

RAD: 12-13015- -5	FECHA: 2013-11-19 15:43:24
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 9
ACT: 519 PRESENTACION	

Abogado(a)/Señor(a)
JUAN PABLO CADENA SARMIENTO

Asunto:	Radicación:	12-13015- -5
	Trámite:	11
	Evento:	378
	Actuación:	519
	Oficio:	21487
	Folios:	9

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/IB2010/53895				
Fecha de Presentación Internacional	31/08/2010				

Descripción:	Radicado N° 12-013015-00000-0000	27/01/2012
Reivindicaciones:	Radicado N° 12-013015-00000-0000	27/01/2012
	Radicado N° 12-013015-00004-0000	24/09/2012
Prioridad:	FR 0955944	01/09/2009
	US 61/336,992	29/01/2010

Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

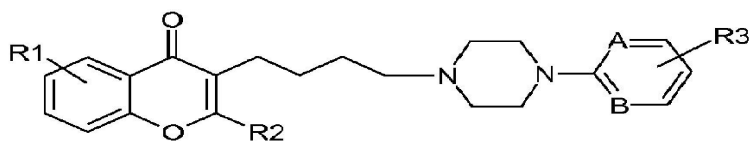
De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Título de la solicitud: "DERIVADOS DE CROMONA, PROCESOS PARA SU PREPARACIÓN Y

APLICACIONES TERAPEÚTICAS DE LOS MISMOS

El problema planteado en esta solicitud consiste en la necesidad de proporcionar compuestos alternativos como agonistas, agonistas parciales o antagonistas del receptor de dopamina D₃ (DRD₃) útiles para el tratamiento de varias condiciones neurológicas y psiquiátricas.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de la fórmula 1, composiciones que lo contienen y procesos para su preparación.



general formula 1

(Ver pág. 4 Radicado 12-013015-00004-0000)

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K31/496 // 31/506; A61P15/10 // 25/16 // 25/18 // 25/22; C07D311/22 // 407/12

4. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es concisa porque las definiciones de R₁, R₂ y R₃, conllevan a un número indefinido de compuestos que no encuentran soporte en la descripción. Al revisar en la descripción se observa según los ejemplos que R₁ corresponde a uno o dos sustituyentes metoxi o junto con los átomos de carbono al cual se une forma un anillo dioxolano, R₂ es hidrógeno, y R₃ se selecciona del grupo consistente en metoxi, cloro, hidroxilo, flúor, nitro, amino, acetamida, metanosulfonamida, etanosulfonamida o junto con los átomos de carbono del anillo aromático al cual se une forma un anillo fusionado seleccionado de benzimidazol, indol, dihidrobenzimidazol y bensoxazol. Por lo tanto, se sugiere restringir la solicitud a una razonable generalización de tales ejemplos o teniendo en cuenta los compuestos que se reclaman en la reivindicación 8.

La reivindicación 9 no es clara porque no especifica en qué condiciones deben reaccionar los compuestos de fórmulas 4 y 5 para obtener un compuesto de fórmula 1. Se requiere al solicitante hacer las correcciones necesarias.

5. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de la cual se invoca prioridad, es decir, el 01 de septiembre de 2009.

Consultadas las fuentes de información con que se cuenta, se encontró (encontraron) el (los) siguiente(s) documento(s) que anticipa(n) el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1 ^[1]	Journal of Medicinal Chemistry, 2003, Vol. 46, No. 18	"N-(ω-(4-(2-Methoxyphenyl)piperazin-1-yl)alkyl)carboxamides as Dopamine D ₂ and D ₃ Receptor Ligands"	28/Agosto/2003
D2 ^[2]	Journal of Medicinal Chemistry, 2009, Vol. 52, No. 1	"Discovery of a New Class of Potential Multifunctional Atypical Antipsychotic Agents Targeting Dopamine D ₃ and Serotonin 5-HT _{1A} and 5-HT _{2A} Receptors: Design, Synthesis, and Effects on Behavior"	08/Enero/2009
D3 ^[3]	US5089497	"Substituted piperazines as central nervous system agents "	18/Febrero/1992

1 URL: <http://pubs.acs.org.ezproxy.unal.edu.co/doi/abs/10.1021/jm030836n>

2 URL: <http://pubs.acs.org.ezproxy.unal.edu.co/doi/abs/10.1021/jm800689g>

El documento D1 divulga un compuesto derivado de cromona útil como ligando del receptor D3 de dopamina (Pág. 3885 Tabla 2)

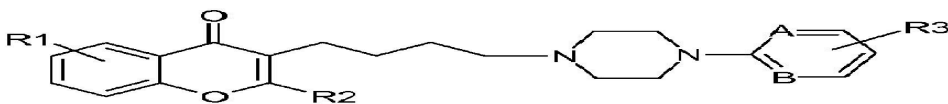
El documento D2 divulga un compuesto derivado de aril-piperazina útil como antagonista del receptor D3 de dopamina (Pág. 153 Tabla 1)

El documento D3 divulga un compuesto dopaminérgico derivado de piperazina (Col. 1, línea 50 a Col. 2 línea 48; Cols. 8 y 9 Ej. 1 a 13)

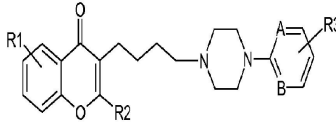
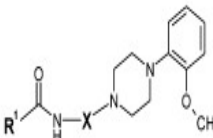
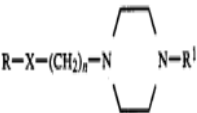
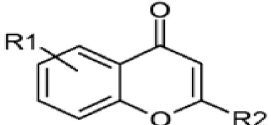
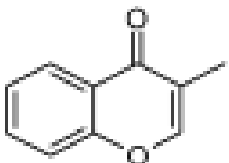
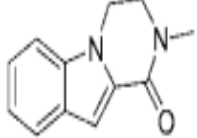
6 . Examen de Patentabilidad (Art 14 D486)

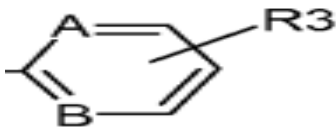
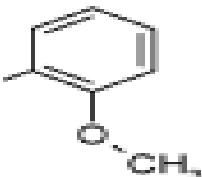
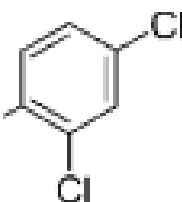
Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en un compuesto de fórmula general 1(Ver pág. 4 Radicado 12-013015-00004-0000)



Comparación de las características técnicas esenciales, con las del estado de la técnica:

SOLICITUD	D1	D2	D3
Compuesto de fórmula general 1  (Ver pág. 4 Radicado 12-013015-00004-0000)	 (Pág. 3885 Tabla 2)	(Ver Pág. 53, Tabla 1)	 (Col. 1, línea 50 a Col. 2 línea 48; Cols. 8 y 9 Ej. 1 a 13)
 R1 representa uno o mas sustituyente/s idéntico/s o diferente/s en el anillo de benceno, cada uno representa independientemente un átomo de hidrógeno o un átomo de halógeno, o un grupo C ₁₋₄ alcoxi o un grupo OH, o un grupo C ₁₋₄ alquilo o un grupo O-(CH ₂) _n -O en el cual n es 1 o 2. R2 representa un átomo de hidrógeno, o un grupo C ₁₋₄ alquilo	 (Pág. 3885, Tabla 2, Ej. 22)	 (Pág. 53, Tabla 1, Ej. 5g)	Piridina, Pirimidina, Quinolina (Col. 9, Ej. 9, 10 y 12)
-(CH ₂) ₄ -	CO-NH--(CH ₂) ₄ - (Pág. 3885, Tabla 2, Ej. 22)	-(CH ₂) ₄ - (Pág. 53, Tabla 1, Ej. 5g)	-(CH ₂) ₄ - (Col. 9, Ej. 9, 10 y 12)

SOLICITUD	D1	D2	D3
	 (Pág. 3885, Tabla 2, Ej. 22)	 (Pág. 53, Tabla 1, Ej. 5g)	Piperazinil (Col. 9, Ej. 9, 10 y 12)
 A y B representan independientemente un átomo de nitrógeno o un átomo de Carbono R3 representa un átomo de hidrógeno o uno o mas sustituyente/s idéntico/s seleccionados del grupo integrado por: un átomo de halógeno, un grupo C₁₋₄ alquilo, un grupo C₁₋₄ alcoxi, un grupo C₁₋₄ tioalcoxi, un grupo O-(CH₂)_n-O en el cual n es 1 o 2, un grupo NO₂, un grupo NHSO₂R4, un grupo NHR5, un grupo OH, un grupo C₁₋₄ haloalquilo, un grupo CN, un grupo C₁₋₄ alcoxycarbonilo, un grupo C₁₋₄ alquilcarbonilo, un grupo C₁₋₄ hidroxialquilo, y un sustituyente bencilo o fenilo opcionalmente sustituido por un grupo C₁₋₄ alcoxi o un grupo C₁₋₄ alquilo o un átomo de halógeno, o R3 constituye un anillo fusionado con el anillo de benceno que lo porta (Ver pág. 4 Radicado 12-013015-00004-0000)	 (Pág. 3885, Tabla 2, Ej. 22)	 (Pág. 53, Tabla 1, Ej. 5g)	Piridina Pirimidina (Col. 9, Ej. 9, 10 y 12)

El documento D1 divulga un compuesto derivado de cromona útil como ligando del receptor D3 de dopamina (Pág. 3885 Tabla 2)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D1 consiste en la presencia de un grupo butil carboxamida como enlazante entre los anillos de cromona y piperazina en lugar del sustituyente butilo reclamado en la solicitud

El documento D2 divulga un compuesto derivado de aril-piperazina útil como antagonista del receptor D3 de dopamina (Pág. 153 Tabla 1)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D2 consiste en que este documento no divulga específicamente el anillo de cromona reclamado en la solicitud

El documento D3 divulga un compuesto dopaminérgico derivado de piperazina (Col. 1, línea 50 a Col. 2 línea 48; Cols. 8 y 9 Ej. 1 a 13)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D3 consiste en que este documento no divulga específicamente el anillo de cromona reclamado en la solicitud

El efecto que logra el incluir el sustituyente butilo como enlazante entre los anillos de cromona y

piperazina es que proporciona nuevos compuestos alternativos como agonistas, agonistas parciales o antagonistas del receptor de dopamina D3 (DRD3) útiles para el tratamiento de varias condiciones neurológicas y psiquiátricas

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el compuesto conocido en el documento D1 para proporcionar nuevos compuestos alternativos como agonistas, agonistas parciales o antagonistas del receptor de dopamina D3 (DRD3) útiles para el tratamiento de varias condiciones neurológicas y psiquiátricas”.

Sin embargo, la utilización del sustituyente butilo sustituyendo el anillo de piperazina en compuestos que actúan sobre el receptor de dopamina D3, ya está sugerida en los documentos D2 y D3. El documento D2 sugiere la utilización de un sustituyente butilo enlazando el anillo de piperazina a un anillo heterocíclico benzo-fusionado (un compuesto oxo-pirazino[1,2-a]indol) (Pág. 53, Tabla 1, Ej. 5g). El documento D3 sugiere la utilización de un sustituyente butilo enlazando el anillo de piperazina a un anillo heteroaromático como lo son los anillos de piridina, pirimidina y quinolina (Col. 9, Ej. 9, 10 y 12)

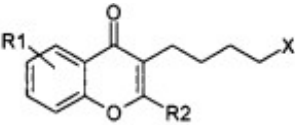
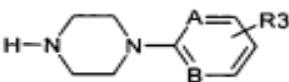
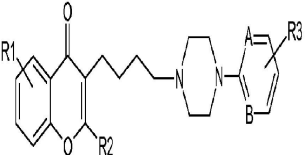
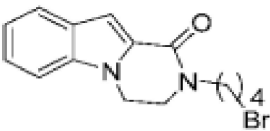
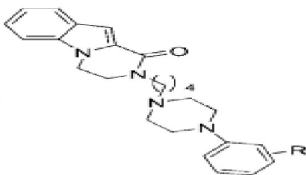
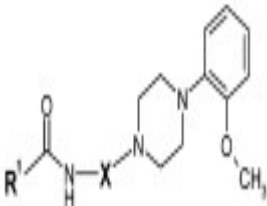
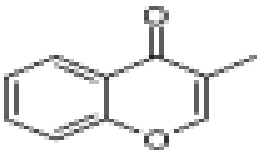
En consecuencia, la persona del oficio normalmente versada en la materia, mantendría la estructura del compuesto sugerido en el documento D1 reemplazando el sustituyente butil-carboxamida por un sustituyente butilo enlazando los anillos de cromona y de piperazina de acuerdo a las enseñanzas proporcionadas en los documentos D2 y D3 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia a la luz de los documentos D1 con D2 y D1 con D3

En conclusión la reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones dependientes 2 – 8 y 11 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

La reivindicación 9 consiste en un proceso para preparación de compuestos de la fórmula general 1 (Ver pág. 7 Radicado 12-013015-00004-0000)

Comparación de las características técnicas esenciales, con las del estado de la técnica:

SOLICITUD	D2	D1
Reacción del compuesto  Con un compuesto  Para obtener un compuesto 	Reacción del compuesto  Con una aril piperazina para obtener el compuesto  (Pág. 155 Esquema 3)	Preparación del compuesto  Donde R¹ es  y X es -(CH₂)₄- (Pág. 3885, Tabla 2, Ej. 22)

El documento D2 divulga un proceso para obtener un compuesto derivado de aril-piperazina útil como antagonista del receptor D3 de dopamina (Pág. 155 Esquema 3)

La diferencia entre la invención, definida en la reivindicación 9 y el proceso del documento D2 consiste en que en el proceso divulgado en este documento se utiliza un anillo oxo-pirazino[1,2-a]indol en lugar del anillo de cromona presente en el compuesto preparado según la reacción reclamada.

El documento D1 divulga la preparación de un compuesto derivado de cromona útil como ligando del receptor D3 de dopamina (Pág. 3885 Tabla 2; Pág. 3887 Esquema 1)

La diferencia entre la invención, definida en la reivindicación 9 y el proceso del documento D1 consiste en que no se divulga específicamente la reacción entre un anillo de cromona haloalquilsustituido y una aril-piperazina

El efecto que logra el utilizar un anillo de cromona haloalquil sustituido como material de partida es que permite obtener un compuesto derivado de cromona aril-piperazinil-alquil sustituida como agonista, agonista parcial o antagonista del receptor de dopamina D3 (DRD3) útil para el tratamiento de varias condiciones neurológicas y psiquiátricas.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el proceso conocido en el documento D2 para obtener nuevos compuestos alternativos como agonistas, agonistas parciales o antagonistas del receptor de dopamina D3 (DRD3) útiles para el tratamiento de varias condiciones neurológicas y psiquiátricas”.

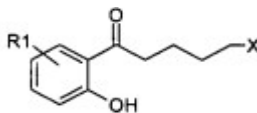
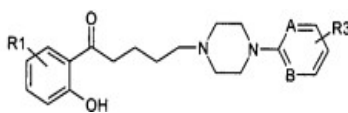
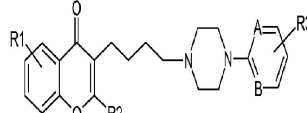
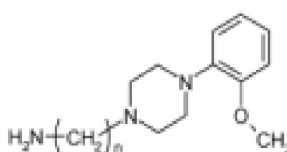
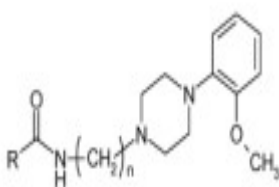
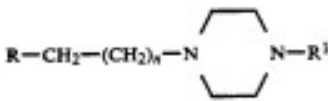
Sin embargo, el documento D1 sugiere la preparación de un compuesto derivado de cromona aril-piperazinil-carboxamidoalquil sustituida lo cual constituye una sugerencia junto con el documento D2 según se analizó previamente para la reivindicación 1 para la preparación de un compuesto derivado de cromona aril-piperazinil-alquil sustituida (Pág. 3885, Tabla 2, Ej. 22)

En consecuencia, la persona del oficio normalmente versada en la materia, reproduciría el proceso divulgado en el documento D2 (Pág. 155 Esquema 3) mantendría la estructura del compuesto sugerido en el documento D1 reemplazando el compuesto oxo-pirazino[1,2-a]indol por un anillo de cromona según las sugerencias del documento D1 para llegar así al objeto de la reivindicación 9 de la solicitud. Por lo cual se considera obvia.

En conclusión la reivindicación 9 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

La reivindicación 10 consiste en un proceso para preparación de compuestos de la fórmula general 1 (Ver pág. 7 Radicado 12-013015-00004-0000)

Comparación de las características técnicas esenciales, con las del estado de la técnica:

SOLICITUD	D1	D3
Reacción del compuesto  Con una aril piperazina en presencia de una base como K₂CO₃, CS₂CO₃ o NEt₃, en un solvente como acetonitrilo o metil etil cetona Para obtener el compuesto  y posterior reacción con DMF o el dimetilacetal de DMF o de DMA Para obtener el compuesto 	Reacción del compuesto  con un cloruro de ácido para obtener el compuesto 	Reacción del compuesto R-CH₂-(CH₂)_n-L Donde L es un halógeno o un grupo saliente. Con el compuesto  En presencia de una base como el carbonato de potasio para obtener el compuesto 

El documento D1 divulga un proceso para obtener un compuesto derivado de aril-piperazina activo sobre el receptor D3 de dopamina (Pág. 3887 Esquema 1)

La diferencia entre la invención, definida en la reivindicación 10 y el proceso del documento D1 consiste en que este documento no divulga la reacción de alquilación del compuesto derivado de cetona aromática protegida por halógeno con una piperazina aril sustituida

El documento D3 divulga la preparación de un compuesto aromático aril-piperazinil-alquil sustituido activo sobre el receptor D3 de dopamina (Cols. 5 y 6)

La diferencia entre la invención, definida en la reivindicación 10 y el proceso del documento D3 consiste en que este documento no divulga la reacción de ciclación con DMF para obtener el compuesto derivado de cromona.

El efecto que logra la reacción del compuesto aromático protegido con una aril piperazina y posterior ciclación en presencia de DMF o el dimetilacetal de DMF o de DMA es que permite obtener un compuesto derivado de cromona aril-piperazinil-alquil sustituida como agonista, agonista parcial o antagonista del receptor de dopamina D3 (DRD3) útil para el tratamiento de varias condiciones neurológicas y psiquiátricas.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el proceso conocido en el documento D1 para obtener nuevos compuestos alternativos como agonistas, agonistas parciales o antagonistas del receptor de dopamina D3 (DRD3) útiles para el tratamiento de varias condiciones neurológicas y psiquiátricas”.

Sin embargo, el documento D3 sugiere la reacción entre un compuesto aromático (piridina o

quinolina) alquil sustituida con una aril-piperazina

En consecuencia, la persona del oficio normalmente versada en la materia teniendo en cuenta el compuesto derivado de cromona divulgado en el documento D1 haría reaccionar un compuesto aromático con una aril-piperazina como sugiere el documento D3 y posteriormente lo haría reaccionar con un cloruro como lo sugiere el documento D1 para obtener el compuesto derivado de cromona y llegar así al objeto de la reivindicación 10 de la solicitud. Por lo cual se considera obvia.

En conclusión la reivindicación 10 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

En consecuencia las reivindicaciones 1 – 11 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

7. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	"N-(ω-(4-(2-Methoxyphenyl)piperazin-1-yl)alkyl)carboxamides as Dopamine D ₂ and D ₃ Receptor Ligands"	1 – 11	Nivel Inventivo
D2	"Discovery of a New Class of Potential Multifunctional Atypical Antipsychotic Agents Targeting Dopamine D ₃ and Serotonin 5-HT _{1A} and 5-HT _{2A} Receptors: Design, Synthesis, and Effects on Behavior"	1 – 9 y 11	Nivel Inventivo
D3	US5089497	1 – 8, 10 y 11	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-11-26

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N-(ω -(4-(2-Methoxyphenyl)piperazin-1-yl)alkyl)carboxamides as Dopamine D₂ and D₃ Receptor Ligands

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The dopamine D₃ receptor is recognized as a potential therapeutic target for the treatment of various neurological and psychiatric disorders. Targeting high affinity and D₃ versus D₂ receptor-preferring ligands, the partial agonist BP 897 was taken as a lead structure. Variations in the spacer and the aryl moiety led to *N*-alkylated 1-(2-methoxyphenyl)piperazines with markedly improved affinity and selectivity. Molecular modeling studies supported the structural development. Pharmacophore models for dopamine D₂ and D₃ receptor ligands were developed from their potentially bioactive conformation and were compared in order to get insight into molecular properties of importance for D₂/D₃ receptor selectivity. For the 72 compounds presented here, an extended and more linear conformation in the aliphatic or aryl spacers turned out to be crucial for dopamine D₃ receptor selectivity. Structural diversity in the aryl moiety (benzamides, heteroarylamides, arylimides) had a major influence on (sub)nanomolar D₃ receptor affinity, which was optimized with more rigid aryl acrylamide derivatives. Compound **38** (ST 280, (*E*)-4-iodo-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoyl-amide) displayed a most promising pharmacological profile (K_i (hD₃) = 0.5 nM; K_i (hD_{2L}) = 76.4 nM; selectivity ratio of 153), and above that, compound **38** offered the prospect of a novel radioligand as a pharmacological tool for various D₃ receptor-related in vitro and in vivo investigation.

Introduction

The neurotransmitter dopamine is implicated in various physiological and pathophysiological processes. The dopaminergic system regulates brain functions such as motion, emotion, and cognition. Its effects are mediated by dopamine receptors, which belong to the family of G protein-coupled receptors and share the characteristic of seven transmembrane domains. Dopamine receptors can be divided into five different receptor subtypes and are further classified into two families, the D₁-like (D₁ and D₅) and the D₂-like (D₂, D₃, and D₄), with related pharmacological, structural, and genetic properties, respectively.^{1,2} An imbalance within the dopaminergic system is related to several psychiatric and neurological disorders, e.g., Parkinson's disease, Huntington's disease, and schizophrenia. Medical treatment of dopamine related disorders is often limited by side effects as a consequence of binding to various dopamine sub-receptors or other related monoamine receptors. Therefore, effective therapy calls for selective dopamine sub-receptor ligands.³ Dopamine D₃ receptors are relatively few in number but display a discrete localization in

special limbic areas of the central nervous system, which are thought to control emotional and cognitive but not locomotor functions.^{4,5} For a long time dopamine D₂-like receptor antagonists have been used to treat schizophrenia and related psychiatric disorders.⁶ A suitably selective dopamine D₃ receptor antagonist may provide antipsychotic properties in the relative absence of limiting extrapyramidal side effects.⁷ Recent findings suggest that dopamine D₃ receptor agonists may have beneficial effects for Parkinson's patients.^{8,9} Furthermore, dopamine D₃ receptor partial agonists are supposed to be beneficial in the treatment of L-DOPA-induced dyskinesia in Parkinson's patients. It has been impressively demonstrated in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-treated monkeys, a primate model for longtime Parkinson's disease treatment, that D₃ receptor partial agonists normalize exacerbated D₃ receptor function.^{10,11} Moreover, the treatment of drug addiction has been considered a relevant therapeutic target for dopamine D₃ receptor partial agonists.¹² These ligands are thought to modulate the long-term effects caused by the reuptake-inhibitor cocaine (second-order schedule of reinforcement).¹³ At the receptor site partial agonists act as agonists or antagonists, depending on dopaminergic activation and on receptor state. Partial agonists are supposed to regulate receptor activation within physiological limits. Consequently they should not exhibit any rewarding activity themselves. In a recent review,

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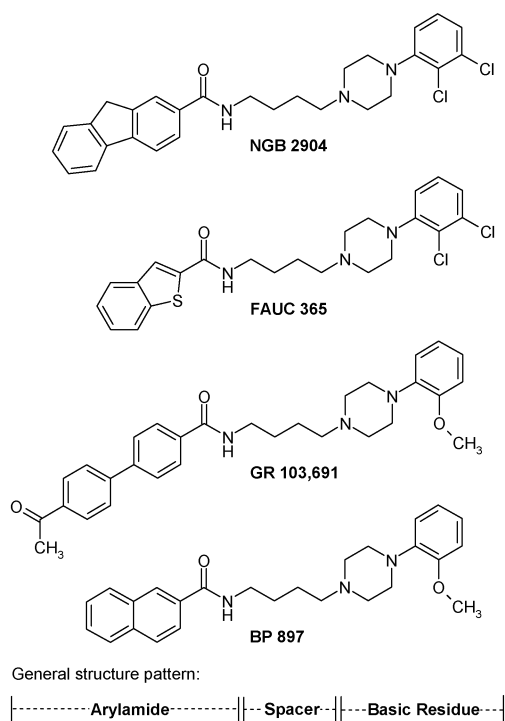
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[§] Laboratoire Bioprojet.

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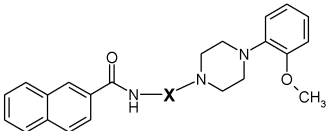
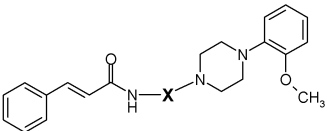
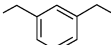
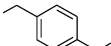
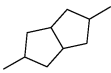
Chart 1



structure–activity relationships for dopamine D₃ receptor partial agonists and antagonists and their potential therapeutic application have been comprehensively

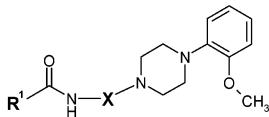
discussed.¹⁴ For many dopamine D₃ receptor ligands with antagonist properties, a general structure pattern can be applied, which is divided into three subunits: an aryl moiety, which is connected to an amide (first subunit) and further linked via an alkyl or aryl spacer (second subunit) to a basic residue with aryl substituent (third subunit) (Chart 1). Among the structural development, compounds with a basic 4-phenylpiperazino residue have turned out to provide prominent selectivity for dopamine D₃ receptors (Chart 1). Compounds with 4-(2,3-dichlorophenyl)piperazino residue, e.g., NGB 2904¹⁵ or FAUC 365,¹⁶ mostly provided an antagonist profile, while compounds with 4-(2-methoxyphenyl)piperazino residue showed antagonism, e.g., GR 103 691,¹⁷ or partial agonism, e.g., BP 897.¹⁸ The acknowledged *n*-butyl spacer has been exchanged to a cyclohexylethyl spacer in some compounds.^{19,20} In many cases the spacer has been linked to an aryl moiety via an amide bond or has been coupled directly,²¹ but ether bridges have been built up as well.^{22,23} The lipophilic residue on the arylamide moiety allowed diverse modifications including aryl, biphenyl, heteroaryl, or cycloalkyl substituents.^{16,17,19,24} Our lead structure BP 897 combined high affinity with pronounced preference for the human dopamine D₃ receptor compared to that for the human D₂ receptor²⁵ (cf. Table 2) and has shown inhibition of cocaine-seeking behavior in animal models.¹⁸ In this study we present a pharmacophore model for dopamine D₃ receptor ligands and one for dopamine D₂ receptor

Table 1. Structures, Physical Data, and Pharmacological Screening Results of Compounds with Spacer Variations

 1 – 4					 5				
No.	X	formula	M_r	mp (°C)	D ₃ ^a K_i (nM) ± SEM	D ₂ ^a K_i (nM) ± SEM	ratio ^b D ₂ /D ₃		
1		C ₃₀ H ₃₁ N ₃ O ₂ ·C ₂ H ₂ O ₄	555.6	219.0 ^c	750 ±190	28.4 ±7.4	0.04		
2		C ₃₀ H ₃₁ N ₃ O ₂ ·C ₂ H ₂ O ₄	555.6	183.0 ^c	≈100	45 ±17	0.5		
3		C ₃₀ H ₃₁ N ₃ O ₂ ·1.25C ₂ H ₂ O ₄	578.1	183-184 ^c	40.0 ±6	200 ±14	5.0		
4		C ₃₀ H ₃₁ N ₃ O ₂ ·C ₂ H ₂ O ₄ ·0.75H ₂ O	483.1	156-158 ^d	37.2 ±1.9	178 ±12	4.8		
5		C ₂₈ H ₃₁ N ₃ O ₂	441.6	188.0 ^d	≈325	18 ±4	0.06		
6^e		C ₃₀ H ₄₂ N ₄ O ₂ ·2C ₂ H ₂ O ₄	670.8	190-191 ^c	700 ±85	630 ±115	0.9		
7^e		C ₃₈ H ₅₅ N ₅ O ₂ ·3C ₂ H ₂ O ₄ ·2H ₂ O	922.0	>215 ^f	358 ±10	103 ±6	0.3		

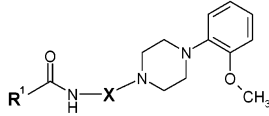
^a Binding assays using [¹²⁵I]iodosulpiride, in CHO cells expressing hD_{2L} and hD₃ receptors. ^b Ratio K_i (D₂)/ K_i (D₃). ^c Crystallized from EtOH. ^d Crystallized from 2-propanol. ^e For structure see Scheme 2. ^f Crystallized from EtOH/Et₂O.

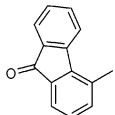
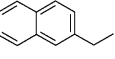
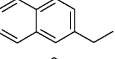
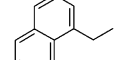
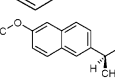
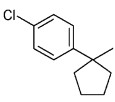
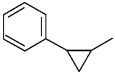
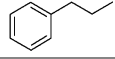
Table 2. Structures, Physical Data, and Pharmacological Screening Results of Aryl, Heteroaryl, and Arylalkyl Carboxylic Acid Derivatives



No.	R ¹	X	formula	M _r	mp (°C)	D ₃ ^a K _i (nM) ± SEM	D ₂ ^a K _i (nM) ± SEM	ratio ^b D ₂ /D ₃
BP 897		(CH ₂) ₄	C ₂₆ H ₃₁ N ₃ O ₂	417.6	121	0.92 ±0.2	61 ±0.2	66
8 ^c		(CH ₂) ₄	C ₂₂ H ₂₈ ClN ₃ O ₂ ·C ₂ H ₂ O ₄	492.0	161.5 ^d	0.50 ±0.09	19.5 ±1.2	39
9 ^c		(CH ₂) ₄	C ₂₂ H ₂₈ I N ₃ O ₂	493.4	167-168 ^e	3.9 ±0.2	146 ±15	37
10		(CH ₂) ₃	C ₂₁ H ₂₆ I N ₃ O ₂	479.4	134-135 ^f	396 ±48	117 ±12	0.3
11		(CH ₂) ₄	C ₂₄ H ₃₁ N ₃ O ₃	409.5	143.5 ^g	1 ±0.08	17.5 ±1	18
12		(CH ₂) ₄	C ₂₆ H ₃₇ N ₃ O ₂	423.6	136 ^h	7.99 ±0.94	26.5 ±3.5	3.3
13		(CH ₂) ₄	C ₂₈ H ₃₃ N ₃ O ₂ ·C ₂ H ₂ O ₄	533.6	167.5 ^j	2.85 ±0.33	75 ±4	26
14		(CH ₂) ₄	C ₂₉ H ₃₃ N ₃ O ₃ ·C ₂ H ₂ O ₄ ·H ₂ O	579.7	127-128 ^k	2.5 ±0.2	63 ±2.7	25
15		(CH ₂) ₄	C ₂₂ H ₂₈ I N ₃ O ₂ ·C ₂ H ₂ O ₄	583.4	146.5 ^d	9.2 ±1	68 ±10	7.4
16		(CH ₂) ₃	C ₂₁ H ₂₆ I N ₃ O ₂ ·C ₄ H ₄ O ₄	595.4	94-95 ^d	300 ±32	235 ±16	0.8
17		(CH ₂) ₄	C ₂₂ H ₂₈ I N ₃ O ₂	493.4	117.5 ^f	7.8 ±2	15.2 ±1.2	1.9
18		(CH ₂) ₃	C ₂₁ H ₂₆ I N ₃ O ₂ ·C ₂ H ₂ O ₄	569.4	185-186 ^j	213 ±13	126 ±8.9	0.6
19		(CH ₂) ₄	C ₂₅ H ₃₀ N ₄ O ₂ ·C ₂ H ₂ O ₄ ·0.5H ₂ O	517.6	170.5 ^l	6.6 ±0.98	53.2 ±4.0	8.1
20		(CH ₂) ₄	C ₂₅ H ₃₀ N ₄ O ₂ ·C ₂ H ₂ O ₄ ·0.3H ₂ O	514.6	172-173 ^h	1.48 ±0.19	27.5 ±2	19
21		(CH ₂) ₄	C ₂₅ H ₃₀ N ₄ O ₂ ·C ₂ H ₂ O ₄ ·0.5H ₂ O	517.6	143-144 ^l	9.62 ±0.69	20.9 ±2	2.2
22		(CH ₂) ₄	C ₂₅ H ₂₉ N ₃ O ₄ ·C ₂ H ₂ O ₄ ·0.5H ₂ O	534.6	178-179 ^d	3.3 ±0.9	47 ±5	14
23		(CH ₂) ₄	C ₂₅ H ₂₉ N ₃ O ₄	435.5	135-136 ^m	1.8 ±0.3	23 ±4	13
24		(CH ₂) ₄	C ₂₄ H ₂₈ ClN ₃ O ₂ S	458.0	109-110 ^h	6.7 ±1.2	71.9 ±9.3	11
25		(CH ₂) ₄	C ₂₆ H ₃₀ ClN ₃ O ₃ ·2C ₂ H ₂ O ₄	648.1	142.5 ⁿ	6.3 ±0.5	200 ±30	32

Table 2. (Continued)



No.	R ¹	X	formula	M _r	mp (°C)	D ₃ ^a K _i (nM) ± SEM	D ₂ ^a K _i (nM) ± SEM	ratio ^b D ₂ /D ₃
26		(CH ₂) ₄	C ₂₉ H ₃₁ N ₃ O ₃	469.6	181.5 ^j	2.96 ±0.18	18 ±0.6	6.1
27		(CH ₂) ₄	C ₂₇ H ₃₃ N ₃ O ₂ ·C ₂ H ₂ O ₄ ·0.5H ₂ O	530.6	77-78 ^d	32 ±2.4	35 ±1.9	1.1
28		(CH ₂) ₃	C ₂₆ H ₃₁ N ₃ O ₂ ·C ₂ H ₂ O ₄ ·0.5H ₂ O	516.6	173.5 ⁱ	140 ±6	56 ±8	0.4
29		(CH ₂) ₃	C ₂₆ H ₃₁ N ₃ O ₂ ·C ₂ H ₂ O ₄ ·1.25H ₂ O	530.1	125.5 ⁱ	108 ±6.5	44 ±2.9	0.4
30		(CH ₂) ₃	C ₂₈ H ₃₅ N ₃ O ₃ ·C ₂ H ₂ O ₄	551.6	133-134 ^m	262 ±52	94.6 ±10.5	0.4
31		(CH ₂) ₄	C ₂₇ H ₃₆ ClN ₃ O ₂ ·C ₂ H ₂ O	560.1	143.5 ⁿ	42 ±3	56 ±3	1.3
32		(CH ₂) ₄	C ₂₅ H ₃₃ N ₃ O ₂ ·C ₂ H ₂ O ₄ ·0.25H ₂ O	502.1	94-95 ^o	15.5 ±1.6	81 ±10	5.2
33		(CH ₂) ₄	C ₂₄ H ₃₃ N ₃ O ₂ ·C ₂ H ₂ O ₄	485.6	116-118 ^l	4.8 ±0.2	48.7 ±2.5	10

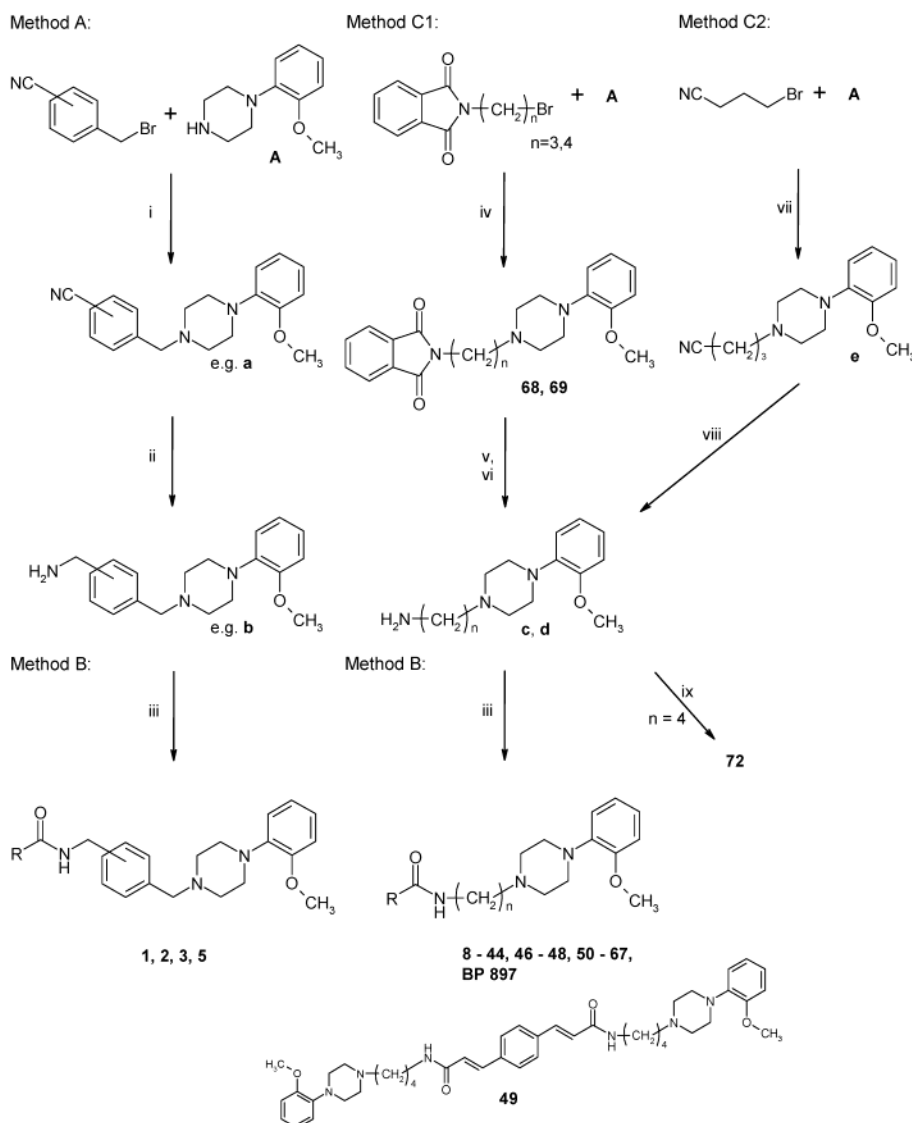
^a Binding assays using [¹²⁵I]iodosulpiride, in CHO cells expressing hD_{2L} and hD₃ receptors. ^b Ratio K_i (D₂)/K_i (D₃). ^c Ref 54. ^d Crystallized from EtOH/Et₂O. ^e Crystallized from MeOH/EtOH. ^f Crystallized from EtOH/H₂O. ^g Crystallized from ethyl acetate. ^h Crystallized from 2-propanol. ⁱ Crystallized from EtOH. ^k Crystallized from EtOH/2-propanol. ^l Crystallized from 2-propanol/Et₂O. ^m Crystallized from 2-propanol/EtOH. ⁿ Crystallized from acetone/Et₂O. ^o Crystallized from acetone.

ligands, as well as a dopamine D₃ receptor model pointing out crucial binding regions between ligand and receptor. In strong correlation to the modeling studies, we developed 72 compounds, which displayed structural modifications in the spacer group and in the aryl moiety. Most of the compounds share the general structure pattern mentioned previously and the 4-(2-methoxyphenyl)piperazino element. To a large extent D₃ receptor affinity was influenced by structural variations in the aryl moiety and selectivity versus the D₂ receptor by structural variations in the spacer group. The iodinated compound **38** (ST 280) provides a promising pharmacological profile and may serve, after further evaluation, as a potential novel radioligand.

Chemistry

All compounds described share a 4-(2-methoxyphenyl)piperazino group. The general preparation pathway is outlined in Scheme 1: divergent methods led to the primary alkanamines (e.g., **b**, **c**, **d**), which were condensed with an activated carboxylic acid to give a carboxamide as final product. According to method A, 1-(2-methoxyphenyl)piperazine was alkylated with isomeric (bromomethyl)benzonitriles by means of procedures known in the literature.²⁶ Subsequent reduction of the benzonitrile (e.g., **a**) to a benzylamine (e.g., **b**) was achieved by treatment with lithium aluminum hydride. Acylation of the amine with 2-naphthoyl chloride or

cinnamoyl chloride provided compounds **1–3** and **5** (method B). For amines with trimethylene or tetramethylene spacers, two methods were applied. For method C1, preparation started with the alkylation of 1-(2-methoxyphenyl)piperazine with *N*-(ω -bromoalkyl)-phthalimide. As an advantage of method C1, the intermediates **68** (NAN 190)²⁷ and **69**²⁸ could be obtained for pharmacological testing. The phthalimides were cleaved by hydrazinolysis to afford the desired alkanamines (**c**, **d**).²⁹ This provided a facile way for the first compounds but was not practical for a greater number of compounds. The alternative and more economic method C2 provided the butanamine derivatives through alkylation of 1-(2-methoxyphenyl)piperazine with 4-bromobutyronitrile and subsequent catalytic reduction of the cyano functionality (**e**).²⁹ This preparative way allowed scaling up as well as reducing time and expenditure of work. The ensuing method B employed the alkanamines, obtained by method C1 or C2, and diverse acid chlorides to afford compounds **8–44**, **46–67**, and BP 897. Furthermore, according to standard procedures the butanamine derivative was added to 3-phenylfuran-2,5-dione and delivered compound **72**. Scheme 2 depicts the reaction way for bicyclo derivatives, which resulted in the additional formation of valuable bisamination byproducts. In the first reaction step, reductive alkylation of 1-(2-methoxyphenyl)piperazine with *cis*-bicyclo[3.3.0]octane-3,7-dione was performed.^{30–32} The major product

Scheme 1. Synthesis of Compounds **1–3**, **5**, **8–44**, **46–69**, **72**, BP 897^a

^a Reagents and conditions. Method A: (i) acetone, K₂CO₃, reflux, 6 h; (ii) dry THF, LiAlH₄, reflux, 3 h. Method B: (iii) acyl chloride, dry CH₂Cl₂, Et₃N, ambient temperature, 3–24 h. Method C1: (iv) acetonitrile or acetone, K₂CO₃, reflux, 14 h; (v) H₂N–NH₂, MeOH, reflux, 2 h; (vi) 2 N HCl, reflux, 1 h. Method C2: (vii) acetonitrile, K₂CO₃, reflux, 6 h; (viii) R_a–Ni, 25 bar H₂, 24 h; (ix) glacial acetic acid, 3-phenylfuran-2,5-dione, reflux, 3 h.

was the desired aminoketone (**f**), accompanied by the octahydropentalene product of bisamination (**6**). A second reductive amination, with ammonia, delivered the primary amine (**g**) and another product of bisamination (**7**). The bisamination products were favorably separated as their salts by crystallization. The primary amine (**g**) was treated with 2-naphthoyl chloride according to method B to give compound **4**. A versatile synthesis for imide derivatives is described in Scheme 3. The reaction of 1-(2-methoxyphenyl)piperazine with 1,4-dibromobutane led to a quaternary spiro structure (**B**), which was obtained in almost quantitative yields by performing the reaction in *n*-butanol.^{33,34} This suitable synthon was treated with imides to provide compounds **70** and **71**.^{35,36}

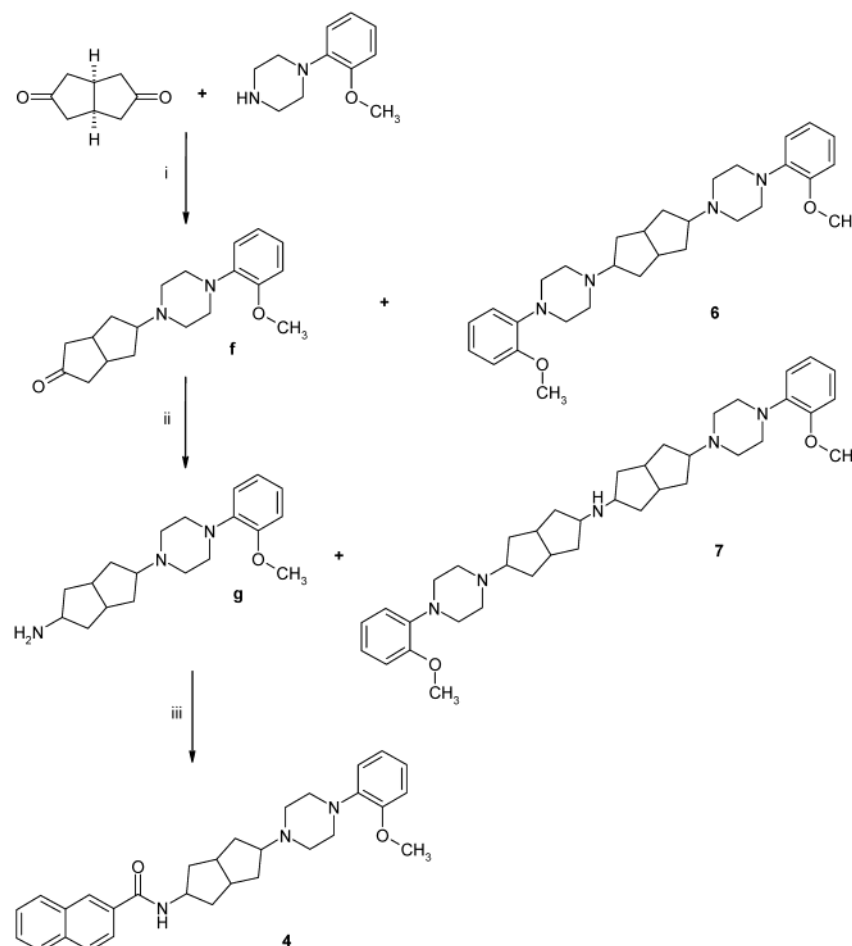
While establishing a method for radiolabeling, phenylstannane compound **45** was obtained as a stable intermediate through cleavage of distannanes by iodine-substituted compound **9** in the presence of palladium catalysts³⁷ (not shown). Compound **45** was reconverted

into aryl iodide **9** using molecular iodine, which was generated in situ from NaI and chloramin T in good yield.³⁸ If [¹²⁵I]NaI were used for this regioselective electrophile *ipso*-substitution, this method would enable facile radiolabeling.

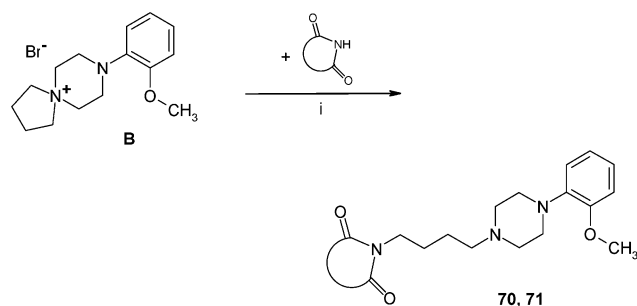
Computational Methods

Ligand Construction. Molecular structures were generated using the SYBYL program.³⁹ Assuming physiological conditions, the basic aliphatic nitrogen atom of the piperazine was taken as protonated. The geometry was optimized using the Tripos force field applying the conjugate gradient method until the energy difference between successive cycles was below 0.01 kcal mol^{−1}. Partial atomic charges were calculated with the Gasteiger–Hückel method, and the dielectric function was set to a constant value of 80.⁴⁰

Pharmacophore Generation. The steric and electrostatic information coming from partially rigid, highly potent ligands was used to generate the pharmacophore

Scheme 2. Synthesis of Compounds **4**, **6**, and **7**^a

^a Reagents and conditions: (i) 1,2-dichloroethane, NaBH(OCOCH₃)₃, glacial acetic acid, ambient temperature, 2 h; (ii) NH₃ in MeOH, Pd/C, H₂, 12 h. Method B: (iii) 2-naphthoyl chloride, dry CH₂Cl₂, Et₃N, ambient temperature, 10 h.

Scheme 3. Synthesis of Compounds **70** and **71**^a

^a Reagents and conditions (for **B**, see refs 28 and 29): (i) xylene, K₂CO₃, 18-crown-6, reflux, 24 h.

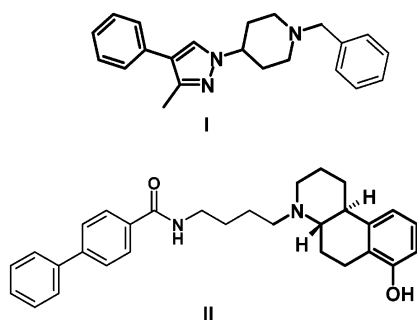
models. To explain the receptor selectivity of the studied ligands, pharmacophore models were developed for dopamine D₂ and dopamine D₃ receptor ligands. The investigated potent dopamine D₃ receptor ligands are characterized by two lipophilic/aromatic moieties, a hydrogen bond acceptor, and a basic aliphatic nitrogen atom.

The molecular alignments were carried out using the FLEXS program.⁴¹ FLEXS approaches flexible superpositioning on the basis of a combinatorial matching procedure. Pairs of molecules are aligned, one of which is treated as rigid and the other one is flexibly fitted. The strategy is to decompose the flexible structure into relatively rigid portions, to start the placement using a

manually selected portion, and to add the remaining portions in an iterative incremental procedure. The scoring functions used to choose appropriate conformations comprise energy-like matching terms for paired intermolecular interactions and overlap terms using Gaussian functions. The Gaussian functions are used to describe different field properties. Therefore, applying the molecular comparison procedure within FLEXS, it is a priori not necessary to define the pharmacophoric features for superimpositioning molecules. This has been demonstrated by Lemmen et al.⁴¹ in an extensive evaluation of the approach based on experimental data.

To determine the D₃ pharmacophore, the following compounds were examined in detail: compound **4**, compound **I**⁴², and compound **II**⁴³ (Chart 2). Critical features for the selected compounds were high binding affinity for the dopamine D₃ receptor and rigid molecule structure, but not necessarily D₃ receptor preference (compound **I** K_i (D₃) = 28 nM, K_i (D₂) = 6.1 nM, K_i (D₄) = 0.39 nM; compound **II** K_i (D₃) = 1 nM, K_i (D₂) = 25 nM). Since none of the ligands examined is completely rigid but some are partially rigid, these three molecules were initially decomposed into fragments (Chart 2), and the conformational space of the restricted fragments were then examined individually. The ring conformations (i.e., octahydrobenzo[f]quinolin-7-ol in **II**) were generated applying the simulated annealing pro-

Chart 2



cedure within SYBYL. The fragments were heated fifty times to 1000 K and were subsequently annealed to 0 K. The generated conformations were compared with corresponding fragments from the Cambridge Structural Database.⁴⁴ Similar conformations were detected by both approaches. The comparison of the 4-(2-methoxyphenyl)piperazino fragment, which is part of compound **4** and all other ligands under study, and the conformationally restricted 4-methyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolin-7-ol fragment of compound **II** determined the common structural properties in the basic residue of the ligands. The distal aryl moiety, which contains the naphthamidine part, was assigned to be planar, referring to known potent imidic ligands, e.g., compound **68**.²⁷ Taking these conformational restrictions into account, a conformational analysis was performed for the whole structure of **4**. The rotatable bonds were scanned using a 10° increment within a 0–350° interval. Using the Tripos force field, 992 energetically favorable conformations were calculated for compound **4** within an energy range of 15 kcal/mol. The conformational space of compounds **I**, **II**, **4**, and **71** was compared in pairs using the FLEXS program. In such a way we succeeded in identifying at least one conformer for each active ligand, which could be superimposed onto at least one conformer of the other ligands. The final superimposition of the selected conformers allowed us to extract the geometric requirements in terms of distances between pharmacophoric points (Figure 1).

Due to their preference for the dopamine D₂ receptor subtype, compounds **1** and **5** were examined in detail to determine the bioactive conformation of D₂ receptor ligands. The four rotatable bonds in the alkyl–aryl spacer, which link the basic and the aryl/acryl moiety, were scanned using a 10° increment within a 0–350° interval. Thus, 417 energetically favorable conformations were obtained, from which the bioactive form had to be determined. Compound **2**, which also possesses affinity for the D₂ receptor, was superimposed onto each possible conformation of **1** and **5** using program FLEXS. The putative bioactive conformation of compound **1** was derived from the top scored superimposition calculated by FLEXS. All the remaining compounds were superimposed onto the predicted bioactive conformation of compound **4** for the dopamine D₃ receptor and onto the predicted bioactive conformation of compound **1** for the D₂ receptor. This procedure was performed using the flexible fitting procedure within FLEXS. In the following step, SYBYL's multifit routine was used for the refinement of the molecular alignment. Each structure was relaxed using the steepest descent method up to a gradient of below 0.1 kcal mol^{−1}.

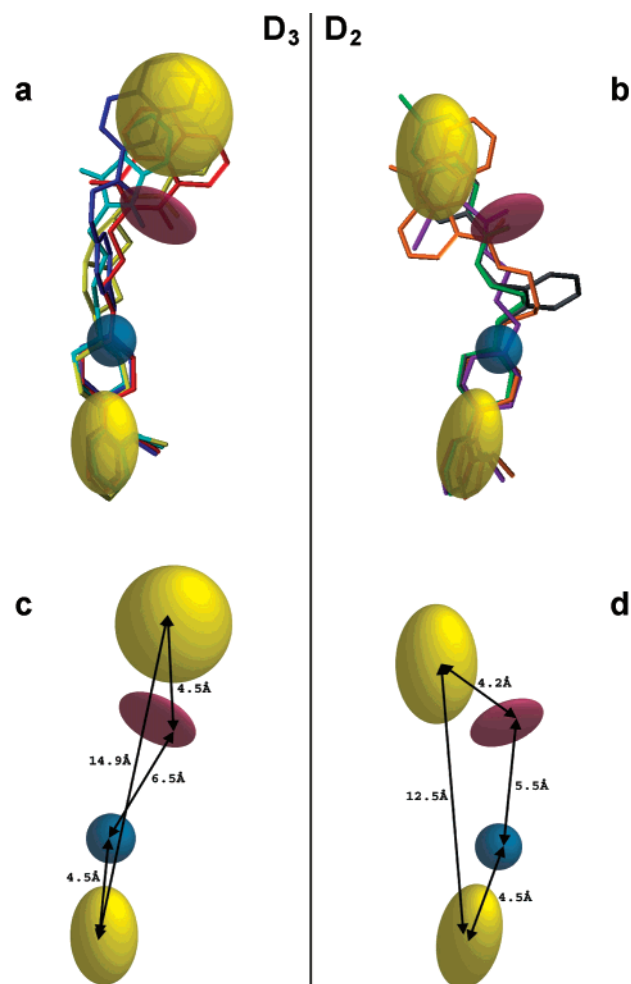


Figure 1. Pharmacophore model for dopamine D₃ and D₂ receptor ligands. Superimposition of ligands **3**, **68**, **71**, and **4** for the D₃ receptor (a) and of **18**, **26**, **37**, and **1** for the D₂ receptor (b). Pharmacophoric points (lipophilic/aromatic moieties, yellow; hydrogen bond acceptor, violet; basic aliphatic nitrogen atom, blue) and their distances within the pharmacophore for dopamine D₃ (c) and D₂ receptor ligands (d). Compounds are colored as follows: (a) **3** blue, **4** yellow, **68** cyan, **71** red; (b) **1** grey, **18** violet, **26** orange, **37** green.

The derived molecular alignments for dopamine D₃ and the D₂ receptor ligands are shown in Figure 1. The superimposition of the potent ligands **3**, **68**, **71**, and **4** onto the D₃ pharmacophore illustrates that the crucial structural features, e.g., positively charged nitrogen atoms, amidic parts, and aryl moieties, occupy a similar area in space. The same is true for the D₂ receptor. The superimposition of ligands **18**, **26**, **37**, and **1** onto the D₂ pharmacophore shows that the crucial features are positioned in a similar area in space. The projection of the D₃ receptor ligands onto the pharmacophore shows clearly that they adopt an extended and more linear conformation. Gmeiner and co-workers very recently confirmed linearity as a crucial structural feature for good D₃ receptor binding properties.¹⁶ The distances between the chemical features are recapitulated in Figure 1. In contrast to the bioactive conformation calculated for the D₃ receptor, ligands at the D₂ receptor adopt a more bent conformation. The corresponding distances between the pharmacophoric features are different compared to those measured in the D₃ pharmacophore. Comparing both pharmacophore models, it

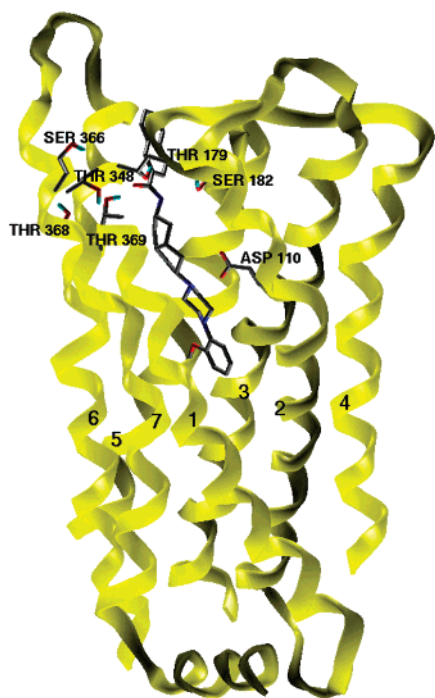


Figure 2. Compound **4** positioned in the dopamine D₃ receptor. The receptor is restricted to its binding relevant fragments. Putative transmembrane domains are numbered 1–7. Additionally, several of the ligand surrounding threonine and serine molecules are pointed out, which allow alternative orientations for the amide oxygen. Asp 110, the counterion to the basic part, is shown for clarity.

can be observed that bulky substitutions in the spacer region are not tolerated with D₃ selective ligands.

GRID Field Calculations. After definition of the arrangement of the pharmacophoric descriptors, the models were validated by closer examination of molecular fields that are produced by the ligands superimposed onto each other in their bioactive conformation. The GRID program⁴⁵ is an approach to predict non-covalent interactions between a molecule of known three-dimensional structure, i.e., the ligand, and a small group as a probe, representing chemical features of corresponding amino acid residues. On a cube enclosing the complete ligand, a series of calculations was performed, searching for potential binding sites complementary to the functional groups. The SYBYL program subsequently showed the contour maps superimposed on the receptor model.

Receptor Modeling. The coordinates of the bovine rhodopsin crystal structure⁴⁶ and a structure–function analysis of rhodopsin-like receptors⁴⁷ delivered the basis for building and optimizing the human dopamine D₃ receptor using the Insight II/Discover modeling package.⁴⁸ The receptor was structurally minimized with the steepest descent method until the RMS value was below 0.1 kcal mol^{−1} Å^{−1} and subsequently minimized with the conjugate gradient method until the RMS value was below 0.01 kcal mol^{−1} Å^{−1}. Minimizations were performed with the Consistent Valence force field, implemented in Insight II/Discover, and a distance depending dielectric constant of 4. In a first approach, compound **4** was docked manually into the binding pocket located between the transmembrane helices 3, 5, 6, and 7. The dock procedure within SYBYL was used for this pur-

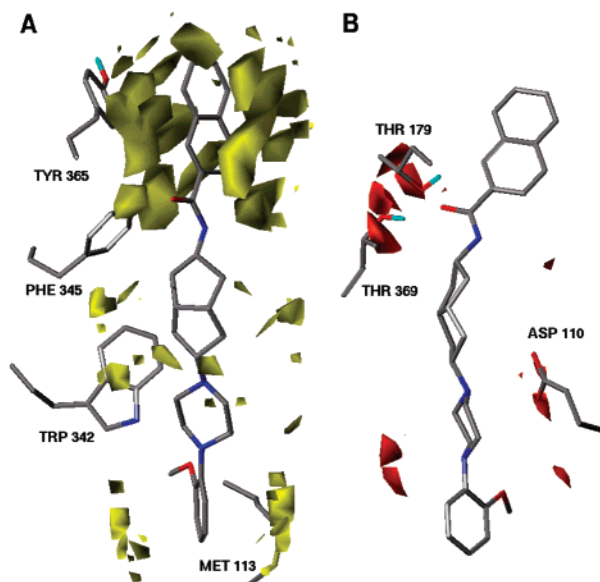


Figure 3. Lipophilic (A) and hydrophilic (B) interactions of compound **4** with discrete amino acids of the dopamine D₃ receptor. Calculations were performed with the GRID DRY-probe (A) displaying interaction zones in yellow, contoured at an energy level of −0.45 kcal mol^{−1}, and with the GRID OH-probe (B) displaying interaction zones in red, contoured at an energy level of −4 kcal mol^{−1}.

pose. The resulting complex was minimized as described above (Figure 2). To further analyze the properties of the binding pocket, the molecular interaction fields for compound **4** were calculated using program GRID and were compared with the amino acid residues of the ligand binding site. The hydrophilic OH probe and the lipophilic DRY probe were chosen to examine favorable corresponding interaction regions. As depicted in Figure 3, lipophilic regions coincided with positions of lipophilic amino acids Met 113, Trp 342, Phe 345, and Tyr 365, whereas hydrophilic areas coincided with positions of hydrophilic amino acids Asp 110, Thr 179, and Thr 369. The FLEXX program,⁴⁹ which was designed to place flexible molecules into active sites of proteins considering their physicochemical properties, was used to dock the ligands into the binding pocket. Since Asp 110 of the dopamine D₃ receptor most probably represented the counterion to the basic part,⁴ a salt-bridge was enabled toward the protonated nitrogen of the ligands. In the aryl moiety, large and bulky substituents, e.g., **48**, **49**, **70**, and **71**, were tolerated, irrespective of amidic or imidic binding. This slight effect on affinity may be due to interactions with various amino acids near the extracellular space (Figure 2), which allow the amide or imide oxygens to point to various directions. Since a number of surrounding amino acids were able to form hydrogen bonds, the carbonyl group was able to undergo several interactions.

Pharmacological Results and Discussion

Binding Studies. Chinese hamster ovary (CHO) cells were stably transfected with cDNA of human D_{2L} and D₃ receptors and cloned.^{50,51} With the cell lines obtained, binding was determined using [¹²⁵I]iodosulpiride in order to measure radioactivity. *K_i* values were calculated from the IC₅₀ values according to the Cheng–Prusoff equation.⁵² All novel compounds showed moder-

ate to high binding affinities for the dopamine D₂ receptor or for the dopamine D₃ receptor (K_i values of 750–0.33 nM) (Tables 1–4). Throughout the whole series an important impact of the chain length and linearity of the spacer was observed. As expected from the modeling study results, flexible spacers showed a good concordance between a tetramethylene chain (C₄) and high dopamine D₃ receptor affinity, whereas a trimethylene spacer (C₃) was favorable for dopamine D₂ receptor binding. Contrary to the shorter trimethylene spacer, the tetramethylene spacer was able to span the distance of about 6.5 Å between the pharmacophoric features basic aliphatic nitrogen and hydrogen bond acceptor (Figure 1). Within pairs of compounds, which only diverged in the length of the chain, the C₄ bridge effected generally lower K_i values than the C₃ analogue and was simultaneously favoring the D₃ receptor (**9** → **10**, **15** → **16**, **17** → **18**, **27** → **28**, **68** → **69**). These results were transferred to compounds with rigid xylene (**1**–**3**, **5**) or octahydropentalene (**4**) spacers (Table 1). D₃ receptor binding was favored by the linear molecules **3** and **4**, and D₂ receptor binding by the angled ones (**1**, **2**, **5**). Obviously the aliphatic structure of the chain was not essential for dopamine D₂-like receptor affinity. Since compounds with C₄ spacer were able to adopt both bioactive conformations that were determined for the D₂ and D₃ receptor and since affinity for both receptors was nanomolar, selectivity toward one or the other receptor could not be determined exclusively by the conformational requirements of these compounds. Detailed comparative molecular field analyses are therefore needed to examine the effects of different C₄ compounds, which cause a change of binding affinity and selectivity toward one receptor. Nevertheless, the qualitative difference in both receptor pharmacophores stressed important points for drug optimization.

Among the three synthesized dimers (compounds with two identical elements in their structure), compound **6** showed low affinity, compound **7** bound moderately, and compound **49** showed improved affinity for both receptors. As outlined in Figure 2, the ligands bound along the helices of the receptor protein with a moiety positioned near the extracellular site. Assuming physiological conditions, the dimers appeared to be 2-fold (**6**, **49**) or 3-fold (**7**) protonated. This induced a strong shift of the physicochemical properties compared to other monoprotinated compounds in this series. Thus, binding properties of compound **6** and **7** may be deteriorated because their protonated nitrogens occupy an area which requires an aryl moiety in the pharmacophore model. Compound **49** comprised all required pharmacophoric features, but the additional, protonated dimeric residue reaches out of the pharmacophore area. This elongated part of the molecule had no negative effect on D₂ and D₃ receptor binding. According to our receptor model, binding deterioration was reduced in correlation to a greater distance between the second proton and the binding pocket. For nonsymmetrical dimeric D₃ receptor ligands with 4-phenylpiperazino and alkylamine residues, a second proximal binding site has been postulated.⁵³

Variations of the naphthoyl residue were performed with benzamides and analogues (Table 2). Divergent benzamide substituents with different requirements in

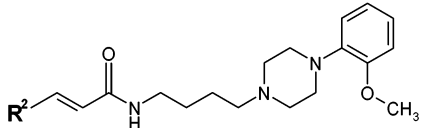
space and interaction possibilities were accepted (**8**–**18**) and the D₃ receptor affinity was maintained below 8 nM for para-substituted compounds with C₄ spacer. The *p*-chlorobenzoyl derivative **8**⁵⁴ displayed significant higher dopamine D₃ receptor affinity than that of the lead compound BP 897 and showed considerable D₃ receptor preference. The low nanomolar affinity values of the three iodobenzoyl structures declined in a sequence of *m*-, *o*-, and *p*-substitution, the latter (**9**)⁵⁴ also giving an enhanced selectivity ratio for the dopamine D₃ receptor. Introduction of voluminous phenyl (**13**) and benzoyl substituents (**14**) was well tolerated for binding and selectivity. Replacement of the aryl moiety into quinoline (**19**), isoquinolines (**20**, **21**), chromone (**22**), and cumarine (**23**) moieties delivered improved binding properties for the isoquinoline **20**. Introduction of 3-chlorobenzo[*b*]thiophene (**24**), related to FAUC 365, or the more bulky fluorenone (**26**) as a potential bioisosteric group led to low nanomolar binding values. A comparable fluorenone moiety was already presented in another series¹⁵ and very recently an analogue, which only diverged in the 4-phenylpiperazino group.⁵⁵ Above that, substituted furan **25** gave an improved selectivity ratio compared to the other heteroaryl compounds **19**–**24** and **26**. Naphthyl acetic acid derivatives **27**–**30** showed no improvement in D₃ receptor affinity, with the C₃ spacer compounds having certain D₂ receptor preference. Compounds selective for the D₂ receptor were not optimized in this investigation. A consequent favorable development of binding properties and selectivity was observed in the series of benzyl compound with steric hindering substituent (**31**), rigid cyclopropane phenyl acetic acid derivative **32**, and the 3-phenylpropionic acid derivative **33**.

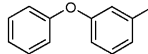
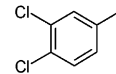
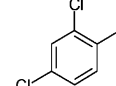
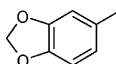
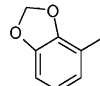
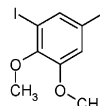
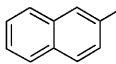
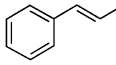
Following these results and the modeling study, molecules were established bearing the amide in a certain distance to the steric fixed aromatic structure (Table 3). Parallel to our development of *E*-cinnamide as a valuable structural basis for further compounds (**34**), some potent *E*-cinnamoyl derivatives have been published.⁵⁶ Isomeric *Z*-derivatives have been reported to possess lower affinities.⁵⁶ The group of mono-, di-, and trisubstituted cinnamoyl amides (**35**–**61**) and analogues (**62**–**67**) consisted of compounds with moderate to enhanced selectivity ratios and partly high affinity. The unsubstituted molecule **34** reached an excellent binding affinity for the dopamine D₃ receptor (K_i (D₃) = 0.33 nM), superior to that of lead BP 897, and considerable D₃ receptor preference. *p*-Fluoro (**35**) or -chloro substitution (**36**) did not lead to further improvement in affinity but increased selectivity for the chloro derivative (ratio_(D₂/D₃) 51), equivalent to that of the *p*-bromo derivative **37**. Best results in this series were obtained with the *p*-iodo derivative **38** (**ST 280**). Selectivity ratio was raised to a value of 153 combined with subnanomolar binding affinity. Substitution with iodine in *o*- (**55**) or *m*-position (**51**) also resulted in low nanomolar D₃ receptor binding and high selectivity ratios but did not exceed those of **38**. Halogen monosubstitution (**36**) was preferable to disubstitution (**57**, **58**) for affinity and selectivity scores. Electron-withdrawing as well as electron-delivering substituents (**43**, **56**) were introduced without great deterioration in affinity. In contrast to the benzamides, space-demanding substituents (**44**–**48**, **53**,

Table 3. Structures, Physical Data, and Pharmacological Screening Results of α,β -Unsaturated Carboxylic Acid Derivatives

No.	R ²	formula	M _r	mp (°C)	D ₃ ^a K _i (nM) ± SEM		D ₂ ^a K _i (nM) ± SEM		ratio ^b D ₂ /D ₃
34		C ₂₄ H ₃₁ N ₃ O ₂ ·C ₂ H ₂ O ₄ ·0.5H ₂ O	492.6	128.5 ^d	0.33	±0.1	13.54	±1.2	41
35		C ₂₄ H ₃₀ FN ₃ O ₂	411.5	149-150 ^j	0.69	±0.11	20.7	±0.8	30
36		C ₂₄ H ₃₀ ClN ₃ O ₂ ·C ₂ H ₂ O ₄ ·0.25H ₂ O	522.5	138.5 ^d	0.44	±0.09	22.6	±1.0	51
37		C ₂₄ H ₃₀ BrN ₃ O ₂ ·C ₂ H ₂ O ₄ ·0.5H ₂ O	571.5	138.5 ^d	0.43	±0.13	23.7		55
38		C ₂₄ H ₃₀ IN ₃ O ₂	519.4	161-162 ^h	0.50	±0.13	76.4		153
39		C ₂₅ H ₃₀ F ₃ N ₃ O ₂	461.5	158.5 ⁱ	2.14	±0.2	36.79	±6.5	17
40		C ₂₅ H ₃₀ N ₄ O ₂ ·2C ₂ H ₂ O ₄	598.6	181-183 ^l	0.46	±0.03	10	±0.7	22
41		C ₂₄ H ₃₀ N ₄ O ₄ ·C ₂ H ₂ O ₄ ·H ₂ O	546.6	117-118 ^l	0.74	±0.1	14	±3	19
42		C ₂₅ H ₃₁ N ₃ O ₃ ·2C ₂ H ₂ O ₄ ·0.5H ₂ O	610.6	128-129 ^l	0.73	±0.1	11.5	±1.9	16
43		C ₂₅ H ₃₃ N ₃ O ₃	423.6	137 ⁱ	0.38	±0.1	12	±1	32
44		C ₂₈ H ₃₉ N ₃ O ₂ ·C ₂ H ₂ O ₄	539.7	88.5 ^d	10.2	±0.94	128	±31.6	13
45		C ₃₆ H ₅₇ N ₃ O ₂ Sn	682.6	oily	92	±6	729	±104	8
46		C ₃₀ H ₃₅ N ₃ O ₂	469.6	185-186 ^p	16	±3	37	±15	2.3
47		C ₃₀ H ₃₅ N ₃ O ₃	485.6	158-159 ^h	10.1	±0.9	65.2	±29.7	6
48		C ₃₁ H ₃₇ N ₃ O ₃	499.7	159-160 ^h	19.5	±1.5	32	±4	1.6
49 ^q		C ₄₂ H ₅₆ N ₆ O ₄	708.9	207-208 ^r	3.6	±0.6	7.2	±0.4	2
50		C ₂₄ H ₃₀ ClN ₃ O ₂ ·C ₂ H ₂ O ₄ ·H ₂ O	536.0	125-127 ^d	0.46	±0.1	20	±4	43
51		C ₂₄ H ₃₀ IN ₃ O ₂	519.4	119-120 ^h	1.2	±0.3	161	±1	134
52		C ₂₅ H ₃₀ F ₃ N ₃ O ₂ ·1.5C ₂ H ₂ O ₄	596.6	120-121 ^h	4.1	±0.4	50.2	±9.3	12

Table 3. (Continued)



No.	R ²	formula	M _r	mp (°C)	D ₃ ^a K _i (nM) ± SEM		D ₂ ^a K _i (nM) ± SEM		ratio ^b D ₂ /D ₃
53		C ₃₀ H ₃₅ N ₃ O ₃ ·C ₂ H ₂ O ₄ ·0.25H ₂ O	580.2	106-107 ^l	19.5	±1.5	114	±27	6
54		C ₂₄ H ₃₀ ClN ₃ O ₂ ·2C ₂ H ₂ O ₄ ·0.5H ₂ O	617.1	86-88 ⁱ	0.52	±0.03	15	±6	29
55		C ₂₄ H ₃₀ IN ₃ O ₂	519.4	143-144 ⁱ	0.75	±0.34	63	±8	84
56		C ₂₅ H ₃₃ N ₃ O ₃	423.6	136 ⁱ	0.56	±0.1	14	±2	25
57		C ₂₄ H ₂₉ Cl ₂ N ₃ O ₂ ·C ₂ H ₂ O ₄ ·H ₂ O	570.5	132-134 ^d	1.31	±0.1	23	±4	18
58		C ₂₄ H ₂₉ Cl ₂ N ₃ O ₂ ·C ₂ H ₂ O ₄ ·1.5H ₂ O	579.5	115-117 ^d	1.28	±0.04	21	±2	16
59		C ₂₅ H ₃₁ N ₃ O ₄	437.5	133-134 ^h	1	±0.2	10.3	±1.3	10
60		C ₂₅ H ₃₁ N ₃ O ₄	437.5	139 ^h	0.55		8		15
61		C ₂₆ H ₃₄ IN ₃ O ₄ ·C ₂ H ₂ O ₄	669.5	189-190 ^l	14.9	±3.9	38.5	±7.4	2.6
62		C ₂₂ H ₂₉ N ₃ O ₂ S·C ₂ H ₂ O ₄ ·0.25H ₂ O	494.1	144-145 ^h	0.59	±0.04	9.5	±0.7	16
63		C ₂₂ H ₂₉ N ₃ O ₂ S·0.25H ₂ O	404.1	134-135 ^h	0.64	±0.04	13	±3	20
64		C ₂₂ H ₂₉ N ₃ O ₃ ·C ₂ H ₂ O ₄ ·C ₂ H ₅ OH	519.6	86 ^d	0.6		11.1		19
65		C ₂₃ H ₃₀ N ₄ O ₂ ·2C ₂ H ₂ O ₄	574.6	154-155 ^h	0.5	±0.02	14.5	±0.4	29
66		C ₂₈ H ₃₃ N ₃ O ₂	443.6	131 ⁱ	0.68	±0.1	32	±2	47
67		C ₂₆ H ₃₃ N ₃ O ₂ ·0.25H ₂ O	424.1	138-139 ^h	1.5	±0.1	19	±2	13

^{a,b,d,g,h,i,l} See corresponding footnotes in Table 2. ^p Free base crystallized while drying in vacuo. ^q For structure, see Scheme 1. ^r Crystallized from MeOH.

61) only moderately deteriorated in binding properties. Interesting exceptions were the benzo[1,3]dioxoles **59** and **60**, which showed structural similarity to the addictive drug MDMA (3,4-methylenedioxy-*N*-methylamphetamine).⁵⁷ All cinnamoyl analogues with aryl heterocycles (**62**–**65**), naphthyl (**66**), and conjugated phenylalkadiene structure (**67**) showed high affinity below 1.5 nM, but unfortunately no improvement in selectivity.

With the amide BP 897 as lead on one hand and with steric demands of the pharmacophore concerning the binding pocket on the other hand, it seemed reasonable to integrate an imide as structural feature (Table 4). But change from amides to imides did not lead to improved binding or selectivity properties. Analogous imides **68** and **71** showed similar binding affinities despite bearing differently bulky aromatic residues. Only **70** revealed slight selectivity for the dopamine D₃

Table 4. Structures, Physical Data, and Pharmacological Screening Results of Imide Compounds

No.	R ³	n	formula	M _r	mp (°C)	D ₃ ^a K _i (nm) ± SEM	D ₂ ^a K _i (nm) ± SEM	ratio ^b D ₂ /D ₃
68 ^s		4	C ₂₃ H ₂₇ N ₃ O ₃ ·C ₂ H ₂ O ₄	483.5	150.5 ⁱ	38 ±5.7	50 ±6	1.3
69 ^t		3	C ₂₂ H ₂₅ N ₃ O ₃ ·1.25C ₂ H ₂ O ₄	541.5	212-213 ^m	≈560	≈300	0.5
70		4	C ₂₇ H ₂₉ N ₃ O ₃	443.6	121-122 ^u	23.3 ±2	145 ±6.5	6.2
71		4	C ₂₇ H ₂₉ N ₃ O ₃	443.6	133.5 ^u	29 ±4	40 ±3	1.4
72		4	C ₂₅ H ₂₉ N ₃ O ₃ ·C ₂ H ₂ O ₄	509.6	131-132 ^t	320 ±40	330 ±80	1.0

^{b,c,i,m,l} See corresponding footnotes in Table 2. ^s NAN 190.²⁷ ^t Ref 28. ^u Crystallized from Et₂O.

receptor. Similar to analogue compounds with C₃ spacer, **69** displayed remarkably high dopamine D₃ receptor K_i values and a dopamine D₂ receptor preference. These results indicated that the related aromatic or lipophilic binding regions of the receptor were tolerant to volume and that the linearity increased the affinity to both receptor subtypes.

Compound **68**²⁷ and other structurally related compounds⁵⁸ have also been described as 5-HT_{1A} receptor and α₁ receptor ligands.⁵⁴ Thus, further pharmacological investigations are required to clarify the ligands' confined cross affinity for related receptors.

Functional Receptor Tests. Dopamine D₃ receptor-based treatment of cocaine abuse with the partial agonist BP 897 was described for monkeys.¹⁸ It cannot be totally excluded that these effects may possibly be related to antagonist properties of BP 897, which have been published for different in vitro tests.⁵⁹ Also for other dopamine D₃ receptor antagonists, inhibition of cocaine-seeking behavior has been reported.⁶⁰ The needed optimal value of intrinsic activity of a partial agonist for the treatment of human cocaine abusers is not known. Therefore, compounds with different intrinsic activity are required for pharmacological screening and evaluation. A mitogenesis test was performed on NG 108-15 cells expressing the dopamine D₃ receptor, measuring [³H]thymidine incorporation.⁶¹ From the most promising compounds with an affinity below 4 nM and a selectivity ratio exceeding 18, structural representatives of comparable affinity and selectivity scores were selected for the mitogenesis test. Lead compound BP 897 displayed an intrinsic activity (α) of 0.6 for the dopamine D₃ receptor (dopamine: α = 1.0) and an EC₅₀ value of 3 nM.¹⁸ The *p*-iodo-substituted benzamide **9** revealed an intrinsic activity of 0.5 with EC₅₀-value of 1.2 ± 0.4 nM. The same intrinsic activity was obtained for benzamide derivative **13**, although bearing a more

bulky phenyl substituent. Due to its high lipophilicity this molecule was supposed to pass the blood-brain barrier rapidly. For heterocyclic compound **20**, partial D₃ receptor agonism was found with diminished intrinsic activity of value 0.3 (EC₅₀ = 2.5 ± 0.8 nM). In the cinnamoyl group, compound **36** was tested and showed with an intrinsic activity of 0.7 (EC₅₀ = 10 ± 3.5 nM) comparable values to BP 897.

Conclusions

Binding and selectivity properties of lead compound BP 897 were improved remarkably by means of variations in distinct structural moieties of the molecule. The development of ligands was supported by molecular modeling methods, based on two pharmacophore models, one for dopamine D₃ receptor and one for dopamine D₂ receptor ligands. The built model for the dopamine D₃ receptor depicted the putative amino acids responsible for binding to the pharmacophore. The modeling results clearly showed a perpendicular positioning of ligands at the D₃ receptor site. According to computational calculations, a stretched dopamine D₃ receptor binding pocket offered ideal binding facilities for a linear ligand with a spacer length of approximately 6.5 Å. This positive effect was determined for both aliphatic chains (tetramethylene or octahydropentalene) and aromatic linkers (*p*-xylene). Steric inflexibility of the aromatic amidic residue resulted in advantageous affinity and selectivity scores. Acryl amides met all these requirements and delivered a structural basis for new dopamine D₃ receptor partial agonists with high affinity and selectivity. These results led to highly potent *p*-substituted 3-phenylacryl amides with tetramethylene spacer, of which the iodo derivative **38** displayed an improved dopamine D₃ receptor selectivity up to a value of 153 combined with a subnanomolar binding affinity. This compound gives perspective for a desired new radio-

ligand, possibly enabling direct visualization of dopamine D₃ receptors in brain. For potential therapeutic application, the selected compounds tested in the functional mitogenesis test showed the pharmacologically desired divergent partial agonism with intrinsic activities of 0.3 (**20**), 0.5 (**9**, **13**), and 0.7 (**36**).

Experimental Section

Chemistry. General Procedures. Melting points were determined on an Electrothermal IA 9000 digital or a Büchi 512 melting point apparatus and are uncorrected. ¹H NMR spectra were recorded on a Bruker DPX 400 Avance (400 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal Me₄Si as reference. ¹H NMR data are reported in the following order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet; m, multiplet); approximate coupling constants in Hertz (Hz); number of protons; *, exchangeable by D₂O; Naph, naphthyl; Xyl, xylenyl; MeOPh, 2-methoxyphenyl; Pipera, piperazinyl; Ph, phenyl; Isoq, isoquinolinyl; Phth, phthalimidyl; Mal, maleic acid. Elemental analyses (C, H, N) were measured on Perkin-Elmer 240 B or Perkin-Elmer 240 C instruments and were within ±0.4% of theoretical values for all compounds. Preparative, centrifugally accelerated, rotary chromatography was performed using a Chromatotron 7924T (Harrison Research) and glass rotors with 4 mm layers of silica gel 60 PF₂₅₄ containing gypsum (Merck). Thin-layer chromatography (TLC) was performed on silica gel PF₂₅₄ plates (Merck). The spots were visualized with fast blue salt B, ninhydrin or by UV absorption at 254 nm. Spectral data and elemental analyses are shown for intermediates (**a–g**, **68**, **69**, **B**) and parent compounds, which were obtained by different reactions or methods, and additionally the most potent compounds (**1**, **3**, **4**, **8**, **9**, **11**, **13**, **14**, **20**, **22**, **25**, **34**, **37**, **38**, **40**, **51**, **55**, **57**, **58**, **60**, **66**, **70**).

General Procedure for Preparation of ((Aminomethyl)phenyl)methyl Derivatives 1–3, 5. Method A. 1-(2-Methoxyphenyl)piperazine·HCl (**A**) (5.5 mmol, 1.26 g) and K₂CO₃ (11 mmol, 1.52 g) were added under ice-cooling to a solution of bromomethyl-benzonitrile (5 mmol, 1 g) in 30 mL of acetone and then heated to reflux for 6 h. After cooling to ambient temperature, the solid was removed by filtration, the residue washed with acetone, and the filtrate was concentrated in vacuo. The residue 4-(4-(2-methoxyphenyl)piperazin-1-ylmethyl)benzonitrile, e.g., (**a**) was dissolved in 15 mL of freshly distilled THF and slowly added under ice-cooling to a suspension of LiAlH₄ (95%) (10 mmol, 0.38 g) in 15 mL of freshly distilled THF. After stirring at room temperature for 30 min, the mixture was heated to reflux for 3 h. After cooling again to ambient temperature, saturated NH₄Cl solution was added to the mixture to perform hydrolyzation. The organic layer was separated and the aqueous phase extracted with ethyl acetate twice. The combined organic phases were evaporated to dryness. The product obtained 4-(4-(2-methoxyphenyl)piperazin-1-ylmethyl)benzylamine, e.g., (**b**) was pure enough to be used in the following reaction step (method B) without further purification.

4-(4-(2-Methoxyphenyl)piperazin-1-ylmethyl)benzonitrile (a). Method A. Yield: 87%. ¹H NMR (DMSO-*d*₆) δ 7.88 (d, *J* = 8.0 Hz, 2H, NC–Ph–3H, NC–Ph–5H), 7.63 (d, *J* = 8.0 Hz, 2H, NC–Ph–2H, NC–Ph–6H), 7.00 (m, 2H, 2 MeOPh–H), 6.89 (m, 2H, 2 MeOPh–H), 3.92 (s, 2H, 1-Pipera–CH₂), 3.77 (s, 3H, OCH₃), 3.05 (br m, 4H, 4 Pipera–H), 2.80 (br m, 4H, 4 Pipera–H). Anal. (C₁₉H₂₁N₃O·C₂H₂O₄) C, H, N.

4-(4-(2-Methoxyphenyl)piperazin-1-ylmethyl)benzylamine (b). Method A. Yield: 83%. ¹H NMR (DMSO-*d*₆) δ 7.36 (m, 4H, 4 Xyl–H), 6.96 (m, 2H, 2 MeOPh–H), 6.87 (m, 2H, 2 MeOPh–H), 3.87 (s, 2H, 1-Pipera–CH₂), 3.81 (s, 3H, OCH₃), 3.51 (s, 2H, NH₂–CH₂), 2.95 (br m, 4H, 4 Pipera–H), 2.63 (br m, 4H, 4 Pipera–H). C₁₉H₂₅N₃O.

General Procedure for Preparation of Amides 1–3, 5, 8–44, and 46–67. Method B. A mixture of the amine (2 mmol), triethylamine (6 mmol, 0.84 mL), and 10 mL of dry CH₂Cl₂ was stirred under argon for 10 min. Then the corre-

sponding carboxylic acid chloride (2.1 mmol) was added and the stirring continued for another 3–24 h. An additional amount of carboxylic acid chloride (0.5–1 mmol) was added if any residual amine was detected by TLC control (ninhydrin). The solvent was removed under reduced pressure and the mixture stirred with water. Water was decanted from semisolids and the procedure repeated to give solid crystals. Oily products were extracted with CH₂Cl₂ or ethyl acetate. If necessary, the product was purified by rotatory chromatography and crystallized as salt of oxalic acid.

N-(2-(4-(2-Methoxyphenyl)piperazin-1-ylmethyl)phenylmethyl)-2-naphthalenecarboxamide (1). Method A, method B. Yield for last reaction step: 77%. ¹H NMR (DMSO-*d*₆) δ 9.35 (m, 1H*, NH), 8.47 (s, 1H, Naph–1H), 8.00 (m, 4H, 4 Naph–H), 7.59 (m, 2H, 2 Naph–H), 7.48 (m, 1H, Xyl–H), 7.39 (m, 2H, 2 Xyl–H), 7.31 (m, 1H, Xyl–H), 6.94 (m, 2H, 2 MeOPh–H), 6.84 (m, 2H, 2 MeOPh–H), 4.70 (d, *J* = 5.5 Hz, 2H, NH–CH₂), 4.05 (br m, 2H, 1-Pipera–CH₂), 3.89 (s, 3H, OCH₃), 3.17 (br m, 4H, 4 Pipera–H), 2.92 (br m, 4H, 4 Pipera–H). Anal. (C₃₀H₃₁N₃O₂·C₂H₂O₄) C, H, N.

N-(4-(4-(2-Methoxyphenyl)piperazin-1-ylmethyl)phenylmethyl)-2-naphthalenecarboxamide (3). Method A, method B. Yield for last reaction step: 20%. ¹H NMR (DMSO-*d*₆) δ 9.23 (t, *J* = 5.6 Hz, 1H*, NH), 8.52 (s, 1H, Naph–1H), 8.00 (m, 4H, 4 Naph–H), 7.61 (m, 2H, 2 Naph–H), 7.38 (m, 4H, 4 Xyl–H), 6.95 (m, 2H, 2 MeOPh–H), 6.86 (m, 2H, 2 MeOPh–H), 4.57 (d, *J* = 5.6 Hz, 2H, NH–CH₂), 3.97 (br m, 2H, 1-Pipera–CH₂), 3.75 (s, 3H, OCH₃), 3.06 (br m, 4H, 4 Pipera–H), 2.92 (br m, 4H, 4 Pipera–H). Anal. (C₃₀H₃₁N₃O₂·1.25C₂H₂O₄) C, H, N.

General Procedure for Preparation of Amine Precursors for 8–44, 46–67, and 72. Method C1. A mixture of 1-(2-methoxyphenyl)piperazine·HCl (**A**) (20 mmol, 4.57 g), *N*-(*ω*-bromoalkyl)phthalimide (20 mmol), and K₂CO₃ (80 mmol, 11.06 g) in 60 mL of acetonitrile or acetone was heated to reflux for 6 h. After further addition of *N*-(*ω*-bromoalkyl)phthalimide (2 mmol), the mixture was heated for another 8 h. To achieve higher reaction temperatures, the use of dimethylformamide was recommended for less reactive components. The hot suspension was filtrated and the residue washed with acetone several times. The filtrates were concentrated under reduced pressure to give the phthalimide intermediate (**68**, **69**).

N-(3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl)phthalimide (68). Method C1. Yield: 98%. ¹H NMR (DMSO-*d*₆) δ 7.88 (m, 4H, 4 Phth–H), 6.97 (m, 4H, 4 MeOPh–H), 3.76 (s, 3H, OCH₃), 3.66 (t, *J* = 6.0 Hz, 2H, Phth–CH₂), 3.28–2.74 (m, 10H, 8 Pipera–H, 1-Pipera–CH₂), 1.98 (m, 2H, CH₂–CH₂–CH₂). Anal. (C₂₂H₂₅N₃O₃·1.5C₂H₂O₄) C, H, N.

N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)phthalimide (69). Method C1. Yield: 75%. ¹H NMR (DMSO-*d*₆) δ 7.88 (m, 4H, 4 Phth–H), 7.00 (m, 4H, 4 MeOPh–H), 3.79 (s, 3H, OCH₃), 3.63 (t, *J* = 6.3 Hz, 2H, Phth–CH₂), 3.28–2.74 (m, 10H, 8 Pipera–H, 1-Pipera–CH₂), 1.65 (m, 4H, CH₂–CH₂–CH₂–CH₂). Anal. (C₂₃H₂₇N₃O₃·C₂H₂O₄) C, H, N.

The *N*-(*ω*-(4-(2-methoxyphenyl)piperazin-1-yl)alkyl)phthalimide (18 mmol) and hydrazine hydrate (22 mmol, 1.07 g) in 30 mL of methanol were heated to reflux for 2 h. To the hot solution was added 20 mL of 2N HCl, and reflux was continued for one more hour. After cooling to ambient temperature, the mixture was filtrated, the residue washed with methanol, and the filtrate evaporated to dryness. This residue was suspended in water and alkalinized with 2N NaOH. Extraction with ethyl acetate (or CH₂Cl₂) delivered an oily product (**c**, **d**), which was pure enough for the following reaction step (method B).

3-(4-(2-Methoxyphenyl)piperazin-1-yl)propylamine (c).²⁹ Method C1. Yield: 91%. ¹H NMR (DMSO-*d*₆) δ 11.25 (s, 1H*, Pipera+–H), 8.18 (s, 1H*, H₃N+), 7.03 (m, 4H, 4 MeOPh–H), 3.78 (s, 3H, OCH₃), 3.51 (m, 4H, 4 Pipera–H), 3.22 (m, 2H, 1-Pipera–CH₂), 3.14 (m, 4H, 4 Pipera–H), 2.94 (m, 2H, H₃N+CH₂), 2.12 (m, 2H, CH₂–CH₂–CH₂). C₁₄H₂₃N₃O·2HCl.

4-(4-(2-Methoxyphenyl)piperazin-1-yl)butylamine (d).²⁹ Method C1. Yield: 93%. ¹H NMR (DMSO-*d*₆) δ 6.98 (m, 4H, 4 MeOPh–H), 6.05 (s, 4H, 4 Mal), 3.79 (s, 3H, OCH₃), 3.35 (m, 8H, 8 Pipera–H), 2.92 (t, *J* = 7.0 Hz, 2H, 1-Pipera–CH₂), 2.84

(t, $J = 7.1$ Hz, 2H, $\text{H}_3\text{N}^+\text{CH}_2$), 1.70 (m, 2H, CH_2), 1.58 (m, 2H, CH_2). Anal. ($\text{C}_{15}\text{H}_{25}\text{N}_3\text{O} \cdot 2\text{C}_2\text{H}_4\text{O}_4$) C, H, N.

Method C2. A mixture of 1-(2-methoxyphenyl)piperazine-HCl (**A**) (20 mmol, 4.57 g), 4-bromobutyronitrile (25 mmol, 2.5 mL), and K_2CO_3 (44 mmol, 6.14 g) in 50 mL of acetonitrile was heated to reflux for 6 h. After further addition of 10 mmol (1 mL) of 4-bromobutyronitrile, the mixture was heated for another 6 h. The cooled mixture was filtrated, the residue washed with acetone, and the combined filtrates evaporated to dryness to afford a yellow oil, which was pure enough for the following reaction step. The 4-(4-(2-methoxyphenyl)piperazin-1-yl)butyronitrile (**e**)²⁹ obtained (17.4 mmol, 4.5 g) was subjected to catalytic hydrogenation using freshly prepared Raney nickel (from 5 g of aluminum-nickel alloy, according to standard procedures) in 150 mL of aqueous solution of ammonia and 20 bar hydrogen for at least 24 h. Cautious filtration and concentration under reduced pressure delivered an oily product (**d**), which crystallized after some time.

4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyronitrile (e).²⁹ Method C2. Yield: 93%. ^1H NMR ($\text{DMSO}-d_6$) δ 6.99 (m, 4H, 4 MeOPh-H), 3.78 (s, 3H, OCH_3), 3.02 (t, $J = 4.7$ Hz, 4H, 4 Pipera-H), 2.54 (t, $J = 4.6$ Hz, 4H, 4 Pipera-H), 2.49 (m, partially covered by DMSO 2H, $\text{NC}-\text{CH}_2$), 2.43 (t, $J = 6.9$ Hz, 2H, 1-Pipera- CH_2), 1.79 (m, 2H, $\text{CH}_2-\text{CH}_2-\text{CH}_2$). $\text{C}_{15}\text{H}_{21}\text{N}_3\text{O}$.

4-Chloro-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)-butyl)benzamide (8). Method C, method B. Yield for last reaction step: 39%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.60 (m, 1H*, NH), 7.87 (d, $J = 7.9$ Hz, 2H, Cl-Ph-2H, Cl-Ph-6H), 7.54 (d, 2H, Cl-Ph-3H, Cl-Ph-5H), 6.96 (m, 4H, 4 MeOPh-H), 3.79 (s, 3H, OCH_3), 3.29 (m, 2H, $\text{CONH}-\text{CH}_2$), 3.19–2.90 (m, 10H, 8 Pipera-H, 1-Pipera- CH_2), 1.69 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.57 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{22}\text{H}_{28}\text{ClN}_3\text{O}_2 \cdot \text{C}_2\text{H}_2\text{O}_4$) C, H, N.

4-Iodo-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-benzamide (9). Method C, method B. Yield for last reaction step: 39%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.49 (m, 1H*, NH), 7.82 (d, $J = 7.0$ Hz, 2H, I-Ph-2H, I-Ph-6H), 7.60 (d, $J = 7.0$ Hz, 2H, I-Ph-3H, I-Ph-5H), 6.90 (m, 2H, 2 MeOPh-H), 6.85 (m, 2H, 2 MeOPh-H), 3.74 (s, 3H, OCH_3), 3.29 (br m, 2H, $\text{CONH}-\text{CH}_2$), 2.92 (br m, 4H, $\text{PhN}(\text{CH}_2)_2$), 2.5 (hidden by DMSO 4H, $\text{PhN}(\text{CH}_2-\text{CH}_2)_2$), 2.32 (m, 2H, 1-Pipera- CH_2), 1.50 (m, 4H, $\text{CONH}-\text{CH}_2-\text{CH}_2-\text{CH}_2$). Anal. ($\text{C}_{22}\text{H}_{28}\text{IN}_3\text{O}_2$) C, H, N.

4-Acetyl-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)-butyl)benzamide (11). Method C, method B. Yield for last reaction step: 26%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.49 (t, $J = 5.4$ Hz, 1H*, NH), 8.03 (d, $J = 8.4$ Hz, 2H, Ph-2H, Ph-6H), 7.06 (d, $J = 8.4$ Hz, 2H, Ph-3H, Ph-5H), 6.87 (m, 4H, 4 MeOPh-H), 3.77 (s, 3H, OCH_3), 3.31 (m, 2H, $\text{CONH}-\text{CH}_2$), 2.94 (br m, 4H, $\text{PhN}(\text{CH}_2)_2$), 2.62 (s, 3H, COCH_3), 2.5 (hidden by DMSO 4H, $\text{PhN}(\text{CH}_2-\text{CH}_2)_2$), 2.35 (t, $J = 7.1$ Hz, 2H, 1-Pipera- CH_2), 1.57 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.53 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{24}\text{H}_{31}\text{N}_3\text{O}_3$) C, H, N.

N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)bi-phenyl-4-carboxamide (13). Method C, method B. Yield for last reaction step: 44%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.57 (m, 1H*, NH), 7.95 (d, $J = 8.2$ Hz, 2H, $\text{NCOPh}-2\text{H}$, $\text{NCOPh}-6\text{H}$), 7.77 (d, $J = 8.2$ Hz, 2H, $\text{NCOPh}-3\text{H}$, $\text{NCOPh}-5\text{H}$), 7.72 (m, 2H, $\text{OCPhPh}-2\text{H}$, $\text{OCPhPh}-6\text{H}$), 7.48 (m, 2H, $\text{OCPhPh}-3\text{H}$, $\text{OCPhPh}-5\text{H}$), 7.41 (m, 1H, $\text{OCPhPh}-4\text{H}$), 6.97 (m, 4H, 4 MeOPh-H), 3.79 (s, 3H, OCH_3), 3.35 (m, 12H, $\text{CONH}-\text{CH}_2$, 8 Pipera-H, 1-Pipera- CH_2), 1.72 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.60 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{28}\text{H}_{33}\text{N}_3\text{O}_2 \cdot \text{C}_2\text{H}_2\text{O}_4$) C, H, N.

N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)benzo-phenone-4-carboxamide (14). Method C, method B. Yield for last reaction step: 10%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.75 (t, $J = 5.3$ Hz, 1H*, NH), 8.01 (d, $J = 8.2$ Hz, 2H, $\text{NCOPh}-2\text{H}$, $\text{NCOPh}-6\text{H}$), 7.81 (d, $J = 8.2$ Hz, 2H, $\text{NCOPh}-3\text{H}$, $\text{NCOPh}-5\text{H}$), 7.76 (d, $J = 7.4$ Hz, 2H, $\text{PhCOPh}-2\text{H}$, $\text{PhCOPh}-6\text{H}$), 7.72 (m, 1H, $\text{PhCOPh}-4\text{H}$), 7.59 (m, 2H, $\text{PhCOPh}-3\text{H}$, $\text{PhCOPh}-5\text{H}$), 6.97 (m, 4H, 4 MeOPh-H), 3.79 (s, 3H, OCH_3), 3.35 (m, 2H, $\text{CONH}-\text{CH}_2$), 3.25–2.82 (10H, 8 Pipera-H,

1-Pipera- CH_2), 1.70 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.58 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{29}\text{H}_{33}\text{N}_3\text{O}_3 \cdot \text{C}_2\text{H}_2\text{O}_4 \cdot \text{H}_2\text{O}$) C, H, N.

N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)isoquinoline-3-carboxamide (20). Method C, method B. Yield for last reaction step: 25%. ^1H NMR ($\text{DMSO}-d_6$) δ 9.38 (s, 1H, Isoq-4H), 9.01 (t, $J = 6.0$ Hz, 1H*, NH), 8.50 (s, 1H, Isoq-1H), 8.24 (d, $J = 8.1$ Hz, 1H, Isoq-H), 8.18 (d, $J = 8.1$ Hz, 1H, Isoq-H), 7.87 (m, 1H, Isoq-H), 7.80 (m, 1H, Isoq-H), 6.96 (m, 4H, 4 MeOPh-H), 3.77 (s, 3H, OCH_3), 3.40 (m, 2H, $\text{CONH}-\text{CH}_2$), 3.38–3.02 (m, 10H, 8 Pipera-H, 1-Pipera- CH_2), 1.65 (m, 4H, Pipera- $\text{CH}_2-\text{CH}_2-\text{CH}_2$). Anal. ($\text{C}_{25}\text{H}_{30}\text{N}_4\text{O}_2 \cdot \text{C}_2\text{H}_2\text{O}_4 \cdot 0.3\text{H}_2\text{O}$) C, H, N.

N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-4-oxo-4H-chromene-3-carboxamide (22). Method C, method B. Yield for last reaction step: 29%. ^1H NMR ($\text{DMSO}-d_6$) δ 9.23 (t, $J = 5.7$ Hz, 1H*, NH), 8.09 (m, 1H, Chromene-H), 7.93 (m, 1H, Chromene-H), 7.77 (m, 1H, Chromene-H), 7.58 (m, 1H, Chromene-H), 6.97 (m, 4H, 4 MeOPh-H), 6.87 (s, 1H, Chromene-2H), 3.81 (s, 3H, OCH_3), 3.44 (m, 2H, $\text{CONH}-\text{CH}_2$), 3.30 (m, 10H, 8 Pipera-H, 1-Pipera- CH_2), 1.72 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.64 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_4 \cdot \text{C}_2\text{H}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

5-(4-Chlorophenyl)-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)furan-2-carboxamide (25). Method C, method B. Yield for last reaction step: 9%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.61 (t, $J = 5.4$ Hz, 1H*, NH), 7.96 (d, $J = 8.4$ Hz, 2H, ClPh-2H, ClPh-6H), 7.54 (d, $J = 8.4$ Hz, 2H, ClPh-3H, ClPh-5H), 7.16 (m, 2H, Furan-H), 6.97 (m, 4H, 4 MeOPh-H), 3.78 (s, 3H, OCH_3), 3.41 (m, 12H, $\text{CONH}-\text{CH}_2$, 8 Pipera-H, 1-Pipera- CH_2), 1.72 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.58 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{26}\text{H}_{30}\text{ClN}_3\text{O}_3 \cdot 2\text{C}_2\text{H}_2\text{O}_4$) C, H, N.

(E)-N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-cinnamoylamide (34). Method C, method B. Yield for last reaction step: 20%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.21 (t, $J = 5.5$ Hz, 1H*, NH), 7.56 (m, 2H, 2 Ph-H), 7.41 (m, 4H, 3 Ph-H, Ph-CH), 6.96 (m, 4H, 4 MeOPh-H), 6.64 (d, $J = 15.9$ Hz, 1H, CO-CH), 3.79 (s, 3H, OCH_3), 3.40 (m, 12H, $\text{CONH}-\text{CH}_2$, 8 Pipera-H, 1-Pipera- CH_2), 1.68 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.51 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{24}\text{H}_{31}\text{N}_3\text{O}_2 \cdot \text{C}_2\text{H}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

(E)-4-Bromo-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide (37). Method C, method B. Yield for last reaction step: 12%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.22 (t, $J = 5.4$ Hz, 1H*, NH), 7.61 (d, $J = 8.4$ Hz, 2H, Ph-2H, Ph-6H), 7.52 (d, $J = 8.4$ Hz, 2H, Ph-3H, Ph-5H), 7.39 (d, $J = 15.8$ Hz, 1H, Ph-CH), 6.98 (m, 2H, 2 MeOPh-H), 6.89 (m, 2H, 2 MeOPh-H), 6.65 (d, $J = 15.8$ Hz, 1H, CO-CH), 3.79 (s, 3H, OCH_3), 3.22 (br m, 10H, $\text{CONH}-\text{CH}_2$, 8 Pipera-H), 3.01 (br m, 2H, 1-Pipera- CH_2), 1.67 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.50 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{24}\text{H}_{30}\text{BrN}_3\text{O}_2 \cdot \text{C}_2\text{H}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

(E)-4-Iodo-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide (38). Method C, method B. Yield for last reaction step: 56%. ^1H NMR (CDCl_3) δ 7.67 (d, $J = 7.9$ Hz, 2H, I-Ph-2H, I-Ph-6H), 7.55 (d, $J = 15.6$ Hz, 1H, Ph-CH), 7.21 (d, $J = 7.9$ Hz, 2H, I-Ph-3H, I-Ph-5H), 7.02 (s, 1H, NH), 6.92 (m, 4H, 4 MeOPh-H), 6.48 (d, $J = 15.6$ Hz, 1H, CO-CH), 3.86 (s, 3H, OCH_3), 3.43 (s, 2H, $\text{CONH}-\text{CH}_2$), 3.20 (s, 4H, $\text{PhN}(\text{CH}_2)_2$), 2.80 (s, 4H, $\text{PhN}(\text{CH}_2-\text{CH}_2)_2$), 2.57 (s, 2H, 1-Pipera- CH_2), 1.69 (s, 4H, $\text{CONH}-\text{CH}_2-\text{CH}_2-\text{CH}_2$). Anal. ($\text{C}_{24}\text{H}_{30}\text{IN}_3\text{O}_2$) C, H, N.

(E)-4-Cyano-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide (40). Method C, method B. Yield for last reaction step: 3%. ^1H NMR ($\text{DMSO}-d_6$) δ 8.31 (t, $J = 5.4$ Hz, 1H*, NH), 7.87 (d, $J = 7.8$ Hz, 2H, Ph-2H, Ph-6H), 7.46 (d, $J = 7.8$ Hz, 2H, Ph-3H, Ph-5H), 7.48 (d, $J = 15.8$ Hz, 1H, Ph-CH), 6.98 (m, 2H, 2 MeOPh-H), 6.89 (m, 2H, 2 MeOPh-H), 6.77 (d, $J = 15.8$ Hz, 1H, CO-CH), 3.79 (s, 3H, OCH_3), 3.24 (br m, 10H, $\text{CONH}-\text{CH}_2$, 8 Pipera-H), 3.08 (br m, 2H, 1-Pipera- CH_2), 1.68 (m, 2H, $\text{CONH}-\text{CH}_2-\text{CH}_2$), 1.52 (m, 2H, 1-Pipera- CH_2-CH_2). Anal. ($\text{C}_{25}\text{H}_{30}\text{N}_4\text{O}_2 \cdot 2\text{C}_2\text{H}_2\text{O}_4$) C, H, N.

(E)-3-(3-Iodophenyl)-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)acrylamide (51). Method C, method B. Yield for last reaction step: 30%. ^1H NMR (CDCl_3) δ 7.85 (s, 1H,

I-Ph-2H), 7.66 (d, $J = 7.9$ Hz, 1H, I-Ph-H), 7.53 (d, $J = 15.6$ Hz, 1H, Ph-CH), 7.45 (d, $J = 7.8$ Hz, 1H, I-Ph-H), 7.09 (br m, 6H, I-Ph-H, NH, 4 MeOPh-H), 6.49 (d, $J = 15.6$ Hz, 1H, CO-CH), 3.86 (s, 3H, OCH₃), 3.46 (m, 2H, CONH-CH₂), 3.26 (s, 4H, PhN(CH₂)₂), 2.84 (s, 4H, PhN(CH₂-CH₂)₂), 2.67 (s, 2H, 1-Pipera-CH₂), 1.81 (m, 4H, CONH-CH₂-CH₂-CH₂). Anal. (C₂₄H₃₀IN₃O₂) C, H, N.

(E)-3-(2-Iodophenyl)-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)acrylamide (55). Method C, method B. Yield for last reaction step: 39%. ¹H NMR (CDCl₃) δ 7.88 (d, $J = 7.9$ Hz, 1H, I-Ph-H), 7.82 (d, $J = 15.4$ Hz, 1H, Ph-CH), 7.54 (d, $J = 7.8$ Hz, 1H, I-Ph-H), 7.32 (m, 2H, I-Ph-H), 7.05 (m, 2H, 2 MeOPh-H), 6.93 (m, 3H, 2 MeOPh-H, NH), 6.43 (d, $J = 15.4$ Hz, 1H, CO-CH), 3.86 (s, 3H, OCH₃), 3.48 (m, 2H, CONH-CH₂), 3.28 (s, 4H, PhN(CH₂)₂), 2.97 (s, 4H, PhN(CH₂-CH₂)₂), 2.73 (s, 2H, 1-Pipera-CH₂), 1.85 (m, 2H, CONH-CH₂-CH₂), 1.74 (m, 2H, Pipera-CH₂-CH₂). Anal. (C₂₄H₃₀IN₃O₂) C, H, N.

(E)-3,4-Dichloro-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide (57). Method C, method B. Yield for last reaction step: 5%. ¹H NMR (DMSO-*d*₆) δ 8.19 (m, 1H*, NH), 7.85 (s, 1H, Ph-2H), 7.67 (d, $J = 8.4$ Hz, 1H, Ph-6H), 7.57 (d, $J = 8.4$ Hz, 1H, Ph-5H), 7.40 (d, $J = 15.8$ Hz, 1H, Ph-CH), 6.99 (m, 2H, 2 MeOPh-H), 6.89 (m, 2H, 2 MeOPh-H), 6.70 (d, $J = 15.7$ Hz, 1H, CO-CH), 3.79 (s, 3H, OCH₃), 3.22 (m, 12H, CONH-CH₂, 8 Pipera-H, 1-Pipera-CH₂), 1.66 (m, 2H, CONH-CH₂-CH₂), 1.54 (m, 2H, 1-Pipera-CH₂-CH₂). Anal. (C₂₄H₂₉Cl₂N₃O₂·C₂H₄O₄) C, H, N.

(E)-2,4-Dichloro-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)cinnamoylamide (58). Method C, method B. Yield for last reaction step: 3%. ¹H NMR (DMSO-*d*₆) δ 8.32 (m, 1H*, NH), 7.70 (m, 3H, 2 Ph-2H, Ph-CH), 7.50 (d, $J = 7.9$ Hz, 1H, Ph-H), 6.99 (m, 2H, 2 MeOPh-H), 6.89 (m, 2H, 2 MeOPh-H), 6.70 (d, $J = 15.7$ Hz, 1H, CO-CH), 3.79 (s, 3H, OCH₃), 3.22 (m, 12H, CONH-CH₂, 8 Pipera-H, 1-Pipera-CH₂), 1.68 (m, 2H, CONH-CH₂-CH₂), 1.52 (m, 2H, 1-Pipera-CH₂-CH₂). Anal. (C₂₄H₂₉Cl₂N₃O₂·C₂H₄O₄·1.5H₂O) C, H, N.

(E)-3-(Benzo[1,3]dioxol-4-yl)-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)acrylamide (60). Method C, method B. Yield for last reaction step: 37%. ¹H NMR (CDCl₃) δ 7.55 (d, $J = 15.7$ Hz, 1H, Ph-CH), 7.05 (m, 2H, (CH₂O)₂Ph-H, NH), 6.94 (m, 4H, 2 (CH₂O)₂Ph-H, 2 MeOPh-H), 6.84 (m, 2H, 2 MeOPh-H), 6.73 (d, $J = 15.8$ Hz, 1H, CO-CH), 5.88 (s, 2H, (CH₂O)₂Ph), 3.87 (s, 3H, OCH₃), 3.46 (m, 2H, CONH-CH₂), 3.27 (s, 4H, PhN(CH₂)₂), 2.93 (s, 4H, PhN(CH₂-CH₂)₂), 2.71 (s, 2H, 1-Pipera-CH₂), 1.83 (m, 2H, CONH-CH₂-CH₂), 1.72 (m, 2H, Pipera-CH₂-CH₂). Anal. (C₂₅H₃₁N₃O₄) C, H, N.

N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-3-(2-naphthyl)acrylamide (66). Method C, method B. Yield for last reaction step: 10%. ¹H NMR (DMSO-*d*₆) δ 8.14 (t, $J = 5.4$ Hz, 1H*, NH), 8.06 (s, 1H, Naph-1H), 7.93 (m, 3H, 3 Naph-H), 7.72 (d, $J = 8.6$ Hz, 1H, Naph-H), 7.56 (m, 3H, 2 Naph-H, Naph-CH), 6.92 (m, 4H, 4 MeOPh-H), 6.76 (d, $J = 15.7$ Hz, 1H, CO-CH), 3.76 (s, 3H, OCH₃), 3.23 (m, 2H, CONH-CH₂), 2.95 (m, 4H, PhN(CH₂)₂), 2.5 (hidden by DMSO 4H, PhN(CH₂-CH₂)₂), 2.34 (m, 2H, 1-Pipera-CH₂), 1.51 (m, 4H, CONH-CH₂-CH₂-CH₂). Anal. (C₂₈H₃₃N₃O₂) C, H, N.

Preparation of Bicyclo Derivatives 4, 6, and 7. Reductive alkylation of 1-(2-methoxyphenyl)piperazine·HCl (5 mmol, 0.96 g) was performed with *cis*-bicyclo[3.3.0]octane-3,7-dione (5 mmol, 0.69 g), triacetoxymethylborohydride (5 mmol, 1.06 g), and 1 mL of glacial acetic acid in 80 mL of dichloroethane. The mixture was stirred at ambient temperature for 2 h. Then, a half-saturated solution of NaHCO₃ was added, followed by extraction with dichloromethane. The unified organic phases were washed, dried, and concentrated in vacuo. The oily product was dissolved in ethanol. Addition of oxalic acid led to fractionated crystallization. In minor yields the dimer 3,7-bis(4-(2-methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane (6) was obtained, but the major product was 7-(4-(2-methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane-3-one (f). This ketone (1.4 mmol) was dissolved in 50 mL of a methanolic solution of NH₃ with a catalytic amount of palladium on activated charcoal (200 mg). The mixture was hydrogenated

with 1 bar H₂ for 12 h under TLC control. The catalyst was removed by filtration and the filtrate evaporated in vacuo. The residue was suspended in water and extracted with dichloromethane. Again fractionated crystallization with oxalic acid delivered a bis-adduct (bis(7-(4-(2-methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]oct-3-yl)amine, 7) and a distinct major product (7-(4-(2-methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane-3-amine, g). The free base (ca. 0.5 mmol, 0.15 g) of this amine was treated according to method B with 2-naphthoyl chloride (0.53 mmol, 0.1 g) to provide the amide 4. For compound 4, absolute conformation has not been confirmed yet, and minor percentage of diastereomers cannot be excluded.

7-(4-(2-Methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane-3-one (f). Yield: 32%. ¹H NMR (DMSO-*d*₆) δ 6.98 (m, 2H, 2 MeOPh-H), 6.90 (m, 2H, 2 MeOPh-H), 3.79 (s, 3H, OCH₃), 3.51 (br m, 1H, NCH), 3.21 (br m, 8H, 8 Pipera-H), 2.68 (m, 2H), 2.46 (m, partially covered by DMSO 2H), 2.39 (m, 2H), 2.13 (m, 1H), 2.08 (m, 1H), 1.63 (m, 2H). Anal. (C₁₅H₂₆N₂O₂·C₂H₄O₄·H₂O) C, H, N.

7-(4-(2-Methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane-3-amine (g). Yield: 49%. ¹H NMR (DMSO-*d*₆) δ 6.97 (m, 2H, 2 MeOPh-H), 6.93 (m, 2H, 2 MeOPh-H), 3.79 (m, 4H, 3 OCH₃, NCH), 3.10 (m, 1H), 2.95 (m, 4H, 4 Pipera-H), 2.54 (m, partially covered by DMSO 4H, 4 Pipera-H), 2.29 (m, 2H), 2.10 (m, 2H), 2.03 (m, 2H), 1.57 (m, 1H), 1.38 (m, 1H), 1.21 (m, 2H). C₁₉H₂₉N₃O.

N-(7-(4-(2-Methoxyphenyl)piperazin-1-yl)bicyclo[3.3.0]octane-3-yl)-2-naphthalenecarboxamide (4). Yield for last reaction step: 26%. ¹H NMR (DMSO-*d*₆) δ 8.52+8.43 (d, $J = 7.3$ Hz, 1H, NH), 7.43 (s, 1H, Naph-1H), 7.96 (m, 4H, 4 Naph-H), 7.60 (m, 2H, 2 Naph-H), 6.93 (m, 4H, 4 MeOPh-H), 6.87 (m, 4H, 4 MeOPh-H), 4.47+4.31 (m, 1H, NH-CH) 3.77 (s, 6H, 2 OCH₃), 2.95 (br m, 4H, 4Pipera-H), 2.5 (hidden by DMSO 4H, 4 Pipera-H), 2.39 (m, 1H, 1-Pipera-CH), 2.19 (m, 4H, 4 CHH), 1.72 (m, 2H, HC-CH), 1.45–1.00 (4H, 4 CHH). Anal. (C₃₀H₃₅N₃O₂·0.75H₂O) C, H, N.

General Procedure for Preparation of N-Alkylated Imides 70, 71. A mixture of 1-(2-methoxyphenyl)piperazine·HCl (10 mmol, 2.29 g), K₂CO₃ (20 mmol, 2.76 g), and 1,4-dibromobutane (15 mmol, 1.79 mL) in 100 mL *n*-butanol was heated to reflux for 3 h. The hot suspension was filtrated and concentrated under reduced pressure to deliver 8-(2-methoxyphenyl)-8-aza-5-azoniaspiro[4,5]decane-bromide (B). This intermediate (4 mmol, 1.31 g), K₂CO₃ (5 mmol, 0.69 g), the dicarboximide (4 mmol), and a catalytic amount of 18-crown-6 were dissolved in 20 mL of xylene and heated to reflux under argon for 24 h. After filtration of the hot mixture, the filtrate was evaporated to dryness. The obtained colored oil was stirred in diethyl ether and crystallized.

8-(2-Methoxyphenyl)-8-aza-5-azoniaspiro[4,5]decane-bromide (B). Yield: 70%. ¹H NMR (DMSO-*d*₆) δ 6.99 (m, 4H, 4 MeOPh-H), 3.81 (s, 3H, OCH₃), 3.63 (m, 4H, N⁺(CH₂)₂), 3.59 (m, 4H, N⁺(CH₂)₂), 3.30 (br m, 4H, Ph-N(CH₂)₂), 2.10 (m, 4H, N⁺(CH₂-CH₂)₂). Anal. (C₁₅H₂₃BrN₂O·0.75H₂O) C, H, N.

N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)-2,3-naphthalenedicarboximide (70). Yield for last reaction step: 59%. ¹H NMR (DMSO-*d*₆) δ 8.52 (s, 2H, Naph-1H, Naph-4H), 8.27 (m, 2H, Naph-5H, Naph-8H), 7.79 (m, 2H, Naph-6H, Naph-7H), 6.87 (m, 4H, 4 MeOPh-H), 3.75 (s, 3H, OCH₃), 3.69 (t, $J = 6.7$ Hz, 2H, Phth-N-CH₂), 2.92 (m, 4H, Ph-N(CH₂)₂), 2.47 (partially hidden by DMSO 4H, Ph-N(CH₂-CH₂)₂), 2.34 (t, $J = 6.7$ Hz, 2H, 1-Pipera-CH₂), 1.68 (m, 2H, Phth-N-CH₂-CH₂), 1.49 (m, 2H, 1-Pipera-CH₂-CH₂). Anal. (C₂₇H₂₉N₃O₃) C, H, N.

Pharmacological Testing. Binding Studies.⁶² Human D_{2L} and D₃ receptors were expressed in stably transfected Chinese hamster ovary (CHO) cells.^{50,51} These cell lines were cultured in Dulbecco's Modified Eagle Medium supplemented in 10% fetal calf serum in an atmosphere of 5% CO₂. Cells were harvested from culture dishes in the presence of 0.2% trypsin, centrifuged at 2000g for 5 min and homogenized in 10 mM Tris-HCl, pH 7.4, containing 5 mM MgCl₂ using a Polytron. The homogenate was centrifuged at 20 000g for 15 min at 4°C, and the pellet was resuspended by sonication in

50 mM Tris-HCl, pH 7.4, containing (in millimolar): NaCl, 120; KCl, 5; CaCl₂, 2; and MgCl₂, 8 (incubation buffer). Membranes were used either immediately or after storage at -70 °C. A membrane volume of 200 μ L, diluted in incubation buffer supplemented with 0.2% bovine serum albumin, was added to polystyrene tubes containing (in 100 μ L) 0.1 nM [¹²⁵I]iodosulpiride and drug diluted in 100 μ L of incubation buffer. Nonspecific binding was determined in the presence of 1 μ M enomapride. Incubations were run at 30 °C for 30 min. Reactions were stopped by vacuum filtration through Whatman GF/B glass-fiber filters coated in 0.3% polyethylenimine with automated cell harvester (Brandel-Beckman, Gaithersburg, MD). Filters were rinsed three times with 5 mL of ice-cold incubation buffer and counted by liquid scintillation in 5 mL of ACS II (Amersham).

Functional Receptor Tests.⁶² NG 108-15 cells expressing the human D₃ receptor were cultured in Dulbecco's Modified Eagle Medium supplemented in 10% fetal calf serum in an atmosphere of 5% CO₂ and plated in collagen-coated 96-well plates. After a 24-hour culture, cells were washed twice with culture medium without fetal calf serum and incubated for 16 h with 1 μ M forskoline and quinpirole in increasing concentrations, in the absence or presence of compounds at 1.5, 3, 30, or 300 nM. Then, [³H]thymidine (1 μ Ci/well) was added for 2 h and cells were harvested by vacuum filtration through Whatman GF/C glass-fiber filters using an automated cell harvester. The filters were rinsed 15 times with 200 μ L of phosphate buffered saline. Radioactivity was counted by liquid scintigraphy in 5 mL of ACS (Amersham).

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References

- Jackson, D. M.; Westlind-Danielson, A. Dopamine Receptors: Molecular Biology, Biochemistry and Behavioural Aspects. *Pharmacol. Ther.* **1994**, *64*, 291-370.
- Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. Dopamine Receptors: From Structure to Function. *Physiol. Rev.* **1998**, *78*, 189-225.
- Strange, P. G. Antipsychotic Drugs: Importance of Dopamine Receptors for Mechanisms of Therapeutic Actions and Side Effects. *Pharmacol. Rev.* **2001**, *53*, 119-133.
- Sokoloff, P.; Giros, B.; Martes, M. P.; Bouthenet, M. L.; Schwartz, J.-C. Molecular Cloning and Characterization of a Novel Dopamine Receptor (D₃) as a Target for Neuroleptics. *Nature* **1990**, *347*, 146-151.
- Levant, B. The D₃ Dopamine Receptor: Neurology and Potential Clinical Relevance. *Pharmacol. Rev.* **1997**, *49*, 231-252.
- Luedtke, R. R.; Mach, R. H. Progress in Developing D₃ Dopamine Receptor Ligands as Potential Therapeutic Agents for Neurological and Neuropsychiatric Disorders. *Curr. Pharm. Des.* **2003**, *9*, 643-671.
- Schwartz, J.-C.; Diaz, J.; Pilon, C.; Sokoloff, P. Possible Implications of the Dopamine D₃ Receptor in Schizophrenia and in Antipsychotic Drug Actions. *Brain Res. Brain Res. Rev.* **2000**, *31*, 277-287.
- Pinter, M. M.; Rutgers, A. W.; Hebenstreit, E. An Open-Label, Multicentre Clinical Trial to Determine the Levodopa Dose-Sparing Capacity of Pramipexole in Patients with Idiopathic Parkinson's Disease. *J. Neural. Transm.* **2000**, *107*, 1307-1323.
- Möller, J. C.; Oertel, W. H. Pramipexole in the Treatment of Advanced Parkinson's Disease. *Eur. J. Neurol.* **2000**, *7 Suppl 1*, 21-25.
- Bézar, E.; Ferry, S.; Mach, U.; Stark, H.; Leriche, L.; Boraud, T.; Groß, C.; Sokoloff, P. Attenuation of Levodopa-Induced Dyskinesia by Normalizing Dopamine D₃ Receptor Function. *Nat. Med.* **2003**, *9*, 762-767.
- Sokoloff, P.; Bézar, E.; Ferry, S.; Stark, H.; Groß, C. DOPAMINE 2002 Meeting, Portland, OR, July 10-14, 2002; Abstract book, S10.4.
- Le Foll, B.; Schwartz, J.-C.; Sokoloff, P. Dopamine D₃ Receptor Agents as Potential New Medications for Drug Addiction. *Eur. Psychiatry* **2000**, *15*, 140-146.
- Everitt, B. J.; Robbins, T. W. Second-order Schedules of Drug Reinforcement in Rats and Monkeys: Measurement of Reinforcing Efficacy and Drug-seeking Behaviour. *Psychopharmacology (Berlin)* **2000**, *153*, 17-30.
- Hackling, A. E.; Stark, H. Dopamine D₃ Receptor Ligands with Antagonist Properties. *ChemBioChem* **2002**, *3*, 946-961.
- Yuan, J.; Chen, X.; Brodbeck, R.; Primus, R.; Braun, J.; Wasley, J. W. F.; Thurkauf, A. NGB 2904 and NGB 2849: Two Highly Selective Dopamine D₃ Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2715-2718.
- Bettinetti, L.; Schlotter, K.; Hübner, H.; Gmeiner, P. Interactive SAR Studies: Rational Discovery of Super-Potent and Highly Selective Dopamine D₃ Receptor Antagonists and Partial Agonists. *J. Med. Chem.* **2002**, *45*, 4594-4597.
- Murray, P. J.; Harrison, L. A.; Johnson, M. R.; Robertson, G. M.; Scopes, D. I. C.; Bull, D. R.; Graham, E. A.; Hayes, A. G.; Kilpatrick, G. J.; Daas, I. D.; Large, C.; Sheehan, M. J.; Stubbs, C. M.; Turpin, M. P. A Novel Series of Arylpiperazines with High Affinity and Selectivity for the Dopamine D₃ Receptor. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 219-222.
- Pilla, M.; Perachon, S.; Sautel, F.; Garridol, F.; Mann, A.; Wermuth, C. G.; Schwartz, J.-C.; Everitt, B. J.; Sokoloff, P. Selective Inhibition of Cocaine-Seeking Behaviour by a Partial Dopamine D₃ Receptor Agonist. *Nature* **1999**, *400*, 371-375.
- Belliotti, T. R.; Kesten, S. R.; Rubin, J. R.; Wustrow, D. J.; Georgic, L. M.; Zoski, K. T.; Akunne, H. C.; Wise, L. D. Novel Cyclohexyl Amides as Potent and Selective D₃ Dopamine Receptor Ligands. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2403-2408.
- Wustrow, D.; Belliotti, T.; Glase, S.; Kesten, S. R.; Johnson, D.; Colby, N.; Rubin, R.; Blackburn, A.; Akunne, H.; Corbin, A.; Davis, M. D.; Georgic, L.; Whetzel, S.; Zoski, K.; Heffner, T.; Pugsley, T.; Wise, L. Aminopyrimidines with High Affinity for Both Serotonin and Dopamine Receptors. *J. Med. Chem.* **1998**, *41*, 760-771.
- Cha, M. Y.; Choi, B. C.; Kang, K. H.; Pae, A. N.; Choi, K. I.; Cho, Y. S.; Koh, H. Y.; Lee, H. Y.; Jung, D.; Kong, J. Y. Design and Synthesis of a Piperazinylalkylisoxazole Library for Subtype Selective Dopamine Receptor Ligands. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1327-1330.
- Wright, J.; Heffner, T.; Pugsley, T.; MacKenzie, R.; Wise, L. Discovery of Selective Dopamine D₃ Ligands: II. 2-[4-[3-(4-Aryl-1-piperazinyl)propoxy]phenyl]benzimidazole Partial Agonists. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2547-2550.
- Laszlovsky, I.; Ács, T.; Kiss, B.; Domány, G. Substituted Phenoxyalkylpiperazines as Dopamine D₃ Receptor Ligands. *Pharmazie* **2001**, *56*, 287-289.
- Leopoldo, M.; Berardi, F.; Colabufio, N. A.; De Giorgio, P.; Lacivita, E.; Perrone, R.; Rubin, R.; Tortorella, V. Structure-Affinity Relationship Study on N-[4-(4-Arylpiperazin-1-yl)butyl]-arylcarboxamides as Potent and Selective Dopamine D₃ Receptor Ligands. *J. Med. Chem.* **2002**, *45*, 5727-5735.
- Preti, A. BP-897 Bioprojet. *Curr. Opin. Invest. Drugs* **2000**, *1*, 110-115.
- Spielberger, G. In *Houben-Weyl*, 11/1 ed.; Büchel, K. H., Falbe, J., Hagemann, H., Hanack, M., Klamann, D., Kreher, R., Kropf, H., Reglitz, M., Schaumann, E., Eds.; Thieme: Stuttgart, 1957; pp 24-108.
- Glennon, R. A.; Naimann, N. A.; Pierson, M. E.; Titeler, M.; Lyon, R. A.; Weisberg, E. NAN-190: An Arylpiperazine Analog that Antagonizes the Stimulus Effects of the 5-HT_{1A} Agonist 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). *Eur. J. Pharmacol.* **1988**, *154*, 339-341.
- Lavrinovics, E.; Grinsteins, V. Preparation of Phthalimidoalkyl Piperazines. Patent SU 193524 March 13, 1967.
- Wu, Y.-H.; Smith, K. R.; Rayburn, J. W.; Kissel, J. W. Psycho-sedative Agents. N-(4-Phenyl-1-piperazinylalkyl)-Substituted Cyclic Imides. *J. Med. Chem.* **1969**, *12*, 876-881.
- Gribble, G. W.; Lord, P. D.; Skotnicki, J.; Dietz, S. E.; Eaton, J. T.; Johnson, J. L. Reactions of Sodium Borohydride in Acidic Media. I. Reduction of Indoles and Alkylation of Aromatic Amines with Carboxylic Acids. *J. Am. Chem. Soc.* **1974**, *96*, 7812-7814.
- Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures. *J. Org. Chem.* **1996**, *61*, 3849-3862.
- Ramanjulu, J. M.; Joullie, M. M. N-Alkylation of Amino Acid Esters Using Sodium Triacetoxyborohydride. *Synth. Commun.* **1996**, *26*, 1379-1384.
- Clarke, D. E.; Elworthy, T. R.; Morgans, D. J. Jr.; Pfister, J. R.; Repke, D. B.; Sjorgren, E. B.; Wu, H. Y. H. Preparation of 3-(4-Phenylpiperazin-1-yl)propyl-Amino, Thio- and Oxy-Pyridine, Pyrimidine and Benzene Derivatives as α_1 -Adrenoreceptor Antagonists. *Eur. Pat. Appl. EP* **1996**, *711*, 757; *Chem. Abstr.* **1996**, *125*, 86679n.
- Depreux, P.; Aichaoui, H.; Lesieur, I. Synthesis of Indanyl Analogs of 3-[3-(4-Arylpiperazin-1-yl)propyl]-2-oxo-2,3-dihydro-[1,3]benzoxazoles. *Heterocycles* **1993**, *36*, 1051-1058.
- Saji, I.; Muto, M.; Tanno, N.; Yoshigai, M. Preparation of [[[Carboximidomethyl]cycloalkyl]methyl]aziny]arenes as Antipsychotics. *Eur. Pat. Appl.* **1992**, *464*, 846; *Chem. Abstr.* **1992**, *116*, 151794b.

- (36) Yevich, J. P.; New, J. S.; Smith, D. W.; Lobeck, W. G.; Catt, J. D.; Minelli, J. L.; Eison, M. S.; Taylor, D. P.; Riblet, L. A.; Temple, D. L. Jr. Synthesis and Biological Evaluation of 1-(1,2-Benzisothiazol-3-yl)- and (1,2-Benzisoxazol-3-yl)piperazine Derivatives as Potential Antipsychotic Agents. *J. Med. Chem.* **1986**, 29, 359–369.
- (37) Azizian, H.; Eaborn, C.; Pidcock, A. Synthesis of Organotrialkylstannanes. The Reaction between Organic Halides and Hexaalkyldistannanes in the Presence of Palladium Complexes. *J. Organomet. Chem.* **1981**, 215, 49–58.
- (38) Krause, M.; Stark, H.; Schunack, W. Iododestannylation: An Improved Synthesis of [¹²⁵I]Iodoproxyfan, a Specific Radioligand of the Histamine H₃-Receptor. *J. Labelled. Compd. Radiopharm.* **1997**, 39, 601–606.
- (39) SYBYL, version 6.5; Tripos Associates, Inc.: St. Louis, MO, 2001.
- (40) Gasteiger, J.; Marsili, M. Iterative Partial Equalization of Orbital Electronegativity – A Rapid Access to Atomic Charges. *Tetrahedron* **1980**, 36, 3219–3288.
- (41) Lemmen, C.; Lengauer, T.; Klebe, G. FLEXS: A Method for Fast Flexible Ligand Superposition. *J. Med. Chem.* **1998**, 41, 4502–4520.
- (42) Moore, K. W.; Bonner, K.; Jones, E. A.; Emms, F.; Leeson, P. D.; Marywood, R.; Patel, S.; Patel, S.; Rowley, M.; Thomas, S.; Carling, R. W. 4-*N*-Linked-Heterocyclic Piperidine Derivatives with High Affinity and Selectivity for Human Dopamine D₄ Receptors. *Bioorg. Med. Chem. Lett.* **1999**, 9, 1285–1290.
- (43) Avenell, K. Y.; Boyfield, I.; Coldwell, M. C.; Hadley, M. S.; Healy, M. A. M.; Jeffrey, P. M.; Johnson, C. N.; Nash, D. J.; Riley, G. J.; Scott, E. E.; Smith, S. A.; Stacey, R.; Stemp, G.; Thewlis, K. M. Fused Aminotetralins: Novel Antagonists with High Selectivity for the Dopamine D₃ Receptor. *Bioorg. Med. Chem. Lett.* **1998**, 8, 2859–2864.
- (44) Cambridge Structural Database, Cambridge Crystallographic Data Center, Cambridge, U.K.
- (45) GRID, version 16; Molecular Discovery Ltd.: Oxford, 2000.
- (46) Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor. *Science* **2000**, 289, 739–745.
- (47) Ballesteros, J. A.; Shi, L.; Javitch, J. A. Structural Mimicry in G Protein-Coupled Receptors: Implications of the High-Resolution Structure of Rhodopsin for Structure–Function Analysis of Rhodopsin-Like Receptors. *Mol. Pharmacol.* **2001**, 60, 1–19.
- (48) *Insight II/Discover*, Version 2000; Accelrys Inc.: San Diego, 2000.
- (49) Rarey, M.; Kramer, B.; Lengauer, T. Time-Efficient Docking of Flexible Ligands into Active Sites of Proteins. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **1995**, 3, 300–308.
- (50) Sokoloff, P.; Andrieux, M.; Besancon, R.; Pilon, C.; Martres, M. P.; Giros, B.; Schwartz, J.-C. Pharmacology of Human Dopamine D₃ Receptor Expressed in a Mammalian Cell Line: Comparison with D₂ Receptor. *Eur. J. Pharmacol. [Mol. Pharmacol. Sec.]* **1992**, 225, 331–337.
- (51) Lévesque, D.; Diaz, J.; Pilon, C.; Martres, J. M.; Giros, B.; Souil, E.; Schott, D.; Morgat, J. L.; Schwartz, J.-C.; Sokoloff, P. Identification, Characterization, and Localization of the Dopamine D₃ Receptor in Rat Brain Using 7-[³H]hydroxy-*NN*-di-n-propyl-2-aminotetralin. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, 89, 8155–8159.
- (52) Cheng, Y. C.; Prusoff, W. H. Relationship between the Inhibition Constant (K_i) and the Concentration of Inhibitor which Causes 50 per Cent Inhibition (I₅₀) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, 22, 3099–3108.
- (53) Wright, J.; Downing, D.; Heffner, T.; Pugsley, T.; MacKenzie, R.; Wise, L. Discovery of Selective Dopamine D₃ Ligands: I. Dimeric 2-[4-[3-Aminopropoxy]phenyl]benzimidazole Antagonists. *Bioorg. Med. Chem. Lett.* **1995**, 5, 2541–2546.
- (54) Raghupathi, R. K.; Rydelek-Fitzgerald, L.; Teitler, M.; Glennon, R. A. Analogues of the 5-HT_{1A} Serotonin Antagonist 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl]-piperazine with Reduced α1-Adrenergic Affinity. *J. Med. Chem.* **1991**, 34, 2633–2638.
- (55) Robarge, M. J.; Husbands, S. M.; Kielytyka, A.; Brodbeck, R.; Thurkauf, A.; Newman, A. H. Design and Synthesis of [(2,3-Dichlorophenyl)piperazin-1-yl]alkylfluorenylcarboxamides as Novel Ligands Selective for the Dopamine D₃ Receptor Subtype. *J. Med. Chem.* **2001**, 44, 3175–3186.
- (56) Austin, N. E.; Avenell, K. Y.; Boyfield, I.; Branch, C. L.; Coldwell, M. C.; Hadley, M. S.; Jeffrey, P.; Johns, A.; Johnson, C. N.; Nash, D. J.; Riley, G. J.; Smith, S. A.; Stacey, R. C.; Stemp, G.; Thewlis, K. M.; Vong, A. K. K. Novel 1,2,3,4-Tetrahydroisoquinolines with High Affinity and Selectivity for the Dopamine D₃ Receptor. *Bioorg. Med. Chem. Lett.* **1999**, 9, 179–184.
- (57) Gowing, L. R.; Henry-Edwards, S. M.; Irvine, R. J.; Ali, R. L. The Health Effects of Ecstasy: A Literature Review. *Drug Alcohol Rev.* **2002**, 21, 53–63.
- (58) López-Rodríguez, M. L.; Morcillo, M. J.; Rovat, T. K.; Fernández, E.; Vicente, B.; Sanz, A. M.; Hernández, M.; Orensanz, L. Synthesis and Structure–Activity Relationships of a New Model of Arylpiperazines. 4. 1-[ω-(4-Arylpiperazin-1-yl)alkyl]-3-(diphenylmethylene)-2,5-pyrrolidinediones and -3-(9H-fluoren-9-ylidene)-2,5-pyrrolidinediones: Study of the Steric Requirements of the Terminal Amide Fragment on 5-HT_{1A} Affinity/Selectivity. *J. Med. Chem.* **1999**, 42, 36–49.
- (59) Wicke, K.; Garcia-Ladona, J. The dopamine D₃ receptor partial agonist, BP 897, is an antagonist at human dopamine D₃ receptors and at rat somatodendritic dopamine D₃ receptors. *Eur. J. Pharmacol.* **2001**, 424, 85–90.
- (60) Vorel, S. R.; Ashby, C. R. Jr.; Paul, M.; Liu, X.; Hayes, R.; Hagan, J. J.; Middlemiss, D. N.; Stemp, G.; Gardner, E. L. Dopamine D₃ receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J. Neurosci.* **2002**, 22, 9595–9603.
- (61) Pilon, C.; Lévesque, D.; Dimitriadou, V.; Griffon, N.; Mertres, M. P.; Schwartz, J.-C.; Sokoloff, P. Functional Coupling of the Human Dopamine D₃ Receptor in a Transfected NG 108–15 Neuroblastoma-Glioma Hybrid Cell Line. *Eur. J. Pharmacol.* **1994**, 268, 129–139.
- (62) Sautel, F.; Griffon, N.; Sokoloff, P.; Schwartz, J.-C.; Launay, C.; Simon, P.; Costentin, J.; Schoenfelder, A.; Garrido, F.; Mann, A.; Wermuth, C. G. Nafadotride, a Potent Preferential Dopamine D₃ Receptor Antagonist, Activates Locomotion in Rodents. *J. Pharmacol. Exp. Ther.* **1995**, 275, 1239–1246.

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Discovery of a New Class of Potential Multifunctional Atypical Antipsychotic Agents Targeting Dopamine D₃ and Serotonin 5-HT_{1A} and 5-HT_{2A} Receptors: Design, Synthesis, and Effects on Behavior

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Dopamine D₃ antagonism combined with serotonin 5-HT_{1A} and 5-HT_{2A} receptor occupancy may represent a novel paradigm for developing innovative antipsychotics. The unique pharmacological features of **5i** are a high affinity for dopamine D₃, serotonin 5-HT_{1A} and 5-HT_{2A} receptors, together with a low affinity for dopamine D₂ receptors (to minimize extrapyramidal side effects), serotonin 5-HT_{2C} receptors (to reduce the risk of obesity under chronic treatment), and for hERG channels (to reduce incidence of torsade des pointes). Pharmacological and biochemical data, including specific *c-fos* expression in mesocorticolimbic areas, confirmed an atypical antipsychotic profile of **5i** in vivo, characterized by the absence of catalepsy at antipsychotic dose.

Introduction

Schizophrenia is among the most serious mental illnesses; it has a considerable social and economic impact and globally it affects approximately 1% of the world population.¹

Antipsychotic medication is the main therapeutic intervention for schizophrenia. Although the pathophysiology of the disease has yet to be clearly defined, the development of antipsychotic drugs in recent decades has been heavily influenced by the dopamine hypothesis, mainly supported by the capability of antipsychotic drugs of interfering with dopamine receptors in vivo and in vitro and by evidence that the clinical efficacy of typical antipsychotic drugs is correlated with their occupancy at dopamine D₂ receptors (D₂R).²

The early agents for the treatment of psychosis, the “typical” antipsychotics (haloperidol, **1**, Chart 1), were breakthrough therapies for the positive symptoms of schizophrenia, but they failed to manage its negative symptoms and cognitive impairment. Nevertheless, typical antipsychotics carry a heavy side-effect burden (i.e., extrapyramidal symptoms (EPS) and hyperprolactinemia) and are ineffective in one-third of schizophrenic patients.³

The “atypical” antipsychotics (e.g., clozapine (**2**) and olanzapine (**3**)), are characterized by a multireceptor affinity profile, which combines a potent antagonism for serotonin 5-HT_{2A} with a dopamine D₂ and D₃ receptors blockade.⁴ Among the atypical antipsychotic agents approved by regulatory authorities, cloza-

pine still remains invaluable for psychosis, presenting high clinical efficacy and a reduced incidence of EPS and hyperprolactinemia. Olanzapine may precipitate or unmask diabetes in susceptible patients,⁵ and its use was associated with a 12% increase in excessive appetite compared to haloperidol.⁶ Meanwhile, ziprasidone, risperidone, and quetiapine may be responsible for drug-induced long QT syndrome (risk of malignant ventricular arrhythmia).

A new antipsychotic agent (aripiprazole **4**, Chart 1) has recently been launched. It acts as a partial agonist at D₂R.^{7,8} Its unique mechanism of action might underlie its efficacy and low risk of side effects seen with other antipsychotics. However, unmet clinical needs still remain and include (i) more effective antipsychotic therapy for treatment of refractory patients, (ii) improved treatment of negative symptoms, and (iii) improved treatment of cognitive dysfunction.

Several strategies for the development of novel antipsychotics have started to provide additional tools for relieving the symptoms of schizophrenia. In recent years, the pharmaceutical industry and academia have shown significant interest in the development of novel antipsychotics characterized by interaction with less obvious receptors such as metabotropic glutamate receptors and tachykinin receptors.⁹

Second-generation antipsychotics combine D₂R occupancy with activity at serotonergic receptors (such as 5-HT_{1A} receptors (5-HT_{1A}R) and 5-HT_{2A} receptors (5-HT_{2A}R), i.e., clozapine) to provide drug therapies for resistant schizophrenic patients, with prompter therapeutic benefits and the improvement of cognitive symptoms.¹⁰ In contrast, we decided to exploit a novel multireceptor affinity profile approach, which combines antagonism at dopamine D₃ receptors (D₃R) and at 5-HT_{2A}R and partial agonism at serotonin 5-HT_{1A}R, with a low affinity for D₂R (no liability of EPS at antipsychotic doses) and 5-HT_{2C} receptors (5-HT_{2C}R) (reducing the risk of obesity under chronic treatment).

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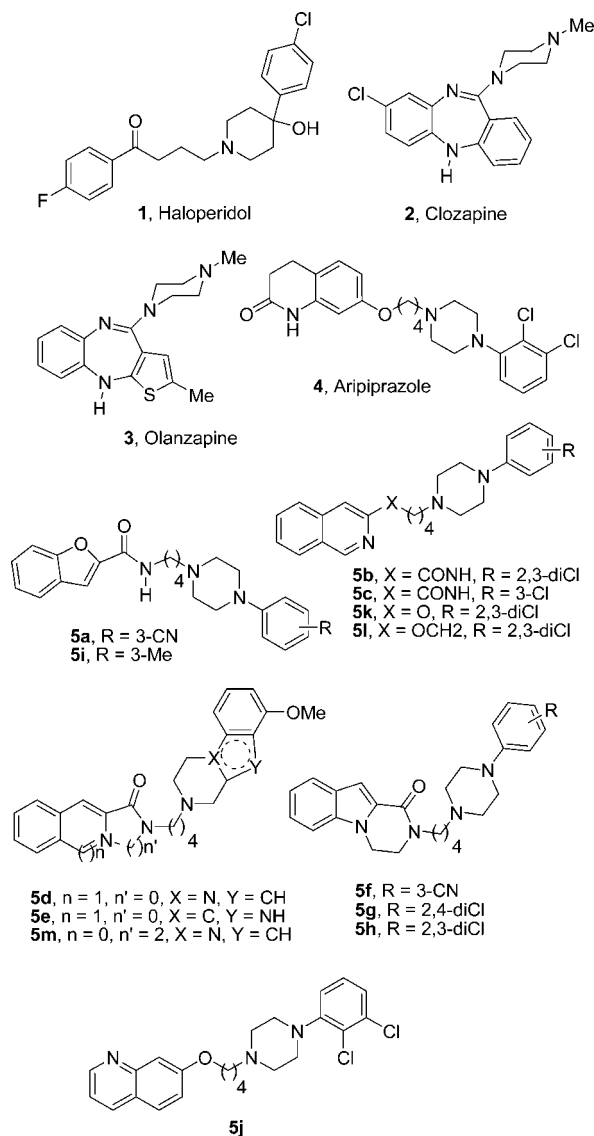
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Chart 1. Title and Reference Compounds

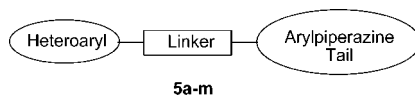


The serotonergic system plays an important role in the regulation of prefrontal cortex (PFC) functions, including emotional control, cognitive behavior, and working memory. PFC pyramidal neurons and GABA interneurons contain several serotonin receptor subtypes with a particularly high density of 5-HT_{1A}R and 5-HT_{2A}R. It has recently been demonstrated in PFC that NMDA receptor channels are the target of 5-HT_{1A}R and that both receptors modulate the excitability of cortical neurons, thus affecting cognitive functions.¹¹ Indeed, a variety of preclinical data has suggested that the 5-HT_{1A}R may be a therapeutic target for the development of improved antipsychotic drugs. Although the role of 5-HT_{1A}R in antipsychotic drug efficacy profile remains speculative, 5-HT_{1A}R affinity contributes to the clinical efficacy of most of the atypical antipsychotic drugs (clozapine, olanzapine, aripiprazole) and their low liability for EPS.¹² A growing number of studies show that 5-HT_{1A}R activation attenuates antipsychotic-induced side effects in humans,¹³ nonhuman primates,¹⁴ and antipsychotic-induced catalepsy in rats. An association has indeed been postulated between agonist activity at 5-HT_{1A}R and anxiolytic or antidepressant effects, improvements in cognitive and negative symptoms, and decreased development of EPS in schizophrenia.¹⁵ Recent studies have provided the first in vivo

evidence that activation of 5-HT_{1A}R can play a role in aripiprazole-mediated behavior in rats.^{16,17} Furthermore, because glutamatergic transmission is dysfunctional in schizophrenia and because glutamate release is decreased by 5-HT_{1A}R activation,¹⁸ agonist properties at postsynaptic 5-HT_{1A}R may be relevant to the therapeutic profile of atypical antipsychotic agents, improving negative symptoms and cognitive deficits. This topic has recently been reviewed by Meltzer and Sumiyoshi,¹⁹ who underline the additional beneficial effects found in many preclinical models based on the reciprocal interaction with the 5-HT_{1A}R (agonists) and 5-HT_{2A}R (antagonists). Serotonin, through its interaction with 5-HT_{2A}R, inhibits neuronal activity in the substantia nigra and ventral tegmental area. 5-HT_{2A}R antagonists may increase the firing rate of midbrain dopaminergic neurons in a state-dependent manner and potentiate the increase in the activity of nigrostriatal dopamine-containing neurons in response to moderate D₂R antagonism by antipsychotics. Therefore, 5-HT_{2A}R antagonism may prevent or alleviate EPS induced by acute or long-term treatment with typical drugs such as 1.

Although the role of dopamine D₂R and serotonin 5-HT_{2A}R has been defined, the role of D₃R is still controversial. Recently, D₃R has been claimed as a potential target for antischizophrenic drug development.²⁰ This is mainly based on the specific distribution of D₃R in the mesolimbic dopaminergic system, along with the observation of elevated levels of these receptors in patients that are off antipsychotics. D₃R functions are primarily related to the mesolimbic, rather than the nigrostriatal, dopaminergic system, thus the blockade of D₃R does not elicit EPS. Furthermore, because dopamine through D₃R modulates the cholinergic system at the prefrontal cortex level, D₃R antagonists (devoid of muscarinic effects) may robustly enhance acetylcholine release in frontal cortex.²¹ Consequently, D₃R antagonists might improve cognitive deficits poorly treated by currently available agents, including clozapine. The persistent concern is whether selective D₃R blockade can relieve positive symptoms of schizophrenia. Investigation of dopamine D₃R function was limited because of the lack of highly selective ligands.

To validate the novel approach to antipsychotics and to achieve an optimum interaction with dopamine and serotonin receptors, we exploited our knowledge acquired in the development of tricyclic atypical antipsychotics.²² Thus, we selected the arylalkylpiperazine system as a flexible scaffold for achieving a fine balancing of dopamine D₃R and serotonin 5-HT_{1A}R and 5-HT_{2A}R affinity, reducing D₁R, D₂R, and 5-HT_{2C}R occupancy. Starting from highly selective D₃R hits²³ that lack significant behavioral effects, the present article deals with the design, synthesis, and behavioral investigation of a rationally designed set of arylpiperazines as novel and potent antipsychotics, characterized by high affinity for D₃R, 5-HT_{1A}R, and 5-HT_{2A}R. Their structure–activity relationships (SARs) for dopamine and serotonin receptors were associated with variation of the aromatic system and the substituents on the arylpiperazine moiety. Among the analogues synthesized and tested (5a–m), compound 5i, a selective antagonist at D₃R and 5-HT_{2A}R, and partial agonist at 5-HT_{1A}R, was selected for further biological investigation. Its atypical antipsychotic profile is herein discussed, together with the molecular modeling study. Despite its structural similarity to aripiprazole (nanomolar affinity for D₂R, D₃R, 5-HT_{1A}R, and 5-HT_{2A}R, Table 2), 5i shows a unique pharmacological profile, being designed to validate the novel approach to atypical antipsychotics based on a D₃, 5-HT_{1A}, and 5-HT_{2A} multireceptor affinity profile.

Table 1. Physical and Chemical Data for Compounds **5a–m**

Cpd	Heteroaryl	Linker	Arylpiperazine Tail	Yield% ^a	mp (°C)	Formula	Anal ^c
5a		CONH(CH ₂) ₄		90.0	oil	C ₂₄ H ₂₆ N ₄ O ₂	C, H, N
5b		CONH(CH ₂) ₄		60.2	oil	C ₂₄ H ₂₆ Cl ₂ N ₄ O	C, H, N
5c		CONH(CH ₂) ₄		50.4	156–157 ^b	C ₂₄ H ₂₇ ClN ₄ O	C, H, N
5d		CONH(CH ₂) ₄		55.0	oil	C ₂₆ H ₂₈ N ₄ O ₂	C, H, N
5e		CONH(CH ₂) ₄		30.0	oil	C ₂₆ H ₂₈ N ₄ O ₂	C, H, N
5f		(CH ₂) ₄		60.0	oil	C ₂₆ H ₂₉ N ₅ O	C, H, N
5g		(CH ₂) ₄		87.4	oil	C ₂₅ H ₂₈ Cl ₂ N ₄ O	C, H, N
5h		(CH ₂) ₄		78.2	160–161 ^b	C ₂₅ H ₂₈ Cl ₂ N ₄ O	C, H, N
5i		CONH(CH ₂) ₄		52.0	119–120 ^b	C ₂₄ H ₂₉ N ₃ O ₂	C, H, N
5j		O(CH ₂) ₄		62.4	oil	C ₂₃ H ₂₅ Cl ₂ N ₃ O	C, H, N
5k		O(CH ₂) ₄		61.0	Amorphous solid	C ₂₃ H ₂₅ Cl ₂ N ₃ O	C, H, N
5l		O(CH ₂) ₅		54.3	133–134 ^b	C ₂₄ H ₂₇ Cl ₂ N ₃ O	C, H, N
5m		(CH ₂) ₄		62.0	oil	C ₂₇ H ₃₀ N ₄ O ₂	C, H, N

^a Yields refer to isolated and purified materials. ^b Recrystallization solvent: methanol. ^c All the compounds were analyzed within $\pm 0.4\%$ of the theoretical values.

Furthermore, long QT syndrome, experienced with several antipsychotics, has prompted considerable efforts in trying to define the molecular basis of this phenomenon. Consequently, efforts to predict long QT syndrome risk have been focused on assays testing hERG channel activities. Investiga-

tion of hERG channel blockade is now a key step along the drug discovery trajectory of the pharmaceutical industry. Therefore, hERG channel interaction was introduced as a further parameter in our design strategy of the novel antipsychotics.

Table 2. Binding Affinities for D₁, D₂, D₃, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} Receptors (K_i nM $\pm$ SD)^a and hERG Channels (K_i μ M) of Compounds **5a–m** and Reference Antipsychotics

compd	D ₁ ^b	D ₂ ^c	D ₃ ^d	5-HT _{1A} ^e	5-HT _{2A} ^f	5-HT _{2C} ^g	hERG ^h
5a	>10000	859 $\pm$ 121	0.54 $\pm$ 0.07	876 $\pm$ 22	NT ⁱ	NT ⁱ	0.39 (2)
5b	4600 $\pm$ 870	>10000	0.099 $\pm$ 0.002	5.0 $\pm$ 0.3	41 $\pm$ 5	136 $\pm$ 25	0.20 (3)
5c	5500 $\pm$ 1130	1039 $\pm$ 190	0.27 $\pm$ 0.02	14.7 $\pm$ 0.6	23.4 $\pm$ 6	173 $\pm$ 23	0.30 (4)
5d	>10000	1012 $\pm$ 29	33.5 $\pm$ 3.5	28.0 $\pm$ 1.3	164 $\pm$ 12	1086 $\pm$ 190	1.84 (2)
5e	>10000	6475 $\pm$ 646	6.0 $\pm$ 0.5	118 $\pm$ 16	149 $\pm$ 39	434 $\pm$ 47	NT ⁱ
5f	>10000	339 $\pm$ 86	3.7 $\pm$ 0.5	NT ⁱ	389 $\pm$ 34	NT ⁱ	NT ⁱ
5g	4315 $\pm$ 880	229 $\pm$ 76	3.2 $\pm$ 0.4	NT ⁱ	300 $\pm$ 63	NT ⁱ	NT ⁱ
5h	4370 $\pm$ 852	122 $\pm$ 37	0.105 $\pm$ 0.002	9.3 $\pm$ 1.9	83 $\pm$ 28	18.4 $\pm$ 3.0	0.20 (4)
5i	3290 $\pm$ 418	263 $\pm$ 31	4.5 $\pm$ 0.8	11.9 $\pm$ 1.7	15.3 $\pm$ 3.2	206 $\pm$ 14	0.93 (2)
5j	NA ^j	63 $\pm$ 6	8.2 $\pm$ 0.9	4.0 $\pm$ 0.7	127 $\pm$ 28	NT ⁱ	NT ⁱ
5k	NT ⁱ	215 $\pm$ 15	32.92 $\pm$ 3	24.2 $\pm$ 2	122.76 $\pm$ 15	NT ⁱ	0.16 (2)
5l	7124 $\pm$ 488	75 $\pm$ 7	0.14 $\pm$ 0.03	18.0 $\pm$ 4.3	57 $\pm$ 11	154 $\pm$ 14	0.21 (2)
5m	>10000	3530 $\pm$ 918	8.6 $\pm$ 0.9	33 $\pm$ 3	200 $\pm$ 40	1360 $\pm$ 191	0.26 (2)
1	318 $\pm$ 22	2.6 $\pm$ 0.5	0.9 $\pm$ 0.04	1800 $\pm$ 310	164 $\pm$ 88	4700 $\pm$ 500	0.12 (8)
2	353 $\pm$ 67	210 $\pm$ 31	319 $\pm$ 55	160 $\pm$ 30	10 $\pm$ 2	4.8 $\pm$ 0.4	17 (3)
3	25 $\pm$ 3.5	20 $\pm$ 17	39 $\pm$ 5.9	610 $\pm$ 85	4.0 $\pm$ 1.0	4.1 $\pm$ 0.9	36 (2)
4	1960 $\pm$ 180	0.8 $\pm$ 0.07	3.3 $\pm$ 0.5	5.6 $\pm$ 0.2	8.7 $\pm$ 0.9	22 $\pm$ 1.7	1.02 (2)
Risperidone	50 $\pm$ 2	3.8 $\pm$ 0.3	6.7 $\pm$ 0.7	190 $\pm$ 15	0.15 $\pm$ 0.02	32 $\pm$ 2.2	0.92 (2)

^a Each value is the mean $\pm$ SD of three determinations and represents nM K_i value. ^b [³H]SCH 23390, rat striatum. ^c [³H]spiperone, rat striatum. ^d [³H]-7-OH-DPAT, Sf9 cells. ^e [³H]-8-OH-DPAT, rat hippocampus. ^f [³H]ketanserin, rat cortex. ^g [³H]mesulergine, guinea pig cortex. ^h Each value is the mean of 2–8 determinations (number of determinations are given in parentheses), SD were all within 10% of the mean. ⁱ NT not tested. ^j NA not active at 100 μ M.

Table 3. Multiple Sequence Alignment of the Trans-Membrane Ligand-Binding Domain of Considered Human DAR and 5-HTR Subtypes

TM1–7 similarity (%)	receptor subtype	TM2 sequence ^{a,b}	TM3 sequence ^{a,b}	TM4 sequence ^{a,b}	TM5 sequence ^{a,b}	TM6 sequence ^{a,b}	TM7 sequence ^{a,b}
	D ₃	LVMPWVVYL	FVTLDVMMC	ITAVWVLAF	FVIYSSVVSFYLP	FIVCWLPFFL	SATTWLGYY
81	D ₂	LVMPWVVYL	FVTLDVMMC	ISIVWVLSF	FVVYSSIVSFYVP	FIICWLPFFI	SAFTWLGYY
63	5-HT _{2C}	LVMPLSLLA	WISLDVLF	IAIVWAISI	FVLIGSEVAFIP	FLIMWCPFFI	NVEVWIGYY
61	5-HT _{1A}	LVLPMALY	FIALDVLC	ISLTWLIGF	YTIYSTFGAFYIP	FILCWLPFFI	AIINWLGYS
59	5-HT _{2A}	LVMPSMLT	WYLDVLF	IIAVWTISV	FVLIGSEVSEFIP	FVVMWCPFFI	NVEVWIGYL
57	D ₁	LVMPWKAVA	WVAFDIMCS	ISVAWTLVS	YAISSSVISFYIP	FVCCWLPFFI	DVFVWFGWA

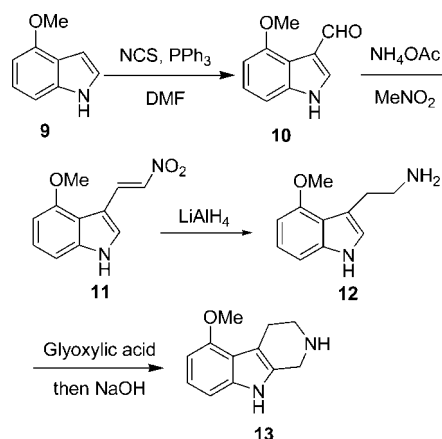
^a Bold: conserved Asp residue on TM3, serine residues on TM5, and aromatic residues lining the ligand binding pocket. ^b Underlined: residues displayed in Figure 1A,C.

Chemistry

The synthesis of compounds **5a–m** (Chart 1 and Table 1) is described in the Schemes 1–4.

The secondary amine synthons **8** and **13** were synthesized following the synthetic strategies described in Schemes 1 and 2. Compound **8** was prepared starting from previously described ethyl 7-methoxyindole-2-carboxylate **6**,²⁴ which was *N*-alkylated with bromoacetonitrile to afford the cyano-derivative **7**.²⁵ Reduction of nitrile **7** using an excess of lithium aluminum hydride gave rise to a cyclic lactam intermediate that was further reduced in situ to afford the target amine **8**.²⁵

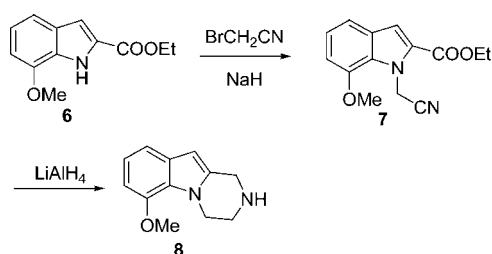
The synthesis of the known β -carboline **13** was realized by a modification of the literature procedure²⁶ as described in Scheme 2. Formylation of the indole-derivative **9** at C3 was performed using *N*-chlorosuccinimide (NCS), triphenylphosphine, and *N,N*-dimethylformamide (DMF).²⁷ The aldehyde **10** thus obtained was treated with nitromethane and ammonium acetate to afford 4-methoxy-3-(2-nitrovinyl)-1*H*-indole **11**.²⁸ This latter was subsequently reduced by lithium aluminum

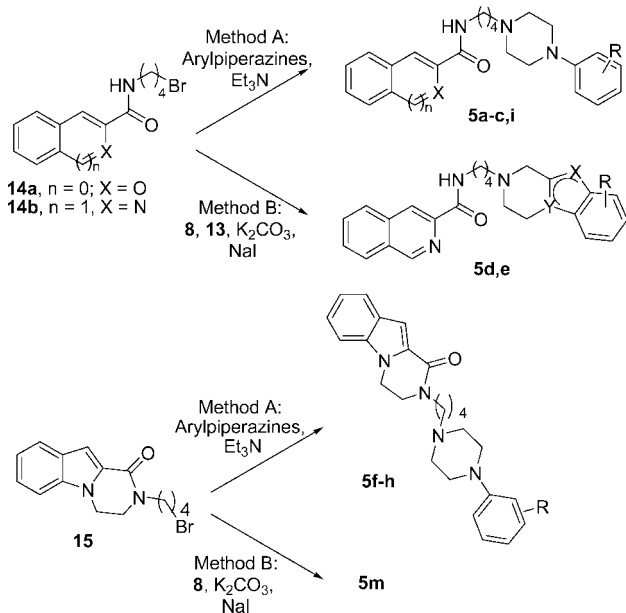
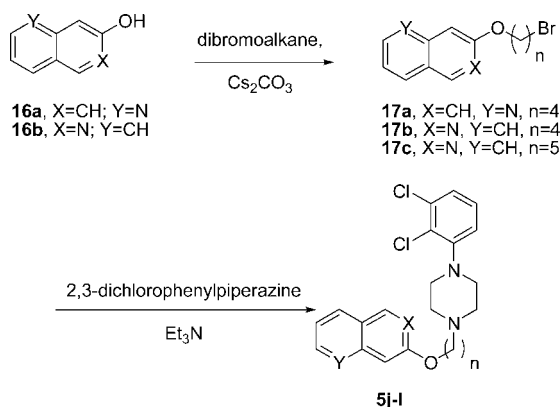
Scheme 2. Synthesis of Intermediate **13**

hydride, and the corresponding tryptamine derivative **12** was submitted to a classic Pictet–Spengler reaction, affording the β -carboline **13**.²⁶

The synthetic pathway for obtaining compounds **5a–i,m** is shown in Scheme 3. The bromo-derivatives **14a,b** and **15** were obtained according to the procedure previously reported in the literature.²³ Subsequent alkylation of suitable arylpiperazines (commercially available, or obtained by a previously reported methodology,²³ or according to Schemes 1 and 2) gave the final compounds **5a–i,m**.

The ether-derivatives **5j–l** (Scheme 4) were synthesized starting from the aromatic hydroxy-derivatives **16a,b**, which were *O*-alkylated with the appropriate commercially available dibromoalkane in the presence of cesium carbonate as a base.

Scheme 1. Synthesis of Intermediate **8**

Scheme 3. Synthesis of Compounds **5a–i,m****Scheme 4.** Synthesis of Compounds **5j–l**

The bromo-derivatives **17a–c** were reacted with 2,3-dichlorophenylpiperazine to give compounds **5j–l**.²³

Results and Discussion

For in vitro and in vivo testing, compounds **5a–m** were used as mono-, di-, or trihydrochloride salts.

The binding affinities for 5-HT_{1A}, 5-HT_{2C}, 5-HT_{2A}, D₁, D₂, and D₃ receptors of compounds **5a–m**, together with clozapine, olanzapine, and haloperidol, are given in Table 2. Furthermore, Table 2 lists the affinity of the tested compounds for hERG channels. Figure 3 summarizes the effects of **5i** in animal models sensitive to mesolimbic mediated antipsychotic activity (methamphetamine (MAMP)) and phencyclidine (PCP) induced hyperactivity) and striatal mediated side effects (catalepsy). Figure 4 reports the effect of **5i** on Fos protein induction in the nucleus accumbens shell subregion, and core subregion and in the dorsolateral part of the rostral striatum. Table 4 reports a summary of the behavioral effects of **5c**, **5h**, **5i**, **5l**, and the reference compounds.

1. Rational Design, Binding Studies, and Structure–Activity Relationships (SARs). Design Strategy. The challenge in designing new potential antipsychotics is to balance, in a single molecular structure, the required activity and selectivity toward specific multiple receptor subtypes (multireceptor affinity

profile). We had previously acquired experience in tricyclic antipsychotics²² and in arylpiperazines designed to selectively interact with D₃R to modulate cocaine-seeking behavior.²³ In this work, we therefore decided to exploit specific modifications of the versatile arylpiperazine scaffold. We were following an innovative pharmacological hypothesis aimed at modulating activity toward highly homologous dopamine receptors, such as D₂R and D₃R, while still maintaining a good affinity for serotonin 5-HT_{1A} and 5-HT_{2A} receptors and minimizing affinity for the D₁R and 5-HT_{2C}R subtypes. Furthermore, to improve the drugability of the novel antipsychotics, we directed our design strategy at limiting hERG potassium channel inhibition by up to 1 μM, thus minimizing the risk of long QT syndrome and cardiac side effects.

To drive the structural modifications of this new series of arylpiperazine derivatives, we performed a conformational and electronic analysis of the ligands, along with a structural and bioinformatic analysis of the above-mentioned serotonin and dopamine receptor subtypes (see Experimental Section for details). The amino acid (AA) sequences of the receptors were aligned²⁹ with the aim of mapping their active sites' homology and a model of the putative binding site for our competitive ligands was produced (Homology, Insight2005, Accelrys, San Diego) (Table 3, Figure 1A,C). Indeed, all the considered receptors (i.e., D₁, D₂, D₃, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C}) belong to the 7tm_1 family of G-protein coupled receptors (GPCRs). In particular, the two endogenous aromatic amines, dopamine and serotonin, share a homologous binding site.³⁰ Many GPCR models have been proposed, derived from analogies with high-resolution structures of rhodopsin. Despite this, the actual seven trans-membrane (TMs) helices connectivity, arrangement, and 3D structure in the different GPCR subtypes is still unknown. However, biophysical and mutagenesis studies on rhodopsin and a number of ligand-activated GPCRs provided evidence that the formation of the receptor active state involves movements of TM5, TM6, and TM7 with respect to TM3.^{31–34} In particular, different clusters of aromatic residues, critical to receptor structure and ligand recognition, were identified.^{31,35,36} The very recently solved structure of the β₂-adrenergic receptor in complex with the inverse agonist carazolol^{37–39} confirmed the data obtained from mutagenesis studies³⁵ and offered a new template for the generation of receptor 3D models (see Experimental Section).

The generated receptor models provided the rational bases for driving structural modification of the arylpiperazine skeleton in order to achieve the desired dopamine/serotonin receptor subtype selectivity. Consequently, we first identified, in the arylpiperazine skeleton, different pharmacophoric moieties (Chart 2) aimed at binding to the corresponding interaction sites identified in different secondary structures of the receptor models (Figure 1). In this view, the polymethylene tether length is crucial for guaranteeing the required structural flexibility in order to adapt the different pharmacophoric moieties to multiple receptors' binding sites. Indeed, in agreement with the reported structural and biochemical data on dopamine and serotonin receptors, we hypothesized that our competitive ligands bind by placing their heteroaromatic moiety (namely the “head”) into the “relevant aromatic pocket”^{22c} formed by the motif W_i, F_{i+3}, F_{i+4} on the TM6 helix, which is conserved throughout the biogenic amine receptors (Table 3, Figure 1).^{35,38} At the same time, the H-bonding group of our compounds is thought to interact with the equally conserved Ser/Thr residues on TM5 helix, reported to be important for receptor interaction/activation as well as for ligand specificity (see the discussion below for

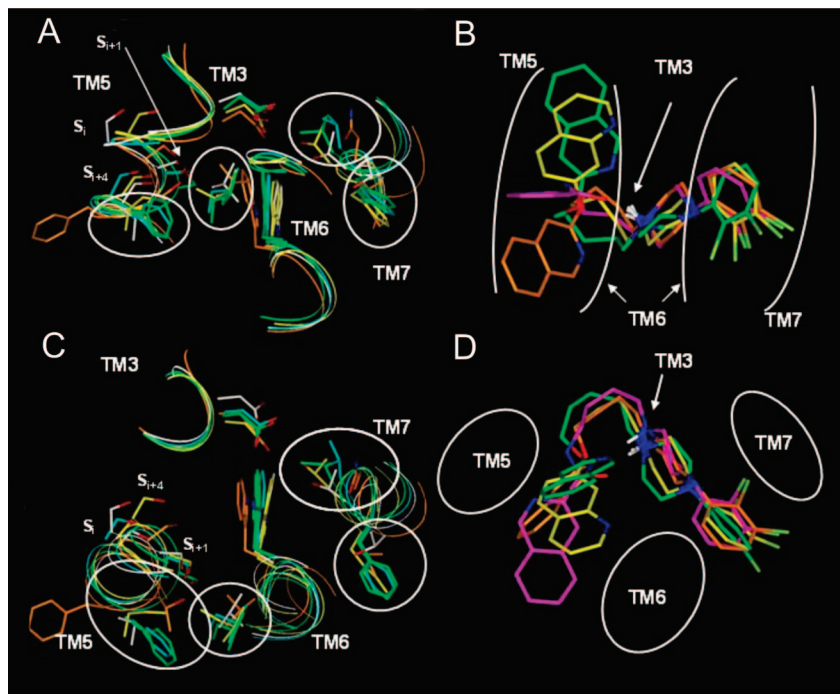


Figure 1. Binding sites (A: longitudinal view; C: transversal view) of 5-HTR and DAR 3D models. The inactive state (template: β_2 adrenergic receptor; PDB code: 2RH1) of D₂R (yellow), D₃R (white), 5-HT_{2A}R (cyan), and 5-HT_{2C}R (green), and the active state (template: bovine rhodopsin; PDB code: 2I37) of 5-HT_{1A}R (orange) are superimposed by C α atoms. Key residues on TM3, TM5, TM6, and TM7 are displayed. AA involved in subtype selectivity are highlighted by white circles. Serine residues on TM5 are labeled as discussed in the main text. Longitudinal (B) and transversal (D) view of **5b** (magenta), **5j** (yellow), **5k** (orange), and **5l** (green) AM1 global minimum conformers, superimposed by fitting their pharmacophoric moieties (i.e., “head” ring centroid; protonated piperazine nitrogen hydrogen; H-bond donor group; “tail” ring centroid). The hypothesized interactions with TM3, TM5, TM6, and TM7 receptor helices are evidenced.

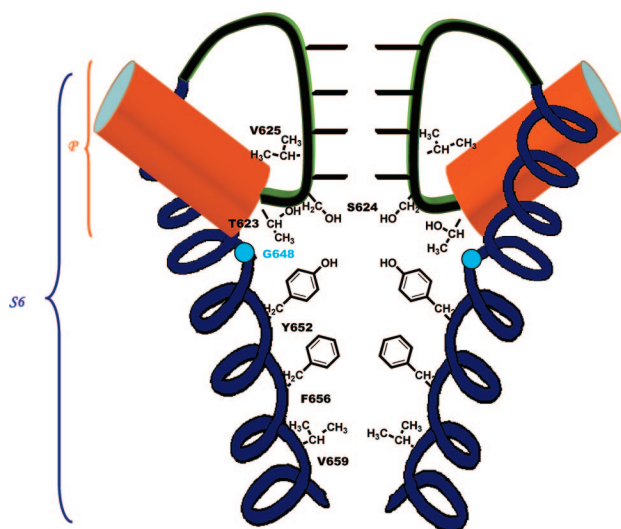


Figure 2. Illustrated representation of the central pore cavity of hERG channels according to the model reported by Stansfeld et al.⁴⁶ The central pore helix P and the S6 helix are indicated by orange and blue brackets, respectively. Key binding residues for blockers identified by mutagenesis studies are evidenced.

details).^{31,40} Finally, the Asp residue on TM3, present in all GPCRs amine receptors, is likely to interact with the protonated piperazine nitrogen of our ligands (Table 1 and 3, Figure 1). Moreover, because the binding sites of both dopamine and serotonin contain additional aromatic pockets, modification of the chemical-physical features of the “tail” of our compounds could drive the molecule to assume alternative binding modes in the active site, adapting (or not) to different receptor subtypes. Indeed, in addition to the above-mentioned Trp residue on TM6,

all the considered receptors have in common a further Trp residue on TM4 and TM7 helices (dopamine subtypes also on TM2), lining the substrate binding site (Figure 1A,C and Table 3). Mutagenesis studies in the 5-HT_{2A}R also support an interaction of serotonergic ligands with the conserved Trp on TM7.⁴¹ However, crucial changes in the AA composition of dopamine and serotonin binding sites determine the molecular bases for substrate and ligand selectivity (Figure 1 and Table 3). In particular, residues that are critical for pharmacological specificity are often one turn of helix away from the critical “conserved” residues.^{35,38}

Hence, taking into account the structural homology among our dopamine and serotonin receptor models, as well as the structural comparison with other known dopamine/serotonin competitive ligands, we rationally modified the electronic and conformational features of our ligands on the basis of our experience in the field of antipsychotics²² and in the development of selective D₃R ligands.²³ Accordingly, we maintained the 4-methylene linker in the amide series of compounds, because it was previously demonstrated to be the optimal spacer for high D₃R affinity,⁴² while we tested 4- and 5-methylene linkers in the ether series (**5j–l**, Table 1). However, we varied the chemical-physical parameters of: (i) the heteroaromatic system at the “head”, (ii) the functional group (X) connecting the “head” to the methylene linker, (iii) the phenyl ring at the “tail”, by modifying the nature, position, and number of substituents (Chart 2 and Table 1).

Following this rationale, we were able to obtain the optimal affinity profile for entering into further in vivo experiments.

Dopamine Receptors. According to dopamine receptors’ classification (D₁-like and D₂-like subfamilies), dopamine D₁R showed the lowest degree of sequence homology with both D₂R and D₃R, and with 5-HTRs, presenting relevant AA changes in

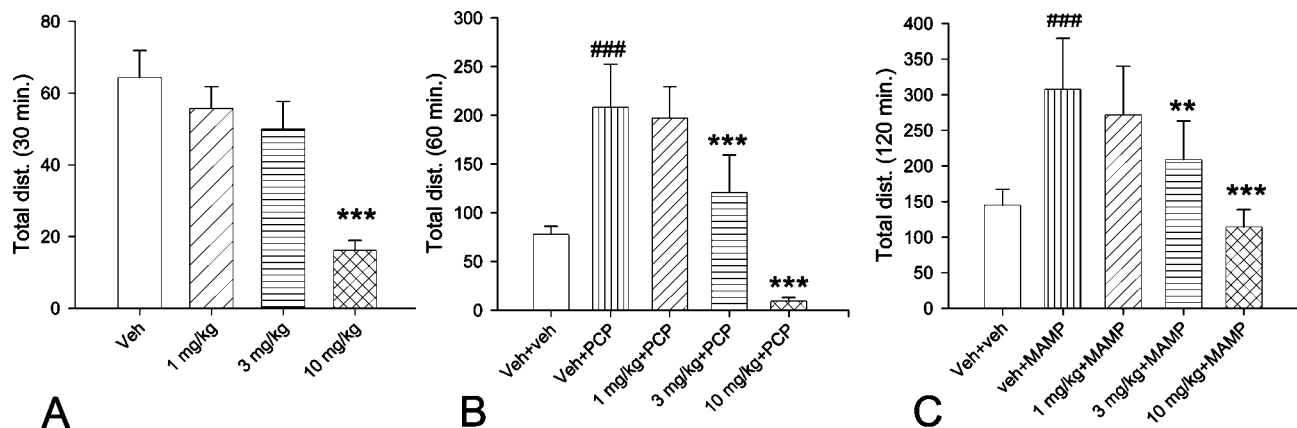


Figure 3. Effect of compound **5i** on spontaneous exploratory locomotor activity (A), MAMP (B), and PCP-induced hyperactivity (C). (A) Compound **5i** caused a dose-dependent reduction in spontaneous exploratory locomotor activity, marginally reducing activity at 3 mg/kg ($p < 0.05$) and potentially reducing activity at 10 mg/kg ($p < 0.001$). (B) Compound **5i** dose-dependently reduced MAMP-induced hyperactivity in doses of 3 and 10 mg/kg when compared to VEH + MAMP ($p < 0.01$ and $p < 0.001$ respectively). (C) Likewise, PCP-induced hyperactivity was also significantly reduced by compound **5i**, reaching statistical significance at 3 and 10 mg/kg ($p < 0.001$ for both) when compared to VEH + PCP. $n = 7$ pr dose group. Results are expressed as means $\pm$ SEM of distance traveled. Statistical evaluation was performed by two-way ANOVA followed by Tukey test for multiple comparisons. ###: $p < 0.001$ versus vehicle treatment; **: $p < 0.01$ versus MAMP/PCP treatment; ***: $p < 0.001$ versus MAMP/PCP treatment.

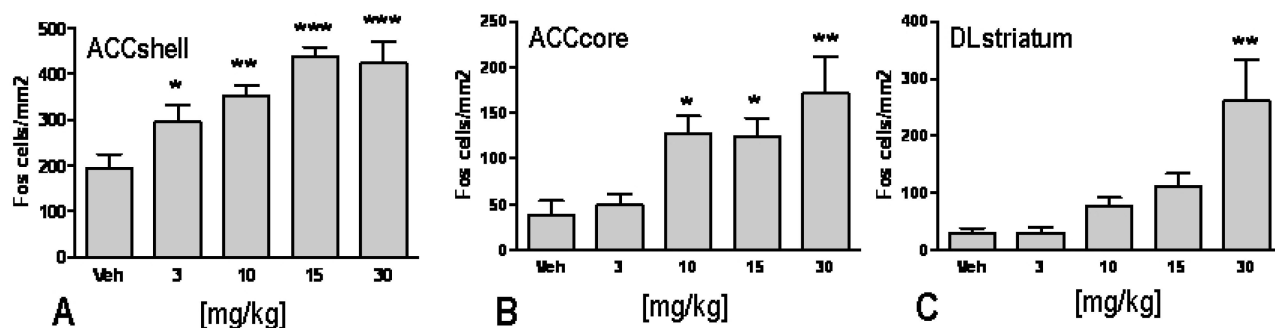


Figure 4. Effect of a single intraperitoneal dose of **5i** on Fos induction 60 min after the treatment in the nucleus accumbens shell subregion (A), the nucleus accumbens core subregion (B), and the dorsolateral part of the rostral striatum (C). The results are expressed as means $\pm$ SEM of the number of Fos-immunoreactive cells/mm² in all regions. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared to respective vehicle-treated group.

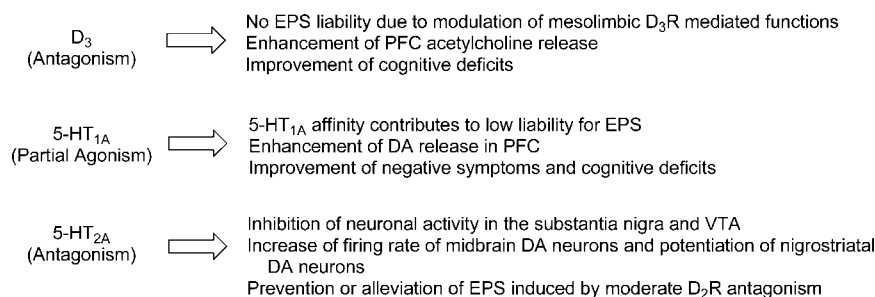
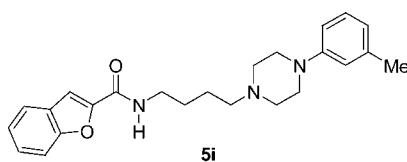


Figure 5. Outline of **5i** multireceptor affinity profile and its significance for optimizing the atypical antipsychotic profile.

TM1, TM2, TM5, TM6, and TM7 helices involved in ligand–receptor interaction/activation (Table 3).^{35,40} Accordingly, we easily designed arylpiperazines devoid of D₁R affinity (Table 2). However, despite the high degree of identity between D₂R and D₃R active sites, we were able to selectively modulate D₂R occupancy in our series of compounds.

The D₂R and D₃R substrate binding site both present on TM2, TM4, and TM7 helices, an aromatic residue placed one turn of helix away (i.e., at the i+3/i+4 position) with respect to the conserved Trp residue, thus forming additional aromatic pockets (Table 3). Nevertheless, on D₃R TM7 helix, there is a Thr residue, not conserved in D₂R, just before the conserved Trp

Table 4. Summary of Behavioral Effects of **5c**, **5h**, **5i**, and **5l** Compared to the Effects of **18**, **19**, Aripiprazole, Clozapine, and Haloperidol Tested Under Identical Conditions

compd	expl. locom. ^a ED ₅₀ (mg/kg)	MAMP locom. ^b ED ₅₀ (mg/kg)	PCP locom. ^c ED ₅₀ (mg/kg)	cataplexy
5c	14	4.2	6.7	
5h	>30	3.7	>3	
5i	5.5	2.1	2.6	>30
5l	>30	>10	>10	
18			>30	
19			>30	
Aripiprazole	0.6	<0.3	0.2	>30
Clozapine	2.4	2.5	1.1	>30
Haloperidol	0.08	0.06	0.09	0.19

^a Expl. Locom.: exploratory locomotor activity; ^b MAMP locom.: methamphetamine-induced locomotor activity; ^c PCP locom.: PCP-induced locomotor activity.

Chart 2. General Structure of Title Compounds

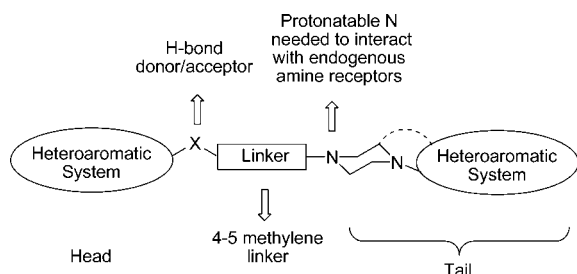
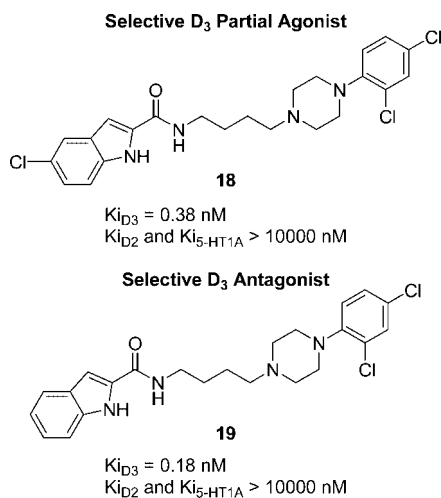


Chart 3. Structure and Binding Profile of Compounds **18** and **19**



residue (T369, human D₃ numbering; Table 3). This could offer a further H-bond interaction site (i.e., besides the Ser stretch S_i, S_{i+1}, S_{i+4} on TM5) close to the relevant aromatic pocket on TM6 and the Asp residue on TM3 (Figure 1A,C). Consequently, the arylpiperazine skeleton is suitable for high D₃R affinity, likely placing (i) the protonated N in contact with the Asp residue on TM3, (ii) the heteroaromatic "head" (Chart 2) on the relevant aromatic pocket on TM6, establishing H-bond interactions with TM5 or TM7, and (iii) the aromatic "tail" in one of the additional aromatic pockets on TM2, TM4, and TM7 (Figure 1). It cannot be excluded that significant structural changes in the molecule, such as the introduction of further H-bond donor/acceptor groups at the "tail", could drive this latter into the relevant aromatic pocket on TM6, leading to putative additional binding modes.^{22c} To this end, we succeeded in

maintaining high D₃R affinity through the whole series of compounds (Table 2).

The crucial role in D₂R/D₃R selectivity played by the orientation of H-bond donor/acceptor group(s) (supposed to interact with the serine stretch S_i, S_{i+1}, S_{i+4} on TM5) with respect to the extra volume occupied by the heteroaromatic ring (supposed to contact TM6) (Figure 1) was already observed by us,²³ and it is confirmed by the affinity profile of the newly designed compounds (Table 2). In fact, when an ether function (X = O) is replaced by an amide bond (X = CONH) (Charts 1, 2 and Table 1), D₂R affinity dramatically decreased and the compound become extremely selective toward D₃R (**5k** vs **5b**, Table 2). Indeed, in addition to the above-mentioned Thr residue on TM7, D₂R and D₃R present further differences in the AA composition of their binding site. In particular, in the hydrophobic cleft between TM5 and TM6, bulky Ile residues are present in D₂R (I195(TM5) and I391(TM6), human numbering; Figure 1, Table 3), while at the D₃R level, these isoleucines are replaced by the less hindered Val residues. This may account for the potency and selectivity of the designed compounds toward D₃R. Accordingly, the different amino acid composition of the regions shaped between TM5/TM6 and TM6/TM7 helices was exploited by us to obtain the desired D₃R/D₂R selectivity profile by modifying the "head" of title compounds, (Chart 2, Tables 1–3, Figure 1A–D). In particular, the electronic partial charge distribution of **5j**–**1**, in their AM1 global minimum conformer (Figure 1B,D), evidenced the bridging oxygen (full optimized AM1 partial charge = −0.26) and the heteroaromatic nitrogen (full optimized AM1 partial charge ∼−0.20) lone pairs as putative H-bond acceptor groups. Our computational analysis also highlighted that the high flexibility due to the ether linker (i.e., at least five sp³ atoms), together with the formation of an H-bond interaction between the electron-dense ether oxygen (conjugated to an heteroaromatic ring) and the protonated piperazine nitrogen (Figure 1B,D), strongly stabilized a "butterfly-shaped" conformation, fitting the D₂R pharmacophoric requirements reported for tricyclic D₂R antagonists.^{22c} Thus, the ether derivatives may be able to: (i) interact with the serine stretch S_i, S_{i+1}, S_{i+4} on TM5, (ii) accommodate the heteroaromatic "head" in the region between TM5 and TM6 establishing favorable interactions with the relevant aromatic pocket on TM6 (W_i, F_{i+3}, F_{i+4} motif), (iii) interact with the Asp residue on TM3, and (iv) place the "tail" in the additional aromatic pocket on TM7 (Figure 1). Because the flexibility of the alkyl tether determines the disposition of the pharmacophoric moieties, it plays a key role in dopamine receptors (DAR) affinity.²³ In the present series, this is confirmed by the ether derivative **5l** (**5l** vs **5k**, Figure 1B,D, Table 1, 2). However, when an amide bond is in place (**5b**), our calculations revealed the occurrence of intramolecular H-bonds between: (i) the amide hydrogen and the isoquinoline nitrogen, and (ii) the carbonyl oxygen and the protonated piperazine nitrogen. Accordingly, we calculated a strong conformational preference for this family of "H-bonded" conformers. In consequence, the amide derivatives are characterized by a different orientation of the heteroaromatic "head" with respect to the H-bonding groups (Chart 2, Figure 1B,D). Indeed, in the case of the amide bridged analogues, the carbonyl oxygen (full optimized AM1 partial charge = −0.46), the aromatic heteroatom (full optimized AM1 partial charge ∼−0.15), and the amide hydrogen (partial charge = 0.26) represent the most probable H-bond donor/acceptor to interact with the above-mentioned serine stretch on TM5 helix (Figure 1). To establish this latter interaction and, at the same time, to accommodate the heteroaromatic "head" in the "relevant aromatic pocket" on

TM6, the molecule should project toward the above-mentioned hydrophobic region between TM5 and TM6, where bulky Ile residues are present in D₂R (Figure 1, Table 3). This may account for the very low affinity of **5b** vs **5k/5l**. According to these observations, the conformationally constrained analogues **5f** and **5h**, lacking the H-bond acceptor group (NH), proved to be more potent than **5a** and **5b** on D₂R, although the potency on D₃R was comparable (Table 2).

Once we had selected the amidic function at the “head” (X group, Chart 2) to get the best affinity pattern at D₂R and D₃R, we explored the influence of specific substitutions at the “tail” level in order to obtain the desired pharmacological profile. On the basis of our previous findings,²³ different electron withdrawing groups were introduced on the phenyl ring at the “tail” to lower D₂R affinity (Table 1). Among the arylpiperazines tested in the present series, the 2,3-dichlorophenyl piperazine (**5b**, **5g**, **5h**, **5j–l**) provided analogues with significant D₂R occupancy and the best D₃R affinity. However, they differed, as predicted, in the D₂R/D₃R affinity ratio by 1–5 orders of magnitude (Table 2). By shifting the *meta*-chlorine atom at the *para* position, the affinity decreased on both subtypes, although this effect was more pronounced on D₃R than on D₂R (**5h** vs **5g**, Table 2). Contrary to the trend previously observed for D₂R on chlorine-substituted arylpiperazines,²³ the introduction of electron-withdrawing substituents at the *meta* position of the phenyl ring increased D₃R affinity (**5a** vs **5i**, Table 2). On these bases, compound **5i**, bearing a methyl group at the *meta* position, despite showing a decreased D₃R receptor affinity with respect to **5a**, still maintained a great selectivity profile, exhibiting, in addition, low affinity for hERG channel (see below).

Finally, in agreement with our design hypothesis, the D₃R binding site can tolerate significant structural changes. In particular, according to the presence of T198 on TM7, which may allow alternative binding modes, introducing further H-bonding groups at the “tail” of the arylpiperazine skeleton (i.e., compounds **5d**, **5e**, and **5m**) we maintained good D₃R affinity and D₂R/D₃R selectivity ratios.

Serotonin Receptors. 5-HT_{1A}R affinity is not greatly influenced by the position of the H-bonding group at the “head” of the arylpiperazine skeleton (Chart 2, Table 2). Indeed, compounds **5b,c**, **5h–j**, and **5l**, characterized by different heteroaromatic moieties and X groups (Chart 2, Table 1), presented a 5-HT_{1A}R affinity ranging from 5.0 to 18.0 nM (Table 2). However, the affinity of the above-cited set of compounds on 5-HT_{2A}R (ranging from 15.3 to 127 nM, Table 2) demonstrated the different sensitivity of the two serotonin receptor subtypes to structural modifications at the “head” of our arylpiperazine scaffold. Similarly, the variation of the alkyl tether length in the ether series differently affected serotonin receptor subtype affinity (**5l** vs **5k**, Tables 1 and 2). In fact, these serotonin receptor subtypes present critical AA changes in the TM5 helix (Figure 1A,C, Table 3), which can account for the observed SARs.^{34,43,44} In particular, the DAR serine stretch (S_i, S_{i+1}, S_{i+4}) on TM5 is not conserved in 5-HT_{1A}R, where S_i is still present, but S_{i+1} and S_{i+4} are replaced by a Thr and an Ala residue, respectively. In contrast, in the 5-HT_{2A}R subtype S_i is replaced by a Gly residue, while S_{i+1} and S_{i+4} are conserved (Table 3).

Moreover, 5-HT_{1A}R binding site presents an Asn residue on TM7 (N386, human 5-HT_{1A}R human numbering, Table 3), corresponding to the β -adrenergic receptor TM7 Asn residue involved in carazolol binding.^{37–39} As with D₃R (reported above), this could represent a putative additional H-bond interaction site besides the Ser stretch on TM5 (Figure 1A,C).

It could also allow in 5-HTR subtypes alternative binding modes for the arylpiperazine-like skeleton.

The intrinsic activity showed by compound **5i** (see next paragraph) indicated an agonist like effect on 5-HT_{1A}R only. Interestingly, in the activated state of GPCRs (Figure 1 protein orange), TM5 turns clockwise, shifting one residue away. Although in 5-HT_{1A}R S_{i+1} is missing, the clockwise shifting during the receptor activation pushes the S_i serine residue (conserved in 5-HT_{1A}R) to occupy the S_{i+1} serine position of the inactivated state. The position of the S_{i+1} serine residue of the inactivated state now corresponds to the position of the S_i serine residue of the activated state (Figure 1A,C). Thus **5i** binds 5-HT_{1A}R in the activated state. This observation may explain the intrinsic activity of **5i** at 5-HT_{1A}R.

The higher tolerance of 5-HT_{2A}R with respect to D₂R has already been evidenced and exploited by us in the design of new atypical antipsychotics based on a tricyclic scaffold.²² It is noteworthy that the pharmacophoric requirements reported to be responsible for D₂R/5-HT_{2A}R selectivity in the tricyclic series, such as the distance between the aromatic ring binding to the relevant aromatic pocket on TM6 and the protonated nitrogen binding to TM3, are similarly related to the selectivity profile of our new arylpiperazine-like derivatives (e.g., **5b**, distance = 7.42 Å, K_{ID2} > 10000 nM, K_{i5-HT2A} = 41 nM vs **5j**, distance = 5.63 Å, K_{ID2} = 63 nM, K_{i5-HT2A} = 127 nM).

The analogues **5b–e**, **5h,i**, and **5l,m** were also tested on 5-HT_{2C}R. Of these, only the indole-based ligand **5h**, characterized by the presence of alkyl-substituted nitrogens at the “head” of the structure (Table 1), showed a significant affinity (K_i = 18.4 nM, Table 2). Accordingly, in 5-HT_{2C}R only S_{i+1} is conserved, while S_i and S_{i+4} are replaced by a Gly and an Ala residue, respectively. Moreover, bulky hydrophobic residues are present around S_{i+1} on TM5 and TM6 (Table 3, Figure 1). Biochemical studies^{4,34,46} demonstrated that mutation of these residues causes a substantial alteration of ligand specificity and selectivity.

In conclusion, we based our design strategy on the differences among receptor subtypes’ composition of TM5/6/7 helices. We have thus been able to design compounds with weak affinity for 5-HT_{2C}R while maintaining the desired affinity and activity profile on 5-HT_{1A}R and 5-HT_{2A}R.

Following our rationale, the optimal dopamine/serotonin receptors’ affinity balance was obtained for compounds **5b**, **5c**, and **5i** (Table 2) characterized by: (i) the isoquinoline (**5b** and **5c**) or benzofuran (**5i**) rings as heteroaromatic systems (Table 1), (ii) the amide bond as X group, and (iii) the presence of small electron-withdrawing (**5b** and **5c**) and/or -donating (**5i**) substituents at 2 and/or 3 phenyl ring position of the arylpiperazine moiety.

Ether-a-gogo-Related Gene (hERG) KC Channels. Reducing the risk of drug-induced cardiac arrhythmia is recognized as a major hurdle in the successful development of new drugs. To date, investigation of hERG channel blockade has been a significant step along the drug discovery trajectory of the pharmaceutical industry. The most common problem is acquired long QT syndrome. This is caused by drugs that block human ether-a-gogo-related-gene (hERG) KC channels, delay cardiac repolarization, and increase the risk of torsades de pointes arrhythmia (TdP). Indeed, efforts to predict long QT syndrome risk have been focused on assays testing hERG channel activities. This is because hERG channel blockade is an important indicator of potential pro-arrhythmic liability. Drug-induced arrhythmia by noncardiac drugs is rare. However, commonly used medications can induce ventricular arrhythmia.

These include antipsychotics, antimalarials, and antidepressants. Although these drugs represent different therapeutic classes and chemical structures, they share the ability to prolong the QT interval, which is an indirect measure of ventricular-repolarization time measured from the ECG and a known risk factor for TdP. Sertindole and thioridazine were recently withdrawn from the market for this reason.

To date, long QT syndrome has alarmed regulatory authorities and has been most often associated with drug-induced ventricular arrhythmia. We therefore believe that weeding out potential hERG channel blocker activity as early as possible, preferably in the drug-design stage, will eliminate a lot of safety concerns that have previously accompanied the development of numerous drugs, especially antipsychotics. To this end, we decided to investigate hERG channel interaction at the earliest stages of the drug design and discovery process. Starting from the published protein-based and ligand-based pharmacophore models (hERG cartoon at Figure 2), we exploited our experience in drug design and, consequently, developed our own strategy for the structural class of compounds under investigation.

Scanning alanine mutagenesis studies have identified key binding-sensitive residues for a growing list of known hERG blockers in the inner helix (S6) and in the loop connecting S6 to the pore helix, close to the potassium selectivity filter.^{47–50} In particular, the binding site of drugs blocking hERG functionality is contained within the central pore cavity of the pore domain, located below the selectivity filter and flanked by the four S6 helices of the tetrameric channel. Ligand-binding sensitivity to Ala mutations has been demonstrated for (i) T623, S624, and V625 residues, at the base of the selectivity filter and for (ii) G648, Y652, F656, and V659 residues, all of which lie on the same face of S6 helix (i, i + 4 positions) (Figure 2).

All potent hERG blockers are highly sensitive to Y652A and F656A mutations. The presence of these two aromatic amino acids (Y652 and F656) in S6 of hERG is unique to the ether-a-go-go family of K⁺ channels. Indeed, despite the lack of negative charged residue on the hERG S6 inner helix, the binding of the cationic species is guaranteed by the overall negative electrostatic field inside the hERG cavity. This is due to Tyr and Phe side chains lining the inner pore in a 4-fold symmetry. However, the tetrameric assembling of the S6 and pore helices suggests the possibility of alternative binding modes for blockers. This is also supported by data obtained from mutagenesis studies.⁵¹ Therefore, hERG pore structure can account for the observed unintentional binding of biogenic amine competitive agonists/antagonists, such as antipsychotics. Accordingly, published hERG blockers pharmacophores^{52,53} share similar features with the dopamine/serotonin pharmacophores, consisting of two or more hydrophobic/aromatic groups surrounding a basic center. Thus, it is not surprising that our arylpiperazine derivatives showed blocking properties on hERG, although at high nanomolar concentrations (Table 2). Our strategy, to lower hERG affinity to the micromolar level, was based on the principle that, although hERG channels and dopamine/serotonin receptors share a similar and highly polarized binding site for cationic species (reflected in the similarity of the predicted hERG blockers and antipsychotics pharmacophores), the polarization of the endogenous ligand and, consequently, the polarization of the active site itself, must differ. Accordingly, once we had established the key pharmacophoric features necessary for obtaining the desired receptor binding profile, we tried to modulate hERG affinity by changing the polarization of the phenyl group at the “tail” of the compounds.

This strategy led to the discovery of **5i**, a potential atypical antipsychotic agent characterized by low affinity for hERG (Table 2).

Following this approach, we have been able to modulate hERG channel occupancy within the same structural class of compounds while maintaining the desired receptor binding profile (**5a** vs **5b** and **5i**, Tables 1 and 2) and in vivo pharmacological properties.

Miscellaneous Receptor Binding and 5i Intrinsic Activity at Selected Receptors. On the basis of its unique binding profile, **5i** was therefore tested against a panel of other receptors including histamine H₁ receptors (K_i = 15 nM), central imidazole I₂ receptors (K_i = 138 nM) and adrenergic receptors α_1 and α_2 (K_i of 6 and 43 nM, respectively). The affinity for α_1 receptors is a critical aspect of clozapine/olanzapine binding profile. The α_2 or 5-HT_{1A}R⁵⁴ occupancy might explain the marked increase in dopamine output in the prefrontal cortex induced by clozapine, which is beneficial to cognitive functions. The α_2 affinity of **5i** might enhance its clinical efficacy, while its α_1 occupancy might protect against dopamine deficits.

The intrinsic activity of compound **5i**, at D_{2L}, D_{2S}, and D₃ receptors was determined in [³⁵S]-GTP γ S binding assays, at 5-HT_{2A}R was determined in rat aortic ring contraction model, and at 5-HT_{1A}R in guinea pig ileum relaxation model. Compound **5i** behaves as antagonist at D_{2L} receptors (IC₅₀ = 584 nM), at D_{2S} receptors (IC₅₀ = 1240 nM), and at D₃R (IC₅₀ = 143 nM). On 5-HT_{2A}R, **5i** displayed antagonist-like effects (IC₅₀ = 230 nM), while on 5-HT_{1A}R displayed agonist like effects (IC₅₀ = 180 nM).

Taking into account the great potency of **5i** on serotonin, dopamine, and adrenergic receptors, this compound, together with **5c,h,k**, was selected as a promising atypical antipsychotic agent and subjected to in vivo pharmacological characterization.

2. Behavioral Studies. 2.1. Can Selective D₃R Blockade Relieve Positive Symptoms? To validate the approach herein proposed for the treatment of schizophrenia, the first question to be addressed was whether selective D₃R occupancy alone relieves positive symptoms of schizophrenia. Previously, we reported the synthesis of the arylpiperazines **18** and **19** (Chart 3) designed to investigate the role of D₂R/D₃R on cocaine-seeking behavior.²³ Arylpiperazines **18** and **19** proved to be two of the most potent and selective D₃R ligands showing partial agonist properties (**18**) or full antagonism (**19**) toward D₃R.²³ Both compounds were used in the present study as pharmacological tools to investigate the role played by D₃R occupancy on schizophrenia models. They did not show significant behavioral effect in the animal models herein described.

Together with the arylpiperazine aripiprazole (**4**), an analogous arylpiperazine characterized by high potency against D₂R, D₃R, 5-HT_{1A}R, and 5-HT_{2A}R, compounds **18** and **19** were tested in the PCP-induced hyperactivity animal model for psychosis. While aripiprazole showed a dose-dependent reduction of hyperactivity (Table 4), compounds **18** and **19** did not significantly reduce hyperactivity (ED₅₀ > 30 mg/kg, Table 4), indicating that D₃R occupancy alone lacks efficacy in animal models predictive for antipsychotic properties. We concluded that D₃R selective ligands may not relieve positive symptoms of schizophrenia. Accordingly, we designed arylpiperazines capable of interacting with D₃R, 5-HT_{1A}R, and 5-HT_{2A}R.

2.2. Behavioral Effects of a Selected Set of Novel Arylpiperazines. An initial behavioral screening was performed on compounds **5c**, **5h**, **5i**, and **5l**, selected on the basis of their multireceptor affinity profile. Clinically, the term “atypical antipsychotic” has been used for drugs, like clozapine, that

relieve positive symptoms at doses that do not cause side effects such as prolactin increase and extrapyramidal effects. Preclinically, this translates into drugs that show effect in models of psychosis at doses that do not cause dopamine D₂R related side effects. In this study, side-effect liability was evaluated by the horizontal bar test, which is very sensitive for catalepsy induced by dopamine D₂R blockade. Antipsychotic potential of the compounds was assessed in mice rendered hyperactive by MAMP and PCP. Both tests are sensitive to mesolimbic mediated antipsychotic activity. Meth- (or D-) amphetamine-induced hyperactivity are potently reduced by D₂R antagonists, such as haloperidol, whereas PCP-induced hyperactivity has been reported to be more sensitive to atypical antipsychotic compounds when compared to typical antipsychotics.⁵⁵

2.3. Effects of 5c, 5h, 5i, 5l, and Reference Compounds on Spontaneous Exploratory Locomotor Activity. In the exploratory locomotor activity test, the mice are transferred to new cages immediately before the test began and consequently used the first 30 min or so to explore their new environment. Compounds that cause sedation or induce motor disturbances will result in reduction in spontaneous exploratory locomotor activity. Pretreatment with 3, 10, and 30 mg/kg of compound **5h** and **5l** only mildly reduced exploratory locomotor activity with ED₅₀ values >30 mg/kg (Table 4). Compounds **5c** and **5i** dose-dependently reduced spontaneous locomotor activity with ED₅₀ values at 13.8 and 5.5 mg/kg, respectively (Table 4, and Figure 3 for **5i**). For comparison, aripiprazole, clozapine, and haloperidol were tested. All three compounds reduced exploratory locomotor activity with ED₅₀ values of 0.6, 2.4, and 0.08 mg/kg, respectively.

2.4. Effects of 5c, 5h, 5i, and 5l on Methamphetamine-Induced Locomotor Activity. As shown in Table 4 and Figure 3 for **5i**, pretreatment with 1, 3, and 10 mg/kg of compound **5l** did not reduce MAMP-induced hyperactivity when compared to MAMP alone (ED₅₀ value >10 mg/kg). In contrast, pretreatment with 1, 3, and 10 mg/kg of compounds **5c**, **5h**, and **5i** all caused a dose-related reduction in MAMP-induced hyperactivity, with ED₅₀ values of 4.2, 3.7, and 2.1 mg/kg, respectively. These effects cannot be accounted for by drug-induced sedation because sedative effects were only observed in the exploratory locomotor activity test at ED₅₀ values ranging from 2.6- to 8.1-fold higher doses (see above). For comparison, aripiprazole, clozapine, and haloperidol were also tested. All three compounds dose-dependently reduced MAMP-induced hyperactivity. Aripiprazole reduced MAMP-induced hyperactivity with ED₅₀ < 0.3 mg/kg, i.e., at least 2-fold lower than the ED₅₀ value for sedative effects in the exploratory locomotor activity test (ED₅₀ = 0.6 mg/kg). Clozapine reduced MAMP-induced hyperactivity with ED₅₀ = 2.5 mg/kg, which was in the same dose range as needed to cause sedation in the exploratory locomotor test (ED₅₀ = 2.4 mg/kg). Haloperidol reduced MAMP-induced hyperactivity with ED₅₀ = 0.06 mg/kg, also in the same dose range as needed to cause sedation (ED₅₀ = 0.08 in exploratory locomotor activity). Accordingly, compounds **5c**, **5h**, and **5i** showed a favorable profile when compared to both atypical and typical reference compounds in this test, with a 2–8 fold separation between doses causing sedation and doses reducing MAMP-induced hyperactivity.

2.5. Effects of Compounds 5c, 5h, 5i, and 5l on PCP-Induced Locomotor Activity. Pretreatment with 0.3, 1, and 3 mg/kg of compound **5h** and 1, 3, and 10 mg/kg of compound **5l** was not able to reduce PCP-induced increase in locomotor activity (ED₅₀ > 3 and > 10 mg/kg, respectively). In contrast, compounds **5c** and **5i**, both dosed in 1, 3, and 10 mg/kg, dose-

dependently reduced hyperactivity induced by PCP with ED₅₀ values at 6.7 and 2.6 mg/kg, respectively. Sedative effects were observed at approximately 2-fold higher ED₅₀ values in the exploratory locomotor activity test (13.8 mg/kg for compound **5c** and 5.5 mg/kg for compound **5i**). The atypical antipsychotic compounds aripiprazole and clozapine both dose-dependently reduced PCP-induced hyperactivity. Aripiprazole was administered at doses of 0.1, 0.3, and 1 mg/kg and inhibited PCP-induced hyperactivity with an ED₅₀ value of 0.2 mg/kg. Clozapine, dosed in 0.3, 1, and 3 mg/kg, reduced PCP-induced hyperactivity with an ED₅₀ value of 1.1 mg/kg. Both compounds showed sedative effects in the exploratory locomotor activity test with ED₅₀ values approximately twice the ED₅₀ values necessary to reduce PCP-induced hyperactivity. Furthermore, the typical antipsychotic compound haloperidol, tested at doses of 0.01, 0.025, and 0.05 mg/kg, reduced PCP-induced hyperactivity, reaching ED₅₀ at 0.09 mg/kg. This is in the same dose range as the ED₅₀ for causing sedation in the exploratory locomotor activity test (ED₅₀ = 0.08 mg/kg). In summary, compounds **5c** and **5i** both showed favorable profiles in MAMP- and PCP-induced hyperactivity when compared to typical as well as atypical antipsychotic reference compounds (Table 4, and Figure 3 for **5i**). On the basis of these results, **5i** was selected as the lead compound of the new series and was subjected to side-effect evaluation to further characterize its atypical antipsychotic profile.

2.6. Comparison of Cataleptogenic Effects of Aripiprazole, Clozapine, Haloperidol and Compound 5i. Compound **5i** did not cause catalepsy in the horizontal bar test at doses up to 30 mg/kg (Table 4). As expected, the typical antipsychotic compound haloperidol dose-dependently resulted in catalepsy with an ED₅₀ value of 0.19 mg/kg, while the atypical antipsychotic compounds aripiprazole and clozapine did not induce catalepsy at doses up to 30 mg/kg.

2.7. Mesocorticolimbic Selectivity and Atypical Antipsychotic Profile of 5i. The immunohistochemistry of the Fos protein has been shown to be useful in mapping functional pathways in the central nervous system and especially in identifying brain areas that are targets for antipsychotics. Moreover, the ability of these drugs to increase Fos protein expression in the striatal complex has been considered useful in discriminating between typical and atypical antipsychotic compounds.⁵⁶ In contrast to haloperidol, but like most atypical antipsychotics, **5i** did not affect Fos immunoreactivity in the regions implicated in the control of extrapyramidal motor function, such as the dorsolateral striatum. However, like clozapine, it markedly enhanced the expression of Fos protein in the mesocorticolimbic regions, which are involved in the control of affective and motivational behaviors, i.e., the shell subregion of the nucleus accumbens (Figure 4). In this respect, **5i** clearly behaves as an atypical antipsychotic. However, it is worth noting that not all of the atypical antipsychotics, which similarly increase Fos expression in limbic areas, can enhance this expression in the medial prefrontal cortex too.^{56a}

A single systemic injection of **5i** produced a dose-dependent and site-selective induction of the transcription factor Fos in the limbic striatum (Figure 4). There were clear topographical differences in the induction of Fos in different striatal areas. While **5i** markedly increased the number of Fos-immunoreactive cells in the nucleus accumbens (ACC) ACCshell at a dose of 3 mg/kg, higher doses were necessary to produce an induction in the ACCcore and an even higher in the dorsolateral (DL) DLstriatum (Figure 4). In the ACCcore, **5i** had no effect at a low dose, but at 10 mg/kg and higher, the compound induced

Fos in this region. Similarly, in the DLstriatum, **5i** increased the Fos induction, but only at the extremely high dose of 30 mg/kg.

Interestingly, it has been recently shown⁵⁷ that the 5-HT_{1A}R agonist 8-OH-DPAT is able to convert the “typical” pattern of haloperidol on *c-fos* expression into a pattern resembling that of clozapine, suggesting that the profile of Fos protein expression of **5i** might be linked to partial agonist properties at 5-HT_{1A}R.

3. Summary of the in Vivo Characterization of the Atypical Antipsychotic Agent 5i and Its Therapeutic Potential. As already discussed, **5i** (1, 3, 10 mg/kg, sc) caused a dose-dependent reduction in MAMP- and PCP- induced hyperactivity in mice, reaching ED₅₀ values of 2.1 and 2.6 mg/kg, respectively. In the exploratory locomotor activity test, sedation was only observed with ED₅₀ values approximately twice the value needed to reduce psychostimulant-induced hyperactivity. This is in the same range observed in this study by marketed atypical antipsychotics such as aripiprazole and clozapine. The ratio between ED₅₀ for reducing MAMP-/PCP-induced hyperactivity and ED₅₀ for sedation ranges between 1 and 3. D₂R-related side-effects liability was evaluated by the horizontal bar test. Neither compound **5i** nor aripiprazole nor clozapine caused catalepsy up to the highest dose tested (30 mg/kg). In contrast, haloperidol potently induced catalepsy with an ED₅₀ value of 0.19 mg/kg. In conclusion, **5i** has an approximate 200-fold selectivity toward the D₃R ($K_{iD2} = 764$ nM; $K_{iD3} = 4.5$ nM) and high affinity for 5-HT_{1A}R and 5-HT_{2A}R with ($K_i = 12$ and 15 nM, respectively). Taking this into account, **5i** was found effective in psychosis models without showing effect in the striatal-mediated side-effect models, suggesting antipsychotic activity with no propensity for causing D₂R-related side effects.

The in vivo studies, after acute administration of **5i**, clearly showed that this compound has an antipsychotic potential. As with all other antipsychotics tested so far, **5i** induces Fos in the nucleus accumbens shell.^{58,59} Furthermore, **5i** displays an atypical profile, activating neurons exclusively in the limbic/ventral striatal subdivisions rather than the sensory-motor portions in the dorsolateral striatum.

Conclusions

In summary, starting from highly potent and selective dopamine D₃R ligands previously described, with no overt behavioral effects in animal model for schizophrenia, we discovered novel arylpiperazine atypical antipsychotic agents characterized by specific occupancy of D₃R, 5-HT_{1A}R, and 5-HT_{2A}R. Interaction with D₂R and 5-HT_{2C}R was minimized in order to reduce any liability of EPS and to reduce the risk of obesity under chronic treatment, respectively. The analogue **5i**, which has unique receptor affinity properties on dopamine, serotonin, and adrenergic receptors, was identified as a potential atypical drug candidate. The binding profile of **5i** suggests a complex interaction on the cortical receptors involved in the regulation of the activity of prefrontal cortical cells innervated by ventral tegmental area neurons (5-HT_{2A}, dopaminergic, and α adrenergic receptor subtypes). Moreover, **5i** showed a low affinity for hERG channels. **5i** validated the hypothesis based on specific interaction with D₃R, 5-HT_{1A}R, and 5-HT_{2A}R for the discovery of innovative antipsychotic drugs (Figure 5). **5i** reduced methamphetamine as well as PCP-induced locomotor activity without causing catalepsy (up to 30 mg/kg, sc). Moreover, ED₅₀ for reducing MAMP- and PCP-induced hyperactivity was approximately a factor 2-fold lower than the ED₅₀ for causing sedation. This was found to be in the same range for the two marketed atypical antipsychotics, aripiprazole and

clozapine. Accordingly, **5i** shows an “atypical” antipsychotic activity without liability for side effects as measured by standard behavioral testing paradigms. This is supported by the preferred increase in expression of Fos immunoreactive cells in the ACCshell when compared to ACCcore and DLstriatum. Moreover, the data would suggest a therapeutic index of at least 10–15-fold between efficacy (methamphetamine or PCP models) and side effects (catalepsy). These findings indirectly illustrate the high and selective activity of **5i** toward the mesolimbic and mesocortical dopaminergic system. **5i** may pave the way for the development of a novel class of drugs for the treatment of neuropsychiatric disorders.

Experimental Section

Reagents were purchased from Aldrich and were used as received. Reaction progress was monitored by TLC using Merck silica gel 60 F₂₅₄ (0.040–0.063 mm) with detection by UV. Merck silica gel 60 (0.040–0.063 mm) was used for column chromatography.

Melting points were determined in Pyrex capillary tubes using an Electrothermal 8103 apparatus and are uncorrected. ¹H NMR and ¹³C NMR spectra were recorded on Bruker 200 MHz or Varian 300 MHz spectrometer with TMS as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); the value of chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hertz (Hz).

GC-MS were performed on a Saturn 3 (Varian) or Saturn 2000 (Varian) GC-MS System using a Chrompack DB5 capillary column (30 mm $\times$ 0.25 mm i.d.; 0.25 μ m film thickness). FAB-MS spectra were performed using a VG 70-250S spectrometer. ESI-MS, APCI-MS spectra were performed by an Agilent 1100 series LC/MSD spectrometer and by LCQDeca-THERMOFINNIGAN spectrometer.

Elemental analyses were performed in a Perkin-Elmer 240C elemental analyzer, and the results were within $\pm 0.4\%$ of the theoretical values, unless otherwise noted.

Yields refer to purified products and are not optimized. All moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents. All the organic layers were dried using anhydrous sodium sulfate.

1-(2,3-Dichlorophenyl)piperazine, 1-(3-methylphenyl)piperazine, and 1-(3-chlorophenyl)piperazine were commercially available, 1-(2,4-dichlorophenyl)piperazine was synthesized as previously described,²³ and 1-(3-cyanophenyl)piperazine was obtained starting from 3-cyanobromobenzene and piperazine following the same procedure described for the synthesis of 1-(2,4-dichlorophenyl)piperazine (spectroscopic data are consistent with those reported in the literature).⁶⁰

For testing, compounds **5a–m** were transformed into the corresponding hydrochloride salts by a standard procedure.

Ethyl 1-(Cyanomethyl)-7-methoxy-1H-indol-2-carboxylate (7). A mixture of sodium hydride (60% dispersion in mineral oil, (849.2 mg, 21.23 mmol) and ethyl 7-methoxyindole-2-carboxylate **6** (3.1 g, 14.15 mmol) in dry DMF (15.0 mL) was stirred at room temperature for 30 min before the addition of bromoacetonitrile (2.0 mL, 28.3 mmol) in dry DMF (2.0 mL). The reaction mixture was then heated to 65 °C for 30 min and stirred for further 6 h at room temperature, and treated with ice. The separated solid was filtered and purified by means of flash chromatography (33% *n*-hexane in dichloromethane) to give **7** (30.1% yield) as a white solid: mp (ethanol) 99–101 °C. ¹H NMR (CDCl₃) δ 1.41 (t, 3H, *J* = 7.2 Hz), 3.99 (s, 3H), 4.40 (q, 2H, *J* = 7.1 Hz), 5.96 (s, 2H), 6.80 (d, 1H, *J* = 7.7 Hz), 7.10 (t, 1H, *J* = 7.9 Hz), 7.25 (m, 1H), 7.33 (s, 1H). GC-MS *m/z* 258 (100) [M]⁺, 232, 213, 201, 187, 172, 144, 130, 114, 89.

1,2,3,4-Tetrahydro-6-methoxypyrazino[1,2-*a*]indole (8). A suspension of **7** (500.0 mg, 1.93 mmol) in dry diethyl ether (Et₂O) (20.0 mL) was added slowly to a well-stirred slurry of lithium aluminum hydride (LiAlH₄) (293.4 mg, 7.72 mmol) in dry Et₂O (10.0 mL). The mixture was refluxed for 4 h and then poured into ice–water. 1 N NaOH (10.0 mL) was added and the aqueous phase

was extracted with EtOAc (3 × 30 mL). The collected organic layers were dried and evaporated. The crude product was chromatographed (10% methanol in chloroform) to afford **8** as a yellow solid (40.6% yield): mp (methanol) 120–122 °C. ¹H NMR (CDCl₃) δ 1.89 (br s, 1H), 3.26 (t, 2H, *J* = 5.7 Hz), 3.90 (s, 3H), 4.17 (s, 2H), 4.47 (t, 2H, *J* = 5.8 Hz), 6.14 (s, 1H), 6.58 (d, 1H, *J* = 7.7 Hz), 6.97 (t, 1H, *J* = 7.7 Hz), 7.14 (d, 1H, *J* = 7.8 Hz). ESI-MS *m/z* 405 [2 M + H]⁺, 203 (100) [M + H]⁺.

1,2,3,4-Tetrahydro-5-methoxy-β-carboline (13). 4-Methoxytryptamine **12** (375.0 mg, 1.97 mmol) was transformed into the corresponding hydrochloride salt by a standard procedure. To a solution of 4-methoxytryptamine hydrochloride (445.0 mg, 1.97 mmol) in water (50.0 mL) glyoxylic acid monohydrate (181.2 mg, 1.97 mmol) was added and the mixture was stirred under reflux for 1 h. After cooling to room temperature, a 20% solution of NaOH was added and the mixture was extracted with EtOAc (3 × 30 mL). The organic layers were dried and evaporated. The crude product was chromatographed (CHCl₃/MeOH/NH₄OH 20:5:0.5 v/v) to give **13** as an amorphous solid (63.2% yield). ¹H NMR (CDCl₃) δ 1.67 (br s, 1H), 2.96 (m, 2H), 3.13 (m, 2H), 3.88 (s, 3H), 3.98 (s, 2H), 6.47 (d, 1H, *J* = 7.6 Hz), 6.89 (d, 1H, *J* = 8.1 Hz), 7.01 (t, 1H, *J* = 7.9 Hz), 7.75 (br s, 1H).

7-(4-Bromobutoxy)quinoline (17a). To a solution of 7-hydroxyquinoline **16a** (500.0 mg, 3.45 mmol) in dry DMF (15.0 mL), 1,4-dibromobutane (2.22 mL, 10.34 mmol) was added and the mixture was stirred at room temperature for 10 min. Then cesium carbonate (1.12 g, 3.45 mmol) was added and the mixture was heated to 65 °C for 12 h. After cooling to room temperature, methyl-*tert*-butylether (MTBE) (40.0 mL) and water (30.0 mL) were added and the mixture was extracted with MTBE (3 × 35 mL). The collected organic layers were dried, filtered, and evaporated. The residue was chromatographed (dichloromethane) to afford 645.0 mg of pure **17a** as a yellow oil (67.0% yield). ¹H NMR (CDCl₃) δ 2.00 (m, 4H), 3.44 (t, 2H, *J* = 5.9 Hz), 4.09 (t, 2H, *J* = 5.1 Hz), 7.16 (m, 2H), 7.35 (d, 1H, *J* = 2.5 Hz), 7.63 (d, 1H, *J* = 8.9 Hz), 7.99 (m, 1H), 8.77 (m, 1H). ESI-MS *m/z* 280, (100) [M + H]⁺, 198.

3-(4-Bromobutoxy)isoquinoline (17b). Compound **17b** was obtained starting from isosquinolin-3-ol **16b** (250.0 mg, 1.72 mmol) and following the above-described procedure for **17a**. Compound **17b** was obtained as a yellow oil (52.0% yield). ¹H NMR (CDCl₃) δ 2.07 (m, 4H), 3.50 (t, 2H, *J* = 6.3 Hz), 4.38 (t, 2H, *J* = 5.8 Hz), 6.36 (s, 1H), 7.35 (m, 1H), 7.55 (m, 1H), 7.67 (d, 1H, *J* = 8.2 Hz), 7.86 (d, 1H, *J* = 8.2 Hz), 8.92 (s, 1H). ESI-MS *m/z* 302 [M + Na]⁺, 280 [M + H]⁺, 199 (100).

3-(5-Bromopentyloxy)isoquinoline (17c). Compound **17c** was obtained starting from isosquinolin-3-ol **16b** (200.0 mg, 1.37 mmol) and 1,5-dibromopentane following the above-described procedure for **17a**. Compound **17c** was obtained as a yellow oil (49.8% yield). ¹H NMR (CDCl₃) δ 1.64 (m, 2H), 1.89 (m, 4H), 3.43 (t, 2H, *J* = 6.5 Hz), 4.34 (t, 2H, *J* = 6.3 Hz), 6.97 (s, 1H), 7.33 (m, 1H), 7.54 (t, 1H, *J* = 7.2 Hz), 7.66 (d, 1H, *J* = 8.3 Hz), 7.85 (d, 1H, *J* = 8.2 Hz), 8.92 (s, 1H). ESI-MS *m/z* 295 (100) [M + H]⁺, 146.

N-[4-[4-(3-Cyanophenyl)piperazin-1-yl]butyl]benzo[b]furan-2-carboxamide (5a). To a stirred solution of *N*-[4-(1-bromo)butyl]benzo[b]furan-2-carboxamide (**14a**) (50.0 mg, 0.17 mmol) in dry acetonitrile (3.0 mL), 1-(3-cyanophenyl)piperazine (31.7 mg, 0.17 mmol) and triethylamine (38.2 μL, 0.27 mmol) were added; the solution was refluxed for 12 h under stirring. The solvent was removed under reduced pressure, water was added, and the mixture was extracted with dichloromethane (3 × 10 mL). The organic layers were dried and concentrated and the crude product was chromatographed (10% MeOH in CHCl₃) to give 60.0 mg of **5a** (90.0% yield) as colorless oil. ¹H NMR (CDCl₃) δ 1.72 (m, 4H), 2.46 (t, 2H, *J* = 6.7 Hz), 2.61 (t, 4H, *J* = 4.9 Hz), 3.24 (t, 4H, *J* = 5.0 Hz), 3.52 (q, 2H, *J* = 6.1 Hz), 6.89 (br s, 1H), 7.09 (m, 3H), 7.36 (m, 5H), 7.66 (d, 1H, *J* = 7.5 Hz). FAB-MS *m/z* 403 [M + H]⁺, 147. Anal. (C₂₄H₂₆N₄O₂) C, H, N.

N-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl]isoquinoline-3-carboxamide (5b). To a stirred solution of *N*-[1-(4-bromo)butyl]isoquinoline-3-carboxamide (**14b**) (100.0 mg, 0.33 mmol) in

dry acetonitrile (10.0 mL), 1-(2,3-dichlorophenyl)piperazine hydrochloride (87.7 mg, 0.33 mmol) and triethylamine (74.0 μL, 0.53 mmol) were added and the solution was refluxed overnight under stirring. The solvent was removed under reduced pressure, water was added, and the mixture was extracted with dichloromethane (3 × 10 mL). The organic layers were dried and concentrated and the crude product was chromatographed (10% MeOH in CHCl₃) to give **5b** (60.2% yield) as a colorless oil. ¹H NMR (CDCl₃) δ 1.67 (m, 4H), 2.47 (t, 2H, *J* = 6.9 Hz), 2.64 (m, 4H), 3.06 (m, 4H), 3.57 (q, 2H, *J* = 6.1 Hz), 6.92 (m, 1H), 7.11 (m, 2H), 7.70 (m, 2H), 7.98 (t, 2H, *J* = 8.3 Hz), 8.33 (br s, 1H), 8.60 (s, 1H), 9.12 (s, 1H). ¹³C NMR (CDCl₃) δ 24.3, 27.6, 39.3, 51.3, 53.3, 58.0, 118.5, 120.1, 124.4, 127.3, 127.4, 127.5, 128.0, 128.7, 129.6, 130.9, 133.9, 136.0, 143.7, 150.9, 151.3, 164.7. ESI-MS *m/z* 457 [M + H]⁺, ESI-MS/MS of [M + H]⁺ 285, 227 (100). Anal. (C₂₄H₂₆Cl₂N₄O) C, H, N.

N-[4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl]isoquinoline-3-carboxamide (5c). The title compound was prepared starting from **14b** (190.0 mg, 0.62 mmol) and 1-(3-chlorophenyl)piperazine hydrochloride (144.0 mg, 0.62 mmol) following the above-described procedure for **5b**. Compound **5c** was obtained as a white solid (50.4% yield): mp (methanol) 156–157 °C. ¹H NMR (CDCl₃) δ 1.65 (m, 4H), 2.46 (t, 2H, *J* = 6.7 Hz), 2.60 (t, 4H, *J* = 4.9 Hz), 3.21 (t, 4H, *J* = 5.0 Hz), 3.57 (q, 2H, *J* = 6.5 Hz), 6.78 (m, 2H), 6.86 (d, 1H, *J* = 1.6 Hz), 7.14 (t, 1H, *J* = 8.0 Hz), 7.72 (m, 2H), 8.00 (t, 2H, *J* = 8.2 Hz), 8.33 (br s, 1H), 8.61 (s, 1H), 9.14 (s, 1H). ¹³C NMR (CDCl₃) δ 24.6, 27.9, 39.5, 48.7, 53.1, 58.2, 114.0, 115.9, 119.4, 120.4, 127.8, 128.3, 128.9, 129.8, 130.2, 131.2, 135.1, 136.2, 144.0, 151.2, 152.5, 165.0. ESI-MS *m/z* 445 (100) [M + Na]⁺, 423 [M + H]⁺, ESI-MS/MS of [M + H]⁺ 251, 227 (100). Anal. (C₂₄H₂₇ClN₄O) C, H, N.

N-[4-(3,4-Dihydro-6-methoxypyrazino[1,2-*a*]indol-2(1H)-yl)-butyl]isoquinoline-3-carboxamide (5d). To a suspension of 1,2,3,4-tetrahydro-6-methoxypyrazino[1,2-*a*]indole (**8**) (120.0 mg, 0.32 mmol) and K₂CO₃ (160.0 mg, 1.22 mmol) in dry acetonitrile (5.0 mL), bromo-derivative **14b** (98.0 mg, 0.32 mmol) and a catalytic amount of NaI were added and the resulting mixture was heated under reflux for 18 h. Thereafter the mixture was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was suspended in water (10.0 mL) and extracted with Et₂O (2 × 25 mL) and dichloromethane (1 × 25 mL). The combined organic layers were evaporated under reduced pressure, and the crude product was purified by means of flash chromatography (0.5% MeOH in CHCl₃) affording to **5d** as a yellow oil (55.0% yield). ¹H NMR (CDCl₃) δ 1.75 (m, 4H), 2.60 (m, 2H), 2.89 (t, 2H, *J* = 5.5 Hz), 3.57 (q, 2H, *J* = 6.1 Hz), 3.79 (s, 2H), 3.86 (s, 3H), 4.49 (t, 2H, *J* = 5.6 Hz), 6.12 (s, 1H), 6.53 (d, 1H, *J* = 7.6 Hz), 6.93 (t, 1H, *J* = 7.7 Hz), 7.10 (d, 1H, *J* = 7.9 Hz), 7.70 (m, 2H), 7.97 (m, 2H), 8.39 (br s, 1H), 8.59 (s, 1H), 9.05 (s, 1H). ESI-MS *m/z* 879 [2 M + Na]⁺, 451 [M + Na]⁺, 429 (100) [M + H]⁺. Anal. (C₂₆H₂₈N₄O₂ · 1/2 H₂O) C, H, N.

N-[4-(1,2,3,4-Tetrahydro-5-methoxy-β-carboline-2-yl)butyl]isoquinoline-3-carboxamide (5e). The title compound was obtained following the procedure described for **5d**, starting from 1,2,3,4-tetrahydro-5-methoxy-β-carboline (**13**) (94.0 mg, 0.55 mmol) and **14b** (137.0 mg, 0.45 mmol). Compound **5e** was obtained as a yellow oil (30.0% yield). ¹H NMR (CDCl₃) δ 1.74 (m, 4H), 2.64 (m, 2H), 2.82 (m, 2H), 3.03 (m, 2H), 3.57 (m, 4H), 3.86 (s, 3H), 6.44 (d, 1H, *J* = 7.5 Hz), 6.92 (m, 2H), 7.72 (m, 2H), 7.97 (m, 3H), 8.39 (br s, 1H), 8.59 (s, 1H), 9.07 (s, 1H). ESI-MS *m/z* 429 (100) [M + H]⁺, 256, 227. Anal. (C₂₆H₂₈N₄O₂) C, H, N.

N-[4-[4-(3-Cyanophenyl)piperazin-1-yl]butyl]3,4-dihydropyrazino[1,2-*a*]indol-1(2H)-one (5f). The title compound was prepared starting from **15** (190.0 mg, 0.59 mmol) and 1-(3-cyanophenyl)piperazine (110.3 mg, 0.59 mmol) following the same procedure described for compound **5a**. Compound **5f** was obtained as a colorless oil (60.0% yield). ¹H NMR (CD₃OD) δ 1.83 (m, 4H), 3.24 (m, 6H), 3.70 (m, 4H), 3.92 (m, 4H), 4.37 (m, 2H), 7.13 (m, 2H), 7.23 (m, 1H), 7.33 (m, 3H), 7.44 (m, 2H), 7.66 (m, 1H). ESI-MS *m/z* 428, (100) [M + H]⁺, 241, 199. Anal. (C₂₆H₂₉N₅O) C, H, N.

N-[4-[4-(2,4-Dichlorophenyl)piperazin-1-yl]butyl]3,4-dihydropyrazino[1,2-*a*]indol-1(2H)-one (5g). The title compound was prepared starting from bromoderivative **15** (200.0 mg, 0.63 mmol) and 1-(2,4-dichlorophenyl)piperazine (145.0 mg, 0.63 mmol) following the same procedure described for **5a**. Compound **5g** was obtained as a yellow oil (87.4% yield). ¹H NMR (CDCl₃) δ 1.68 (m, 4H), 2.51 (m, 2H), 2.66 (m, 4H), 3.05 (m, 4H), 3.65 (t, 2H, *J* = 7.1 Hz), 3.78 (t, 2H, *J* = 5.7 Hz), 4.28 (m, 2H), 6.94 (d, 1H, *J* = 8.4 Hz), 7.16 (m, 2H), 7.31 (m, 4H), 7.71 (d, 1H, *J* = 7.8 Hz). ESI-MS *m/z* 471, (100) [M + H]⁺, 285, 241, 199. Anal. (C₂₅H₂₈Cl₂N₄O·1/2H₂O) C, H, N.

N-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl]3,4-dihydropyrazino[1,2-*a*]indol-1(2H)-one (5h). The title compound was prepared starting from **15** (270.0 mg, 0.84 mmol) and 1-(2,3-dichlorophenyl)piperazine (193.0 mg, 0.84 mmol) following the same procedure described for **5a**. Compound **5h** was obtained as a white solid (78.2% yield): mp (methanol) 160–161 °C. ¹H NMR (CDCl₃) δ 1.65 (m, 4H), 2.46 (t, 2H, *J* = 7.2 Hz), 2.62 (m, 4H), 3.06 (m, 4H), 3.64 (t, 2H, *J* = 6.5 Hz), 3.78 (t, 2H, *J* = 6.0 Hz), 4.26 (t, 2H, *J* = 5.3 Hz), 6.93 (m, 2H), 7.14 (m, 3H), 7.31 (m, 2H), 7.70 (d, 1H, *J* = 8.0 Hz). ESI-MS *m/z* 472 (100) [M + H]⁺, 285, 241, 199, 172, 144. Anal. (C₂₅H₂₈Cl₂N₄O) C, H, N.

N-(4-(4-(*m*-Tolyl)piperazin-1-yl)butyl)benzo[*b*]furan-2-carboxamide (5i). To a stirred solution of **14a** (620.0 mg, 2.09 mmol) in dry acetonitrile (30.0 mL) under argon, 1-(*m*-tolyl)piperazine dihydrochloride (443.0 mg, 2.09 mmol) and triethylamine (620 μL, 4.60 mmol) were added and the solution was refluxed overnight under stirring. The solvent was removed under reduced pressure, water was added, and the mixture was extracted with dichloromethane (3 × 30 mL). The organic layers were dried and concentrated, and the crude product was chromatographed (6% MeOH in CHCl₃) to give 0.42 g of **5i** (52.0% yield) as white solid: mp (methanol) 119–120 °C. ¹H NMR (CDCl₃) δ 1.70 (m, 4H), 2.31 (s, 3H), 2.46 (t, 2H, *J* = 6.6 Hz), 2.62 (t, 4H, *J* = 4.9 Hz), 3.23 (t, 4H, *J* = 4.9 Hz), 3.52 (q, 2H, *J* = 6.1 Hz), 6.70 (m, 3H), 7.00 (br s, 1H), 7.13 (t, 1H, *J* = 4.4 Hz), 7.33 (m, 4H), 7.66 (d, 1H, *J* = 7.7 Hz). ¹³C NMR (CDCl₃) δ 22.0, 24.5, 27.7, 39.4, 49.3, 53.5, 58.1, 110.5, 111.9, 113.4, 117.1, 120.9, 122.9, 123.9, 127.0, 127.9, 129.2, 139.0, 149.2, 151.5, 154.9, 159.1. ESI-MS *m/z* 805 (100) [2 M + Na]⁺, 414 [M + Na]⁺, 392 [M + H]⁺. Anal. (C₂₄H₂₉N₃O₂) C, H, N.

7-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy]isoquinoline (5j). The title compound was prepared starting from **17a** (160.0 mg, 0.57 mmol) and 1-(2,3-dichlorophenyl)piperazine hydrochloride (153.4 mg, 0.57 mmol) following the procedure described for the synthesis of **5b**. Compound **5j** was obtained as a colorless oil (62.4% yield). ¹H NMR (CDCl₃) δ 1.85 (m, 4H), 2.50 (t, 2H, *J* = 7.4 Hz), 2.66 (m, 4H), 3.06 (t, 4H, *J* = 4.3 Hz), 4.16 (t, 2H, *J* = 6.0 Hz), 6.93 (m, 2H), 7.14 (m, 1H), 7.24 (m, 2H), 7.40 (d, 1H, *J* = 2.3 Hz), 7.68 (d, 1H, *J* = 8.9 Hz), 8.05 (d, 1H, *J* = 8.0 Hz), 8.81 (m, 1H). ESI-MS *m/z* 430 [M + H]⁺. Anal. (C₂₃H₂₅Cl₂N₃O) C, H, N.

3-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy]isoquinoline (5k). The title compound was prepared starting from **17b** (250.0 mg, 0.89 mmol) and 1-(2,3-dichlorophenyl)piperazine hydrochloride (237.0 mg, 0.89 mmol) following the procedure described for the synthesis of **5b**. Compound **5k** was obtained as a white amorphous solid (61.0% yield). ¹H NMR (CDCl₃) δ 1.81 (m, 4H), 2.52 (t, 2H, *J* = 7.3 Hz), 2.66 (m, 4H), 3.06 (m, 4H), 4.38 (t, 2H, *J* = 6.3 Hz), 6.94 (m, 2H), 7.13 (m, 2H), 7.36 (m, 1H), 7.56 (t, 1H, *J* = 7.3 Hz), 7.67 (d, 1H, *J* = 8.2 Hz), 7.87 (d, 1H, *J* = 8.1 Hz), 8.94 (s, 1H). ESI-MS *m/z* 452 [M + Na]⁺, 430 (100) [M + H]⁺. Anal. (C₂₃H₂₅Cl₂N₃O) C, H, N.

3-[5-[4-(2,3-Dichlorophenyl)piperazin-1-yl]pentyloxy]isoquinoline (5l). The title compound was prepared starting from **17c** (325.0 mg, 1.11 mmol) and 1-(2,3-dichlorophenyl)piperazine hydrochloride (295.0 mg, 1.11 mmol) following the procedure described for synthesis of **5b**. Compound **5l** was obtained as a white amorphous solid (54.3% yield), mp (methanol) 133–134 °C. ¹H NMR (CDCl₃) δ 1.53 (m, 4H), 1.86 (m, 2H), 2.42 (m, 2H), 2.61 (m, 4H), 3.03 (m, 4H), 4.32 (m, 2H), 6.92 (m, 2H), 7.10 (m, 2H), 7.32 (t, 1H, *J*

= 7.4 Hz), 7.52 (t, 1H, *J* = 7.5 Hz), 7.64 (d, 1H, *J* = 8.4 Hz), 7.83 (d, 1H, *J* = 8.2 Hz), 8.91 (s, 1H). ESI-MS *m/z* 466 [M + Na]⁺, 444 (100) [M + H]⁺, 299. Anal. (C₂₄H₂₇Cl₂N₃O) C, H, N.

3,4-Dihydro-2-[4-(3,4-dihydro-6-methoxypyrazino[1,2-*a*]indol-2(1H)-yl)butyl]pyrazino[1,2-*a*]indol-1(2H)-one (5m). The title compound was prepared starting from bromo-derivative **15** (47.7 mg, 0.15 mmol) and 1,2,3,4-tetrahydro-6-methoxypyrazino[1,2-*a*]indole **8** (30.0 mg, 0.15 mmol) following the procedure described for the synthesis of compound **5d**. Compound **5m** was obtained as a yellow oil (62.0% yield). ¹H NMR (CDCl₃) δ 1.69 (m, 4H), 2.58 (t, 2H, *J* = 6.6 Hz), 2.88 (t, 2H, *J* = 5.5 Hz), 3.66 (t, 2H, *J* = 6.7 Hz), 3.77 (m, 4H), 3.88 (s, 3H), 4.24 (t, 2H, *J* = 5.8 Hz), 4.45 (t, 2H, *J* = 5.6 Hz), 6.10 (s, 1H), 6.54 (d, 1H, *J* = 7.6 Hz), 6.93 (t, 1H, *J* = 7.8 Hz), 7.13 (m, 3H), 7.29 (m, 2H), 7.70 (d, 1H, *J* = 7.9 Hz). ¹³C NMR (CDCl₃) δ 24.3, 25.3, 29.6, 40.2, 45.5, 45.9, 46.1, 51.2, 51.7, 55.3, 57.2, 97.1, 101.8, 106.0, 109.5, 112.9, 120.6, 122.6, 124.3, 125.9, 127.5, 129.4, 130.3, 134.5, 136.3, 147.7, 159.9. ESI-MS *m/z* 907 [2 M + Na]⁺, 884 [2 M + H]⁺, 443 (100) [M + H]⁺. Anal. (C₂₇H₃₀N₄O₂·1/3H₂O) C, H, N.

Molecular Modeling. Molecular modeling calculations were performed on SGI Origin 200 8XR12000, while molecular modeling graphics were carried out on SGI Octane 2 and Octane workstations.

Homology Modeling. Originally we choose the bovine rhodopsin's crystal structures in the inactivated and partially activated states downloaded from the PDB data bank (<http://www.rcsb.org/pdb/>; PDB IDs: 1U19 and 2I37, respectively) as templates for the modeling of the 7TMs region of human D₂R and D₃R and human 5HT_{1A}R, 5HT_{2A}R, and 5HT_{2C}R. Subsequently, after the publication of the X-ray structures of the human β₂-adrenergic receptor, we also used this receptor as template, choosing the structure with the higher resolution³⁸ (PDB ID: 2RH1, *R* = 2.4 Å) for the modeling of dopaminergic and serotonergic receptors TMs in the partially inactivated state. Hereafter, we will refer to a common computational procedure applied for the generation of all receptor models in each activation state.

To be sure of correctly predicting the “7TMs” region of our receptor models, we first performed a secondary structure prediction test on the template sequences (i.e., bovine rhodopsin and β₂-adrenergic receptors) by using the Structure Prediction and Sequence Analysis (PredictProtein) server (<http://www.predictprotein.org/>). The correctness of the prediction was then verified against the X-ray structures. On the basis of the results obtained, D₂R, D₃R, 5-HT_{1A}R, 5-HT_{2A}R, and 5-HT_{2C}R sequences were subjected to the same PredictProtein server (<http://www.predictprotein.org/>) calculation. Accordingly, we modeled the 7TMs regions of the dopamine and serotonin receptors by aligning the following sequence portions of the templates (PDB numbering). Bovine rhodopsin: TM1 = 33–66, TM2 = 70–100, TM3 = 106–141, TM4 = 151–173, TM5 = 199–230, TM6 = 244–278, TM7 = 285–310; human β₂-adrenergic receptor: TM1 = 29–62, TM2 = 66–96, TM3 = 102–137, TM4 = 148–170, TM5 = 195–225, TM6 = 265–299, TM7 = 305–330, with the dopamine/serotonin receptors segments predicted to assume an α-helix secondary structure and to have a transmembranarian location.

Second, to be sure of properly aligning the sequences of the templates and of the receptors to be modeled, we performed an alignment test on the template sequences by using either Bioinfo (<http://cheminfo.u-strasbg.fr>) or ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2/>).

The ligands, 11-*cis*-retinal (1U19) and carazolol (2RH1), were removed using the *Unmerge* command in the Biopolymer module of Insight2005 (Accelrys, San Diego). A.car and an.mdf file were generated for the coordinates of the 7TMs region of each template. Template sequences were extracted from the 3D structures by using the *Extract* command in the *Sequence* pulldown of the Homology module (Insight2005) and manually aligned to that of each receptor subtype, taking into account the results obtained from: (a) PredictProtein (<http://www.predictprotein.org/>) analysis, (b) ConSeq (<http://conseq.bioinfo.tau.ac.il/>) analysis, (c) ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2/>) multiple alignment, (d) Bioinfo (<http://cheminfo.u-strasbg.fr>) multiple alignment, (e) mutagenesis studies,³⁵ and (f)

Eukaryotic Linear Motif server sequence analysis (<http://elm.eu.org/>) and protein structural domains analysis,⁶¹ aimed at identifying linear functional motifs and structural domains possibly involved in protein–protein interactions. The resulting multiple sequence alignment is reported in the Supporting Information.

The coordinates of the 7TMs for each model were assigned as follows. Boxes were created and frozen around each sequence segment of the model aligned with the corresponding TM of the template protein (Homology module; Insight2005). These sequence segments represented the putative 7TMs of the receptor models and were defined as structural conserved regions (SCR). Coordinates were assigned using the *SCR-AssignCoords* command of the *Sequence* pulldown. In particular, for template/model identical side chains, the coordinates for the whole amino acid were transferred. However, when the side chain of the model differed from that of the template, backbone coordinates were transferred while side chain atoms coordinates were assigned by aligning the side chain dihedral angles with the corresponding template residue. This was to preserve the conformation of the reference side chain as much as possible. Eventual bumps were removed by manually changing the torsion angles or by means of the *Manual_Rotamer* command of the *Residue* pulldown of the Homology module. Hydrogens were added to all amino acids considering a pH value of 7.2. The C- and N-terminus of each helix were capped with an aldehydic and an N_{sp2}H₂ group, respectively, to avoid unrealistic ionic contacts.

The receptor model optimization procedure was carried out by using the Discover3 module of Insight2005. Every structure was progressively relaxed (Cell Multipole summation method for nonbond interactions,⁶² $\epsilon = 4r$, CVFF force field).⁶³ First, an initial optimization of side chain interactions was performed, keeping the backbone atoms fixed (algorithms: Steepest Descent until the maximum rms derivative was less than 0.5 kcal/Å, Conjugate Gradient from a derivative of 0.5 to less than 0.1 kcal/Å). Subsequently, the backbone was relaxed through six minimization cycles by using the above-reported combination of Steepest Descent and Conjugate Gradient algorithms and applying different tether force values for each minimization cycle: from an initial value of 5 kcal/Å² to a final value of 0.1 kcal/Å². A final energy minimization round was performed with a residual tether force of 0.1 Kcal/Å² applied only to backbone Cα atoms. Each energy minimization cycle was followed by a structural check by using the *Struct_Check* command of the *ProStat* pulldown in the Homology module to verify the correctness of the geometry optimization procedure before moving to the next minimization cycle. Checks included φ , Ψ , χ_1 , χ_2 , χ_3 , and ω dihedral angles, Cα virtual torsions, and Kabsch and Sander main chain H-bond energy evaluation. A full unconstrained geometry optimization has been attempted but produced unrealistic results considering that the system upon study is lacking in structural elements (i.e., loops, lipid bilayer, water molecules, etc.).

Conformational Analysis for Compounds 5a–m. Compounds 5a–m were built using the Insight2005 Builder module. The apparent pK_a values were estimated using the ACD/pK_a DB version 11.00 software (Advanced Chemistry Development Inc., Toronto, Canada). According to the resulting values (Table 2 of the Supporting Information), in all subsequent calculations, all compounds were considered protonated at pH = 7.2 (cytoplasmic value) on the aliphatic nitrogen of the piperazine ring. Partial charges were assigned by using the CFF91 force field.⁶⁴

The conformational space of compounds 5a–m was sampled through 200 cycles of simulated annealing. An initial temperature of 1000 K was applied to the system for 1000 fs with the aim of surmounting torsional barriers. Successively, temperature was linearly reduced to 200 K with a decrement of 0.5 K/fs. The resulting structures were subjected to two protocols of energy minimization within Insight2005 Discover module (CFF91 force field, Conjugate Gradient algorithm; maximum rms derivative < 0.001 kcal/Å), which differed in the dielectric constant value, i.e. $\epsilon = 80r$ and $\epsilon = 1$, in order to maximize inter- or intramolecular interactions, respectively. All the resulting conformers were subsequently ranked in different families taking into account their conformational energy as well as some geometric parameters, such

as: (a) the distance between the centroid of the phenyl ring at the tail and the centroid of the heteroaromatic ring at the head, (b) the orientation of the piperazine nitrogen hydrogen with respect to the position of the aromatic rings, (c) the conformation of the piperazine ring (i.e., *chair*, *half-chair*, and *boat*), (d) the dihedral angle value between the heteroatom of the heteroaryl moiety and the heteroatom X (amid or ether oxygen) of the linker, (e) the rotation of the phenyl ring with respect to the piperazine ring (i.e., dihedral angle value), and (f) the configuration of the amide group (i.e., *cis* or *trans*).

To properly analyze the electronic properties of the compounds, the most stable conformer of each family, within the range of 5 kcal/mol from the global minimum conformer ($\Delta E_{GM} < 5$ kcal/mol), was subjected to a full geometry optimization through semiempirical calculations, using the quantum mechanical method AM1 in the Mopac2007 package.⁶⁵ The EF (eigenvector Following routine) algorithm of geometry optimization was used, with a GNORM value set to 0.01. The max step size parameter was set to 0.05 to tackle the convergence problems and to properly reach a full geometry optimization. With the same aim, the criteria for terminating all optimizations was increased by a factor of 100 using the keyword PRECISE. All the resulting conformers were subsequently ranked in different families using the same energy and geometry criteria reported above.

The dipole moments were calculated using partial charges obtained by the quantum mechanical method AM1 (Mopac2007) and visualized as vector by using the Decipher module of Insight2005.

Pharmacology. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U. suppl. 40, 18 Febbraio 1992, Circolare no. 8, G.U. Fourteen Luglio 1994) and international laws and policies (EEC Council Directives 86/609, OJL 358, 1, Dec. 12, 1987; Guide for the Care and Use of laboratory Animals, U.S. National Research Council, 1996).

1. In Vitro Binding Assays on GPCRs.^{22a,b,23} **Serotonin and Dopamine Receptors.** Male CRL:CD(SD)BR-COBS rats and male CRL:(HA) BR albino guinea pig (Charles River, Italy) were killed by decapitation; their brains were rapidly dissected into the various areas (rat striatum for D₁R and D₂R, rat hippocampus for 5-HT_{1A}R, rat cortex for 5-HT_{2A}R and guinea pig cortex for 5-HT_{2C}R) and stored at – 80 °C until assay. Binding was calculated on rat tissue homogenates (D₁, D₂, 5-HT_{2A}, and 5-HT_{2C} receptors) and on Sf9 cell membranes (D₃). Tissues were homogenized in about 50 volumes of ice-cold Tris HCl, 50 mM, pH 7.4 (for D₁R, D₂R, and 5-HT_{2A}R) using an Ultra-Turrax TP-1810 homogenizer (2 × 20 s) and centrifuged at 50000g for 10 min at 4 °C (Beckman J-25 centrifuge). Each pellet was resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min, and centrifuged again at 50000g for 10 min at 4 °C. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The resulting pellets were resuspended just before the binding assay in the appropriate incubation buffer 50 mM Tris HCl, pH 7.4, containing 10 μM pargyline, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ for D₁R and D₂R; 50 mM Tris HCl, pH 7.4, containing 10 μM pargyline and 4 mM CaCl₂ for 5-HT_{1A}R; 50 mM Tris HCl, pH 7.7 for 5-HT_{2A}R; 50 mM Tris HCl, pH 7.4, containing 10 μM pargyline, 4 mM CaCl₂; and 0.1% ascorbic acid for 5-HT_{2C}R). For D₃ receptors, Sf9 cells membranes expressing D₃ dopamine receptors (Signal Screen) were resuspended just before the binding assay in 50 mM Tris HCl, pH 7.4, containing 5 mM EDTA, 5 mM MgCl₂, 5 mM KCl, 1.5 mM CaCl₂, 120 mM NaCl.

[³H]-SCH 23390 (specific activity, 75.5 Ci/mmol; NEN) binding to D₁R was assayed in a final incubation volume of 0.5 mL, consisting of 0.25 mL of membrane suspension (striatum 2.5 mg of tissue/sample), 0.25 mL of [³H]ligand (0.4 nM) and 10 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 10 μM (–)-*cis*-flupentixol.

[³H]-Spiperone (specific activity, 25 Ci/mmol; NEN) binding to D₂R was assayed in a final incubation volume of 1 mL, consisting

of 0.5 mL of membrane suspension (striatum 2.5 mg of tissue/sample), 0.5 mL of [^3H]ligand (0.2 nM), and 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 100 μM (–)sulpiride.

[^3H]-7-OH-DPAT (specific activity, 159 Ci/mmol; Amersham) binding to $\text{D}_{3\text{r}}$ receptors was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (12 μg protein/sample), 0.5 mL of [^3H]ligand (0.7 nM), and 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 1 μM dopamine.

[^3H]-8-OH-DPAT (specific activity, 217 Ci/mmol; Amersham) binding to 5-HT $_{1\text{A}}$ R was assayed in a final incubation volume of 0.5 mL, consisting of 0.25 mL of membrane suspension (5 mg tissue/sample), 0.25 mL of [^3H]ligand (1 nM), and 10 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 10 μM serotonin.

[^3H]-Ketanserin (specific activity, 88 Ci/mmol; Amersham) binding to 5-HT $_{2\text{R}}$ was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (5 mg of tissue/sample), 0.5 mL of [^3H]ligand (0.7 nM), and 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 1 μM methysergide.

[^3H]-Mesulergine (specific activity, 90 Ci/mmol; Amersham) binding to 5-HT $_{2\text{C}}$ R was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (30 mg of tissue/sample), 0.5 mL of [^3H]ligand (1 nM), and 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 10 μM mesulergine.

Incubations (15 min at 25 $^{\circ}\text{C}$ for $\text{D}_{1\text{R}}$, 15 min at 37 $^{\circ}\text{C}$ for $\text{D}_{2\text{R}}$ and 5-HT $_{2\text{A}}$ R, 30 min at 25 $^{\circ}\text{C}$ for 5-HT $_{1\text{A}}$ R, 60 min at 25 $^{\circ}\text{C}$ for $\text{D}_{3\text{r}}$ receptors, 30 min at 37 $^{\circ}\text{C}$ for 5-HT $_{2\text{C}}$ R) were stopped by rapid filtration under vacuum through GF/B (for $\text{D}_{1\text{R}}$, $\text{D}_{2\text{R}}$, 5-HT $_{1\text{A}}$ R, 5-HT $_{2\text{A}}$ R, and 5-HT $_{2\text{C}}$ R) or GF/B presoaked with 0.3% polyethyleneimine for $\text{D}_{3\text{r}}$ filters which were then washed with 12 mL (4 mL $\times$ 3 times) of ice-cold buffer (50 mM Tris HCl, pH 7.4) using a Brandel M-48R cell harvester. The radioactivity trapped on the filters was counted in 4 mL of Ultima Gold MV (Packard) in a β counter (Wallac) with a counting efficiency of 50%. For all binding assays the radioactivity trapped on the filters was counted in 4 mL of Ultima Gold MV (Packard) in a LKB 1214 rack beta liquid scintillation spectrometer with a counting efficiency of 50%.

Histamine H_1 Receptor. Histamine H_1 receptor. Binding affinity was tested according to the procedure of Dini et al.⁶⁶ Binding was determined using membranes prepared from guinea pig cerebellum with [^3H]pyrilamine (0.5 nM) as radioligand.

Adrenergic α_1 Receptors. Binding was determined using membranes prepared from rat cerebral cortex homogenized in phosphate buffer. The homogenate was centrifuged at 44000g for 10 min, and the pellet was suspended in phosphate buffer and washed two times more; 500 μL of this homogenate was incubated with 500 μL of 0.2 nM [^3H]prazosin and 20 μL of test compound for 30 min at 25 $^{\circ}\text{C}$ and then filtered through a Whatman GF/B filter (Whatman International Ltd.). Radioactivity on the filter was measured with a liquid scintillation counter. Complete (100%) inhibition of [^3H]prazosin binding was determined in the presence of 10 μM prazosin. Nonspecific binding was determined in the presence of 10 μM prazosin.

Adrenergic α_2 Receptors. Binding was determined using membranes prepared from rat cerebral cortex homogenized in phosphate buffer. The homogenate was centrifuged at 44000g for 10 min, and the pellet was suspended in phosphate buffer and washed two times more; 500 μL of this homogenate was incubated with 500 μL of 1 nM [^3H]clonidine and 20 μL of test compound for 30 min at 25 $^{\circ}\text{C}$ and then filtered through a Whatman GF/B filter (Whatman International Ltd.). Nonspecific binding was determined in the presence of 10 μM clonidine.

Imidazole I_2 Receptors. Rabbit kidney was homogenized in 10 volumes of Tris-HCl buffer (50 mM, pH 7.4) and 250 mM sucrose and centrifuged at 500g for 10 min. The supernatant was centrifuged at 28000g for 30 min, and the resulting pellet was washed twice with the same buffer without sucrose. The final pellet was

resuspended in Tris-HCl buffer (50 mM, pH 7.4) and stored at -80°C until use. Rabbit kidney membranes (200 μg of protein) were incubated with 5 nM [^3H]idazoxan (Amersham, 43 Ci/mmol) in the absence or presence of a range of 10–12 concentrations of competing ligand drug in a total volume of 400 μL of assay buffer. To mask adrenoreceptors, 10 μM (–)norepinephrine (in the presence of 0.005% ascorbic acid) was added to all tubes. Nonspecific binding was determined with 10 μM of cirazoline. Specific binding represented about 90% of the total binding at 5 nM [^3H]idazoxan. Following equilibrium (45 min at 25 $^{\circ}\text{C}$), bound radioactivity was separated from free by filtration as described above. Each point was performed in triplicate.

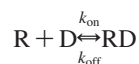
Dose–inhibition curves were analyzed by the Allfit⁶⁷ program to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding. The K_i values were derived from the IC_{50} values according to the method of Cheng and Prusoff.⁶⁸

2. In Vitro Assays for hERG Affinity Evaluation. Plasmids. The human ERG and KCNE1 (potassium voltage-gated channel, Isk-related family, member 1) were subcloned into the mammalian expression vectors pNS1n and pNS1z, respectively, to produce the plasmid constructs pNS1n_hERG and pNS1z-minK.

HEK 293 cells stably expressing hERG + KCNQ1 (potassium voltage-gated channel, KQT-like subfamily, member 1). HEK 293 tissue culture cells were transfected with equal amounts of the plasmids pNS1n_hERG and pNS1z-minK using lipofection (Lipofectamin, Life Technologies). Expression of functional hERG channels was verified by patch-clamp measurements.

Whole-Cell Recordings. Cells plated on coverslips were placed in a 15 μL perfusion chamber (flowrate ~ 1 mL/min = full exchange every 1 s). All experiments were performed at room temperature (20–22 $^{\circ}\text{C}$) using EPC-9 patch-clamp amplifiers (HEKA-electronics, Lambrecht, Germany). Series resistances as well as capacitance compensation were updated before each stimulus. Usually the cell capacitance ranged from 5 to 20 pF and the series resistances were in the range 3–6 M Ω .

Fitting Procedure. We assumed that the drugs (D) interacted with the receptors (R) in the following way:



This is a simple bimolecular reaction, which integrated under nonequilibrium conditions are described by equation 1:

$$I_t = I_0(1 - (C/C + K_i * (1 - \exp^{-t(C*k_{\text{on}} + k_{\text{off}})}))) \quad (1)$$

Where I_t = current at time t in nA, I_0 = unblocked current in nA, C = drug concentration in M, k_{off} = off-rate in s^{-1} , k_{on} = on-rate in $\text{M}^{-1}\text{s}^{-1}$, $K_i = k_{\text{off}}/k_{\text{on}}$. At $t = \infty$ eq 1 simplifies to Michaelis–Menten equation with $K_i = \text{IC}_{50}$.

The peak tail-current was plotted versus time. The blocker-induced decrease in current versus time was fitted to eq (1) to give $k_{\text{on}}*k_{\text{off}}$ and thereby k_i . Each value is the mean $\pm$ SD of 2–8 determinations and represents μM values.

3. Experimental In Vitro Pharmacology for Intrinsic Activity Assessment. 3.1. Cell-Based Assays. Dopamine $\text{D}_{2\text{L}}$ Receptors.

For evaluation of [^{35}S]-GTP γS binding to $\text{D}_{2\text{L}}$ receptors, human CHO cells Chinese hamster ovary in DMSO (0.4%) vehicle were resuspended in Hepes 20 mM, pH 7.4, containing 100 mM NaCl, 1 mM EDTA, 10 mM MgCl_2 , 1 mM DTT, in a glass/Teflon potter. The binding assay was carried out in a final incubation volume of 120 μL , consisting of 100 μL of membrane suspension (about 15 μg of protein/sample), 10 μL of [^{35}S]-GTP γS (final concentration 1 nM), and 10 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 10 μM GTP γS ; samples were preincubated for 15 min at 30 $^{\circ}\text{C}$ without [^{35}S]-GTP γS and then for 15 min at 30 $^{\circ}\text{C}$ with [^{35}S]-GTP γS .

Dopamine $\text{D}_{2\text{S}}$ Receptors. For evaluation of [^{35}S]-GTP γS binding to $\text{D}_{2\text{S}}$ receptors, human CHO cells Chinese hamster ovary in DMSO (1%) vehicle were resuspended in Hepes 20 mM, pH 7.4, containing 100 mM NaCl, 1 mM EDTA, 10 mM MgCl_2 , 1 mM DTT, in a glass/Teflon potter. The binding assay was carried

out in a final incubation volume of 120 μ L, consisting of 100 μ L of membrane suspension (about 15 μ g of protein/sample), 10 μ L of [³⁵S]-GTP γ S (final concentration 1 nM), and 10 μ L of displacing agent or solvent.

Nonspecific binding was obtained in the presence of 10 μ M GTP γ S; samples were preincubated for 15 min at 30 °C without [³⁵S]-GTP γ S and then for 30 min at 30 °C with [³⁵S]-GTP γ S.

Dopamine D₃ Receptors. For evaluation of [³⁵S]-GTP γ S binding to D₃ receptors, human CHO-K1 cells Chinese hamster ovary in DMSO (1%) vehicle were resuspended in Hepes 20 mM, pH 7.4, containing 100 mM NaCl, 1 mM EDTA, 10 mM MgCl₂, 1 mM DTT, in a glass/Teflon potter. The binding assay was carried out in a final incubation volume of 120 μ L, consisting of 100 μ L of membrane suspension (about 15 μ g of protein/sample), 10 μ L of [³⁵S]-GTP γ S (final concentration 1 nM), and 10 μ L of displacing agent or solvent.

Nonspecific binding was obtained in the presence of 10 μ M GTP γ S; samples were preincubated for 15 min at 30 °C without [³⁵S]-GTP γ S and then for 30 min at 30 °C with [³⁵S]-GTP γ S.

3.2. Tissue Assays. Serotonin 5-HT_{1A} Receptors. For 5-HT_{1A}R intrinsic activity assessment, Duncan Hartley guinea pig ileum 325 $\pm$ 25 g were taken in DMSO (0.1%) vehicle and were put in the incubation buffer (Krebs, pH 7.4) and were incubated 5 min at 32 °C. The final volume of the bath was brought to 10 mL. After 5 min, the quantitation method was isometric (gram changes). Significant criteria: agonism $\geq$ 50% reduction of neurogenic twitch relative to 0.12 μ M 8-OHDPAT response. Significant criteria: antagonism $\geq$ 50% inhibition of 0.12 μ M 8-OHDPAT-induced relaxation.

Serotonin 5-HT_{2A} Receptors. For 5-HT_{2A}R intrinsic activity assessment, Wistar rat 275 $\pm$ 25 g aortic ring was taken in DMSO (0.1%) vehicle and was put in the incubation buffer (Krebs at pH 7.4) and was incubated 5 min at 37 °C. The final volume of the bath was brought to 10 mL. After 5 min, the quantitation method was isometric (gram changes). Significant criteria: agonism $\geq$ 50% contraction response relative to 3 μ M 5-HT response. Significant criteria: antagonism $\geq$ 50% inhibition of 3 μ M 5-HT induced contraction.

4. Behavioral Tests. All experimental procedures carried out in this study were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Danish Animal Welfare Committee, appointed by the Danish Ministry of Justice.

Male Wistar rats (200–225 gr) or female NMRI mice (Taconic M&B, P.O. Box 1079, DK-8680 Ry, Denmark) kept in a ventilated closed rack (Scantainer, Scanbur Ltd., Denmark) at constant temperature (21 °C) and humidity (60–70%) with a 7:00 a.m. light/7:00 p.m. dark cycle were used in these studies. Food (Altromin rat pellets) and water were available ad libitum. Experiments were conducted during daytime in the light phase. The animals were habituated to the experimental room approximately 24 h before start of the experiments.

5c, 5h, 5i, 5l. Aripiprazole (Sequoia Research Products Ltd.) and Clozapine (RBI) were dissolved in 10% Tween 80 and diluted to final concentration by adding appropriate volume of 0.9% NaCl. Haloperidol (Seranase, Janssen-Cilag, ampules of 5 mg/mL), S-(+)-Methamphetamine-HCl (RBI) and Phencyclidine (PCP, synthesized at NeuroSearch A/S) were dissolved in 0.9% NaCl. All test- and reference compounds were administered subcutaneously (sc) 30 min before test start in a dose volume of 10 mL/kg. MAMP and PCP were dosed intraperitoneally (ip) and sc, respectively, and administered in a dose volume of 10 mL/kg immediately before test start. Appropriate doses of **5c**, **5h**, **5i**, and **5l** were chosen based on initial behavioral observations.

Exploratory Locomotor Activity. Exploratory locomotor activity was assessed in 14 automated activity frames (TSE Home Cage Activity Monitoring System, MoTil, TSE Technical and Scientific Equipment GmbH, Germany) equipped with infrared photobeam emitters and sensors. To assess drug effect on exploratory locomotor activity, the mice were transferred to new home cages immediately before test start and activity was measured for 30 min. Test or

reference compounds were administered 30 min before test start at the following doses: **5c**, **5h**, and **5l**: 3, 10, 30 mg/kg; **5i**: 1, 3, 10 mg/kg; aripiprazole: 0.1, 0.3, 1.0, 3.0 mg/kg; clozapine: 0.3, 1, 3 mg/kg; haloperidol: 0.025, 0.05, 0.1, 0.2 mg/kg.

MAMP- and PCP-Induced Hyperactivity. MAMP- and PCP-induced hyperactivity was measured in automated activity frames as described above. MAMP and PCP were administered in a dose of 2 and 4 mg/kg, respectively. Both compounds were dosed immediately before test start. In the MAMP study, test or reference compounds were pretreated 30 min before test start at the following doses: **5h**, **5c**, **5i**, and **5l**: 1, 3, and 10 mg/kg; aripiprazole: 0.1, 0.3, and 1.0 mg/kg; clozapine: 0.3, 1, and 3 mg/kg; haloperidol: 0.025, 0.05, and 0.1 mg/kg. Locomotor activity was measured for a total of 120 min. In the PCP study, test or reference compounds were administered 30 min before test start at the following doses: **5h**: 0.3, 1, 3 mg/kg; **5c**, **5i**, and **5l**: 1, 3, and 10 mg/kg; aripiprazole: 0.1, 0.3, and 1.0 mg/kg; clozapine: 0.3, 1.0, and 3.0 mg/kg; haloperidol: 0.01, 0.025, and 0.05 mg/kg. Locomotor activity was measured for 60 min.

Catalepsy. Cataleptogenic potential of the compounds was assessed in the horizontal bar test consisting of a horizontal bar with a diameter of 2 mm and elevated 4 cm above ground. Test or reference compound was administered sc 30 min before the first catalepsy assessment. Hereafter, the degree of catalepsy was scored every 15 min for a total of 45 min.

Statistics. Drug effects on exploratory and stimulant-induced locomotor activity were analyzed by applying a two-way ANOVA with drug effect and time interval as factors. Drug-induced catalepsy was evaluated by one-way ANOVA with treatment as factor. *P* values < 0.05 were considered statistically significant.

5. Fos Induction After Acute Administration of 5i. Rats (*n* = 5 pr group) were injected i.p. with a single dose of **5i** (3, 10, 15, or 30 mg/kg) or vehicle (5% Chremophor) and returned to their home-cage. One hour after drug administration, the rat was deeply anesthetized with mebumal (50 mg/mL, 3 mL/kg) and perfused transcardially with 0.1 M phosphate buffered saline (PBS; pH = 7.4) followed by fixation in 4% paraformaldehyde-PBS for 10 min.⁶⁹ Then 40 μ m serial coronal sections were cut through the forebrain in series of four and processed for c-Fos immunoreactivity according to earlier report.⁶⁹ Prior to the immunocytochemical steps, the sections were rinsed for 3 $\times$ 10 min in 0.01 M PBS, incubated for 10 min in 1% H₂O₂–PBS to block endogenous peroxidase activity, and for a minimum of 20 min in 0.01 M PBS with 0.3% Triton X-100 (TX), 5% swine serum, and 1% bovine serum albumin (BSA) to block nonspecific binding sites. The sections were then incubated at 4 °C for 24 h in the primary antiserum diluted 1:4000 in 0.01 M PBS with 0.3% Triton X-100 and 1% BSA. The primary polyclonal antiserum (1:4000) was generated in a rabbit against the N-terminal peptide similar to amino acids 2–17 of the rat c-Fos protein in our laboratory and characterized previously.⁷⁰ After incubation in primary antiserum, immunoreactivity was detected by means of the avidin–biotin method using diaminobenzidine as chromagen as previously described.⁶⁹ The number of c-Fos positive cells were counted by means of light microscopy (20 $\times$ magnification) using a counting grid (500 μ m $\times$ 500 μ m) placed over either the shell of nucleus accumbens (ACCshell), core of nucleus accumbens (ACCcore), or dorsolateral part of the rostral striatum (DLstriatum) (Figure 4) by an observer blind to treatment regimens. The number of c-Fos positive cells was averaged from two adjacent sections of each animal, and statistical analysis was performed on group means $\pm$ SEM using a one-way ANOVA with Newman–Keul's posthoc test.

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Supporting Information Available: Multiple sequence alignment of the 7TMs region of bovine rhodopsin, human β_2 -adrenergic receptors, between them and with the 7TMs region modelled of human D₂R, D₃R, 5-HT_{1A}R, 5-HT_{2A}R, and 5HT_{2C}R, percentage of neutral and protonated form on the aliphatic nitrogen of the

piperazine ring at pH = 7.2 of compounds **5a–m** accordingly to apparent pK_a value estimated with ACD/ pK_a DB, experimental procedure for the synthesis of compounds **10–12**, and elemental analysis data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Practice guideline for the treatment of patients with schizophrenia. American Psychiatric Association. *Am. J. Psychiatry* **1997**, *154*, 1–63.
- (2) Carlsson, A. The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* **1988**, *1*, 179–186.
- (3) Meltzer, H. Y. Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. *Psychopharmacology (Berlin)* **1989**, *99*, S18–S27.
- (4) Campbell, M.; Young, P. I.; Bateman, D. N.; Smith, J. M.; Thomas, S. H. The use of atypical antipsychotics in the management of schizophrenia. *Br. J. Clin. Pharmacol.* **1999**, *47*, 13–22.
- (5) Koller, E. A.; Doraiswamy, P. M. Olanzapine-associated diabetes mellitus. *Pharmacotherapy* **2002**, *22*, 841–852.
- (6) Geddes, J.; Freemantle, N.; Harrison, P.; Bebbington, P. Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. *Br. Med. J.* **2000**, *321*, 1371–1376.
- (7) McGawin, J. K.; Goa, K. L. Aripiprazole. *CNS Drugs* **2002**, *16*, 779–786.
- (8) Buckley, P. F. Aripiprazole: Efficacy and Tolerability Profile of a Novel-Acting Atypical Antipsychotic. *Drugs Today* **2003**, *2*, 145–151.
- (9) Butini, S.; Campiani, G.; De Angelis, M.; Fattorusso, C.; Nacci, V.; Fiorini, I. Novel antipsychotic agents: recent advances in the drug treatment of schizophrenia. *Expert Opin. Ther. Patents* **2003**, *13*, 425–448.
- (10) Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* **2005**, *48*, 6523–6543.
- (11) Zhong, P.; Yuen, E. Y.; Yan, Z. Modulation of Neuronal Excitability by Serotonin–NMDA Interactions in Prefrontal Cortex. *Mol. Cell. Neurosci.* **2008**, *38*, 290–299.
- (12) (a) Mason, S. L.; Reynolds, G. P. Clozapine has submicromolar affinity for 5-HT_{1A} receptors in human brain tissue. *Eur. J. Pharmacol.* **1992**, *221*, 397–398. (b) Elliott, J.; Reynolds, G. P. Agonist-stimulated GTP γ [³⁵S] binding to 5-HT_{1A} receptors in human post-mortem brain. *Eur. J. Pharmacol.* **1999**, *386*, 313–315. (c) Richelson, E.; Souder, T. Binding of antipsychotic drugs to human brain receptors. Focus on newer generation compounds. *Life Sci.* **2000**, *68*, 29–39.
- (13) Yoshida, K.; Sugita, T.; Higuchi, H.; Hishikawa, Y. Effect of tandospirone on tardive dyskinesia and parkinsonian symptoms. *Eur. Psychiatry* **1998**, *13*, 421–422.
- (14) Christoffersen, C. L.; Meltzer, L. T. Reversal of haloperidol-induced extrapyramidal side effects in cebus monkeys by 8-hydroxy-2-(di-*N*-propylamino)tetralin and its enantiomers. *Neuropsychopharmacology* **1998**, *18*, 399–402.
- (15) Millan, M. J. Improving the treatment of schizophrenia: focus on serotonin 5-HT_{1A} receptors. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 853–861.
- (16) Marona-Lewicka, D.; Nichols, D. E. Aripiprazole (OPC-14597) fully substitutes for the 5-HT_{1A} receptor agonist LY293284 in the drug discrimination assay in rats. *Psychopharmacology (Berlin)* **2004**, *172*, 415–421.
- (17) Jordan, S.; Koprivica, V.; Chen, R.; Tottori, K.; Kikuchi, T.; Altar, C. A. The antipsychotic aripiprazole is a potent, partial agonist at the human 5-HT_{1A} receptor. *Eur. J. Pharmacol.* **2002**, *441*, 137–140.
- (18) Mauler, F.; Fahrig, T.; Horvath, E.; Jork, R. Inhibition of evoked glutamate release by the neuroprotective 5-HT_{1A} receptor agonist BAY X 3702 in vitro and in vivo. *Brain Res.* **2001**, *888*, 150–157.
- (19) Meltzer, H. Y.; Sumiyoshi, T. Does stimulation of 5-HT_{1A} receptors improve cognition in schizophrenia. *Behav. Brain Res.* **2008**, *195*, 98–102.
- (20) (a) Schwartz, J. C.; Diaz, J.; Pilon, C.; Sokoloff, P. Possible implications of the dopamine D₃ receptor in schizophrenia and in antipsychotic drug actions. *Brain Res. Brain Res. Rev.* **2000**, *31*, 277–287. (b) Joyce, J. N.; Millan, M. J. Dopamine D₃ receptor antagonists as therapeutic agents. *Drug Discovery Today* **2005**, *10*, 917–925.
- (21) Millan, M. J.; Loiseau, F.; Dekeyne, A.; Gobert, A.; Flik, G.; Cremers, T. I.; Rivet, J. M.; Sicard, D.; Billiras, R.; Brocco, M. S33138 (*N*-[4-[2-[(3a*S*,9*bR*)-8-cyano-1,3a,4,9*b*-tetrahydro[1] benzopyrrolo[3,4-*c*]pyrrol-2(3*H*)-yl]-ethyl]phenyl]-acetamide), a preferential dopamine D₃ versus D₂ receptor antagonist and potential antipsychotic agent: III. Actions in models of therapeutic activity and induction of side effects. *J. Pharmacol. Exp. Ther.* **2008**, *324*, 1212–1226.
- (22) (a) Campiani, G.; Nacci, V.; Bechelli, S.; Ciani, S. M.; Garofalo, A.; Fiorini, I.; Wikstrom, H.; de Boer, P.; Liao, Y.; Tepper, P. G.; Cagnotto, A.; Mennini, T. New Antipsychotic Agents with Serotonin and Dopamine Antagonist Properties Based on a Pyrrolo[2,1-*b*][1,3]benzothiazepine Structure. *J. Med. Chem.* **1998**, *41*, 3763–3772. (b) Campiani, G.; Butini, S.; Gemma, S.; Nacci, V.; Fattorusso, C.; Catalanotti, B.; Giorgi, G.; Cagnotto, A.; Goegan, M.; Mennini, T.; Minetti, P.; Di Cesare, M. A.; Mastroianni, D.; Scafetta, N.; Galletti, B.; Stasi, M. A.; Castorina, M.; Pacifici, L.; Ghirardi, O.; Tinti, O.; Carminati, P. Pyrrolo[1,3]benzothiazepine atypical antipsychotic agents. Synthesis, structure–activity relationships, molecular-modelling, and biological studies. *J. Med. Chem.* **2002**, *45*, 344–359. (c) Campiani, G.; Butini, S.; Fattorusso, C.; Catalanotti, B.; Gemma, S.; Nacci, V.; Morelli, E.; Cagnotto, A.; Mereghetti, I.; Mennini, T.; Carli, M.; Minetti, P.; Di Cesare, M. A.; Mastroianni, D.; Scafetta, N.; Galletti, B.; Stasi, M. A.; Castorina, M.; Pacifici, L.; Veretichy, M.; Di Serio, S.; Ghirardi, O.; Tinti, O.; Carminati, P. Pyrrolo[1,3]benzothiazepine-based serotonin and dopamine receptor antagonists. Molecular modeling, further structure–activity relationship studies, and identification of novel atypical antipsychotic agents. *J. Med. Chem.* **2004**, *47*, 143–157. (d) Campiani, G.; Butini, S.; Fattorusso, C.; Trotta, F.; Gemma, S.; Catalanotti, B.; Nacci, V.; Fiorini, I.; Cagnotto, A.; Mereghetti, I.; Mennini, T.; Minetti, P.; Di Cesare, M. A.; Stasi, M. A.; Di Serio, S.; Ghirardi, O.; Tinti, O.; Carminati, P. Novel atypical antipsychotic agents: rational design, an efficient palladium-catalyzed route, and pharmacological studies. *J. Med. Chem.* **2005**, *48*, 1705–1708.
- (23) Campiani, G.; Butini, S.; Trotta, F.; Fattorusso, C.; Catalanotti, B.; Aiello, F.; Gemma, S.; Nacci, V.; Novellino, E.; Stark, J. A.; Cagnotto, A.; Fumagalli, E.; Carnovali, F.; Cervo, L.; Mennini, T. Synthesis and pharmacological evaluation of potent and highly selective D₃ receptor ligands: inhibition of cocaine-seeking behavior and the role of dopamine D₃/D₂ receptors. *J. Med. Chem.* **2003**, *46*, 3822–3839.
- (24) Suzuki, H.; Gyoutoku, H.; Yokoo, H.; Shinba, M.; Sato, Y.; Yamada, H.; Murakami, Y. Unexpected formation of quinolone derivatives in Reissert indole synthesis. *Synlett* **2000**, *8*, 1196–1199.
- (25) Rajur, S. B.; Merwade, A. Y.; Flendi, S. B.; Basanagoudar, I. D. Synthesis of 1,2,3,4-tetrahydropyrazino[1,2-*a*]indoles and ethyl 1-(2-amino-ethyl)indole-2-carboxylates. *Indian J. Chem.* **1989**, *28B*, 1065–1068.
- (26) Repke, D. B., III. Synthesis of 5-methoxy- and 5-hydroxy-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indoles. *J. Heterocycl. Chem.* **1982**, *19*, 845–848.
- (27) Sugimoto, O.; Mori, M.; Moriya, K.; Tanji, K. Application of phosphonium salts to the reactions of various kinds of amides. *Helv. Chim. Acta* **2001**, *84*, 1112–1118.
- (28) Robertson, A. A. B.; Botting, N. P. Synthesis of deuterium labelled desulfolucosinolates as internal standards for LC-MS analysis. *Tetrahedron* **1999**, *55*, 13269–13284.
- (29) GPCR alignments; www.bioinfo-pharma.u-strasbg.fr/gpcrdb/jss.html.
- (30) Terakita, A. The opsins. *Genome Biol.* **2005**, *6*, 213.
- (31) Gether, U.; Kobilka, B. K. G protein-coupled receptors. II. Mechanism of agonist activation. *J. Biol. Chem.* **1998**, *273*, 17979–17982.
- (32) Ballesteros, J. A.; Jensen, A. D.; Liapakis, G.; Rasmussen, S. G.; Shi, L.; Gether, U.; Javitch, J. A. Activation of the beta 2-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. *J. Biol. Chem.* **2001**, *276*, 29171–29177.
- (33) Shapiro, D. A.; Kristiansen, K.; Weiner, D. M.; Kroeze, W. K.; Roth, B. L. Evidence for a model of agonist-induced activation of 5-hydroxytryptamine 2A serotonin receptors that involves the disruption of a strong ionic interaction between helices 3 and 6. *J. Biol. Chem.* **2002**, *277*, 11441–11449.
- (34) Schwartz, T. W.; Frimurer, T. M.; Holst, B.; Rosenkilde, M. M.; Elling, C. E. Molecular mechanism of 7TM receptor activation—a global toggle switch model. *Annu. Rev. Pharmacol. Toxicol.* **2006**, *46*, 481–519.
- (35) Shi, L.; Javitch, J. A. The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 437–467.
- (36) Javitch, J. A.; Ballesteros, J. A.; Weinstein, H.; Chen, J. A cluster of aromatic residues in the sixth membrane-spanning segment of the dopamine D₂ receptor is accessible in the binding-site crevice. *Biochemistry* **1998**, *37*, 998–1006.
- (37) Rasmussen, S. G.; Choi, H. J.; Rosenbaum, D. M.; Kobilka, T. S.; Thian, F. S.; Edwards, P. C.; Burghammer, M.; Ratnala, V. R.; Sanishvili, R.; Fischetti, R. F.; Schertler, G. F.; Weis, W. I.; Kobilka, B. K. Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature* **2007**, *450*, 383–387.
- (38) Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H. J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K.; Stevens, R. C. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. *Science* **2007**, *318*, 1258–1265.
- (39) Rosenbaum, D. M.; Cherezov, V.; Hanson, M. A.; Rasmussen, S. G.; Thian, F. S.; Kobilka, T. S.; Choi, H. J.; Yao, X. J.; Weis, W. I.;

- Stevens, R. C.; Kobilka, B. K. GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. *Science* **2007**, *318*, 1266–1273.
- (40) Kalani, M. Y.; Vaidehi, N.; Hall, S. E.; Trabanino, R. J.; Freddolino, P. L.; Kalani, M. A.; Floriano, W. B.; Kam, V. W.; Goddard, W. A., 3rd. The predicted 3D structure of the human D₂ dopamine receptor and the binding site and binding affinities for agonists and antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 3815–3820.
- (41) Roth, B. L.; Shoham, M.; Choudhary, M. S.; Khan, N. Identification of conserved aromatic residues essential for agonist binding and second messenger production at 5-hydroxytryptamine_{2A} receptors. *Mol. Pharmacol.* **1997**, *52*, 259–266.
- (42) Boeckler, F.; Gmeiner, P. The structural evolution of dopamine D₃ receptor ligands: structure–activity relationships and selected neuropharmacological aspects. *Pharmacol. Ther.* **2006**, *112*, 281–333.
- (43) Boess, F. G., Jr.; Meyer, V.; Zwingelstein, C.; Sleight, A. J. Interaction of tryptamine and ergoline compounds with threonine 196 in the ligand binding site of the 5-hydroxytryptamine₆ receptor. *Mol. Pharmacol.* **1997**, *52*, 515–523.
- (44) Shapiro, D. A.; Kristiansen, K.; Kroeze, W. K.; Roth, B. L. Differential modes of agonist binding to 5-hydroxytryptamine(2A) serotonin receptors revealed by mutation and molecular modeling of conserved residues in transmembrane region 5. *Mol. Pharmacol.* **2000**, *58*, 877–886.
- (45) Johnson, M. P.; Loncharich, R. J.; Baez, M.; Nelson, D. L. Species variations in transmembrane region V of the 5-hydroxytryptamine type 2A receptor alter the structure–activity relationship of certain ergolines and tryptamines. *Mol. Pharmacol.* **1994**, *45*, 277–286.
- (46) Almaula, N.; Ebersole, B. J.; Ballesteros, J. A.; Weinstein, H.; Sealfon, S. C. Contribution of a helix 5 locus to selectivity of hallucinogenic and nonhallucinogenic ligands for the human 5-hydroxytryptamine_{2A} and 5-hydroxytryptamine_{2C} receptors: direct and indirect effects on ligand affinity mediated by the same locus. *Mol. Pharmacol.* **1996**, *50*, 34–42.
- (47) Recanatini, M.; Cavalli, A.; Masetti, M. Modeling hERG and its Interactions with Drugs: Recent Advances in Light of Current Potassium Channel Simulations. *ChemMedChem* **2008**, *3*, 523–535.
- (48) Stansfeld, P. J.; Gedeck, P.; Gosling, M.; Cox, B.; Mitcheson, J. S.; Sutcliffe, M. J. Drug block of the hERG potassium channel: insight from modelling. *Proteins* **2007**, *68*, 568–580.
- (49) Farid, R.; Day, T.; Friesner, R. A.; Pearlstein, R. A. New insights about hERG blockade obtained from protein modeling, potential energy mapping, and docking studies. *Bioorg. Med. Chem.* **2006**, *14*, 3160–3173.
- (50) Kamiya, K.; Niwa, R.; Mitcheson, J. S.; Sanguinetti, M. C. Molecular determinants of hERG channel block. *Mol. Pharmacol.* **2006**, *69*, 1709–1716.
- (51) Myokai, T.; Ryu, S.; Shimizu, H.; Oiki, S. Topological mapping of the asymmetric drug binding to the human ether-à-go-go-related gene product (hERG) potassium channel by use of tandem dimers. *Mol. Pharmacol.* **2008**, *73*, 1643–1651.
- (52) Pearlstein, R. A.; Vaz, R. J.; Kang, J.; Chen, X. L.; Preobrazhenskaya, M.; Shchekotikhin, A. E.; Korolev, A. M.; Lysenkova, L. N.; Miroshnikova, O. V.; Hendrix, J.; Rampe, D. Characterization of hERG potassium channel inhibition using CoMSiA 3D QSAR and homology modeling approaches. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1829–1835.
- (53) Cavalli, A.; Poluzzi, E.; De Ponti, F.; Recanatini, M. Toward a pharmacophore for drugs inducing the long QT syndrome: insights from a CoMFA study of hERG K(+) channel blockers. *J. Med. Chem.* **2002**, *45*, 3844–3853.
- (54) Ichikawa, J.; Ishii, H.; Bonaccorso, S.; Fowler, W. L.; O’Laughlin, I. A.; Meltzer, H. Y. 5-HT_{2A} and D₂ receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J. Neurochem.* **2001**, *76*, 1521–1531.
- (55) (a) Maurel-Remy, S.; Bervoets, K.; Millan, M. J. Blockade of phencyclidine-induced hyperlocomotion by clozapine and MDL 100907 in rats reflects antagonism of 5-HT_{2A} receptors. *Eur. J. Pharmacol.* **1995**, *280*, R9–R11. (b) Gleason, S. D.; Shannon, H. E. Blockade of phencyclidine-induced hyperlocomotion by olanzapine, clozapine and serotonin receptor subtype selective antagonists in mice. *Psychopharmacology (Berlin)* **1997**, *129*, 79–84.
- (56) (a) Deutch, A. Y.; Lee, M. C.; Iadarola, M. J. Regionally specific effects of atypical antipsychotic drugs on striatal fos expression: the nucleus accumbens shell as a locus of antipsychotic action. *Mol. Cell. Neurosci.* **1992**, *3*, 332–341. (b) Robertson, G. S.; Fibiger, H. C. Neuroleptics increase c-fos expression in the forebrain: contrasting effects of haloperidol and clozapine. *Neuroscience* **1992**, *46*, 315–328.
- (57) Tremblay, P. O.; Gervais, J.; Rouillard, C. Modification of haloperidol-induced pattern of c-fos expression by serotonin agonists. *Eur. J. Neurosci.* **1998**, *10*, 3546–3555.
- (58) Robertson, G. S.; Matsumura, H.; Fibiger, H. C. Induction Patterns of Fos-Like Immunoreactivity in the Forebrain as Predictors of Atypical Antipsychotic Activity. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1058–1066.
- (59) Sumner, B. E.; Cruise, L. A.; Slatery, D. A.; Hill, D. R.; Shahid, M.; Henry, B. Testing the validity of c-fos expression profiling to aid the therapeutic classification of psychoactive drugs. *Psychopharmacology (Berlin)* **2004**, *171*, 306–321.
- (60) Belfield, A. J.; Brown, G. R.; Foubister, A. J.; Ratcliffe, P. D. Synthesis of meta-substituted aniline derivatives by nucleophilic substitution. *Tetrahedron* **1999**, *55*, 13285–13300.
- (61) Butini, S.; Campiani, G.; Borriello, M.; Gemma, S.; Panico, A.; Persico, M.; Catalanotti, B.; Ros, S.; Brindisi, M.; Agnusdei, M.; Fiorini, I.; Nacci, V.; Novellino, E.; Belinskaya, T.; Saxena, A.; Fattorusso, C. Exploiting Protein Fluctuations at the Active-Site Gorge of Human Cholinesterases: Further Optimization of the Design Strategy to Develop Extremely Potent Inhibitors. *J. Med. Chem.* **2008**, *51*, 3154–3170.
- (62) Ding, H. Q.; Karasawa, N.; Goddard, W. A. III atomic level simulations on a million particles: the cell multipole method for Coulomb and London nonbond interactions. *J. Chem. Phys.* **1992**, *97*, 4309–4315.
- (63) Dauber-Osguthorpe, P.; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J.; Genest, M.; Hagler, A. T. Structure and energetics of ligand binding to proteins: *Escherichia coli* dihydrofolate reductase-trimethoprim, a drug-receptor system. *Proteins* **1988**, *4*, 31–47.
- (64) Maple, J. R.; Hwang, M. J.; Stockfisch, T. P.; Dinur, U.; Waldman, M.; Ewig, C. S.; Hagler, A. T. Derivation of class II force fields. I. Methodology and quantum force field for the alkyl functional group and alkane molecules. *J. Comput. Chem.* **1994**, *15*, 162–182.
- (65) Stewart, J. P. MOPAC; Stewart Computational Chemistry: Colorado Springs, CO, 2007; <http://OpenMOPAC.net>.
- (66) Dini, S.; Caselli, G. F.; Ferrari, M. P.; Giani, R.; Clavenna, G. Heterogeneity of [³H]-mepyramine binding sites in guinea pig cerebellum and lung. *Agents Actions* **1991**, *33*, 181–184.
- (67) DeLean, K. W.; Munson, P. J.; Rodbard, D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves. *Am. J. Physiol.* **1978**, *235*, E97–E102.
- (68) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC₅₀) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
- (69) Hansen, H. H.; Timmermann, D. B.; Peters, D.; Walters, C.; Damaj, M. I.; Mikkelsen, J. D. Alpha-7 nicotinic acetylcholine receptor agonists selectively activate limbic regions of the rat forebrain: an effect similar to antipsychotics. *J. Neurosci. Res.* **2007**, *85*, 1810–1818.
- (70) Mikkelsen, J. D.; Vrang, N.; Mrosovsky, N. Expression of Fos in the circadian system following nonphotic stimulation. *Brain Res. Bull.* **1998**, *47*, 367–376.

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