A', straight chained or branched C₁-C₇ alkyl, aryl, heteroaryl, aryl(C₁-C₆)alkyl or heteroaryl(C₁-C₆)alkyl; and

A' is

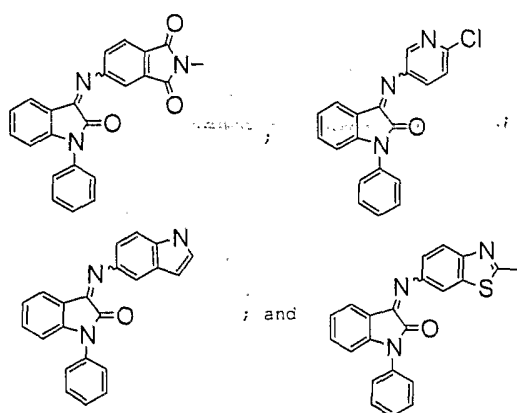


The present invention further provides for a compound wherein each of Y₁, Y₂, Y₃, and Y₄ is independently -H;

straight chained or branched C_1 - C_7 alkyl, $-CF_3$, $-F$, $-Cl$, $-Br$, $-I$, $-OR_4$, $-N(R_4)_2$, or $-CON(R_4)_2$. In a further embodiment of the present invention, A is aryl or aryl(C_1 - C_6)alkyl.

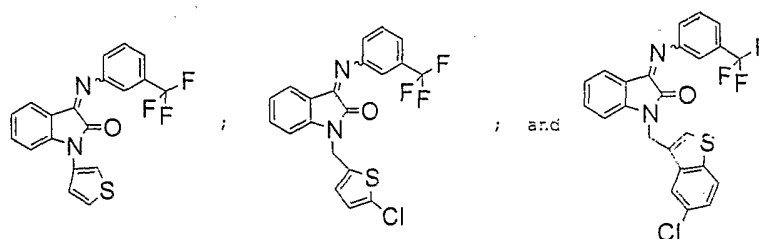
5

In another embodiment, the aforementioned compound is selected from the group consisting of:



In an embodiment of the aforementioned compound, B is
 10 aryl. In a further embodiment of the aforementioned compound, each of Y_1 , Y_2 , Y_3 , and Y_4 is independently $-H$; straight chained or branched C_1 - C_7 alkyl, $-CF_3$, $-F$, $-Cl$, $-Br$, $-I$, $-OR_4$, $-N(R_4)_2$, or $-CON(R_4)_2$.

15 In another embodiment, the compound is selected from the group consisting of:



Experimental Details

Synthesis of Chemical Compounds

5

The following examples are for the purpose of illustrating methods useful for making compounds of this invention.

- 10 **General Methods:** All reactions were performed under an Argon atmosphere and the reagents, neat or in appropriate solvents, were transferred to the reaction vessel via syringe and cannula techniques. Anhydrous solvents were purchased from the Aldrich Chemical Company and used as
- 15 received. The examples described in the patent were named using the ACD/Name Program (version 4.01, Advanced Chemistry Development Inc., Toronto, Ontario, M5H2L3, Canada). The ^1H NMR and ^{13}C NMR spectra were recorded at either 300 MHz (GEQE Plus) or 400 MHz (Bruker Avance) in
- 20 CDCl_3 as solvent and tetramethylsilane as the internal standard unless otherwise noted. Chemical shifts (δ) are expressed in ppm, coupling constants (J) are expressed in Hz, and splitting patterns are described as follows: s = singlet; d = doublet; t = triplet; q = quartet; quintet;
- 25 sextet; septet; br = broad; m = multiplet; dd = doublet of doublets; dt = doublet of triplets. Elemental analyses were performed by Robertson Microlit Laboratories, Inc. Unless otherwise, mass spectra were obtained using electrospray ionization (ESI, Micromass Platform II) and
- 30 MH^+ is reported. Thin-layer Chromatography (TLC) was carried out on glass plates pre-coated with silica gel 60 F₂₅₄ (0.25 mm, EM Separations Tech.). Preparative TLC was carried out on glass sheets pre-coated with silica gel GF

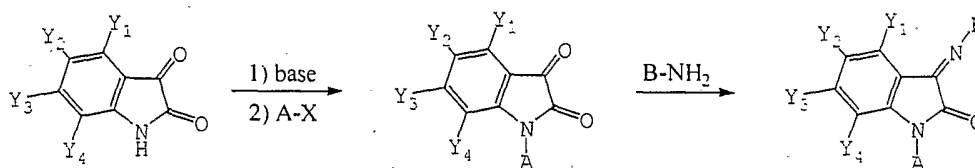
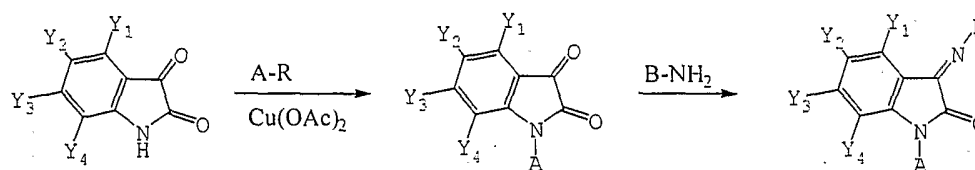
(2 mm, Analtech). Flash column chromatography was performed on Merck silica gel 60 (230 -400 mesh). Melting points (mp) were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected.

5

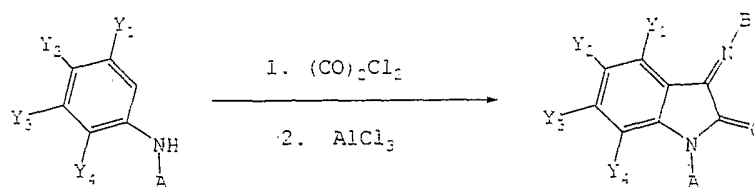
General Procedure for Synthesis of Iminoisatins.

The appropriately substituted isatin (10 mg - 10 g) was placed in a flask and the appropriate aniline (1.0 - 1.1 equivalents) was added and the mixture was stirred to homogeneity. The mixture was then heated to 110 °C for 2-7 hours and then cooled. Solids were crystallized from hot methanol and filtered, giving the desired products (usually as an inseparable interconverting mixture of E/Z isomers).

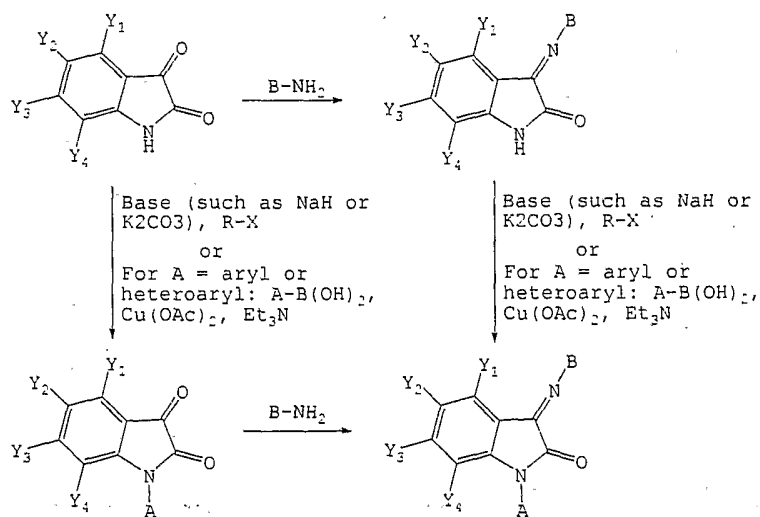
15

Scheme 6^aScheme 7^a

^aY₁, Y₂, Y₃, Y₄, A, and B are defined as described in the specification. X is a leaving group such as Cl, Br, I, or OTs. R is boric acid or a dialkylborate group.

Scheme 8^a. Synthesis of Isatins

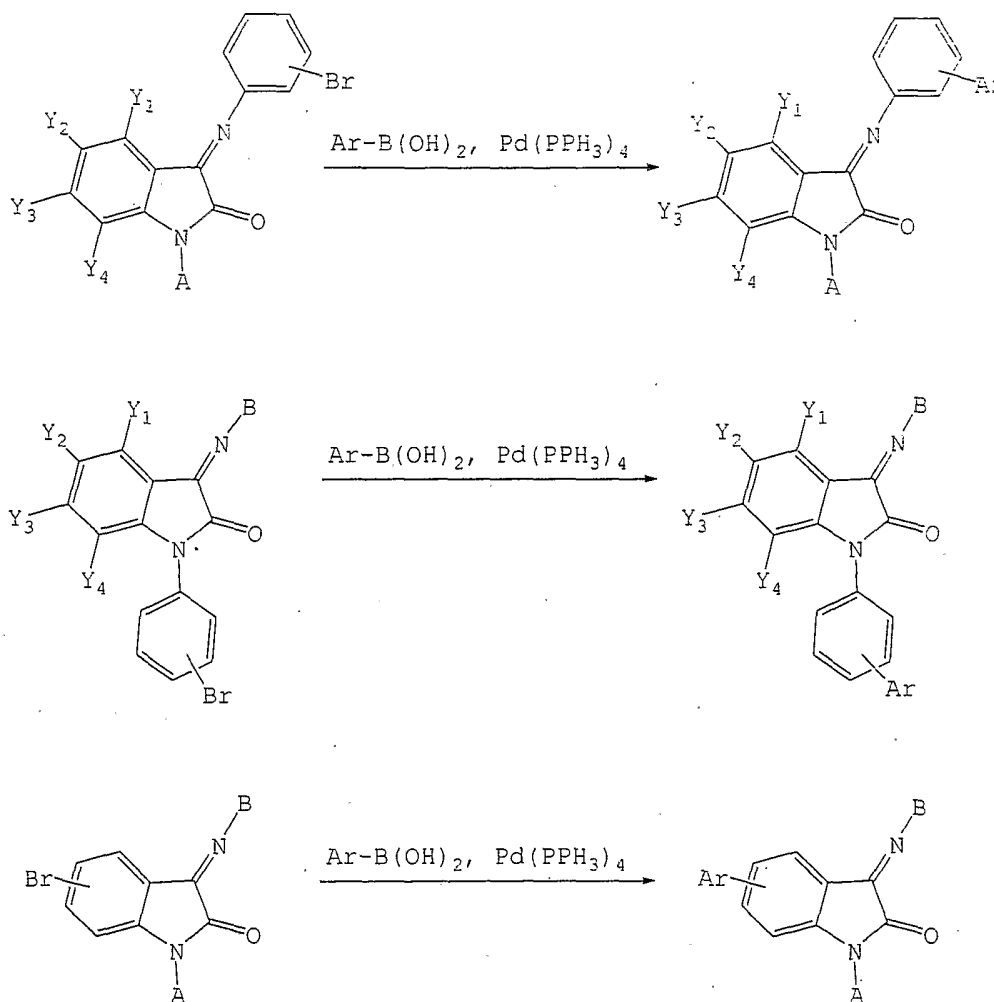
^a Y_1, Y_2, Y_3, Y_4, A , and B are defined as described in the specification.
 X is a leaving group such as Cl, Br, I , or OTs . R is boric acid or a dialkylborate group.

Scheme 9^a. Synthesis of Substituted Iminoindolones

X is a leaving group such as a halogen or tosylate.

^a Y_1, Y_2, Y_3, Y_4, A , and B are defined as described in the specification.
 X is a leaving group such as Cl, Br, I , or OTs . R is boric acid or a dialkylborate group.

Scheme 10^a. Synthesis of Aryl or Heteroaryl-Substituted Iminoindolones



^aY₁, Y₂, Y₃, Y₄, A, and B are defined as described in the specification. X is a leaving group such as Cl, Br, I, or OTs. R is boric acid or a dialkylborate group.

General Procedure for Synthesis of Iminoisatins. The
 5 appropriately substituted isatin (10 mg - 10 g) was placed in a flask and the appropriate aniline (1.0 - 1.1 equivalents) was added and the mixture was stirred to

homogeneity. The mixture was then heated to 110 °C for 2-7 hours and then cooled. Solids were crystallized from hot methanol and filtered, giving the desired products (usually as an inseparable interconverting mixture of E/Z isomers).

Procedure A:

1-(3-THIENYL)-1H-INDOLE-2,3-DIONE: Triethylamine (56.9 mL, 0.408 mol), was added to a mixture of 1H-indole-2,3-dione (15.0 g, 0.102 mol), copper (II) acetate (46.0 g, 0.255 mol), and 3-thienylboronic acid (19.6 g, 0.153 mol) in CH₂Cl₂ (500 mL). The reaction mixture was stirred overnight, filtered through Celite, rinsed with EtOAc/hexane (1:1, 300 mL), and concentrated *in vacuo*. The crude product was purified by column chromatography on silica using Hexane/EtOAc (1:1), giving the desired product (1.1 g, 50 %).

Procedure B:

(3E)-3-[(4-METHYLPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: A solution of 1-(3-Thienyl)-1H-indole-2,3-dione (20 mg, 0.087 mmol) in 1% HOAc/MeOH (8 mL) was added to a solution of *p*-toluidine (19 mg, 0.18 mmol) in 1% HOAc/MeOH (8 mL). The reaction mixture was stirred for 12 h at room temperature, heated at 50 °C for 1 h, and concentrated *in vacuo*. The residue was purified by preparative TLC on silica using EtOAc/hexanes (3:7, 0.1 % TEA) giving the desired product (14 mg, 50%).

Procedure C:

(3Z)-1-PHENYL-3-{[4-(3-THIENYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: A mixture of (3Z)-3-[(4-

bromophenyl)imino]-1-phenyl-1,3-dihydro-2H-indol-2-one
(50.0 mg, 0.133 mmol), thiophene-3-boronic acid (26.0 mg,
0.199 mmol), tetrakis(triphenylphosphine)palladium(0)
(31.0 mg, 0.0268 mmol in THF (5 mL), and aqueous Na₂CO₃
5 (2M, 100 µL) was heated at 67 °C for 24 h. The crude
product was concentrated *in vacuo* and the residue was
extracted with CH₂Cl₂ (3 x 1 mL), and concentrated. The
crude product was purified by preparative TLC using 10 %
methanol in CHCl₃, giving the desired product (18 mg,
10 35%).

Procedure D:

(3Z)-5-BROMO-3-{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-
DIHYDRO-2H-INDOL-2-ONE: A mixture of 5-bromo-1H-indole-
15 2,3-dione (1.0 g, 0.442 mmol) and 3-
trifluoromethylaniline (0.993 g, 6.2 mmol) in a solution
of 1% acetic acid in methanol was stirred at 50 °C for 12
h. The crude product was concentrated *in vacuo*, giving
the desired crude product (640 mg, 40%).

20

Procedure E:

(3Z)-5-BROMO-1-PHENYL-3-{[3-
(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-
ONE: A mixture of (3Z)-5-bromo-3-{[3-
25 (trifluoromethyl)phenyl]imino}-1,3-dihydro-2H-indol-2-one
(100 mg, 0.272 mmol), copper (II) acetate (54 mg, 0.33
mmol), triethylamine (82.8 mg, 0.817 mmol), and benzene
boronic acid (40 mg, 0.325 mmol) in 5 mL of CH₂Cl₂ was
stirred at room temperature for 12 h. The crude mixture
30 was concentrated *in vacuo* and purified by preparative TLC
using EtOAc:hexane (3:7, 1% triethylamine), giving the
desired product (22 mg, 20%).

Procedure F:

(3Z)-1,5-DIPHENYL-3-([3-(TRIFLUOROMETHYL)PHENYL]IMINO)-
1,3-DIHYDRO-2H-INDOL-2-ONE: A mixture of (3z)-5-bromo-1-
5 phenyl-3-([3-(trifluoromethyl)phenyl]imino)-1,3-dihydro-
2H-indol-2-one (22 mg, 0.05 mmol),
tetrakis(triphenylphosphine)palladium(0) (12.0 mg, 0.01
mmol), benzene boronic acid (10 mg, 0.08 mmol) in THF (5
mL), and aqueous Na₂CO₃ (2M, 100 µL) was heated at 67 °C
10 for 24 h. The crude product was concentrated *in vacuo*
and the residue was extracted with CH₂Cl₂ (3 x 1 ml),
concentrated, and purified by preparative TLC using 10 %
methanol in CHCl₃, giving the desired product (4 mg, 18%).

15 Procedure G:

ETHYL 5-[(2,3-DIOXO-2,3-DIHYDRO-1H-INDOL-1-YL)METHYL]-2-
FUROATE: A mixture of ethyl 5-(chloromethyl)-2-furoate
(148 mg, 1.01 mmol) in dioxane (15 ml) was added to a
mixture of NaH (48 mg, 1.20 mmol) in dioxane (10 mL)
20 under argon at 0 °C. The mixture was stirred for 1 h at
room temperature, refluxed under argon for 16 h, cooled
to room temperature, and then concentrated *in vacuo*. The
residue was purified by preparative TLC using
EtOAc/hexane (3:7), giving the desired product (56 mg, 19
25 %).

Procedure H:

ETHYL 5-[(3Z)-2-OXO-3-([3-(TRIFLUOROMETHYL)PHENYL]
IMINO)-2,3-DIHYDRO-1H-INDOL-1-YL)METHYL]-2-FUROATE: A
30 mixture of ethyl 5-[(2,3-dioxo-2,3-dihydro-1H-indol-1-
yl)methyl]-2-furoate (60 mg, 0.200 mmol) and 3-
trifluoromethylaniline (32 mg, 0.200 mmol) was heated at

140 °C for 2 h. The residue was dissolved in CHCl₃ (1 mL) and purified by preparative TLC using EtOAc/hexane (6:4), giving the desired product (20 mg, 23 %).

5 Procedure I:

6-METHOXY-1-PHENYL-1H-INDOLE-2,3-DIONE: A solution of *N*-(3-methoxyphenyl)-*N*-phenylamine (1.14 g, 5.72 mmol) in ether (3 mL) was added to a solution of oxalyl chloride (728 g, 5.75 mmol) and heated at reflux for 1 h. The resulting mixture was cooled to room temperature, concentrated to dryness, and redissolved in nitrobenzene (35 mL). The solution was added to a solution of AlCl₃ in nitrobenzene (0.762 g, 5.72 mmol), and the resulting mixture was heated at 70 °C for 16 h. The crude product was concentrated in vacuo and purified by column chromatography using EtOAc/hexane (1:1), giving the desired product 60, mg, 50 %).

Procedure J:

20 (3Z)-1-(4-BROMOPHENYL)-3-([3-(TRIFLUOROMETHYL) PHENYL] IMINO)-1,3-DIHYDRO-2H-INDOL-2-ONE: A solution of (3Z)-3-([3-(trifluoromethyl)phenyl]imino)-1,3-dihydro-2H-indol-2-one (100 mg, 0.344 mmol), copper (II) acetate (93 mg, 0.516 mmol), triethylamine (105 mg, 1.03 mmol), and 4-bromobenzene boronic acid (104 mg, 0.516 mmol) in 5 mL of CH₂Cl₂ was stirred at room temperature for 12 h. The crude mixture was concentrated in vacuo and purified by preparative TLC using EtOAc:hexane (3:7, 1% triethylamine), giving the desired product (65 mg, 42%).

30

Procedure K:

A solution of (3Z)-1-(4-bromophenyl)-3-([3-(trifluoromethyl)phenyl]imino)-1,3-dihydro-2H-indol-2-one (30 mg, 0.068), tetrakis(triphenylphosphine)palladium(0) (16.0 mg, 0.014 mmol), benzene boronic acid (13 mg, 5 0.101 mmol) in THF (5 mL), and aqueous Na₂CO₃ (0.45 M, 300 µL) was heated at 67 °C for 40 h. The crude product was concentrated in vacuo and the residue was extracted with CH₂Cl₂ (3 x 1 ml), concentrated, and purified by preparative TLC using 10 % methanol in CHCl₃, giving the 10 desired product (5 mg, 16%).

The compounds of Examples 92 - 107, inclusive, were purchased from Bionet Research Ltd., 3 Highfield Industrial Estate, Camelford, Cornwall PL32 9QZ, UK. 15 These compounds can also be synthesized using the procedure described above.

Example 91: 3-[(2-METHOXYPHENYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

20

Example 92: 1-PHENYL-3-[[3-(TRIFLUOROMETHYL)PHENYL]IMINO]-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 93: 3-[(3-METHYLPHENYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

25

Example 94: 3-[(3-CHLOROPHENYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 95: 1-PHENYL-3-[4-(TRIFLUOROMETHYL)PHENYL]IMINO]-1,3-DIHYDRO-2H-INDOL-2-ONE

30

Example 96: 3-[(4-METHYLPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 97: 3-[(4-CHLOROPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 98: 3-[(4-BROMOPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 99: 3-[(4-FLUOROPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 100: 3-[(4-PHENOXYPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 101: 3-[(4-ETHOXYPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 102: 3-[(4-METHOXYPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 103: 3-[(3,5-DICHLOROPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 104: 3-[(3,5-DIMETHYLPHENYL) IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 105: 1-ALLYL-3-[(3,4-DICHLOROPHENYL) IMINO]-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 106: 1-ALLYL-3-[(3,5-DICHLOROPHENYL) IMINO]-1,3-DIHYDRO-2H-INDOL-2-ONE

Example 107: 3-[(4-BROMOPHENYL)IMINO]-1-ISOPROPYL-1,3-DIHYDRO-2H-INDOL-2-ONE

5 The methods that follow demonstrate procedures useful for synthesizing compounds of this invention (illustrated in Schemes 6 and 7). Substituted isatins useful for synthesizing compounds of this invention can alternatively be obtained using the procedures described
10 in the following references:

Garden, S. J.; Da Silva, L. E.; Pinto, A.C.; *Synthetic Communications*, 1998, 28, 1679 - 1689.

Coppola, G.M.; *Journal of Heterocyclic Chemistry*, 1987,
15 24, 1249.

Hess, B.A. Jr; Corbino, S.; *Journal of Heterocyclic Chemistry*, 1971, 8, 161.

Bryant, W. M. III; Huhn, G.F.; Jensen, J.H.; Pierce, M. E.; Stambach, C.; *Synthetic Communications*, 1993, 23,
20 1617 - 1625.

Example 108: 1-[(5-CHLORO-2-THIENYL)METHYL]-3-([3-(TRIFLUOROMETHYL)PHENYL]IMINO)-1,3-DIHYDRO-2H-INDOL-2-

ONE: A mixture of 1-[(5-chloro-2-thienyl)methyl]-2H-
25 indole-2,3-dione (25 mg, 0.09 mmol) (prepared as described below) and 3-trifluoromethylaniline (11.3 μ L, 0.09 mmol) was heated neat at 140 °C for 2 h. The crude material was purified by preparative TLC using a mixture of 3:7 ethyl acetate in hexane as the eluent, giving the
30 desired product (23 mg 0.05 mmol, 61 %). ^1H NMR (400 MHz): δ (major isomer) 7.57 (t, J = 7.7, 1H), 7.53 (t, J = 7.8, 1H), 7.33 (t, J = 7.8, 1H), 7.28 (s, 1H), 7.19 (d,

$J = 7.6$, 2H), 6.94 – 6.72 (m, 4H), 6.56 (d, $J = 7.7$, 1H), 5.02 (s, 2H); ESI-MS m/z found 421 (MH^+).

1-[(5-CHLORO-2-THIENYL)METHYL]-2H-INDOLE-2,3-DIONE: A

5 solution of isatin (125 mg, 0.85 mmol) in anhydrous dioxane (10 mL) was added dropwise to a solution of sodium hydride (60% dispersion in mineral oil, 24 mg, 0.62 mmol) in anhydrous dioxane (10 mL) at 0 °C under argon. The mixture was allowed to stir for 5 minutes and
10 then 2-chloro-5-(chloromethyl)thiophene (0.12 mL, 1.02 mmol) in dioxane (10 mL) was added dropwise to the resulting mixture. The reaction mixture was heated at reflux under argon for 16 h and concentrated in vacuo. The crude material was purified preparative TLC using
15 1:24 methanol in chloroform as the eluent, giving the desired product as a yellow solid (53 mg, 0.19 mmol, 22 %). 1H NMR (400 MHz): δ 7.62 (d, $J = 7.4$, 1H), 7.56 (t, $J = 7.8$, 1H), 7.14 (t, $J = 7.7$, 1H), 6.94 (d, $J = 8.0$, 1H), 6.90 (d, $J = 3.2$, 1H), 6.78 (d, $J = 3.7$, 1H), 4.90 (s,
20 2H).

Example 109: 1-(3-THIENYL)-3-{[3-(TRIFLUOROMETHYL)

PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: A mixture of 1-(3-thienyl)-2H-indole-2,3-dione (25 mg, 0.11 mmol)
25 (prepared as described below) and 3-trifluoromethylaniline (14 μ L, 0.11 mmol) was heated neat at 140 °C for 2 h. The crude material was purified by preparative TLC using a mixture of 3:7 ethyl acetate and hexane as the eluent, giving the desired product as a
30 yellow solid (7.3 mg, 0.02 mmol, 22 %). 1H NMR (400 MHz) δ 7.62 – 7.19 (m, 9H), 6.94 (d, $J = 8.0$, 1H), 6.76 (t, $J = 7.6$, 1H); ESI-MS m/z found 373 (MH^+).

1-(3-THIENYL)-2H-INDOLE-2,3-DIONE: Copper(II) acetate monohydrate (4.25 g, 23.4 mmol) was heated at reflux in acetic anhydride (30 mL) for 2 h. The mixture was
5 filtered and washed with anhydrous ether (500 mL). The solid was dried in vacuo at 55 °C for 16 h. Dichloromethane (1 mL) was added to a mixture of copper(II) acetate (62 mg, 0.34 mmol), isatin (50 mg, 0.34 mmol), and thiophene-3-boronic acid (87 mg, 0.68
10 mmol), followed by triethylamine (0.10 mL, 0.68 mmol) under argon. The resulting solution was stirred for 16 h at room temperature. The reaction mixture was then recharged with 0.10 mmol copper(II) acetate, 0.10 mmol of 3-thiophene boronic acid, and 1 drop of triethylamine,
15 and the mixture was heated at 50 °C for 6 h. The crude material was purified by preparative TLC using 3:97 methanol in chloroform as the eluent, giving the desired product as a yellow solid (25 mg, 0.11 mmol, 33 %). ¹H NMR (400 MHz): δ 7.70 (d, J = 7.5, 1H), 7.58 (t, J = 7.8, 1H), 7.50 (d, J = 5.1, 1H), 7.48 (s, 1H), 7.24 (d, J = 5.1, 1H), 7.18 (t, J = 7.51, 1H), 7.05 (d, J = 8.0, 1H).

Example 110: 2-METHYL-5-[(2-OXO-1-PHENYL-1,2-DIHYDRO-3H-INDOL-3-YLIDENE)AMINO]-2H-ISOINDOLE-1,3(2H)-DIONE: A
25 mixture of 1-phenylisatin (50 mg, 0.22 mmol) and 4-amino-N-methylphthalimide (40 mg, 0.22 mmol) was heated neat at 215 °C for 2 h. The crude material was purified by preparative TLC using a mixture of 3:7 ethyl acetate and hexane as the eluent, giving the desired product as a
30 yellow solid (8 mg, 0.02 mmol, 10 %). ¹H NMR (400 MHz): δ 7.88 (d, J = 7.8, 1H), 7.83 - 7.80 (m, 1H), 7.51 (t, J = 7.5, 1H), 7.47 - 7.18 (m, 6H), 7.02 (t, J = 8.0, 1H),

6.91 - 6.79 (m, 2H), 6.58 (d, $J = 7.5$, 1H), 3.22 (s, 3H);
ESI-MS m/z found 382 (MH^+).

5 Example 111: 1-[(5-CHLORO-1-BENZOTHIEN-3-YL)METHYL]-3-
{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-
2-ONE: A mixture of 1-[(5-chloro-1-benzothien-3-
yl)methyl]-2H-indole-2,3-dione (50 mg, 0.15 mmol)
(prepared as described below) and 3-
10 trifluoromethylaniline (0.020 mL, 0.15 mmol) was heated
neat at 140 °C for 2 h. The crude material was purified
by preparative TLC using a mixture of 1:3 ethyl acetate
and hexane as the eluent giving the desired product as a
yellow solid (13 mg, 0.030 mmol, 18%). 1H NMR (400 MHz):
15 δ 7.98 (d, $J = 2.0$, 1H), 7.80 (d, $J = 8.6$, 1H), 7.58 (t,
 $J = 7.7$, 1H), 7.52 (d, $J = 8.1$, 1H), 7.43 (s, 1H), 7.38
(dd, $J = 8.6$, 1.9, 1H), 7.31 (overlapping singlet and dt,
 $J = 1.2$, 7.8, 2H), 7.24 (d, $J = 7.8$, 1H), 6.87 (d, $J =$
7.9, 1H), 6.77 (t, $J = 7.7$, 1H), 6.59 (d, $J = 7.7$, 1H),
20 5.20 (s, 2H). ESI-MS m/z found 471 (MH^+ with ^{35}Cl), 473
(MH^+ with ^{37}Cl).

1-[(5-CHLORO-1-BENZOTHIEN-3-YL)METHYL]-2H-INDOLE-2,3-
dione: A solution of isatin (125mg, 0.85 mmol) in
25 anhydrous dioxane (10 mL) was added dropwise to a
solution of sodium hydride (60% dispersion in mineral
oil, 25 mg, 0.62 mmol) in anhydrous dioxane (10 mL) at 0
°C under argon. The mixture was allowed to stir for 5
minutes and then a solution of 3-(bromomethyl)-5-
30 chlorobenzo[b]thiophene (267 mg, 1.02 mmol) in dioxane
(10 mL) was added dropwise to the reaction mixture. The
reaction mixture was heated at reflux under argon for 16

h and concentrated in *vacuo*. The crude material was purified by preparative TLC using 1:24 methanol in chloroform as the eluent, giving the desired product as a yellow solid (125 mg, 0.38 mmol, 45%). ¹H NMR (400 MHz): δ 7.89 (s, 1H), 7.79 (d, *J* = 8.5, 1H), 7.65 (d, *J* = 7.5, 1H), 7.54 (t, *J* = 8.0, 1H), 7.42 (s, 1H), 7.38 (d, *J* = 8.5, 1H), 7.14 (t, *J* = 7.5, 1H), 6.88 (d, *J* = 7.8, 1H), 5.13 (s, 2H).

10 Example 112: 3-(1H-INDOL-5-YLIMINO)-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: 1-phenylisatin (51.8 mg, 0.23 mmol) and 5-aminoindole (31 mg, 0.23 mmol) were mixed and heated at 140 °C for 2 h. The resulting crude product was purified by preparative TLC using ethyl acetate/hexane (6:4) as the eluent, giving the desired product as a yellow solid (10.8 mg, 14%). ¹H NMR (400 MHz): δ 8.28 (s, 1H), 7.57 (t, *J* = 7.7, 2H), 7.49 - 7.40 (m, 6H), 7.29 - 7.23 (m, 1H), 7.03 (dd, *J* = 8.5, 1.7, 1H), 6.98 (d, *J* = 7.6, 1H), 6.83 (d, *J* = 8.0, 1H), 6.74, *J* = 7.6, 1H), 6.59 (s, 1H); ESI-MS *m/z* found 338 (MH⁺).

Example 113: 3-[(6-CHLORO-3-PYRIDINYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: 1-phenylisatin (23.0 mg, 0.10 mmol) and 5-amino-2-chloropyridine (12.8 mg, 0.10 mmol) were mixed and heated at 140 °C for 7 h. The resulting crude product was purified by preparative TLC using hexane/ethyl acetate (8:2) as the eluent, giving the desired product as a yellow solid (19.7 mg, 59%). ¹H NMR (400 MHz) δ 8.15 (d, *J* = 8, 1H), 7.6 - 7.2 (m, 9H), 6.85 - 6.75 (m, 2H); ESI-MS *m/z* found 334 (MH⁺).

Example 114: 3-[(2-METHYL-1,3-BENZOTHAZOL-5-YL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: 5-amino-2-methylbenzothiazole (52.2 mg, 0.31 mmol) was mixed with 1-phenylisatin (69.7 mg, 0.31 mmol) and heated at 140 °C for 3 h. The resulting crude product was purified by preparative TLC using ethyl acetate/hexane (6:4) as the eluent to give the desired product as a yellow solid (36.9 mg, 32.3 %). ¹H NMR Data: δ 7.9-6.7 (m, 12H), 2.9 (s, 3H). ESI-MS m/z found 370 (MH⁺).

10

Example 254: (3Z)-3-[(3,4-DICHLOROPHENYL)IMINO]-1-(2-PYRIDINYLMETHYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures H and K (for substitution of 2-picoly chloride). ¹H NMR (400 MHz, CDCl₃) δ 8.51 - 8.46 (m, 1H), 7.87 - 7.78 (m, 1H), 7.64 (d, 1H, J = 7.1), 7.53 - 7.31 (m, 5H), 7.28 (d, 1H, J = 4.1), 7.12 (d, 1H, J = 8.1), 6.58-6.53 (m, 1H), 5.51 (s, 2H); ESI-MS m/z 381 (MH⁺).

Example 255: (3Z)-3-[(3,4-DICHLOROPHENYL)IMINO]-1-[(3,5-DIMETHYL-4-ISOXAZOLYL)METHYL]-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B (microwave heating). ¹H NMR (400 MHz, CDCl₃) δ 7.63 (d, 1H, J = 9.1), 7.46 (dt, 1H, J = 8.1, 2.0), 7.28 (d, 1H, J = 2.1), 7.02 (d, 1H, J = 2.0), 6.88 (dt, 1H, J = 8.0, 2.1), 6.74 - 6.72 (m, 1H), 6.72 - 6.70 (m, 1H), 5.53 (s, 2H), 2.50 (s, 3H), 2.24 (s, 3H); ESI-MS m/z 399 (MH⁺).

Example 256: (3Z)-3-[(3,4-DICHLOROPHENYL)IMINO]-1-[3-(TRIFLUOROMETHYL)PHENYL]-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B. ¹H NMR (400 MHz, CDCl₃) δ 7.90 - 7.87 (m, 1H), 7.83 - 7.79 (m, 1H), 7.67 (d, 1H, J

= 8), 7.46 - 7.40 (m, 1H), 7.33 (d, 1H, $J = 2$), 7.08 - 7.05 (m, 1H), 6.96 - 6.80 (m, 5H); ESI-MS m/z 435 (MH^+).

Example 257: (3Z)-1-(3,5-DICHLOROPHENYL)-3-[(3,4-DICHLOROPHENYL)IMINO]-1,3-DIHYDRO-2H-INDOL-2-ONE:

Prepared by Procedures A and B. 1H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, 1H, $J = 8.1$), 7.79 (d, 1H, $J = 6.0$), 7.72 - 7.68 (m, 1H), 7.59 - 7.45 (m, 1H), 7.46 (d, 1H, $J = 8.1$), 7.32 (dt, 1H, $J = 8.0, 2.1$), 7.23 (d, 1H, $J = 2.5$), 6.97 (dd, 1H, $J = 8.0, 2.1$), 6.92 - 6.87 (m, 1H), 6.85 - 6.81 (m, 1H); ESI-MS m/z 435 (MH^+).

Example 258: (3Z)-3-[(3,4-DICHLOROPHENYL)IMINO]-6-METHOXY-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures K, L, and B. 1H NMR (400 MHz, $CDCl_3$) δ 7.69 - 7.54 (m, 1H), 7.53 - 7.38 (m, 3H), 7.29 (d, 1H, $J = 2.0$), 7.17 (d, 1H, $J = 8.1$), 7.12 (d, 1H, $J = 8.0$), 6.84 (d, 1H, $J = 2.5$), 6.78 (d, 1H, $J = 8$), 6.6 (dd, 2H, $J = 8.0, 2.0$), 6.55 (dd, 2H, $J = 8.1, 2.5$); ESI-MS m/z (398 MH^+).

20

Example 259: (3Z)-3-[(4-CHLORO-3-METHYLPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (80 °C). 1H NMR (400 MHz, $CDCl_3$) δ 7.69 - 7.62 (m, 2H), 7.49 (s, 1H), 7.47 (s, 1H), 7.41 (dt, 1H, $J = 7.1, 1.6$), 7.3 (dd, 1H, $J = 5.0, 1.6$), 7.05 - 6.97 (m, 1H), 6.93 - 6.86 (m, 1H), 6.77 (m, 1H), 6.56 (m, 1H), 2.53 (s, 3H); ESI-MS m/z 353 (MH^+).

Example 260: (3Z)-3-(2-NAPHTHYLIMINO)-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (80 °C). 1H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, 1H, $J = 9.1$), 8.06 - 7.99 (m, 1H), 7.89 - 7.80 (m, 1H), 7.78 - 7.71 (m,

30

1H), 7.71 - 7.47 (m, 4H), 7.41 - 7.35 (m, 1H), 7.33 (d, 1H, $J = 5.2$), 7.28 (d, 1H, $J = 6.8.1$), 7.00 (d, 1H, $J = 8.0$), 6.76 (t, 1H, $J = 7.8$), 6.67 (d, 1H, $J = 7.9$); ESI-MS m/z 355 (MH^+).

5

Example 261: 3-[(4-CHLOROPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (80 °C). 1H NMR (400 MHz, $CDCl_3$) δ 7.69 - 7.56 (m, 2H), 7.54 - 7.48 (m, 1H), 7.41 (dt, 1H, $J = 8, 2$), 7.32 - 7.28 (m, 1H), 7.11 - 6.99 (m, 3H), 6.89 (dt, 1H, $J = 8$), 6.77 - 6.73 (m, 1H), 6.66 - 6.33 (m, 1H); ESI-MS m/z 339 (MH^+).

10

Example 262: 3-[(4-IODOPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (1% HOAc in MeOH). 1H NMR (400 MHz, $CDCl_3$) δ 7.79 - 7.74 (m, 2H), 7.53 - 7.48 (m, 2H), 7.35 (dt, 1H, $J = 8.0, 1.2$), 7.29 - 7.24 (m, 1H), 6.98 (d, 1H, $J = 8.0$), 6.89 - 6.75 (m, 4H); ESI-MS m/z 431 (MH^+).

15

Example 263: 3-[(4-METHYLPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (1% HOAc in MeOH). 1H NMR (400 MHz, $CDCl_3$) δ 7.52 - 7.44 (m, 2H), 7.35 - 7.22 (m, 4H), 6.99 - 6.93 (m, 3H), 6.87 - 6.78 (m, 2H), 2.42 (s, 3H); ESI-MS m/z 319 (MH^+).

20

Example 264: 3-[(3,5-DIFLUOROPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (1% HOAc in MeOH). 1H NMR (400 MHz, $CDCl_3$) δ 7.54 - 7.16 (m, 4H), 6.99 (dt, 1H, $J = 8.2, 0.8$), 6.89 (dt, 1H, $J = 7.7, 1.1$), 6.76 (d, 1H, $J = 7.5$), 6.71 (tt, 1H, $J = 9.3, 2.3$), 6.64 - 6.57 (m, 2H); ESI-MS m/z 341 (MH^+).

25

30

Example 265: 3-([1,1'-BIPHENYL]-4-YLIMINO)-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (1% HOAc in MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.73 - 7.12 (m, 13H), 6.99 (d, 1H, J = 8.0), 6.89 (d, 1H, J = 8.0),
5 6.82 (dt, 1H, J = 7.6, 1.0); ESI-MS m/z 381 (MH⁺).

Example 266: ETHYL 3-([(3Z)-2-OXO-1-(3-THIENYL)-1,2-DIHYDRO-3H-INDOL-3-YLIDENE]AMINO)BENZOATE: Prepared by Procedures A and B (1% HOAc in MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.96 (d, 1H, J = 7.4), 7.75 - 7.17 (m, 6H), 6.98
10 (d, 1H, J = 8.0), 6.87 - 6.78 (m, 2H), 6.63 (d, 1H, J = 7.8), 4.45 - 4.32 (m, 2H), 1.43 - 1.33 (m, 3H); ESI-MS m/z 377 (MH⁺).

Example 267: 3-[(6-CHLORO-3-PYRIDINYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by
15 Procedures A and B (1% HOAc in MeOH). ¹H NMR (400 MHz, CDCl₃) δ 8.21 - 6.81 (m, 10H); ESI-MS m/z 340.13 (MH⁺).

Example 268: 3-[(4-PHENOXYPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (1% HOAc in MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.85 - 6.70
20 (m, 16H); ESI-MS m/z 397 (MH⁺).

Example 269: 3-[(4-BROMOPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and H. ¹H NMR (400 MHz, CDCl₃) δ 7.82 - 6.55 (m, 11H); ESI-MS m/z 383 (MH⁺).

25 Example 270: 3-[(3-CHLOROPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and H. ¹H NMR (400 MHz, CDCl₃) δ 7.55 - 6.50 (m, 11H); ESI-MS m/z 339 (MH⁺).

Example 271: 3-[(3-METHYLPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: : Prepared by Procedures A and B (1% HOAc in MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.67 - 6.78 (m, 11H), 2.39 (s, 3H); ESI-MS m/z 319 (MH⁺).

- 5 Example 272: 3-[(3,4-DICHLOROPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: : Prepared by Procedures A and B (1% HOAc in MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.82 - 6.80 (m, 10H); ESI-MS m/z 373 (MH⁺).

- 10 Example 273: 1-(2-PYRIDINYLMETHYL)-3-{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: : Prepared by Procedure B. ESI-MS m/z 382 (MH⁺).

Example 274: 3-[(3,5-DICHLOROPHENYL)IMINO]-1-(2-PYRIDINYLMETHYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B. ESI-MS m/z 382 (MH⁺).

- 15 Example 275: 1-[(3,5-DIMETHYL-4-ISOXAZOLYL)METHYL]-3-{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B. ESI-MS m/z 400 (MH⁺).

- 20 Example 276: 3-[(3,4-DIFLUOROPHENYL)IMINO]-1-(3-PYRIDINYLMETHYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B. ESI-MS m/z 350 (MH⁺).

Example 277: 1-(3-PYRIDINYLMETHYL)-3-{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B. ESI-MS m/z 382 ((MH⁺)).

- 25 Example 278: 3-[(3,4-DIFLUOROPHENYL)IMINO]-1-(2-PYRIDINYLMETHYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B. ESI-MS m/z 350 (MH⁺).

Example 279: 3-[(3,5-DICHLOROPHENYL)IMINO]-1-(3-PYRIDINYLMETHYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B. ESI-MS m/z 384 (MH^+).

Example 280: 3-[(3,5-DICHLOROPHENYL)IMINO]-1-[(3,5-DIMETHYL-4-ISOXAZOLYL)METHYL]-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B. ESI-MS m/z 402 (MH^+).

Example 281: 3-[(9-ETHYL-9H-CARBAZOL-3-YL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure H. 1H NMR (400 MHz, $CDCl_3$) δ 8.28 - 6.66 (m, 16H), 4.47 - 4.35 (m, 2H), 1.55 - 1.44 (m, 3H); ESI-MS m/z 416 (MH^+).

Example 282: 1-PHENYL-3-(5-QUINOLINYLMIMINO)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure H. 1H NMR (400 MHz, $CDCl_3$) δ 9.38 - 9.32 (m, 1H), 8.55 - 8.50 (m, 1H), 8.01 - 6.62 (m, 12H), 6.43 - 6.35 (m, 1H); ESI-MS m/z 350 (MH^+).

Example 283: 3-[(4-IODOPHENYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B (0.1 % HOAc, 80 °C, 92 h, 4 eq RNH_2 , 3 Å molecular sieves). ESI-MS m/z 425 (MH^+).

Example 285: 3-[(3,4-DIFLUOROPHENYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B (0.1 % HOAc, 80 °C, 92 h, 4 eq RNH_2 , 3 Å molecular sieves). ESI-MS m/z 335 (MH^+).

Example 286: 3-[(2-CHLORO-4-METHYLPHENYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B (0.1 % HOAc, 80 °C, 92 h, 4 eq RNH₂, 3 Å molecular sieves). ESI-MS *m/z* 347 (MH⁺ with ³⁵Cl), 349 (MH⁺ with ³⁷Cl).

5

Example 287: 3-[(2,4-DIMETHOXYPHENYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B (0.1 % HOAc, 80 °C, 92 h, 4 eq RNH₂, 3 Å molecular sieves). ESI-MS *m/z* 359 (MH⁺).

10

Example 288: 3-[(3Z)-2-OXO-1-PHENYL-1,2-DIHYDRO-3H-INDOL-3-YLIDENE]AMINO)BENZONITRILE: Prepared by Procedure B (0.1 % HOAc, 80 °C, 92 h, 4 eq RNH₂, 3 Å molecular sieves). ESI-MS *m/z* 324 (MH⁺).

15

Example 289: 3-[[2-METHYL-5-(TRIFLUOROMETHYL)PHENYL]IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B (0.1 % HOAc, 80 °C, 92 h, 4 eq RNH₂, 3 Å molecular sieves). ESI-MS *m/z* 381 (MH⁺).

20

Example 290: 3-[(4-CHLORO-3-METHYLPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (80 °C). ESI-MS *m/z* 353 (MH⁺).

25

Example 291: 3-(6-QUINOLINYLMINO)-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (80 °C). ESI-MS *m/z* 356 (MH⁺).

30

Example 292: 3-[(4-CHLOROPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (80 °C). ESI-MS *m/z* 339 (MH⁺).

Example 295: 3-[(3-ISOPROPYLPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (80 °C). ESI-MS m/z 347 (MH^+).

5 Example 296: 3-[(4-CYCLOHEXYLPHENYL)IMINO]-1-(3-THIENYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures A and B (80 °C). ESI-MS m/z 387 (MH^+).

10 Example 297: (4-[(3Z)-2-OXO-1-PHENYL-1,2-DIHYDRO-3H-INDOL-3-YLIDENE]AMINO)PHENYL)ACETONITRILE: Prepared by Procedure B (0.1 % HOAc, 80 °C, 92 h, 4 eq RNH_2 , 3 Å molecular sieves). ESI-MS m/z 339 (MH^+).

15 Example 298: 3-[(2,2-DIFLUORO-1,3-BENZODIOXOL-5-YL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B (0.1 % HOAc, 80 °C, 92 h, 4 eq RNH_2 , 3 Å molecular sieves). ESI-MS m/z 379 (MH^+).

20 Example 299: 3-(1,3-BENZOTHAZOL-6-YLIMINO)-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure H. ESI-MS m/z 356 (MH^+).

25 Example 300: 1-TETRAHYDRO-2H-PYRAN-4-YL-3-[(3-(TRIFLUOROMETHYL)PHENYL)IMINO]-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures G and H. ESI-MS m/z 375 (MH^+).

Example 301: 3-(1H-INDAZOL-6-YLIMINO)-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure H. ESI-MS m/z 339 (MH^+).

Example 302: 3-[(3-CHLOROPHENYL)IMINO]-6-METHOXY-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures I and H. ESI-MS m/z 363 (MH^+).

5 Example 303: 6-METHOXY-1-PHENYL-3-{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures I and H. ESI-MS m/z 397 (MH^+).

10 Example 304: 1-PHENYL-3-{[4-(3-THIENYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures H and C. ESI-MS m/z 381 (MH^+).

15 Example 305: 1-PHENYL-3-{[3'-(TRIFLUOROMETHYL)[1,1'-BIPHENYL]-4-YL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures H and C. ESI-MS m/z 443 (MH^+).

20 Example 306: 1-PHENYL-3-{[4-(3-PYRIDINYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures H and C. ESI-MS m/z 376 (MH^+).

25 Example 307: 3-[(3-BROMOPHENYL)IMINO]-1-PHENYL-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedure B. ESI-MS m/z 378 (MH^+).

Example 308: 1,5-DIPHENYL-3-{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures D, E, and F. ESI-MS m/z 443 (MH^+).

30 Example 309: 1-[1,1'-BIPHENYL]-4-YL-3-{[3-(TRIFLUOROMETHYL)PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared

by Procedures H (6 eq of aniline), J, and K. ESI-MS m/z 443 (MH^+).

Example 310: 1-(4-HYDROXYPHENYL)-3-{[3-(TRIFLUOROMETHYL) PHENYL]IMINO}-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures H (6 eq of aniline) and E. ESI-MS m/z 383 (MH^+).

Example 311: 3-[(3,4-DICHLOROPHENYL)IMINO]-1-(3-PYRIDINYLMETHYL)-1,3-DIHYDRO-2H-INDOL-2-ONE: Prepared by Procedures H (75 °C, 2 h), K (3-picolyl chloride), and B. ESI-MS m/z 383 (MH

Examples 91-114 and 254-311 as described above are merely illustrative of the methods used to synthesize indolone derivatives. Further derivatives may be obtained utilizing methods shown in Schemes 6a, 7a and 8-10. The substituents in Schemes 6a, 7a and 8-10 are described in the Detailed Description.

It may be necessary to incorporate protection and deprotection strategies for substituents such as amino, amido, carboxylic acid, and hydroxyl groups in the synthetic methods described above to form indolone derivatives. Methods for protection and deprotection of such groups are well-known in the art, and may be found, for example in Green, T. W. and Wuts, P.G. M. (1991) Protection Groups in Organic Synthesis, 2nd Edition John Wiley & Sons, New York.

Radioligand Binding of Indolones at Cloned Galanin

Receptors

The binding properties of the indolones of the present invention were evaluated at the cloned human galanin receptors, GAL1, GAL2, and GALR3, using protocols
5 described herein.

Radioligand Binding Assay Results

The indolones described in Examples 91-114 and 254-311 were assayed using cloned human galanin receptors. The
10 compounds were found to be selective for the GALR3 receptor. The binding affinities of the compounds of Examples 91-114 and 254-311 are illustrated in Tables 4 and 4a.

Table 4

Example						Ki (nM)		
	R1	R2	R3	R4	R5	GalR1	GalR2	GalR3
91	Ph	OMe	H	H	H	>10000	>10000	527
92	Ph	H	CF ₃	H	H	>10000	>10000	
93	Ph	H	Me	H	H	>10000	>10000	171
94	Ph	H	Cl	H	H	>10000	>10000	49
95	Ph	H	H	CF ₃	H	>10000	>10000	29
96	Ph	H	H	Me	H	>10000	>10000	111
97	Ph	H	H	Cl	H	>10000	>10000	51
98	Ph	H	H	Br	H	>10000	>10000	38
99	Ph	H	H	F	H	>10000	>10000	229
100	Ph	H	H	OPh	H	>10000	>10000	
101	Ph	H	H	OE _t	H	>10000	>10000	305
102	Ph	H	H	OMe	H	>10000	>10000	429
103	Ph	H	Cl	H	Cl	>10000	>10000	68
104	Ph	H	Me	H	Me	>10000	>10000	143
105	allyl	H	Cl	Cl	H	>10000	>10000	97
106	allyl	H	Cl	H	Cl	>10000	>10000	62
107	isopropyl	H	H	Br	H	>10000	>10000	126

Key: Ph= Phenyl

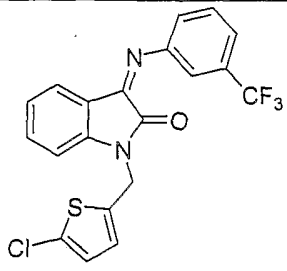
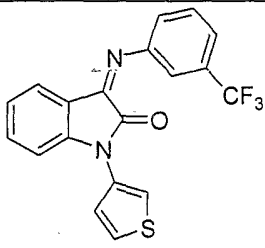
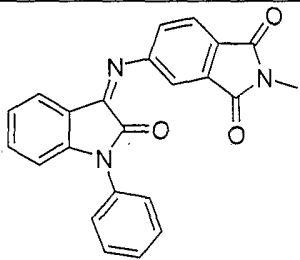
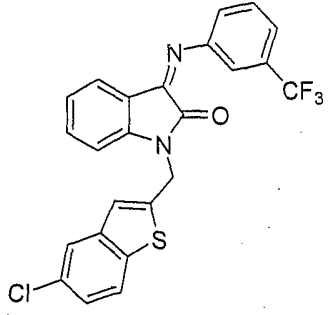
OMe= Methoxy

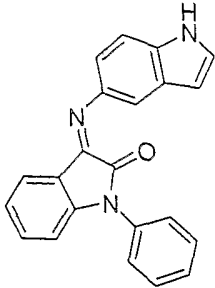
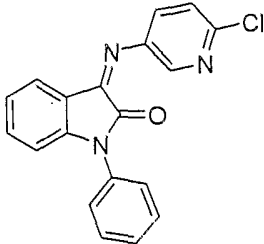
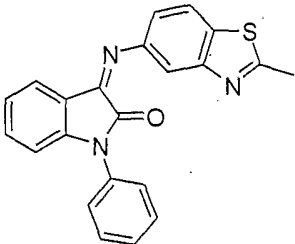
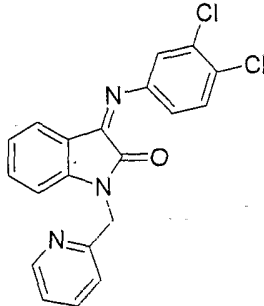
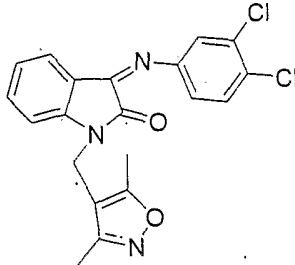
OE_t= Ethoxy

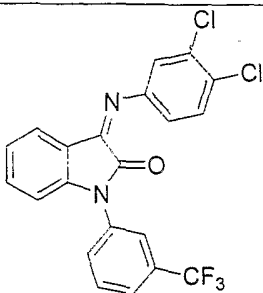
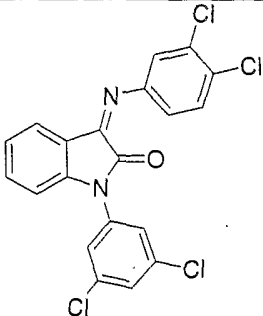
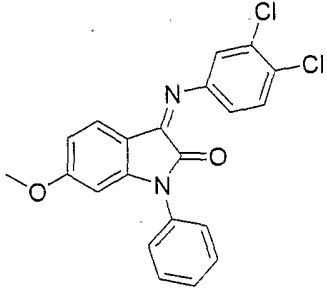
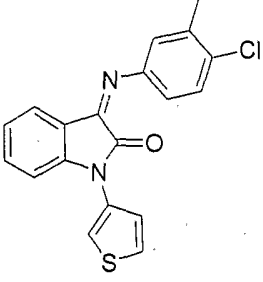
Me= Methyl

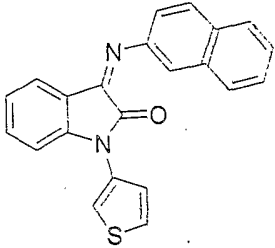
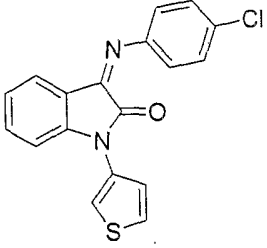
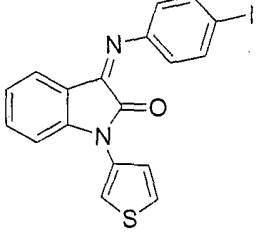
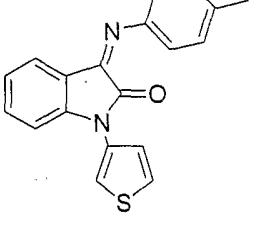
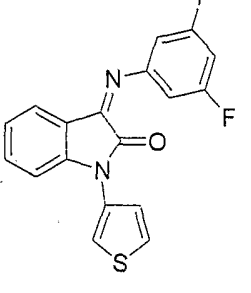
OPh= Phenoxy

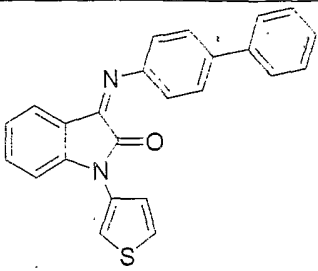
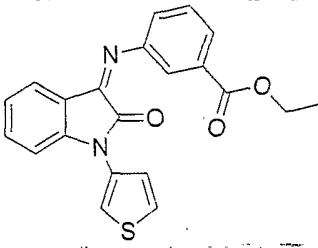
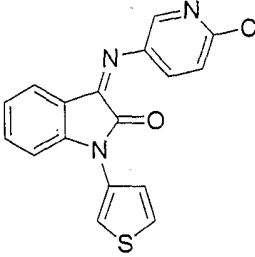
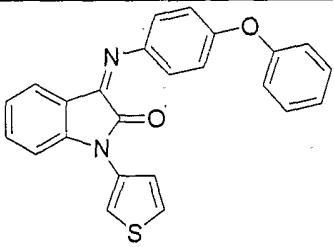
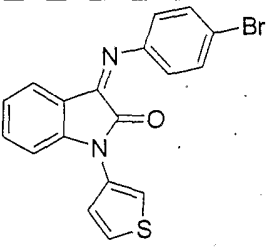
Table 4a.

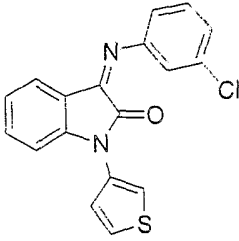
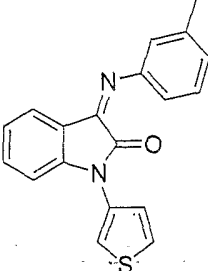
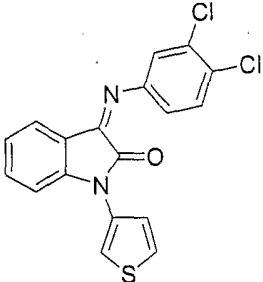
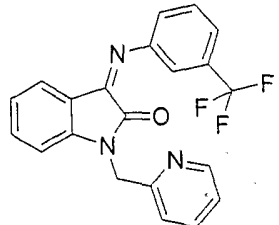
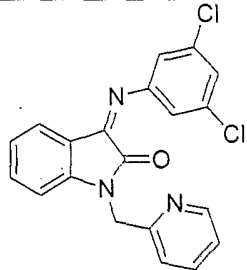
Example	Structure	Ki (nM) GalR3
108		84
109		103
110		138
111		1178

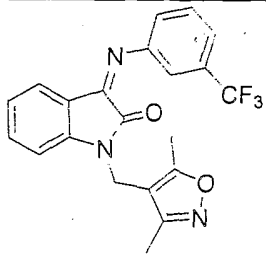
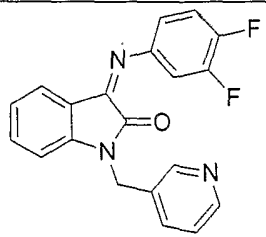
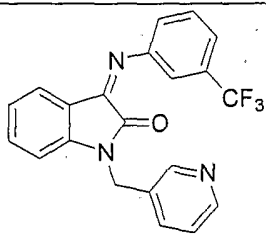
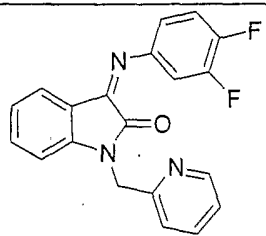
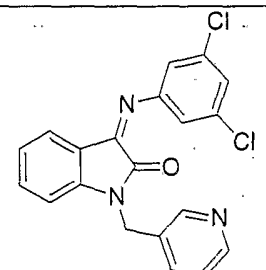
112		2324
113		136
114		569
254		64
255		49

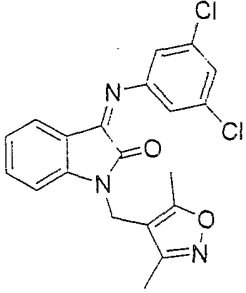
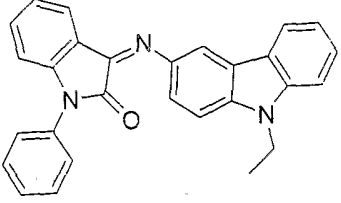
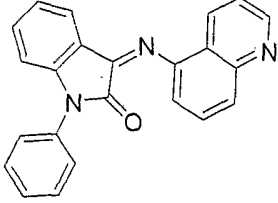
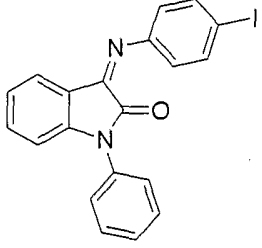
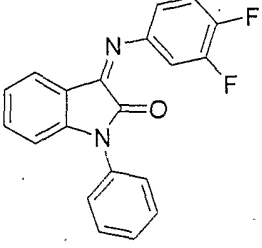
256		18
257		33
258		67
259		55

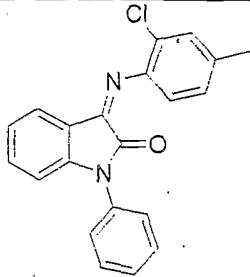
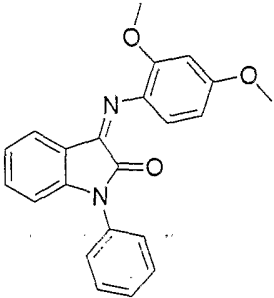
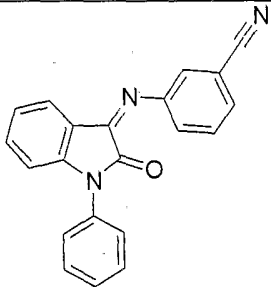
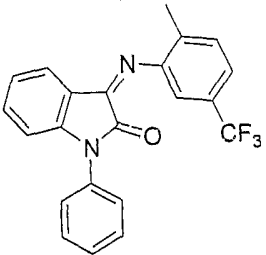
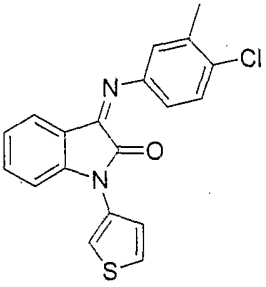
260		60
261		34
262		46
263		136
264		27

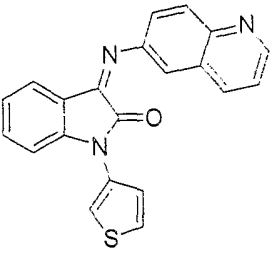
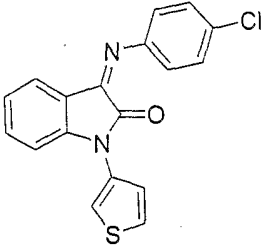
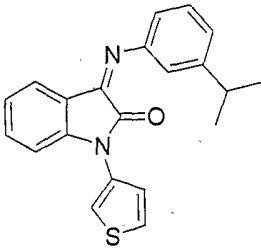
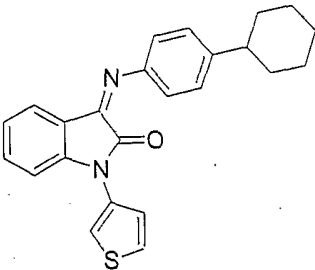
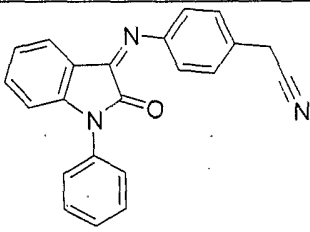
265		80
266		236
267		234
268		57
269		46

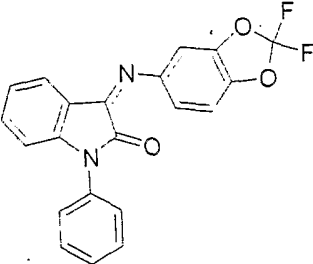
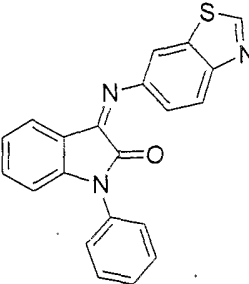
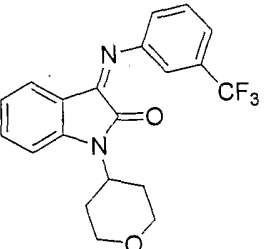
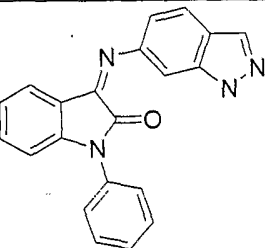
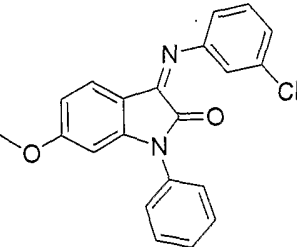
270		42
271		114
272		26
273		202
274		174

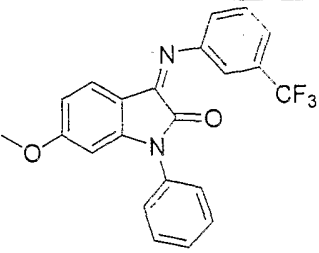
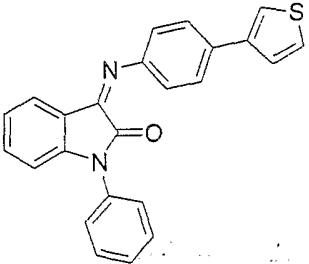
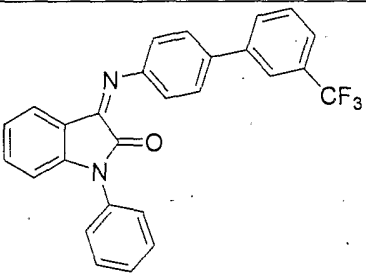
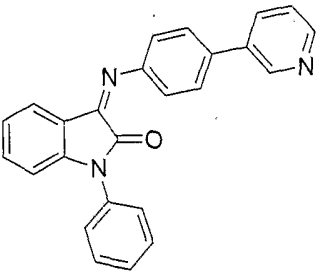
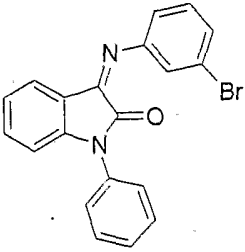
275		595
276		192
277		198
278		340
279		81

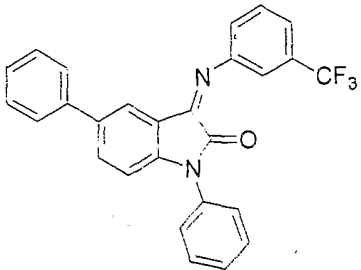
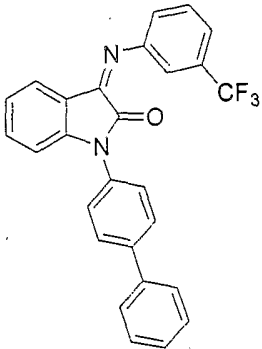
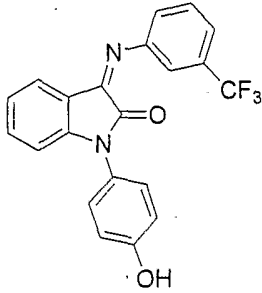
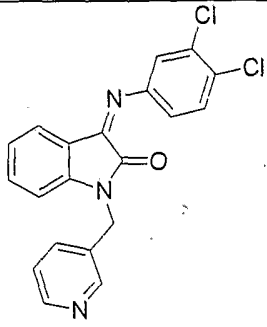
280		521
281		150
282		333
283		33
285		26

286		36
287		260
288		39
289		59
290		55

291		271
292		34
295		242
296		82
297		226

298		22
299		377
300		742
301		875
302		150

303		214
304		728
305		638
306		160
307		41

308		98
309		224
310		126
311		32

5

I. Oral Compositions

As a specific embodiment of an oral composition of a compound of this invention, 100 mg of one of the
10 compounds described herein is formulated with sufficient finely divided lactose to provide a total amount of 580 to 590 mg to fill a size O hard gel capsule.

II. In-Vivo Models

15

A. Materials and Methods

1. Forced Swim Test (FST)

The procedure used in this study was similar to that
20 previously described (Porsolt, et al., 1978), except the water depth (30 cm in this procedure). The greater depth in this test prevented the rats from supporting themselves by touching the bottom of the cylinder with their feet. Swim sessions were conducted by placing rats
25 in individual plexiglass cylinders (46 cm tall x 20 cm in diameter) containing 23-25°C water 30 cm deep (Porsolt, et al. used a depth of only 15 cm; also, see Detke, et al., 1995). Two swim tests were conducted always between 1200 and 1800 hours: an initial 15-min pretest followed
30 24 h later by a 5-minute test. Drug treatments were administered 60 minutes before the 5-minute test period. All other test sessions were conducted between 1300 to 1700 hours. Following all swim sessions, rats were removed from the cylinders, dried with paper towels and

placed in a heated cage for 15 minutes and returned to their home cages. All test sessions were videotaped using a Panasonic color video camera and recorder for scoring later.

5

Animals

Male Sprague-Dawley rats (Taconic Farms, NY) were used in all experiments. Rats were housed in pairs and maintained on a 12:12-h light-dark cycle. Rats were
.0 handled for 5 minutes each day for 5 days prior to behavioral testing.

Behavioral Scoring

The rat's behavior was rated at 5 second intervals during
.5 the 5 minute test as one of the following:

1. Immobility- rat remained floating in the water without struggling and was only making those movements necessary to keep its head above water;
20
2. Climbing - rat was making active movements with its forepaws in and out of the water, usually directed against the walls;
- 15 3. Swimming - rat was making active swimming motions, more than necessary to merely maintain its head above water, e.g. moving around in the cylinder; and
4. Diving - entire body of the rat was submerged.

10

All of the behavior scoring was done by a single rater, who was blind to the treatment condition. The rater was

also present in the room throughout the entire test period.

Drug Administration

5 Animals were randomly assigned to receive a single i.p. administration of Example 92 (1, 3, 10 or 30 mg/kg, dissolved in 100% DMSO), fluoxetine (10 mg/kg, dissolved in distilled water) or vehicle (equal mixture of DMSO and distilled water) 30 minutes before the start of the 5
10 minute test period. All injections were given using 1 cc tuberculin syringe with 26 3/8 gauge needles (Becton-Dickinson, VWR Scientific, Bridgeport, NJ). The volume of injection was 1 ml/kg.

15 In another set of experiments, animals were randomly assigned to receive a single p.o. administration of one of the following treatments: Example 151 (1, 3 or 10 mg/kg), fluoxetine (5 or 10 mg/kg) or vehicle (1 ml/kg of 100% N,N-dimethylacetamide) 60 minutes before the start
20 of the 5 minute test period. The drugs were dissolved in 100% N,N-dimethylacetamide. All administrations were given using 1 cc tuberculin syringes, to which a 3 inch, curved, stainless steel gavage needle was attached. The volume of administration was 1 ml/kg.

25 In other sets of experiments, animals were randomly assigned to receive a single p.o. administration of one of the following treatments: Example 103 (3, 10 and 30 mg/kg), fluoxetine (10 mg/kg) or vehicle (1 ml/kg of 100%
30 N,N-dimethylacetamide) 60 minutes before the start of the 5 minute test period; or Example 272 (3 mg/kg), fluoxetine (10 mg/kg) or vehicle (1 ml/kg of 100% N,N-

dimethylacetamide) 24 hours before the start of the 5 minute test period; or Example 98 (3, 10 and 30 mg/kg), fluoxetine (10 mg/kg) or vehicle (1 ml/kg of 100% N,N-dimethylacetamide) 60 minutes before the start of the 5 minute test period; or Example 34 (0.3, 1, 3 and 10 mg/kg), fluoxetine (10 mg/kg) or vehicle (1 ml/kg of a 100% solution of dimethylacetamide) 60 minutes before the start of the 5 minute test period; or Example 49 (3, 10 and 30 mg/kg), fluoxetine (10 mg/kg) or vehicle (1 ml/kg of 100% N,N-dimethylacetamide) 60 minutes before the start of the 5 minute test period; or Example 22 (3, 10 and 30 mg/kg), fluoxetine (10 mg/kg) or vehicle (1 ml/kg of 100% N,N-dimethylacetamide) 60 minutes before the start of the 5 minute test period. The compounds were dissolved in 100% N,N-dimethylacetamide. All administrations were given using 1 cc tuberculin syringes, to which a 3 inch, curved, stainless steel gavage needle was attached. The volume of administration was 1 ml/kg.

20

The effect of 5 or 10 mg/kg of fluoxetine was utilized in the FST as a positive control.

Data Analysis

25 The forced swim test data (immobility, swimming, climbing, diving) were subjected to a randomized, one-way ANOVA and post hoc tests conducted using the Student-Newman-Keuls test. The data were analyzed using the GBSTAT program, version 6.5 (Dynamics Microsystems, Inc., Silver Spring, MD, 1997). All data are presented as means $\pm$ S.E.M.

2. Social Interaction Test (SIT)

Rats were allowed to acclimate to the animal care facility for 5 days and were housed singly for 5 days prior to testing. Animals were handled for 5 minutes per day. The design and procedure for the Social Interaction Test was carried out as previously described by Kennett, et al. (1997). On the test day, weight matched pairs of rats ($\pm$ 5%), unfamiliar to each other, were given identical treatments and returned to their home cages.

Animals were randomly divided into 5 treatment groups, with 5 pairs per group, and were given one of the following i.p. treatments: Example 92 (10, 30 or 100 mg/kg), vehicle (1 ml/kg) or chlordiazepoxide (5 mg/kg). Dosing was 1 hour prior to testing. Rats were subsequently placed in a white perspex test box or arena (54 x 37 x 26 cm), whose floor was divided up into 24 equal squares, for 15 minutes. An air conditioner was used to generate background noise and to keep the room at approximately 74°F. All sessions were videotaped using a JVC camcorder (model GR-SZ1, Elmwood Park, NJ) with either TDK (HG ultimate brand) or Sony 30 minute videocassettes. All sessions were conducted between 1:00 - 4:30 P.M. Active social interaction, defined as grooming, sniffing, biting, boxing, wrestling, following and crawling over or under, was scored using a stopwatch (Sportsline model no. 226, 1/100 sec. discriminability). The number of episodes of rearing (animal completely raises up its body on its hind limbs), grooming (licking, biting, scratching of body), and face washing (i.e. hands are moved repeatedly over face), and number of squares crossed were scored. Passive social interaction (animals are lying beside or on top of each other) was not scored.

All behaviors were assessed later by an observer who was blind as to the treatment of each pair. At the end of each test, the box was thoroughly wiped with moistened paper towels.

5

Animals

Male albino Sprague-Dawley rats (Taconic Farms, NY) were housed in pairs under a 12 hr light dark cycle (lights on at 0700 hrs.) with free access to food and water.

10

Drug Administration

Example 92 was dissolved in 100% DMSO (Sigma Chemical Co., St. Louis, MO). Chlordiazepoxide (purchased from Sigma Chemical Co., St. Louis, MO) was dissolved in double distilled water. The vehicle consisted of 50% DMSO (v/v). All drug solutions were made up 10 minutes prior to injection and the solutions were discarded.

15

Data Analysis

The social interaction data (time interacting, rearing and squares crossed) were subjected to a randomized, one-way ANOVA and post hoc tests conducted using the Student-Newman-Keuls test. The data were subjected to a test of normality (Shapiro-Wilk test). The data were analyzed using the GBSTAT program, version 6.5 (Dynamics Microsystems, Inc., Silver Spring, MD, 1997). All data are presented as means $\pm$ S.E.M.

25

30 B. Results

1. Forced Swim Test

A. The Effect Of Vehicle, Fluoxetine and Example 92 On Immobility, Climbing and Swimming In The Forced Swim Test

5 Immobility

Statistical analysis indicated that there was a significant drug effect [$F(4,45) = 12.1$, $p < 0.0001$] on immobility. Subsequent post hoc analysis revealed that a single injection of 10 mg/kg i.p. of fluoxetine significantly decreased immobility to 21.0 ± 0.9 (Student-Newman-Keuls value was 36.5, $p < 0.01$) compared to vehicle-treated controls (Table 5 and Figure 1). In addition, a single injection of either 3 or 10 mg/kg i.p. of Example 92 significantly decreased immobility (24 ± 1.1 & 24 ± 0.8 counts at each dose, respectively) compared to vehicle-treated controls 30 ± 1.2 (Student-Newman-Keuls values of 16.8 and 15.7, respectively) (Table 5 and Figure 1). No significant effects on immobility were observed with Example 92 at 30 mg/kg i.p. (Table 5 and Figure 1).

Climbing

The statistical analysis of the climbing counts indicated that there was a significant drug effect [$F(4,45) = 4.4$, $p = 0.004$]. Post hoc analysis indicated that a single injection of 10 mg/kg of fluoxetine did not significantly alter climbing counts compared to vehicle-treated animals (Table 5 and Figure 2). In contrast, a single injection of 10 mg/kg of Example 92 produced a significant increase (16.8 ± 0.6) in climbing counts (Student-Newman-Keuls value = 11.6, $p < 0.01$) compared to vehicle-treated

animals (12 ± 0.8). Example 92 dosed at 1, 3 & 30 mg/kg did not significantly alter climbing.

Swimming

5 The statistical analysis of the swimming data indicated that there was a significant drug effect [$F(4,45) = 6.6$, $p < 0.0001$] (Table 5 and Figure 3). The post hoc test showed that a single injection of 10 mg/kg i.p. of fluoxetine produced a significant increase (25 ± 1.2) in
10 swimming counts over the vehicle treated animals, 18 ± 1 (Student-Newman-Keuls value of 19.9, $p < 0.01$). In contrast, a single injection of 1, 3 or 10 mg/kg i.p. of Example 92 did not significantly alter swimming counts 20 ± 1.1 , 21 ± 0.9 , & 18 ± 0.9 , respectively (Table 5 and
15 Figure 3). (However, at 30 mg/kg i.p. Example 92 significantly increased swim behavior in the rat, comparable to fluoxetine at 10 mg/kg i.p. (27 ± 2.5 vs. 25 ± 1.2 , Table 5 and Figure 3).

20 Diving

This behavior was rarely observed following a single injection of vehicle (0.1 ± 0.1 , one animal dove once), fluoxetine (0.1 ± 0.1 , one animal out of 10 dove once), 1 mg/kg of Example 92 (0.6 ± 0.2 ; 5 animals had counts of
25 2, 1, 1, 1, and 1), 3 mg/kg of Example 92 (0.6 ± 0.3 ; 3 animals had counts of 3, 2 and 1) or 10 mg/kg of Example 92 (0.5 ± 0.5 ; note: only one animal at this dose showed diving behavior and the score was 5). At 30 mg/kg i.p. of Example 92 diving behavior was only observed in two
30 animals (mean = 0.2 ± 0.2). Thus there was no significant drug effect on diving [$F(4,45) = 0.77$, $p = 0.55$].

Table 5. The effect of a single injection of vehicle, fluoxetine and Example 92 on immobility, climbing and swimming in the rat Forced Swim Test.

5	Treatment	Dose (mg/kg)	Immobility	Climbing	Swimming
	Vehicle	-----	30 ± 1.2	12.0 ± 0.8	18 ± 1
	Fluoxetine	10	21 ± 0.9^a	14.3 ± 0.9	25 ± 1.2^b
	Example 92	1	28 ± 1.0	11.7 ± 1.1	20 ± 1.1
	Example 92	3	24 ± 1.1^a	14.6 ± 1.5	21 ± 0.9
	Example 92	10	24 ± 0.8^a	16.8 ± 0.6^c	18 ± 0.9
	Example 92	30	25 ± 3.5	8.6 ± 1.7	27 ± 2.5^d

Each value represents the mean number of counts per 5 seconds $\pm$ S.E.M in a 5 minute observation period.

^a Significantly less than Vehicle on immobility scores, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

^b Significantly greater than Vehicle and 1, 3 & 10 of Example 92, on swim scores, $p < 0.01$, ANOVA and Student-Newman-Keuls.

^c Significantly greater than vehicle and 1, 3 & 30 mg/kg dose of Example 92 on climbing scores, $p < 0.01$, ANOVA and Student-Newman-Keuls

^d Significantly greater than Vehicle, 1, 3 and 10 mg/kg i.p. of Example 92 on swim scores, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

The results of the Forced Swim Test indicate that using a modified version of the Lucki forced swim test, a single injection of 10 mg/kg i.p. of fluoxetine produced a significant decrease in immobility and an increase in swimming in male Sprague-Dawley rats. This is consistent with findings from previous studies using the Lucki version (Detke, et al., 1995; Kirby and Lucki, 1997; Lucki, 1997; Page, et al., 1999; Reneric and Lucki, 1998). In addition, the results obtained using fluoxetine are consistent with those using other SSRIs (Detke, et al., 1995). Thus, a modified version of the Lucki forced swim test can consistently detect the antidepressant action of SSRIs such as fluoxetine.

Interestingly, at doses of 3 and 10 mg/kg i.p., Example 92, significantly decreased immobility compared to vehicle-treated animals. The magnitude of the decrease was not significantly different than that of fluoxetine. Thus, based on past interpretations of the Forced Swim Test, our results suggest that Example 92 has antidepressant-like properties.

A single injection of either 1, 3 or 10 mg/kg i.p. of Example 92 did not significantly alter swimming behavior. This is in contrast to the results obtained with fluoxetine, which increased swimming at 10 mg/kg i.p. Previously, it has been reported that compounds which selectively block serotonin uptake significantly increase swimming but not climbing whereas selective NE uptake blockers significantly increase climbing but not swimming behavior (Reneric and Lucki, 1998). Thus, the present

findings suggest that Example 92 exhibits a profile similar to NE and selective serotonin reuptake inhibitors (SSRIs) depending on the dose tested.

5 Finally, as previously reported by Lucki, diving behavior was rarely observed in vehicle or fluoxetine-treated animals (1 dive in one rat for each group). Example 92 at all doses tested did not produce a significant effect on diving behavior. It is possible that antidepressant
10 drugs do not induce diving behavior.

In conclusion, compared to vehicle-treated animals, Example 92, at doses of 3 and 10 mg/kg, produced a significant decrease in immobility and a significant
15 increase in climbing at the 10 mg/kg dose. At 30mg/kg i.p. Example 92 produced a significant increase in swimming behavior comparable with that observed with the antidepressant fluoxetine, thus supporting the antidepressant-like profile of Example 92.

20

B. The Effect of a Single P.O. Administration of Example 103, Fluoxetine and Vehicle on Swimming, Immobility, Climbing and Diving in the Forced Swim Test

25

Immobility

Statistical analysis indicated a significant effect of treatment on immobility (ANOVA, $F(4,40) = 6.3$, $p = 0.0005$). Post hoc analyses revealed that a single p.o.
30 administration of 10 mg/kg of fluoxetine significantly decreased immobility (Student-Newman-Keuls value of 8.3) compared to vehicle-treated animals (Table 5b). The

decrease in immobility produced by fluoxetine was significantly greater than that of either 3 or 10 mg/kg p.o. of Example 103 (Student-Newman-Keuls values of 9.1 and 6.1, respectively).

5

A single p.o. administration of either 3 or 10 mg/kg of Example 103 did not significantly alter immobility compared to vehicle-treated animals. However, the 30 mg/kg dose of Example 103 produced a significant decrease
10 in immobility (Student-Newman-Keuls values of 13.9) compared to vehicle-treated animals. In addition, the decrease in immobility produced by 30 mg/kg of Example 103 was significantly greater than that of 3 and 10 mg/kg of Example 103 (Student-Newman-Keuls values of 14.4 and
15 10.6, respectively). There was no significant difference between fluoxetine and 30 mg/kg of Example 103 in the reduction of immobility.

Swimming

20 Statistical analysis indicated a significant treatment effect on swimming behavior (ANOVA, $F(4,40) = 9.2$, $p < 0.0001$). Post hoc analyses revealed that a single p.o. administration of 10 mg/kg of fluoxetine produced a significant increase in swimming behavior compared to
25 animals treated with vehicle, 3 or 10 mg/kg p.o. of Example 103 (Student-Newman-Keuls values of 14.9, 15.3 and 11.6, respectively) (Table 5b).

A single p.o. administration of either 3 or 10 mg/kg of
30 Example 103 did not significantly alter swimming behavior compared to vehicle-treated animals. A single p.o. administration of 30 mg/kg of Example 103 produced a

significantly greater increase in swimming behavior compared to animals treated with either vehicle, 3 or 10 mg/kg of Example 103 (Student-Newman-Keuls values of 18, 18.6 and 14.5 respectively).

5

Climbing behavior

Statistical analysis revealed that diving was not significantly altered by a single p.o. administration of 3, 10 or 30 mg/kg of Example 103 or 10 mg/kg of
10 fluoxetine compared to vehicle-treated animals (ANOVA, $F(4,40) = 1.2$, $p = 0.31$) (Table 5b).

Diving

Statistical analysis revealed that diving was not
15 significantly altered by a single p.o. administration of 3, 10 or 30 mg/kg of Example 103 or 10 mg/kg of fluoxetine compared to vehicle-treated animals (ANOVA, $F(4,40) = 1.1$, $p = 0.36$) (Table 5b).

20

TABLE 5b. The effect of a single p.o. administration of vehicle, 10 mg/kg of fluoxetine and 3, 10 or 30 mg/kg of Example 103 on immobility, climbing, diving and swimming in the forced swim test in male Sprague-Dawley rats.

5

<u>Treatment</u>	<u>Immobility</u>	<u>Climbing</u>	<u>Swimming</u>	<u>Diving</u>
Vehicle	44 ± 1.7	2.9 ± 0.7	13.1 ± 1.2	0.4 ± 0.2
3 mg/kg EX103	44 ± 2.7	2.8 ± 0.6	13.2 ± 1.9	0.5 ± 0.4
10 mg/kg EX103	42 ± 2.2	3.5 ± 0.6	14.3 ± 1.6	0.4 ± 0.2
30mg/kg EX103	32 ± 1.8 ^a	4.8 ± 0.7	22.7 ± 1.1 ^c	1.1 ± 0.5
10 mg/kg Fluox	34 ± 2.3 ^b	3.8 ± 0.8	21.8 ± 1.4 ^c	0.1 ± 0.1

Each value represents the mean ± S.E.M. A total of 8-10 animals were examined for each treatment group. Fluox = 10 Fluoxetine, EX103 = Example 103. Experiments were conducted 1 hr. after the appropriate treatment.

^aSignificantly less than Vehicle, 3 and 10 mg/kg of Example 103, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

^bSignificantly less than Vehicle, 3 and 10 mg/kg of Example 103, $p < 0.05$, ANOVA and Student-Newman-Keuls test.

20

^cSignificantly greater than Vehicle, 3 and 10 mg/kg of Example 103, $P < 0.01$, ANOVA and Student-Newman-Keuls test.

5 The results of this study indicated that as previously reported, a single p.o. administration of 10 mg/kg of fluoxetine produced a significant increase in swimming and a significant decrease in immobility in male rats in the FST compared to vehicle-treated animals. The
10 magnitude of these changes are similar to those reported of our past studies with 10 mg/kg p.o. of fluoxetine. In contrast, neither climbing nor diving behavior was significantly altered by a single p.o. administration of 10 mg/kg of fluoxetine.

15

A single p.o. administration of either 3 or 10 mg/kg of Example 103 did not significantly alter swimming, climbing, immobility or diving in male rats in the FST, indicating that at these doses using the p.o. route,
20 Example 103 does not exhibit antidepressant action in the FST. In contrast, a single p.o. administration of 30 mg/kg of Example 103 produced a significant increase in swimming and a significant decrease in immobility compared to animals treated with vehicle or 10 mg/kg of
25 Example 103. However, the 30 mg/kg p.o. dose of Example 103 did not significantly alter diving or climbing counts compared to vehicle-treated animals. The increase in swimming counts produced by 30 mg/kg p.o. of Example 103 was comparable to that for 10 mg/kg of fluoxetine.

30

In conclusion, a single p.o. administration of 30 mg/kg of Example 103 (one hour before the last swim test)

increases swimming and decreases immobility counts in the FST, suggesting that Example 103 has antidepressant properties.

- 5 C. Effect of a single p.o. administration of Example 272, fluoxetine and vehicle on swimming, climbing, immobility and diving in the forced swim test

Immobility

- 10 Statistical analysis indicated a significant effect of treatment on immobility (ANOVA, $F(2,27) = 5.2$, $p = 0.0126$). Post hoc analyses revealed that a single p.o. administration of 10 mg/kg of fluoxetine and 3 mg/kg of Example 272 significantly decreased immobility (Student-
15 Newman-Keuls values of 5.4 and 9.8, respectively) compared to vehicle-treated animals (Table 5c). There was no significant difference between fluoxetine and 3 mg/kg of Example 272 in the reduction of immobility (Student-Newman-Keuls value of 0.53).

20

Swimming

- Statistical analysis indicated a significant treatment effect on swimming behavior (ANOVA, $F(2,27) = 9.9$, $p < 0.0007$). Post hoc analyses revealed that a single p.o.
25 administration of 10 mg/kg of fluoxetine and Example 272 produced a significant increase in swimming behavior compared to animals treated with vehicle (Student-Newman-Keuls values of 11.9 and 17.5, respectively) (Table 5c). There was no significant difference in the
30 increase in swimming between 10 mg/kg of fluoxetine and 3 mg/kg of Example 272 (Student-Newman-Keuls value of 0.42).

Climbing behavior

Statistical analysis revealed that diving was not significantly altered by a single p.o. administration of either 3 mg/kg of Example 272 or 10 mg/kg of fluoxetine compared to vehicle-treated animals (ANOVA, $F(2,27) = 1.8$, $p = 0.19$) (Table 5c).

Diving

Statistical analysis revealed that diving was not significantly altered by a single p.o. administration of 3 mg/kg of Example 272 or 10 mg/kg of fluoxetine compared to vehicle-treated animals (ANOVA, $F(2,27) = 0.65$, $p = 0.53$) (Table 5c).

TABLE 5c. The effect of a single p.o. administration of vehicle, fluoxetine and Example 272 on immobility, climbing, diving and swimming in the forced swim test in male Sprague-Dawley rats.

5

<u>Treatment</u>	<u>Immobility</u>	<u>Climbing</u>	<u>Swimming</u>	<u>Diving</u>
Vehicle	43 $\pm$ 3.3	2.4 $\pm$ 0.4	13.4 $\pm$ 2.2	0.2 $\pm$ 0.1
3 mg/kg EX272	33 $\pm$ 1.8 ^a	3.9 $\pm$ 0.6	22.9 $\pm$ 1.3 ^b	0.6 $\pm$ 0.4
10 mg/kg FLUOX	35 $\pm$ 1.7 ^a	3.3 $\pm$ 0.6	21.4 $\pm$ 1.0 ^b	0.2 $\pm$ 0.1

Each value represents the mean $\pm$ S.E.M. A total of 9-10 animals were examined for each treatment group.

10 Abbreviations: FLUOX = Fluoxetine, EX272 = Example 272. Animals received 1 p.o. administration of the appropriate treatment 24 hours before the test day.

^aSignificantly less than Vehicle, $p < 0.05$, ANOVA and
15 Student-Newman-Keuls test.

^bSignificantly less than Vehicle, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

20 The finding of this study indicate that a single p.o. administration of 3 mg/kg of the compound Example 272 produced a significant increase in swimming and a significant decrease in immobility 24 hours after administration compared to vehicle-treated animals.
25 However, the administration of Example 272 did not

significantly alter climbing or diving compared to vehicle-treated animals. These results are similar to those of a single p.o. administration of 10 mg/kg of fluoxetine. Our finding suggest that a single p.o.
5 administration of 3 mg/kg of Example 272 has the profile of an antidepressant in male Sprague-Dawley rats in the Lucki version of the FST.

E. Effect of a single p.o. administration of Example 98,
10 fluoxetine and vehicle on swimming, climbing, immobility and diving in the forced swim test.

Immobility

Statistical analysis indicated a significant effect of
15 treatment on immobility (ANOVA, $F(4,43) = 7.5$, $p = 0.0001$). Post hoc analyses revealed that a single p.o. administration of 10 mg/kg of fluoxetine significantly decreased immobility (Student-Newman-Keuls value of 23.8) compared to vehicle-treated animals (Table 5d).

20

A single p.o. administration of 3, 10 or 30 mg/kg of Example 98 significantly decreased immobility compared to vehicle-treated animals (Student-Newman-Keuls values of 19.3, 9.7 and 13.7, respectively). There was no
25 significant difference between fluoxetine and 3, 10 or 30 mg/kg of Example 98 in the magnitude of the reduction of immobility. There were no significant differences between the doses of Example 98 regarding the magnitude of the decrease in immobility.

30

Swimming

Statistical analysis indicated a significant treatment effect on swimming behavior (ANOVA, $F(4,43) = 11$, $p < 0.0001$). Post hoc analyses revealed that a single p.o. administration of 10 mg/kg of fluoxetine produced a significant increase in swimming behavior compared to vehicle-treated animals (Student-Newman-Keuls value of 35.1) (Table 5d).

A single p.o. administration of 3, 10 or 30 mg/kg of Example 98 significantly increased swimming compared to vehicle-treated animals (Student-Newman-Keuls values of 24.4, 14.7 and 25.1, respectively) (Table 5d). There was no significant difference between fluoxetine and 3, 10 or 30 mg/kg of Example 98 in the magnitude of the increase in swimming. There were no significant differences between the doses of Example 98 regarding the magnitude of the increase in immobility.

Climbing behavior

There was a significant treatment effect on climbing behavior (ANOVA, $F(4,43) = 2.8$, $p = 0.04$) (Table 5d). Post hoc tests indicated that this was the result of the 3 mg/kg dose of Example 98 producing a significantly greater increase in climbing compared to 30 mg/kg of Example 98 (Table 5d; Student-Newman-Keuls value of 8.6). There was no significant difference in the number of climbing counts between animals treated with vehicle and Example 98.

Diving

Statistical analysis revealed that diving was not

significantly altered by a single p.o. administration of 3, 10 or 30 mg/kg of Example 98 or 10 mg/kg of fluoxetine compared to vehicle-treated animals (ANOVA, $F(4,43) = 1.29$, $p = 0.29$) (Table 5d).

5

TABLE 5d. The effect of a single p.o. administration of vehicle, 10 mg/kg of fluoxetine and 3, 10 or 30 mg/kg of Example 98 on immobility, climbing, diving and swimming in the forced swim test in male Sprague-Dawley rats.

10

<u>Treatment</u>	<u>Immobility</u>	<u>Climbing</u>	<u>Swimming</u>	<u>Diving</u>
Vehicle	48 ± 1.2	2.5 ± 0.5	8.8 ± 0.9	0.4 ± 0.3
3 mg/kg EX98	35 ± 2.6 ^a	4.3 ± 0.9 ^b	20.4 ± 1.9 ^c	0.1 ± 0.1
10 mg/kg EX98	39 ± 1.1 ^a	2.4 ± 0.3	17.6 ± 1.0 ^c	0.8 ± 0.4
30 mg/kg EX98	38 ± 2.3 ^a	2.0 ± 0.3	20.3 ± 2.1 ^c	0.2 ± 0.2
10 mg/kg Fluox	34 ± 3.0 ^a	3.4 ± 0.8	22.8 ± 2.2 ^c	0.1 ± 0.1

Each value represents the mean ± S.E.M. A total of 10 animals were examined for each treatment group, except for the fluoxetine and 3 mg/kg groups, where a total of 9 animals were examined. Vehicle = 100% DMA.

Fluox = Fluoxetine, EX98 = Example 98. Experiments were conducted 1 hr. after the appropriate treatment.

20

^aSignificantly less than Vehicle, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

^bSignificantly greater than 30 mg/kg of Example 98, $p < 0.05$, ANOVA and Student-Newman-Keuls test.

^cSignificantly greater than Vehicle, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

10 The results of this study clearly indicate that in male Sprague-Dawley rats, a single p.o. administration of 3, 10 or 30 mg/kg of Example 98 produces a significant increase in swimming and a significant decrease in immobility compared to vehicle-treated animals in the
15 FST. In addition, the Example 98 induced alterations were similar in magnitude to that of a single p.o. administration of 10 mg/kg p.o. of fluoxetine. However, neither fluoxetine nor Example 98 produced a significant alteration in climbing or diving compared to vehicle-
20 treated animals.

In conclusion, these results indicate that a single p.o. administration of Example 98 produces a profile in the modified Lucki version of the FST resembling that of the
25 clinically established antidepressant fluoxetine.

I. Effect of a single p.o. administration of Example 95, fluoxetine and vehicle on swimming, climbing, immobility
30 and diving in the forced swim test

Statistical analysis indicated that a single . p.o. administration of 10 or 30 mg/kg Example 95 significantly increased rat immobility and significantly decreased swim behavior in the rat forced swim test at both doses (Table 5 5h, $p < 0.01$, ANOVA and Student-Newman-Keuls, respectively).

TABLE 5h. The effect of a single p.o. administration of vehicle, 10 mg/kg of fluoxetine and 3, 10 or 30 mg/kg of Example 95 on immobility, climbing, diving and swimming in the forced swim test in male Sprague-Dawley rats.

5

Treatment	Immobility	Climbing	Swimming	Diving
Vehicle	42 ± 1.7	2.3 ± 0.5	14.7 ± 1.0	0.1 ± 0.1
3 mg/kg EX95	40 ± 3.3	2.6 ± 0.8	17.1 ± 2.5	0.0 ± 0.0
10 mg/kg EX95	52 ± 1.2 ^a	1.3 ± 0.5	6.9 ± 0.9 ^b	0.1 ± 0.1
30mg/kg EX95	54 ± 0.9 ^a	1.0 ± 0.3	4.8 ± 0.7 ^b	0.0 ± 0.0
10 mg/kg Fluox	38 ± 2.2	1.9 ± 0.6	20.0 ± 1.5 ^c	0.1 ± 0.1

Each value represents the mean ± S.E.M. A total of 8 animals were examined for each treatment group, except for the vehicle, where a total of 10 animals were examined. Fluox = Fluoxetine; EX95 = Example 95. Experiments were conducted 1 hr. after the appropriate treatment.

^aSignificantly less than Vehicle, 3 mg/kg of Example 95 and 10 mg/kg of fluoxetine, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

^bSignificantly less than Vehicle, 3 mg/kg of Example 95 and 10 mg/kg of fluoxetine, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

^cSignificantly greater than Vehicle ($p < 0.05$), 10 and 30 mg/kg of Example 95 ($p < 0.01$), ANOVA and Student-Newman-Keuls test.

5

A single p.o. administration of 10 mg/kg of fluoxetine produced a significant increase in swimming behavior compared to vehicle-treated animals. In addition, fluoxetine significantly decreased immobility compared to vehicle-treated animals. A single p.o. administration of 10 3 mg/kg of Example 95 did not significantly alter swimming, climbing, immobility or diving behavior compared to vehicle-treated animals. In contrast, a single p.o. administration of either 10 or 30 mg/kg of 15 Example 95 produced a significant increase in immobility and a significant decrease in swimming behavior compared to vehicle-treated animals. There was no significant difference in the magnitude of change in swimming and immobility between the 10 and 30 mg/kg doses of Example 20 95.

These data indicate that at a doses of 10 and 30 mg/kg p.o., Example 95 produced effects opposite of that seen with antidepressants in the rat forced swim test, 25 suggesting that Example 95 does not produce antidepressant-like actions in the forced swim test in male Sprague-Dawley rats.

F. Effect of a single p.o. administration of Example 98, 30 fluoxetine and vehicle on swimming, climbing, immobility and diving in the forced swim test. See Table 7 for data.

TABLE 7. The effect of a single p.o. administration of vehicle, 10 mg/kg of fluoxetine or 1, 3 or 10 mg/kg of Example 310 on immobility, climbing, diving and swimming in the forced swim test in male Sprague-Dawley rats.

5

p.o. Treatment	Immobility	Climbing	Swimming	Diving
Vehicle	46 ± 2.7	3.8 ± 1.3	11.0 ± 1.8	0.0 ± 0.0
1 mg/kg Example 310	32 ± 2.4^a	6.2 ± 1.0	21.3 ± 1.6^b	0.1 ± 0.1
3 mg/kg Example 310	33 ± 2.0^a	5.0 ± 0.9	21.0 ± 1.3^b	0.8 ± 0.6
10 mg/kg Example 310	36 ± 1.7^a	4.4 ± 0.7	19.6 ± 1.2^b	0.0 ± 0.0
10 mg/kg Fluox	36 ± 1.8^a	4.2 ± 0.8	19.9 ± 1.1^b	0.1 ± 0.1

Each value represents the mean $\pm$ S.E.M. A total of 8-10
 10 animals were examined for each treatment group. Fluox =
 Fluoxetine. Experiments were conducted 2 hrs. after the
 appropriate treatment. Vehicle = 100% DMA.

^aSignificantly less than Vehicle, $p < 0.01$, ANOVA and
 15 Student-Newman-Keuls test.

^bSignificantly greater than Vehicle, $p < 0.01$, ANOVA and
 Student-Newman-Keuls test.

20

2. Social Interaction Test

A. The Effect Of Example 92 And Chlordiazepoxide On Behavior In The Rat Social Interaction Test

5 A single i.p. administration of either 10 or 30 mg/kg of Example 92 significantly increased social interaction (Table 6 and Figure 4), as did the benzodiazepine anxiolytic, chlordiazepoxide (Student-Newman-Keuls value of 31.3) compared to vehicle-treated animals [ANOVA, 10 $F(4,45) = 10.3$, $p < 0.0001$; Student-Newman-Keuls values for the 10 and 30 mg/kg doses were 8.61 and 19.55, respectively]. However, the 100 mg/kg i.p. dose of Example 92 did not significantly alter social interaction time compared to vehicle-treated animals (Table 6 and 15 Figure 4). The degree of social interaction was greater in the chlordiazepoxide-treated animals compared to those that received either the 10 or 30 mg/kg doses of Example 92.

Table 6. The Effect Of A Single Injection Of Vehicle, Chlordiazepoxide And Example 92 On The Social Interaction And Rearing Of Unfamiliar Cage Mates In A Familiar Arena

5

Drug Social Treatment (i.p.) Interaction (sec)^a

Vehicle, 1 ml/kg	96 ± 12
Chlordiazepoxide, 5 mg/kg	188 ± 15 ^b
Example 92, 10 mg/kg	144 ± 12 ^b
Example 92, 30 mg/kg	169 ± 13 ^c
Example 92, 100 mg/kg	117 ± 6 ^d

^a Each value represents the mean seconds of social interaction ± S.E.M.

^b Significantly greater than Vehicle, $p < 0.05$, ANOVA and Student-Newman-Keuls test.

15 ^c Significantly greater than Vehicle, $p < 0.01$, ANOVA and Student-Newman-Keuls test.

20 ^d Significantly less than 30 mg/kg dose and chlordiazepoxide, $p < 0.01$, ANOVA and Student-Newman-Keuls.

B. The Effect Of Example 92 And Chlordiazepoxide On Rearing Behavior, Locomotor Activity And Grooming In The Rat Social Interaction Test

5 The administration of 10 and 30 mg/kg, but not 100 mg/kg of Example 92, significantly increased rearing behavior compared to either vehicle or chlordiazepoxide [ANOVA, $F(4,45) = 2.6$, $p = 0.046$; See Table 13]. In addition, the number of rearings at the 10 mg/kg dose of Example 92
10 was significantly greater than that produced by chlordiazepoxide (Table 13).

The administration of either Example 92 or chlordiazepoxide did not significantly alter the number
15 of grooming bouts compared to vehicle-treated animals [$F(4,45) = .67$, $p = 0.62$].

A single injection of either 10 or 30 mg/kg i.p. of Example 92 or 5 mg/kg i.p. of chlordiazepoxide did not
20 significantly alter the number of squares crossed (Table 13). However, the number of squares crossed following the 100 mg/kg dose of Example 92 was significantly lower than animals treated with either vehicle, 10 mg/kg i.p. of Example 92, 30 mg/kg i.p. of Example 92 or 5 mg/kg
25 i.p. of chlordiazepoxide. [ANOVA, $F(4,43) = 6.94$, $p = 0.0002$]

Table 13. The Effect of a Single Injection of Vehicle, Chlordiazepoxide and Example 92 on the Number of Rearings, Squares Crossed and Grooming Bouts in the Rat Social Interaction Test.

Drug Treatment (i.p.)	Rearings	Squares Crossed	Grooming Bouts
Vehicle, 1 ml/kg	33 $\pm$ 4	393 $\pm$ 26	5.1 $\pm$ 1.1
Chlordiazepoxide, 5 mg/kg	30 $\pm$ 2	287 $\pm$ 28	7.3 $\pm$ 1.3
Example 92, 10 mg/kg	47 $\pm$ 8 ^a	298 $\pm$ 40	6.1 $\pm$ 0.5
Example 92, 30 mg/kg	45 $\pm$ 5 ^b	368 $\pm$ 36	6.2 $\pm$ 0.7
Example 92, 100 mg/kg	31 $\pm$ 4	195 $\pm$ 19 ^c	6.8 $\pm$ 1.3

All values represent the mean $\pm$ S.E.M.

^a Significantly greater than chlordiazepoxide, $p < 0.05$, ANOVA and Student-Newman-Keuls test

^b Significantly greater than vehicle and chlordiazepoxide, $p < 0.05$, ANOVA and Student-Newman-Keuls test.

^c Significantly less than 10 & 30 mg/kg of Example 92 ($p < 0.01$), vehicle ($p < 0.01$) and chlordiazepoxide ($p < 0.05$), ANOVA and Student-Newman-Keuls test.

At doses of 10 and 30 mg/kg i.p., Example 92 produced a significant increase in social interaction time in male

rats compared to vehicle-treated animals. Also, the anxiolytic agent (5 mg/kg i.p. chlordiazepoxide) produced a significant increase in social interaction time compared to vehicle-treated animals. The response produced by the 30 mg/kg dose of Example 92 was comparable to that of the positive control, chlordiazepoxide. The 30 mg/kg dose of Example 92 produced a significant increase in rearing compared to vehicle- and chlordiazepoxide-treated animals. Previously, it has been shown that in the Social Interaction Test, psychostimulants such as amphetamine and caffeine, increase social interaction and locomotor activity, whereas anxiolytics increase social interaction time. (File, 1985; File and Hyde, 1979; Guy and Gardner, 1985). Consistent with an increase in social interaction, Example 92 increased rearing behavior. However, it did not produce an increase in horizontal locomotor activity or grooming bouts. In addition, Example 92 did not elicit stereotypes or produce aggressive behaviors. In fact, locomotor activity as measured by squares crossed was significantly reduced at the 100 mg/kg i.p. dose of Example 92 compared to vehicle-treated animals. This decrease in locomotor activity did not appear to be accompanied by ataxia or sedation. Thus, it is unlikely that Example 92 is producing a non-specific effect on social interaction through motor stimulation. In order to justify this claim, in another study (not reported), the effect of Example 92 was dosed to familiar cage mates in the social interaction test and no significant increase in interaction in this variation of the Social Interaction Test was observed. In this test, the

anxiogenic stimulus of a novel partner is removed and therefore only locomotor activity and normal behavior are observed (Guy and Gardner, 1985). In conclusion, the results of this study indicate that Example 92, at doses of 10 and 30 mg/kg i.p., significantly increases social interaction time without producing an increase in horizontal locomotor activity or grooming bouts. Furthermore, the effect produced by the 30 mg/kg of Example 92 was comparable to that observed for 5 mg/kg of chlordiazepoxide, the active control. No increase in social interaction was observed at the 100 mg/kg dose of Example 92. However, a decrease in the number of squares crossed was observed. In summary, Example 92 has the profile of an anxiolytic drug in the Social Interaction Test.

II. Binding Properties of Compounds at Cloned Receptors

A. Materials and Methods

The binding properties of the compounds of the present invention were evaluated at one or more cloned receptors or native, tissue-derived transporters, using protocols described below.

Cell Culture

COS-7 cells were grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin) at 37°C with 5% CO₂. Stock plates of COS-7 cells were trypsinized and split 1:6 every 3-4 days. Human embryonic kidney 293 cells were grown on 150 mm plates in D-MEM with supplements (minimal essential medium) with Hanks' salts and

supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin, 100 µg /ml streptomycin) at 37°C with 5% CO₂. Stock plates of 293 cells were trypsinized and split 1:6 every 3-4 days. Mouse fibroblast LM(tk-) cells were grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin) at 37°C with 5% CO₂. Stock plates of LM(tk-) cells were trypsinized and split 1:10 every 3-4 days. Chinese Hamster Ovary (CHO) cells were grown on 150 mm plates in HAM's F12 medium with (HAM's F-12 with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin) at 37°C with 5% CO₂. Stock plates of CHO cells were trypsinized and split 1:8 every 3-4 days.

LM(tk-) cells were stably transfected with the human GAL1 or GALR3 receptor. CHO cells were stably transfected with the human GAL2 receptor.

Stable Transfection

cDNAs for the human and rat GAL1, and human and rat GALR3 receptors were transfected with a G-418 resistant gene into the mouse fibroblast LM(tk-) cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells were selected with G-418. Human and rat GAL2 receptors were similarly transfected into CHO cells.

Membrane Harvest

Membranes were harvested from stably transfected LM(tk-

) cells. Adherent cells were washed twice in ice-cold phosphate buffered saline (138 mM NaCl, 8.1 mM Na₂HPO₄, 2.5 mM KCl, 1.2 mM KH₂PO₄, 0.9 mM CaCl₂, 0.5 mM MgCl₂, pH 7.4) and lysed by sonication in ice-cold sonication buffer (20 mM Tris-HCl, 5 mM EDTA, pH 7.7). Large particles and debris were cleared by low speed centrifugation (200 x g, 5 min, 4°C). Membranes were collected from the supernatant fraction by centrifugation (32,000 x g, 18 min, 4°C), washed with ice-cold hypotonic buffer, and collected again by centrifugation (32,000 x g, 18 min, 4°C). The final membrane pellet was resuspended by sonication into a small volume of ice-cold binding buffer (~1 ml for every 5 plates: 10 mM NaCl, 20 mM HEPES, 0.22 mM KH₂PO₄, 1.26 mM CaCl₂, 0.81 mM MgSO₄, pH 7.4). Protein concentration was measured by the Bradford method (Bradford, 1976) using Bio-Rad Reagent, with bovine serum albumin as a standard. Membranes were held on ice for up to one hour and used fresh, or flash frozen and stored in liquid nitrogen. Membranes were prepared similarly from CHO cells.

As described in the Background of the Invention, compounds that block the effects of galanin on the GALR3 receptor subtype can potentially be used for the treatment of depression and anxiety. Biogenic amine transmitter molecules that mediate neuronal signals are currently known in the art and include among others serotonin (5HT), norepinephrine (NE), and dopamine (DA). Recent advances in the molecular studies of the mechanisms for these transmitter molecules, together with the characterization of their pharmacological

properties, has enabled the identification of numerous potential targets for therapeutic intervention. Inhibitors of the 5HT, NE and DA transporter systems, and inhibitors of the enzyme, monoamine oxidase, have been widely studied and are known to enhance the action of biogenic amine neurotransmitters. The resultant clinically effective antidepressant drugs are known today as TCAs, SSRIs and MAOIs. (Tatsumi et al., 1997; Iversen, 2000).

In the case of galanin, the evidence presented in this invention suggests that GPCR-targeted molecules that bind to and antagonize the GALR3 receptor may be used for the treatment of depression and/or anxiety disorders. Another approach could involve the administration of an antagonist of the GALR3 receptor, such as those described herein, which also possesses 5HT₄ receptor antagonist properties (Kennett et al., 1997). A further approach could involve the administration of a GALR3 antagonist, such as those described herein, which also possesses 5HT_{1A} receptor binding properties (Razani et al., 1997). However, in any case the GALR3 antagonist(s) should be free of activity at the human GAL1 receptor and the 5HT, NE and DA transporters. Furthermore, the GALR3 antagonist(s) should not inhibit the enzymatic activity of monoamine oxidase A (MAO_A) or monoamine oxidase B (MAO_B) present in the brain (i.e. central MAO). The design of such compounds can be optimized by determining their binding affinity at the recombinant GALR3, GAL1, 5HT₄, and 5HT_{1A} receptors; and the native 5HT, NE and DA transporters. The design of such compounds can be further optimized

by determining their interaction with central MAO_A and central MAO_B.

5 Additionally, the GALR3 antagonist(s) would optimally not bind at the following receptors due to possible side effects: human GAL2; human H₁ histamine; human α_{1A} adrenergic, human α_{1B} adrenergic, human α_{1D} adrenergic, human α_{2A} adrenergic, human α_{2B} adrenergic, and human α_{2C} adrenergic; human dopamine D₁, D₂, D₃, D₄, and D₅; and
10 the human 5HT_{1B}, human 5HT_{1D}, human 5HT_{1E}, human 5HT_{1F}, human 5HT_{2A}, rat 5HT_{2C}, human 5HT₆, and human 5HT₇ receptors.

Radioligand Binding Assays and Enzymatic Assays

15 The methods to obtain the cDNA of the receptors, express said receptors in heterologous systems, and carry out assays to determine binding affinity are described as follows.

20 Galanin Receptors: Binding assays were performed according to the following published methods: human GALR3 (PCT International Publication No. WO 98/15570), human GAL1 (PCT International Publication No. WO 95/2260), human GAL2 (PCT International Publication No.
25 WO 97/26853).

Human 5HT_{1B}, 5HT_{1D}, 5HT_{1E}, 5HT_{1F}, and 5HT₇ Receptors: The cell lysates of LM(tk-) clonal cell line stably transfected with the genes encoding each of these 5HT
30 receptor-subtypes were prepared as described above. Cell membranes were suspended in 50mM Tris-HCl buffer (pH 7.4 at 37°C) containing 10 mM MgCl₂, 0.2 mM EDTA, 10

M pargyline, and 0.1% ascorbate. The affinities of compounds were determined in equilibrium competition binding assays by incubation for 30 minutes at 37 °C in the presence of 5 nM [³H]-serotonin. Nonspecific binding was determined in the presence of 10 μM serotonin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human 5HT_{2A} Receptor: The coding sequence of the human 5HT_{2A} receptor was obtained from a human brain cortex cDNA library, and cloned into the cloning site of pCEXV-3 eukaryotic expression vector. This construct was transfected into COS-7 cells by the DEAE-dextran method (Cullen, 1987). Cells were harvested after 72 hours and lysed by sonication in 5 mM Tris-HCl, 5 mM EDTA, pH 7.5. The cell lysates were subjected to centrifugation at 1000 rpm for 5 minutes at 4°C, and the supernatant was subjected to centrifugation at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50 mM Tris-HCl buffer (pH 7.7 at room temperature) containing 10 mM MgSO₄, 0.5 mM EDTA, and 0.1% ascorbate. The affinity of compounds at 5HT_{2A} receptors were determined in equilibrium competition binding assays using [³H]ketanserin (1 nM). Nonspecific binding was defined by the addition of 10 μM mianserin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

5-HT_{1A} Receptor: The cDNA corresponding to the 5-HT_{1A} receptor open reading frames and variable non-coding 5'- and 3'-regions, was cloned into the eukaryotic expression vector pCEXV-3. These constructs were

transfected transiently into COS-7 cells by the DEAE-dextran method (Cullen, 1987), and harvested after 72 hours. Radioligand binding assays were performed as described above for the 5-HT_{2A} receptor, except that
5 ³H-8-OH-DPAT was used as the radioligand and nonspecific binding was determined by the addition of 10 μM mianserin.

Other 5-HT Receptors: Other serotonin receptor binding
10 assays were performed according to published methods: rat 5HT_{2C} receptor (Julius et al., 1988); and 5-HT₆
(Monsma, et al., 1993). The binding assays using the
5-HT₄ receptor were performed according to the procedures described in U.S. Patent No. 5,766,879, the
15 disclosure of which is hereby incorporated by reference in its entirety into this application.

Other receptors: Cell membranes expressing human
20 dopamine D₁, D₂, D₄ and rat D₃ receptors were purchased through BioSignal, Inc. (Montreal, Canada). Binding assays using the histamine H₁ receptor; dopamine receptors; and α_{1A}, α_{1B}, and α₂ adrenergic receptors may be carried out according to the procedures described in
25 U.S. Patent No. 5,780,485, the disclosure of which is hereby incorporated by reference in its entirety into this application. Binding assays using the dopamine D₅ receptor may be carried out according to the procedures described in U.S. Patent No. 5,882,855, the disclosure
30 of which is hereby incorporated by reference in its entirety into this application. Binding assays for the human α_{1D} adrenergic receptor may be carried out

according to the procedures described in U.S. Patent No. 6,156,518, the disclosure of which is hereby incorporated by reference in its entirety into this application.

5

The methods to determine binding affinity at native transporters are described in the following publications: 5HT transporter and NE transporter (Owens et al., 1997), and DA transporter (Javitch et al, 10 1984).

The methods to determine activity at monoamine oxidase enzymes (for example, central MAO_A and MAO_B) are described by Otsuka and Kobayashi, 1964, and were 15 performed by NovaScreen (Hanover, MD) with the following modifications.

Central Monoamine Oxidase A Enzyme Assay: Rat brain was used as the enzyme source. The enzyme source was 20 pre-incubated with reference compound (RO 41-1049), test compound (Example 92), and subtype selective blocker (100nM deprenyl) for 60 minutes at 37°C in 50 mM KPO₄ containing 50 µM EDTA and 10 µM dithiothreitol (pH 7.2 at 25°C). Substrate ([¹⁴C]Serotonin, 45-60 Ci/mmol) 25 was then added and incubated for 30 minutes. The reaction was stopped by the addition of 0.5 ml of 1-2M citric acid. Radioactive product was extracted into xylene/ethyl acetate fluor and compared to control values by scintillation spectrophotometry in order to 30 ascertain any interactions of test compound with central MAO_A.

Central Monoamine Oxidase B Enzyme Assay: Rat brain was used as the enzyme source. The assay was performed as described above for central MAO_A, except the reference compound was RO 166491 and the subtype selective blocker was 100 nM clorgyline. Also, the substrate ([¹⁴C]Phenylethylamine, 0.056 Ci/mmol) was added and incubated for 10 minutes.

Materials

Cell culture media and supplements were from Specialty Media (Lavallette, NJ). Cell culture plates (150 mm and ~~96-well microtiter~~) ~~were from Corning~~ (Corning, NY). Polypropylene 96-well microtiter plates were from Costar (Cambridge, MA). Bovine serum albumin (ultra-fat free, A-7511) was from Sigma (St. Louis, MO). All radioligands were from New England Nuclear (Boston, MA). Commercially available peptides and peptide analogs were either from Bachem California (Torrance, CA) or Peninsula (Belmont, CA). All other materials were reagent grade.

Data Analysis

Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA). Enzymatic assay data were derived from a standard curve of reference compound data.

The selectivity ratios for compounds of the claimed invention were calculated from the binding data presented in Tables 1-4 of the subject application. More specifically, these ratios were calculated by

dividing (a) the binding affinity (K_i value) of said compound to a particular receptor or transporter by (b) the binding affinity (K_i value) of said compound to the human GALR3 receptor.

5

B. Results

The compounds described in the claimed invention were assayed using a panel of cloned receptors and native transporters. The preferred compounds were found to be selective GALR3 antagonists. The binding affinities and selectivity ratios of several compounds are illustrated in Tables 1-2.

10

The activity of Example 92 was determined for central MAO_A and central MAO_B using the methods described hereinabove. The results, expressed as percent inhibition, are illustrated in Table 11.

15

Table 11: Percent inhibition of Example 92 in the central monoamine oxidase enzyme assay

20

TARGET	SPECIES	% INHIBITION
Monoamine Oxidase A (central)	Rat	10
Monoamine Oxidase B (central)	Rat	1

IV. GALR3 Receptor Localization

A. Materials And Methods

5 Preparation of the anti-GALR3 Antiserum

BioSource International, Hopkinton, MA performed the immunization and maintenance of rabbits. Following a pre-immune bleed, one peptide for each GAL receptor was injected into a pair of New Zealand white rabbits. The peptide sequences was chosen based on sequence specificity and immunogenicity. The rabbit anti-GALR3 antiserum were raised against C-terminal epitopes corresponding to amino acids 357 - 370 (Genbank accession number AF073798). The peptides were conjugated to the carrier KLH (keyhole limpet hemocyanin) by a cross linker and subcutaneously injected into the rabbits. The generation of the anti-GALR3 antiserum required OVA followed by a third series of injections with the GALR3 peptide conjugated to tetanus toxoid (TTOX). All injections were done using the Freund's Adjuvant System. Once immunoreactivity was established (see below) the antiserum was affinity purified by passing it over an agarose based column thiol coupled to its antigenic peptide. The column was washed and the antiserum was eluted using a low pH glycine buffer. The purified material was dialyzed, the optical density is taken at 280 λ and the purified antiserum was frozen.

30 Characterization of the anti-GALR3 antiserum

Recombinant GAL1, GAL2, and GALR3 receptor transfected cells

To determine the ability of the GALR3 antiserum to recognize only the GALR3 receptor protein in vitro, COS-7 cells were grown on poly-L-lysine-coated plastic chamber slides (Nalge Nunc International, Naperville, IL) and transfected with recombinant rat GAL receptors (Genbank accession numbers U30290, AF010318, AF073798, respectively) or expression vector only (for mock-transfected cells) as previously described by Borowsky et al. (1999). Receptor expression was confirmed by radioligand binding. Briefly, a subset of slides was washed three times in binding buffer (50 mM Tris, pH 7.5, 5 mM MgCl₂, 1 mM EDTA, 0.1% bovine serum albumin, and 0.1% bacitracin) and incubated in 500 µl binding buffer containing porcine ¹²⁵I-galanin (625,000 dpm) plus or minus 10 µM porcine galanin. After incubation at room temperature for 1 hour, the binding buffer was aspirated and slides were rinsed three times in ice cold 50 mM Tris, pH 7.5. Cells were solubilized in 1 ml of 0.1 N NaOH and 0.05% sodium deoxycholate for 30 minutes then transferred to test tubes for gamma counting of ¹²⁵I. To evaluate antibody activity another subset of slides were washed with phosphate buffered saline (PBS) (Sigma, St. Louis, MO) to remove the medium and fixed with 4% paraformaldehyde (PFA) (Sigma, St. Louis, MO) then permeabilized using 0.2% Triton X-100/PBS and incubated in 3% normal goat serum for 30 minutes to minimize nonspecific binding of the primary antibody. Cells were incubated overnight at 4°C with the anti-GALR3 antiserum (1:1000 dilution). The cells were rinsed three times with PBS, incubated for 30

minutes at 25°C with goat anti-rabbit IgG (1:200 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA), rinsed and processed using the peroxidase-antiperoxidase (PAP) reaction of Sternberger et al. (1982). Control experiments for antibody specificity were (1) incubation of the cells in primary antiserum that had been preabsorbed with the respective antigenic peptide (20 µg/ml), (2) incubation without the primary antiserum, or (3) incubation with the primary antiserum replaced by normal goat serum.

Western Blotting

Membranes were prepared from COS-7 cells transiently transfected with the rat recombinant receptors GAL1, GAL2, and GALR3 as previously described (Borowsky et al., 1999). Transfected cells were lysed by sonication in ice-cold sonication buffer (20 mM Tris-HCl, pH 7.7, 5 mM EDTA). Cell lysates were subjected to centrifugation at 4°C for 10 minutes at 200 g. The supernatant was then fractionated by centrifugation at 4°C for 18 minutes at 32,000 g. The resulting membrane pellet was suspended into 50 mM Tris, pH 7.5, 5 mM MgCl₂, 1 mM EDTA. Protein samples (1-10 µg) were solubilized in 2 X Laemmli buffer (Bio-Rad, Hercules, CA) and fractionated by SDS-PAGE in 10% polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membranes for immunoblot analysis in ice-cold 25 mM Tris, pH 8, 192 mM glycine, 20% methanol as previously described by Harlow and Lane (1999). Blots were incubated for 1 hour at 25°C in blocking buffer composed of 5% non-fat dried milk in TTBS (0.1% Tween-20, 500 mM NaCl, 20 mM Tris, pH 7.5) then for 16 hours

at 25°C with the receptor-specific polyclonal antibody (1:1000 dilution in blocking buffer) (0.25 mg/ml for GAL2 or 1.5 mg/ml for GALR3). Immunoreactive bands were detected with the Phototope-HRP Detection Kit for Western Blotting (New England BioLab, Beverly, MA) according to the protocol. Briefly, the blots were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG then developed with a mixture of LumiGLO plus hydrogen peroxide and recorded by chemiluminescence on Kodak Biomax-ML film (Kodak, Rochester, NY).

Immunohistochemistry

Male Sprague-Dawley rats, (200-250 g; Charles Rivers, Rochester, NY) were anesthetized by intraperitoneal injection of ketamine 20 mg/kg (RBI, Natick, MA) and xylazine 0.2 mg/kg (Bayer, Shawnee Mission, KS) then transcardially perfused with 200 ml PBS, pH 7.4 followed by 200 ml 4% PFA in PBS. The brains and spinal cords were removed, blocked, and postfixed in the same fixative for 4 hours at 4°C then cryoprotected in 30% sucrose in PBS at 4°C for 48 hours before freezing on dry ice. Coronal brain sections and transverse spinal cord sections were cut at 30 µm using a freezing microtome. Tissue sections were immediately immersed in PBS and stored at 4°C until use. Sections were processed free-floating according to the protocol outlined in NEN Life Science Products TSA (Tyramide Signal Amplification) Indirect Kit. Briefly, tissue sections were permeabilized in 0.2% Triton X-100 (Sigma, St. Louis, MO)/PBS, incubated in 1% hydrogen peroxide (Sigma, St. Louis, MO)/PBS to remove

endogenous peroxidase activity then blocked in TNB Buffer (0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl, and 0.5% Blocking Reagent. Sections were incubated for 24 hours at 4°C in either the anti-GAL2 or anti-GALR3 antiserum (1:100). Following incubation with the primary antiserum, the tissue sections were washed in TNT Buffer (0.1 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween 20) followed by incubation at 25°C for 30 minutes with horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin (1:200) (Sternberger Monoclonals Inc., Lutherville, MD). Tissue sections were rinsed in TNT Buffer and incubated in a solution containing biotinylated tyramide to amplify the signal then rinsed in TNT buffer and incubated with HRP-conjugated streptavidin at 25°C for 30 minutes. An immunoperoxidase reaction was done by incubating the section in 3,3'-diaminobenzidine (DAB) (0.05%) in 0.1 mM Tris, pH 7.4 and adding hydrogen peroxide to 0.006% immediately before use. The reaction was stopped in water and the sections mounted on microscopic slide with mounting medium (40% ethanol: gelatin) and counterstained with Cresyl violet then coverslipped for light microscopy.

Optimal GALR3 antibody concentrations (1:200) for rat brain sections were determined in preliminary titration experiments. Experimental controls in the tissue sections included (1) incubation in normal rabbit serum or (2) omission of the primary antiserum.

30 Analysis

COS-7 cells and tissue sections were examined using a Zeiss Axioscope. A total of 6 male rats were examined

with the anti-GALR3 antiserum. The identification of GALR3-LI in the transfected cells and brain regions was based on the presence of immunoreactivity appearing as a brownish precipitate in individual cells and their projections or in the neuropil of the tissue by light microscopy. The descriptions of neuroanatomic boundaries are based on the atlas of Paxinos and Watson (1998).

10 B. Results

Characterization of the GALR3 antiserum

15 Recombinant GAL1, GAL2, and GALR3 receptor transfected cells

The ability of the anti-GALR3 antiserum to recognize only the GALR3 receptor protein in vitro was established by performing immunocytochemistry on COS-7 cells transiently transfected with the recombinant receptor proteins for the rat GAL1, GAL2, and GALR3, or mock-transfected with vector only. Specific porcine ¹²⁵I-galanin binding was detected for all transfectants except mock-transfected cells. An immune response was detected only in the COS-7 cells incubated with the antiserum generated for the particular recombinant receptor. Specifically, no immune reaction was observed with the anti-GALR3 antiserum (1:1000) in GAL1 or GAL2 transfected cells. Furthermore, no visible immune reaction was detected in the mock-transfected cells. Incubation of the cells in primary antiserum that had been preabsorbed with the antigenic peptide (20 µg/ml) or without the primary antiserum or with the

primary replaced by normal goat serum did not result in an immune response.

Taken together, these data demonstrate that the anti-GALR3 antiserum recognizes the receptor against which it was generated and does not show cross reactivity with other known GAL receptors.

Western Blots

To determine the specificity of the anti-GALR3 antiserum, COS-7 cells were transiently transfected either with recombinant rat GAL2 or GALR3 receptors or with expression vector only; membranes were then isolated for evaluation by immunoblotting (see Figure 5). The anti-GALR3 antiserum labeled proteins in membranes only from rat GALR3-transfected cells; a predominant band was evident with an apparent molecular weight of approximately 56 kDa (Figure 5), somewhat higher than the amino acid-derived value of 40.4 kDa. (For comparison, apparent molecular weights determined by SDS-PAGE are 56 kDa (Servin et al., 1987) or 54 kDa (Chen et al., 1992) for native GAL receptors purified from rat brain and 54 kDa (Amiranoff et al., 1989) for native GAL receptors purified from Rin m 5F cells. These values are all higher than the amino acid-derived value any known GAL receptor subtype, including the value of 38.9 kDa for rat GAL1 (Parker et al., 1995). The apparently high molecular weight observed for rat GALR3 very likely reflects post-translational processing such as glycosylation; note that rat GALR3 contains multiple N-terminal glycosylation sites (Smith et al., 1998). Relative to the predominant

band, additional species of higher molecular weight as well as lower molecular weight were labeled by the corresponding antiserum (Figure 5). These are presumably receptor-related species composed of protein aggregates of C-terminal fragments, as they are absent in mock-transfected cells.

Immunohistochemical distribution of GALR3-LI in the CNS

GALR3-like immunoreactivity (GALR3-LI) was observed in many regions of the brain, specifically, the neocortex, septum, hippocampus, amygdala, and brainstem (see Table 12). Throughout the brain and spinal cord GALR3-LI was found to be associated with neuronal profiles however, there was neuropil staining observed in several brain regions. Several regions of the CNS almost exclusively expressed GALR3-LI, specifically the accumbens nucleus, dorsal raphe, ventral tegmental area (Table 12). There was no observable staining of the fiber tracts.

The specificity of the anti-GALR3 antiserum was determined in tissue sections by (1) omission of the primary antiserum or (2) incubation with normal rabbit serum. No specific staining was observed in either condition. Preabsorption of the GALR3 primary antiserum with the antigenic peptide (10 µg/ml) decreased but did not completely block staining in the tissue sections as in the transfected cells. This was most likely related to the different localization approaches. In the transiently transfected COS-7 cells the expression of GALR3 receptor protein was relatively high therefore, indirect immunocytochemistry with no

amplification was used. In contrast, GALR3 receptor protein expression is presumed to be relatively lower in the tissue sections and for that reason the TSA (amplification) technique was employed. It is possible that because of the amplification (1000-fold) in the TSA technique even small amounts of unabsorbed antiserum may result in a signal.

Distribution of GALR3-LI in the rat CNS

Cerebral cortex

GALR3-LI was widespread in the cerebral cortex and the distribution pattern extended rostrocaudally. A weak to moderate GALR3-LI was seen in numerous cell bodies in the anterior cingulate cortex.

Septal region

An extensive and densely stained fiber network was seen throughout the entire lateral, intermediate and medial septal nuclei. The dorsal division of the lateral septum contained scarce moderately GALR3-like immunoreactive somata.

Basal ganglia

Numerous moderately GALR3-like immunoreactive cell bodies and fibers were present in the shell and core of the accumbens nucleus. The cell bodies of the subthalamic nucleus, a relay nucleus in the basal ganglia, contained weak GALR3-LI.

Amygdala and Extended Amygdala

In general, GALR3-LI was weak throughout the amygdala. Scattered cell bodies and fibers exhibited weak staining in several nuclei. Very fine GALR3-like immunoreactive fibers with scattered moderately labeled cells were detected in the central amygdaloid nucleus.

Midbrain/Mesencephalon

Labeled cells were detected within the dorsal raphe and projections from these cells were seen converging toward the midline of the raphe. Moderately immunoreactive-scattered cells were evident in the ventral tegmental area.

Brain stem

Intense staining was observed in cell bodies in the locus coeruleus.

The distribution of rat GALR3 protein in the CNS using receptor subtype selective polyclonal antibodies and tyramide signal amplification (TSA) immunocytochemistry is illustrated in Table 12. These were qualitative evaluations for the rat GALR3 receptor protein distribution based on the relative intensity of the chromogen (3,3'-diaminobenzidine) observed in individual cells at the microscopic level.

A total of 4 rat brains were analyzed for this study. As shown in Table 12, the strength of the signal obtained in various regions of the rat brain was graded as weak (+), or moderate (++) or intense(+++). Table 12

REGION	cells	fibers	Potential Therapeutic Application
Telencephalon			
Frontal cortex	++		Anxiety/Depression
Cingulate cortex	++		Anxiety/Depression
Basal ganglia			
Accumbens nucleus	++	-	Treatment of the positive symptoms of schizophrenia Treatment of drug addiction. This region is particularly sensitive to psychoactive drugs. Anxiety/Depression
Septal Region			Relief of fear
Lateral septal nucleus, dorsal	+	++	
Lateral septal nucleus, ventral	+	++	
Intermediate septal nucleus	-	++	
Medial septal nucleus		++	
Amygdala and extended Amygdala			Treatment of anxiety, panic attack, and depression. Treatment of disorders of integrated behaviors such as defense, ingestion, reproduction, and learning.
Central nucleus	++	++	Fear and anxiety

Mesencephalon			
Dorsal raphe	++	-	Depression/Analgesia
Ventral tegmental area	++	-	Depression
Brainstem/Pons/Medulla			
Locus coeruleus	+++	-	Modulation of noradrenergic transmission. Treatment of depression

The GALR3 antiserum was characterized using recombinant GAL receptors in transiently transfected COS-7 cells and Western blot analysis and the specificity of the GALR3 antiserum to recognize only the cognate receptor in vitro was established. The anatomical distribution of the GALR3 receptor protein in the rat CNS was determined using a modified immunohistochemical technique to enhance sensitivity and detectability via tyramide signal amplification (Toda et al., 1999).

The results indicate that the expression GALR3-LI was primarily found in neuronal profiles with neuropil labeling detectable in several areas. In general, the distribution of GALR3-LI is in good agreement with the reported distribution for galanin-LI, galanin binding sites, and GALR3 mRNA in the rat brain (for recent review, Branchek et al., 2000). Overall, GALR3-LI was extensively distributed throughout the brain. Paralleling the distribution of galanin binding sites GALR3-LI was observed in ventral regions of the brain.

The localization of the GALR3 protein in the dorsal raphe and locus coeruleus suggests a potential therapeutic application of galanin receptor antagonists in the treatment of depression by attenuating galanin's inhibitory tone on both of these regions.

A decrease in central serotonin (5-HT) neurotransmission has been implicated in depression. GALR3 antagonists could possibly act via GALR3 receptors on the cell bodies of dorsal raphe neurons to increase firing rate of raphe neurons thus increasing 5-HT release in the telencephalon and diencephalon. Another possible site of action for a GALR3 antagonist could be on postsynaptic GALR3 receptors in the limbic forebrain to block the putative ability of galanin to negatively regulate 5-HT_{1A} receptor transmission (Misane et al, 1998).

Unlike the dorsal raphe cells, the cells of the locus coeruleus express abundant galanin under normal conditions and it has been proposed that galanin may be released from dendrites and soma of the noradrenergic cell bodies (for review, Hökfelt et al., 1998). The ascending afferent projections of the locus coeruleus are extensive throughout the brain. Changes in the noradrenergic system have been hypothesized to be involved in depression-related behaviors and symptoms (for review, Weiss et al., 1998). The ventral tegmental area (VTA) receives projections from the locus coeruleus that have been reported to co-localize galanin and noradrenaline. It has been proposed that in certain pathological states (ex. stress induced

depression) galanin . released from noradrenergic terminals in the VTA inhibits dopaminergic neurons in the region that results in decreased dopamine release in the forebrain regions, particularly the accumbens nucleus and prefrontal cortex. This decrease in dopamine release produces a decreased motor activation and anhedonia. GALR3 has been identified in all of these regions and thus presents itself as a potential therapeutic target in the treatment of depression.

5 Drugs that would effectively decrease galanin's release in the VTA whether at the level of the locus coeruleus (somatodendritic GALR3 receptors to decrease the activity of LC cells) or in the VTA itself (presynaptically on NE/GAL terminals in the VTA or via

10 GALR3 receptors on VTA-DA neurons to prevent the hyperpolarization VTA-DA cells by released galanin)

15 would produce an antidepressant effect.

REFERENCES

- American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.
5 American Psychiatric Association, Washington, DC.
- Amiranoff, B., et al., (1989) Galanin receptor in the rat pancreatic beta cell line Rin m 5F. Molecular characterization by chemical cross-linking. *J. Biol. Chem.*, 264(34): 20714-20717.
10
- ~~Asymmetric Synthesis (1983) Vol.: 2-5, Academic Press.~~
Editor Morrison, J.
- 15 Bakker, R.A., et al., (2000) Constitutive activity of the histamine H1 receptor reveals inverse agonism of histamine H1 receptor antagonists. *Eur. J. Pharmacol.*, 387: R5-R7.
- 20 Borowsky, B., et al., (1999) Cloning and characterization of the human galanin GALR2 receptor. *Peptides*, 19: 1771-1781.
- Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of the protein-dye binding.
25 *Anal. Biochem.*, 72: 248-254.
- Branchek, T.A., et al., (2000) Galanin receptor subtypes. *Trends in Pharm. Sci.*, 21: 109-116.
30
- Bryant, W.M.III, et al., (1993) *Synthetic*

Communications, 23: 1617-1625.

Chen, Y., et al., (1992) Solubilization and molecular
characterization of active galanin receptors from rat
5 brain. *Biochemistry*, 31(8): 2415-2422.

Choshi, T., et al., (1997) New developments of
carbazole syntheses by thermal dlectrocyclic reaction.
Fukuyama Daigaku Yakugakubu Kenkyu Nenpo, 15: 1-24.

10

Coppola, G.M. (1987) *Journal of Heterocyclic Chemistry*,
24: 1249

Cullen, B. (1987) Use of eukaryotic expression
15 technology in the functional analysis of cloned genes.
Methods Enzymol., 152: 685-704.

deLigt, R.A., et al., (2000) Inverse agonism at G
protein-coupled receptors: (patho)physiological
20 relevance and implications for drug discovery. *Br. J.*
Pharmacol., 130(1): 1-12.

De Weille, J.R., et al., (1989) Galanin inhibits
dopamine secretion and activates a potassium channel in
25 pheochromocytoma cells. *Brain Res.*, 485: 199-203.

Detke, M.J., et al., (1995) Active behaviors in the rat
forced swim test differentially produced by
serotonergic and noradrenergic antidepressants.
30 *Psychopharmacology*, 121: 66-72.

Ennis, M. D. and Ghazal, N. B., (1992) The synthesis of (+) and (-)-flesinoxan: Application of enzymatic resolution methodology. *Tetrahedron Lett.*, 33: 6287-6290.

5

File, S.E. (1985) Animal models for predicting clinical efficacy of anxiolytic drugs: social behaviour. *Neuropsychobiology*, 13: 55-62.

10

File, S.E. and Pellow, S. (1984) The anxiogenic action of FG 7142 in the social interaction test is reversed by chlordiazepoxide and Ro-15-1788 but not by CGS 8216. *Archs. Int. Pharmacodyn. Ther.*, 271: 198-205.

15

File, S.E. and Pellow, S. (1983) The anxiogenic action of a convulsant benzodiazepine: reversal by chlordiazepoxide. *Brain Res.*, 278: 370-372.

20

File, S.E., et al., (1982) The anxiogenic action of benzodiazepine-like antagonists. *Neuropharmacology*, 21: 1033-1037.

25

File, S.E. (1980) The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *J. Neurosci. Methods*, 2: 219-238.

30

File, S.E. and Hyde, J.R.G. (1979) A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilisers and of stimulants. *Pharmacol. Behav. Biochem.*, 11: 65-69.

File, S.E. and Hyde, J.R.G. (1978) Can social interaction be used to measure anxiety? *Br. J. Pharmacol.*, 62: 19-24.

5

Fusco, R., et al., (1978) Fischer synthesis of inoles from 2,6-disubstituted arylhydrazones. *Khom. Geterotsikl. Soedin.* 2: 200-216.

10 Garden, S.J., et al., (1998). *Synthetic Communications*, 28: 1679-1689.

Glover, V. (1998) Function of endogenous monoamine oxidase inhibitors (tribulin). *J. Neural. Transm. Suppl.*, 52: 307-13.

15

Gopalan, C., et al., (1993) Neurochemical evidence that the inhibitory effect of galanin on tuberoinfundibular dopamine neurons is activity dependent.

20

Green, T.W. and Wuts, P.G.M. (1991) *Protection groups in Organic Synthesis*, second Edition John Wiley & Sons, New York.

25

Guy, A.P. and Gardner, C.R. (1985) Pharmacological characterisation of a modified social interaction model of anxiety. *Neuropsychobiology*, 13: 194-200.

30 Harlow, E. and Lane, D. (1999) *Immunoblotting*. In: Barker, P. editor. *Using Antibodies: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press.

p 267-309.

Harrison, T. (1996) Saturated nitrogen heterocycles.
3(4): 259-275.

5

Herrick-Davis, K., et al., (2000) Inverse agonist
activity of atypical antipsychotic drugs at human 5-
Hydroxytryptamine_{2C} receptors. *J. Pharmacol. Exp.*
Ther., 295(1): 226-32.

10

Hess, B.A. Jr. and Corbino, S. (1971) *Journal of*
Heterocyclic Chemistry, 8: 161.

15 Hökfelt, T., et al., (1998) Galanin in Ascending
Systems. *Annals of the N.Y. Acad. Sci.*, Ed. T.
Hökfelt, Tamas Bartfai and J. Crawley p. 252-263.

20 Iversen, L. (2000) Neurotransmitter transporters:
fruitful targets for CNS drug discovery. *Mol.*
Psychiatry, 5(4): 357-62.

25 Jansson, A., et al., (1989) Centrally administered
galanin reduces dopamine utilization in the median
eminence and increases dopamine utilization in the
medial neostriatum of the male rat. *Acta Physiol.*
Scand., 135: 199-200.

30 Javitch, J.A., et al, (1984) ³H-Mazindol binding
associated with neuronal dopamine and norepinephrine
uptake sites. *Molecular Pharmacology*, 26: 35-44.

Jaques, J., et al., (1981) *Enantiomer, Racemates and Resolutions*. John Wiley & Sons.

5 Julius, D., et al., (1988) Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. *Science*, 241: 558-564.

10 Kenakin, T. (1996) The classification of seven transmembrane receptors in recombinant expression systems. *Pharmacol. Rev.*, 48(3): 413-63.

~~Kennett, G.A., et al., (1997)~~ Anxiolytic-like actions of the selective 5-HT₄ receptor antagonist SB-20470-A and SB-20766-A in rats. *Neuropharmacology*, 36(4-5): 15 707-712.

20 Kirby, L.G. and Lucki, I. (1997) Interaction between the forced swimming test and fluoxetine treatment on extracellular 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in the rat. *Stress*, 2(4): 251-263.

25 Katritzky, A.R., (1996) Recent progress in the synthesis of 1,2,3,4-Tetradyroquinolines. *Tetrahedron*, 52(48): 15031-15070.

Kouznetsov, V. et al., (1998) Some Aspects of Reduced Quionline chemistry. *J. Het. Chem.*, 35(4): 761-785.

30 Leonard BE. (1996) New approaches to the treatment of depression. *J Clin Psychiatry*. 57(4): 26-33.

- Lightowler, S., et al., (1994) Anxiolytic-like effect of paroxetine in a rat social interaction test. *Pharmacol. Behav. Biochem.*, 49: 281-285.
- 5 Lucki, I. (1997) The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav. Pharmacol.*, 8: 523-528.
- Lutz, M. and Kenakin, T. (1999) *Quantitative Molecular Pharmacology and Informatics in Drug Discovery*, John Wiley & Sons, LTD, West Sussex, England. p. 153.
- 10
- Misane, I., et al., (1998) Modulation of a 5-HT_{1A} receptor-mediated behavioral response by the neuropeptide galanin. *Ann. N.Y. Acad. Sci.*, 863: 442-444.
- 15
- Monsma, F.J. Jr., et al., (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.*, 43: 320-327.
- 20
- Nógrádi, M. (1987) *Stereoselective Synthesis*, VCH, Editor Ebel, H.
- 25
- Nordstrom, O., et al., (1987) Evidence for an inhibitory effect of the peptide galanin on dopamine release from the rat median eminence. *Neurosci. Lett.*, 73: 21-26.
- 30
- Owens, M.J. (1997) Neurotransmitter receptor and transporter binding profile of antidepressants and

their metabolites. *J. Pharm. Exp. Ther.*, 283: 1305-1322.

5 Otsuka, S. and Kobayashi, Y. (1964) A radioisotopic assay for monoamine oxidase determinations in human plasma. *Biochem. Pharmacol.*, 13: 995-1006.

10 Page, M.E., et al., (1999) Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swim test. *Psychopharmacology*, 147: 162-167.

15 Parker, E.M., et al., (1995) Cloning and characterization of the rat GALR1 galanin receptor from Rin14B insulinoma cells. *Mol. Brain Res.*, 34: 179-189.

20 Paxinos, G. and Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates*. San Diego: Academic Press, Inc.

Pindu, U. (1990) Recent developments in the syntheses of carbazole alkaloids. *Chimia* 44(12): 406-412.

25 Porsolt, R.D. (1981) Behavioral despair. In Enna, SJ (ed) *Antidepressants: neurochemical, behavioral and clinical perspectives*. Raven Press, New York, pp. 121-139.

30 Porsolt, R.D., et al., (1978) Behavioral despair in rats: a new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.*, 47: 379-391.

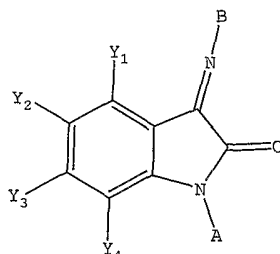
- Porsolt, R.D., et al., (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature*, 266: 730-732.
- 5
- Preobrazhenskaya, M.N., et al., (1967) Synthesis of substituted indoles through indolines. *Usp. Khim.* 36(10): 1760-1798.
- 10
- Razani, H., et al., (1997) 5-HT_{1A} receptor activation: short-term effects on the mRNA expression of the 5-HT_{1A} receptor and galanin in the raphe nuclei. *Neuroreport*, 8(16): 3565-3570.
- 15
- Reneric, J.P. and Lucki, I. (1998) Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swim test. *Psychopharmacology*, 136: 190-197.
- 20
- Rodgers, R.J., et al., (1997) Animal models of anxiety: an ethological perspective. *Braz. J. Med. Biol. Res.*, 30: 289-304.
- 25
- Servin, A.L., et al., (1987) Identification and molecular characterization of galanin receptor sites in rat brain. *Biochem. Biophys. Res. Commun.*, 144(1): 298-306.
- 30
- Seutin, V., et al., (1989) Galanin decreases the activity of locus coeruleus neurons in vitro. *Euro. J. Pharmacol.* 164: 373-376.

- Smith, K.E., et al., (1998) Cloned human and rat galanin GALR3 receptors Pharmacology and activation of G-protein inwardly rectifying K⁺ channels. *J. Biol. Chem.*, 273(36): 23321-223326.
- 5 Sternberger, L.A. (1982) Neurotypy: regional individuality in rat brain detected by immunocytochemistry with monoclonal antibodies. *Proc. Natl. Acad. Sci. USA*, 79: 1326-1330.
- 10 Sukhomlinov, A.K. et al., (1987) Acridine: A base for synthesis of pharmaceuticals. *Farm. Zh.* 4: 34-38.
- 15 Tatsumi, M., et al., (1997) Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur. J. Pharmacol.*, 340(2-3): 249-258.
- 20 Toda, Y., et al., (1999) Application of tyramide signal amplification system to immunohistochemistry: a potent method to localize antigens that are not detectable by ordinary method. *Pathol. Int.*, 49(5): 479-483.
- 25 Treit, D. (1985) Animal models for the study of anti-anxiety agents: a review. *Neurosci. Biobehav. Rev.*, 9: 203-222.
- 30 Weiss, J.M., et al., (1998) *Annals of the N.Y. Acad. Sci.*, (Ed. T. Hökfelt, Tamas Bartfai and J. Crawley) p. 364-382.
- Xu, Z., et al., (1998) Galanin-5-hydroxytryptamine

interactions: Electrophysiological, immunohistochemical and in situ hybridization studies on rat dorsal raphe neurons with a note on galanin R1 and R2 receptors. *Neuroscience*, 87: 79-94.

What is claimed is:

1. A compound having the structure:



wherein each of Y_1 , Y_2 , Y_3 , and Y_4 is independently -H; straight chained or branched C_1 - C_7 alkyl, $-CF_3$, -F, -Cl, -Br, -I, $-OR_4$, $-N(R_4)_2$ or $-CON(R_4)_2$;

wherein each R_4 is independently -H; straight chained or branched C_1 - C_7 alkyl, monofluoroalkyl or polyfluoroalkyl; straight chained or branched C_2 - C_7 alkenyl or alkynyl; C_3 - C_7 cycloalkyl, C_5 - C_7 cycloalkenyl, aryl or aryl(C_1 - C_6)alkyl;

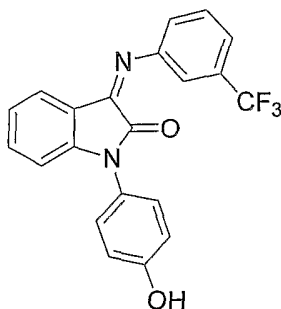
wherein B is aryl;

wherein A is aryl, substituted with -OH;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 24, wherein each of Y_1 , Y_2 , Y_3 and Y_4 is independently H or $-OR_4$;
3. The compound of claim 25, wherein A is naphthyl substituted with -OH.

4. The compound of claim 25, wherein A is phenyl substituted with -OH.
5. The compound of claim 27, wherein the compound has the following structure:

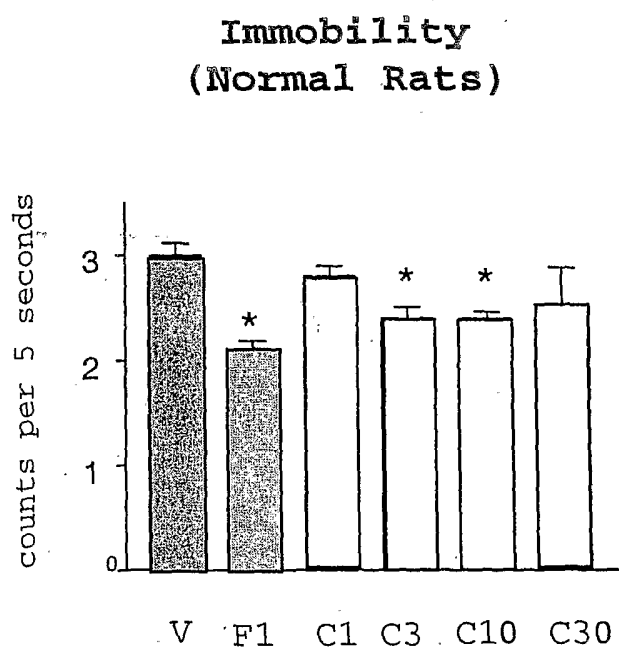


6. A pure Z imine isomer of the compound of claim 24.
7. A pure E imine isomer of the compound of claim 24.
8. A pharmaceutical composition comprising a therapeutically effective amount of the compound of claim 24 and a pharmaceutically acceptable carrier.
9. A pharmaceutical composition for treating depression made by combining a therapeutically effective amount of the compound of claim 24 and a pharmaceutically acceptable carrier.
10. A process for making a pharmaceutical composition comprising combining a therapeutically effective amount of the compound of claim 24 and a pharmaceutically acceptable carrier.
11. A method of treating a subject suffering from depression

which comprises administering to the subject an amount of the compound of claim 24 effective to treat the subject.

12. A method of treating a subject suffering from anxiety which comprises administering to the subject an amount of the compound of claim 24 effective to treat the subject.

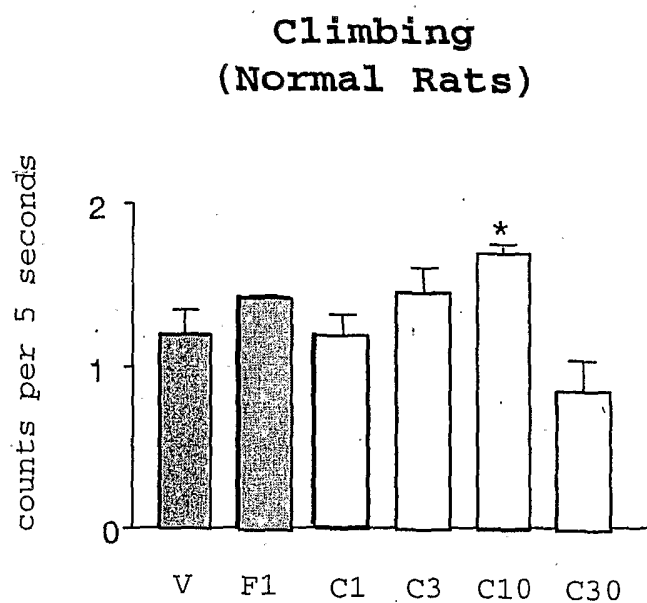
1/5



*. Significantly less than
Vehicle, $p < 0.01$, ANOVA and
Student-Newman-Keuls

FIGURE 1

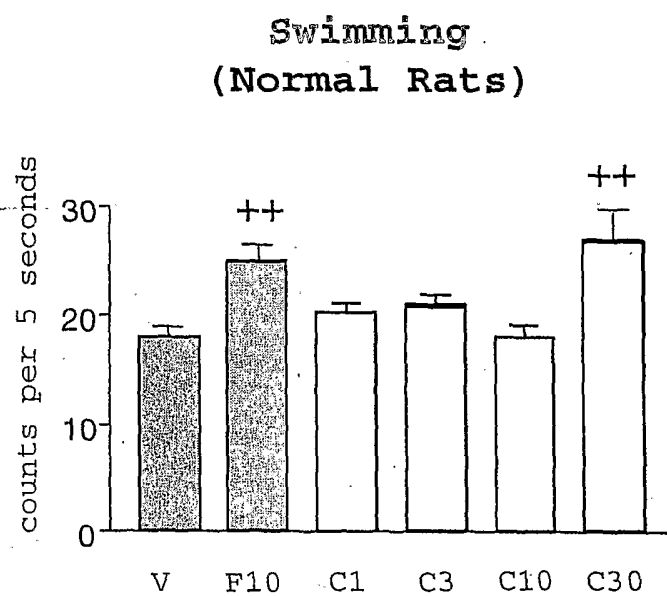
2/5



*Significantly greater than
Vehicle, $p < 0.01$, ANOVA and Student-
Newman-Keuls

FIGURE 2

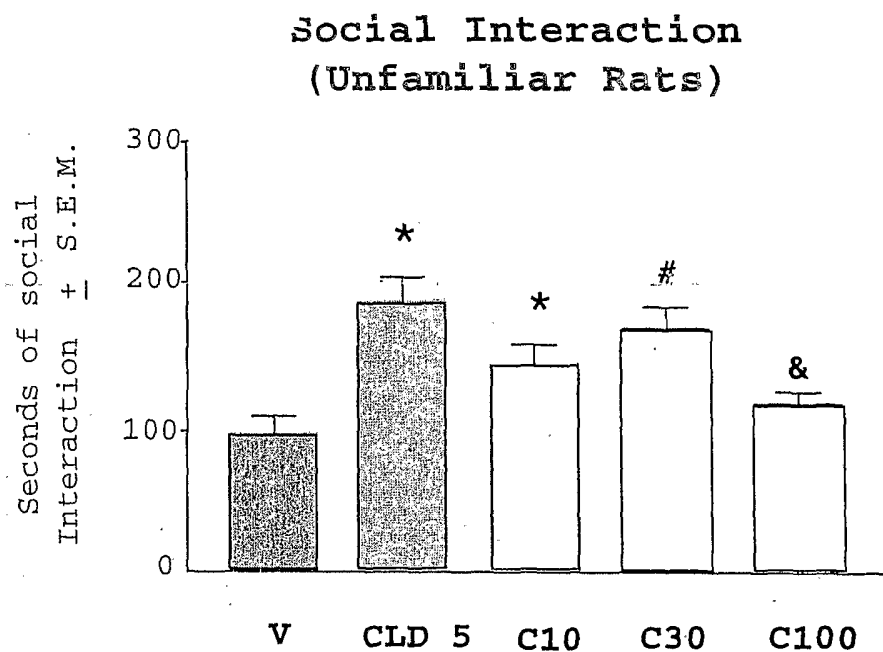
3/5



++Significantly greater than
Vehicle, $p < 0.01$, ANOVA and
Student-Newman-Keuls

FIGURE 3

4/5



- * Significantly greater than vehicle, $p < 0.05$, ANOVA and Student-Newman-Keuls test
- # Significantly greater than vehicle, $p < 0.01$, ANOVA and Student-Newman-Keuls test
- & Significantly less than 30 mg/kg dose and CLD5, $p < 0.01$, ANOVA and Student-Newman-Keuls test

FIGURE 4

5/5

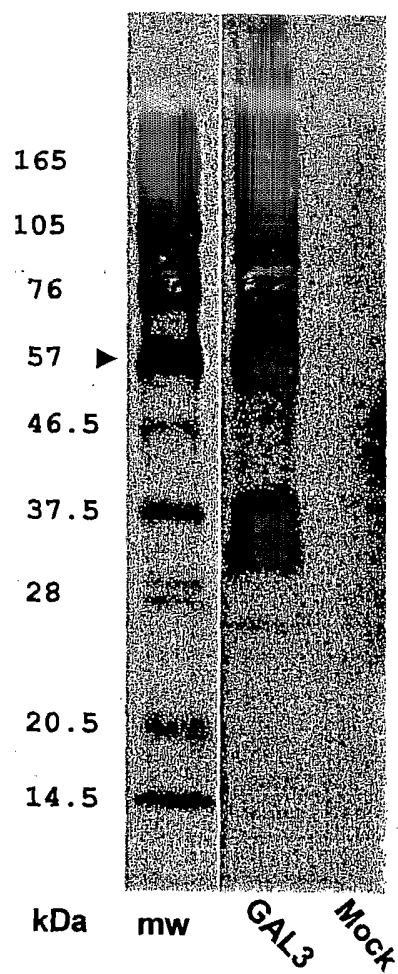


FIGURE 5