

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Act. 519 TRASLADOINFORME Folios: 16

ASUNTO Radicación 09-23386
Trámite 011
Evento 378
Actuación 519
Oficio **E - 19187**
Folios 016

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (Fase Nacional)	☒	Cap. I	☒
		Cap. II	☐

No. Solicitud Internacional PCT/EP2007/008042
Fecha de Presentación Internacional 07 de Septiembre de 2007

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 258 a 551	06/Marzo/2009
Reivindicaciones:	Folios 552 a 586	06/Marzo/2009
Dibujos:	Folios 588 a 599	06/Marzo/2009
Prioridad:	EP 06090166.7	08/Septiembre/2006
	EP 07090079.0	23/Abril/2007

2. Análisis de la Prioridad

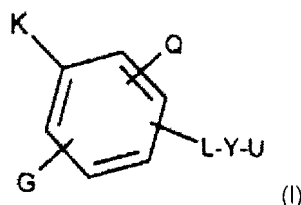
Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486), y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Agentes que comprenden compuestos marcados con ¹⁸F y métodos relacionados".

El problema planteado en esta solicitud consiste en la necesidad de proporcionar nuevos compuestos apropiados para radiomarcarse o que ya están radiomarcados con halógenos, mas específicamente con ^{18}F , un método para preparar estos compuestos, útiles como agentes de diagnóstico por imágenes y composiciones que comprenden estos compuestos.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de la fórmula (I) y una sal aceptable para uso farmacéutico de un ácido inorgánico u orgánico del mismo, un hidrato, complejo, éster, amida, solvato, o prodroga del mismo.



El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 61 K 51/08, 51/04; C 07 K 7/06, 255/58.

4. Excepción a la Patentabilidad (Art 20 D 486 literales (a) – (d))

El objeto de las reivindicaciones 38 no es patentable de acuerdo con el Art citado.

5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 43 - 44 no es patentable, de acuerdo con el Art 14.

6. De la Solicitud de Patente

Título

El título es muy general y no define el objeto de la solicitud. Se sugiere al solicitante hacer la corrección necesaria, la cual corresponda a la específica definición de la materia reivindicada.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio no es conciso porque contiene un excesivo número de reivindicaciones independientes, lo cual hace difícil definir cuál es el objeto que se busca proteger.

El capítulo reivindicatorio no es claro ni conciso porque la definición de la invención en términos de sustituyentes "alquilo", "arilo", "heteroarilo", entre otros, son géneros funcionales que no permiten distinguir la invención del estado de la técnica. Esto debido a que no se especifican el número de átomos de carbono que los conforman, en el caso de los anillos, ni el número y la clase de heteroátomos que los pueden

llegar a conformar en el caso de los “hetero”, motivo por el cual se obtienen un sin número de compuestos que no encuentran soporte en la descripción.

El capítulo reivindicatorio no es conciso, ni está sustentado en la descripción porque no hay suficientes ejemplos que demuestren que se hayan obtenido los compuestos con los sustituyentes tal como se encuentran allí definidos. Se encuentra que los compuestos obtenidos corresponden a los que comprenden preferiblemente los siguientes sustituyentes:

Radical	Radical soportado en la descripción o realización preferida
K:	¹⁸ F, N(R1)(R2)(R3)
(R1)(R2)(R3):	Grupo metilo
G:	CN, CF3,
Q:	Átomo de hidrógeno, átomo de Cl
L:	C(O), C(O)NR
Y:	Un enlace, NH(CH2)pC(O), p= 2 - 10 NH(CH2CH2O)qCH2CH2C(O), q= 0 - 5 Secuencia de aminoácidos de 2 a 10 unidades.
U:	Secuencia de aminoácidos de 4 a 11 unidades

La reivindicación 1 no es clara debido que contiene un gran numero de posibles sustituyentes lo cual no permite diferenciar la invención del estado de la técnica ni evidenciar el alcance de la invención.

La reivindicación 1 no es clara debido que emplea termino “espaciador”, “agente direccionador”, los cuales no definen la invención y no permite diferenciarlo del estado de la técnica.

Las reivindicaciones 23 y 30 no son claras debido que hacen referencia a un número muy amplio de unidades de aminoácidos, los cuales no permiten diferenciar la invención del estado de la técnica.

Las reivindicaciones 35 y 36 hacer referencia a un método para prepara un compuesto de fórmula A, no son claras debido que un método de preparación debe estar definido por una secuencia lógica de pasos, especificando las condiciones particulares para realizarlo, para que la persona normalmente versada en la materia pueda llevarlo a cabo.

La reivindicación 39 no es clara debido que reclama un kit el cual debe estar caracterizado por un medicamento o combinación de los mismos, forma de administración de cada medicamento o combinación, instrucciones para la administración del medicamento para conseguir un efecto deseado y la cantidad de la forma de dosificación a ser administrado e un tiempo determinado y por un envase, recipiente o contenedor

Se requiere al Solicitante hacer las aclaraciones necesarias.

7. Unidad de invención (Art 25 D 486)

La Solicitud no cumple con el Requisito de Unidad de Invención porque se refiere a 5 grupos inventivos.

Los Grupos Inventivos están definidos por las siguientes características **esenciales**:

- 1. Compuestos y métodos en los cuales U corresponde a un péptido o un compuesto peptidomimético. Reivindicaciones 1 – 22, 27 – 29,32, 33, 35 -45 (todas en parte) y 23 – 26, 36 (todas completas). Divulgado en los documentos Artículo "New PET imaging for the serotonin transporter: (18)ACF (2-((2-amino-4-chloro-5-fluorophenyl)thio) -N,N-dimethyl-benzenmethanamin" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY y en el documento patente WO 02/44144.
- 2. Compuestos y métodos en los cuales U corresponde a una molécula pequeña. Reivindicaciones 1 – 22, 27 – 29, 32, 33, 35 – 45 (todas en parte) y 30 (completa). Divulgado en los documentos Artículo "New PET imaging for the serotonin transporter: (18)ACF (2-((2-amino-4-chloro-5-fluorophenyl)thio) -N,N-dimethyl-benzenmethanamin" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY y en el documento patente WO 02/44144.
- 3. Compuestos métodos en los cuales U corresponde a un oligonucleótido. Reivindicaciones 1 – 22, 27 – 29, 35 -45 (todas en parte) y 31 (completa). Divulgado en los documentos Artículo "New PET imaging for the serotonin transporter: (18)ACF (2-((2-amino-4-chloro-5-fluorophenyl)thio) -N,N-dimethyl-benzenmethanamin" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY y en el documento patente WO 02/44144.
- 4. Compuesto y métodos de fórmula V. Reivindicaciones 46 – 48.
- 5. Péptidos correspondientes a la reivindicación 49. Reivindicación 49 (completa). Divulgado en los documentos patente WO 92/09626 y WO 91/02746.

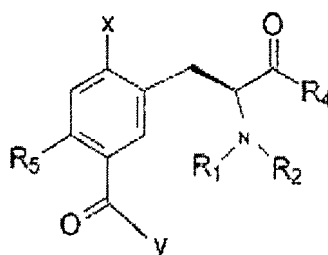
Se sugiere presentar las Solicitudes Divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

8. Determinación del Estado de la Técnica

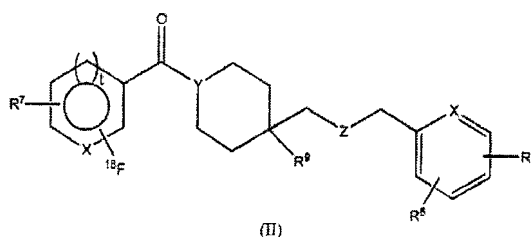
El Estado de la Técnica se determinó con base en la fecha de presentación de la Solicitud de Prioridad: Septiembre 08 de 2006, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	WO 02/44144	Nucleophilic aproach for preparing radiolabeled imaging agents and associated compound	06/Junio/2002
D2	WO 2006083424	Radiolabeled compounds and uses thereof	10/Agosto/2006
D3	New PET imaging agent for the serotonin transporter: [18F] ACF (2-[(2-amino-4-chloro-5-fluorophenyl)thiol]-N,N-dimethyl-benzenmethanamine	Journal of Medicinal Chemistry, 2002, Vol 45, N° 21, Pág 4716 - 4723	19/Abril/2002
D4	US 6639076	Growth Hormone Secretagogues	28/Octubre/2003
D5	WO 03/002157	Solid-phase Nucleophilic fluoration	09/Enero/2003

D1 <http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=0244144A2&KC=A2&FT=D&ND=&date=20020606&DB=&locale=en> EP divulga (página 5) un compuesto de la fórmula



D2 <http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2006083424A2&KC=A2&FT=D&ND=&date=20060810&DB=&locale=en> EP divulga (página 16, línea 17) Un compuesto de fórmula (II)



D4 <http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=6639076B1&KC=B1&FT=D&ND=3&date=20031028&DB=EPODOC&locale=en> EP Divulga compuestos específicos Columnas 104-106.

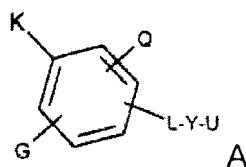
D5 <http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=03002157A1&KC=A1&FT=D&ND=&date=20030109&DB=&locale=en> EP Divulga la fluoración de péptidos.

9. Examen de Patentabilidad (Art 14 D486)

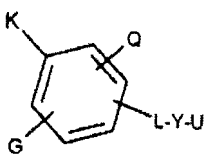
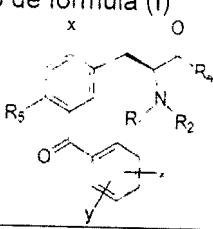
El examen de patentabilidad se realiza sobre el grupo inventivo 1.

Novedad (Art 16 D486)

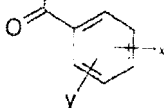
La reivindicación 1 consiste en un compuesto de formula general (A). (Fl. 552).



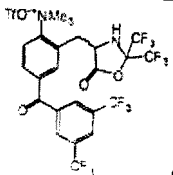
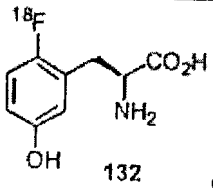
Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
Compuesto de fórmula 	Compuesto de fórmula (I) 
K: ¹⁸ F	X: ¹⁸ F
Q: Átomo del hidrógeno, Cl	Átomo de hidrógeno
G: Átomo de cloro, C(O)R	R5: halógeno, acilo
L: C(O)	C(O)
Y: Un enlace	Un enlace
U: Agente direccionador	Un compuesto aromático sustituido con x y Y.

Las características **esenciales** del compuesto de la reivindicación 1 habían sido divulgadas previamente en D1, el cual da a conocer un compuesto el cual presenta en su estructura un compuesto aromático sustituido, en posición 1 del anillo por un isotopo de flúor, ¹⁸F, en posición 4 se encuentra enlazado a compuesto de estructura



, opcionalmente sustituido con los compuesto y Y, en la posición 5 se encuentra sustituido con R5 el cual es seleccionado de forma independiente de un halógeno o un grupo acilo. Adicionalmente divulga los siguientes compuestos específicos:

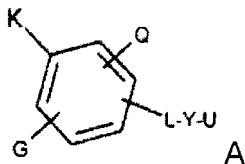
 Compuesto 39 (pág 112)	 132 Compuesto 32 (fig 62)
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En consecuencia, la reivindicación 1 no es nueva, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 2- 10 y 37 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

Novedad frente a D3 y D4.

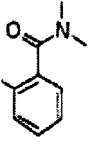
La reivindicación 1 consiste en un compuesto de formula general (A). (Fl. 552).

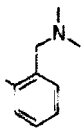


Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

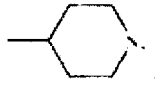
Características esenciales	D3	D4
Compuesto de fórmula 	Divulga los compuestos específicos 4 5 7	Compuestos derivados de específicos Ejemplo 21 22 Ejemplo
K: es W o N+(R1)(R2)(R3) R1, R2, R3: Alquilo. W: es un isótopo de flúor	F, N+(CH3)3	Átomo de Flúor
Q: Átomo de hidrógeno	Átomo de hidrógeno	Átomo de hidrógeno
G: Átomo de cloro, C(O)R, CF3	Átomo de cloro	CF3
L: (S)p, (CH2)dC(O)	Átomo se S	C(O)
Y: Un enlace	Un enlace	Un enlace
U: Agente direccionador	Compuestos y	Compuesto y

Las características **esenciales** del compuesto de la reivindicación 1 habían sido divulgadas previamente en los documentos D3 y D4:
El documento D3 da a conocer los compuestos específicos 4,5 y 7, los cuales presentan en su estructura un fenilo sustituido con un átomo de flúor, en el caso de los compuestos 4 y 5, en el compuesto 7 con un compuesto de N(CH3)3, de igual forma los compuestos específicos se encuentran sustituidos con un átomo de

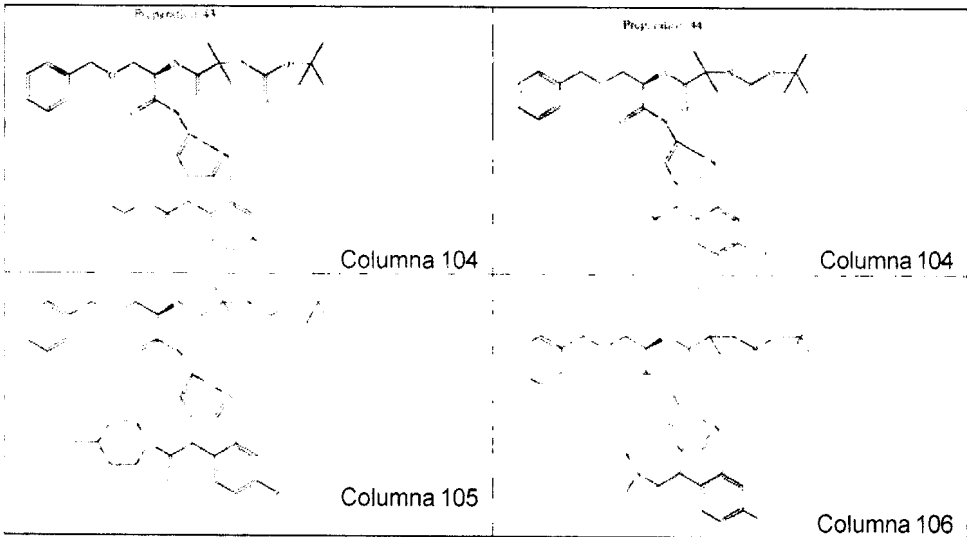
halógeno, enlazados a un átomo de S y éstos unidos a los compuestos  para

los compuesto 4 y 7 , y  para el compuesto 5.

Por su parte el documento D4 da a conocer los compuestos específicos los cuales presentan en su estructura un fenilo sustituido con un átomo de flúor, átomo de hidrógeno y un CF3, enlazado a un compuesto (CH2)dC(O) y unidos a los

compuestos  y .

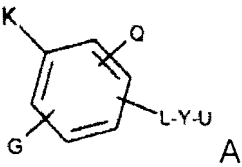
En consecuencia, la reivindicación 1 no es nueva, según lo divulgado en los documentos D3 y D4. Adicionalmente, D4 divulga los siguientes compuestos:



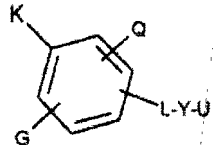
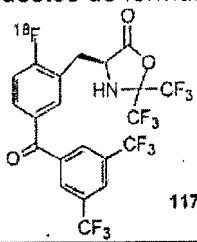
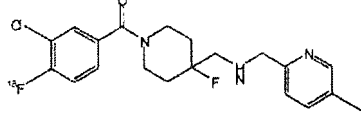
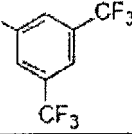
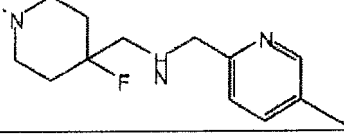
Las reivindicaciones dependientes 3 - 16 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en un compuesto de formula general (A). (Fl. 552).



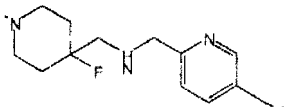
Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Compuesto de fórmula 	Compuestos de fórmula 	Compuesto de fórmula (D) 
K: ¹⁸ F	¹⁸ F	¹⁸ F
Q: Átomo del hidrógeno, Cl	Átomo del hidrógeno	Átomo del hidrógeno
G: Átomo de cloro	No Menciona	Átomo de Cloro
L: C(O)	C(O)	C(O)
Y: Un enlace	Enlace	Un enlace
U: Agente direccionador	Compuesto 	Compuesto 

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

El documento D1 da a conocer un compuestos radio marcados, útiles como agentes diagnósticos, en particular D1 divulga un compuesto de fórmula 117, el cual presenta en su estructura un compuesto aromático sustituido en la posición 1 del anillo con un isotopo de flúor, ¹⁸F, en la posición 4 se encuentra sustituido con un compuesto C(O) unido a un benceno doblemente sustituido con CF3.

Por su parte, el documento D2 da a conocer compuestos radios marcados y sus usos como medio de contraste con fines diagnósticos. El documento D1 da a conocer en particular el compuesto de fórmula (D), el cual presenta en su estructura un anillo aromático, benceno, sustituido con un isotopo de flúor, en posición orto respecto al átomo de flúor se encuentra sustituido con un átomo de cloro, y en posición para con



un grupo C(O) sustituido con el compuesto

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto 117 del documento D1, consiste en la presencia del sustituyente G, el cual corresponde a un átomo halógeno, Cl.

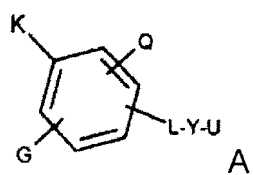
El hecho de incluir un átomo de un compuesto halógeno correspondiente a un átomo de cloro, no evidencia un efecto técnico inesperado o sorprendente asociado a la utilidad de dichos compuestos como agentes de diagnostico por imágenes. De igual manera el empleo de péptidos como agentes de direccionamiento no demuestra un efecto técnico sorprendente.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el compuesto de fórmula 117 conocido en D1 para obtener compuestos radio marcados con ¹⁸F alternativos, útiles como agentes diagnósticos”.

Sin embargo, la utilización de un átomo de halógeno, Cloro, como un sustituyente del compuesto aromático y el empleo de compuestos como para la obtención de compuestos útiles como agentes diagnósticos, ya está divulgada en el documento D2.

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría un átomo de halógeno, Cloro divulgado en el documento D2 en el compuesto 117 del documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

La reivindicación 1 consiste en un compuesto de formula general (A). (Fl. 552).

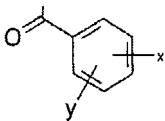


Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica

Características esenciales	D1	D5
Compuesto de fórmula	Compuesto de formula (I)	Compuesto de fórmula (Ia)
K: ^{18}F o F	X: ^{18}F	Linker (agente enlazante): Compuesto
Q: Átomo del hidrógeno, Cl	Átomo de hidrógeno	No menciona
G: Átomo de F o C(O)	R5: halógeno, acilo	F
L: C(O)	C(O)	No menciona
Y: Un enlace	Un enlace	Un enlace
U: Agente direccionador	Un compuesto aromático sustituido con x y Y.	Tracer: Péptido o proteína

Los documentos D1 y D5 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

El documento D1 da a conocer un compuesto el cual presenta en su estructura un compuesto aromático sustituido, en posición 1 del anillo por un isotopo de flúor, ^{18}F ,



en posición 4 se encuentra enlazado a compuesto de estructura , opcionalmente sustituido con los compuesto y Y, en la posición 5 se encuentra sustituido con R5 el cual es seleccionado de forma independiente de un halógeno o un grupo acilo.

Por su parte el documento D5 da a conocer el compuesto de fórmula (Ia), el cual presenta un grupo enlazante que corresponde a un compuesto aromático sustituido con átomos de flúor, unido a un grupo S(O)3 y este a su vez enlazado a un péptido o proteína, preferiblemente un péptido que comprende de 2 a 1000 aminoácidos (Pág. 10, línea 10 – 14)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D1, consiste en la presencia en el sustituyente U el cual corresponde a un agente direccionador, seleccionado de un grupo que comprende péptidos.

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto de documento D5, consiste en la sustitución del anillo aromático por un átomo de flúor o un isótopo del mismo y la presencia en el sustituyente L un carbono carbonílico, C(O).

El hecho de incluir en el sustituyente U una agente direccionador que corresponde a un péptido, no evidencia un efecto técnico sorprendente o asociado a la obtención de compuestos radiomarcados con halógenos, útiles como agentes diagnósticos por imágenes.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el compuesto de fórmula (I) conocido en el documento D1 para compuestos radiomarcados alternativos útiles como agentes diagnósticos por imágenes”.

Sin embargo, la de péptidos que comprenden 2 a 1000 aminoácidos, para obtener compuestos radiomarcados con fines diagnósticos ya ha sido divulgada en el documento D5.

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría péptidos que comprenden cadenas de aminoácidos del documento D5 en el compuesto de fórmula (I) del documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

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

10. Conclusión

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

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO 02/44144	1 – 10. 37 1	Novedad Nivel Inventivo
D2	WO 2006083424	1	Nivel Inventivo
D3	New PET imaging agent	1, 3 - 16	Novedad

	for the serotonin transporter: [18F] ACF (2-[(2-amino-4-chloro-5-fluorophenyl)thio]-N,N-dimethyl-benzenmethanamine		
D4	US 6639076	1 3 16	Novedad
D5	WO 03/002157	1 22 24	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 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.

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 13 0 OCT. 2012

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Aprobado por: José Luis Salazar López

New PET Imaging Agent for the Serotonin Transporter: [¹⁸F]ACF (2-[(2-Amino-4-chloro-5-fluorophenyl)thio]-N,N-dimethyl-benzenethanamine)

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A new F-18 labeled phenylthiophenyl derivative specific for imaging of serotonin transporters (SERT) in the brain by positron emission tomography (PET) is described. Fluorinated phenylthiophenyl derivative, ACF, 2-[(2-amino-4-chloro-5-fluorophenyl)thio]-N,N-dimethyl-benzenethanamine, was prepared by first coupling 2,5-dichloro-4-nitroaniline with 2-mercapto-N,N-dimethylbenzamide. The amino group of the coupled adduct was converted to a fluoro group through a Schemes reaction. Subsequently, a one pot reduction of both nitro and amide groups by H₂/tetrahydrate yielded the nonradioactive ACF (yield 25%). In vitro binding assays using cell membrane homogenates of LLC cells expressing SERT, dopamine transporters (DAT), or norepinephrine transporters (NET) showed excellent binding affinity and selectivity for SERT (K_i = 0.05, 3020, and 650 nM for SERT, DAT, and NET, respectively). For preparation of the [¹⁸F]ACF, the NH₂ group of the initially coupled adduct was converted to the trimethylammonium salt, which was replaced by [¹⁸F]fluoride in the presence of Kryptofix 222 and potassium carbonate. The final product, [¹⁸F]ACF, was obtained after a borane and stannous chloride reduction reaction. The combined two-step reaction gave a radiochemical yield of 40–15% (R₁/R₂) and a radiochemical purity of >99%. Synthesis of the novel PET tracer, [¹⁸F]ACF, as a probe for binding to SERT in the brain was successfully achieved. The new tracer [¹⁸F]ACF showed excellent brain penetration and selective localization after an injection in rats (brain uptake at 2, 30, 60, 120, and 240 min was 3.27, 1.28, 0.69, 0.74, and 0.08% dose/gm, respectively). The hypothalamocerebellum ratio at 60 min post injection was 3.55. This specific localization in the hypothalamus was blocked by pretreatment of (+)McN5652. This novel ligand is a potential PET tracer for in vivo evaluation of SERT in the brain.

Introduction

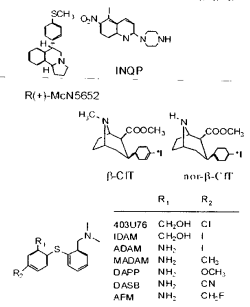
Alterations in serotonergic neuronal function in the central nervous system often occur in patients with major depression.¹ Recent reviews have summarized the important associations between serotonergic functions and depression.^{1–3} A series of antidepressants, i.e., citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline, have been marketed. Selective serotonin reuptake inhibitors (SSRIs) are drugs, which are designed to preferentially increase 5-HT transmission by inhibiting the serotonin transporter (SERT). They have no or only a slight effect on other uptake mechanisms, neurotransmitter receptors, enzymes, etc. These SSRIs have revolutionized the management of depression for millions of patients, and they are widely prescribed for treating various affective disorders.

Imaging of SERT in humans would provide a useful tool to understand how alterations of this system are related to depressive illness and other psychiatric disorders; therefore, it potentially can benefit millions of patients who are being treated with SSRIs. The first successful radioligand was [¹²⁵I]McN5652 for positron emission tomography (PET) imaging (Chart 1).^{4–9} It showed excellent inhibition of 5-HT reuptake in rat

brain synaptosomes (K_i = 0.40 nM for inhibition of SERT) and moderate selectivity toward other monoamine transporters (DAT, dopamine, and NET; norepinephrine transporters: K_i = 235 and 182 nM, respectively).¹⁰ Specific binding of [¹²⁵I]McN5652 correlates well with the known density of SERT sites in the human brain.^{11–13} Recent reports using [¹²⁵I]McN5652 for imaging SERT as an indicator of serotonin neurons, have suggested that MDMA (methylendioxyamphetamin) “ecstasy” may cause an irreversible decrease of SERT binding sites.^{14,15} Despite its successful demonstration in imaging SERTs in humans, it has been reported previously that [¹²⁵I]McN5652 has several limitations.¹⁶ The uptake in the specific binding area is slow requiring at least 120 min of data acquisition. The nonspecific binding is relatively high, which precludes the measurements of lower SERT density regions. The plasma free fraction is very low (F₁ = 0.3), and it interferes with the known modeling using this tracer.

In search of a “superior” ligand for SERTs, many compounds have been evaluated (Chart 1).^{16–19} Among these tracers, only [¹²⁵I]6-bromo-6-nitroazepine (INQ)^{16–18} showed promising properties for imaging SERT sites in monkey's brain.¹⁶ No human study of [¹²⁵I]INQ has been reported. Previously, it has been suggested that [¹²⁵I]β-CIT (Chart 1), a SPECT imaging agent, binding to both DAT and SERT, would be able to clarify pathological changes in both dopaminergic and serotonergic systems. However, overlapping uptake

Chart 1. Chemical Structures of SERT Imaging Agents



regions and differential kinetics of [¹²⁵I]β-CIT binding to DAT and SERT were observed.^{16–18} Nonetheless, the effect of a selective SSRI in human brain in vivo has been directly measured by [¹²⁵I]β-CIT/SPECT imaging of SERT sites in depressed patients undergoing treatment with citalopram.¹⁹ Recently, [¹²⁵I]β-CIT/SPECT imaging showed a significant reduction (30%) of SERT binding in the midbrain area in depressed subjects as compared with controls.^{19–21} A recent report on [¹²⁵I]β-CIT/SPECT imaging of a group of patients suggests that schizophrenia is generally not associated with alterations of DAT in the striatum or SERT in the brain stem.²² A more selective compound, non-β-CIT (X demethylated analogue of β-CIT)²³ and its related derivatives,²⁴ have recently been reported as improved SPECT imaging agents for SERT. It is suggested that [¹²⁵I]non-β-CIT might be a suitable alternative tracer for visualization of SERT sites in the human brain with SPECT.^{25–28} However, [¹²⁵I]non-β-CIT still binds to both DAT and SERT, and the selectivity is only marginally improved, not sufficient to distinguish between these two monoamine transporter sites in vivo.

A phenylthiophenyl derivative, 403U76 (Chart 1), was reported as an inhibitor of serotonin and norepinephrine uptake in rat brain synaptosomes (K_i = 2.1 and 55 nM, respectively). No inhibition of dopamine uptake was observed for this series of compounds.²⁹ It appeared that 403U76 was one of the candidates of antidepressants developed by Glaxo Wellcome.^{30–32} Surprisingly, no other reports on 403U76 have appeared after 1996.³³ To develop a new SERT selective imaging agent, a series of derivatives based on the substituted phenylthiophenyl structure were prepared.^{34–37} Methods for synthesis of phenylthiophenyl or the phenoxypheyl derivatives resulted in different types of coupling reactions to form the desired ring system for developing specific SERT

imaging agents.³³ Among the compounds prepared, 1-(4-fluorophenyl)-4-methylpiperazine (IDAM) (Chart 1), are the most promising candidates for SPECT imaging agents with several attractive properties: high binding affinity and selectivity.^{33,34,35} In vitro binding studies showed that IDAM displayed an excellent affinity to SERT sites (K_i = 0.097 nM, using membrane preparations of LLC PK1 cells expressing the specific transporter) and showed more than 1000-fold selectivity for SERT over NET and DAT (expressed in the same LLC PK1 cells). IDAM also displayed an extremely potent binding affinity toward SERT (K_d = 0.013 nM). It showed more than 1000-fold selectivity for SERT over NET and DAT (K_d = 699 and 840 nM, for NET and DAT, respectively). A preliminary imaging study of [¹²⁵I]ADAM in the brain of a baboon by SPECT at 180–240 min post injection indicated a specific uptake in the midbrain region rich in SERT. It is apparent that [¹²⁵I]ADAM showed a significant improvement over [¹²⁵I]IDAM as a SPECT imaging agent for SERT in the brain.^{34,36,37} Initial imaging studies in humans suggest that the agent clearly localized in the region of hypothalamus region of the brain where the concentration of SERT is the highest (Dr. A. Altonen, private communication).

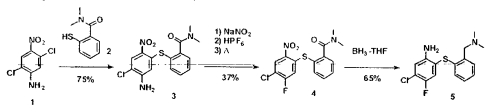
A group of ¹²⁵I-labeled compounds, based on a similar phenylthiophenyl ring system, was reported, among which DASB, DAPP, AFM, and MADAM showed the most promising properties as PET imaging agents for SERT.^{38–40} Similar to that of IDAM or ADAM, DASB displayed an excellent binding affinity toward SERT (K_i = 1.0 nM), while the binding affinity to the other monoamine transporters was a few 1000-fold lower. After an iv injection in rats, [¹²⁵I]DASB showed good brain uptake and selective localization in the hypothalamus region of the brain suggesting selective binding to the SERT. Initial human studies showed that [¹²⁵I]DASB had a good brain penetration and localized in the hypothalamus region rich in SERT binding sites. The specific binding in the striatum and hypothalamus regions can be blocked by pretreatment of SSRI, such as paroxetine or citalopram.⁴¹ Subsequently, using the same ligand, it was reported that a significant decrease in striatal SERT binding potential was found after either treatment, as compared to changes found over a 4-week period in healthy subjects. For patients treated with 20 mg/day of paroxetine and citalopram, the mean proportions of SERT sites occupied were 83 and 77%, respectively.^{42–44} A direct measurement of drug occupancy for SSRI on SERT binding sites, an important factor on drug efficacy,⁴⁵ was clearly demonstrated by the in vivo imaging technique.

In general, C-11 labeled PET tracers (T_{1/2} = 20 min) have a limited useful window of opportunity for data collection. To improve the feasibility of a full kinetic data analysis and provide a useful alternative PET tracer for imaging SERT with PET, F-18 labeled phenylthiophenyl derivatives were investigated. An initial attempt in preparation of [¹⁸F]AFM was reported.⁴⁶ To improve the stability of the fluorinated compounds, the fluorine was directly attached to one of the phenyl rings. Reported herein is the synthesis, radiochemistry, and characterization of a novel PET tracer, [¹⁸F]ACF (Chart 1), for imaging SERT in the brain.

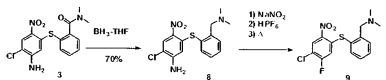
To whom all correspondence should be addressed. Tel.: 610-368-2066; Fax: (610) 361-3671; e-mail: hank@pharmacy.upenn.edu.
¹Department of Radiology, University of Pennsylvania.
²Institute for Nuclear Chemistry.
³Department of Pharmacology, University of Pennsylvania.

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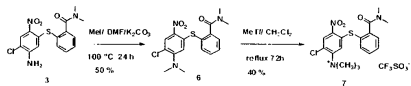
Scheme 1. Synthesis of Neuroinactive ALF (5)



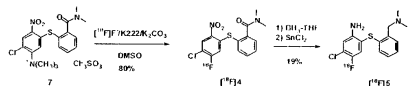
Scheme 2



Scheme 3. Synthesis of Precursor 7



Scheme 4. Synthesis of F-18 labeled ACF ([¹⁸F]5)



Chemistry

Preparations of neuro-inactive compounds are illustrated in Schemes 1 and 2. 2,5-Dichloro-4-nitrophenylamine (**1**) was coupled with thiodiphenylamine (**2**) to produce a phenylthioether compound **3**. The amino group of compound **3** was converted to fluoride **4** by quenching the diazo intermediate with H₂PtCl₆ and subsequent thermal decomposition. Nitro and amide groups of fluoride **4** were reduced to amines simultaneously with borane-tetrahydrofuran (THF) to yield 5-chloro-2,4-dimethylaniline (5-chloro-2,4-dimethylaniline) (**5**). The amino group of fluoride **4** was reduced to amines simultaneously with borane-tetrahydrofuran (THF) to yield 5-chloro-2,4-dimethylaniline (5-chloro-2,4-dimethylaniline) (**5**). The amino group of fluoride **4** was reduced to amines simultaneously with borane-tetrahydrofuran (THF) to yield 5-chloro-2,4-dimethylaniline (5-chloro-2,4-dimethylaniline) (**5**).

To obtain [¹⁸F]ACF ([¹⁸F]5), trimethylammonium salt **7** was prepared as a precursor (Scheme 3), through which the [¹⁸F]fluoride displacement was utilized for the radiolabeling reaction. Initially, compound **3** was converted to *N,N*-dimethylbenzamide derivative **6** by refluxing with methyl iodide and potassium carbonate in dimethylformamide (DMF). The third *N*-methyl group was introduced by a reaction with methyl iodide to give compound **7**, which was highly hygroscopic. However,

it was sufficiently pure after several recrystallizations, and it was used directly for the radiolabeling reaction without further purification (Scheme 3). Compound **7** was mixed with [¹⁸F]fluoride in dimethyl sulfoxide (DMSO) and heated at 55 °C for 10 min. The desired intermediate, [¹⁸F]4, was purified from the reaction mixture by a Sep-Pak silica cartridge, and the crude product was reduced by a sequential reduction reaction with BH₃·THF and SnCl₄ to provide [¹⁸F]ACF. At dilution, reducing agent SnCl₄ was needed to reduce the reaction time. The final product, [¹⁸F]ACF ([¹⁸F]5), was purified by a silica cartridge and high-performance liquid chromatography (HPLC). The entire procedure required approximately 3 h, and the radiochemical yield (decay corrected) was 15%. The specific activity was 1.0 Ci/nmol (measured by comparing to the diluted cold compound by HPLC by UV detector) at the end of the synthesis (Scheme 4).

Biological Evaluation

In vitro binding assays were performed by using LLC-PK1 cells overexpressing three different types of monoamine transporters (SERT, DAT, or NET, respectively). Well characterized ligands, [³H]PAC and [³H]

PET Imaging Agent for the Serotonin Transporter

Table 1. Selectivity of Compounds for Monoamine Transporters: SERT, DAT, or NET (K_i, nM)

compound	SERT	DAT	NET
1	0.05 ± 0.01	3020 ± 110	650 ± 80
2	0.05 ± 0.01	3020 ± 110	650 ± 80
3	0.05 ± 0.01	3020 ± 110	650 ± 80
4	0.05 ± 0.01	3020 ± 110	650 ± 80
5	0.05 ± 0.01	3020 ± 110	650 ± 80
6	0.05 ± 0.01	3020 ± 110	650 ± 80
7	0.05 ± 0.01	3020 ± 110	650 ± 80
8	0.05 ± 0.01	3020 ± 110	650 ± 80
9	0.05 ± 0.01	3020 ± 110	650 ± 80

* Values are the mean ± SEM of three determinations performed in duplicate. Cell membrane homogenates of LLC-PK1 cells overexpressing either SERT, NET, or DAT ([³H]PAC) was used as the ligand for the DAT and NET binding assay.^{45,47} For the SERT binding assay, [³H]DASB was used.

DASB, were used.^{45,47} Similar to that observed for the other phenylthioether derivatives, IDAM, ADAM, DASB, and MADAM, the target compound, **5** (ACF), displayed excellent binding affinity to SERT (K_i = 0.05 ± 0.01 nM). Binding affinities to the other monoamine transporters were more than 1000-fold lower, suggesting that ACF is a highly potent and selective ligand for SERT. Two other nitro groups containing intermediates, **3** and **9**, also showed good binding affinity and selectivity.

When the no carrier added [¹⁸F]ACF ([¹⁸F]5) was injected (ex vivo) into rats, it showed excellent initial brain uptake (3.27 ± 0.79% dose/organ or 1.71 ± 0.45% dose/g). The target also localized in muscle, lungs, and liver, organs where initial blood flow is high (Table 2).

Table 2. Organ Distribution (% Dose/Organ) and Brain Regional Uptake (% Dose/g) of [¹⁸F]5 in Rats (Average of Three Rats ± SD)

A. Organ Distribution (% Dose/Organ)					
organ	2 min	30 min	60 min	120 min	240 min
heart	6.56 ± 0.64	4.52 ± 0.22	1.65 ± 0.16	2.20 ± 0.53	2.33 ± 0.34
liver	1.69 ± 0.24	0.18 ± 0.02	0.11 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
muscle	14.34 ± 9.78	10.70 ± 1.68	7.09 ± 0.28	1.02 ± 0.22	2.08 ± 0.05
lung	14.89 ± 2.28	1.76 ± 0.20	0.73 ± 0.08	0.52 ± 0.06	0.15 ± 0.02
kidney	6.97 ± 0.73	2.64 ± 0.79	1.38 ± 0.25	0.78 ± 0.15	0.51 ± 0.07
spleen	0.64 ± 0.20	0.29 ± 0.09	0.11 ± 0.02	0.06 ± 0.02	0.03 ± 0.00
liver	9.11 ± 1.82	15.20 ± 1.20	10.40 ± 2.50	5.90 ± 1.03	3.55 ± 0.35
skin	4.76 ± 0.89	6.51 ± 0.66	5.97 ± 1.64	2.61 ± 0.17	1.53 ± 0.12
brain	3.27 ± 0.79	1.28 ± 0.11	0.69 ± 0.05	0.21 ± 0.03	0.06 ± 0.01
bone	0.01 ± 0.00	0.01 ± 0.03	0.08 ± 0.02	0.28 ± 0.06	0.27 ± 0.02

B. Regional Brain Distribution (% Dose/g)				
region	2 min	30 min	60 min	240 min
cerebellum	1.33 ± 0.51	0.37 ± 0.07	0.15 ± 0.03	0.05 ± 0.01
striatum	1.78 ± 0.36	0.76 ± 0.08	0.45 ± 0.14	0.15 ± 0.02
hippocampus	1.60 ± 0.46	0.70 ± 0.06	0.45 ± 0.13	0.14 ± 0.02
cortex	2.31 ± 0.46	0.62 ± 0.07	0.33 ± 0.13	0.09 ± 0.01
neocortex	1.69 ± 0.43	0.62 ± 0.06	0.39 ± 0.03	0.12 ± 0.01
hypothalamus	1.76 ± 0.48	0.65 ± 0.11	0.51 ± 0.13	0.17 ± 0.01

C. Ratio to Cerebellum				
region	2 min	30 min	60 min	240 min
cerebellum	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
striatum	1.01 ± 0.31	2.04 ± 0.19	2.98 ± 0.52	2.97 ± 0.16
hippocampus	1.24 ± 0.15	1.89 ± 0.21	2.91 ± 0.61	2.67 ± 0.15
cortex	1.84 ± 0.43	1.69 ± 0.20	2.10 ± 0.17	1.77 ± 0.09
neocortex	1.32 ± 0.21	1.80 ± 0.11	2.56 ± 0.11	2.34 ± 0.09
hypothalamus	1.37 ± 0.19	2.29 ± 0.06	3.53 ± 0.33	3.23 ± 0.21

* Rest of the brain.

Regional distribution in the brain showed an expected high uptake and retention in the striatum, hippocampus, and hypothalamus regions, where the serotonin neurons are highly concentrated (Table 2). It is also observed that the specific uptake for [¹⁸F]ACF ([¹⁸F]5) in the hypothalamus region, reached the peak between 60 and 120 min after injection as compared to [¹⁸F]ADAM, which reached the peak uptake at 4 h. The relatively fast kinetics of reaching peak uptake is highly desirable for future kinetic modeling study. The ratio of hypothalamus/cerebellum was 3.53 at 60 min and slowly decreased with time. At 4 h after injection, the ratio of hypothalamus/cerebellum was 2.37. In comparison, at 1 h after the iv injection into rats, [¹⁸F]DASB showed brain uptake of 0.62% dose/g and a hypothalamus/cerebellum ratio of 8.86. To demonstrate that the uptake of [¹⁸F]ACF ([¹⁸F]5) in the retention in the striatum, hippocampus, and hypothalamus regions was related to selective SERT, rats were pretreated with specific monoamine transporter inhibitors (MNS552 for SERT, mianserin for NET, and methamphetamine for DAT) (Table 3). There were significant changes in the brain regions where the serotonin neurons (SERT) were highly concentrated. There was a significant decrease in specific retention at 60 min in the hypothalamus, striatum, and hippocampus regions after the pretreatment of (1) MNS552 (2 mg/kg, at 3 min prior to tracer injection), suggesting that the [¹⁸F]ACF was competing for the same SERT binding sites as those for (1) MNS552. No significant differences were observed in rats pretreated with mianserin or methamphetamine since these drugs are not binding to the SERT. The in vivo competition experiment strongly established that the binding of [¹⁸F]ACF ([¹⁸F]5) was directly related to

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Table 3. Effects of Pretreatment with Monoamine Reuptake Inhibitors on the Specific Binding of [³H]5 in Rat Brain Regions

A. Regional Brain Uptake (5-Dose)				
region	control	1 μM McN5652	reserpine	methylphenidate
cerebellum	0.21 ± 0.02	0.17 ± 0.03	0.19 ± 0.01	0.17 ± 0.02
striatum	0.56 ± 0.09	0.25 ± 0.03 ^a	0.47 ± 0.03	0.47 ± 0.06
hippocampus	0.53 ± 0.03	0.34 ± 0.08 ^a	0.41 ± 0.11	0.50 ± 0.15
cortex	0.39 ± 0.03	0.24 ± 0.02 ^a	0.37 ± 0.04	0.35 ± 0.04
retinales ^b	2.28 ± 0.04	1.37 ± 0.10 ^a	1.94 ± 0.16	2.62 ± 0.21
hypothalamus	0.63 ± 0.05	0.24 ± 0.03 ^a	0.55 ± 0.02	0.58 ± 0.07
blot ^c	0.65 ± 0.03	0.26 ± 0.03 ^a	0.26 ± 0.01	0.28 ± 0.02
B. Same as in table 1a				
region	control	1 μM McN5652	reserpine ^a	methylphenidate ^a
cerebellum	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
striatum	2.63 ± 0.23	1.48 ± 0.32	2.50 ± 0.12	2.75 ± 0.09
hippocampus	2.52 ± 0.13	1.34 ± 0.21 ^a	2.41 ± 0.11	2.50 ± 0.15
cortex	1.88 ± 0.05	1.43 ± 0.23 ^a	1.94 ± 0.16	2.62 ± 0.21
retinales ^b	2.28 ± 0.04	1.37 ± 0.10 ^a	2.17 ± 0.04	2.23 ± 0.05
hypothalamus	2.98 ± 0.03	1.36 ± 0.15	2.89 ± 0.11	2.95 ± 0.11

^a Rats were pretreated with drugs with a dose of 2 mg/kg, i.v. 5 min prior to the tracer administration (iv). One hour after the tracer injection, uptake in each brain region was compared between saline-pretreated (control) and drug-pretreated rats. Values are presented as the average ± SD of three rats in each point. ^b Rest of the brain. ^c *p* < 0.05. 1 μM McN5652, SERT ligand; reserpine, vesicular transporter ligand; methylphenidate, dopamine transporter ligand.

the binding of SERT sites. In vivo metabolism of [³H] ACF ([³H]5) in three rats was evaluated at 1 h after iv injection. It was found that essentially all of the activity (95 ± 1.0–40%) extracted from the brain was the original [³H]ACF (over 98% by thin-layer chromatography (TLC) analysis). The other tissues showed more extensive metabolism; the percentages of organic extractable material were 6.76 ± 1.00, 20.25 ± 0.60, and 20.03 ± 0.60 from plasma, kidney, and liver, respectively. It is likely that the metabolites in the peripheral tissues may not play an important role on uptake and retention of [³H]ACF in the brain of rats. Further studies will be needed to identify the chemical structures of possible metabolites in the peripheral tissues and organs.

In conclusion, a novel PET tracer, [³H]ACF ([³H]5), for imaging SERT sites in the brain was developed. It is a phenylthiophenyl derivative with a fluorine atom directly attached to one of the phenyl ring. In vitro binding studies showed that ACF binds to the SERT sites with high affinity and selectivity. After an injection into rats, [³H]ACF ([³H]5), showed high uptake and specific binding to hypothalamus, striatum, and hippocampus regions of the brain where serotonin neurons are highly concentrated. This ligand is a potentially useful agent in conjunction with PET for imaging SERTs in the brain.

Experimental Section

IR spectra were recorded on a Mattson Polaris Fourier transform FT-IR spectrometer. ¹H and NMR spectra were performed on a Bruker DPX 200 spectrometer using tetra-*methylsilane* as an internal standard. Elemental analyses and mass spectrometry were performed at Department of Chemistry, University of Pennsylvania. THF was distilled immediately before use from sodium benzophenone ketyl. All other chemicals were purchased from Aldrich Chemical Co. and used without further purification. 2-Mercapto-*N*,*N*,*N*,*N*-tetra-methylbenzamide (2) was prepared by using a reported procedure mandatorily prior to the reaction and used without purification. 18 sodium fluoride was provided by Caledon Chemicals, University of Pennsylvania, Male Springs. Twelve rats weighing 250–300 g were used in all of the studies. 1,1-McN5652 was kindly provided by Research Triangle Institute (Durham, NC) through a drug synthesis program supported by the National Institute of Mental Health. Synthesis and

methylphenidate were purchased from Research Triangle Institute. All other chemicals used were of reagent grade.

2-(2-Amino-4-chloro-2-nitro-phenylthio)-*N*,*N*-dimethylbenzamide (9). A solution of 1 (3.0 g, 14.5 mmol) and 2 (3.0 g, 16.8 mmol) in DMF (50 mL) was heated to reflux with potassium carbonate (10 g) for 24 h. The mixture was poured into ice water and the precipitate was collected by filtration. A dark colored solid was recrystallized from petroleum ether/methylene chloride, which yielded 3.9 g of 3 as a pale yellow powder (72% IR (cm⁻¹): KBr) 3461, 1726, 1513, 1472, 1576, 1492, 1135. ¹H NMR (200 MHz, CDCl₃) δ 8.52 (s, 1H, 7.3–7.6 (m, 4H), 6.16 (s, 1H), 1.8 (br s, 2H), 3.37 (s, 3H), 2.86 (s, 3H). Anal. Calcd. for C₁₄H₁₄ClN₂O₂S: C, 57.8%.

2-(4-Chloro-5-fluoro-2-nitro-phenylthio)-*N*,*N*-dimethylbenzamide (4). To a solution of 3 (1 g, 2.84 mmol) in 6.5 mL THF (10 mL), a solution of sodium azide (0.3 g, 3.5 mmol) in water (0.5 mL) was added at 5–10 °C. To this mixture, 48% hexachlorophosphoric acid (1.5 mL) was added at 0 °C and the mixture was stirred for 10 min. The precipitate was collected by filtration. The deep red solid was washed with ice water and an dried using a vacuum filter. The solid was further dried under high vacuum for several hours. The red powder was heated at 170 °C using an oil bath until gas evolution was no longer observed. A dark colored residue was purified by PLASMA (Bunce) prepurified silica column (CHL 1) with a eluent: 35/25 to yield 580 mg of 4 (57% IR (cm⁻¹): neat) 3472, 1641, 1521, 1465, 1490, 1336, 1282, 1249, 1094. ¹H NMR (200 MHz, CDCl₃) δ 8.32 (d, 1H), 7.28 (d, 1H), 7.4–7.6 (m, 2H), 6.47 (d, 1H), 5.0 Hz, 1H), 3.04 (s, 3H), 2.84 (s, 3H). Anal. Calcd. for C₁₄H₁₂ClF₂N₂O₂S: C, 57.8%.

2-(2-Amino-4-chloro-5-fluorophenylthio)-*N*,*N*-dimethylbenzethanamine (5). To a solution of 4 (2.00 mg, 0.9 mmol) in 10 mL of anhydrous THF, 1.91 base (THF complex) (0.9 mL) was added at 0 °C under N₂. The mixture was heated to reflux for 2 h. After it was cooled to 0 °C, 1 mL of concentrated HCl was carefully added and the solvent was removed in vacuo. In the residue, 10 mL of water was added and the mixture was heated to reflux for 20 min. The mixture was cooled and 1 N NaOH was added to make the pH of the solution basic (pH = 10). The resulting cloudy aqueous solution was extracted with ethyl acetate (5 mL × 3). The combined organic layer was dried (Na₂SO₄) and the solvent was removed in vacuo. The residue was purified by silica gel plate (eluent: acetone) to yield 170 mg of 5 as colorless oil (80% IR (cm⁻¹): neat) 3457, 3378, 3281, 2960, 2819, 2777, 1606, 1489, 1247, 1049, 117. ¹H NMR (200 MHz, CDCl₃) δ 7.25 (d, 2H), 7.15 (m, 2H), 6.14 (m, 1H), 6.73 (d, 1H), 6.5 Hz, 1H), 3.55 (s, 2H), 2.28 (s, 3H). HRMS (MALDI) Calcd. for C₁₄H₁₄ClFN₂S: 311.0786; found 311.0711. Anal. Calcd. for C₁₄H₁₄ClFN₂S: C, 57.8%.

2-(4-Chloro-5-(dimethylamino)-2-nitro-phenylthio)-*N*,*N*-dimethylbenzamide (6). Compound 3 (1.0 g, 2.84 mmol) was heated to reflux with methyl iodide (10 g) and potassium carbonate (5 g) in 10 mL of *n*-butanol (DMF for 24 h. The mixture was poured into cold water, and the precipitate was collected by filtration and purified by PLASMA (Bunce) prepurified silica column, CHL 1 (eluent: acetone) 5/1 to yield 6 (yellow powder, 0.7 g, 65%) IR (cm⁻¹): neat) 2923, 1636, 1577, 1498, 1322, 1239. ¹H NMR (200 MHz, CDCl₃) δ 8.22 (s, 1H), 7.3–7.6 (m, 4H), 6.20 (s, 1H), 3.04 (s, 3H), 2.82 (s, 3H), 2.71 (s, 3H). Anal. Calcd. for C₁₄H₁₄ClN₄S: 319.133 C, 57.8%.

2-(4-Chloro-5-(2-dimethylaminoethyl-phenylthio)-4-nitro-phenylthio)-*N*,*N*-dimethylbenzamide. Trifluoromethanesulfonate (7). Compound 6 (0.5 g, 1.42 mmol) and methyl trifluoromethanesulfonate (0.3 g, 1.43 mmol) were heated to reflux in 5 mL of methylene chloride for 72 h. After it was cooled, ethyl ether was added to the mixture. The precipitate was collected and recrystallized from CHCl₃/ethyl ether repeatedly. Highly hygroscopic pale yellow powder was dried in vacuo and used directly for PET radiolabeling without further purification (yellow powder, 0.31 g, 40%) IR (cm⁻¹): neat) 3560, 1623, 1553, 1515, 1256, 1151, 1027, 794, 640. ¹H NMR (200 MHz, CDCl₃) δ 8.33 (s, 1H), 7.74–7.90 (m, 5H), 7.03 (s, 1H), 3.69 (s, 9H), 3.40 (s, 3H), 3.06 (s, 3H). HRMS (calcd. for C₂₁H₂₄N₄O₄S₂) 584.0902; found 584.0905.

2-Chloro-5-(2-dimethylaminoethyl-phenylthio)-4-nitrobenzamide (8). Compound 8 was obtained from 3 (0.5 g, 1.4 mmol) using the same procedure for the preparation of 5 (high brown oil, 350 mg, 74%) IR (cm⁻¹): neat) 3449, 3376, 1608, 1599, 1521, 1417, 1379, 1319, 1184, 1285, 1251, 1133, 725, 41. ¹H NMR (200 MHz, CDCl₃) δ 8.29 (s, 1H), 7.67 (d, 1H), 6.8 Hz, 1H), 7.54 (m, 2H), 7.34 (m, 1H), 3.55 (s, 2H), 3.53 (s, 2H), 2.20 (s, 3H). Anal. Calcd. for C₁₄H₁₄ClN₂O₂: 282.043 C, 57.8%.

2-(4-Chloro-5-fluoro-2-nitro-phenylthio)-*N*,*N*-dimethylbenzethanamine (9). The desired product 9 was prepared from 4 (0.5 g, 0.88 mmol) using the procedure for 4 (colorless, 150 mg, 50%) IR (cm⁻¹): neat) 2927, 2849, 1550, 1425, 1421, 1340, 1278, 1252. ¹H NMR (200 MHz, CDCl₃) δ 8.72 (d, 1H), 7.22 Hz, 1H), 7.3–7.6 (m, 4H), 6.62 (d, 1H), 5.8 Hz, 1H), 3.52 (s, 2H), 2.18 (s, 3H). Anal. Calcd. for C₁₄H₁₄ClFN₂S: 311.0786; found 311.0711. Anal. Calcd. for C₁₄H₁₄ClFN₂S: C, 57.8%.

[³H]-182 (2-Amino-4-chloro-5-fluorophenylthio)-*N*,*N*-dimethylbenzethanamine ([³H]5). [³H]iodide, produced by a cyclotron using ²⁰Ne on ¹⁹F reaction, was passed through a Sep Pak Light OMA cartridge as an aqueous solution (pH 5) in deionized water. The cartridge was dried by air flow, and the activity was eluted with 2 mL of Rspitox 222-322 (K₂CO₃) solution (2 mg of 8,222 and 4.6 mg of K₂CO₃ in CHCl₃/N₂O₄) 1/7/0.2/0. The solvent was removed at 110 °C under an argon stream. The residue was azeotropically dried with 1 mL of anhydrous CHCl₃ twice. A solution of precursor 7 (5 mg in DMSO 0.5 mL) was added to the reaction vessel containing dried [³H]-182 activities. The solution was heated at 55 °C for 10 min. Water (2 mL) was added, and the mixture was extracted with ethyl acetate (1 mL × 2). The combined organic layer was dried (Na₂SO₄) and the solvent was removed using an argon stream with gentle heating (55–60 °C). The residue was dissolved in CHCl₃ (2 mL) and injected into a Sep Pak Plus silica cartridge. The cartridge was washed with 10 mL of CHCl₃, and eluted activity was discarded. Related activity was eluted with 5 mL of CHCl₃/ethyl acetate 2/3. Solvent was removed using an argon stream with gentle heating. Compound [³H]5 was obtained with a radiochemical purity of 100% (radiochemical yield: 30%). Compound [³H]5 was dissolved in THF (0.5 mL) and 0.2 mL of 1 N HCl. THF was added. The mixture was heated at 55 °C under an argon for 25 min. The solvent was concentrated to near dryness by an argon stream at the end of reaction. To this mixture, 0.1 mL of concentrated HCl and 0.5 mL of ethanol were added with external elution. In the mixture, 1 mg of NaCl was added and the reaction was kept at room temperature for 20 min. An amount of 55.2 μM HCl (2 mL) was added to the reaction with cooling and extracted with ethyl acetate (2 mL × 2). The combined organic layer was filtered

and dried (Na₂SO₄). The solvent was removed using an argon stream. The residue was dissolved in CHCl₃ (2 mL) and injected into a Sep Pak Plus silica cartridge. The cartridge was washed with CHCl₃/ethyl acetate (20/1) 10 mL. Related activity was eluted with ethyl acetate/McCl₃ (2/1) 5 mL. The solvent was removed, and [³H]5 was obtained with an RCP of 30%, which was further purified with HPLC (TSP, 1 column, CHCl₃/dimethylacetate buffer (pH 10.9), 1 mL/min at 0–8.1 min) to attain RCP > 99%. The average product was approximately 3 h, and radiochemical yield was 15% (decay corrected).

In Vitro Binding. The membrane homogenates of three monoamine transporters (referred to as LLC-DAT, LLC-NEET, and LLC-SERT), which were expressed in a common parental cell line (LLC-PK1) were prepared and used for the binding assays. Competitive binding assays were performed in a final volume of 0.2 mL. Aliquots of membrane suspensions (100 μL), corresponding to 30–40 mg of protein, were mixed with 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 0.1% bovine serum albumin, 0.1 mM [³H]HTF or [³H]DAM, and 5–10 concentrations (10⁻⁹ to 10⁻⁶ M) of competing drugs. Nonspecific binding was defined with 10 μM 25 carbomethoxy 3(4-fluorophenyl) tropane (CFT) for DAT and NEET assays and 1 μM DAM for SERT assays. Incubation was carried out for 1 h at room temperature, and the bound ligand was collected on glass fiber filters presoaked with 1% polyvinylpyrrolidone (Sigma St. Louis, MO) and counted in a gamma counter (Packard 5000 Downer, Grove, IL). Results of competition experiments were subjected to nonlinear regression analysis using LIGAND software (Biosoft Biosoft, Cambridge, U.K.).

Biodistribution in Rats. These rats per group were used for each biodistribution study. While under ether anesthesia (0.2 mL of a saline solution containing 30 μL of radioactive tracer was injected into the femoral vein). The rats were sacrificed at the time indicated by cardiac arrest while under ether anesthesia. Organs of interest were removed and weighed, and the radioactivity was counted. The percent dose per organ was calculated by comparing the tissue counts to that of 1% of the initial dose measured at the same time. Regional brain distribution in rats was measured after an injection of the radioactive tracer. Samples from different brain regions (cortex, striatum, hippocampus, cerebellum, and hypothalamus) were dissected, weighed, and counted. The percentage dosage of each sample was calculated by comparing sample counts with the counts of the diluted initial dose described above.

In vivo competitive binding of various compounds in the regional uptake of [³H]5 was investigated by pretreating animals, respectively, with various competing drugs (2 μg/kg, each, iv) at 5 min prior to injection of [³H]5 (0.1 μg). The competing drugs included methylphenidate, reserpine, and 1 μM McN5652. The reduction of regional brain uptake in the drug-pretreated rats was compared to the control animals with saline pretreatment.

To investigate in vivo metabolism, 1 h after an iv injection of 200 μCi of [³H]ACF into three rats, samples of brain, liver, kidney, and blood were removed individually. They were homogenized in 10 volume buffer (50 mM Tris buffer, pH 7.4). The tissue homogenates were extracted with ethyl acetate (3 × 2 mL). Combined ethyl acetate layers were evaporated to near dryness, and the purity of the combined extracts was analyzed by TLC (silica gel plates, ethyl acetate/McCl₃ 2/3). The spots on the TLC plates were counted by Phosphor Imager ST (Molecular Dynamics).

Statistics. Effects of drug on the radioactivity in the various regions of brain after [³H]5 injection were examined using analysis of variance procedures. Data of regional brain uptake of [³H]5 in pretreated rats were compared against those of control rats using Dunnett's test for multiple comparisons, with the level of significance set at *p* < 0.05.

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