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Señor

**SUPERINTENDENTE DE INDUSTRIA Y
SUPERINTENDENCIA DE INDUSTRIA Y
E. S. D.**

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 11-121527- -00014-0000

Fecha: 2014-07-24 13:59:34 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 412 REPOSPRESRECU Folios: 72 21

Re: Expediente No.
Patente de Invención:

11-121527

**“COMPOSICIONES, SÍNTESIS, Y
MÉTODOS DE UTILIZACIÓN DE
DERIVADOS DE
ARILOPIPERAZINA”**

Solicitud Internacional:
Titular:

**PCT/US2010/025687
REVIVA PHARMACEUTICALS,
INC.**

**RECURSO DE REPOSICIÓN CONTRA LA RESOLUCIÓN
NO. 62673 DE 28 DE OCTUBRE DE 2013, NOTIFICADA
POR FIJACION EN LISTA DE 17 DE JUNIO DE 2014,
DESEFIJADA EL 17 DE JULIO DE 2014.**

HUMBERTO RUBIO CAMACHO, mayor de edad, domiciliado en Bogotá, D.C., con la Oficina 506 de la Carrera 7 No. 74-64 de ésta ciudad, Abogado en Ejercicio, portador de la Tarjeta Profesional No. 50.007 expedida por el Consejo Superior de la Judicatura, actuando en carácter de apoderado de la sociedad **REVIVA PHARMACEUTICALS, INC.**, domiciliada en San José Biocenter 5941, Optical Court, Suite 215, San José, California 95138, Estados Unidos de América; reconocido por su despacho y encontrándome dentro de la debida oportunidad procesal, por medio del presente escrito interpongo **RECURSO DE REPOSICIÓN** contra la Resolución No. 62673 de 28 de Octubre de 2013, notificada por fijación en lista de 17 de Junio de 2014; con el propósito que sea revocada y en su lugar proferir otra, en que se **OTORGUE** el privilegio de la Patente de Invención denominada: **“COMPOSICIONES, SÍNTESIS, Y MÉTODOS DE UTILIZACIÓN DE DERIVADOS DE ARILOPIPERAZINA”**, conforme a lo siguiente:

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1. MOTIVOS DE INCONFORMIDAD.-

Base de la Negativa

- 1.1. Mediante la Resolución objeto del presente recurso, su Despacho deniega el privilegio de patente de invención denominada: **“COMPOSICIONES, SÍNTESIS, Y MÉTODOS DE UTILIZACIÓN DE DERIVADOS DE ARILOPIPERAZINA”** solicitado por mí representada la sociedad **REVIVA PHARMACEUTICALS, INC.** al considerar que no cumple con los requisitos dispuestos en la legislación comunitaria vigente.
- 1.2. La División de Nuevas Creaciones decidió negar el derecho de patente de invención porque consideró que las reivindicaciones 1 a 11 no cumplen con el requisito de nivel inventivo. Se puede establecer con la simple lectura de la Resolución que esa división de ninguna manera considera la inversión, la importancia y el alcance del objeto de la presente solicitud y no obstante a tener novedad y nivel inventivo decide negarla, haciéndose una interpretación equivocada.
- 1.3. Considera ese Despacho que la presente solicitud está afectada por los documentos D1 US5919784 y D2 US2008/293736, precisión con la que estamos en total desacuerdo.
- 1.4. No obstante lo anterior y con el objeto de facilitar el trámite de la presente solicitud, y conforme lo establece el Artículo 34 de la Decisión 486, el solicitante radico ante su Despacho el 23 de julio de 2014, una Modificación Voluntaria al Capítulo Reivindicatorio, para limitar el compuesto de la presente solicitud para que dicho compuesto cumplan con los requisitos de nivel inventivo, sean nuevas y sean susceptibles de aplicación industrial y así sean consideradas inventivas frente al arte previo citado por el examinador, sin que esto implique ninguna ampliación a la materia. Por tanto, respetuosamente solicito tener en cuenta el nuevo pliego de reivindicaciones presentado al momento de resolver el presente recurso.
- 1.5. Nos permitimos respetuosamente indicar que no compartimos la interpretación realizada por el examinador con respecto a los

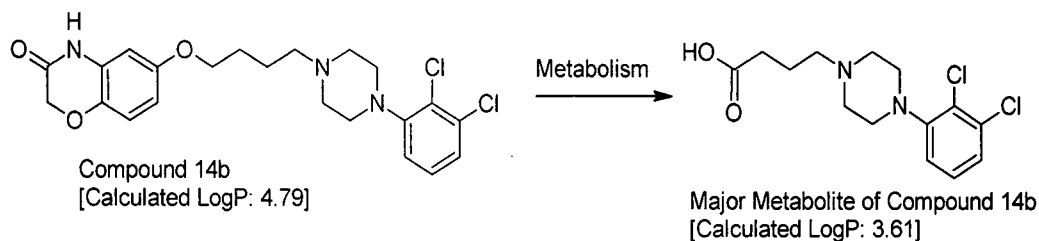
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documentos citados, en los cuales se fundamentó el rechazo de la presente solicitud, interpretación equivocada teniendo en cuenta los siguiente argumentos:

1.5.1. D1 (US 5,919,784)

Los compuestos de **D1** son diferentes a los compuestos reivindicados, al menos en A. En las presentes reivindicaciones, A es $-O-(CH_2)_4-$. In **D1**, A es $-CH_2-$.

Los compuestos de la Reivindicación 1 (por ejemplo, el Compuesto 14b) se adherir de manera diferente en el cuerpo humano, en comparación con los compuestos descritos en D1.

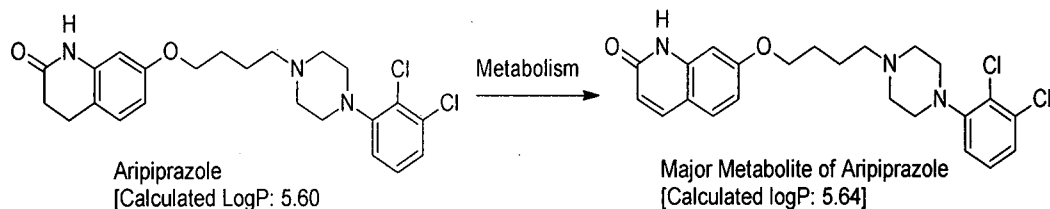


Los compuestos divulgados en **D1** no tienen el átomo de oxígeno (O) unido al grupo funcional arilo [i.e. benzo(b)1,4-oxazin-3(4H)-ona] y por lo tanto no se pueden adherir de la misma forma que el Compuesto 14b.

1.5.2. D2 (US2008/293736)

D2 describe compuestos que son muy diferentes a los compuestos de la presente. En los compuestos de la presente, B= O; mientras que en D2, B = carbono. Los anillos de dos miembros del lado izquierdo son diferentes en D2 y en las presentes reivindicaciones.

Los compuestos de la Reivindicación 1 (por ejemplo, el Compuesto 14b) se adhieren de manera diferente en el cuerpo humano en comparación con los compuestos divulgados en **D2** o el aripiprazol.



Una persona experta en la materia puede entender fácilmente que el principal metabolito formado por el Compuesto 14b es menos lipófilo que los metabolitos formados por los compuestos divulgados en D1 y D2, incluyendo el aripiprazol. Los metabolitos menos lipófilos, en especial los ácidos carboxílicos como el metabolito del Compuesto 14b-metabolito, son conocidos por ser excretados del cuerpo de manera más sencilla que los metabolitos lipófilos. El metabolito del Compuesto 14b no es farmacológicamente activo, mientras que el metabolito de aripiprazol es farmacológicamente activo. El metabolito activo farmacológicamente puede contribuir a efectos secundarios derivados de la interacción droga-a-droga ya que muchos pacientes con esquizofrenia toman más de un medicamento. En general, el perfil farmacocinético de los compuestos de la Reivindicación 1 (por ejemplo, el Compuesto 14b) contribuye a una mejor seguridad que los compuestos divulgados en D1 y D2.

1.5.3. No existen enseñanzas o sugerencias en el arte previo sobre la combinación de las estructuras químicas de D1 y D2 de acuerdo con la invención del Solicitante.

El examinador dice que una persona experta en la materia estaría motivada a substituir el grupo 3,4-dihidroquinolina-2(1H)-ona de los compuestos divulgados en el Documento D2 por un bioisóestero tal como el grupo benzo ([b]1,4)oxazin-3(4H)-ona porque los dos grupos son útiles en el tratamiento de esquizofrenia, tal como se revela en el documento D1. El solicitante no está de acuerdo con el examinador porque es claramente una retrospección después de leer la invención del Solicitante. No hay bases para que al leer D1 y D2 dicha substitución única y específica lleve a una molécula que tenga propiedades fisicoquímicas, farmacodinámicas y farmacocinéticas favorables.

1.5.4. El Compuesto 14 no es menos activo sobre los receptores de dopamina que el compuesto del arte previo

El examinador dice que el Compuesto 14 de la solicitud es menos activo sobre los receptores de dopamina que el Compuesto más cercano en el estado del arte, aripiprazol, y por lo tanto es menos potente que el Compuesto descrito por el documento D2 de acuerdo con los resultados de los estudios de relaciones entre estructura y actividad revelados en Avram, et al., Monatsh Chem, 139, 407-426 (2008), página 422, Tabla 5; y Salama, Bioorganic & Medicinal Chemistry, 14: 5898-5912 (2006), página 5899, Esquema 1. El solicitante no está de acuerdo por las siguientes razones:

Las afinidades (K_i) de enlace de los receptores de los compuestos son muy sensibles a las condiciones del ensayo y por lo tanto, uno puede esperar variaciones en los valores K_i generados en diferentes laboratorios en diferentes momentos, incluso bajo condiciones similares. A menos que los componentes sean probados en el mismo experimento y comparados uno al lado del otro, no tiene sentido comparar valores históricos.

En Avram et al, Tabla 5 muestra el pK_{iD1} , pK_{iD2} (9.95), pK_{iD3} , pK_{iD4} del aripiprazol. En Salama et al, Esquema 1 muestra el valor pK_i de D2 (8.35) del aripiprazol. Estas cifras están basadas en la literatura de afinidades de enlace (pK_i) tomadas de diferentes fuentes. Tal como se muestra en Avram y Salama, los valores pK_i se diferencian en más de un punto. No hay evidencia de que el Compuesto 14b de la invención es menos potente que los compuestos del arte previo. También es muy conocido que la actividad farmacológica calculada del Compuesto (Avram et al) puede ser diferente a su actividad real en condiciones fisiológicas.

1.5.5. El Compuesto 14b tiene un perfil farmacológico diferente que los compuestos del arte previo

El Compuesto 14b es un modulador de dopamina-serotonina y su perfil farmacológico es diferente al de los compuestos divulgados en D1y D2, incluyendo la droga aprobada aripiprazol.

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La mayoría de condiciones comórbidas prevalecientes de la esquizofrenia son síntomas negativos, depresión severa, y disfunción cognitiva. Además de las actividades agonistas parciales en el receptor de dopamina D2 y el receptor de serotonina 5-HT_{1A}, los compuestos con actividad agonista parcial en el receptor D4 de dopamina y actividad antagónica en el receptor de serotonina 5-HT₇ son reportados como efectivos para el tratamiento de condiciones comórbidas tales como disfunción cognitiva, síntomas negativos y depresión. Favor véase Sarkisyan et al (Behavioural Brain Research, 209: 99-108, 2010), el cual reporta que el receptor 5-HT₇ es un mediador y modulador del comportamiento de manera similar al antidepresivo (véase Título y Resumen). Por favor también véase Depootere et al, British J. Pharmacology, 151: 266-277, 2007), el cual reporta que el equilibrio las interacciones entre los receptores D2/D3, D4, y 5-HT_{1a} de F15063 producen un perfil prometedor en cuanto a la actividad en modelos de déficits cognitivos y síntomas negativos de esquizofrenia.

Ningún compuesto reportado en la literatura hasta la fecha tiene la combinación de las fuertes actividades de enlace de los receptores D4 de dopamina y el receptor de serotonina 5-HT₇. El Compuesto 14b ha mostrado fuertes actividades de enlace de los receptores D4 de dopamina y el receptor de serotonina 5-HT₇, y esta es una de las diferencias más relevantes en el perfil farmacológico. El perfil farmacológico in vitro, y los datos de las fases 1 y 2 de las pruebas clínicas en humanos del Compuesto 14b fueron presentados en la Reunión Anual de la Unidad de Evaluación Clínica de Nuevas Drogas (NCDEU), 28 al 31 de mayo, Hollywood, Florida, USA y una copia del anuncio se adjunta aquí. Por favor tenga en cuenta que en el anuncio se menciona RP5063 el cual es el mismo Compuesto 14b.

Como se muestra en la diapositiva #3, el Compuesto 14b es un potente antagonista parcial de los receptores de dopamina (D₂, D₃ y D₄) y de serotonina (5-HT_{1A} y 5HT_{2A}), y antagonista de los receptores de serotonina 5-HT₆ y 5-HT₇. Tiene una alta afinidad con los principales receptores, K_i (nM): D_{2L} (0.45, IA = 43%), D_{2S} (0.61, IA = 52%), D₃ (3.7, IA = 44%), D₄ (6.0, IA = 51%), 5-HT_{1A} (1.5, IA = 19%), 5-HT_{2A} (4.1, IA = 52%) D₃ (3.7, IA=44%), D₄ (6.0, IA=51%), 5-HT_{1A} (1.5, IA=19%), 5-

HT_{2A} (4.1, IA=33%), 5-HT₇ (2.7), y H₁ (2.5). Tiene una afinidad moderada con los receptores, K_i (nM): nicotina nAChR-- $\alpha_4\beta_2$ (36.3), 5-HT_{2C} (39), 5-HT₆ (51), adrenérgicos α_{1B} (47), D₁ (100), y SERT (106). Por favor tenga en cuenta que IA es actividad intrínseca. En este perfil farmacológico en particular, la combinación de las fuertes actividades de enlace del receptor de dopamina D₄ y el receptor de serotonina 5-HT₇, es diferente a la de D₁, D₂, y el aripiprazol.

1.5.6. El Compuesto 14 b es más potente que la droga aprobada aripiprazol

Por favor véase las diapositivas # 7: 189 sobre pacientes con esquizofrenia tratados con 15 mg, 30 mg, 50 mg por día durante 4 semanas con el Compuesto 14b, placebo, o 15 mg de aripiprazol. En la diapositiva #9, el puntaje total PANSS (Escala Positiva y Negativa del Síndrome) se mejoró significativamente con el Compuesto 14b con 15 mg, 30 mg, y 50 mg/por día; y el efecto fue mucho mejor que el del aripiprazol. La diapositiva #10 muestra que la Escala total PANSS-Positiva se mejoró significativamente con el Compuesto 14b con 15 mg, 30 mg, y 50 mg/por día; y no se mejoró con aripiprazol. La diapositiva #12 muestra que la Escala total PANSS-negativa fue mejorada significativamente con el Compuesto 14b con 15 mg, 30 mg, y 50 mg/por día; y también fue mejorada con aripiprazol.

Como se presenta en el anuncio señalado anteriormente, el Compuesto 14b mostró una mayor eficacia y perfil de seguridad cuando se compara con la droga antisicótica aprobada aripiprazol que se usa ampliamente a nivel global para tratar la esquizofrenia. La eficacia superior del Compuesto 14b especialmente para los síntomas negativos, depresión y disfunción cognitiva pueden atribuirse a sus actividades en los receptores de dopamina D₄ y 5-HT₇ además de su actividad en los receptores D₂, y 5-HT_{1A}.

En vista de lo anterior y dadas las ventajas inesperadas ya presentadas, las Reivindicaciones 1-7 no son obvias frente a D₁, D₂, y el aripiprazol.

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- 1.6. Respetuosamente consideramos que los documentos D1 y D2 pueden enseñar ciertas técnicas comunes en el estado de la técnica relacionada, pero ninguno de estos documentos anticipa el conjunto de la presente solicitud, y por tanto para una persona versada en la materia NO le resultaría obvia ni derivada de manera evidente del estado de la técnica, como equivocadamente lo sostiene esa entidad. En consecuencia, el solicitante respetuosamente considera que las referencias citadas D1 y D2, ni separadamente ni de forma combinada, muestran y menos aún sugieren “todas” las características técnicas incluidas en las reivindicaciones de la presente solicitud tal y como fueron modificadas. Por lo tanto la presente solicitud no es solo nueva, sino también inventiva a la luz del estado de la técnica citado.
- 1.7. Adicionalmente queremos reiterar y resaltar para que el examinador lo tenga en cuenta y valore al momento de estudiar el expediente, que esta solicitud de Patente específicamente, las reivindicaciones de la presente solicitud se han adaptado conforme a las reivindicaciones aceptadas para la patente análoga concedida en Estados Unidos de America bajo el número USPN 8,188,076 (copia adjunta). Estas modificaciones están enteramente sustentadas en el contenido de la solicitud originalmente presentada. Entendemos el criterio de esa entidad, que las reivindicaciones deben cumplir con las disposiciones legales vigentes y conforme lo establece la Decisión 486, sin embargo, teniendo en cuenta que con las nuevas reivindicaciones tal y como se han enmendado, cumplen con lo dispuesto en la norma señalada, es importante y necesario en esa etapa, señalar que también ha sido concedida la patente análoga, es decir, la solicitud inicial de donde se desprende esta fase nacional.
- 1.8. Teniendo en cuenta lo anteriormente expuesto, consideramos que la decisión contenida en la resolución, motivo de la actual impugnación, resulta equivocada e injusta, por tanto y teniendo en cuenta el nuevo capítulo reivindicatorio y lo que se expuso anteriormente, debe considerarse que la presente solicitud cumple con lo estipulado en los Artículo 14, 16 y 18 de la Decisión 486, y se refiere a una invención patentable a la luz de lo dispuesto por la mencionada norma comunitaria. Pues todo lo aquí demostrado nos está indicando que la presente solicitud cumple con los presupuestos señalados en la

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Decisión 486 de la Comunidad Andina, para que sea otorgada como Patente de Privilegio de Invención.

2. PETICIONES.

2.1. Pretensión Principal.

De acuerdo a lo señalado en el transcurso de este recurso, solicito respetuosamente a su despacho, tener en cuenta los argumentos anteriormente propuestos y con base a ello, proceder con la concesión total o parcial de la Patente de Privilegio de Invención: **“COMPOSICIONES, SÍNTESIS, Y MÉTODOS DE UTILIZACIÓN DE DERIVADOS DE ARILOPIPERAZINA”**, a favor de la sociedad **REVIVA PHARMACEUTICALS, INC.**

2.2. Pretensiones Subsidiarias.

- a). **REVOCAR** la Resolución No. 62673 de 28 de Octubre de 2013, y en su lugar proferir otra en la que otorguen en forma total o parcial las reivindicaciones solicitadas, todo de conformidad con los documentos que hacen parte esencial del trámite.
- b). **REVOCAR** la Resolución No. 62673 de 28 de Octubre de 2013, y en su lugar proferir otra en la que si su Despacho, no obstante la claridad de la invención, considera que es necesaria alguna precisión, se requiera a mi representada con las explicaciones pertinentes, pues reiteramos que la invención solicitada encaja dentro de las normas vigentes, por lo tanto en aras de los principios de equidad y justicia deberá ser otorgada total o parcialmente, como respetuosamente lo solicito.

3. PRUEBAS.

- 3.1. Todos y cada uno de los documentos que reposan en el expediente citado en la referencia.
- 3.2. Resolución No. 62673 de 28 de Octubre de 2013.
- 3.3. Modificación presentada el 23 de Julio de 2014.

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- 3.4. Copia de la solicitud concedida en Estados Unidos de America bajo USPN 8,188,076.
- 3.5. Sarkisyan et al (Behavioural Brain Research, 209: 99-108, 2010).
- 3.6. Depootere et al, British J. Pharmacology, 151: 266-277, 2007).
- 3.7. Poster Presentación: New Clinical Drug Evaluation Unit (NCDEU) Annual Meeting, May 28-31, Hollywood, FL
- 3.8. La actuación surtida.

4.- FUNDAMENTOS DE DERECHO.

Las disposiciones legales que invoco como fundamento para promover el presente recurso los artículos 14, 16, 18, y 34 y demás aplicables de la Decisión 486 de la Comunidad Andina, en las normas aplicables del Código de Procedimiento Administrativo y de lo Contencioso Administrativo contenidas en la Ley 1437 de 2011, y en las demás disposiciones vigentes.

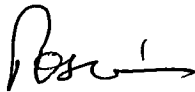
5.- OPORTUNIDAD.

La Resolución recurrida fue notificada por fijación en lista de 17 de Junio de 2014, desfijada el 17 de Julio de 2014, por lo tanto me encuentro dentro de la debida oportunidad legal, consagrada en el Código Contencioso Administrativo.

6.- NOTIFICACIONES.

Mí representada y el suscrito recibiremos notificaciones en la Secretaría de su Despacho o en mi Oficina de Abogado, situada en la Carrera 7 No. 74-64 Oficina 506 de Bogotá.

Atentamente,



HUMBERTO RUBIO CAMACHO

C.C. 17'192.062 de Bogotá

**T.P. 50.007 Consejo Superior
de la Judicatura**

COD. 4134

 Industria y Comercio SUPERINTENDENCIA	Espacio reservado para el adhesivo de radicación SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO  No. 11-121527- -00013-0000 Fecha: 2014-07-23 13:16:18 Dep. 2020 DIR.NUEVASCR Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI Act. 608 MODIFICASOLICIT Folios: 5
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DIRECCIÓN DE NUEVAS CREACIONES MODIFICACIONES Y/O CORRECCIONES

1. Identificación del Trámite

☐ Error material
 ☒ Modificación a una Solicitud en trámite

☒ Patente de Invención
☐ Patente de Modelo de Utilidad
☐ Registro de Diseño Industrial
☐ Esquema de Trazado de Circuitos Integrados

2. Datos del solicitante

Persona Natural ☐
Persona Jurídica ☒

REVIVA PHARMACEUTICALS, INC.

Nombre o denominación / Nombre o razón social completa

Nombre representante legal

Tipo de empresa Micro ☐ Pequeña ☐ Mediana ☐ Otra ☐

Documento de identificación: ☐ C.C. ☐ C.E. ☐ NIT ☐ Otro Número _____

Nacionalidad/País de constitución	Domicilio Titular
Estados Unidos de América	San Jose Biocenter 5941 Optical Court, Suite 215 San Jose, California 95138, Estados Unidos de América

☒ Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico

3. Datos del apoderado:

RUBIO CAMACHO HUMBERTO	17'192.062	50.007 C.S.J.
Apellidos y Nombre	Documento de identificación	Tarjeta profesional

Dirección y domicilio		Ciudad
Carrera 7 # 74-64 Oficina 506		Bogotá, D.C.
Dirección electrónica	No. Fax	Número telefónico
mail@rubiolawyers.com	3131647	3132408

En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación o protocolo de poder general.

4. Datos del trámite

Expediente No.: 11-121527

Corrección:

Aparece _____

Debe ser _____

Modificación:

☒ Modificación al capítulo reivindicatorio.

☐ Modificación al capítulo descriptivo.

☐ Modificación al resumen.

☐ Modificación a los dibujos.

☐ Modificación al solicitante.

5. Anexos

- ☒ Comprobante de pago de la tasa para la presentación de la corrección y/o modificación. **Recibo No. 11-00777-02 de 23/07/2014**
- ☐ Comprobante de pago de la tasa por excedente de Reivindicaciones.
- ☐ Poder, si fuere el caso.
- ☒ Documento que contiene las modificaciones o correcciones.
- ☐ Copia de la solicitud y sus anexos en formato magnético.

6. Firma

	HUMBERTO RUBIO CAMACHO
Firma	Nombre y apellidos
50.007 C.S.J.	17'192.062
Tarjeta Profesional	C.C.



US008188076B2

(12) **United States Patent**
Bhat et al.

(10) **Patent No.:** **US 8,188,076 B2**
(45) Date of Patent: **May 29, 2012**

(54) **COMPOSITIONS, SYNTHESIS, AND METHODS OF UTILIZING ARYLPIPERAZINE DERIVATIVES**

WO WO 2004/063162 A1 7/2004
 WO WO 2004/099152 A1 11/2004
 WO WO 2006/030446 A1 3/2006

(75) **Inventors:** **Laxminarayan Bhat**, San Jose, CA (US); **Prabhu Prasad Mohapatra**, San Jose, CA (US); **Kouacou Adiey**, San Jose, CA (US)

(73) **Assignee:** **Reviva Pharmaceuticals, Inc.**, San Jose, CA (US)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 241 days.

(21) **Appl. No.:** **12/714,406**

(22) **Filed:** **Feb. 26, 2010**

(65) **Prior Publication Data**
 US 2010/0216783 A1 Aug. 26, 2010

Related U.S. Application Data

(60) Provisional application No. 61/155,791, filed on Feb. 26, 2009.

(51) **Int. Cl.**
C07D 413/12 (2006.01)
A61K 31/538 (2006.01)

(52) **U.S. Cl.** **514/230.5; 544/105**

(58) **Field of Classification Search** **514/230.5**

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

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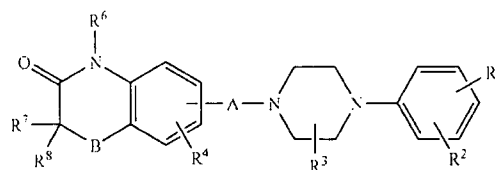
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(57) **ABSTRACT**

The present invention provides arylpiperazine derivatives having Formula I which can be advantageously used for treating schizophrenia and related psychoses such as acute manic, bipolar disorder, autistic disorder and depression.

Formula 1



7 Claims, No Drawings

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COMPOSITIONS, SYNTHESIS, AND METHODS OF UTILIZING ARYLPIPERAZINE DERIVATIVES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to, U.S. Provisional Application No. 61/155,791, filed Feb. 26, 2009, the contents of which are incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to compositions of arylpiperazine derivatives, synthesis of arylpiperazine derivatives, and methods of utilizing arylpiperazine derivatives. The present invention more particularly relates to synthesis, compositions and methods of utilizing arylpiperazine based compounds which are useful for the pharmacological treatment of schizophrenia and related psychoses such as acute manic, bipolar disorder, autistic disorder and depression.

BACKGROUND OF THE INVENTION

Medications used to treat psychotic disorders are called antipsychotics. Typical antipsychotics (sometimes referred to as conventional antipsychotics) are class of first generation antipsychotic drugs and used to treat psychosis including schizophrenia. The typical antipsychotics include chlorpromazine (THORAZINE®), fluphenazine (PROLIXIN®), haloperidol (HALDOL®), thiorixene (NAVANE®), trifluoroperazine (STELAZINE®), perphenazine (TRILAFON®), and thioridazine (MELLARIL®). The second generation antipsychotics introduced in the 1990's are called atypical antipsychotics. Compared to the first generation antipsychotics, the atypical antipsychotics appear to be equally effective in reducing the positive symptoms like hallucinations and delusions but may be better than the typical antipsychotics at relieving the negative symptoms of schizophrenia such as apathy, withdrawal, emotional depression and the like. The atypical antipsychotics currently in clinical use include Aripiprazole (ABILIFY®), clozapine (CLOZARIL®), risperidone (RISPERDAL®), olanzapine (ZYPREXA®), quetiapine (SEROQUEL®), and ziprasidone (GEODON®).

Atypical antipsychotics have diminished propensity to cause extrapyramidal symptoms (EPS) and tardive dyskinesia (TD) than typical antipsychotics. Additional benefits associated with the atypical antipsychotics include better treatment of negative symptoms, better compliance, possible benefits for cognitive impairments, and lower rates of relapse. Within the class of atypical antipsychotics, however, differences exist both in efficacy and side effects. Clozapine does not cause EPS, and is clearly more effective than all other antipsychotics used in humans to date. It is however a life-altering drug, because of its side effects and need for continual medical monitoring, in some countries, for agranulocytosis. This has markedly limited its use. The other atypical antipsychotics with the greatest amount of efficacy data are risperidone and olanzapine. These drugs are the most commonly used first-line antipsychotics today. This is warranted because they are more clinically effective than conventional drugs and much easier to use than clozapine. However, both risperidone and olanzapine are limited by side effects. Risperidone causes prolactin elevations, weight gain and dose-dependant EPS. Olanzapine use is associated with much more weight gain in addition to lipid and glucose abnormalities.

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Quetiapine and Ziprasidone may be safer alternatives to risperidone and olanzapine but these drugs do not appear to be as clinically effective as the other atypical antipsychotics. Aripiprazole is one of a new generation of atypical antipsychotic drugs approved by the FDA for the treatment of schizophrenia in November 2002 (Satyanarayana, C. et al. WO 2006/030446; Tsujimori, H. et al. WO 2004/063162; Salama, P. et al. WO 2004/099152; Wikstorm, H. et al. WO 2003/064393). It was approved for the treatment of acute mania and mixed episode associated with bipolar disorder in March 2005. Aripiprazole does not differ greatly from other atypical antipsychotics with respect to treatment response, efficacy and tolerability.

Atypical antipsychotics are increasingly being used in children and adolescents for a variety of psychiatric conditions. Conditions for which atypical antipsychotics are prescribed include bipolar disorder, psychotic depression, schizophrenia, pervasive developmental disorders, attention-deficit/hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and conduct disorder. They are also used symptomatically to treat rage, insomnia, and anorexia. Younger patients appear to be at a higher risk of adverse effects associated with the treatment of atypical antipsychotics especially weight gain and drug induced diabetes mellitus.

In general, atypical antipsychotics share many of the side effects of typical antipsychotics, including sedation, akathisia, weight gain, extrapyramidal symptoms (EPS), neuromalignant syndrome, and tardive dyskinesia; longer experience with them have shown that new risks need to be considered, such as metabolic syndromes and QTc prolongation. QTc prolongation is known to have potential liability to produce fatal cardiac arrhythmias of Torsades de Pointes (TdP). Drug induced adverse metabolic effects such as weight gain, lipid abnormalities, and diabetes mellitus have been identified as a major risk factor for various medical disorders that might be responsible for some of the increased morbidity and mortality rates in psychotic patients treated with atypical antipsychotics.

Off-target pharmacology and drug to drug interactions are mainly responsible for most of the adverse side effects associated with the atypical antipsychotics. All the atypical antipsychotic drugs currently being used for the treatment of schizophrenia and related psychotic disorders have poor therapeutic target selectivity. For example, one of the most widely prescribed atypical antipsychotic drugs, Olanzapine and the most effective atypical antipsychotic drug, clozapine are reported to have significant activities against more than 12 receptors such as dopamine (D₁, D₂, D₃ and D₄), serotonin (5-HT_{2A}, 5-HT_{2C}, 5-HT₆, and 5-HT₇), adrenergic (alpha 1 and alpha 2), histamine (H₁), muscarinic (M₁), Dopamine transporter (DAT) and norepinephrine transporter (NET) receptors (Miyamoto et al., Molecular Psychiatry, 2005, 10, 79). Similarly, the other FDA approved atypical antipsychotics such as risperidone and aripiprazole are also reported to have significant activities against more than nine of the receptors mentioned above. The current research suggests that compounds exhibiting activity against dopamine (D₂) and serotonin (5-HT_{1A} and 5-HT_{2A}) receptors may have the intended antipsychotic effect (Snyder, S. H., Nature 2008, 452, 38-39; Di Pietro, N. C., Seamans, J. K., Pharmacopsychiatry 2007, 40(S1), S27-S33; Stark, A. D. et al., Psychopharmacology 2007, 190, 373-382) while compounds exhibiting activity against other receptors like serotonin, 5HT_{2C}, histamine (H₁), and adrenergic (alpha 1) may cause adverse side effects such as cardiac arrhythmias.

Although, the atypical antipsychotics (aripiprazole, clozapine, risperidone, olanzapine, quetiapine, and ziprasidone)

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currently in clinical use represent significant advances in treatment of people with schizophrenia, there is a need for new psychotropic drugs with improved safety profiles.

Therefore, development of a novel antipsychotic that has improved therapeutic target selectivity than the currently available therapies would provide effective and safer medicines for the treatment of schizophrenia and related psychotic disorders.

SUMMARY OF THE INVENTION

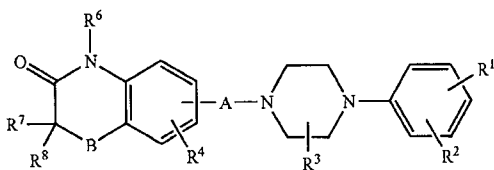
The present invention provides compounds, synthesis of the compounds, compositions and methods of using the compounds for treating schizophrenia and related psychoses such as acute manic, bipolar disorder, autistic disorder and depression, where the compounds are arylpiperazine derivatives. The present invention provides methods for synthesizing such arylpiperazine compounds. The present invention also provides methods for using arylpiperazine based atypical antipsychotics, and composition of arylpiperazine based atypical antipsychotics for treating schizophrenia and related psychoses such as acute manic, bipolar disorder, autistic disorder and depression.

The compounds of the subject invention provide next generation novel antipsychotics that are particularly effective and safer for the treatment of schizophrenia. They are advantageous because of their highly desirable pharmacological, metabolic, and pharmacokinetics profiles. The compounds of the invention are designed:

- 1) to exhibit affinity for dopamine 2 (D_2) receptor;
- 2) to exhibit affinity for serotonin 1A ($5-HT_{1A}$) receptor;
- 3) to exhibit affinity for serotonin 2A ($5-HT_{2A}$) receptors
- 4) to form therapeutically inactive or least active metabolite(s).

In one aspect, the present invention provides arylpiperazine derivatives comprising compounds of Formula (1):

Formula 1



wherein:

A is $-(CH_2)_n-$, $-O-(CH_2)_n-$, $-S-(CH_2)_n-$, $-S(O)$
 $(O)-(CH_2)_n-$, $-NH-(CH_2)_n-$, $-CH_2-O-(CH_2)_n-$,
 $-(CH_2)_n-O-CH_2-CH_2-$, $-CH_2-S-(CH_2)_n-$,
 $-(CH_2)_n-S-CH_2-CH_2-$, $-CH_2-S(O)(O)-$
 $(CH_2)_n-$, $-(CH_2)_n-S(O)(O)-CH_2-CH_2-$, $-O-C$
 $(O)-(CH_2)_n-$, $-S-C(O)-(CH_2)_n-$, $-NH-C(O)-$
 $(CH_2)_n-$, $-CH_2-C(O)-O-(CH_2)_n-$, $-CH_2-C$
 $(O)-NH-(CH_2)_n-$, $-CH_2-C(O)-S-(CH_2)_n-$,
 $-(CH_2)_n-C(O)-O-CH_2-CH_2-$, $-(CH_2)_n-C$
 $(O)-NH-CH_2-CH_2-$, $-(CH_2)_n-C(O)-S-$
 CH_2-CH_2- , $-CH_2-O-C(O)-(CH_2)_n-$, $-CH_2-$
 $NH-C(O)-(CH_2)_n-$, $-CH_2-S-C(O)-(CH_2)_n-$,
 $(CH_2)_n-O-C(O)-CH_2-CH_2-$, $(CH_2)_n-NH-C$
 $(O)-CH_2-CH_2-$, or $(CH_2)_n-S-C(O)-CH_2-$
 CH_2- , wherein n is an integer from 1 to 7;

B is O, S, S(O)(O), or NR^5 ; and
 each of R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , and R^8 is independently
 hydrogen, alkyl, substituted alkyl, aryl, substituted aryl,

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arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, acylalkyloxycarbonyl, acyloxyalkyloxycarbonyl, acylalkyloxycarbonylamino, acyloxyalkyloxycarbonylamino, alkoxy, alkoxyalkyl, alkoxyalkylalkoxy, alkoxyalkylalkylamino, alkylsulfinyl, alkylsulfonyl, alkylthio, amino, alkylamino, arylalkylamino, dialkylamino, arylalkoxy, arylalkoxyalkoxy, arylalkoxyalkylalkylamino, aryloxyalkoxy, arylalkoxyalkoxy, aryloxyalkoxyalkylalkylamino, carboxy, carbamoyl, carbamate, carbonate, cyano, halo, heteroaryloxycarbonyl, hydroxy, phosphate, phosphonate, sulfate, sulfonate, or sulfonamide, wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 and R^8 and A may optionally be substituted with isotopes that include, but not limited to ^2H (deuterium), ^3H (tritium), ^{13}C , ^{36}Cl , ^{18}F , ^{15}N , ^{17}O , ^{18}O , ^{31}P , ^{32}P , and ^{35}S ;

or a pharmaceutically acceptable salt, racemate or diastereomeric mixtures thereof

In one aspect of the invention, pharmaceutical compositions are provided comprising the compounds of the present disclosure.

In one aspect of the invention, methods of treating one of psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression are described, comprising administering to a patient in need thereof the compounds of the present disclosure.

In one aspect of the invention, compounds of the present disclosure are used to treat psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression.

In one aspect of the invention, compounds of present disclosure are used in the manufacture of a medicament for use in the treatment of psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression treat psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to synthesis, compositions and methods of using arylpiperazine derivatives which are useful for treating schizophrenia and related psychoses such as acute manic, bipolar disorder, autistic disorder and depression. The present invention provides compounds, compositions and methods for pharmacological treatment of schizophrenia and related psychoses such as acute manic, bipolar disorder, autistic disorder, and depression.

Definitions

Unless otherwise stated, the following terms used in this application, including the specification and claims, have the definitions given below. Any terms not directly defined herein shall be understood to have the meanings commonly associated with them as understood within the art of the present disclosure.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

Definition of standard chemistry terms may be found in reference works, including Carey and Sundberg (1992) "Advanced Organic Chemistry 3rd Ed." Vols. A and B, Plenum Press, New York. The practice of the present invention will employ, unless otherwise indicated, conventional methods of mass spectroscopy, protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. The compositions and formulations described

herein can be practiced employing the pharmaceutically acceptable excipients and salts available in *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pa.: Mack Publishing Company, 1990).

"Compounds of the invention" refers to compounds encompassed by structural Formula (1) as disclosed herein. The compounds of the invention can be identified either by their chemical structure and/or chemical name. When the chemical structure and chemical name conflict, the chemical structures is determinative of the identity of the compound. The compounds of the invention may contain one or more chiral centers and/or double bonds and therefore, may exist as stereoisomers, such as double-bond isomers (i.e., geometric isomers), enantiomers or diastereoisomers. Accordingly, the chemical structures depicted herein encompass all possible enantiomers and stereoisomers of the illustrated compounds including the stereoisomerically pure form (e.g., geometrically pure, enantiomerically pure or diastereomerically pure) and enantiomeric and stereoisomeric mixtures. Enantiomeric and stereoisomeric mixtures can be resolved into their component enantiomers or stereoisomers using separation techniques or chiral synthesis techniques well known to the skilled artisan. The compounds of the invention may also exist in several tautomeric forms including the enol form, the keto form and mixtures thereof. Accordingly, the chemical structures depicted herein encompass all possible tautomeric forms of the illustrated compounds. The compounds of the invention also include isotopically labeled compounds where one or more atoms have an atomic mass different from the atomic mass of conventionally found in nature. Examples of isotopes that may be incorporated into the compounds of the invention include, but are not limited to ²H, ³H, ¹³C, ¹⁵N, ¹⁸O, ¹⁷O, ³¹P, ³²P, ³⁵S, ¹⁸F and ³⁶Cl. Further, it should be understood, when partial structures of the compounds of the invention are illustrated, that brackets of dashes indicate the point of attachment of the partial structure to the rest of the molecule.

"Composition of the invention" refers to at least one compound of the invention and a pharmaceutically acceptable vehicle, with which the compound is administered to a patient. When administered to a patient, the compounds of the invention are administered in isolated form, which means separated from a synthetic organic reaction mixture.

"Alkyl" refers to a saturated or unsaturated, branched, straight-chain or cyclic monovalent hydrocarbon radical derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene or alkyne. Typical alkyl groups include, but are not limited to methyl; ethyls such as ethanyl, ethenyl, ethynyl; propyls such as propan-1-yl, propan-2-yl, cyclopropan-1-yl, prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl(allyl), cycloprop-1-en-1-yl, cycloprop-2-en-1-yl, prop-1-yn-1-yl, prop-2-yn-1-yl, etc.; butyls such as butan-1-yl, butan-2-yl, 2-methyl-propan-1-yl, 2-methyl-propan-2-yl, cyclobutan-1-yl, but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, etc.; and the like.

The term "alkyl" specifically intended to include radicals having any degree or level of saturation, i.e., groups having exclusively single carbon-carbon bonds, groups having one or more double carbon-carbon bonds, groups having one or more triple carbon-carbon bonds and groups having mixtures of single, double and triple carbon-carbon bonds. Where a specific level of saturation is intended, the expressions "alka-

nyl," "alkenyl," and "alkynyl," are used. Preferably, an alkyl group comprises from 1-20 carbon atoms, more preferably, from 1 to 10 carbon atoms.

"Alkanyl" refers to a saturated branched, straight-chain or cyclic alkyl radical derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane. Typical alkanyl groups include but are not limited to, methanyl; ethanyl; propanyl such as propan-1-yl, propan-2-yl(isopropyl), cyclopropan-1-yl, etc.; butanyl such as butan-1-yl, butan-2-yl(sec-butyl), 2-methyl-propan-1-yl(isobutyl), 2-methyl-propan-2-yl(t-butyl), cyclobutan-1-yl, etc.; and the like.

"Alkenyl" refers to an unsaturated branched, straight-chain or cyclic alkyl radical having at least one carbon-carbon double bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkene. The group may be in either the cis or trans conformation about the double bond(s). Typical alkenyl groups include, but are not limited to, ethenyl; propenyls such as prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl(allyl), prop-2-en-2-yl, cycloprop-1-en-1-yl, cycloprop-2-en-1-yl; butenyls such as but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, etc.; and the like.

"Alkynyl" refers to an unsaturated branched, straight-chain or cyclic alkyl radical having at least one carbon-carbon triple bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkyne. Typical alkynyl groups include, but are not limited to, ethynyl; propynyls such as prop-1-yn-1-yl, prop-2-yn-1-yl, etc.; butynyls such as but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, etc.; and the like.

"Acy" refers to a radical —C(O)R , where R is hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to formyl, acetyl, cyclohexylcarbonyl, cyclohexylmethylcarbonyl, benzoyl, benzylcarbonyl and the like.

"Acyloxyalkyloxycarbonyl" refers to a radical $\text{—C(O)OCR'R''OC(O)R''}$, where R', R'', and R''' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but not limited to $\text{—C(O)OCH}_2\text{OC(O)CH}_3$, $\text{—C(O)OCH}_2\text{OC(O)CH}_2\text{CH}_3$, $\text{—C(O)OCH(CH}_3\text{)OC(O)CH}_2\text{CH}_3$, $\text{—C(O)OCH(CH}_3\text{)OC(O)C}_6\text{H}_5$, and the like.

"Acyloxyalkyloxycarbonyl" refers to a radical $\text{—C(O)OCR'R''C(O)R'''}$, where R', R'', and R''' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but not limited to $\text{—C(O)OCH}_2\text{C(O)CH}_3$, $\text{—C(O)OCH}_2\text{C(O)CH}_2\text{CH}_3$, $\text{—C(O)OCH(CH}_3\text{)C(O)CH}_2\text{CH}_3$, $\text{—C(O)OCH(CH}_3\text{)C(O)C}_6\text{H}_5$, and the like.

"Acyloxyalkyloxycarbonylamino" refers to a radical $\text{—NRC(O)OCR'R''OC(O)R'''}$, where R, R', R'', and R''' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but not limited to $\text{—NHC(O)OCH}_2\text{OC(O)CH}_3$, $\text{—NHC(O)OCH}_2\text{OC(O)CH}_2\text{CH}_3$, $\text{—NHC(O)OCH(CH}_3\text{)OC(O)CH}_2\text{CH}_3$, $\text{—NHC(O)OCH(CH}_3\text{)OC(O)C}_6\text{H}_5$, and the like.

"Acylalkyloxycarbonylamino" refers to a radical $\text{—NRC(O)OCR'R"C(O)R"}$, where R, R', R", and R''' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but not limited to $\text{—NHC(O)OCH}_2\text{C(O)CH}_3$, $\text{—NHC(O)OCH}_2\text{C(O)CH}_2\text{CH}_3$, $\text{—NHC(O)OCH(CH}_3\text{)C(O)CH}_2\text{CH}_3$, $\text{—NHC(O)OCH(CH}_3\text{)C(O)C}_6\text{H}_5$, and the like.

"Acylamino" refers to "amide" as defined herein.

"Alkylamino" means a radical —NHR where R represents an alkyl, or cycloalkyl group as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to, methylamino, ethylamino, 1-methylethylamino, cyclohexylamino and the like.

"Alkoxy" refers to a radical —OR where R represents an alkyl, or cycloalkyl group as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to methoxy, ethoxy, propoxy, butoxy, cyclohexyloxy and the like.

"Alkoxy carbonyl" refers to a radical —C(O)—alkoxy where alkoxy is as defined herein.

"Alkoxy carbonylalkoxy" refers to a radical $\text{—OCR'R"C(O)—alkoxy}$ where alkoxy is as defined herein. Similarly, where R' and R" are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to $\text{—OCH}_2\text{C(O)OCH}_3$, $\text{—OCH}_2\text{C(O)OCH}_2\text{CH}_3$, $\text{—OCH(CH}_3\text{)C(O)OCH}_2\text{CH}_3$, $\text{—OCH(CH}_3\text{)C(O)OCH}_2\text{CH}_3$, $\text{—OCH(CH}_2\text{C}_6\text{H}_5\text{)C(O)OCH}_2\text{CH}_3$, $\text{—OC(CH}_3\text{)(CH}_3\text{)C(O)OCH}_2\text{CH}_3$, and the like.

"Alkoxy carbonylalkylamino" refers to a radical $\text{—NRCR'R"C(O)—alkoxy}$ where alkoxy is as defined herein. Similarly, where R, R', R" and R''' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to $\text{—NHCH}_2\text{C(O)OCH}_3$, $\text{—N(CH}_3\text{)CH}_2\text{C(O)OCH}_2\text{CH}_3$, $\text{—NHCH(CH}_3\text{)C(O)OCH}_2\text{CH}_3$, $\text{—NHCH(CH}_3\text{)C(O)OCH}_2\text{CH}_3$, $\text{—NHCH(CH}_2\text{C}_6\text{H}_5\text{)C(O)OCH}_2\text{CH}_3$, $\text{—NHC(CH}_3\text{)(CH}_3\text{)C(O)OCH}_2\text{CH}_3$, and the like.

"Alkylsulfonyl" refers to a radical $\text{—S(O)}_2\text{R}$ where R is an alkyl, or cycloalkyl group as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to, methylsulfonyl, ethylsulfonyl, propylsulfonyl, butylsulfonyl, and the like.

"Alkylsulfinyl" refers to a radical —S(O)R where R is an alkyl, or cycloalkyl group as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to, methylsulfinyl, ethylsulfinyl, propylsulfinyl, butylsulfinyl, and the like.

"Alkylthio" refers to a radical —SR where R is an alkyl or cycloalkyl group as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to methylthio, ethylthio, propylthio, butylthio, and the like.

"Amide" or "acylamino" refers to a radical —NR'C(O)R' , where R' and R" are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, het-

eroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to, formylamino, acetylamino, cyclohexylcarbonylamino, cyclohexylmethylcarbonyl-amino, benzoylamino, benzylcarbonylamino and the like.

"Amino" refers to the radical —NH_2 .

"Aryl" refers to a monovalent aromatic hydrocarbon radical derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, groups derived from acenaphthylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, coronene, fluoranthene, fluorine, hexacene, hexaphene, hexalene, as-indacene, s-indacene, indane, indene, naphthalene, octacene, octaphene, octalene, ovalene, penta-2,4-diene, pentacene, pentalene, pentaphene, perylene, phenalene, phenanthrene, picene, pleidene, pyrene, pyranthrene, rubicene, triphenylene, trinaphthalene, and the like. Preferable, an aryl group comprises from 6 to 20 carbon atoms, more preferably, between 6 to 12 carbon atoms.

"Arylalkyl" refers to an acyclic alkyl in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl group. Typically arylalkyl groups include, but not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethene-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. Where specific alkyl moieties are intended, the nomenclature arylalkany, arylalkenyl and/or arylalkynyl is used. Preferably, an arylalkyl group is $(\text{C}_6\text{—C}_{30})$ arylalkyl, e.g., the alkanyl, alkenyl or alkynyl moiety of the arylalkyl group is $(\text{C}_1\text{—C}_{10})$ and the aryl moiety is $(\text{C}_6\text{—C}_{20})$, more preferably, an arylalkyl group is $(\text{C}_6\text{—C}_{20})$ arylalkyl, e.g., the alkanyl, alkenyl or alkynyl moiety of the arylalkyl group is $(\text{C}_1\text{—C}_8)$ and the aryl moiety is $(\text{C}_6\text{—C}_{12})$.

"Arylalkoxy" refers to an —O—arylalkyl radical where arylalkyl is as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Arylalkoxycarbonylalkoxy" refers to a radical $\text{—OCR'R"C(O)—arylalkoxy}$ where arylalkoxy is as defined herein. Similarly, where R' and R" are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to $\text{—OCH}_2\text{C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—OCH(CH}_3\text{)C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—OCH(CH}_3\text{)C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—OCH(CH}_2\text{C}_6\text{H}_5\text{)C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—OC(CH}_3\text{)(CH}_3\text{)C(O)OCH}_2\text{C}_6\text{H}_5$, and the like.

"Arylalkoxycarbonylalkylamino" refers to a radical $\text{—NRCR'R"C(O)—arylalkoxy}$ where arylalkoxy is as defined herein. Similarly, where R, R', R" and R''' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to $\text{—NHCH}_2\text{C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—N(CH}_3\text{)CH}_2\text{C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—NHCH(CH}_3\text{)C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—NHCH(CH}_3\text{)C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—NHCH(CH}_2\text{C}_6\text{H}_5\text{)C(O)OCH}_2\text{C}_6\text{H}_5$, $\text{—NHC(CH}_3\text{)(CH}_3\text{)C(O)OCH}_2\text{C}_6\text{H}_5$, and the like.

"Aryloxycarbonyl" refers to radical C(O)—O—aryl where aryl is defined herein that may be optionally substituted by one or more substituents as defined herein.

"Aryloxycarbonylalkoxy" refers to a radical $\text{—OCR'R"C(O)—aryloxy}$ where aryloxy is as defined herein. Similarly, where R' and R" are each independently hydrogen, alkyl,

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cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to $-\text{OCH}_2\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{OCH}(\text{CH}_3)\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{OCH}(\text{C}_6\text{H}_5)\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{OCH}(\text{CH}_2\text{C}_6\text{H}_5)\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{OC}(\text{CH}_3)(\text{CH}_3)\text{C}(\text{O})\text{OC}_6\text{H}_5$, and the like.

"Aryloxy carbonylalkylamino" refers to a radical $-\text{NRCR}'\text{R}''\text{C}(\text{O})\text{-aryloxy}$ where aryloxy is as defined herein. Similarly, where R, R', R'' and R''' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to $-\text{NHCH}_2\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{N}(\text{CH}_3)\text{CH}_2\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{NHCH}(\text{CH}_3)\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{NHCH}(\text{C}_6\text{H}_5)\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{NHCH}(\text{CH}_2\text{C}_6\text{H}_5)\text{C}(\text{O})\text{OC}_6\text{H}_5$, $-\text{NHC}(\text{CH}_3)(\text{CH}_3)\text{C}(\text{O})\text{OC}_6\text{H}_5$, and the like.

"Carbamoyl" refers to the radical $-\text{C}(\text{O})\text{NRR}$ where each R group is independently, hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Carbamate" refers to a radical $-\text{NR}'\text{C}(\text{O})\text{OR}$, where R' and R are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to, methylcarbamate ($-\text{NHC}(\text{O})\text{OCH}_3$), ethylcarbamate ($-\text{NHC}(\text{O})\text{OCH}_2\text{CH}_3$), benzylcarbamate ($-\text{NHC}(\text{O})\text{OCH}_2\text{C}_6\text{H}_5$), and the like.

"Carbonate" refers to a radical $-\text{OC}(\text{O})\text{OR}$, where R is alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to, methyl carbonate ($-\text{C}(\text{O})\text{OCH}_3$), cyclohexyl carbonate ($-\text{C}(\text{O})\text{OC}_6\text{H}_{11}$), phenyl carbonate ($-\text{C}(\text{O})\text{OC}_6\text{H}_5$), benzyl carbonate ($\text{C}(\text{O})\text{OCH}_2\text{C}_6\text{H}_5$), and the like.

"Carboxy" means the radical $-\text{C}(\text{O})\text{OH}$.

"Cyano" means the radical $-\text{CN}$.

"Cycloalkyl" refers to a substituted or unsubstituted cyclic alkyl radical. Where a specific level of saturation is intended, the nomenclature "cycloalkanyl" or "cycloalkenyl" is used. Typical cycloalkyl groups include, but are not limited to, groups derived from cyclopropane, cyclobutane, cyclopentane, cyclohexane, and the like. In a preferred embodiment, the cycloalkyl group is ($\text{C}_3\text{-C}_{10}$) cycloalkyl, more preferably ($\text{C}_3\text{-C}_7$) cycloalkyl.

"Cycloheteroalkyl" refers to a saturated or unsaturated cyclic alkyl radical in which one or more carbon atoms (and any associated hydrogen atoms) are independently replaced with the same or different heteroatom. Typical heteroatoms to replace the carbon atom(s) include, but are not limited to, N, P, O, S, Si, etc. Where a specific level of saturation is intended, the nomenclature "cycloheteroalkanyl" or "cycloheteroalkenyl" is used. Typical cycloheteroalkyl groups include, but are not limited to, groups derived from epoxides, imidazolidine, morpholine, piperazine, piperidine, pyrazolidine, pyrrolidine, quinuclidine, and the like.

"Cycloheteroalkoxycarbonyl" refers to a radical $\text{C}(\text{O})\text{OR}$ where R is cycloheteroalkyl as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Dialkylamino" means a radical $-\text{NRR}'$ where R and R' independently represent an alkyl or cycloalkyl group as

defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to dimethylamino, methylethylamino, di-(1-methylethyl)amino, (cyclohexyl)(methyl)amino, (cyclohexyl)(ethyl)amino, (cyclohexyl)(propyl)amino, and the like.

"Derived from a drug" refers to a fragment that is structurally related to such a drug. The structure of the fragment is identical to the drug except where a hydrogen atom attached to a heteroatom (N or O) has been replaced with a covalent bond to another group (typically, a promoity). Note that when a drug is a salt form of a carboxylic, phosphonic or phosphoric acid, the corresponding structural fragment derived from such a drug is considered to be derived from the protonated acid form.

"Drug" refers to a compound that exhibits therapeutic and/or prophylactic and/or diagnostic utility when administered in effective amounts to a patient or a mammal.

"Ester" refers to a radical $-\text{C}(\text{O})\text{OR}$, where R is alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, heteroalkyl, substituted heteroalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl as defined herein that may be optionally substituted by one or more substituents as defined herein. Representative examples include, but are not limited to, methyl ester ($-\text{C}(\text{O})\text{OCH}_3$), cyclohexyl ester ($-\text{C}(\text{O})\text{OC}_6\text{H}_{11}$), phenyl ester ($-\text{C}(\text{O})\text{OC}_6\text{H}_5$), benzyl ester ($-\text{C}(\text{O})\text{OCH}_2\text{C}_6\text{H}_5$), and the like.

"Ether" refers to a radical $-\text{OR}$, where R is alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Halo" means fluoro, chloro, bromo, or iodo.

"Heteroalkoxy" means an $-\text{O-heteroalkyl}$ radical where heteroalkyl is as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Heteroalkyl, Heteroalkanyl, Heteroalkenyl, Heteroalkynyl" refer to alkyl, alkanyl, alkenyl and alkynyl groups, respectively, in which one or more of the carbon atoms (and any associated hydrogen atoms) are each independently replaced with the same or different heteroatomic groups. Typical heteroatomic groups include, but are not limited to $-\text{O}-$, $-\text{S}-$, $-\text{O}-\text{O}-$, $-\text{S}-\text{S}-$, $-\text{OS}-$, $-\text{NR}'-$, $=\text{N}-$, $-\text{N}=\text{N}-$, $-\text{N}=\text{N}-\text{NR}'-$, $-\text{PH}-$, $-\text{P}(\text{O})_2$, $-\text{O}-\text{P}(\text{O})-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{SnH}_2$, and the like, wherein R' is hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl or substituted aryl that may be optionally substituted by one or more substituents as defined herein.

"Heteroaryl" refers to a monovalent heteroaromatic radical derived by the removal of one hydrogen atom from a single atom of a parent heteroaromatic ring system. Typical heteroaryl groups include, but are not limited to, groups derived from acridine, arindole, carbazole, carboline, chromane, chromene, cinnoline, furan, imidazole, indazole, indole, indoline, indolizine, isobenzofuran, isochromene, isoindole, isoindoline, isoquinoline, isothiazole, isoxazole, naphthyridine, oxadiazole, oxazole, perimidine, phenanthridine, phenanthroline, phenazine, phthalazine, pteridine, purine, pyran, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolizine, quinazoline, quinoxaline, quinolizine, quinoxaline, tetrazole, thiadiazole, thiazole, thiophene, triazole, xanthene, and the like. Preferably, the heteroaryl group is between 5-20 membered heteroaryl, with 5-10 membered heteroaryl being particularly preferred. Preferred heteroaryl

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groups are those derived from thiophene, pyrrole, benzothiofene, benzofuran, indole, pyridine, quinoline, imidazole, oxazole and pyrazine.

"Heteroarylloxycarbonyl" refers to a radical —C(O)—OR where R is heteroaryl as defined that may be optionally substituted by one or more substituents as defined herein.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl group. Where specific alkyl moieties are intended, the nomenclature heteroarylalkanyl, heteroarylalkenyl and/or heteroarylalkynyl is used. Preferably, the heteroarylalkyl radical is a 6-30 carbon membered heteroarylalkyl, e.g., the alkanyl, alkenyl or alkynyl moiety of the heteroarylalkyl is 1-10 membered and the heteroaryl moiety is a 5-20 membered heteroaryl, more preferably, a 6-20 membered heteroarylalkyl, e.g., the alkanyl, alkenyl or alkynyl moiety of the heteroarylalkyl is 1-8 membered and the heteroaryl moiety is a 5-12 membered heteroaryl.

"Hydroxy" means the radical —OH .

"Oxo" means the divalent radical =O .

As used herein, the term "patient" encompasses mammals and non-mammals.

Examples of mammals include, but are not limited to, any member of the Mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish and the like. The term does not denote a particular age or gender.

"Pharmaceutically acceptable" means approved or approvable by a regulatory agency of the Federal or state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly in humans.

"Pharmaceutically acceptable salt" refers to a salt of a compound of the invention, which is pharmaceutically acceptable and possesses the desired pharmacological activity of the parent compound. Such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentane propionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedithiosulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2,2,2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, laurylsulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine and the like.

"Pharmaceutically acceptable vehicle" refers to a diluent, adjuvant, excipient or carrier with which a compound of the invention is administered.

"Phosphate" refers to a radical —OP(O)(OR')(OR'') , where R' and R'' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Phosphonate" refers to a radical —P(O)(OR')(OR'') , where R' and R'' are each independently hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Preventing" or "Prevention" refers to a reduction in risk of acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease).

"Protecting group" refers to a group of atoms that when attached to a reactive group in a molecule masks, reduces or prevents that reactivity. Examples of protecting groups can be found in Green et al., "Protective Groups in Organic Chemistry", (Wiley, 2nd ed. 1991) and Harrison et al., "Compendium of Synthetic Organic Methods", vols. 1-8 (John Wiley and Sons, 1971-1996). Representative amino protecting groups include, but are not limited to, formyl, acetyl, trifluoroacetyl, benzyl, benzyloxycarbonyl ("CBZ"), tert-butoxycarbonyl ("Boc"), trimethylsilyl ("TMS"), 2-trimethylsilyl-ethanesulfonyl ("SES"), trityl and substituted trityl groups, allyloxycarbonyl, 9-fluorenylmethyloxy-carbonyl ("FMOC"), nitroveratryloxycarbonyl ("NVOC") and the like. Representative hydroxyl protecting groups include, but are not limited to, those where the hydroxyl group is either acylated or alkylated such as benzyl, and trialkylsilyl ethers and allyl ethers.

"Racemate" refers to an equimolar mixture of enantiomers of a chiral molecule.

"Substituted" refers to a group in which one or more hydrogen atoms are each independently replaced with the same or different substituents(s). Typical substituents include, but are not limited to, —X , —R^{54} , —O^- , =O , —OR^{54} , —SR^{54} , —S , =S , $\text{—NR}^{54}\text{R}^{55}$, =NR^{54} , —CX_3 , —CF_3 , —CN , —OCN , —SCN , —NO , —NO_2 , =N_2 , —N_3 , $\text{—S(O)}_2\text{O}^-$, $\text{—S(O)}_2\text{OH}$, $\text{—S(O)}_2\text{OR}^{54}$, $\text{—OS(O)}_2\text{O}^{31}$, $\text{—OS(O)}_2\text{R}^{54}$, —P(O)(O—)_2 , $\text{—P(O)(OR}^{14})(\text{O}^{31})$, $\text{—OP(O)(OR}^{54})(\text{OR}^{55})$, —C(O)R^{54} , —C(S)R^{54} , —C(O)OR^{54} , $\text{—C(O)NR}^{54}\text{R}^{55}$, —C(O)O^- , —C(S)OR^{54} , $\text{—NR}^{56}\text{C(O)NR}^{54}\text{R}^{55}$, $\text{—NR}^{56}\text{C(S)NR}^{54}\text{R}^{55}$, $\text{—NR}^{57}\text{C(NR}^{56})\text{NR}^{54}\text{R}^{55}$, and $\text{—C(NR}^{56})\text{NR}^{54}\text{R}^{55}$, where each X is independently a halogen; each R^{54} , R^{55} , R^{56} and R^{57} are independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, heteroalkyl, substituted heteroalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, $\text{—NR}^{58}\text{R}^{59}$, —C(O)R^{58} or $\text{—S(O)}_2\text{R}^{58}$ or optionally R^{58} and R^{59} together with the atom to which they are both attached form a cycloheteroalkyl or substituted cycloheteroalkyl ring; and R^{58} and R^{59} are independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, heteroalkyl, substituted heteroalkyl, heteroaryl, substituted heteroarylalkyl, substituted heteroarylalkyl.

"Sulfate" refers to a radical —OS(O)(O)OR , where R is hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Sulfonamide" refers to a radical —S(O)(O)NR'R'' , where R' and R'' are independently hydrogen, alkyl, cycloalkyl,

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cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein or optionally R' and R'' together with the atom to which they are both attached form a cycloheteroalkyl or substituted cycloheteroalkyl ring. Representative examples include but not limited to azetidiny, pyrrolidinyl, piperidinyl, morpholinyl, 4-(NR''')-piperazinyl or imidazolyl group wherein said group may be optionally substituted by one or more substituents as defined herein. R''' hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Sulfonate" refers to a radical $-\text{S}(\text{O})(\text{O})\text{R}$, where R is hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein.

"Thio" means the radical $-\text{SH}$.

"Thioether" refers to a radical $-\text{SR}$, where R is alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl, as defined herein that may be optionally substituted by one or more substituents as defined herein.

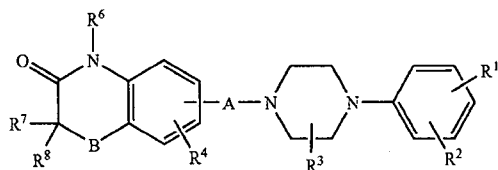
"Treating" or "treatment" of any disease or disorder refers, in one embodiment, to ameliorating the disease or disorder (i.e., arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another embodiment "treating" or "treatment" refers to ameliorating at least one physical parameter, which may not be discernible by the patient. In yet another embodiment, "treating" or "treatment" refers to inhibiting the disease or disorder, either physically (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In yet another embodiment, "treating" or "treatment" refers to delaying the onset of the disease or disorder.

"Therapeutically effective amount" means the amount of a compound that, when administered to a patient for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the compound, the disease and is severity and the age, weight, etc., of the patient to be treated, and can be determined by one of skill in the art without undue experimentation.

Reference now will be made in detail to preferred embodiments of the invention. While the invention will be described in conjunction with preferred embodiments, it will be understood that it is not intended to limit the invention to those preferred embodiments. To the contrary, it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

Compounds of the Invention

In one aspect of the present invention, compounds of Formula (1) are described:



wherein:

A is $-(\text{CH}_2)_n-$, $-\text{O}-(\text{CH}_2)_n-$, $-\text{S}-(\text{CH}_2)_n-$, $-\text{S}(\text{O})(\text{O})-(\text{CH}_2)_n-$, $-\text{NH}-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{O}-$

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$(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{O}-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{S}-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{S}-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{S}(\text{O})(\text{O})-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{S}(\text{O})(\text{O})-\text{CH}_2-\text{CH}_2-$, $-\text{O}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-\text{S}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{C}(\text{O})-\text{O}-$
 $(\text{CH}_2)_n-$, $-\text{CH}_2-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{C}(\text{O})-\text{S}-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{C}(\text{O})-\text{O}-\text{CH}_2-$
 CH_2- , $-(\text{CH}_2)_n-\text{C}(\text{O})-\text{NH}-\text{CH}_2-\text{CH}_2-$, $-(\text{CH}_2)_n-\text{C}(\text{O})-\text{S}-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{O}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{S}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{O}-\text{C}(\text{O})-\text{CH}_2-\text{CH}_2-$, $(\text{CH}_2)_n-\text{NH}-\text{C}(\text{O})-\text{CH}_2-\text{CH}_2-$, or $(\text{CH}_2)_n-\text{S}-\text{C}(\text{O})-\text{CH}_2-\text{CH}_2-$, wherein n is an integer from 1 to 7;

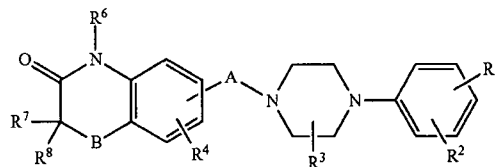
B is O, S, $\text{S}(\text{O})(\text{O})$, or NR^5 ; and

each of R¹, R², R³, R⁴, R⁵, R⁶, R⁷, and R⁸ is independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, acylalkyloxycarbonyl, acyloxyalkyloxycarbonyl, acylalkyloxycarbonylamino, acyloxyalkyloxycarbonylamino, alkoxy, alkoxyalkyl, alkoxyalkylalkoxy, alkoxyalkylalkylamino, alkylsulfinyl, alkylsulfonyl, alkylthio, amino, alkylamino, arylalkylamino, dialkylamino, arylalkoxy, arylalkoxyalkylalkoxy, arylalkoxyalkylalkylamino, arylalkoxyalkylalkylalkoxy, arylalkoxyalkylalkylalkylamino, carboxy, carbamoyl, carbamate, carbonate, cyano, halo, heteroarylalkoxy, hydroxy, phosphate, phosphonate, sulfate, sulfonate, or sulfonamide, wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and R⁸ and A may optionally be substituted with isotopes that include, but not limited to ²H (deuterium), ³H (tritium), ¹³C, ³⁶Cl, ¹⁸F, ¹⁵N, ¹⁷O, ¹⁸O, ³¹P, ³²P, and ³⁵S;

or a pharmaceutically acceptable salt, racemate or diastereomeric mixtures thereof

In another aspect, compounds of Formula (1a) are described:

Formula 1a



Formula 1

wherein:

A is $-(\text{CH}_2)_n-$, $-\text{O}-(\text{CH}_2)_n-$, $-\text{S}-(\text{CH}_2)_n-$, $-\text{S}(\text{O})(\text{O})-(\text{CH}_2)_n-$, $-\text{NH}-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{O}-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{O}-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{S}-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{S}-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-\text{S}(\text{O})(\text{O})-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{S}(\text{O})(\text{O})-\text{CH}_2-\text{CH}_2-$, $-\text{O}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-\text{S}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-\text{NH}-\text{C}(\text{O})-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{C}(\text{O})-\text{O}-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{C}(\text{O})-\text{NH}-(\text{CH}_2)_n-$, $-\text{CH}_2-\text{C}(\text{O})-\text{S}-(\text{CH}_2)_n-$, $-(\text{CH}_2)_n-\text{C}(\text{O})-\text{O}-\text{CH}_2-\text{CH}_2-$, $-(\text{CH}_2)_n-\text{C}(\text{O})-\text{NH}-\text{CH}_2-\text{CH}_2-$, or $(\text{CH}_2)_n-\text{S}-\text{C}(\text{O})-\text{CH}_2-\text{CH}_2-$, wherein n is an integer from 1 to 7;

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(O)—NH—CH₂—CH₂—, —(CH₂)_n—C(O)—S—
CH₂—CH₂—, —CH₂—O—C(O)—(CH₂)_n—, —CH₂—
NH—C(O)—(CH₂)_n—, —CH₂—S—C(O)—(CH₂)_n—,
—(CH₂)_n—O—C(O)—CH₂—CH₂—, (CH₂)_n—NH—C
(O)—CH₂—CH₂—, or (CH₂)_n—S—C(O)—CH₂—
CH₂—, wherein n is an integer from 1 to 7;

B is O, S, S(O)(O), or NR⁵; and

each of R¹, R², R³, R⁴, R⁵, R⁶, R⁷, and R⁸ is independently

hydrogen, alkyl, substituted alkyl, aryl, substituted aryl,
arylalkyl, substituted arylalkyl, cycloalkyl, substituted
cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl,
heteroaryl, substituted heteroaryl, heteroarylalkyl, substi-
tuted heteroarylalkyl, acylalkyloxycarbonyl, acyloxyalky-
loxycarbonyl, acylalkyloxycarbonylamino, acyloxyalky-
loxycarbonylamino, alkoxy, alkoxy carbonyl,
alkoxy carbonylalkoxy, alkoxy carbonylalkylamino, alkyl-
sulfinyl, alkylsulfonyle, alkylthio, amino, alkylamino, ary-
lalkylamino, dialkylamino, arylalkoxy, arylalkoxy carbo-
nylalkoxy, arylalkoxy carbonylalkylamino,
aryloxycarbonyl, aryloxycarbonylalkoxy, aryloxycarbo-
nylalkylamino, carboxy, carbamoyl, carbamate, carbonate,
cyano, halo, heteroaryloxycarbonyl, hydroxy, phosphate,
phosphonate, sulfate, sulfonate, or sulfonamide, wherein
R¹, R², R³, R⁴, R⁵, R⁶, R⁷ and R⁸ and A may optionally be
substituted with isotopes that include, but not limited to ²H
(deuterium), ³H (tritium), ¹³C, ³⁶Cl, ¹⁸F, ¹⁵N, ¹⁷O, ¹⁸O, ³¹P,
³²P, and ³⁵S;

or a pharmaceutically acceptable salt, racemate or diastereo-
meric mixtures thereof

In another aspect of the invention, A is —(CH₂)_n—.

In another aspect of the invention, A is —O—(CH₂)_n—,
—S—(CH₂)_n—, —CH₂—O—(CH₂)_n—, —(CH₂)_n—O—
CH₂—CH₂—, —CH₂—S—(CH₂)_n—, or —(CH₂)_n—S—
CH₂—CH₂—.

In another aspect of the invention, A is —NH—C(O)—
(CH₂)_n—, —CH₂—NH—C(O)—(CH₂)_n—, —CH₂—C
(O) NH (CH₂)_n or (CH₂)_n C(O) NH CH₂
CH₂—.

In another aspect of the invention, B is O.

In another aspect of the invention, R⁴ is H.

In another aspect of the invention, each of R¹ and R² is
independently H, halogen, haloalkyl or alkoxy.

The compounds of this invention described herein can have
one or more of the following characteristics or properties:

- Compounds of the invention can have affinity for
dopamine D₂ receptors;
- Compounds of the invention can have affinity for sero-
tonin 5-HT_{1A} receptors;
- Compounds of the invention can have affinity for sero-
tonin 5-HT_{2A} receptors;
- The primary metabolite(s), regardless of the electro-
physiological properties of the parent drug, has, or have,
negligible inhibitory activity at the HERG (human ether-
a-go-go related gene) potassium channel at the normal
therapeutic concentration of the parent drug in plasma
(e.g. the concentration of the metabolite must be at least
five times higher than the normal therapeutic concentra-
tion of the parent compound before activity at the HERG
potassium channel is observed);

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(e) Compounds of the invention, as well as the metabolites
thereof, do not cause, or have reduced incidence of meta-
bolic drug-drug interaction (DDI) when co-adminis-
tered with other drugs;

(f) Compounds of the invention, as well as metabolites
thereof, do not substantially elevate liver function test
(LFT) values when administered alone;

(g) Oral bioavailability of the compounds is consistent with
oral administration using standard pharmacological oral
formulations; however, the compounds, and composi-
tions thereof, can also be administered using any deliv-
ery system that produces constant and controllable
blood levels over time.

In one aspect, the invention provides compounds having
any two or more of the above-identified characteristics or
properties. In another aspect, the invention provides for com-
pounds having at least any three or more of the above-iden-
tified properties or characteristics. Preferably, the compounds
of the invention have all seven characteristics or properties.

Additional modifications of the compounds disclosed
herein can readily be made by those skilled in the art. Thus,
analog and salts of the exemplified compounds are within the
scope of the subject invention. With knowledge of the com-
pounds of the subject invention skilled artisans can use known
procedures to synthesize these compounds from available
substrates. As used in this application, the term "analog"
refers to compounds which are substantially the same as
another compound but which may have been modified by, for
example, adding additional side groups. The term "analog"
as used in this application also may refer to compounds which
are substantially the same as another compound but which
have atomic or molecular substitution at certain locations in
the compound.

The subject invention further pertains to enantiomerically
isolated compounds, and compositions comprising the com-
pounds. The isolated enantiomeric forms of the compounds
of the invention are substantially free from one another (i.e.,
in enantiomeric excess). In other words, the "R" forms of the
compounds are substantially free from the "S" forms of the
compounds and are, thus, in enantiomeric excess of the "S"
forms. Conversely, "S" forms of the compounds are substan-
tially free of "R" forms of the compounds and are, thus, in
enantiomeric excess of the "R" forms. In one embodiment of
the invention, the isolated enantiomeric compounds are at
least about in 80% enantiomeric excess. Thus, for example,
the compounds are at least about 90% enantiomeric excess,
preferably at least about 95% enantiomeric excess, more pre-
ferably at least about 97% enantiomeric excess, or even more
preferably, at least 99% or greater than 99% enantiomeric
excess.

Synthesis of the Compounds of the Invention

The compounds of the invention can be obtained via the
synthetic methods illustrated in Schemes 1-2. Several meth-
ods have been described in the art for the synthesis of arylpip-
erazine derivatives. The starting materials and building
blocks useful for preparing compounds of the invention and
intermediates thereof are commercially available or can be
prepared by well-known synthetic methods (see e.g., Green et
al., "Protective Groups in Organic Chemistry," (Wiley, 4th
ed., 2006); Harrison et al. "Compendium of Synthetic Organic
Methods," vols. 1-8 (John Wiley and Sons, 1971-1996);
"Beilstein Handbook of Organic Chemistry, Frankfurt, Ger-
many; Feiser et al., "Reagents for Organic Synthesis," Vol-

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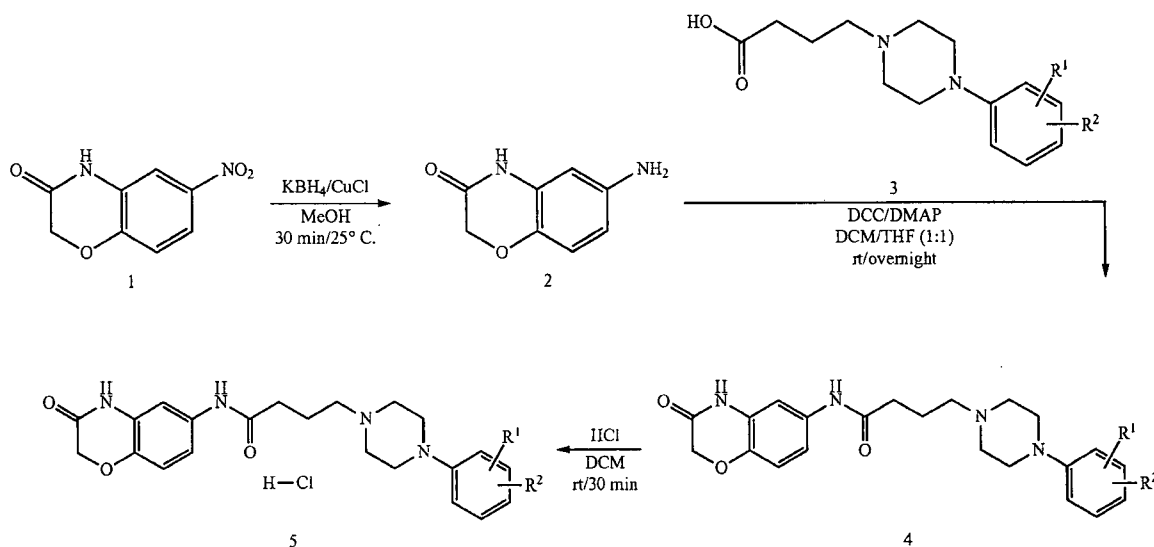
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umes 1-45, Karger, 1991; March, Advanced Organic Chemistry," Wiley Interscience, 4th ed., 1991; Larock "Comprehensive Organic Transformations," Wiley-VCH Publishers, 2nd ed., 1999; Paquette, "Encyclopedia of Reagents for Organic Synthesis," John Wiley and Sons, 1st ed., 1995). Other methods for the synthesis of arylpiperazine derivatives described herein are either described in the art or will be readily apparent to the skilled artisan in view of the references provided above and may be used to synthesize the compounds of the invention. Accordingly, the methods presented in the Schemes herein are illustrative rather than comprehensive.

In one method arylpiperazine derivatives comprising Formula (1) was prepared as described in Scheme 1. The starting building block 6-nitrobenzoxazinone 1 was purchased from the commercial source Sigma-Aldrich. The compound 1 can also be synthesized from a method well known in the literature. The reduction of nitro moiety in compound 1 using a reducing agent like potassium borohydride (KBH₄) in presence of a mild Lewis acid copper (I) chloride (CuCl) in a protic solvent such as methanol gave 6-aminobenzoxazinone 2. The target benzoxazinone 4 was prepared by coupling the amine 2 with a suitable carboxylic acid 3 under standard coupling conditions using dicyclohexylcarbodiimide (DCC) as coupling agent in presence of a mild base 4-(N,N-dimethylamino)pyridine (DMAP) in a polar aprotic solvent medium. The carboxylic acid 3 was prepared by the alkylation of a suitable arylpiperazine with an appropriate bromocarboxylic acid followed by saponification. The benzoxazinone derivative 4 was converted into hydrochloride salt 5 by treating with hydrogen chloride under standard conditions. The benzoxazinones 4 can also be converted into other form of pharmaceutically acceptable salts such as methanesulfonic acid salts and lower aliphatic carboxylic acid salts using well known methods in the field.

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In another method, arylpiperazine derivatives comprising Formula (1) were prepared as described in Scheme 2. The starting building block 4-methoxy-2-nitrophenol 6 was purchased from the commercial source Sigma-Aldrich. The nitrophenol 6 was alkylated with ethylbromoacetate 7 by heating in acetone in presence of a mild base potassium carbonate (K₂CO₃) to give the ester 8. The ester 8 was treated with aluminum chloride (AlCl₃) in anhydrous dichloromethane at reflux temperature to get the corresponding nitrophenol derivative 9. The compound 11 was prepared by alkylating the nitrophenol 9 with 1,4-dibromobutane 10 under identical reaction conditions described for preparing the compound 8 (Scheme 2). The reaction of compound 11 with arylpiperazine 12 in presence of N,N-diisopropylethylamine (DIEA) in acetonitrile at approximately 60-70° C. for 8 to 16 h gave the compound 13. The compound 13 when subjected to reduction conditions using iron(III)chloride in presence of metallic iron in ethanol and acetic acid solvent mixture at reflux temperature afforded the corresponding benzoxazinone 14 which was converted into hydrochloride salt 15 by treating with hydrogen chloride under standard conditions. Other standard nitro group reduction conditions like hydrogenation in presence of catalyst palladium on activated carbon (Pd/C) also gave corresponding cyclized product 14. The benzoxazinones 14 can also be converted into other form of pharmaceutically acceptable salts such as methanesulfonic acid salts and lower aliphatic carboxylic acid salts using well known methods in the field.

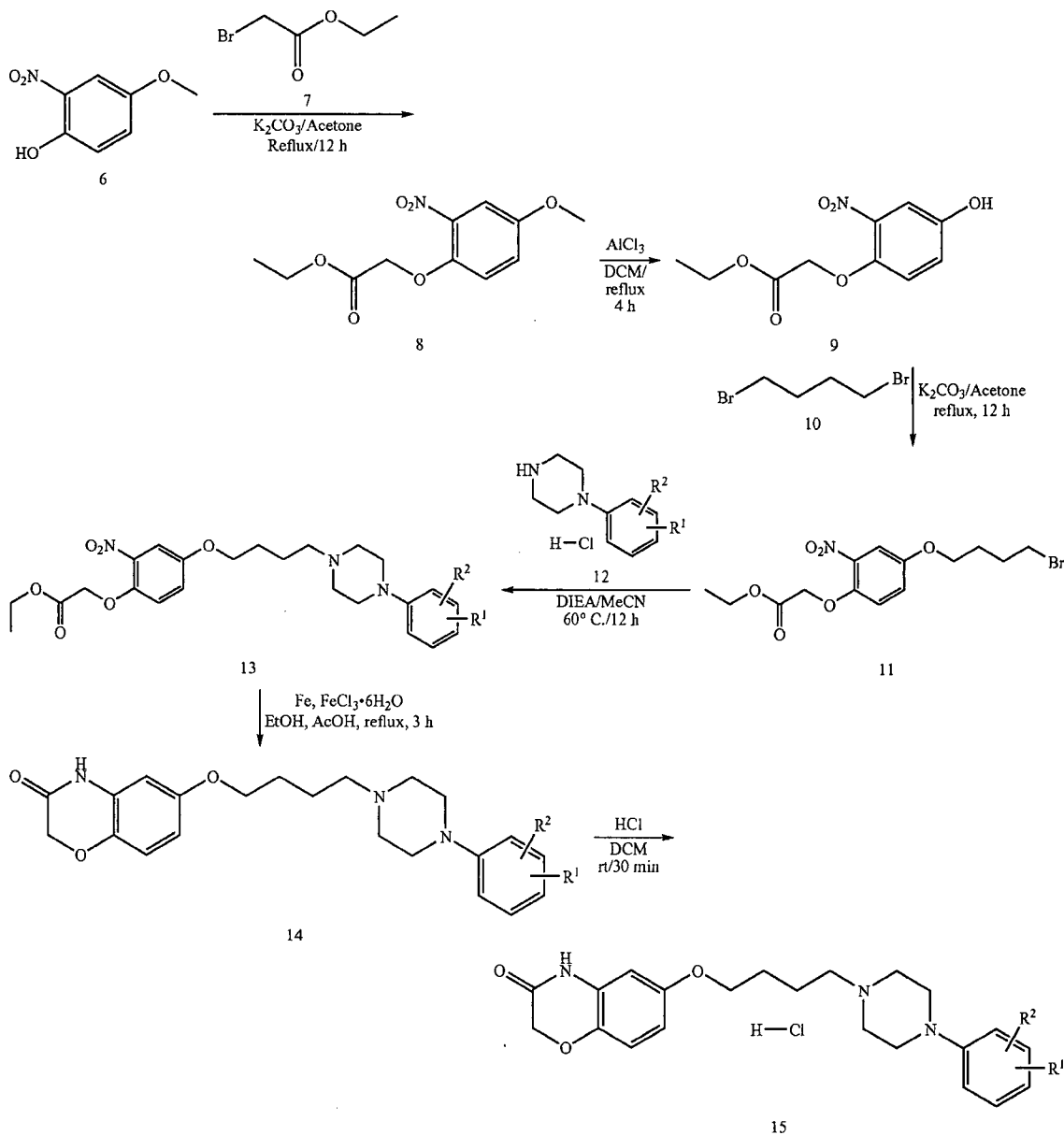


a) R¹ - 2-OMe, R² - H;
b) R¹ = 2-Cl, R² = 3-Cl

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Scheme 2



Therapeutic Uses of Compounds of Structural Formula (1)

The present invention relates to synthesis, compositions and methods of using arylpiperazine based compounds which are useful for treating schizophrenia and related psychoses such as acute maniac, bipolar disorder, autistic disorder and depression. The present invention provides methods for synthesizing such arylpiperazine based antipsychotic agents. The present invention also provides methods for using arylpiperazine based antipsychotic agents and composition of arylpiperazine based antipsychotic agents for treating schizophrenia and related psychoses such as acute maniac, bipolar disorder, autistic disorder and depression.

In accordance with the invention, a compound and/or a composition containing a compound of structural Formula (1) is administered to a patient, preferably a human, suffering

from schizophrenia. Further, in certain embodiments, the compounds and/or compositions of the invention are administered to a patient, preferably a human, as a treatment or preventive measure against acute manic, bipolar disorder, autistic disorder and depression.

Thus, those of skill in the art may readily assay and use the compounds and/or compositions containing compound(s) of structural Formula (1) to treat a medical condition for which an antipsychotic is desired.

Therapeutic/Prophylactic Administration

The compounds, and/or compositions containing compounds(s), of structural Formula (1) can be advantageously used in human medicine. As previously described in detail above, compounds and compositions containing compound(s) of structural Formula (1) are useful for the

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treatment of schizophrenia and related psychoses such as acute manic, bipolar disorder, autistic disorder and depression.

When used to treat or prevent the above disease or disorders compounds and/or compositions of the invention can be administered or applied singly, in combination with other agents. The compounds and/or compositions of the invention can also be administered or applied singly, in combination with other pharmaceutically active agents, including other compounds and/or compositions of the invention.

The current invention provides methods of treatment and prophylaxis by administration to a patient of a therapeutically effective amount of a composition and/or compound of the invention. The patient may be an animal, is more preferably a mammal, and most preferably a human.

The present compounds and/or compositions of the invention, which comprise one or more compounds and/or compositions of the invention are preferably administered orally. The compounds and/or compositions of the invention may also be administered by any other convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.). Administration can be systemic or local. Various delivery systems are known, (e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, etc.) that can be used to administer a compound and/or composition of the invention. Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes or skin.

In particularly, preferred embodiments, the compounds and/or compositions of the invention can be delivered via sustained release systems, preferably oral sustained release systems. In one embodiment, a pump may be used (see, Langer, supra; Sefton, 1987, CRC Crit. Rev Biomed. Eng. 14:201; Saudek et al., 1989, N. Engl. J. Med. 321:574).

In another embodiment, polymeric materials can be used (see "Medical Applications of Controlled Release," Langer and Wise (eds.), Wiley, N.Y. (1984); Ranger and Peppas, 1983, J. Macromol. Sci. Rev. Macromol Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105). In a preferred embodiment, polymeric materials are used for oral sustained release delivery. Preferred polymers include sodium carboxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose and hydroxyethylcellulose (most preferred, hydroxypropylmethylcellulose). Other preferred cellulose ethers have been described in the art (Bamba et al., Int. J. Pharm., 1979, 2, 307).

In another embodiment, enteric-coated preparations can be used for oral sustained release administration. Preferred coating materials include polymers with a pH-dependent solubility (i.e., pH-controlled release), polymers with a slow or pH-dependent rate of swelling, dissolution or erosion (i.e., time controlled release), polymers that are degraded by enzymes (i.e., enzyme controlled release) and polymers that form firm layers that are destroyed by an increase in pressure (i.e., pressure-controlled release).

In still another embodiment, osmotic delivery systems are used for oral sustained release administration (Verma et al., Drug Dev. Ind. Pharm., 2000, 26:695-708). In a preferred embodiment, OROS® osmotic delivery systems are used for oral sustained release delivery devices (See for example, Theeuwes et al., U.S. Pat. No. 3,845,770; and Theeuwes et al., U.S. Pat. No. 3,916,899).

In yet another embodiment, a controlled-release system can be placed in proximity of the target of the compounds and/or composition of the invention, thus requiring only a fraction of the systemic dose (See, e.g., Goodson, in "Medical Applications of Controlled Release," supra, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in Langer, 1990, Science 249:1527-1533 may also be used.

The compounds, and/or compositions containing compound(s) of structural Formula (1) of the invention may be cleaved either chemically and/or enzymatically. One or more enzymes present in the stomach, intestinal lumen, intestinal tissue, blood, liver, brain or any other suitable tissue of a mammal may enzymatically cleave the compounds and/or compositions of the invention.

15 Compositions of the Invention

In one aspect of the invention, pharmaceutical compositions are provided comprising the compounds of the present disclosure.

The present composition contain a therapeutically effective amount of one or more compounds of the invention, preferably in purified form, together with a suitable amount of a pharmaceutically acceptable vehicle, which so as to provide the form for proper administration to a patient. When administered to a patient, the compounds of the invention and pharmaceutically acceptable vehicles are preferably sterile. Water is preferred vehicle when the compound of the invention is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid vehicles, particularly for injectable solutions. Suitable pharmaceutical vehicles also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present agents, or pH buffering agents. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents may be used.

Pharmaceutical compositions comprising a compound of the invention may be manufactured by means of conventional mixing, dissolving, granulating, dragee-making levigating, and emulsifying, encapsulating, entrapping or lyophilizing process. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries, which facilitate processing of compounds of the invention into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, and capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment, the pharmaceutically acceptable vehicle is a capsule (see e.g., Grosswald et al., U.S. Pat. No. 5,698,155). Other examples of suitable pharmaceutical vehicles have been described in the art (see Remington's Pharmaceutical Sciences, Philadelphia College of Pharmacy and Science, 17th Edition, 1985). Preferred compositions of the invention are formulated for oral delivery, particularly for oral sustained release administration.

Compositions for oral delivery may be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups or elixirs, for example. Orally administered compositions may contain one or more optionally agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry coloring agents and

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preserving agents to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions may be coated to delay disintegration and absorption in the gastrointestinal tract, thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered compounds of the invention. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time delay material such as glycerol monostearate or glycerol stearate may also be used. Oral compositions can include standard vehicles such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such vehicles are preferably of pharmaceutical grade.

For oral liquid preparations such as, for example, suspensions, elixirs and solutions, suitable carriers, excipients or diluents include water, saline, alkylene glycols (e.g., propylene glycol), polyalkylene glycols (e.g., polyethylene glycol) oils, alcohols, slightly acidic buffers between pH 4 and pH 6 (e.g., acetate, citrate, ascorbate at between about mM to about 50 mM) etc. Additionally, flavoring agents, preservatives, coloring agents, bile salts, acylcamitines and the like may be added.

Compositions for administration via other routes may also be contemplated. For buccal administration, the compositions may take the form of tablets, lozenges, etc. formulated in conventional manner. Liquid drug formulations suitable for use with nebulizers and liquid spray devices and FHD aerosol devices will typically include a compound of the invention with a pharmaceutically acceptable vehicle. Preferably, the pharmaceutically acceptable vehicle is a liquid such as alcohol, water, polyethylene glycol or a perfluorocarbon. Optionally, another material may be added to alter the aerosol properties of the solution or suspension of compounds of the invention. Preferably, this material is liquid such as alcohol, glycol, polyglycol or fatty acid. Other methods of formulating liquid drug solutions or suspension suitable for use in aerosol devices are known to those of skill in the art (see, e.g., Biesalski, U.S. Pat. No. 5,112,598; Biesalski, U.S. Pat. No. 5,556,611). A compound of the invention may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa, butter or other glycerides. In addition to the formulations described previously, a compound of the invention may also be formulated as depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, a compound of the invention may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

When a compound of the invention is acidic, it may be included in any of the above-described formulations as the free acid, a pharmaceutically acceptable salt, a solvate or hydrate. Pharmaceutically acceptable salts substantially retain the activity of the free acid, may be prepared by reaction with bases and tend to be more soluble in aqueous and other protic solvents than the corresponding free acid form.

Methods of Use and Doses

A compound of the invention, or compositions thereof, will generally be used in an amount effective to achieve the intended purpose. For use to treat schizophrenia and related psychoses such as acute manic, bipolar disorder, autistic disorder and depression. The compounds of Formula (1) and compositions containing a compound of Formula (1) are administered or applied in a therapeutically effective amount.

The amount of a compound of the invention that will be effective in the treatment of a particular disorder or condition disclosed herein will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques known in the art as previously described. In addition, in vitro or in vivo assays may optionally be employed to help identify optimal dosage ranges. The amount of a compound of the invention administered will, of course, is dependent on, among other factors, the subject being treated, and the weight of the subject, the severity of the affliction, the manner of administration and the judgment of the prescribing physician. For example, the dosage may be delivered in a pharmaceutical composition by a single administration, by multiple applications or controlled release. In a preferred embodiment, the compounds of the invention are delivered by oral sustained release administration. Preferably, in this embodiment, the compounds of the invention are administered twice per day, and more preferably, once per day. Dosing may be repeated intermittently, may be provided alone or in combination with other drugs and may continue as long as required for effective treatment of the disease state or disorder.

The compounds and/or compositions containing compound(s), of structural Formula (1) for the pharmacological treatment of schizophrenia and related psychoses such as acute maniac, bipolar disorder, autistic disorder and depression may be administered in the range 0.1 mg to 500 mg preferably 1 mg to 100 mg per day given in one or more doses and more preferably 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 35 mg or 50 mg per day and most preferably 10 mg.

The compounds of the invention are preferably assayed in vitro and in vivo, for the desired therapeutic or prophylactic activity, prior to use in humans. The compounds of the invention may also be demonstrated to be effective and safe using animal model systems.

Preferably, the therapeutically effective dose of a compound of the invention described herein will provide therapeutic benefit without causing substantial toxicity. Toxicity of compounds of the invention may be determined using standard pharmaceutical procedures and may be readily ascertained by the skilled artisan. The dose ratio between toxic and therapeutic effect is the therapeutic index. A compound of the invention will preferably exhibit particularly high therapeutic indices in treating disease and disorders. The dosage of a compound of the inventions described herein will preferably be within a range of circulating concentrations that include an effective dose with little or no toxicity.

In one aspect of the invention, methods of treating one of psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression are described, comprising administering to a patient in need thereof the compounds the present disclosure.

In another aspect of the invention, the method treats schizophrenia.

In another aspect of the invention, the method treats bipolar disorder.

In one aspect of the invention, compounds of the present disclosure used to treat psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression.

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In another aspect of the invention, the use comprises the treatment of schizophrenia.

In another aspect of the invention, the use comprises the treatment of bipolar disorder.

In one aspect of the invention, compounds of the present disclosure are used in the manufacture of a medicament for use in the treatment of psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression treat psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression.

In another aspect of the invention, the use is for the treatment of schizophrenia.

In another aspect of the invention, the use is for the treatment of bipolar disorder.

Combination Therapy

In certain embodiments of the present invention, the compounds of the invention can be used in combination therapy with at least one other therapeutic agent. The compound of the invention and the therapeutic agent can act additively or, more preferably, synergistically. In a preferred embodiment, composition comprising a compound of the invention is administered concurrently with the administration of another therapeutic agent, which can be part of the same composition. In another embodiment, a composition comprising a compound of the invention is administered prior or subsequent to administration of another therapeutic agent.

EXAMPLES

The invention is further defined by reference to the following examples, which describe in detail preparation of compounds and compositions of the invention and assays for using compounds and compositions of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

Atm=Atmosphere

CDI=1,1'-Carbonyldiimidazole

DCM=dichloromethane

DMAp=4-N,N-dimethylaminopyridine

DMF=N,N-dimethylformamide

g=gram

h=hours

L=liter

LC/MS=liquid chromatography/mass spectroscopy

M=molar

mL=milliliter

mmol=millimols

nM=nanomolar

μ M=micromolar

MTBE=methyl tert-butyl ether

rt=room temperature

TEA=triethylamine

THF=tetrahydrofuran

TFA=trifluoroacetic acid

Example 1

6-Amino-2H-benzo[b][1,4]oxazin-3(4H)-one (2) (Scheme 1). To a suspension of 6-nitro-2H-1,4-benzoxazin-3(4H)-one 1 (0.5 g, 0.0026 mol) and CuCl (0.77 g, 0.0078 mol) in anhydrous methanol (25 mL), stirred at 25° C. was added potassium borohydride (0.98 g, 0.018 mol) in portions (exothermic with evolution of hydrogen gas). The reaction

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mixture was stirred at 25° C. for 30 min. The black precipitate formed was filtered and washed with methanol. The combined filtrate and washings was evaporated to give 6-amino-2H-benzo[b][1,4]oxazin-3(4H)-one which was purified by silica gel column chromatography using ethyl acetate. Brown solid (0.29 g, 67%). ¹H NMR (400 MHz, CDCl₃): δ 4.34 (s, 2H); 4.81 (s, 2H); 6.09 (dd, J=2.8, 8.4 Hz, 1H); 6.14 (d, J=2.8 Hz, 1H); 6.59 (d, J=8.4 Hz, 1H); 10.44 (s, 1H).

Example 2

4-(4-(2-Methoxyphenyl)piperazin-1-yl)-N-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)butanamide (4a) (Scheme 1). A mixture of 6-amino-2H-benzo[b][1,4]oxazin-3(4H)-one 2 (0.08 g, 0.0005 mol), 4-(4-(substituted-phenyl)piperazin-1-yl)butanoic acid 3a (0.0005 mol), dicyclohexylcarbodiimide (0.1 g, 0.0005 mol), 4-(dimethylamino)pyridine (0.006 g, 0.00005 mol) in 10 mL dichloromethane was stirred at room temperature for overnight. The progress of the reaction was monitored by thin layer chromatography (TLC). The reaction mixture was cooled; filtered to remove the urea precipitated, washed with saturated sodium bicarbonate solution, dried over sodium sulfate and evaporated under reduced pressure to give 4-(4-(substituted-phenyl)piperazin-1-yl)-N-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)butanamide 4a which was purified by silica gel column chromatography using 0-10% gradient of ethyl acetate and methanol. The pure product 4a gave satisfactory ¹H NMR and/or Mass spectral data. White solid (0.06 g, 29%).

¹H NMR (400 MHz, CDCl₃): δ 1.94-2.00 (m, 2H); 2.53-2.59 (m, 4H); 2.70 (br s, 4H); 3.14 (br s, 4H); 3.87 (s, 3H); 4.55 (s, 2H); 6.63 (dd, J=2.4, 8.8 Hz, 1H); 6.84-6.88 (m, 2H); 6.93-6.95 (m, 2H); 6.99-7.04 (m, 1H); 7.82 (d, J=2.4 Hz, 1H); 9.04 (br s, 1H); 9.12 (br s, 1H).

MS (ESI): m/z=425.2 (M+H⁺).

Example 3

4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-N-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)butanamide (4b) (Scheme 1). The compound 4b was synthesized using the protocol described for the synthesis of compound 4a in Example 2 (Scheme 1). White solid (0.08 g, 34%). ¹H NMR (400 MHz, CDCl₃): δ 1.93-2.00 (m, 2H); 2.50-2.57 (m, 4H); 2.68 (br s, 4H); 3.09 (br s, 4H); 4.50 (s, 2H); 6.63 (dd, J=2.4, 8.8 Hz, 1H); 6.88 (d, J=8.8 Hz, 1H); 6.99-6.92 (m, 1H); 7.13-7.20 (m, 2H); 7.71-7.72 (m, 1H); 8.46 (br s, 1H); 8.58 (br s, 1H). MS (ESI): m/z=463.2 (M⁺).

Example 4

4-(4-(2-Methoxyphenyl)piperazin-1-yl)-N-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)butanamide hydrochloride (5a) (Scheme 1). To a solution of 4-(4-(substituted-phenyl)piperazin-1-yl)-N-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)butanamide 4a in 5 mL dichloromethane was added 2 mL 2M HCl solution in diethyl ether, and then the solution was evaporated at 25° C. to give 4-(4-(substituted-phenyl)piperazin-1-yl)-N-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)butanamide hydrochloride 5a. The pure products 5a gave satisfactory ¹H NMR and/or Mass spectral data. White solid (60 mg). MS (ESI): m/z=425.2 (M-HCl).

Example 5

4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-N-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)butanamide hydro-

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chloride (5b) (Scheme 1). The compound 5b was synthesized using the protocol described for the synthesis of compound 5a in Example 4 (Scheme 1). White solid (40 mg). MS (ESI): $m/z=463.2$ (M-HCl).

Example 6

Ethyl 2-(4-methoxy-2-nitrophenoxy)acetate (8) (Scheme 2). A mixture of 4-methoxy-2-nitrophenol 6 (1.69 g, 0.01 mol), potassium carbonate (2.76 g, 0.02 mol) and ethyl bromoacetate 7 (1.1 mL, 0.01 mol) in 20 mL anhydrous acetone was refluxed for overnight (12 h). The progress of the reaction was monitored by thin layer chromatography. The reaction mixture was evaporated, the residue was diluted with water and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated to give ethyl 2-(4-methoxy-2-nitrophenoxy)acetate 8 which was purified by silica gel column chromatography using 0-50% gradient of hexane and ethyl acetate. Yellow solid (2.18 g, 85%). ^1H NMR (400 MHz, CDCl_3): δ 1.28 (t, $J=7.2$ Hz, 3H); 3.82 (s, 3H); 4.25 (q, $J=7.2$ Hz, 2H); 4.70 (s, 2H); 7.01 (d, $J=9.2$ Hz, 1H); 7.08 (dd, $J=3.2, 9.2$ Hz, 1H); 7.40 (d, $J=2.8$ Hz, 1H).

Example 7

Ethyl 2-(4-hydroxy-2-nitrophenoxy)acetate (9) (Scheme 2). To a solution of ethyl 2-(4-methoxy-2-nitrophenoxy)acetate 8 (1.0 g, 0.004 mol) in 10 mL dichloromethane cooled in an ice-bath was added aluminum chloride (1.6 g, 0.0133 mol) portion wise. The resulting mixture was gradually warmed to room temperature and then refluxed for 4 h. The progress of the reaction was monitored by thin layer chromatography (TLC). The reaction mixture was washed with saturated sodium bicarbonate solution and dried over sodium sulfate to give ethyl 2-(4-hydroxy-2-nitrophenoxy)acetate 9 which was purified by silica gel column chromatography using a gradient of hexane and ethyl acetate. White solid (0.28 g, 37%). ^1H NMR (400 MHz, CDCl_3): δ 1.26 (t, $J=6.8$ Hz, 3H); 4.27 (q, $J=6.8$ Hz, 2H); 4.71 (s, 2H); 6.32 (s, 1H); 6.90 (d, $J=8.8$ Hz, 1H); 6.99 (dd, $J=3.2$ Hz; 8.8 Hz, 1H); 7.30 (d, $J=2.8$ Hz, 1H).

Example 8

Ethyl 2-(4-(4-bromobutoxy)-2-nitrophenoxy)acetate (11) (Scheme 2). To a solution of ethyl 2-(4-hydroxy-2-nitrophenoxy)acetate 9 (0.17 g, 0.0007 mol) in 10 mL acetone was added potassium carbonate (0.39 g, 0.0028 mol). The reaction mixture was stirred at room temperature for 10 min. Then 1,4-dibromobutane 10 (0.33 mL, 0.0028 mol) was added. The resulting mixture was heated at reflux for 12 h. The progress of the reaction was monitored by thin layer chromatography (TLC). Acetone was evaporated and the residue was diluted with water, extracted with ethyl acetate and dried over sodium sulfate to give ethyl 2-(4-(4-bromobutoxy)-2-nitrophenoxy)acetate 11 which was purified by silica gel column chromatography using 0-20% gradient of hexane and ethyl acetate. Yellow oil (0.52 g, 92%). ^1H NMR (400 MHz, CDCl_3): δ 1.26 (t, $J=6.8$ Hz, 3H); 1.91-1.98 (m, 2H); 2.02-2.09 (m, 2H); 3.48 (t, $J=6.8$ Hz, 2H); 3.99 (t, $J=6.0$ Hz, 2H); 4.26 (q, $J=6.8$ Hz, 2H); 4.70 (s, 2H); 6.99-7.02 (m, 2H); 7.38 (d, $J=3.2$ Hz, 1H).

Example 9

Ethyl 2-(4-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butoxy)-2-nitrophenoxy)acetate 13a (Scheme 2). A mixture of ethyl 2-(4-(4-bromobutoxy)-2-nitrophenoxy)acetate 11

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(0.0007 mol), 1-(2-substituted-phenyl)piperazine hydrochloride 12 (0.16 g, 0.0007 mol), N,N-diisopropylethylamine (0.36 mL, 0.0021 mol) in 10 mL acetonitrile was heated at 60°C. for 12 h. The progress of the reaction was monitored by thin layer chromatography (TLC). The reaction mixture was evaporated to remove the volatiles and the residue was diluted with water, extracted with ethyl acetate, the organic extracts were washed with sodium bicarbonate solution and dried over sodium sulfate to give ethyl 2-(4-(4-(4-(substituted-phenyl)piperazin-1-yl)butoxy)-2-nitrophenoxy)acetate 13a which was purified by silica gel column chromatography using a gradient of hexane and ethyl acetate. The pure product 13a gave satisfactory ^1H NMR and/or Mass spectral data. Colorless oil (0.3 g, 88%). ^1H NMR (400 MHz, CDCl_3): δ 1.26 (t, $J=6.8$ Hz, 3H); 1.70-1.72 (m, 2H); 1.82-1.85 (m, 2H); 2.47 (t, $J=6.4$ Hz, 2H); 2.67 (br s, 4H); 3.10 (br s, 4H); 3.86 (s, 3H); 3.98 (t, $J=6.4$ Hz, 2H); 4.24 (q, $J=6.8$ Hz, 2H); 4.70 (s, 2H); 6.84-6.96-7.08 (m, 6H); 7.38 (d, $J=2.8$ Hz, 1H).

Example 10

Ethyl 2-(4-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butoxy)-2-nitrophenoxy)acetate 13b (Scheme 2). The compound 13b was prepared using the protocol described for the synthesis of compound 13a as described in Example 9 (Scheme 2). Yellow oil (0.3 g, 81%). ^1H NMR (400 MHz, CDCl_3): δ 1.26 (t, $J=6.8$ Hz, 3H); 1.70-1.73 (m, 2H); 1.80-1.85 (m, 2H); 2.48 (t, $J=6.4$ Hz, 2H); 2.65 (br s, 4H); 3.07 (br s, 4H); 3.99 (t, $J=6.4$ Hz, 2H); 4.26 (q, $J=6.8$ Hz, 2H); 4.70 (s, 2H); 6.94-7.08 (m, 3H); 7.13-7.16 (m, 2H); 7.39 (d, $J=3.2$ Hz, 1H).

Example 11

6-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butoxy)-2H-benzo[b][1,4]oxazin-3(4H)-one (14a) (Scheme 2). Ethyl 2-(4-(4-(4-(substituted-phenyl)piperazin-1-yl)butoxy)-2-nitrophenoxy)acetate 13a (0.1 g, 0.0002 mol) was dissolved in a mixture of 10 mL ethanol and 1.2 mL acetic acid in a 100 mL flask equipped with an efficient condenser, and the stirred mixture brought to a gentle reflux. Iron powder (0.084 g, 0.0015 mol) was added, followed immediately by iron(III) chloride hexahydrate (0.01 g, 0.000034 mol). The mixture was refluxed for a further 3 h, then cooled and filtered using a Buchner funnel, washed with ethanol. The combined filtrate and washings were evaporated. To the residue was added ethyl acetate and water, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were dried and concentrated to give 6-(4-(4-(substituted-phenyl)piperazin-1-yl)butoxy)-2H-benzo[b][1,4]oxazin-3(4H)-one 14a which was purified by silica gel column chromatography using a gradient of hexane and ethyl acetate. The pure product 14a gave satisfactory ^1H NMR and/or Mass spectral data. Colorless oil (0.08 g, 97%). ^1H NMR (400 MHz, CDCl_3): δ 1.70 (brs, 2H); 1.78 (brs, 2H); 2.47 (brs, 2H); 2.67 (brs, 4H); 3.10 (brs, 4H); 3.86 (s, 3H); 3.91 (brs, 2H); 4.55 (s, 2H); 6.38 (br s, 1H); 6.48-6.50 (m, 1H); 6.85-6.99 (m, 5H); 9.12 (br s, 1H). MS (ESI): $m/z=412.3$ (M^++H).

Example 12

6-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butoxy)-2H-benzo[b][1,4]oxazin-3(4H)-one (14b) (Scheme 2). The compound 14b was prepared using the protocol described for the synthesis of compound 14a as described in Example 11 (Scheme 2). Colorless oil (0.1 g, 42%). ^1H NMR (400 MHz, CDCl_3): δ 1.79 (brs, 4H); 2.74 (brs, 2H); 2.96 (brs, 4H); 3.17

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(br s, 4H); 3.91 (s, 2H); 4.05 (s, 2H); 6.44 (s, 1H); 6.48 (dd, J=2.4 Hz; 8.8 Hz, 1H); 6.85 (d, J=8.8 Hz, 1H); 6.96 (dd, J=2.0 Hz; 7.2 Hz, 1H); 7.12-7.19 (m, 2H); 9.49 (s, 1H); 9.87 (br s, 1H). MS (ESI): m/z=450.2 (M⁺).

Example 13

6-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butoxy)-2H-benzo[b][1,4]oxazin-3(4H)-one hydrochloride (15a) (Scheme 2). To a solution of 6-(4-(4-(substituted-phenyl)piperazin-1-yl)butoxy)-2H-benzo[b][1,4]oxazin-3(4H)-one 14a in 5 mL dichloromethane was added 2 mL 2M HCl solution in diethyl ether, and then the solution was evaporated at 25° C. to give 6-(4-(4-(substituted-phenyl)piperazin-1-yl)butoxy)-2H-benzo[b][1,4]oxazin-3(4H)-one hydrochloride 15a. The pure products 15a gave satisfactory ¹H NMR and/or Mass spectral data. White solid (0.08 g). MS (ESI): m/z=412.3 (M-HCl).

Example 14

6-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy)-2H-benzo[b][1,4]oxazin-3(4H)-one hydrochloride (15b) (Scheme 2). The compound 15b was prepared using the protocol described for the synthesis of compound 15a as described in Example 13 (Scheme 2). White solid (0.28 g). MS (ESI): m/z=450.2 (M-HCl).

In Vitro Pharmacology Results

The arylpiperazine derivatives comprising Formula (1) described in this invention were tested in the in vitro pharmacological assays to evaluate their activities for dopamine, D_{2S}, serotonin, 5-HT_{1A} and serotonin, 5-HT_{2A} receptors. The in vitro assay protocols and literature references are described herein.

Dopamine, D_{2S} (human recombinant) binding assay

Materials and Methods:

Receptor Source: Human recombinant expressed in CHO cells

Radioligand: [³H]Spiperone (20-60 Ci/mmol)

Control Compound: Haloperidol

Incubation Conditions: The reactions were carried out in 50 mM TRIS-HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 5 mM MgCl₂, 1 mM EDTA for 60 minutes at 25° C. The reaction was terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters was determined and compared to control values in order to ascertain any interactions of test compounds with the cloned dopamine-D₂ short binding site (Literature Reference: Jarvis, K. R. et al. Journal of Receptor Research 1993, 13(1-4), 573-590; Gundlach, A. L. et al. Life Sciences 1984, 35, 1981-1988.)

Serotonin, 5HT_{1A} (human recombinant) binding assay

Materials and Methods:

Receptor Source: Human recombinant expressed in HEK-293 cells

Radioligand: [³H]-8-OH-DPAT (221 Ci/mmol)

Control Compound: 8-OH-DPAT

Incubation Conditions: The reactions were carried out in 50 mM TRIS-HCl (pH 7.4) containing 10 mM MgSO₄, 0.5 mM EDTA and 0.1% Ascorbic acid at room temperature for 1 hour. The reaction was terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters was determined and compared to control values in order to ascertain any interactions of test compounds with the cloned serotonin-5HT_{1A} binding site (Literature Reference: Hoyer, D. et al. Eur. Journal Pharmacol. 1985,

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118, 13-23; Schoeffter, P. and Hoyer, D. Naunyn-Schmiedeberg's Arch. Pharmac. 1989, 340, 135-138)

Serotonin, 5HT_{2A} (human) binding assay

Materials and Methods:

Receptor Source: Human Cortex

Radioligand: [³H]-Ketanserin (60-90 Ci/mmol)

Control Compound: Ketanserin

Incubation Conditions: The reactions were carried out in 50 mM TRIS-HCl (pH 7.6) at room temperature for 90 minutes. The reaction was terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters was determined and compared to control values in order to ascertain any interactions of test compounds with the serotonin-5HT_{2A} binding site (Literature Reference: Lysen, J. E. et al. Mol. Pharmacol. 1982, 21, 301-314; Martin, G. R. and Humphrey, P. P. A. Neuropharmacol. 1994, 33(3/4), 261-273.)

The radioligand binding assays for dopamine-D_{2S}, serotonin-5HT_{1A} and serotonin-5HT_{2A} were carried out at six different concentrations and the test concentrations were 0.5 nM, 1 nM, 10 nM, 100 nM, 300 nM and 1000 nM.

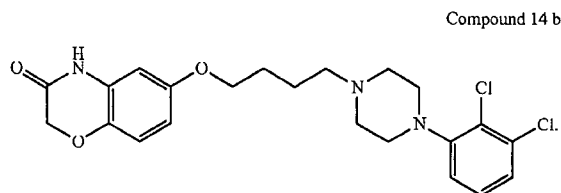
The in vitro pharmacological activities of the selected compounds using radioligand binding assays are reported in the following table.

Compound	Assay	IC ₅₀	K _i
15a (Example 13)	D _{2S}	0.98 nM	0.30 nM
15a (Example 13)	5-HT _{1A}	0.97 nM	0.65 nM
15a (Example 13)	5-HT _{2A}	262 nM	118 nM
15b (Example 14)	D _{2S}	1.96 nM	0.61 nM
15b (Example 14)	5-HT _{1A}	2.25 nM	1.50 nM
15b (Example 14)	5-HT _{2A}	130 nM	58.50 nM

While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention. All printed patents and publications referred to in this application are hereby incorporated herein in their entirety by this reference.

What is claimed is:

1. A compound having the structure of Compound 14b, or a pharmaceutically acceptable salt thereof:

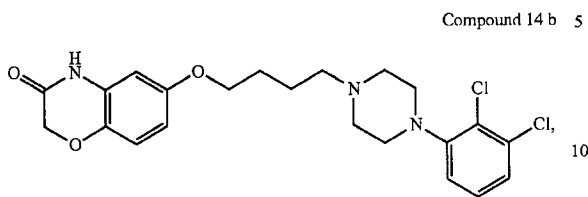


2. The compound of claim 1, wherein the compound is in the form of a hydrochloride salt.

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3. A compound having the structure of Compound 14b, or a pharmaceutically acceptable salt or isomer thereof:



wherein one or more hydrogens of the compound are optionally substituted with ^2H (deuterium).

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4. A pharmaceutical composition comprising the compound according to claim 1 and a pharmaceutically acceptable carrier, excipient, or diluent.

5. A method of treating psychoses, schizophrenia, acute mania, bipolar disorder, autistic disorder or depression, the method comprising administering to a patient in need thereof the pharmaceutical formulation according to claim 4.

6. The method of claim 5, wherein said method treats schizophrenia.

7. The method of claim 5, wherein said method treats bipolar disorder.

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Research report

The 5-HT₇ receptor as a mediator and modulator of antidepressant-like behaviorGor Sarkisyan^{a,1}, Amanda J. Roberts^{b,2}, Peter B. Hedlund^{a,*}^a Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA^b Molecular and Integrative Neurosciences Department, The Scripps Research Institute, MB-18, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

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ABSTRACT

The 5-HT₇ receptor has been suggested as a target for treating depression since inactivation or blockade of the receptor has an antidepressant-like behavioral effect. The present study investigated possible interactions between various classes of drugs with antidepressant properties and blockade or inactivation of the 5-HT₇ receptor. Immobility despair in the tail suspension test and the forced swim test was evaluated in mice lacking the 5-HT₇ receptor (5-HT₇^{-/-}) and in wild-type controls (5-HT₇^{+/+}) following acute drug treatments. Citalopram, a selective serotonin reuptake inhibitor and widely used antidepressant, dose-dependently reduced immobility in the tail suspension test in both 5-HT₇^{+/+} and 5-HT₇^{-/-} mice. Combining doses of citalopram and the 5-HT₇ receptor antagonist SB-269970 that by themselves did not affect behavior, reduced immobility in 5-HT₇^{+/+} mice in both the tail suspension test and the forced swim test. No effect was seen in 5-HT₇^{-/-} mice. Desipramine and reboxetine, two norepinephrine reuptake inhibitors, dose-dependently reduced immobility in the tail suspension test in 5-HT₇^{+/+} mice, but had no effect in 5-HT₇^{-/-} mice. A synergistic effect between desipramine and SB-269970 was found in both behavioral tests in 5-HT₇^{+/+} mice. Reboxetine combined with SB-269970 had effect only in the forced swim test. GBR 12909, a dopamine reuptake inhibitor, dose-dependently reduced tail suspension test immobility in both genotypes. There was no interaction between GBR 12909 and SB-269970. Aripiprazole, an antipsychotic, reduced immobility in both tests in 5-HT₇^{+/+} mice, but not in 5-HT₇^{-/-} mice. The results show that the 5-HT₇ receptor is required for the observed interaction between this receptor and antidepressants such as citalopram. The data furthermore support the hypothesis that the 5-HT₇ receptor might be a suitable target for treating depression.

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1. Introduction

The 5-HT₇ receptor, a member of a more recently discovered group of 5-HT receptors [1–6], has been found to modulate behaviors affected by antidepressant drugs. Behavioral models of significant value for evaluating putative antidepressants and for characterizing transgenic mice [7,8] include the forced swim test [9] and the tail suspension test [10]. In both of these tests, inactivation or blockade of the 5-HT₇ receptor has been shown to lead to antidepressant-like behavior [11–13]. For inhibition of the 5-HT₇ receptor, these studies used the selective 5-HT₇ receptor antagonist

SB-269970 [14,15]. Furthermore, inactivation or pharmacological blockade of the 5-HT₇ receptor lead to changes in rapid eye movement sleep that are opposite to those seen in depression [12,15].

These findings have led to the hypothesis that the 5-HT₇ receptor might be suitable new target for the treatment of depression [16–19]. It has been shown that certain antidepressants might exert some of their effects by acting directly at the 5-HT₇ receptor [20]. More interestingly, recent studies have found a synergistic interaction between SB-269970 and various antidepressants [21,22]. One of the studies found that mice given an ineffective dose of citalopram, a selective serotonin reuptake inhibitor (SSRI), in conjunction with an ineffective dose of SB-269970 exhibited antidepressant-like behavior in the tail suspension test [21]. At a higher dose, it has been demonstrated that SB-269970 alone reduces immobility in the mouse tail suspension test [12,21]. A similar synergistic interaction between SB-269970 and citalopram has also been described in the mouse forced swim test [22]. This study also reported that interactions occur also between SB-269970 and other classes of antidepressants. Thus, ineffective doses of imipramine (a tricyclic antidepressant), desipramine (a tricyclic antidepressant acting mainly as a norepinephrine reuptake inhibitor), and moclobemide (a monoamine oxidase inhibitor)

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all reduced immobility in the mouse forced swim test when given in combination with SB-269970 [22]. The interaction between SB-269970 and imipramine has also been shown in the forced swim test using Wistar rats [23]. The prefrontal cortex has been suggested as an important region for these interactions [21,22], but the hippocampus has also been implicated in the effects of SB-269970 and imipramine on the rat forced swim test [24].

Although the monoamine hypothesis of depression with its focus on 5-HT and norepinephrine has been very important for the understanding of this disorder, there are remaining issues, suggesting that other mechanisms might also be involved [25]. One significant pharmacological issue is that reuptake inhibition following treatment with an antidepressant occur rapidly, but its clinical effects require much longer time [26]. The present study aims to further investigate the relationships between the 5-HT₇ receptor and monoamine reuptake inhibition, and also to investigate possible relationships with other factors deemed to be relevant for the understanding of depression. Specifically, we have evaluated possible modulations of the 5-HT₇ receptor on dopamine reuptake, on the 5-HT_{1A} receptor, on the antidepressant properties of the antipsychotic aripiprazole, and on corticosterone in models of depression.

Dopamine has been implicated in depression as it has been suggested that reduced availability of dopamine, in a similar way as for 5-HT and norepinephrine, might contribute to the pathophysiology of depression. In fact, dopaminergic mechanisms have gained increased interest with the development of combined norepinephrine and dopamine reuptake inhibitors and even triple reuptake inhibitors [27–30]. Selective dopamine reuptake inhibitors have been shown to reduce immobility in the forced swim test in rats. Nomifensine at low doses, which significantly reduced the immobility time, had no significant effect on open field locomotor activity. At higher doses nomifensine increased overall locomotor activity as well as decreased immobility in the forced swim test [31]. GBR 12909 is another dopamine reuptake inhibitor that has recently been shown to reduce immobility in both the tail suspension test and the forced swim test, but also to increase overall locomotor activity [32].

Interestingly, it has recently been found that the dopamine D₂/D₃ antagonist amisulpride, an antipsychotic with known antidepressant properties [33,34], most likely exerts its antidepressant effect through the 5-HT₇ receptor and not, as previously thought, through dopaminergic mechanisms [35]. Thus, amisulpride was shown to have high affinity for the human 5-HT₇ receptor and to reduce immobility in both the tail suspension test and the forced swim test in 5-HT₇^{+/+} mice, but not in 5-HT₇^{-/-} mice. To extend these findings we have in the present study investigated aripiprazole, an atypical antipsychotic with high affinity for the 5-HT₇ receptor [36] that is approved as augmentation therapy to antidepressants [37] and that has recently has been shown to potentiate the effect of fluoxetine [38].

The 5-HT_{1A} receptor and the 5-HT₇ receptor have been shown to modulate the action of each other. After the discovery of the 5-HT₇ receptor it was found that 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin), originally thought to be a selective agonist for the 5-HT_{1A} receptor, had high affinity for the 5-HT₇ receptor [1]. It has subsequently been demonstrated that 5-HT_{1A} and 5-HT₇ receptors both are involved in mechanisms such as thermoregulation [39]. Mice lacking the 5-HT_{1A} receptor show reduced immobility in the forced swim test [40], but it has not been possible to induce such an effect with antagonists for this receptor [41]. Nevertheless, 5-HT_{1A} receptor antagonists such as pindolol have been successfully used to reduce the time needed until clinical effect is achieved with antidepressants [42].

For decades, it has been known that individuals under sustained, elevated stress levels are at a higher risk of developing men-

tal illnesses, especially depression. Stressors induce the secretion of glucocorticoids such as corticosterone in rodents and cortisol in humans through the hypothalamic–pituitary–adrenal axis. The serotonergic system has been shown to modulate the effects of glucocorticoids in mood disorders as well as regulate the expression of glucocorticoid and mineralocorticoid receptors [43,44]. An increase in 5-HT₇ mRNA in the hippocampus was observed following blockade of corticosterone synthesis in male Wistar rats likely due to a reduction in glucocorticoid receptor transmission in the hippocampus [45].

In the present study we have further tested the hypothesis that the 5-HT₇ receptor is a putative new target for the treatment of depressions [16–19]. We have performed an expanded series of experiments on the interaction between reuptake inhibitors for 5-HT, norepinephrine, and dopamine, and antagonists for the 5-HT_{1A} and 5-HT₇ receptors, as well as the antipsychotic aripiprazole. Furthermore, we have investigated possible influences on serum corticosterone levels. In all experiments, comparisons were made between wild-type mice (5-HT₇^{+/+}) and mice lacking the 5-HT₇ receptor (5-HT₇^{-/-}) in order to test the hypothesis that this receptor is required for the observed interactions.

2. Materials and methods

2.1. Animals

Ten-to-twelve week old male 5-HT₇^{-/-} mice and their male 5-HT₇^{+/+} sibling controls were used. The generation of the 5-HT₇^{-/-} mouse strain has been described previously [46]. The mice used in this study had been back-crossed on a C57BL/6J background for at least 16 generations. As the forced swim and tail suspension tests involve learning, the same animal was never used twice for the same behavioral method. A larger number of mice were exposed to the tail suspension test than the forced swim test. Thus, all mice tested in the forced swim test were also used for the tail suspension test, but not vice versa. A minimum of one week separated the two tests. A separate cohort of mice was used for evaluation of locomotor activity. All behavioral experiments were started at 09:00 h. The mice were housed in a 12-h light/dark cycle (lights on at 06:00 and off at 18:00) and had free access to water and food pellets. All the experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health, and were approved by the Animal Care and Use Committee at The Scripps Research Institute. Every effort was made to reduce the number of animals used and to minimize potential suffering.

2.2. Tail suspension test

The tail suspension test was performed as previously described [12]. Briefly, mice were suspended from a metal rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape. The duration of the test was 6 min and immobility was measured during the last 4 min to facilitate comparison with the forced swim test. Immobility was defined as the absence of any limb or body movements, except those caused by respiration.

2.3. Forced swim test

The forced swim test was performed as previously described [12]. Briefly, mice were gently placed in a clear plastic cylinder, diameter of 16 cm, height 25 cm, filled with 10 cm of clear water at 25 °C. Test duration was 6 min and immobility was measured during the last 4 min. Immobility was defined as the absence of any horizontal or vertical movement in the water, but excluded minor movements required for the mouse to keep its head above the surface. The water was replaced before each animal.

2.4. Locomotor activity

Locomotor activity was measured in Plexiglas cages (42 cm × 22 cm × 20 cm) placed into frames (25.5 cm × 47 cm) mounted with two levels of photocell beams at 2 and 7 cm above the bottom of the cage (San Diego Instruments, San Diego, CA, USA). The two sets of beams allowed for the recording of both horizontal (locomotion) and vertical (rearing) behavior. A thin layer of bedding material was applied to the bottom of the cage. Mice were habituated to the activity boxes during three once daily 30-min sessions. The mice were then tested for drug for possible drug effects. For each treatment evaluated a mouse first had a 15-min session in the activity box followed by the injection. The mouse was then tested again 30 min later for 15 min.

2.5. Serum corticosterone

Blood was collected in heparinized capillaries through eye bleeds. Approximately 150 μ l was collected from each animal sampled. The blood was transferred to 1.5 ml plastic tubes and allowed to coagulate. The samples were then centrifuged at 3000 rpm for 15 min. The serum was transferred to a new tube and stored at -80°C until analyzed. Serum corticosterone was measured using a radio-immuno assay kit following the instructions provided by the manufacturer (MP Biomedicals, Solon, OH).

2.6. Drug treatments

For the forced swim test, tail suspension test, and locomotor activity test, single intra-peritoneal injections were given 30 min prior to the test. Citalopram, desipramine, and WAY 100135 were obtained from Sigma (St. Louis, MO). GBR 12909 and SB-269970 were purchased from Tocris (Ellisville, MO). Reboxetine and aripiprazole was bought from Toronto Research Chemicals (North York, ON). All drugs were dissolved in 0.9% NaCl and administered in the doses indicated in a total volume of 8 ml/kg. Although long-term treatment with drugs like citalopram is required in the clinic to obtain therapeutic effect, acute single injections are sufficient when using animal models [7]. The vehicle 0.9% NaCl alone was used as control. Data analysis: All values are expressed as means $\pm$ standard errors of the mean (S.E.M.). Possible differences between genotypes and/or drug treatments were analyzed using Student's *t*-test or two-way analysis of variance (ANOVA) with genotype as one factor and drug treatment as the other factor. The ANOVA was followed by an appropriate Bonferroni posttest. All analyzes were performed using the GraphPad Prism (<http://www.graphpad.com>) software package. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Tail suspension test and forced swim test

As previously reported [12], 5-HT $_7^{-/-}$ mice showed lower immobility than 5-HT $_7^{+/+}$ mice in both the tail suspension test and the forced swim test when given vehicle only.

3.1.1. Serotonin reuptake inhibition

Citalopram dose-dependently reduced immobility in 5-HT $_7^{+/+}$ mice (Fig. 1A) in the tail suspension test. Citalopram also reduced immobility in 5-HT $_7^{-/-}$ mice. A two-way ANOVA revealed significant effects for treatment ($F(4, 58) = 35.56, P < 0.001$), genotype ($F(1, 58) = 40.80, P < 0.001$), and interaction ($F(4, 58) = 4.66, P < 0.05$). A maximal effect (reduction) for the doses tested of approximately 20 s of immobility was reached at 10 mg/kg for 5-HT $_7^{+/+}$ mice and at 3 mg/kg for 5-HT $_7^{-/-}$ mice. When an ineffective dose of citalopram (1 mg/kg) was combined with an ineffective dose of SB-269970 (1 mg/kg), immobility was reduced in 5-HT $_7^{+/+}$ mice ($F_{\text{interaction}}(3, 34) = 3.48, P < 0.05$; Fig. 1B). The dose of SB-269970 was chosen based on initial testing and earlier demonstrations that doses of 3 mg/kg and higher reduce immobility in wild-type mice [12,21]. This combination had no additional effect in 5-HT $_7^{-/-}$ mice. A similar synergistic interaction between citalopram and SB-269970 was observed for 5-HT $_7^{+/+}$ mice in the forced swim test ($F_{\text{interaction}}(3, 64) = 8.41, P < 0.001$; Fig. 1C). Interestingly, in this test, the dose 1 mg/kg of citalopram had an effect of its own in 5-HT $_7^{-/-}$ mice (Fig. 1C).

3.1.2. Norepinephrine reuptake inhibition

Desipramine dose-dependently reduced immobility in the tail suspension test for 5-HT $_7^{+/+}$ mice (Fig. 2A). For the doses tested desipramine did not further affect immobility in 5-HT $_7^{-/-}$ mice. A two-way ANOVA showed significant effects for treatment ($F(4, 71) = 7.75, P < 0.001$), genotype ($F(1, 71) = 7.21, P < 0.01$), and interaction ($F(4, 71) = 4.05, P < 0.01$). When an ineffective dose of desipramine (1 mg/kg) was combined with an ineffective dose of SB-269970 (1 mg/kg), immobility was reduced in 5-HT $_7^{+/+}$ mice ($F_{\text{interaction}}(3, 70) = 4.02, P < 0.05$; Fig. 2B). Such an effect was also seen in the forced swim test where the combination of desipramine (1 mg/kg) and SB-269970 (1 mg/kg) reduced immobility in the 5-

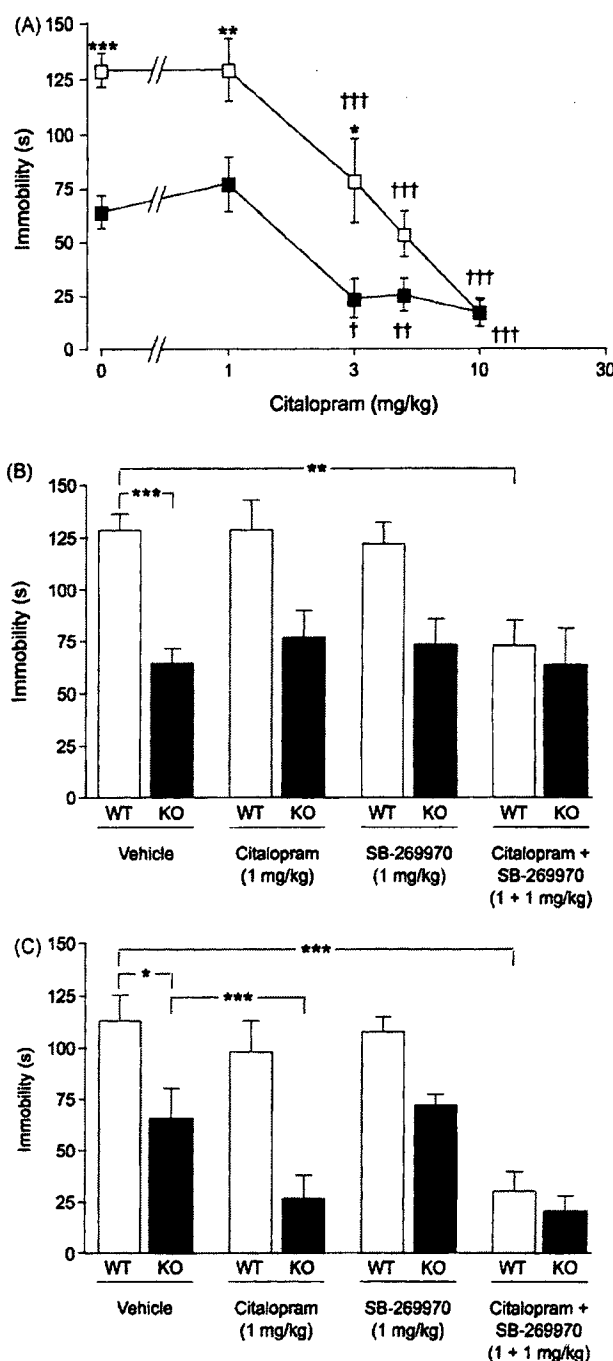


Fig. 1. Effects of citalopram on mouse behavior in the tail suspension and forced swim tests. (A) Dose-response effect of citalopram on the immobility profile of 5-HT $_7^{+/+}$ (□) and 5-HT $_7^{-/-}$ (■) mice in the tail suspension test. (B) Effects of individual and concurrent injections of 1 mg/kg citalopram and 1 mg/kg SB-269970 in the tail suspension test in 5-HT $_7^{+/+}$ (WT) and 5-HT $_7^{-/-}$ (KO) mice. (C) Effects of individual and concurrent injections of 1 mg/kg citalopram and 1 mg/kg SB-269970 in the forced swim test in 5-HT $_7^{+/+}$ (WT) and 5-HT $_7^{-/-}$ (KO) mice. Values are mean $\pm$ S.E.M. $n = 8-10$ animals per genotype per treatment group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ between the genotypes (A) or indicated groups (B and C); † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ within a genotype compared to control; two-way ANOVA followed by Bonferroni's post hoc test.

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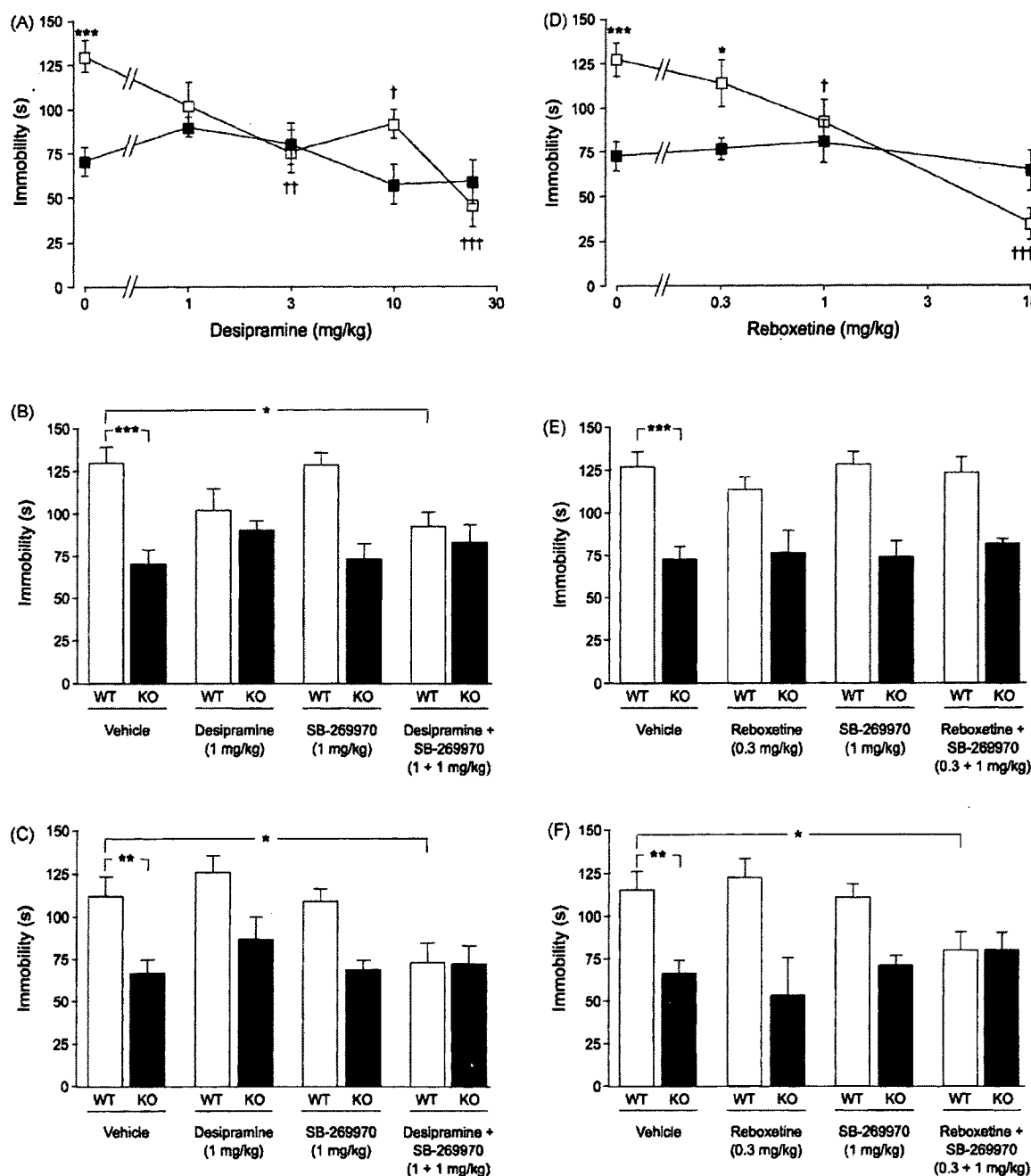


Fig. 2. Effects of desipramine and reboxetine on mouse behavior in the tail suspension and forced swim tests. (A) Dose–response effect of desipramine on the immobility profile of 5-HT₇^{+/+} (□) and 5-HT₇^{-/-} (■) mice in the tail suspension test. (B) Effects of individual and concurrent injections of 1 mg/kg desipramine and 1 mg/kg SB-269970 in the tail suspension test in 5-HT₇^{+/+} (WT) and 5-HT₇^{-/-} (KO) mice. (C) Effects of individual and concurrent injections of 1 mg/kg desipramine and 1 mg/kg SB-269970 in the forced swim test in 5-HT₇^{+/+} (WT) and 5-HT₇^{-/-} (KO) mice. (D) Dose–response effect of reboxetine on the immobility profile of 5-HT₇^{+/+} (□) and 5-HT₇^{-/-} (■) mice in the tail suspension test. (E) Effects of individual and concurrent injections of 0.3 mg/kg reboxetine and 1 mg/kg SB-269970 in the tail suspension test in 5-HT₇^{+/+} (WT) and 5-HT₇^{-/-} (KO) mice. (F) Effects of individual and concurrent injections of 0.3 mg/kg reboxetine and 1 mg/kg SB-269970 in the forced swim test in 5-HT₇^{+/+} (WT) and 5-HT₇^{-/-} (KO) mice. Values are mean ± S.E.M. $n = 8–10$ animals per genotype per treatment group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ between the genotypes (A, D) or indicated groups (B, C, E, F); † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ within a genotype compared to control; two-way ANOVA followed by Bonferroni's post hoc test.

HT₇^{+/+} mice ($F_{\text{interaction}}(3, 56) = 3.00$, $P < 0.05$; Fig. 2C). The dose 1 mg/kg of desipramine had no effect on immobility in the 5-HT₇^{-/-} mice.

In view of the results obtained with desipramine, especially its inability to reduce immobility in the 5-HT₇^{-/-} mice, the norepinephrine reuptake inhibitor reboxetine was also evaluated. The results for reboxetine were similar to those obtained with desipramine. Thus, reboxetine also dose-dependently reduced immobility in the tail suspension test for 5-HT₇^{+/+} mice (Fig. 2D),

but the doses tested did not further affect immobility in 5-HT₇^{-/-} mice. A two-way ANOVA showed significant effects for treatment ($F(3, 49) = 10.09$, $P < 0.001$), genotype ($F(1, 49) = 6.39$, $P < 0.05$), and interaction ($F(3, 49) = 6.68$, $P < 0.001$). When an ineffective dose of reboxetine (0.3 mg/kg) was combined with an ineffective dose of SB-269970 (1 mg/kg), immobility was not altered in the tail suspension test in any of the genotypes ($F_{\text{interaction}}(3, 56) = 0.52$, $P = 0.67$; Fig. 2E). However, in the forced swim test an interaction was observed where the combination of reboxetine (0.3 mg/kg)

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and SB-269970 (1 mg/kg) reduced immobility in the 5-HT₇^{+/+} mice ($F_{\text{interaction}}(3, 54) = 2.99, P < 0.05$; Fig. 2F).

3.1.3. Dopamine reuptake inhibition

GBR 12909 dose-dependently reduced immobility in 5-HT₇^{+/+} mice (Fig. 3A) in the tail suspension test. GBR 12909 also reduced

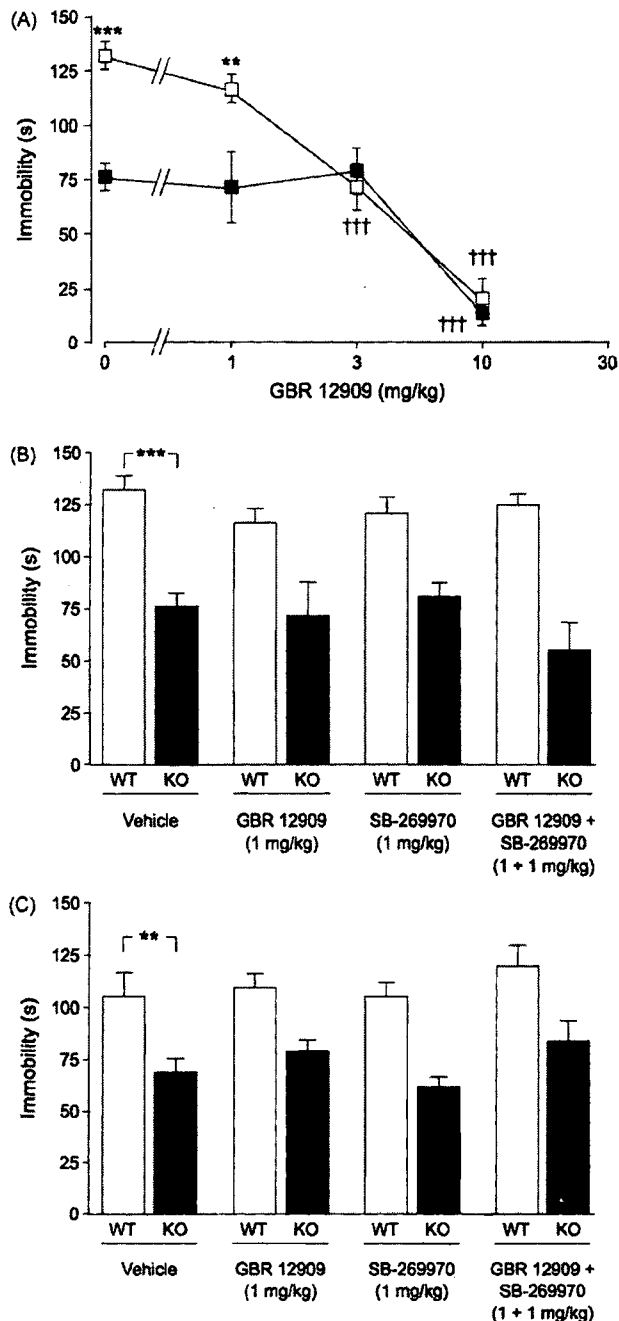


Fig. 3. Effects of GBR 12909 on mouse behavior in the tail suspension and forced swim tests. (A) Dose-response effect of GBR 12909 on the immobility profile of 5-HT₇^{+/+} (□) and 5-HT₇^{-/-} (■) mice in the tail suspension test. (B) Effects of individual and concurrent injections of 1 mg/kg GBR 12909 and 1 mg/kg SB-269970 in the tail suspension test in 5-HT₇^{+/+} (WT) and 5-HT₇^{-/-} (KO) mice. (C) Effects of individual and concurrent injections of 1 mg/kg GBR 12909 and 1 mg/kg SB-269970 in the forced swim test in 5-HT₇^{+/+} (WT) and 5-HT₇^{-/-} (KO) mice. Values are mean $\pm$ S.E.M. $n = 8$ –10 animals per genotype per treatment group. ** $P < 0.01$, *** $P < 0.001$ between the genotypes (A) or indicated groups (B and C); +++ $P < 0.001$ within a genotype compared to control; two-way ANOVA followed by Bonferroni's post hoc test.

immobility in 5-HT₇^{-/-} mice. A two-way ANOVA revealed significant effects for treatment ($F(3, 54) = 46.76, P < 0.001$), genotype ($F(1, 54) = 16.25, P < 0.001$), and interaction ($F(3, 54) = 6.38, P < 0.001$). A maximal effect (reduction) for the doses tested of approximately 15 s of immobility was reached at 10 mg/kg for both genotypes. The dose 1 mg/kg of GBR 12909 had no effect on immobility in either genotype. Interestingly, an intermediate dose (3 mg/kg) reduced immobility in 5-HT₇^{+/+} mice, but had no effect in 5-HT₇^{-/-} mice (Fig. 3A). Combining the dose 1 mg/kg of GBR 12909 with 1 mg/kg of SB-269970 did not influence immobility ($F_{\text{interaction}}(3, 59) = 1.33, P = 0.27$; Fig. 3B). Comparable results were obtained in the forced swim test where 10 mg/kg of GBR 12909 reduced immobility in both genotypes (37.0 ± 14.1 s immobility, $n = 9$, for 5-HT₇^{+/+} and 9.3 ± 6.2 s, $n = 6$ for 5-HT₇^{-/-}), but 1 mg/kg did not and there was no interaction with SB-269970 ($F_{\text{interaction}}(3, 74) = 0.24, P = 0.87$; Fig. 3C).

3.1.4. 5-HT_{1A} receptor inhibition

WAY 100135 (10 mg/kg) did not influence immobility in the tail suspension test for 5-HT₇^{+/+} or 5-HT₇^{-/-} mice. The dose of WAY 100135 was chosen based on its ability to inhibit 8-OH-DPAT-induced hypothermia [39]. As expected, there was an effect for genotype ($F(1, 1) = 30.12, P < 0.001$), but no effect for treatment ($F(1, 1) = 0.31, P = 0.58$). The observed immobility values(s) were 127.70 ± 7.81 (5-HT₇^{+/+}, vehicle, $n = 10$), 75.60 ± 5.16 (5-HT₇^{-/-}, vehicle, $n = 10$), 114.00 ± 12.72 (5-HT₇^{+/+}, WAY 100135, $n = 8$), and 80.56 ± 4.45 (5-HT₇^{-/-}, WAY 100135, $n = 9$).

3.1.5. Aripiprazole

In 5-HT₇^{+/+} mice aripiprazole showed a U-shaped dose-response curve (Fig. 4). In the tail suspension test immobility was reduced at 0.1 mg/kg, but then increased at higher doses to reach durations longer than for vehicle (Fig. 4A). In the forced swim test immobility was reduced at 0.03 mg/kg (Fig. 4B). In 5-HT₇^{-/-} mice aripiprazole dose-dependently increased immobility in the tail suspension test (Fig. 4A). Within the doses tested aripiprazole did not alter immobility in the 5-HT₇^{-/-} mice in the forced swim test (Fig. 4B). The two-way ANOVA values were for the tail suspension test for treatment ($F(4, 58) = 27.53, P < 0.001$), genotype ($F(1, 58) = 11.17, P < 0.01$), and interaction ($F(4, 58) = 7.86, P < 0.001$), and for the forced swim test for treatment ($F(4, 69) = 3.51, P < 0.05$), genotype ($F(1, 69) = 9.8, P < 0.01$), and interaction ($F(4, 69) = 1.32, P = 0.27$).

3.2. Locomotor activity

Citalopram, SB-269970, and their combination were assessed for any possible effect on locomotor activity as such an effect could influence behavior in the tail suspension or forced swim tests. The 1 mg/kg doses of citalopram or SB-269970 or their combination did not affect horizontal locomotor activity (Fig. 5). Rearing was also not affected (data not shown). As the limited availability of 5-HT₇^{-/-} mice required that the same animals were tested for all the different treatments it was noted that repeated testing increased locomotor activity in the 5-HT₇^{-/-} mice but not the 5-HT₇^{+/+} (Fig. 5). The effect was however identical for vehicle treated animals and thus not a drug effect. The two-way ANOVA effects for genotype were $F(1, 36) = 21.23, P < 0.001$ for citalopram, $F(1, 36) = 4.65, P < 0.05$ for SB-269970, and $F(1, 36) = 10.48, P < 0.01$ for citalopram + SB-269970.

3.3. Serum corticosterone

Serum levels of corticosterone showed an expected circadian pattern in both 5-HT₇^{+/+} and 5-HT₇^{-/-} mice that had not been exposed to any behavioral tests (Fig. 6A). Serum corticosterone was

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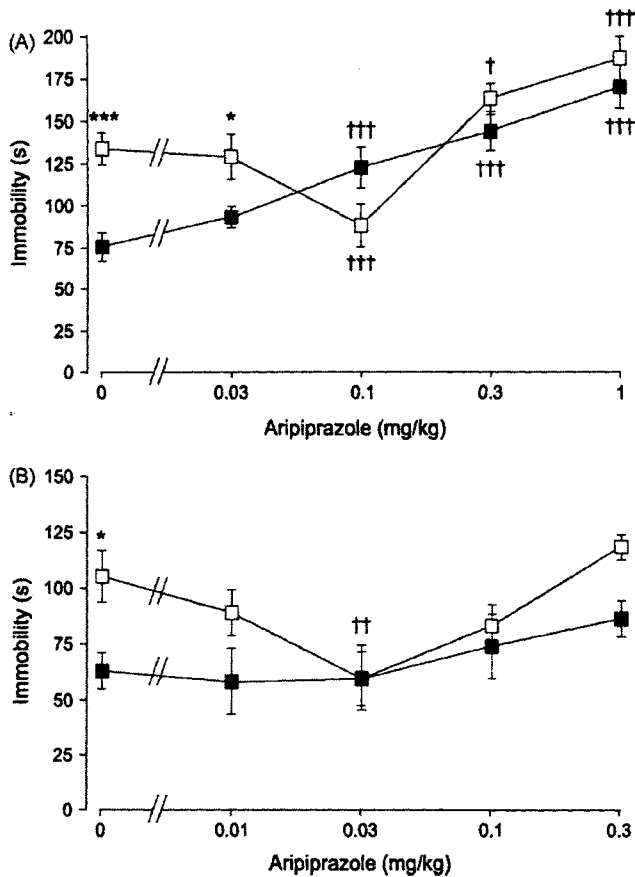


Fig. 4. Effects of aripiprazole on mouse behavior in the tail suspension and forced swim tests. (A) Dose-response effect of aripiprazole on the immobility profile of 5-HT₇^{+/+} (□) and 5-HT₇^{-/-} (■) mice in the tail suspension test. (B) Dose-response effect of aripiprazole on the immobility profile of 5-HT₇^{+/+} (□) and 5-HT₇^{-/-} (■) mice in the forced swim. Values are mean ± S.E.M. *n* = 8–10 animals per genotype per treatment group. **P* < 0.05, ****P* < 0.001 between the genotypes; †*P* < 0.05, ††*P* < 0.01, †††*P* < 0.001 within a genotype compared to control; two-way ANOVA followed by Bonferroni's post hoc test.

also measured in vehicle-treated mice exposed to the tail suspension test and the forced swim test (Fig. 6B). These measurements were taken at approximately 10:30 a.m. during the light cycle. Thus, there appears to be an increase in serum corticosterone due to the

stress of the behavioral tests, but this was not evaluated statistically as the aim was to determine any difference between the genotypes. As no such differences were observed, serum corticosterone levels were not studied further.

4. Discussion

The major finding of the present study was that although drugs representing various classes of antidepressants seem to act independently of the 5-HT₇ receptor in reducing immobility in the tail suspension and forced swim tests, the interaction experiments clearly show a synergistic effect between certain monoamine uptake inhibitors, but not others, and a 5-HT₇ receptor antagonist. Renewed evidence that atypical antipsychotics with high affinity for the 5-HT₇ receptor mediate their clinically established antidepressant effect through the 5-HT₇ receptor is also presented.

Citalopram represents the selective serotonin reuptake inhibitors and is widely used to treat depression [25]. It has been shown to reduce immobility in the forced swim test [47] and the tail suspension test [48]. At least in the tail suspension test the effect is dose-dependent [48]. Thus, the present results confirm and extend previous findings by showing a dose-dependent effect of citalopram in reducing immobility and that the effect is present in both 5-HT₇^{+/+} and 5-HT₇^{-/-} mice. We have previously demonstrated that the selective 5-HT₇ receptor antagonist SB-269970 reduces immobility in these test [12]. This has been confirmed for the tail suspension test by others [21]. The present results revealed a synergistic interaction between citalopram and SB-269970 when both compounds were given in ineffective doses in 5-HT₇^{+/+} mice. The data also showed that the interaction affects both the tail suspension test and the forced swim test. The specificity of the synergism to 5-HT₇ receptors and their requirement for this interaction was confirmed by the lack of such an effect in 5-HT₇^{-/-} mice. A synergistic interaction between citalopram and SB-269970 has previously been demonstrated for the forced swim test [22] and the tail suspension test [21]. The first group also showed similar synergism with imipramine (serotonin and norepinephrine reuptake inhibitor) where an ineffective dose of imipramine concurrently administered with SB-269970 resulted in significant reduction in immobility in the forced swim test.

Using microdialysis, attempts have been made to find correlates between observed behavioral changes in response to antidepressants and possible changes in brain 5-HT concentrations [21,23,49]. A straight forward interpretation of the results has proved to be dif-

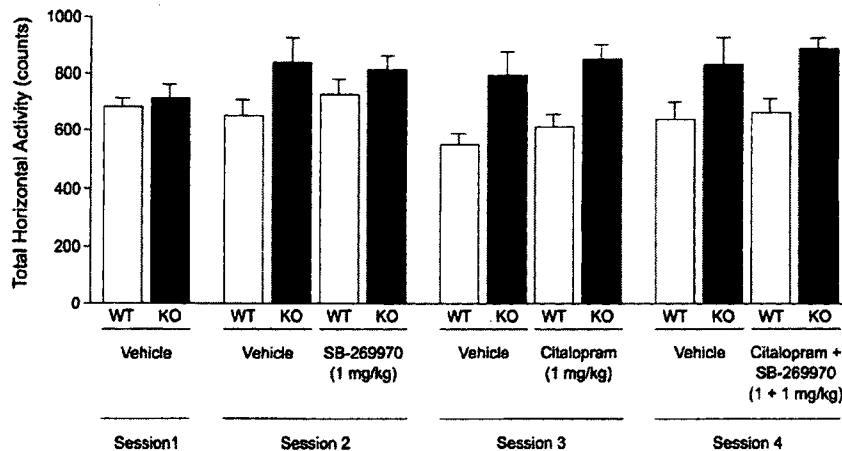


Fig. 5. Effects of citalopram and SB-269970 on mouse locomotor activity. Mice were habituated to the test apparatus for three sessions (not shown). They were then tested in four separate sessions as indicated. In the first session all mice received vehicle. In the subsequent sessions half of the mice were randomly assigned to receive vehicle and the other half drug. Comparisons were made between 5-HT₇^{+/+} (WT) and 5-HT₇^{-/-} (KO) mice. There were no treatment-induced effects, but 5-HT₇^{-/-} mice increased their activity with repeated testing, see text for details. Values are mean ± S.E.M. *n* = 10 (20 in session 1) animals per genotype per treatment group.

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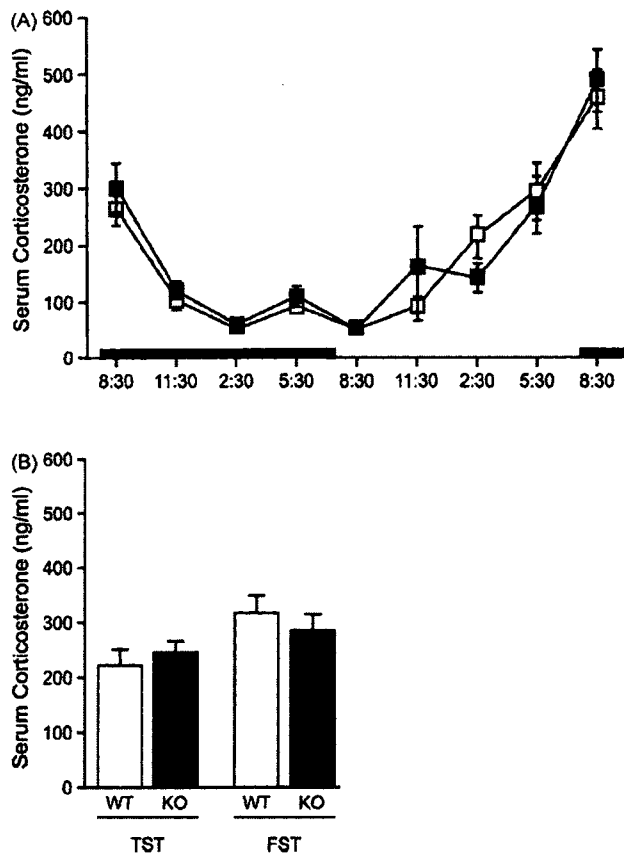


Fig. 6. (A) Corticosterone levels measured in blood serum. Expected corticosterone concentrations changes were observed during a 24-h cycle. There were no differences between 5-HT₇^{+/+} (□) and 5-HT₇^{-/-} (■) mice. The black bar represents the dark period of the day. (B) Serum corticosterone levels after the tail suspension test (TST) and the forced swim test (FST). These measurements were made at approximately 10:30 during the light phase and thus appear to be elevated as a result of the behavioral tests performed although this was not statistically evaluated as the objective was to determine possible changes between the genotypes. There were no differences in serum corticosterone levels between 5-HT₇^{+/+} (WT) and 5-HT₇^{-/-} (KO) mice following either test. Values are mean $\pm$ S.E.M. $n = 8$ animals per genotype for the time-course data; $n = 6$ per genotype for the tail suspension test; $n = 8$ per genotype for the forced swim test.

ficult, most likely due to different experimental designs. One study showed no effect of SB-269970 on 5-HT concentration in the frontal cortex [21]. Another study showed increases in 5-HT concentration in response to SB-269970 treatment in the prefrontal cortex, but this study was done already 24 h after surgery [23]. One possible explanation for the differing results is different stress levels at different post-surgical intervals. It has been shown that 5-HT₇ receptor mRNA expression is upregulated in response to stress [50]. Possibly contradicting results were obtained in a study showing that a selective 5-HT₇ receptor antagonist, SB-258741, could inhibit the increase in 5-HT levels induced by citalopram in the ventral hippocampus, but only if 5-HT_{1A} receptors were simultaneously blocked by WAY 100635 [49].

Desipramine represents a different class of anti-depressants in two ways. It belongs to the so called tricyclic antidepressants and it acts mainly as a norepinephrine reuptake inhibitor [51]. In contrast to citalopram, desipramine only reduced immobility in 5-HT₇^{+/+} mice even at higher doses. The highest dose of desipramine used in the current study has previously been shown to induce a maximal reduction in immobility in C57BL/6J mice [48,52]. A difference in action between citalopram and desipramine in 5-HT₇^{-/-} mice has previously been described in a sleep study [53]. This study showed

that citalopram had a greater ability than desipramine to suppress rapid eye movement sleep in these mice. Nevertheless, a similar interaction as with citalopram was seen with ineffective doses of desipramine in the tail suspension test and the forced swim test. Again, that 5-HT₇ receptors are required for this interaction was confirmed by the lack of such an effect in 5-HT₇^{-/-} mice. A synergistic interaction between desipramine and SB-269970 has previously been demonstrated for the forced swim test [22].

To verify and extend the results obtained with desipramine we also tested the selective norepinephrine reuptake inhibitor reboxetine, a clinically used antidepressant [54]. Reboxetine has been shown to reduce immobility in the tail suspension and forced swim tests including in mice on a C57BL/6J background [54,55]. The results were very similar compared with desipramine since both drugs reduced immobility in wild-type mice but not in 5-HT₇^{-/-} mice in the tail suspension test. A weak or absent interaction with SB-269970 was seen in the tail suspension test, whereas a more distinct interaction was observed for both drugs in the forced swim test. Thus, although both serotonin and norepinephrine reuptake are modulated by the 5-HT₇ receptor there appears to be differences in how the different classes of drugs interact.

GBR 12909 represents a selective dopamine reuptake inhibitor. It has several hundred times higher affinity for the dopamine transporter than for the 5-HT transporter and no affinity for the norepinephrine transporter [56]. Thus, GBR 12909 should not affect synaptic 5-HT levels, at least not in animals with intact 5-HT transporters [57]. GBR 12909 has recently been shown to reduce immobility in both the tail suspension test and the forced swim test, but it is possible that this, at least in part, is due to an increase in overall locomotor activity [32]. The possible impact of changes in locomotor activity is, however, reduced by the fact that there is no difference in ambulatory or rearing activity over a 24-h period between 5-HT₇^{+/+} and 5-HT₇^{-/-} mice [58]. The present data confirm the previous findings and show that the effects on immobility are dose-dependent. GBR 12909 (10 mg/kg) had an effect in both 5-HT₇^{+/+} and 5-HT₇^{-/-} mice. Interestingly, however, the effect was not additive in 5-HT₇^{-/-} mice as 3 mg/kg of GBR 12909 reduced immobility in 5-HT₇^{+/+} mice but had no additional effect in 5-HT₇^{-/-} mice. It is noteworthy that GBR 12909 did not interact with SB-269970 to modulate the immobility response in either test. Thus, even though to our knowledge the affinity of GBR 12909 for the 5-HT₇ receptor is not known, the most probable interpretation of the present results is that GBR 12909 acts independently of the 5-HT₇ receptor, and that as yet undescribed mechanisms in the 5-HT₇^{-/-} mice are responsible for the dose related differences seen between the genotypes. Even though there is evidence supporting the hypothesis that dopamine is involved in depression, the recent finding that the antidepressant effect of amisulpride is most likely mediated by the 5-HT₇ receptor [35] might suggest the need for a reevaluation of some studies, especially those using antipsychotics with high affinity for the 5-HT₇ receptor [59].

In a recent study it was found that the atypical antipsychotic amisulpride had high affinity for the 5-HT₇ receptor and that it was able to reduce immobility in both the tail suspension and forced swim tests in 5-HT₇^{+/+}, but not 5-HT₇^{-/-} mice [35]. These findings provided the first rational explanation for the antidepressant properties of amisulpride [60–62]. Aripiprazole is another atypical antipsychotic with high affinity for the 5-HT₇ receptor [63]. Aripiprazole is approved as augmentation therapy in depression [37,64]. Interestingly it was recently shown that aripiprazole potentiates the effect of fluoxetine in the mouse tail suspension test, an effect the authors were not able to fully explain [38]. With the present findings that aripiprazole reduced immobility in both the tail suspension and forced swim tests in 5-HT₇^{+/+} mice but not in 5-HT₇^{-/-} mice it appears that also the antidepressant properties of aripiprazole are mediated by the 5-HT₇ receptor.

To fully understand the mechanisms regulating the degree of immobility in the tail suspension test and the forced swim test has proven to be difficult. Recent studies have shown that there are pronounced strain differences in the behavioral response [52,65]. One notable finding is that C57BL/6J mice, the background strain for the 5-HT₇^{-/-} mice, generally have a high baseline immobility and do not respond to certain selective serotonin reuptake inhibitors (e.g. fluoxetine) [65]. There is a linear correlation between the amount of 5-HT transporter binding and immobility, but no such correlation exists for the norepinephrine transporter [52]. Interesting differences in response are seen also in the present study. Citalopram and GBR 12909 dose-dependently reduced immobility in the tail suspension test in both 5-HT₇^{+/+} and 5-HT₇^{-/-} mice. In contrast, desipramine and reboxetine failed to further reduce immobility in the 5-HT₇^{-/-} mice. Citalopram, desipramine and reboxetine, but not GBR 12909, interacted synergistically with the 5-HT₇ receptor antagonist SB-269970. Especially the interaction between citalopram and SB-269970 had an interesting profile that it might be possible to exploit therapeutically. With the norepinephrine reuptake inhibitors the interaction was most prominent in the forced swim test, whereas it was weak or absent in the tail suspension test. It should be noted that combining citalopram and SB-269970 synergistically increased prefrontal cortex levels of 5-HT [21], whereas the combination of imipramine and SB-269970 did not [23]. It even appears that blockade of the 5-HT₇ receptor might lead to decreased 5-HT levels [49]. Nevertheless, an explanation based on a mechanism resulting in increased synaptic 5-HT (and possibly norepinephrine) levels for the enhanced antidepressant-like responses observed by combining antidepressants with 5-HT₇ receptor blockade remains closest at hand. Inhibition of 5-HT₇ receptors located on axon terminals might cause such an effect [21,66,67].

One factor that can influence the results in the tail suspension and the forced swim tests is changes in locomotor activity induced by drugs or genetic manipulations. We verified that the interactions seen between low doses of citalopram and SB-269970 were not due to changes in locomotion. This is in agreement with other studies [21,22]. Nevertheless, it was of importance to exclude this possibility since higher doses of citalopram has been shown to increase locomotor activity [68,69]. SB-269970 has been found not to affect locomotion [13]. Due to the increased activity seen in the 5-HT₇^{-/-} mice with repeated testing we did not test any of the other drugs used in this study. The cause and implications of this effect needs further more detailed studies as a previous 24 h comparison between 5-HT₇^{+/+} and 5-HT₇^{-/-} mice did not reveal any differences in locomotor activity [58]. It should be noted that desipramine has been found to reduce locomotor activity [69] and that reboxetine does not affect locomotion [70,69]. Thus, the observations made in the present study for these drugs are likely not due to changes in locomotion. On the other hand, the effects seen with GBR 12909 might be due to increased locomotor activity induced by this compound [71].

Previous studies [11,12] and the present data show a strong correlation between effects seen in the tail suspension test and the forced swim test in 5-HT₇^{-/-} mice and by using a selective 5-HT₇ receptor antagonists. Thus, one can be highly confident that the findings in 5-HT₇^{-/-} mice are due to the lack of 5-HT₇ receptors. With the 5-HT_{1A} receptor the situation is different. Mice lacking the 5-HT_{1A} receptor exhibit reduced immobility in the forced swim test [40]. Interestingly, agonists for the 5-HT_{1A} receptor, where 8-OH-DPAT is the most commonly used, also reduce immobility, an effect that can be blocked by relatively selective antagonists [72]. Selective antagonists for the 5-HT_{1A} receptor by themselves do not influence immobility [41]. It has also been shown that there is no interaction between the 5-HT_{1A} receptor and antidepressants in the forced swim test [41]. Although less studied, the results are

similar for the 5-HT_{1A} receptor and the tail suspension test [73]. Even though 8-OH-DPAT is also an agonist for 5-HT₇ receptors it is unlikely that its ability to reduce immobility is mediated by this receptor since inactivation or blockade reduces immobility. In the present study, using the 5-HT_{1A} receptor antagonist/partial agonist WAY 100135, no indication was found for a direct effect on immobility in the tail suspension test nor an interaction with 5-HT₇ receptors.

Glucocorticoids are believed to play a major role in depression [25]. Several studies have also shown that the 5-HT₇ receptor is involved in the central regulation of glucocorticoids. Serotonin-mediated upregulation of glucocorticoid receptors is mediated by 5-HT₇ receptors [74]. Furthermore, both adrenalectomy and restraint stress has been shown to upregulate 5-HT₇ receptor mRNA expression in the hippocampus [45,50]. Thus, we hypothesized that glucocorticoid homeostasis might be altered in 5-HT₇^{-/-} mice. However, the present data show that serum corticosterone levels were not altered in 5-HT₇^{-/-} mice, and that the serum levels had a normal circadian pattern. Serum corticosterone levels were also not differentially regulated in 5-HT₇^{+/+} and 5-HT₇^{-/-} mice following the tail suspension test or the forced swim test.

The present findings support the hypothesis that the 5-HT₇ receptor is a new target for disorders treated with antidepressants. Possibly a 5-HT₇ receptor antagonist would be beneficial by itself as inactivation or blockade of the 5-HT₇ receptor in itself is sufficient to induce antidepressant-like behavior. Alternatively, the combination of a 5-HT₇ receptor antagonist with an antidepressant might prove to be a treatment with improved efficacy and reduced side-effects as lower doses most likely could be used.

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RESEARCH PAPER

F15063, a compound with D₂/D₃ antagonist, 5-HT_{1A} agonist and D₄ partial agonist properties: (III) Activity in models of cognition and negative symptoms

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Background and purpose: The D₂/D₃ receptor antagonist, D₄ receptor partial agonist, and high efficacy 5-HT_{1A} receptor agonist F15063 was shown to be highly efficacious and potent in rodent models of activity against positive symptoms of schizophrenia. However F15063 induced neither catalepsy nor the 'serotonin syndrome'. Here, we evaluated its profile in rat models predictive of efficacy against negative symptoms/cognitive deficits of schizophrenia.

Experimental approach: F15063, given i.p., was assessed in models of behavioural deficits induced by interference with the NMDA/glutamatergic (phencyclidine: PCP) or cholinergic (scopolamine) systems.

Key results: Through 5-HT_{1A} activation, F15063 partially alleviated (MED: 0.04 mg kg⁻¹) PCP-induced social interaction deficit between two adult rats, without effect by itself, underlining its potential to combat negative symptoms. At doses above 0.16 mg kg⁻¹, F15063 reduced interaction by itself. F15063 (0.16 mg kg⁻¹) selectively re-established PCP-impaired 'cognitive flexibility' in a reversal learning task, suggesting potential against adaptability deficits. F15063 (0.04–0.63 mg kg⁻¹) also reversed scopolamine-induced amnesia in a juvenile-adult rat social recognition test, indicative of a pro-cholinergic influence. Activity in this latter test is consistent with its D₄ partial agonism, as it was blocked by the D₄ antagonist L745,870. Finally, F15063 up to 40 mg kg⁻¹ did not disrupt basal prepulse inhibition of startle reflex in rats, a marker of sensorimotor gating.

Conclusions and implications: The balance of D₂/D₃, D₄ and 5-HT_{1A} receptor interactions of F15063 yields a promising profile of activity in models of cognitive deficits and negative symptoms of schizophrenia.

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Keywords: 5-HT_{1A} agonist; atypical antipsychotic; cognitive deficit; D₂ antagonist; D₄ partial agonist; schizophrenia

Abbreviations: ED₅₀, effective dose for 50% response; EPS, extrapyramidal syndrome; F15063, *N*-[(2,2-dimethyl-2,3-dihydro-benzofuran-7-yloxy)ethyl]-3-(cyclopent-1-enyl)-benzylamine mono-tartrate; LT, learning task; i.p., intraperitoneal; NP, no pulse; PA, pulse alone; PCP, phencyclidine; pp, prepulse; PPI, prepulse inhibition; ppP, prepulse-pulse; p.o., per os; s.c., subcutaneous

Introduction

Currently available antipsychotics control the so-called positive symptoms of schizophrenia (hallucination, delusions, etc) in about two-thirds of patients treated, reflecting their antagonist properties at dopamine D₂ receptors (Kapur and Remington, 2001). Other aspects of the pathology, such as negative symptoms (flat affect, avolition, anergia, etc) and

cognitive dysfunction (including working memory impairment, perseveration and attention deficits) are also alleviated but to a more limited extent (Tandon *et al.*, 1993, Breier *et al.*, 1994, Buchanan *et al.*, 1998, Rosenheck *et al.*, 1999). These cognitive deficits are increasingly considered to be part of the core symptoms of the pathology and the limited efficacy of antipsychotics to combat them hampers proper societal functioning of patients and their reinsertion (Freedman, 2003; Green *et al.*, 2004), and constitutes a real challenge to clinicians.

The search for more effective antipsychotics (i.e. with beneficial effects on negative symptomatology and cognitive deficits) has resulted in the development of numerous

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compounds with a wide variety of target receptors (Sanger, 2004). One approach involves the association of DA D₂ receptor antagonist and 5-HT_{1A} receptor agonist activities. Such a combination has been demonstrated to prevent catalepsy – a preclinical marker of extrapyramidal syndrome (EPS) – that results from blockade of DA D₂ receptors. It has also been proposed to bring additional benefits against negative symptoms and cognitive dysfunction associated with schizophrenia (see Millan, 2000; Bantick *et al.*, 2001; Ichikawa *et al.*, 2001; Bardin *et al.*, 2006a; Depoortere *et al.*, 2007, for more detailed discussion). Indeed, several new compounds in development conform to this profile: bifeprunox (Feenstra *et al.*, 2001; Wolf, 2003), SSR181507 (Claustre *et al.*, 2003; Depoortere *et al.*, 2003; Boulay *et al.*, 2004; Terranova *et al.*, 2005), SLV313 (Feenstra *et al.*, 2002; McCreary *et al.*, 2002) and sarizotan (now developed as an anti-dyskinetic: Bibbiani *et al.*, 2001; Rabiner *et al.*, 2002; Bartoszyk *et al.*, 2004).

We have previously reported on the binding, neurochemical (Newman-Tancredi *et al.*, 2006) and behavioral profile in tests predictive of antipsychotic activity (Depoortere *et al.*, 2006) of F15063 (*N*-[(2,2-dimethyl-2,3-dihydro-benzofuran-7-yloxy)ethyl]-3-(cyclopent-1-enyl)-benzylamine; monotartrate), synthesized by the Medicinal Chemistry department of Pierre Fabre Recherche (Vacher *et al.*, 2002). This compound interacts potently with human DA D_{2L} ($pK_i = 9.44$), D_{2S} ($pK_i = 9.25$), D₃ ($pK_i = 8.95$) and D₄ ($pK_i = 8.81$) receptors, and has somewhat lesser affinity for 5-HT_{1A} receptors ($pK_i = 8.37$). It presents low affinity at a multitude of other receptors, including 5-HT_{2A} and 5-HT_{2C}, α_1 and α_2 adrenoceptors, muscarine M₁ and histamine H₁. In functional *in vitro* tests, it behaves as an antagonist at DA D₂ receptors (contrary to other preferential D₂/5-HT_{1A} antipsychotics such as bifeprunox and SSR181507 that act as partial agonists at these receptors: Bruins Slot *et al.*, 2006; Cosi *et al.*, 2006), and as a partial agonist at D₄ and an high efficacy agonist at 5-HT_{1A} receptors (Newman-Tancredi *et al.*, 2006). F15063 was potently (effective doses for 50% response (ED₅₀s) from 0.23 to 1.10 mg kg⁻¹ intraperitoneal (i.p.)) active in rodent models that detect activity against positive symptoms of schizophrenia: methylphenidate-induced stereotyped behaviors, D-amphetamine- or ketamine-induced hyperlocomotion, apomorphine-induced prepulse inhibition (PPI) deficits, conditioned avoidance test and apomorphine-induced climbing. However, because of its 5-HT_{1A} agonism, F15063 had no cataleptogenic potential (ED₅₀ > 40 mg kg⁻¹, i.p.), suggesting that it should not produce EPS. Finally, F15063 did not induce the 'serotonin syndrome' in rats (ED₅₀ > 32 mg kg⁻¹ i.p.). In conclusion, F15063 has the preclinical profile of a potent atypical antipsychotic (Depoortere *et al.*, 2006, 2007).

However, as emphasized above, controlling positive symptomatology is not sufficient to enable schizophrenic patients to return to normal social activity and there is a considerable unmet need for relieving negative symptoms and memory/cognitive defects associated with the pathology. It is therefore essential to characterize potential antipsychotics in a battery of preclinical tests purported to mimic negative symptoms and cognitive deficits of schizophrenia.

Based on the hypothesis that interference with glutamatergic transmission via the NMDA receptor underlies aspects of schizophrenia (Javitt and Zukin, 1991; Olney and Farber, 1995), we undertook the assessment of F15063 in two models of negative symptoms and deficits of cognition/memory produced by the noncompetitive antagonist of NMDA receptors, phencyclidine (PCP): (1) social interaction between a dyad of adult rats and (2) a reversal learning task (RLT) using a two-lever food-reinforced operant schedule.

Additionally, alterations of cholinergic neurotransmission have been shown to occur in schizophrenia (Bymaster *et al.*, 1999; Hyde and Crook, 2001). For that reason, we assessed the influence of F15063 on memory disruption consecutive to administration of the anticholinergic agent scopolamine, in a model of social recognition between an adult and a juvenile rat. Further, because F15063 is also a partial agonist at the dopamine D₄ receptor, we also conducted antagonist studies with the D₄ receptor blocker L745,870 in this model.

Lastly, because of its 5-HT_{1A} agonist activity, F15063 was evaluated in the PPI of the startle reflex in rats. In effect, activation of 5-HT_{1A} receptors by agonists such as 8-OH-DPAT and buspirone (Nanry and Tilson, 1989; Rigdon and Weatherspoon, 1992) diminishes basal PPI in rats. Such a disruption could be of concern, since schizophrenic patients already suffer from reduced basal PPI levels (Braff *et al.*, 1978; Swerdlow *et al.*, 2000 for review), and it would thus be desirable for an antipsychotic not to further affect basal PPI.

Data presented in this paper have been for the most part presented in abstract forms (Auclair *et al.*, 2006b; Bardin *et al.*, 2006b; Depoortere *et al.*, 2006).

Materials and methods

Animals

Male Sprague-Dawley rats (180–200 g for adults, 70–80 g for juveniles, at the start of the experiments) were supplied by Iffa-Credo (Les Oncins, France). Animals were kept in temperature- and humidity-controlled rooms (21 ± 1°C, relative humidity: 55 ± 5%) on a 12:12 h light:dark cycle (lights on at 0700 h). Food (standard A04 rodent chow, Animal Food and Engineering, Epinay sur Orge, France) and filtered water (0.22 µm pores) were available *ad libitum* (except when specified otherwise below). Animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, USA) and the European Directive 86/609. In addition, the protocols were carried out in compliance with French regulations and the local ethical committee guidelines for animal research.

PCP-induced social interaction deficit between a dyad of adult rats

The social interaction procedure was adapted from that developed by Sams-Dodd (1995) and described in detail by Bruins Slot *et al.* (2005). F15063 or vehicle was administered i.p. daily for 3 days in combination with a subcutaneous (s.c.) injection of either vehicle or 2.5 mg kg⁻¹ PCP. Social interaction was measured on the last (3rd) day of drug treatment, 45 min after the last injection. For the

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antagonism study, rats received a supplementary s.c. injection of either vehicle or 0.63 mg kg^{-1} WAY100,635 90 min before the behavioral observations. Pairs of unfamiliar rats having received the same treatment were placed for 10 min in a wooden open arena ($150 \times 100 \times 40 \text{ cm}$ high) painted in black. The total number of the following behavioral items was scored (one point for each occurrence of behavior in either rat, with no lower cutoff time limit) by an investigator blind to the treatment: investigative sniffing behavior (sniffing the conspecific's snout or parts of the body including the anogenital region), following (moving towards and following the conspecific around the arena), climbing over or under (climbing over the conspecific's back or pushing the head and forepart of the body beneath the conspecific), and aggression (including biting, fighting, mounting and threats). Scores for each behavioural item were added to yield a total score for a given pair of rats (i.e., total number of events).

Data (total scores) were first analyzed by a two-way ANOVA with the dose of F15063 and pretreatment (saline or PCP) as the between-subjects factors, followed by one-way ANOVA on the dose of F15063, and *post hoc* Bonferroni's test. For the antagonism study with WAY100,635, data were analyzed with a one-way ANOVA, followed by a *post hoc* Bonferroni's test.

PCP-induced deficit of reference memory and task reacquisition in a RLT in rats

All rats were tested in operant Skinner boxes ($29 \times 25 \times 32 \text{ cm}$, $W \times L \times H$, Coulbourn Instruments, Lehigh Valley, PA, USA) enclosed in ventilated and sound-attenuating cubicles ($54 \times 40 \times 45 \text{ cm}$, $W \times L \times H$). Each box was fitted with two retractable levers ($3 \times 2 \text{ cm}$ deep) on either side of the magazine where 45 mg food pellets were delivered. A white cue lamp and a buzzer (85 dB, 2 s tone: a high-tone (10 kHz), and a low-tone (2 kHz), associated with presentation of the right and left retractable lever, respectively) served as stimulus cues.

First, rats were shaped (daily sessions of 30 min) to lever-press to receive a reinforcement (45 mg pellet) on a fixed-ratio 1 schedule. Initially, one lever was randomly presented: if the rat pressed this lever, one pellet was delivered, the lever was retracted, and another lever was immediately randomly (left or right) presented. If the rat failed to press within 30 s of lever presentation, the lever was retracted, and a lever was again immediately randomly presented. Each lever was presented in concomitance with its associated cue light and tone combination (see above). This pretraining period lasted between 5 and 11 days. Rats progressed to the learning task 1 (LT1) schedule once they pressed each lever at least 20 times during two consecutive pretraining sessions.

This LT1 schedule lasted for 5 days: each daily session (40 min) started with the introduction of both levers, only one being active, that is delivering a pellet when pressed. The active lever was signalled by the concomitant presentation of the associated cue light and tone. If the rat pressed the active lever within 10 s of presentation, a pellet was delivered, both levers were retracted and reintroduced 10 s later. The active lever was randomly reassigned. If the rat did not press within

10 s or pressed the inactive lever, the house light was turned off, both levers were retracted for 10 s, after which a new trial of presentation was initiated, the active lever remaining the same as in the preceding 'failed' trial. Rats were required at least 70% correct responding on three consecutive sessions to be trained in the learning task 2 (LT2) schedule.

The LT2 schedule was in all points similar to that of the LT1, except that the rules were reversed, that is the active lever was the one *not* associated with its cue-light and tone. Rats were required at least 70% correct responding on three consecutive sessions to enter the phase of pharmacological treatment. This schedule lasted up to 20 days.

This phase lasted for 14 sessions: during the first three sessions (D1–D3), rats were treated with vehicle or F15063 (i.p.) 45 min before vehicle or PCP (2.5 mg kg^{-1} , s.c.). During these 3 days, rats were not tested: this period of treatment was intended to induce tolerance to the motor-disrupting side-effects of PCP, which could interfere with operant responding during tests sessions. On the 4th day (D4), rats were treated as usual and tested under the LT2 schedule 45 min after the second injection: this test was introduced to assess the impact of pharmacological treatment (D1–D4) on the retention of LT2 (reference memory component). From D5–D14, rats were tested under the LT1 schedule: this phase served to assess the influence of pharmacological treatment on the reacquisition of LT1 (second reversal) (Scheme 1).

D1–D3		D4	D5–D14	
Pre-training	LT1	LT2	LT2	LT1
		Treatment		

LT: learning task

Scheme 1 Synopsis of training schedules and pharmacological treatment phase for the RLT experiment.

The percentage of correct responses and the latency time to respond on any lever (in 1/10 s) were recorded during each LT1 and LT2 schedule session. Control (vehicle-vehicle) and vehicle-PCP-treated rats included rats injected with saline i.p. (with and without Tween 80) and with saline s.c. This is because a whole series of reference antipsychotics (data to be published) have been tested in parallel with F15063, with different routes of administration and excipient. Data were analyzed by means of a one-way ANOVA for comparing, across treatment conditions, basal data (average of the last 3 days before starting the pharmacological treatment) and for comparing data obtained during D4. It was followed when appropriate by a *post hoc* Bonferroni's test. Data collected during D5–D14 were collated across all 10 sessions, and were analyzed with a one-way ANOVA, followed when appropriate by *post hoc* Dunnett's test.

Scopolamine-induced deficit of social recognition between an adult and a juvenile rat

The procedure was adapted from that described by Perio *et al.* (1989). Briefly, on the test day, an adult was placed alone in an observation arena for 5 min, after which a juvenile rat was

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introduced for a first 5 min session (T1). The same juvenile was re-exposed to the adult rat 30 min later, for a second session of social interaction (T2). The time (in s) spent by the adult interacting socially with the juvenile (sniffing, following, climbing over or going under, or aggression) was manually recorded during T1 and T2 by an investigator blind to the treatment. The adult rat was treated with vehicle or F15063 i.p. 45 min before the first observation session (T1), followed 15 min later by scopolamine (0.63 mg kg^{-1} , s.c.).

An additional control experiment aimed at assessing the specificity of reversing effects against scopolamine-induced amnesia was undertaken. To that end, the adult rat was exposed to an unfamiliar juvenile during T2, after being injected with F15063 alone (without scopolamine). If F15063 produces a retention score close to that seen in vehicle-treated rats, it will mean that the adult spent as much time investigating the unfamiliar juvenile during T2 as investigating the first juvenile during T1. This would indicate that the reversing effects seen against scopolamine are not secondary to a lack of interest for the juvenile or to aspecific motor effects produced by F15063, which would somehow interfere with proper social interaction. Finally, in order to assess the amnesic activity of F15063 on its own, the compound was also injected alone, 30 min before T1, and the adult rat was presented with the same juvenile during T2.

In a separate experiment, adult rats received an i.p. injection of vehicle or 0.63 mg kg^{-1} of the DA D_4 receptor antagonist L745,870, 15 min before administration of F15063, which was followed by an injection of scopolamine (see above).

Retention scores (difference in the time spent in active social behavior during the second encounter minus the time spent in active social behavior during the first encounter: $T2 - T1$, in second) were analyzed by a one-way ANOVA followed by *post hoc* Bonferroni's test.

Effects on basal PPI of the startle reflex in rats

A detailed description is given in Auclair *et al.* (2006a). Rats were pretested in startle chambers (SR LAB, San Diego Instruments, San Diego, CA, USA) 1 h 45 min before the pharmacological challenge (test) session. This pretest session was used to accustom rats to the procedure. Three different trial types were presented against a continuous 70 dB background noise: no pulse (NP), 118 dB pulse (pulse alone: PA) and 78 dB prepulse (pp) followed by a 118 dB pulse (prepulse-pulse: ppP). The PA duration was 40 ms, the pp duration 20 ms, and the interval between the end of the pp and the onset of the PA 80 ms. Sessions started with a 5 min adaptation period after which the animals were exposed to 10 PA (included to induce habituation to startle, such that habituation during the following PPI assessment would be minimized: these trials were not used for data analysis). These 10 PA trials were followed by 10 PA, 10 ppP and three NP trials presented in a pseudo-random order. The interval between trials was variable but with a median of 15 s.

At the end of the pretest session, animals were injected i.p. with F15063 or its vehicle and 45 min later with vehicle s.c. This second injection was meant to be under conditions

similar to those used in the reversion of apomorphine deficits by antipsychotics, where vehicle was replaced by apomorphine (Auclair *et al.*, 2006a). They were then (15 min later) subjected to a test session, in all respects similar to the pretest session (*vide supra*).

For each test session, the median of the amplitude of the startle responses for the last 10 PA trials and for the 10 ppP was calculated. The percentage PPI was calculated as follows:

$$\frac{(\text{median PA amplitude} - \text{median ppP amplitude}) \times 100}{(\text{median PA amplitude})}$$

Data (percentage PPI and amplitude of PA) were analyzed with a one-way ANOVA with the treatment as the between-subjects factor, followed by a Dunnett's *post hoc* test.

Statistical analyses. Results were analyzed by ANOVA (one- or two-way) with appropriate *post hoc* tests (Dunnett's, Bonferroni's or Newman-Keul's). The details of the statistical treatment of each data set are given at the end of the description of the methods used to generate the data.

Drugs

F15063 ((N-[(2,2-dimethyl-2,3-dihydro-benzofuran-7-yloxy)ethyl]-3-(cyclopent-1-enyl)-benzylamine; mono-tartrate) and WAY100,635 were synthesized by Bernard Vacher/Stephane Cuisat and Jean Louis Maurel (Medicinal Chemistry, Centre de Recherche Pierre Fabre, Castres, France). All other compounds were obtained commercially: scopolamine (Sigma RBI, St Quentin Fallavier, France), L745,870 (Tocris, Illkirch, France) and PCP hydrochloride (Francopia, Paris, France), and like WAY100,635, were dissolved in distilled water and administered s.c. at a volume of 10 ml kg^{-1} . F15063 was administered i.p. in a volume of 10 ml kg^{-1} in distilled water + Tween 80 (0.1% v/v). Doses refer to the weight of the free base.

Results

Attenuation of PCP-induced social interaction deficit between a dyad of adult rats

Treatment with 2.5 mg kg^{-1} s.c. PCP produced a robust reduction of the number of social interaction events, representing a 60% decrease versus control values (compare the two most leftward symbols, Figure 1). F15063, from 0.04 to 0.16 , and at 0.63 mg kg^{-1} , significantly attenuated PCP-induced reduction of social interaction (filled circles). At these doses, it had no significant effect of its own (0.04 and 0.08 mg kg^{-1}) or significantly reduced (0.16 and 0.63 mg kg^{-1}) social interaction in the vehicle-pretreated animals (Figure 1).

Attenuation by F15063 of PCP-induced social interaction deficit is antagonized by the 5-HT_{1A} receptor antagonist WAY100,635

F15063 (0.04 and 0.16 mg kg^{-1} i.p.) significantly attenuated PCP-induced deficit of social interaction (compare 2nd

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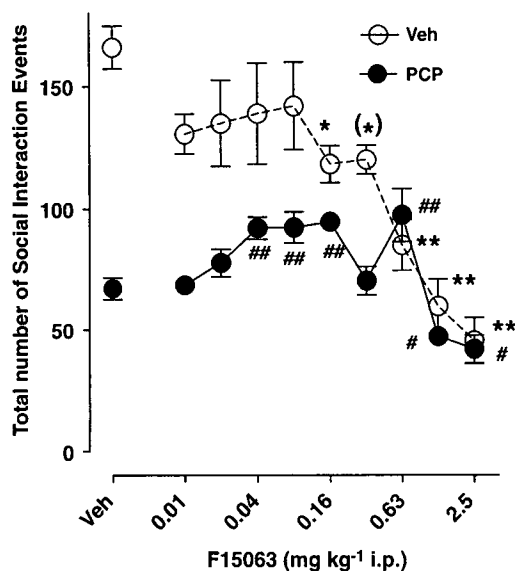


Figure 1 F15063 attenuated a deficit of social interaction in rats produced by PCP. Each symbol/bar represents the mean ($\pm$ s.e.m.) number of social interaction events between a dyad of rats. F15063 or vehicle was administered daily for 3 days in combination with a s.c. injection of either vehicle or 2.5 mg kg⁻¹ PCP. Social interaction (10 min observation period) was measured on the last (3rd) day of drug treatment, 45 min after the last injection. Statistical analysis: two-way ANOVA: $F(9,132) = 14.1$, $P < 0.001$, $F(1,132) = 815$, $P < 0.001$ and $F(9,132) = 6.7$, $P < 0.001$, for the F15063 dose, PCP treatment and F15063 dose $\times$ PCP treatment interaction, respectively. This two-way ANOVA was followed by separate one-way ANOVAs. (*) $P = 0.05$, * $P < 0.05$, ** $P < 0.01$, versus vehicle for vehicle-treated rats, Bonferroni's *post hoc* test, following significant one-way ANOVA: $F(9,69) = 10.3$, $P < 0.001$. # $P < 0.05$, ## $P < 0.01$, versus vehicle for PCP-treated rats, Bonferroni's *post hoc* test, following significant one-way ANOVA: $F(9,69) = 11.4$, $P < 0.001$. $N = 7$ –9 pairs of rats per group.

with 5th and 6th white bars, starting from the left, Figure 2, left panel). By itself, at these doses, F15063 produced a significant reduction of social interaction at 0.16 mg kg⁻¹ only (compare 1st with 4th white bars, starting from the left).

Pretreatment with WAY100,635 (0.63 mg kg⁻¹, s.c.) prevented the attenuating effects of F15063 on disruption of social interaction produced by PCP (compare the 2nd with 5th and 6th black bars, starting from the left, Figure 2, right panel). In combination with WAY100,635, F15063 had a significant effect by itself at 0.16 mg kg⁻¹ only (compare 1st with 4th black bars, starting from the left).

Attenuation of PCP-induced deficit of choice accuracy and augmentation of lever-pressing latency times during a test session for reference memory in the RLT in rats

Basal (before starting pharmacological treatment) values did not significantly differ between groups of rats (compare leftmost cluster of symbols, Figure 3a and b). On the 4th day of treatment (D4), rats treated with 2.5 mg kg⁻¹, s.c. PCP produced a marked and significant decrease of percentage of correct responses in comparison with vehicle-vehicle control rats (compare circles, second cluster of symbols, starting from the left, Figure 3a and b). More precisely, the level of choice accuracy dropped from 80.6 ± 1.6 ($n = 15$) for controls to 50.9 ± 5.1 ($n = 11$) for PCP, a value very close to the chance level. This suggests that reference memory for responding governed by LT2 was greatly affected by PCP. By itself, F15063, at both doses, did not produce changes (compare open symbols). In combination with PCP, F15063 at 0.16 mg kg⁻¹ tended to attenuate the deleterious effect of PCP (compare filled symbols).

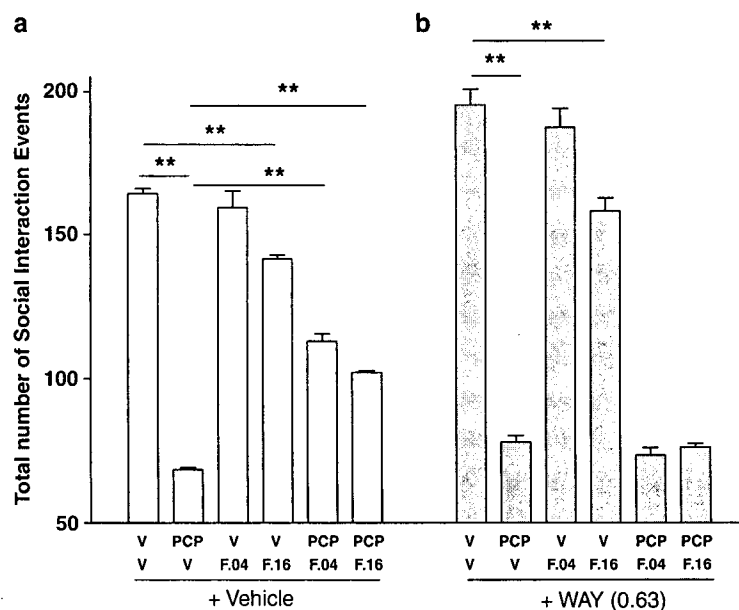


Figure 2 The attenuating effects of F15063 against PCP-induced social interaction deficit (a) were antagonized by the 5-HT_{1A} receptor antagonist WAY100,635 (b). See legend of Figure 1 for details. Rats were given saline (a) or WAY100,635 (0.63 mg kg⁻¹, s.c.) (b), 90 min before the observation period. ** $P < 0.01$, Bonferroni's *post hoc* test, following significant one-way ANOVA; for data in a, $F(5,38) = 210.0$, $P < 0.001$; data in b, $F(5,38) = 188.1$, $P < 0.001$. $N = 7$ pairs of rats per group.

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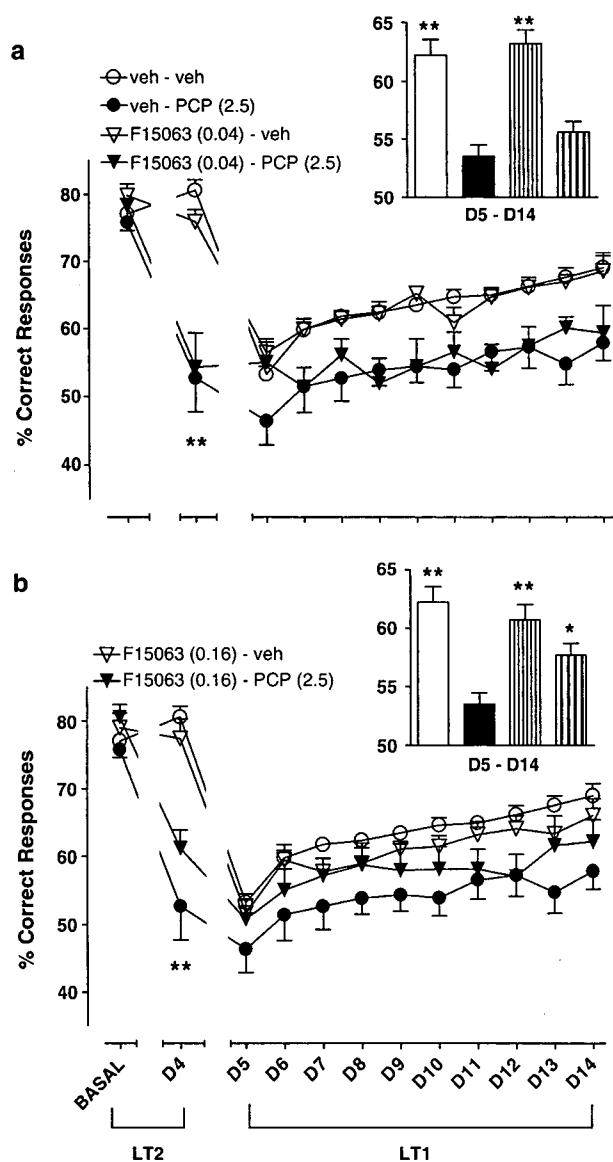


Figure 3 Reduction by F15063 of a deficit of choice accuracy induced by chronic treatment with PCP in the RLT in rats. Each symbol represents the mean ($\pm$ s.e.m.) percentage of correct responses (i.e. responses on the appropriate lever) obtained during the three sessions immediately preceding the start of the pharmacological treatment (BASAL), the 4th day of treatment (D4) and during the 5th to 14th day of treatment (D5–D14). Basal values (lefthand cluster of points) did not differ between groups of rats (one-way ANOVA: $F(3,36)=1.2$, $P>0.05$ and $F(3,39)=1.9$, $P>0.05$, for 0.04 and 0.16 mg kg⁻¹ of F15063, respectively). * $P<0.05$, ** $P<0.01$, compared with vehicle–vehicle value at D4, for the corresponding treatment, Bonferroni's *post hoc* tests following significant one-way ANOVA ($F(3,32)=18.5$, $P<0.001$ and $F(3,33)=18.6$, $P<0.001$, for 0.04 and 0.16 mg kg⁻¹ of F15063, respectively). $N=5-9$ rats per group. Inset: each bar represents the average ($\pm$ s.e.m.) percentage of correct responses collated across the 5th to the 14th day. * $P<0.05$, ** $P<0.01$, compared with vehicle/PCP treated rats, Dunnett's *post hoc* test following significant one-way ANOVA. The order of treatments is the same as that in the legend on the left.

During the basal phase, the latency time to lever-press on any of the two levers did not differ significantly between groups (compare leftmost cluster of symbols, Figure 4a

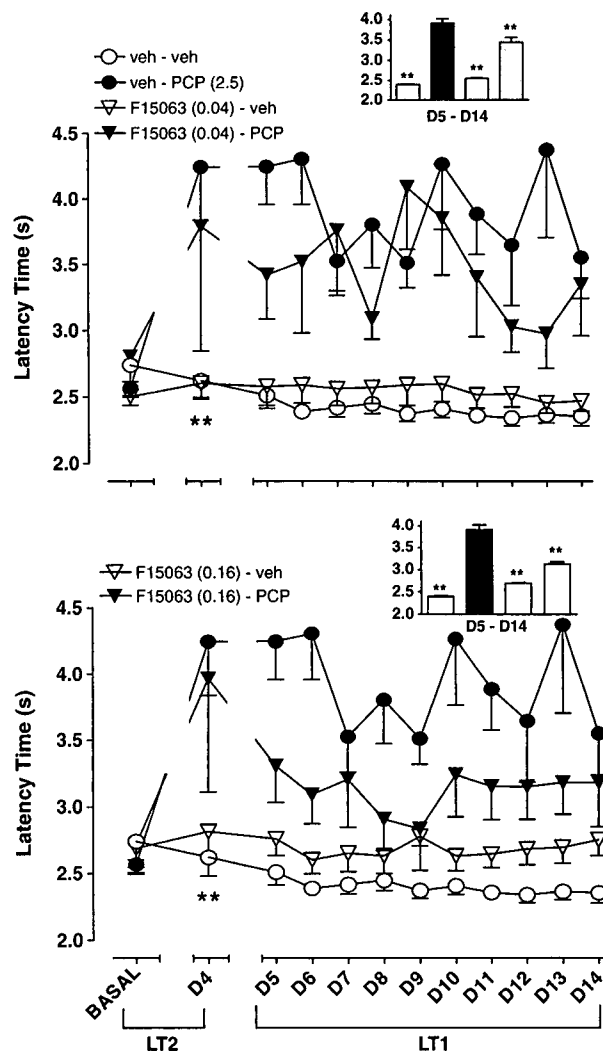


Figure 4 Reduction by F15063 of the increase in the latency time to the first response induced by chronic treatment with PCP in the RLT in rats. Each symbol represents the mean ($\pm$ s.e.m.) latency time to the first response on any lever, obtained during the three sessions immediately preceding the start of the pharmacological treatment (BASAL), the 4th day of treatment (D4) and during the 5th to 14th day of treatment (D5–D14). Basal values did not differ between groups (one-way ANOVAs: $F(3,36)=1.0$, $P>0.05$ and $F(3,39)=0.5$, $P>0.05$, for 0.04 and 0.16 mg kg⁻¹ of F15063, respectively). For day 4, * $P<0.05$, ** $P<0.01$, compared with vehicle/vehicle value at D4, for the corresponding treatment, Bonferroni's *post hoc* tests following significant one-way ANOVA ($F(3,27)=7.1$, $P<0.001$ and $F(3,31)=5.7$, $P<0.05$, for 0.04 and 0.16 mg kg⁻¹ of F15063, respectively). $N=5-9$ rats per group. Inset: each bar represents the average ($\pm$ s.e.m.) latency time collated across the 5th to the 14th day. At the lower dose, F15063 did not reverse PCP-induced effects (Dunnett's *post hoc* test, following significant one-way ANOVA: $F(3,36)=18.6$, $P<0.0001$). At the higher dose (0.16 mg kg⁻¹), ** $P<0.01$, compared to vehicle/PCP-treated rats, Dunnett's *post hoc* test following significant one-way ANOVA ($F(3,36)=10.9$, $P<0.0001$). The order of treatments is the same as that in the legend on the left.

and b). During D4, rats treated with 2.5 mg kg⁻¹ PCP took nearly twice as long before initiating lever-pressing, a significant effect.

Attenuation of PCP-induced deficit of choice accuracy and augmentation of lever-pressing latency times during the reacquisition phase for LT1 (D5–D14) in the RLT in rats

During the reacquisition phase for LT1 (D5–D14 phase, right-hand side of Figure 3a), PCP-injected rats (filled circles) performed less well than their control counterparts. However, the performance of both groups progressed over time, albeit with an apparent steeper slope (faster learning curve) for the vehicle-treated group. Complementary analysis performed on data collated across all 10 sessions showed that F15063 at 0.16 mg kg^{-1} partially but significantly reversed the deficit produced by PCP (compare 2nd and 4th bars, starting from the left, inset of Figure 3b). At 0.04 mg kg^{-1} , F15063 did not significantly reverse PCP-induced disruption (compare 2nd and 4th bars, starting from the left, inset of Figure 3a).

During this phase, the latency time to lever-pressing remained elevated under PCP treatment, (filled circles, right-hand side of Figure 4a and b). Complementary analysis performed on data collated across all 10 trials showed that F15063 at 0.16 mg kg^{-1} partially but significantly reversed the increase in latency produced by PCP (compare 2nd and 4th bars, starting from the left, inset of Figure 4b). However, contrary to what was observed with the percentage of correct responses (*vide supra*), F15063 at 0.04 mg kg^{-1} also significantly attenuated PCP-induced effects on latency (compare 2nd and 4th bars, starting from the left, inset of Figure 4a).

Reversal of scopolamine-induced deficit of social recognition between an adult and a juvenile rat

With respect to those of the control (vehicle/vehicle-injected) group, rats pretreated with 0.63 mg kg^{-1} s.c. of scopolamine presented a highly significant reduction of the retention score (compare first pair of bars, starting from the left, Figure 5a). In other words, under scopolamine, they spent almost as much time interacting with the juvenile during T2 than during T1 (hence a retention score, $T2-T1$, close to 0), consistent with an amnesic effect of scopolamine. F15063 dose-dependently reversed this deleterious effect of scopolamine, with values from 0.04 mg kg^{-1} i.p. not significantly different from those of controls (Bonferroni's *post hoc* test).

On its own, F15063, administered 30 min pretest (before T1) did not modify the retention score (-24.6 ± 3.4 , -27.2 ± 5.5 , -16.3 ± 11.7 , -21.8 ± 5.4 and -29.5 ± 4.4 , for vehicle, 0.01, 0.04, 0.16 and 0.63 mg kg^{-1} , respectively; one-way ANOVA: $F(4,49) = 0.56$, $P > 0.05$).

When confronted with an unfamiliar juvenile during T2, rats treated with F15063 did not show retention scores different from those of vehicle-treated rats (-13.8 ± 2.2 , -13.0 ± 4.7 , -19.9 ± 6.9 , -9.3 ± 8.0 and -29.8 ± 6.6 , for vehicle, 0.01, 0.04, 0.16 and 0.63 mg kg^{-1} , respectively; one-way ANOVA: $F(4,40) = 1.79$, $P > 0.05$). This demonstrates that under these conditions, rats treated with F15063 retain their ability to interact when confronted with an unfamiliar juvenile, and that the reversion observed in combination with scopolamine with a familiar juvenile is not the consequence of nonspecific motor, sedating or attention deficit side-effects produced by F15063.

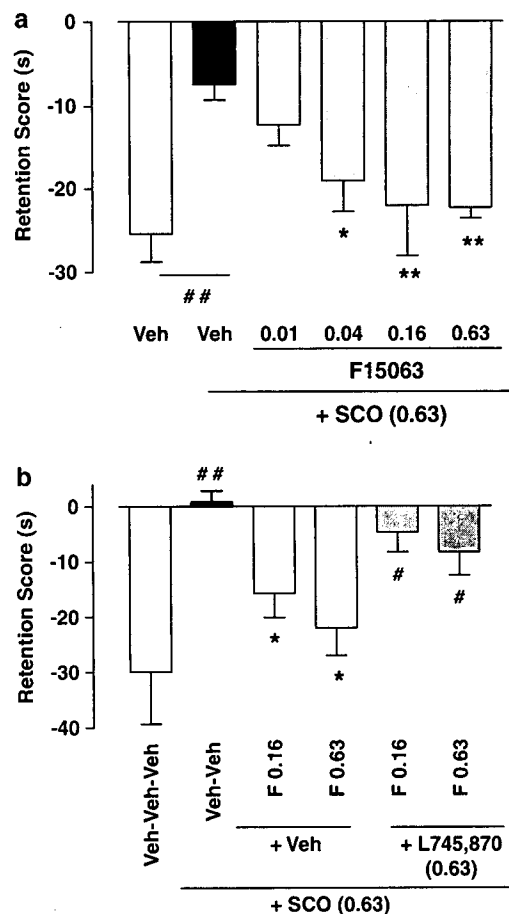


Figure 5 (a) F15063 reversed the decrease of retention score induced by scopolamine. (b) This reversal is antagonized by the dopamine D4 receptor antagonist L745,870. Each bar represents the mean ($\pm$ s.e.m.). difference in the time spent in social interaction during the second encounter (T2) minus the time spent in social interaction during the first encounter (T1). (a) Vehicle or F15063 were administered i.p. to the adult rat 45 min before testing, and scopolamine (Sco: 0.63 mg kg^{-1} , s.c.) or vehicle were administered 30 min before the observation session. Scopolamine caused a reduction of the score (significant one-way ANOVA: $F(5,112) = 6.84$, $P < 0.001$) which was totally reversed by F15063. $N = 9$ adult rats per dose. (b) L745,870 or vehicle were administered i.p. 15 min before vehicle or F15063. The D4 antagonist prevented the effects of F15063 against scopolamine induced amnesia $N = 11$ adult rats per dose. For both panels: * $P < 0.05$, ** $P < 0.01$, compared with the scopolamine-injected group; # $P < 0.05$, ## $P < 0.01$, compared with the vehicle-injected group using Bonferroni's test, following significant ANOVA (one-way, $F(5,58) = 4.62$, $P < 0.001$).

Co-administration of the DA D4 receptor antagonist L745,870 (0.63 mg kg^{-1}) almost completely reversed the beneficial effects of F15063 against scopolamine-induced amnesia (compare the middle and right pairs of bars, Figure 5b). Thus the rats given vehicle/vehicle/scopolamine or L745,870/F15063(0.16)/scopolamine or L745,870/F15063(0.63)/scopolamine behaved differently from those treated with vehicle/vehicle/vehicle. By itself, L745,870 had no effect on scopolamine-induced amnesia (-25.2 ± 3.0 , -6.6 ± 1.8 , -6.0 ± 5.0 , -12.4 ± 4.2 , -8.7 ± 10.6 , for vehicle, scopolamine, 0.04, 0.16 and 0.63 mg kg^{-1} of L745,870, respectively).

Table 1 Effects of F15063 on the percentage of PPI and on the pulse alone amplitude of the startle reflex in rats

Dose	Vehicle	0.0025	0.01	0.04	0.16	0.63	2.5	10
% PPI	48.1 ± 3.7	45.4 ± 4.2	48.1 ± 9.6	50.1 ± 7.3	50.1 ± 15.2	62.3 ± 6.8	52.4 ± 8.4	51.8 ± 7.3
Pulse amplitude	96.6 ± 10.7	104.1 ± 9.5	91.6 ± 15.4	238.7 ± 101.4*	119.8 ± 33.1	78.6 ± 14.9	79.6 ± 10.2	248.1 ± 55.3*

Abbreviations: ANOVA, analysis of variance; i.p., intraperitoneal; PPI, prepulse inhibition; s.e.m., standard error of the mean. Data shown in the Table are the means ± s.e.m.; doses of F15063 are expressed in mg kg⁻¹ i.p. There was no effect of F15063 on % PPI values (one-way ANOVA, $F(7,62) = 0.4$, $P > 0.05$). For the pulse alone amplitude, * $P < 0.05$, compared with the vehicle-injected group using Dunnett's *post hoc* test, following significant one-way ANOVA ($F(7,62) = 3$, $P < 0.01$). $N = 7$ rats per group, except for vehicle ($n = 30$).

Table 2 Summary of pharmacological activity of F15063 in models predictive of activity against negative symptoms and cognitive deficits of schizophrenia

Model	Response	Active dose(s) (mg kg ⁻¹ i.p.)
PCP-induced social interaction impairment	Augments interaction	0.04–0.16 & 0.63
PCP-induced impairment in reversal learning task	Ameliorates correct choice	0.16
	Diminishes latency times to respond	0.04 & 0.16
Scopolamine-induced social recognition deficit	Blocks amnesia	0.04–0.63

Absence of effect on basal PPI of the startle reflex in rats

Over a wide range of doses (0.01. to 10 mg kg⁻¹ i.p.), F15063 did not significantly modify basal PPI levels (Table 1). F15063 significantly affected PA amplitude, with *post hoc* test revealing that the effects were restricted to the 0.04 and 10 mg kg⁻¹ doses (Table 1).

Table 2 summarizes the pharmacological activity of F15063 in tests predictive of activity against memory/cognitive deficits associated with schizophrenia. F15063 did not disrupt ($ED_{50} > 10$ mg kg⁻¹) basal PPI of the startle reflex, suggesting that at therapeutically meaningful doses, F15063 should be free from this potentially troublesome side effect.

Discussion

The present data underline the following points: (1) F15063 reversed or attenuated memory and social-interaction deficits produced by blockade of glutamate/NMDA or muscarinic receptors; (2) Beneficial activity against the latter was mediated by its partial agonist activity at DA D₄ receptors; (3) F15063 had no detrimental effect by itself in these memory/cognition models, except at high doses in the social interaction model where it reduced the score and (4) F15063 did not disrupt basal PPI of the startle reflex.

F15063 is active in models predictive of activity against memory and cognitive deficits associated with schizophrenia

F15063 was, among several reference antipsychotics including clozapine, the only compound with activity in each of two different models of cognitive/memory impairment (present results, Auclair *et al.*, 2006b; Bardin *et al.*, 2006b): (1) PCP-induced memory reacquisition impairment in a 'RLT' operant conditioning paradigm, and (2) scopolamine-induced social memory deficits in the adult/juvenile social recognition model.

In the RLT experiment, semi-chronic treatment with PCP markedly impaired performance on day 4, when rats were tested under the LT2 schedule. This test provides a measure of 'reference' memory, because rats were required to lever-press using a rule that had previously been learned with a high level of accuracy (cf. the 80% or so accuracy score recorded during the basal phase). Furthermore, in the same model, PCP produced a slowing of reacquisition of the first LT1, that is the animals responded at random (i.e. at chance level). Overall, the effects of PCP in this model are reminiscent of the impairment of reference memory and behavioral inflexibility presented by schizophrenic patients (Cridder, 1997). This last aspect is clinically exemplified in the Wisconsin Card Sorting Test, where patients show an impaired ability to learn the new rule and adopt a winning strategy (Fey, 1951). Performance in the WCST is thought to rely heavily on the prefrontal cortex and the poor level of performance in this task displayed by schizophrenic patients is thought to reflect hypofrontality (Deicken *et al.*, 1995; Ragland *et al.*, 1998).

F15063 ameliorated the level of performance in the RLT test. More precisely, it attenuated the deficit of correct responding produced by PCP during day 4. F15063 by itself did not modify this parameter and the latency time to lever-press. During the reversal phase (D5–D14), F15063 ameliorated the level of accuracy and decreased latency times, again with no or very limited effects of its own. In a comparative study (Auclair *et al.*, 2006b) of the effects of reference antipsychotics on deficits of RLT produced by PCP, SSR181507 showed a profile of activity similar to that of F15063. Clozapine also attenuated the deficit, albeit with a longer onset of action: its effects were observable from day 8 of treatment only. Concerning haloperidol and aripiprazole, although both compounds also seemed to reverse the deleterious effects of PCP on task reacquisition, their effects were attributable to deleterious perseveration of responding governed by the initial (LT1) rule. Furthermore, during the retention phase, SSR181507 on its own diminished performance, while aripiprazole and haloperidol caused further

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deterioration of the PCP-induced impairment. There are very few examples in the literature of pharmacological studies on RLT: Ziprasidone, but not haloperidol or clozapine, attenuated a deficit of RLT in an operant conditioning schedule induced by an acute challenge with PCP (Abdul-Monim *et al.*, 2003; Idris *et al.*, 2005).

F15063 attenuates PCP-induced social interaction impairment between a dyad of adult rats, owing to its 5-HT_{1A} agonist activity F15063 alleviated, through activation of 5-HT_{1A} receptors, PCP-induced deficit of social interaction between a pair of adult rats. In a preceding paper (Bruins Slot *et al.*, 2005), we reported that among eight antipsychotics tested under similar experimental conditions, only the mixed DA D₂/5-HT_{1A} compounds SSR181507 and aripiprazole, and the DA D₂ receptor antagonist remoxipride, were able to attenuate this type of deficit. One possible alternative explanation for the beneficial effect of F15063 in this model would be that the compound attenuated hyperlocomotion and motor stereotypies produced by 2.5 mg kg⁻¹ PCP. However, this is improbable, as hyperactivity induced by this dose of PCP was not modified by 0.04 mg kg⁻¹ i.p. F15063 (the most active dose in the social interaction test: cf. Figures 1 and 2). However, when tested in actimeters used for the psychotomimetic-induced hyperlocomotor activity experiments described in the companion paper (Depoortere *et al.*, 2007), motor scores over 15 min were unchanged 67.4 ± 6.1 and 65.0 ± 14.3, for veh/PCP and F15063/PCP-treated rats, respectively ($t = 0.16$, NS).

It should be noted that for all compounds active in this model, the reversal of deterioration of performance following PCP, although significant, was only partial (present data, Bruins Slot *et al.*, 2005). It is possible that under slightly different experimental conditions (such as a lower dose of PCP and/or by habituating rats to the apparatus and to a different companion over several days and/or by measuring the time spent in social contact instead of scoring the total number of social events), a more robust effect might have been observed, as has been reported for SSR181507 by Boulay *et al.* (2004). Also, at doses of 0.16 mg kg⁻¹ and above, F15063 diminished social interaction on its own: a similar response was observed for all antipsychotics that were tested under similar experimental conditions, whether they did (SSR181507 and aripiprazole) or did not (clozapine) attenuate PCP-induced social interaction deficit (Bruins Slot *et al.*, 2005). These effects, which may have indeed limited the extent to which they could reverse PCP-induced deterioration of social behavior (see above) are probably related to sedation: however, patients can develop tachyphylaxis to the sedative effects of antipsychotics. Further, under the present experimental conditions, both rats were treated with an antipsychotic: if only one rat had been treated, interaction with a 'normal' (i.e. vehicle-treated) companion may have been less sensitive to the effects of antipsychotics given alone. This issue deserves further investigation. Similarly to what was observed here, the 5-HT_{1A} receptor antagonist WAY100,635 blocked the effects of SSR181507 and aripiprazole, showing that activation of 5-HT_{1A} receptors is involved in their activity. Furthermore, results with SSR181507, in a variant of the PCP-induced deficit of social interaction used

in this laboratory, led Boulay *et al.* (2004) to formulate the same type of conclusion. The difficulty encountered by schizophrenic patients in establishing social contacts is a major aspect of a cluster of negative symptoms (Dickerson *et al.*, 1996). Activity of F15063 in this present social interaction model suggests potential amelioration of social functioning of patients. This result, in conjunction with the increase in DA in prefrontal cortex of rats (Newman-Tancredi *et al.*, 2006), a surrogate putative marker of activity against negative symptoms of schizophrenia (Kapur and Remington, 1996), additionally underlie the potential of F15063 to combat negative symptomatology.

Efficacy of F15063 in a model of scopolamine-induced memory disruption is mediated by activation of DA D₄ receptors

F15063 fully reversed the deleterious effects of scopolamine in an adult-juvenile social recognition paradigm. Schizophrenic patients show deficit of short-term memory as well as a marked impairment in their ability to initiate and maintain social links (Dickerson *et al.*, 1996); in addition, deficient cholinergic neurotransmission is implicated in schizophrenia (Bymaster *et al.*, 1999, Hyde and Crook, 2001). Both these elements justify the use of this model to probe the promnesic/pro-cognitive activity of a potential antipsychotic. In the case of F15063, its activity against memory perturbation consequent to a pharmacologically induced hypocholinergic state lends credence to the notion that compounds displaying such profile could be useful in pathologies characterized both by a deficient cholinergic system and a high incidence of psychosis. Such pathologies include Alzheimer's disease (AD) and Lewy body dementia. Indeed, up to 41% of AD patients, and 70% of Lewy body dementia patients, present psychosis with hallucinations, delirious thoughts and delusions (Ropacki and Jeste, 2005, Perry and Perry, 1995), and the therapeutic treatment of these patients poses a great challenge to clinicians (Sultzer, 2004).

The beneficial effect of F15063 against scopolamine-induced deficit was dependent on DA D₄ receptor activation, as shown by the blockade of this effect following co-treatment with the DA D₄ antagonist L745,870. In a parallel study, in which we assessed a whole range of antipsychotics in this model, we found that clozapine, olanzapine and SLV313 (another potential antipsychotic with combined DA D₂ antagonist and 5-HT_{1A} agonist and DA D₄ partial agonist activities, with a level of efficacy at the latter receptor somewhat less than that of F15063: (Cussac *et al.*, 2006) reversed scopolamine-induced disruption of social interaction. Further, the positive activity of SLV313 was also antagonized by L745,870 (Bardin *et al.*, 2006b).

In a slightly different version of the paradigm, where social recognition deficits were induced by increasing the time interval between the two presentations of the juvenile to the adult, the DA D₄ receptor agonist A-412997 had a similar beneficial effect (Browman *et al.*, 2005). Other agonists at D₄ receptors have shown positive effects in mice memory models: PD168077 facilitated memory consolidation in a passive avoidance model (Bernaerts and Tirelli, 2003), and RO-10-5824 increased novel object exploration placed in the center of a familiar open field (Powell *et al.*, 2003). None-

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theless, one should note that there are also indications that DA D₄ receptors antagonists, such as NGD 94-1 and L745,870 can have beneficial impact on cognition: Hence, NGD 94-1 reversed PCP-induced cognition deficit in monkeys (Jentsch *et al.*, 1999), and in a delayed alternation task, L745,870 at high doses disrupted working memory in rats with good baseline performance, but improved performance at low dose in poorly performing subjects (Zhang *et al.*, 2004). However, both these ligands have been shown to act as partial agonists under some *in vitro* conditions (Gazi *et al.*, 2000, Zawilska *et al.*, 2003). Nevertheless, under our experimental conditions ([³⁵S]GTPγS binding in CHO cells transfected with hD_{4.4} receptors, manuscript in preparation), in which F15063 acts as a partial agonist, L745,870 behaves as a silent antagonist (NGD94-1 was not tested). In addition, in the delayed alternation task study, results led Zhang and co-workers to conclude that 'Optimal working memory requires an intermediate level of DA D₄ receptor stimulation'. Such a conclusion is consistent with our data suggesting that partial agonism might be the most appropriate means of enhancing memory performance through DA D₄ receptors. It is interesting to note that D₄ receptors are preferentially expressed in the frontal cortex and hippocampus (Tarazi *et al.*, 2004), two brain regions particularly associated with control of memory/cognition.

F15063 does not produce deficit of basal PPI of the startle reflex

In a preceding study, F15063, like haloperidol, risperidone, clozapine and olanzapine (Auclair *et al.*, 2006a), attenuated apomorphine-induced PPI disruption, a model of gating deficits observed in schizophrenic patients, and considered to predict antipsychotic activity (Geyer *et al.*, 2001). Other selective DA D₂/5-HT_{1A} compounds such as sarizotan, SSR181507 and SLV313, failed to reverse apomorphine-induced PPI disruption, due to their excessive 5-HT_{1A} agonist actions (Auclair *et al.*, 2006a). Of interest was the finding that sarizotan, SSR181507, bifeprunox, and to a lesser extent SLV313, disrupted PPI when given alone, probably because of this marked 5-HT_{1A} receptor agonist activity (Auclair *et al.*, 2006b). Such a disruption is potentially problematic, considering that schizophrenic patients present diminished basal PPI levels (Braff *et al.*, 1978; for review, Swerdlow *et al.*, 2000). It would thus be desirable for an antipsychotic not to further affect basal PPI. Interestingly though, F15063, over a wide dose-range (0.01–10 mg kg⁻¹ i.p.), did not modify basal PPI level. This is thought to be related to the preferential affinity of F15063 for DA D₂ over 5-HT_{1A} receptors, as well as to an antagonistic activity at the former. This emphasizes the importance of the precise balance of DA D₂ antagonism versus 5-HT_{1A} agonism for optimal pharmacological activity of new generation antipsychotics targeting both of these receptors (see Newman-Tancredi *et al.* (2007) and Depoortere *et al.* (2007) for further discussion).

Conclusions

F15063 was potentially active in preclinical models of negative symptoms and memory-cognitive dysfunction of schizo-

phrenia, at dose-ranges overlapping with those efficacious in tests detecting activity against positive symptoms (Depoortere *et al.*, 2007). Its partial agonist activity at DA D₄ receptors distinguishes F15063 from most other antipsychotics and was shown to be involved in its activity in the scopolamine-induced deficit of social recognition between an adult and a juvenile rat. Furthermore, F15063 does not produce deficits of basal PPI of the startle reflex, an advantage for a potential antipsychotic. These data also provide compelling evidence that F15063 possesses a profile that distinguishes it from currently commercialized antipsychotic drugs, with a favorable balance of affinity/activity at D₂ and 5-HT_{1A} receptors, and an additional partial agonist profile at DA D₄ receptors. Taken together, the present data suggest that a pharmacological profile exemplified by that of F15063 should be beneficial in alleviating a wide array of manifestations of schizophrenia, ultimately leading to improvement in patients' outcome and rehabilitation.

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Conflict of interest

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REFRESH: RP5063 Phase 2 Efficacy and Safety in Schizophrenia and Schizoaffective Disorder

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NCDEU Annual Meeting, May 28-31, 2013

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Introduction

RP5063, a new chemical entity (NCE), is a dopamine-serotonin system stabilizer and is being developed for treating schizophrenia and schizoaffective disorder.

Schizophrenia is among the most debilitating mental illnesses, affecting approximately 1% of the global population.¹ Symptoms of the disease are divided into three categories^{1,2}: positive symptoms, negative symptoms, and cognitive dysfunction. The predictor of long-term prognosis seems to be the severity of cognitive dysfunction³. Pathology of schizophrenia is not yet fully understood. Dysregulation or disruption of dopaminergic and serotonergic functions have been implicated in its development⁴. Positive symptoms are thought to be a result of dopaminergic hyperactivity in the striatum and while cognitive and some negative symptoms are thought to be caused by hypoactivity in the cortex^{3,5}. Dysregulation of serotonergic function in the brain is reported to contribute to schizoaffective disorders such as bipolar, major depression and mania.^{6,7} Thus, optimal treatment for schizophrenia may not be solely reliant on dopamine blockade but may also depend on stabilization of dopaminergic and serotonergic system. Drugs with the right balance of agonism and antagonism of these receptors are likely to be more effective for treating the broader symptomatology of schizophrenia and schizoaffective disorder.

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RP5063 Pharmacology and Non-Clinical ADME Profile

RP5063 is a potent partial agonist at the dopamine (D_2 , D_3 & D_4) and serotonin ($5-HT_{1A}$ & $5HT_{2A}$) receptors, and antagonist at serotonin $5-HT_6$ & $5-HT_7$ receptors. It has high affinity to key receptors, K_i (nM): D_{2L} (0.45, IA = 43%), D_{2S} (0.61, IA = 52%), D_3 (3.7, IA = 44%), D_4 (6.0, IA = 51%), $5-HT_{1A}$ (1.5, IA = 19%), $5-HT_{2A}$ (4.1, IA = 33%), $5-HT_7$ (2.7), and H_1 (2.5). It has moderate affinity to receptors, K_i (nM): nicotine nAChR- $\alpha_4\beta_2$ (36.3), $5-HT_{2C}$ (39), $5-HT_6$ (51), adrenergic α_{1B} (47), D_1 (100), and SERT (106). RP5063 attenuates MK-801 induced hyperactivity & stereotypy in rats, and apomorphine induced climbing in mice. It also reverses the apomorphine induced PPI in rats.

RP5063 has 85% oral bioavailability in dog and 22% in rat. It has high BBB permeability, ~3.5 brain:plasma ratio in rat with D_2 receptor occupancy, >95%. It is metabolized by CYP 3A4 (62%) and CYP 2D6 (36%) to inactive metabolites.

RP5063 Efficacy and Safety in Early Clinical Studies

Subjects Phase 1a: Caucasians (~70%) & Japanese (~30%) healthy males
Phase 1b: Stable Schizophrenia Patients

Phase 1a
SAD Single ascending dose (10mg, & 15 mg) to MTD, safety & PK,
Double blind, 8-subjects/cohort including 2 placebo

Food Effect Open-label study (n=8) in healthy male subjects, 15mg dose

Phase 1b
MAD Multi ascending dose, once daily for 10 days, safety, PK & PD
10mg, 20mg, 50mg and 100mg, dose per day for 10 days
Double blind, 8-subjects/cohort including 2 placebo

- Well tolerated in schizophrenia patients up to at least 100 mg/day
- Good oral absorption, highly predictable linear pharmacokinetics, once a day dosing
- No dose limiting safety signals in ECG including QT, clinical lab, vital signs, seizures, physical signs including SAS, AIMS Bond and Lader Scale for Subjective Effects
- No increase in weight, triglycerides or glucose. No change in prolactin level.

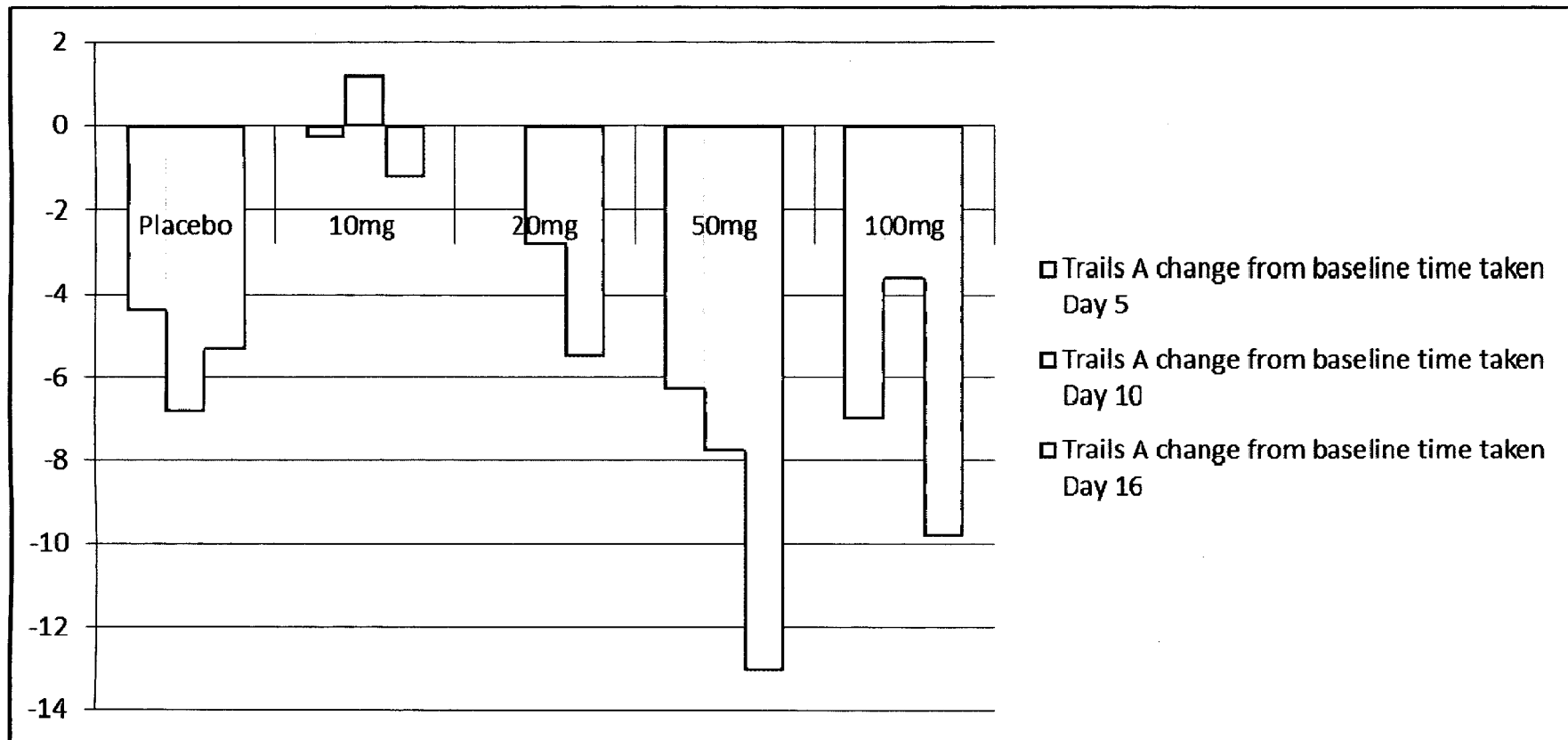
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PANSS in Stable Schizophrenia Patients

- PANSS ns; Marder⁸ factors: positive, negative, disorganized thoughts, hostility/ excitement, anxiety/depression, five factors ANCOVA ns. In PANSS (baseline 39 to 69) ≥ 50 cutoff (Leucht⁹), 19 patients : Positive factor score ($P = 0.024$):
 - 1.00 decrease in scores for the placebo group
 - 1.01 decrease in scores for the treatment group receiving ≤ 20 mg,
 - 4.40 decrease in scores for the treatment group receiving ≥ 50 mg
- Depression/Anxiety factor ($p = 0.043$; with baseline as a covariate $p = 0.040$):
 - < 0.001 decrease in scores for the placebo group
 - 1.37 decrease in scores for the active treatment group

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Trails- A Data for Cognition in Stable Schizophrenia Patients



Trail Making A test effects at higher doses suggest potential executive function improvement, Trail B and DSST positive trends

REFRESH: RP5063 Phase 2 Clinical Study ⁶⁰

Phase 2 Study Title : Randomized double blind, placebo-controlled, multi-center trial to assess the safety and efficacy of RP5063 in subjects with acute schizophrenia or schizoaffective disorder

Geography of Clinical Trial: Global phase 2 trial – USA, Europe and Asia

Study Population: 234 schizophrenia patients enrolled, 189 evaluable patients

Dose and Duration: 15 mg, 30 mg, 50 mg /day for 4 weeks

Comparator Drug: Aripiprazole (15 mg)

Efficacy Scales: PANSS, CGI, depression and cognition

Safety: Clinical, labs including body weight, prolactin, lipids, f-glucose, and EKG

Pharmacokinetics: Population pharmacokinetics

RP5063 Phase 2 Efficacy Remission Data ⁶¹

Efficacy Remission (Andreasen Criteria)

Treatment Arm	Remission
RP5063 (15 mg)	34%
RP5063 (30 mg)	30%
RP5063 (50 mg)	46%
Placebo	22%

- Remission by other criteria as higher bars on PANSS response agree • 30% and above is considered clinically meaningful
- Superior efficacy seen within one week • Remission efficacy across all three RP5063 doses

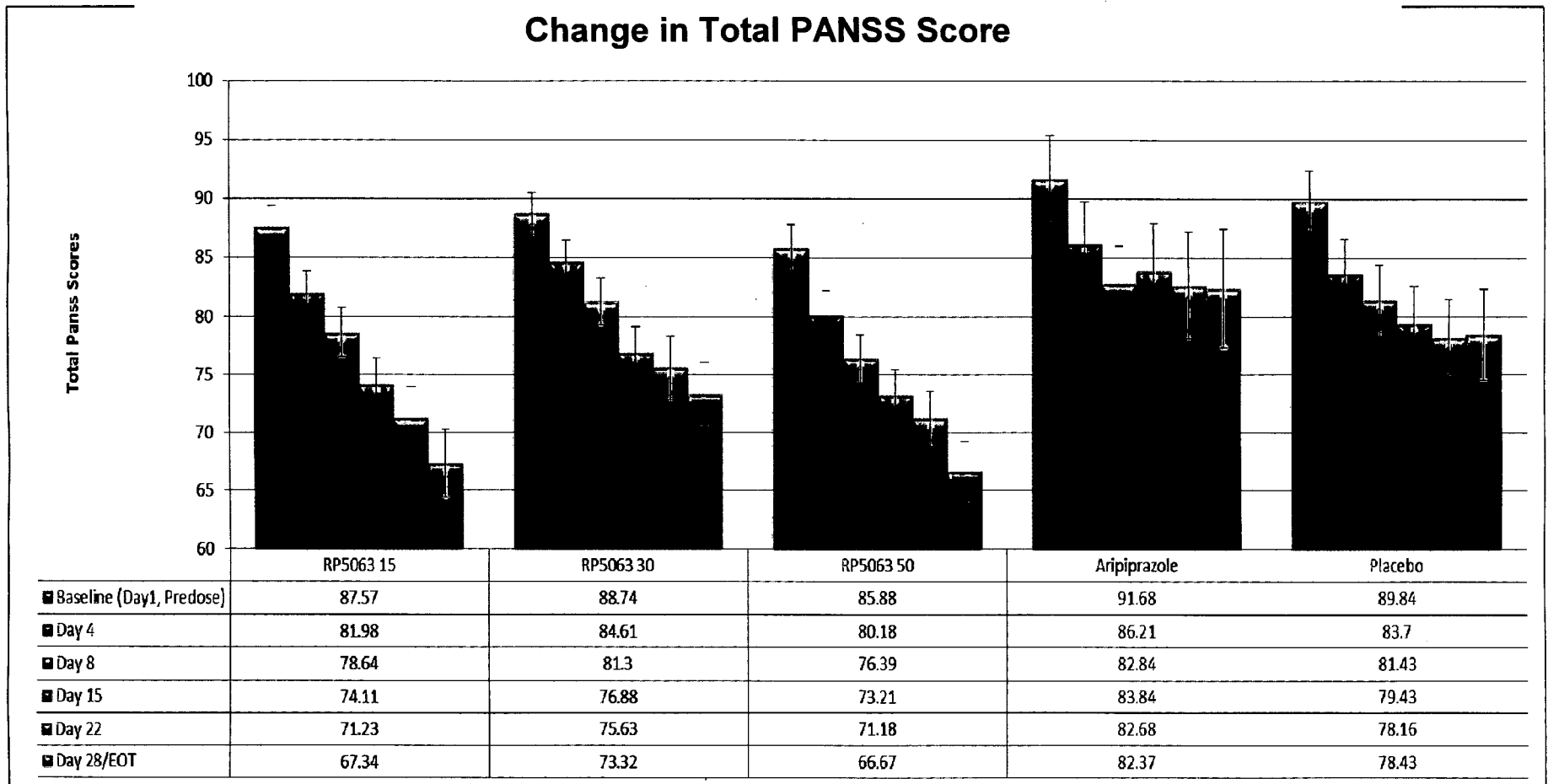
Efficacy Remission (Combination Criteria)

Treatment Arm	CGI 2 pts with 20% Improvement PANSS	CGI 2 pts with 30% Improvement PANSS
RP5063 (15 mg)	45%	36%
RP5063 (30 mg)	32%	21%
RP5063 (50 mg)	33%	23%
Placebo	19%	16%

Remission efficacy levels within such a short study places RP5063 among the most robust antipsychotics

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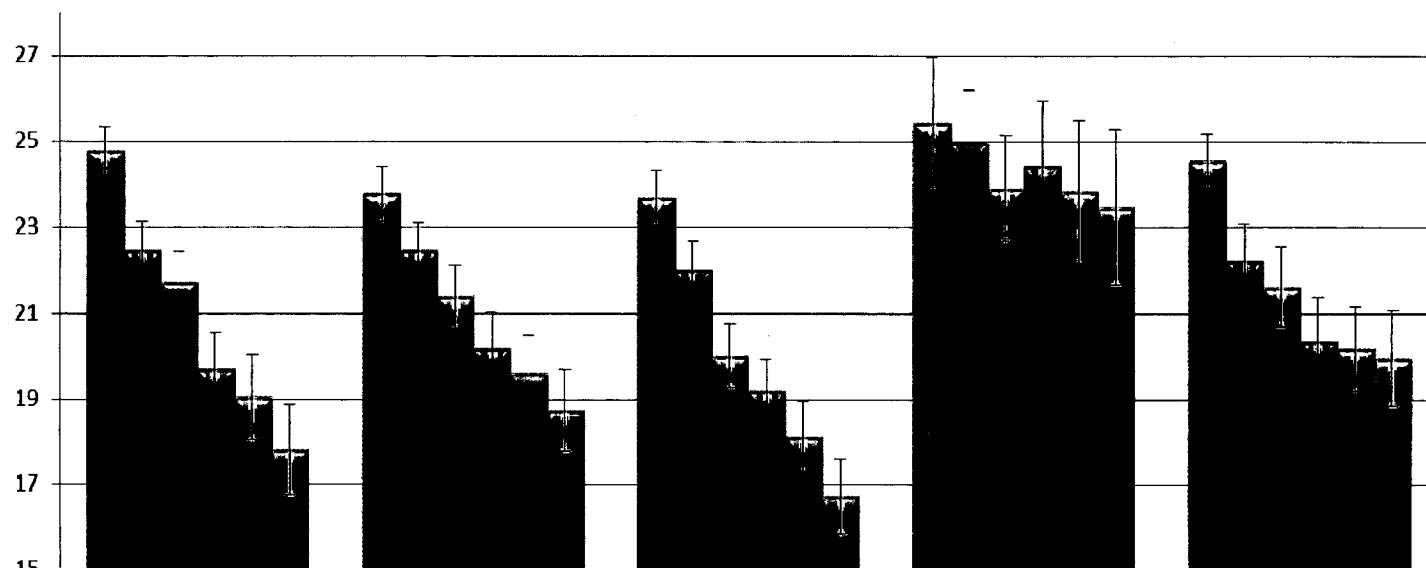
RP5063 Phase 2 Efficacy PANSS Score



RP5063 Phase 2 Efficacy PANSS Score

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Severity Assessment of Total PANSS –Positive Scale



	RP5063 15	RP5063 30	RP5063 50	Aripiprazole	Placebo
■ Baseline (Day1, Predose)	24.79	23.77	23.68	25.42	24.54
■ Day 4	22.46	22.45	22.02	24.95	22.22
■ Day 8	21.73	21.39	20.02	23.89	21.62
■ Day 15	19.7	20.19	19.21	24.42	20.38
■ Day 22	19.04	19.6	18.14	23.84	20.19
■ Day 28/EOT	17.82	18.75	16.72	23.47	19.95

CA

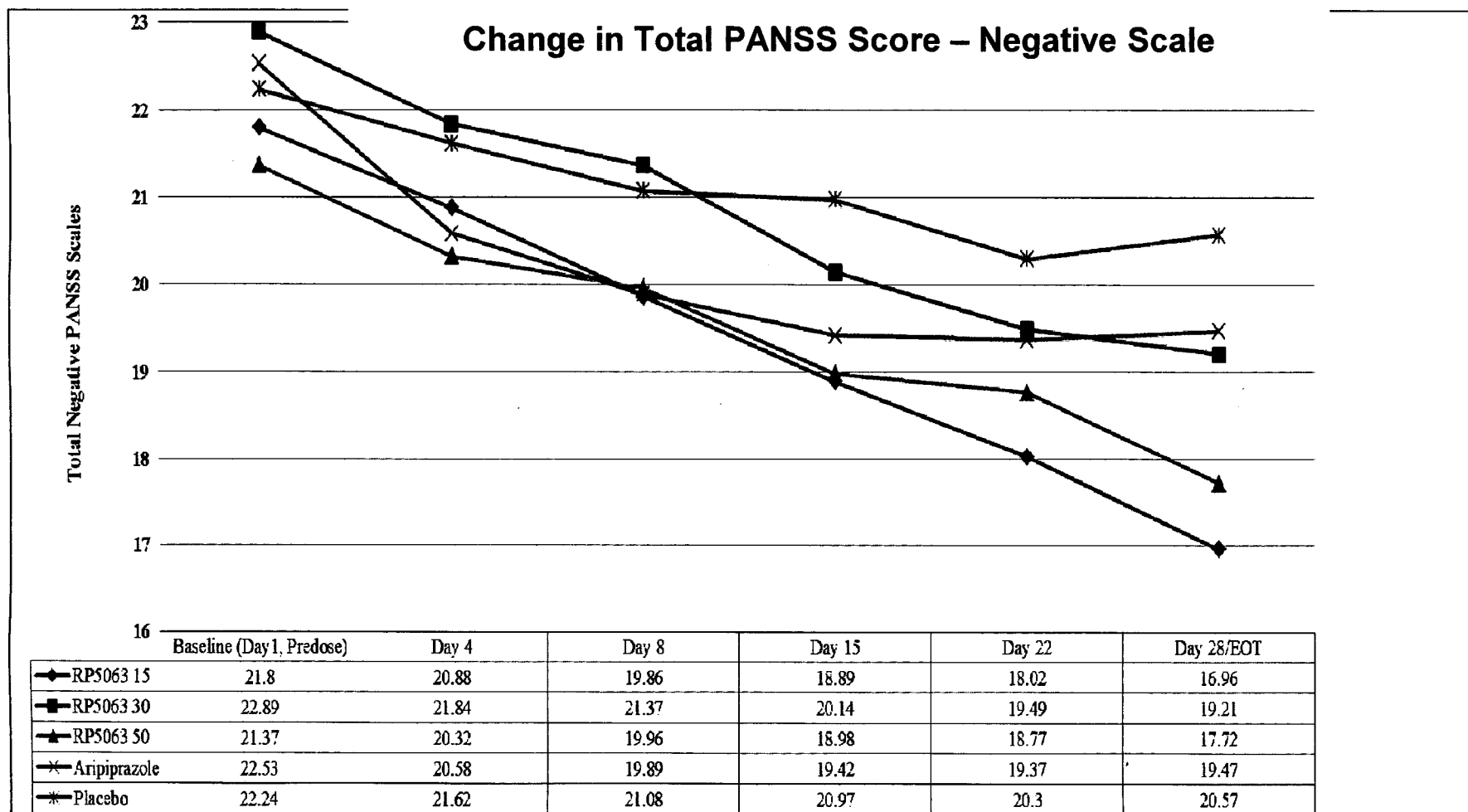
RP5063 Phase 2 Efficacy Comparison of Effect Size PANSS

Cohen's 'd' and Hedges 'g' change compared to placebo group

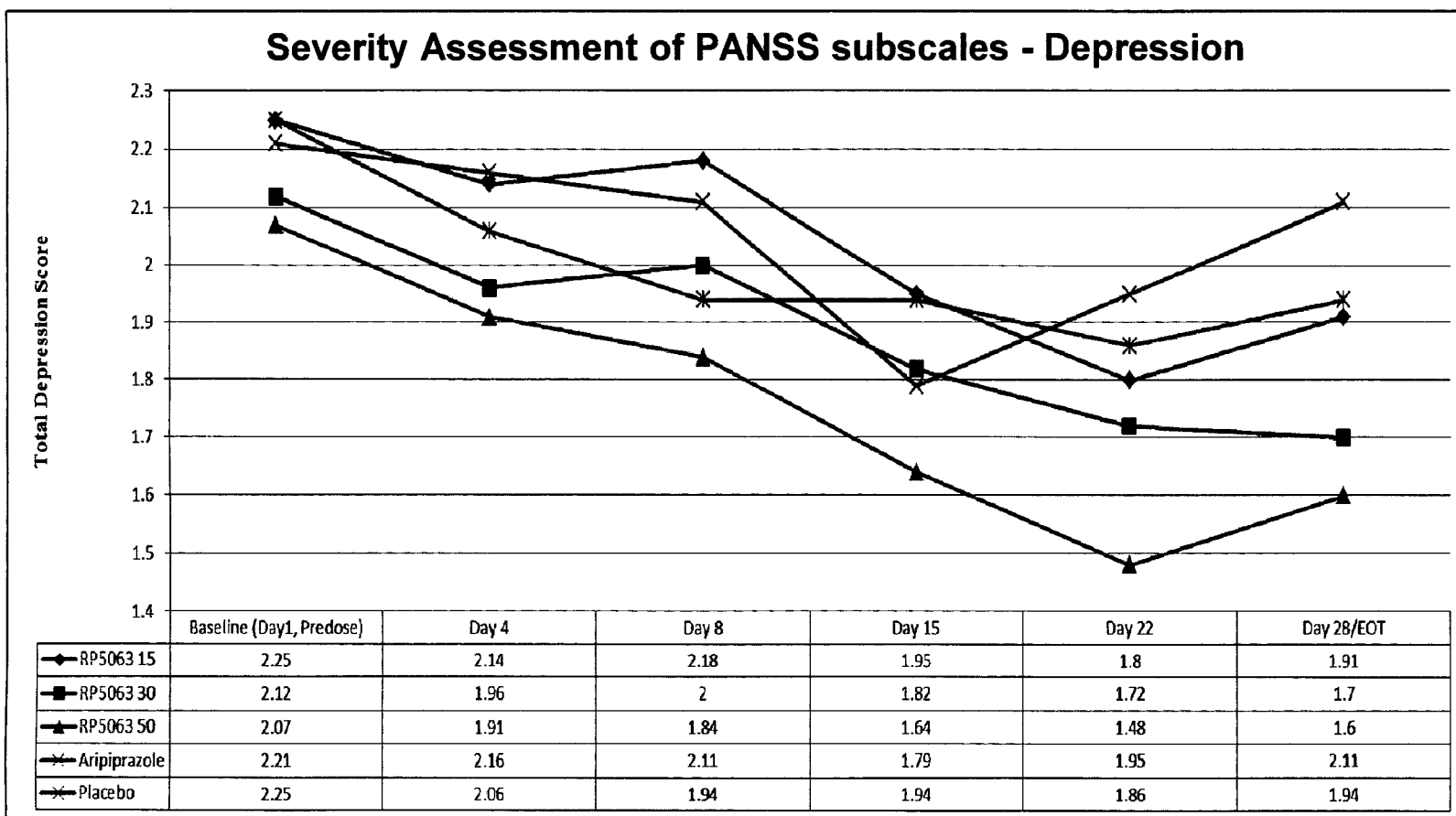
REFRESH Study					Literature ¹⁰	
Effect size	RP5063 15 mg	RP5063 30 mg	RP5063 50 mg	Aripiprazole 15 mg	Lurasidone 80 mg	Lurasidone 120 mg
Cohen's 'd'	0.481	0.230	0.537	0.170	0.44	0.26
Hedges 'g'	0.476	0.228	0.531	0.167	0.438	0.259

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RP5063 Phase 2 Efficacy PANSS Score

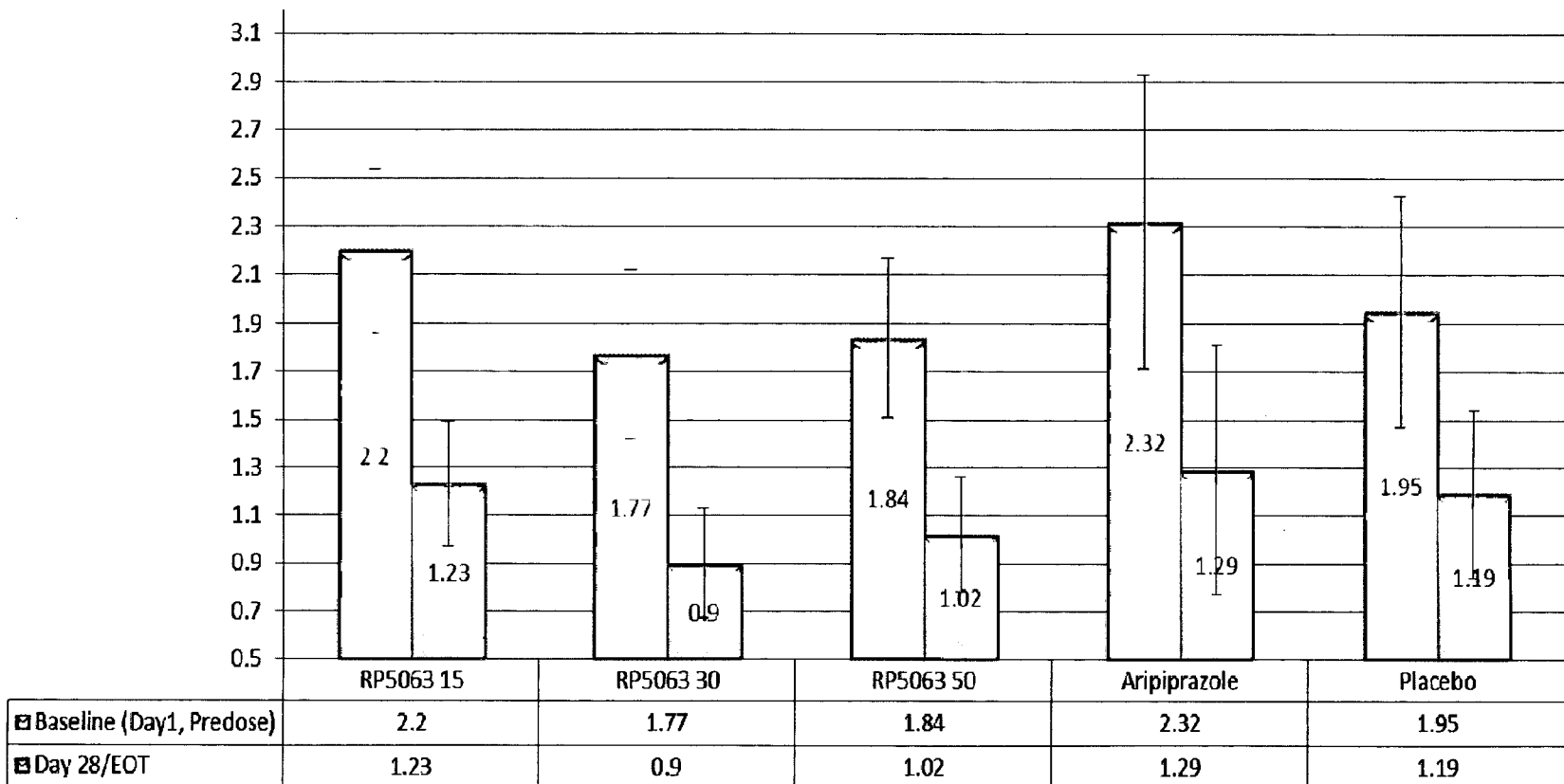


RP5063 Phase 2 Efficacy PANSS Score



RP5063 Phase 2 Efficacy CDSS Depression Scale

14.2.2.8.1.a Assessment of CDSS Scale (Total Score)



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RP5063 Phase 2 Efficacy Summary of CGI-S

At least 1 point improvement in CGI-S score by end of treatment

Group (mg)	No. of Patients	% Patients
RP5063, 15 mg (N=56)	41	73
RP5063, 30 mg (N= 57)	33	58
RP5063, 50 mg (N= 57)	41	72
Aripiprazole, 15 mg (N=19)	6	32
Placebo (N=37)	21	57

RP5063 Phase 2 Safety Summary

- Well tolerated at all 3 dose levels. No new or unexpected adverse effects as compared to phase 1 study. Vital signs and ECG changes within normal limits.
- **Body Weight, Glucose and Lipids:** Stable in all 3 arms (15, 30, and 50 mg) of RP5063, and not different from placebo arms.
- **Prolactin:** Nearly 50% decline by day 28 in all three RP5063 arms, & reversed after restarting routine antipsychotics by day 35 during restabilization.
- **SAEs:** 1 Pyrexia in aripiprazole arm, and 4 AST/ALT increase in 1 each of RP5063 and aripiprazole treated cohorts.

Overall Treatment Emergent Adverse Events (>2%)

Most Common Adverse Events	n (%)
Number of Subjects with Adverse Events	129 (55.36)
Insomnia	53 (22.75)
Agitation	26 (11.16)
Extrapyramidal Disorder	11 (4.72)
Akathisia	10 (4.29)
Restlessness	10 (4.29)
Headache	8 (3.43)
Tremor	7 (3.00)
Pyrexia	7 (3.00)
Nausea	5 (2.15)
ALT/AST Increase	5 (2.15)

Conclusion

- Novel dopamine-serotonin system stabilizer with balanced efficacy potential.
- Remission level efficacy in PANSS and CGI in all doses of RP5063 (15, 30, 50 mg)
- No tolerability / major safety issues: SAS, AIMS, BARS all n.s., compared to placebo
- Pharmacokinetics: Good consistency, predictable dose-dependent and dose-proportional systemic exposure, no evidence of BMI effect on average plasma levels, and no evidence of ethnic or geographic differences.
- Phase 3 clinical study planned for late 2013.

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Disclosures

- Marc Cantillon: Employee of Reviva, shares in Reviva, Merck, Pfizer, Sanofi-Synthelabo, CognRx
- Sarath Kanekal: Employee of Reviva, shares in Reviva
- Robert Ings, Mike Li, and Grace Tung: Consultants to Reviva
- Laxminarayan Bhat: Employee of Reviva, shares in Reviva