SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

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Bogotá, D.C.,

Abogado
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ASUNTO	Radicación	10-37849
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	R 16567
	Folios	027

Patente de Invención Vía Nacional	■	Patente de Modelo de Utilidad		☐
Vía PCT (fase nacional)	■	Cap. I	■	Cap. II ☐

No. Solicitud Internacional PCT/GB2008/050890
Fecha de Presentación Internacional 02/octubre/2008.

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 19 a 333	05/abril/2010
Reivindicaciones:	Folios 334 a 366	05/abril/2010
	Folios 373 a 405	03/junio/2010
	Folios 426 a 458	22/septiembre/2011
Prioridad:	US 60977416	04/octubre/2007

2. Análisis de la Prioridad

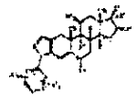
Se acepta la reivindicación de prioridad porque: esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Compuesto de [3,2-C]pirazol esteroides con actividad glucocorticoide"

El problema planteado en esta solicitud consiste en proporcionar compuestos útiles como antagonistas de receptor de glucocorticoides.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de la fórmula (I) y composiciones que lo contienen.



Compuesto I

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07J 71/00, A61K 31/58 y A61P 5/44.

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

El uso de las reivindicaciones 17-18 no es patentable, de acuerdo con el Art 14.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 15 no es clara ni concisa porque un procedimiento para la fabricación de un compuesto se refiere a una serie de etapas ordenadas que indican la totalidad de condiciones necesarias para llevar a cabo la actividad de una manera lógica y secuencial permitiendo a una persona del oficio normalmente versada en la materia a derivar directamente y sin ambigüedad a partir de la divulgación el procedimiento y de esta manera el producto a obtener. Se sugiere al peticionario incluir en la reivindicación 1 las características técnicas esenciales para llevar a cabo el procedimiento que se encuentran soportadas en la descripción y que permiten realizar la preparación del compuesto I

6. Determinación del Estado de la Técnica

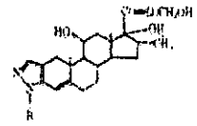
El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: octubre 04 de 2007 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Titulo	Fecha de Publicación*
D1	Journal of Medicinal Chemistry, 1975, Vol. 18, No. 2	Substituted Pyrazolo Corticoids as Topical Antiinflammatory Agents	1975
D2	US2003/ 0092690	Novel anti-inflammatory androstane derivatives	15/mayo/2003
D3	Steroids 63 595-602.1998	Iodinated and fluorinated steroid 2*-aryl-[3,2-c] pyrazoles as potential glucocorticoid receptor imaging agents	1998

D1: refiere (Pág 1. Col. 1) el descubrimiento de cierto heterociclos, especialmente pirazoles que contiene sustituyentes 2'aril, con actividad antiinflamatoria cuando están fusionados con el núcleo corticoide en la posiciones 2,3. Adicionalmente, indica que los pirazolo corticoides pueden tener actividad antiinflamatoria sin requerir todas las típicas funcionalidades de los corticoides.

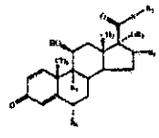
De igual forma, divulga que algunos pirazolo corticoides son 30 veces más potentes en el tratamiento del granuloma local que la hidrocortisona. Por lo que el estudio se enfocó en la búsqueda de un óptimo esteroide antiinflamatorio tópico pirazolo corticoide.

Finalmente, da a conocer (Pág. 3. tab III.) los resultados de su estudio



No.	R_F	Formula[a]	Mp, °C	Rat granuloma assay[b] (passage × hydrocortisone) Local	Systemic	Vasoconstriction,[c] rel potency at ED_{50}
4	C_6H_5[d]	$C_{27}H_{30}N_2O_3$	142–145 dec	100	60	
5	$C_6H_5CH_2$	$C_{28}H_{32}N_2O_3$	190–195	50–75	2–3	8
6	4-FC_6H_4	$C_{34}H_{31}FN_2O_3$	240–249	100–200	110	15
7	4-ClC_6H_4	$C_{34}H_{31}ClN_2O_3$	213–252 dec	40	10	
8	4-$EtOC_6H_4$	$C_{36}H_{34}N_2O_4$	250–255	10–80	3	1
9	4-$CH_3CONHC_6H_4$	$C_{36}H_{35}N_3O_4$	221–232	5–10	4–5	49
10	4-$CH_3SOC_6H_4$	$C_{36}H_{34}N_2O_3S$	246–249		<4	1
11	$CH_2CH_2CH_3$	$C_{32}H_{34}N_2O_3$	198–204	20		4
12	Cyclopropyl	$C_{32}H_{32}N_2O_3$	207–209	5–10		6
13	Cyclohexyl	$C_{36}H_{40}N_2O_3$	179–183	50	6	11
14	Cyclopentyl	$C_{35}H_{38}N_2O_3$	177–186	200–250	50–70	21
15	CH_3OOCCH_2	$C_{30}H_{34}N_2O_5$	118–120	<4	<8	<1
16	$HOCH_2CH_2$	$C_{31}H_{34}N_2O_4$	105–109	20 or >		<1
17	4-Pyridyl	$C_{34}H_{31}N_3O_3$	223–225	70–100	4	28
18	3-Pyridyl	$C_{34}H_{31}N_3O_3$	238–237 dec	5–10		2
19	3-Pyridyl	$C_{33}H_{31}N_3O_3$	250–255	70–100	60	1
20	4-Piperidyl	$C_{34}H_{37}N_3O_3$	Amorphous	5–7	<3	<1

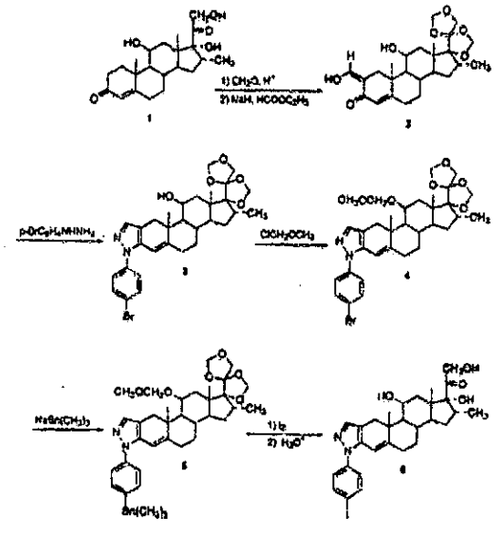
D2: http://www.google.com/patents/US20030092690 divulga una serie de glucocorticoides derivados de la androstano de fórmula I.



Compuesto I.

Adicionalmente, a través de sus realizaciones preferidas da a conocer los compuestos 1 (Pág,1, Par.[0291]) correspondiente al compuesto 6α,9α-difluoro-17α-[(2-funanilcarbonil)oxi]-11β-hidroxi-16α-metil-3-oxo-androsta-1,dieno-17β- ester S-fluorometil del ácido carboxitioco y 6 (Pág,1, Par.[0299]) definido el compuesto 9α-fluoro-17α-[(2-funanilcarbonil)oxi]-11β-hidroxi-16α-metil-3-oxo-androsta-1,dieno-17β-ester S-fluorometil del ácido carboxitioco.

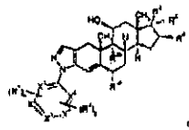
D3. Enseña (Pág. 4, Esq. 1) un esquema para la síntesis de derivados de 2-aril pirazolo corticoide que comprende la reacción de un derivado del pregnano que posea simultáneamente un grupo hidroxialil en la posición 2 y un grupo oxo en la posición 3 con una fenilhidrazina. Luego reaccionan con los siguientes reactivos y en la siguiente secuencia 1.) $\xrightarrow{ClCH_2OCH_3}$ 2.) $\xrightarrow{NaBA(CH_3)_3}$ y 3.) $\xrightarrow[2)\ H_3O^+]{1)\ \Delta}$ para obtener un derivado 2-aril pirazolo corticoides.



7. **Examen de Patentabilidad** (Art 14 D486)

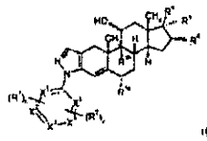
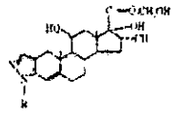
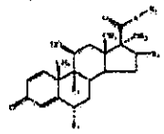
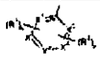
Nivel Inventivo (Art18 D486)

La reivindicación consiste para la reivindicación 1 en un compuesto de fórmula I.



Compuesto I

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

Características esenciales	**D1**	**D2**
Un compuesto de fórmula I  Compuesto I	Un compuesto de fórmula 	Un compuesto de fórmula I  Compuesto I
 donde X^1, X^2, X^3, X^4 y X^5	R^2	
representan cada uno independientemente CH o un átomo de nitrógeno, siempre que no más de dos de X^1, X^2, X^3, X^4 y X^5 pueden representar simultáneamente un átomo de nitrógeno;		
n y p		
representan cada uno independientemente 0 o 1		
R^1	C_6H_5 2-piridil 3-piridil 4-piridil 4-piperidil opcionalmente sustituido por Flúor, Cloro, hidroxilo y metilo	**No refiere**
representa un átomo de halógeno o un grupo metilo o un grupo metoxi;		
R^2		
representa un átomo de halógeno,-C(O)OCH_3,-C(O)-SCH_2CN,-C(O)-heterociclo, SO_2CH_3 un grupo alquenilo C_2–C_6 o un grupo metilo opcionalmente sustituido con halógeno, hidroxilo, metoxi,-$OCH_2CH=CH_2$ o-NR^7R^8		
R^{3a}	hidrógeno	R^4
representa un átomo de hidrógeno o un grupo metilo		hidrógeno o halógeno
R^{3b}	hidrógeno	R^5
representa un hidrógeno o un átomo de flúor		hidrógeno o halógeno
R^7 y R^8	**No refiere**	**No refiere**
representan cada uno independientemente un átomo de hidrógeno,o un grupo alquilo C_1-C_3 o un grupo hidroxialquilo C_1-C_3		
R^7 y R^8		
junto con el átomo de nitrógeno al que están unidos forman un anillo heterocíclico 3 a 8 miembros saturado o parcialmente saturado que opcionalmente contiene un heterogrupo adicional en el anillo seleccionado de nitrógeno, S(O)m y oxígeno, estando el anillo heterocíclico opcionalmente sustituido por al menos un sustituyente seleccionado de hidroxilo, alquilo C_1-C_3 y hidroxialquilo C_1-C_3		
R^4	$C(=O)CH_2OH$	
representa -C(O)-Y-$CH(R^{11})$-R^9 ó -C(O)-$CH(R^{11})$-Y-R^9		

Características esenciales	D1	D2
R^6	OH	
representa $-O-C(O)-R^{10}$		
R^6	metilo	R^3
representa un átomo hidrógeno o halógeno o un grupo metilo o un grupo hidroxilo		Hidrógeno, metilo o un grupo metileno
Y	oxígeno	
oxígeno o azufre o un grupo NH		azufre
R^9	hidrógeno	R^1
hidrógeno, halógeno, ciano,-S-CN,-C(O)N(R^{12})$_2$, alcoxicarbonilo C_1-C_6, alquil C_1-C_6 carbonilo, (opcionalmente sustituido con -OC(O)CH_3), alquilcarbonilo C_1-C_6, alcoxi C_1-C_6, alquil C_1-C_6 tio, C(O)-S-alquilo,-C(=CH_2)-O-CH_2OCH_3,C_1-C_6 alquilo, C_2-C_6 alquenilo, C_2-C_6 alquinilo ,cicloalquilo C_3-C_7 estando los últimos cuatro grupos opcionalmente por uno o más sustituyentes seleccionados independientemente de halógeno, hidroxilo, ciano, hidroximetilo, alcoxi C1-C4 y C 1-C4-carboniloxi;		C_{1-6} alquilo o C_{1-6} haloalquilo
R^{10}	No refiere	R^2
C_1-C_6 alquilo (opcionalmente sustituido por halógeno, C_1-C_4 alcoxi, C_1-C_4-carboniloxi o cicloalquilo C_3-C_7) o un sistema de anillo heterocíclico o carbocíclico saturado ó insaturado de 3 a 10 miembros, pudiendo dicho sistema de anillo estar opcionalmente sustituido con al menos un sustituyente seleccionado de halógeno, oxo, carboxilo, hidroxilo, nitro, ciano, mercapto, C_1-C_6 alquilo, C_2-C_6 alquenilo, haloalquilo C_1-C_6, ,hidroxialquilo C_1-C_6, alcoxi, Cl-C_6 haloalcoxi, Cl-C_6 alquiltio, alquil C_1-C_6 sulfinilo, alquil C_1-C_6 sulfonilo, alquil C_1-C_6 carbonilo alquil C_1-C_6 carboniloxi, alcoxi C_1-C_6 carbonilo, amino (-NH2), carboxamido (-$CONH_2$), (mono) alquil C_1-C_6 amino, dialquil C_1-C_6 amino y fenilo.		Aril o heteroaril
R^{11}	hidrógeno	hidrógeno
un átomo de hidrógeno o un grupo metilo		
R^{12}	No refiere	No refiere
hidrógeno o un grupo metilo; condición de que cuando R^4 representa -C(O)CH_2OH, -C(O)CH_2 OC(O)C_2H_5, X^1, X^2, X^3, X^4 y X^5 representan cada uno CH y R^5 representa-O-C(O)-R^{10} en el que R^{10} representa alquilo C_1-C_6, entonces al menos uno de R^1 y R^2 está presente ;		

El documento D1 se considera el estado más cercano de la técnica porque divulga el una serie de derivados de la aril o heteroaril pirazolo corticoides sustituidos derivados del 17α,21-dihidroxi-20-oxopregn-4-eno-[3,2-c]-2′-(4-fluorofenil)-pirazol como agentes antiinflamatorios tópicos.

La diferencia entre la invención, definida en la reivindicación 1 ejemplificada por el compuesto 122 de la invención y el compuesto 6 divulgado en el documento D1 consiste en que 1.) La invención define en la posición 17 un sustituyente aciloxi y el documento D1 refiere un sustituyente hidroxilo.

Las diferencias entre la invención, definida en la reivindicación 1 ejemplificada por el compuesto 8 de la invención y el compuesto 6 divulgado en el documento D2 consiste en que 1.) La invención define como núcleo central aril o heteroaril pirazolocorticoide y el documento D2 refiere derivados del cortisol , es decir no refiere un anillo fusionado aril o heteroaril (pirazolo) al núcleo central del corticoide y 2.) El compuesto 8 refiere un sustituyente átomo de hidrógeno en la posición 9 del anillo

corticoide y el documento D2 ejemplifica en todas sus realizaciones preferidas sustituyentes flúor en las posiciones 6α y 9α aunque este indica la posibilidad de ser un átomo de hidrógeno.

El efecto que logra al modificar los sustituyentes en el radical R^5 ubicado en la posición 17 del anillo corticoide de los compuestos del documento D1 es obtener compuestos útiles como antagonistas de receptor de glucocorticoides.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "cómo modificar los sustituyentes de los compuestos antagonistas de receptor de glucocorticoides descritos en D1 en la posición 17 del anillo corticoide para así obtener nuevos compuestos alternativos antagonistas de receptor de glucocorticoides"

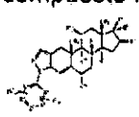
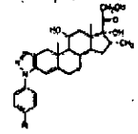
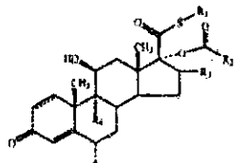
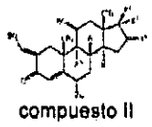
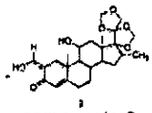
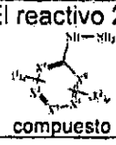
Sin embargo, dado que el documento D2 refiere y ejemplifica una serie de sustituyentes en las posiciones 16 y 17 del anillo corticoide, donde son seleccionados varios de los sustituyentes referidos y ejemplificados por la invención y que el documento D1 divulga que algunos 2-arilpirazolocorticoides poseen hasta 30 veces más potencia que la hidrocortisona (cortisol).

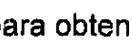
En consecuencia, la persona del oficio normalmente versada en la materia estaría motivada o tendría suficiente expectativa de éxito para modificar los sustituyentes en las posiciones 16 y 17 del anillo corticoide de los compuestos divulgados en D1 por los sustituyentes indicados para esas posiciones en el documento D2 o cualquier otro sustituyente que se pueda considerar una serie bioisostéra de los mismos. Para así obtener compuestos alternativos antagonistas de receptor de glucocorticoides. Por lo tanto la reivindicación 1 se considera obvia.

Dado que las reivindicaciones 2-14 no mencionan características técnicas esenciales a la reivindicación 1, estás tampoco se puede considerar inventivas.

La reivindicación 15 consiste en un proceso para producir el compuesto I que comprende hacer reaccionar un compuesto de fórmula II y un compuesto de fórmula III.

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

Solicitud	D3	D2
Un proceso para producir el compuesto I  compuesto I	Un procedimiento para producir el compuesto 	Un proceso para producir el compuesto II.  compuesto II
El reactivo 1 es compuesto II  compuesto II	El reactivo 1 es compuesto 2  compuesto 2	
El reactivo 2 es  compuesto III	El reactivo 2 es $p\text{-}BrC_6H_4NHNH_2$	

El documento D3 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 15 porque divulgan un método para producir un derivado 2-aril pirazolo corticoide a través de la reacción de un derivado del Pregnano que posea simultáneamente un grupo hidroxialil en la posición 2 y un grupo oxo en la posición 3 del anillo corticoide con una fenilhidrazina. Luego hace reaccionar con los siguientes reactivos y en la secuencia definida a continuación: 1.)  2.) y 3.) para obtener el derivado 2-aril pirazolo corticoides.

La diferencia entre la invención, definida en la reivindicación 15 y el procedimiento de D1 consiste en el reactivo 1 ya que la invención define como reactivo 1 el compuesto II  y D3 refiere como reactivo 1 el compuesto 2  .

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "cómo sustituir el reactivo 1 en el procedimiento divulgado en D3 por otro derivado del Pregnano que posea sustituyentes alternativos en las posiciones 16 y 17 del anillo"

Sin embargo, dado que el documento D2 refiere múltiples series bioisostéras del compuesto I de la invención y dado que la reivindicación no refiere condiciones específicas diferentes a las divulgadas en los documentos D2 y D3.

En consecuencia, la persona del oficio normalmente versada en la materia, sustituiría el reactivo 1 en el procedimiento divulgado en D3 por otro derivado del Pregnano que posea sustituyentes alternativos en las posiciones 16 y 17 del anillo como los divulgados en el documento D2 para llegar así al objeto de la reivindicación 15 de la solicitud. Por lo cual se considera obvia.

8. Conclusión

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

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Journal of Medicinal Chemistry, 1975, Vol. 18, No. 2	1 - 14	Nivel Inventivo
D2	US2003/0092690	1 - 15	Nivel Inventivo
D3	Steroids 63; 595-602.1998	15	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 (60 días) 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 18 SET. 2012

Elaborado por: Lenin Roberto Guerrero.
Revisado Por: Consuelo Leguizamón Leguizamón

Expediente 10-37849 Artículo 45

80% acetone in $CHCl_3$ as solvent. The product recrystallized from aqueous MeOH to afford 11 in a yield of 46%: mp 98–99°; nmr δ 0.81 (s, C-18 H's), 1.21 (s, C-19 H's), and 5.76 (s, C-4 H). *Anal.* ($C_{22}H_{34}O_4$) C, H.

17β-(3'-Hydroxypropyl)androst-4-en-3-one (12). Conditions similar to those used to prepare 7 were used to convert 13 to 12 in a yield of 91%. The product was recrystallized from aqueous acetone: mp 85–86°. *Anal.* ($C_{23}H_{34}O_3$) C, H.

17β-(3'-Hydroxypropoxy)androst-5-ene-3,2'-(1',3'-dioxolane) (13). To a flask which contained 9-borabicyclo[3.3.1]nonane (0.219 g, 1.79 mmol) and which was cooled in an ice bath was added, under N_2, a solution of 3 (0.6 g, 1.34 mmol) in 10 ml of THF. The mixture was stirred at room temperature for 3 hr and under reflux for 1 hr. The solution was cooled to room temperature and a solution of 2 ml of H_2O, 2 ml of 3 *N* aqueous NaOH, and 2 ml of 30% H_2O_2 was added. The mixture was stirred until gas evolution ceased. The product was chromatographed over 17 g of neutral Al_2O_3 using benzene as solvent. Crystallization from aqueous EtOH or acetone gave 13 in a yield of 49%: mp 108–109°; nmr δ 0.80 (s, C-18 H's), 1.05 (s, C-19 H's), and 5.40 (m, C-6 H). *Anal.* ($C_{24}H_{38}O$) C, H.

Acknowledgments. This work was supported, in part, by Grant CA-10116 from the National Cancer Institute of NIH and, in part, by Grant 5 SO1-RR 05464 from NIH. We are also grateful to Carole Haydon for technical services, to Dr. Voigt for assaying our compounds as inhibitors of testosterone 5α-reductase, and to Dr. Ringold for sending us his assay data.

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Substituted Pyrazolo Corticoids as Topical Antiinflammatory Agents

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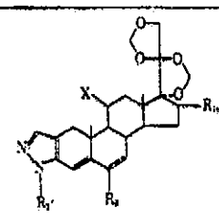
The synthesis of a series of substituted pyrazolo corticoids is described. Of these 11β,17α,21-trihydroxy-6,16α-dimethyl-4,6-pregnadieno[3,2-c]-2'-(4-pyridyl)pyrazole (21) shows an excellent separation of systemic to local activity in the model animal test. Compound 21 exhibits high vasoconstriction activity in human volunteers and is clinically effective in the treatment of psoriasis.

The discovery that certain heterocycles, especially pyrazoles containing 2'-aryl substituents, imparted enhanced antiinflammatory activity when fused to the corticoid nucleus at the 2,3 positions was reported some years ago by Hirschmann, *et al.*,[1a] and has been further explored by the Merck group[1b,2] and by others.[3] The Merck findings have been reviewed.[1c]

To date, there have been no reports that such derivatization raises steroidal androgenic, progestational, or aldosterone antagonist potency. This implies potentially increased selectivity with respect to the antiinflammatory process. Furthermore, preliminary studies indicated that pyrazolo corticoids could be active antiinflammatory agents without requiring all of the typical corticoid functionality and that they could show a high local to systemic ratio relative to hydrocortisone. An early example was 17α,21-dihydroxy-20-oxopregn-4-eno[3,2-c]-2'-(4-fluorophenyl)pyrazole (1) which was 30 times as potent in the local granuloma test as hydrocortisone and which unexpectedly displayed 12 times greater local than systemic inhibition of granuloma formation in the same test system.

With this encouraging data, we initiated a search for an optimal topical antiinflammatory steroid among the pyrazolo corticoids using as criteria high local activity, low systemic activity, and good skin penetration.

Table I



R_2	R_6	X	R_{16}	Method	Mp, °C	Formula[a]
4-FC_6H_4[b]	H	H	H	A	221–225	$C_{30}H_{33}FN_2O_4$
$C_6H_5CH_2$	H	OH	α-CH_3	B	193–196	$C_{32}H_{43}N_2O_4$
4-FC_6H_4	H	OH	α-CH_3	*f*	154.5–156.5	$C_{31}H_{31}FN_2O_4$
4-ClC_6H_4	H	OH	α-CH_3	*g*	156–157	$C_{31}H_{31}ClN_2O_4$
4-HOC_6H_4	H	OH	α-CH_3	B	326–329 dec	$C_{31}H_{32}N_2O_5$
4-$CH_3CONHC_6H_4$[c]	H	OH	α-CH_3	B	199–202 dec	$C_{33}H_{41}N_3O_5$
4-$CH_3SC_6H_4$	H	OH	α-CH_3	B	225–231 dec	$C_{32}H_{40}N_2O_4S$
4-$CH_3SOC_6H_4$[d]	H	OH	α-CH_3		202–207	$C_{32}H_{40}N_2O_5S$
$CH_3CH_2CH_2$	H	OH	α-CH_3	B	183–186	$C_{26}H_{40}N_2O_4$
Cyclopropyl	H	OH	α-CH_3	B	Amorphous	$C_{28}H_{38}N_2O_4$
Cyclopentyl	H	OH	α-CH_3	B	Amorphous	$C_{30}H_{42}N_2O_4$
Cyclohexyl	H	OH	α-CH_3	B	Amorphous	$C_{31}H_{44}N_2O_4$
CH_3OOCCH_2	H	OH	α-CH_3	B	189–194	$C_{28}H_{36}N_2O_6$
$HOCH_2CH_2$	H	OH	α-CH_3	*g*	184–186	$C_{27}H_{38}N_2O_5$
2-Pyridyl	H	OH	α-CH_3	A	270–278 dec	$C_{30}H_{31}N_3O_4$
3-Pyridyl	H	OH	α-CH_3	A	230–235	$C_{30}H_{31}N_3O_4$
4-Pyridyl	H	OH	α-CH_3	A	278–284	$C_{30}H_{31}N_3O_4$
4-Piperidyl	H	OH	α-CH_3	B	Amorphous	$C_{30}H_{43}N_3O_4$
4-Pyridyl	CH_3-Δ^6	OH	α-CH_3	B	279–286	$C_{31}H_{31}N_3O_4$
4-$CH_3CONHC_6H_4$	CH_3-Δ^1	OH	α-CH_3	B	199–202	$C_{34}H_{41}N_3O_5$
4-Pyridyl[e]	CH_3-Δ^1	OH	β-CH_3	*c*	201–205	$C_{31}H_{31}N_3O_4$.

[a] Analytical results were within 0.4% of the theoretical values. [b] 2′ isomer: 58% yield; mp 221–225°; λ_{max} 292 nm (ε 16,820); $[\alpha]^{25}D$ +17.6°. *Anal.* ($C_{30}H_{33}N_2O_4$) C, H, N. 1′ isomer: 11% yield; mp 282–286°; λ_{max} 294 nm (ε 23,470); $[\alpha]^{25}D$ −53.4°. *Anal.* ($C_{30}H_{33}N_2O_4$) C, H, N. [c] Made from 17α,20:20,21-bis(methylenedioxy)-2-hydroxymethylene-16α-methylpregn-4-ene-3,11-dione[1c] *via* pyrazole formation (method B) to yield the product, mp 221–225° [*Anal.* ($C_{33}H_{39}N_3O_5$) C, H, N], further reduced by $NaBH_4$–*i*-PrOH to yield the indicated compound. [d] Prepared from the 4-$CH_3SC_6H_4$ precursor by peracetic acid oxidation. [e] The required 17α,20:20,21-bis(methylenedioxy)-2-hydroxymethylene-6,16β-dimethyl-4,6-pregnadiene-3,11-dione was made in these laboratories by Dr. D. W. Graham and characterized by mp 235–247°; λ_{max} 292 nm (ε 15,300). *Anal.* ($C_{28}H_{32}O_7$) C, H from 17α,20:20,21-bis(methylenedioxy)-11β-hydroxy-6α,16β-dimethylpregn-4-en-3-one (to be published). 17α,20:20,21-Bis(methylenedioxy)-6,16β-dimethyl-11-oxopregna-4,6-dieno[3,2-*c*]-2′-(4-pyridyl)pyrazole was prepared by method A and melted at 315–325° dec. *Anal.* ($C_{31}H_{35}N_3O_3$) C, H, N. It was further reduced by $NaBH_4$–*i*-PrOH to yield the compound indicated in this table. [f] See ref 1a. [g] See ref 1c.

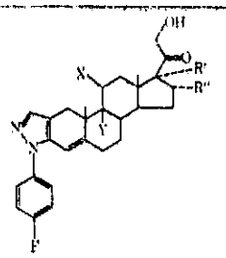
Chemistry. Methods for the synthesis of substituted pyrazolo corticoids were developed by Hirschmann, *et al.*[1a,b] In this investigation we used two procedures for pyrazole formation from the 2-hydroxymethylene-3-keto intermediates. Both are quite broadly applicable although the glacial acetic acid method was not satisfactory in the case of the pyridylpyrazoles. Characterization data for the intermediate, BMD-protected pyrazolo corticoids appear in Table I. Assignments as the desired 2′ isomers were based on characteristic uv differences between the 2′ and 1′ isomers. Only in the case of the 11-desoxy compound 1 was a substantial proportion (11%) of the inactive 1′ isomer formed.

Results

Summary data on several of the pyrazolo corticoids made initially in this project appear in Table II. The intent was to investigate the applicability of the 16,17-acetonide function and the possible usefulness of the 11-desoxy function in reducing systemic effects. Although high potencies and encouraging local/systemic ratios were obtained in the model system, the potency of these steroids did not extrapolate to the vasoconstriction test using human subjects. Perhaps poor dermal penetration was a limiting factor. In any event, it was evident that vasoconstriction activity would have to be an important early criterion in addition to potency and ratio as determined in the rat granuloma systems.

Table III summarizes variations of the 2′ substituent on the pyrazole of 16α-methylhydrocortisone, expanding the work of Hirschmann, *et al.*, in which high systemic activity with 2′-phenyl and 2′-(4-fluorophenyl) substituents was found. Despite its mediocre potency in the granuloma assays, surprisingly high vasoconstriction activity was observed with the 4-acetamidophenyl analog. Good local to systemic activity ratios were apparent for the benzyl, cyclohexyl, and 4-pyridyl derivatives, with the latter appearing to be the most promising in respect to the three preclinical criteria mentioned above. The 2′-(4-pyridyl)- and 2′-(4-acetamido)-[3,2-*c*]pyrazole functions were accordingly combined with other potency enhancing groups in the cortical steroid series. Some of the data which were obtained are summarized in Table IV. It was concluded that compound 21 held special promise for further studies based on both the granuloma and vasoconstriction data. Confirmation was obtained in recent multiclinic studies in

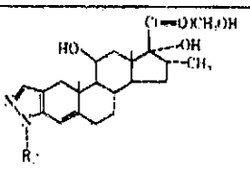
Table II



No.	X	Y	R′	R″	Formula	Mp, °C	Rat granuloma assay[a] (potency × hydrocortisone) Local	Rat granuloma assay[a] (potency × hydrocortisone) Systemic	Vasoconstriction,[b] rel potency at ED_{50}
1[c]	H	H	OH	H	$C_{24}H_{33}FN_2O_3$	225–238 dec	30	2.5	
2	H	H			$C_{31}H_{37}FN_2O_4$	215–221	110	7	<1
3[d]	H	F			$C_{31}H_{36}F_2N_2O_5$	244–258 dec	2000–3000	300	40
Fluocinolone acetonide							100–150	150	100

[a] Inhibition of granuloma was measured in the rat cotton pellet assay.[4] For a local test the steroid was absorbed onto the pellet from a 95% ethanol solution, dried *in vacuo*, and implanted. Systemically, the steroid was administered in the diet. [b] Vasoconstriction was estimated under occlusion on human volunteers using fluocinolone acetonide as the standard (100).[5] The steroids were applied using dilutions of a 95% ethanol solution to groups of ten subjects. Effective dose 50% response was measured graphically from a plot of number of patients responding against – log dilution over a range of $\frac{1}{10,000}$ to $\frac{1}{1,280,000}$. All compounds were submitted for assay under a code number rather than identified by structure. We are grateful to Dr. R. B. Stoughton, Scripps Clinic and Research Foundation, La Jolla, Calif., for these determinations conducted under a grant-in-aid from the Merck Sharp & Dohme Research Laboratories. [c] 2′ isomer: mp 226–238° dec; λ_{max} 260 nm (ε 16,640); $[\alpha]^{21}D$ +116.2°. *Anal.* ($C_{28}H_{33}FN_2O_2$) C, H, N. 1′ isomer: mp 224–236° dec; λ_{max} 295 nm (ε 29,260); $[\alpha]^{22}D$ +46.8°. *Anal.* ($C_{28}H_{33}FN_2O_3$) C, H, N. [d] See ref 3c.

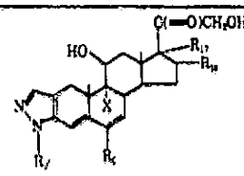
Table III



No.	R_1	Formula[c]	Mp, °C	Rat granuloma assay[a] (potency × hydrocortisone) Local	Rat granuloma assay[a] (potency × hydrocortisone) Systemic	Vasoconstriction,[b] rel potency at ED_{50}
4	C_6H_5[d]	$C_{27}H_{32}N_2O_4$	143–145 dec	100	60	
5	$C_6H_5CH_2$	$C_{28}H_{34}N_2O_4$	180–185	50–70	2–3	2
6	$4\text{-}FC_6H_4$	$C_{27}H_{31}FN_2O_4$	240–248	100–200	110	15
7	$4\text{-}ClC_6H_4$	$C_{27}H_{31}ClN_2O_4$	243–252 dec	40	15	
8	$4\text{-}HOC_6H_4$	$C_{27}H_{32}N_2O_5$	259–263	10–20	3	1
9	$4\text{-}CH_3CONHC_6H_4$	$C_{31}H_{35}N_3O_5$	221–222	5–10	4–5	49
10	$4\text{-}CH_3SOC_6H_4$	$C_{28}H_{34}N_2O_5S$	245–248		<4	1
11	$CH_3CH_2CH_2$	$C_{25}H_{34}N_2O_4$	198–204	20		4
12	Cyclopropyl	$C_{25}H_{34}N_2O_4$	207–209	3–10		6
13	Cyclohexyl	$C_{28}H_{42}N_2O_4$	178–183	50	8	17
14	Cyclopentyl	$C_{27}H_{40}N_2O_4$	177–180	200–250	50–70	21
15	CH_3OOCCH_2	$C_{26}H_{34}N_2O_6$	118–120	<4	<2	<1
16	$HOCH_2CH_2$	$C_{25}H_{34}N_2O_5$	195–199	20 or >		<1
17	4-Pyridyl	$C_{26}H_{31}N_3O_4$	223–232	75–100	4	36
18	2-Pyridyl	$C_{26}H_{31}N_3O_4$	228–237 dec	5–10		2
19	3-Pyridyl	$C_{26}H_{31}N_3O_4$	259–265	75–100	60	7
20	4-Piperidyl	$C_{26}H_{41}N_3O_4$	Amorphous	5–7	<2	<1

[a]–[b] See Table II. [c] Analytical results for C, H, and N were within 0.4% of the theoretical value. [d] See ref 1c.

Table IV



No.	$R_{2'}$	R_6	X	R_{16}	R_{11}	Formula[a]	Mp, °C	Rat granuloma assay[b] (potency × hydrocortisone) Local	Systemic	Vaso-constriction,[b] rel potency at ED_{50}
20	4-Pyridyl	6-CH_2-Δ^6	H	β-CH_3	OH	$C_{31}H_{41}N_3O_4$	214–218	20–30	2–3	2
21	4-Pyridyl[d]	6-CH_2-Δ^6	H	α-CH_3	OH	$C_{29}H_{37}N_3O_4$	209–213	1000–1500	40–50	74
22	4-Pyridyl	H_2	F			$C_{36}H_{38}FN_3O_6$	285–290	300–350	20	2
23	4-$CH_2CONHC_6H_4$	H_2	F			$C_{33}H_{43}FN_2O_6$	200–205		<10	<1

[a,b] See Table II. [c] See Table III. [d] Compound 21 showed no progestational activity at 0.2 and 1.0 mg/kg in the Clauberg-McPhail assay. (We are indebted to Dr. J. R. Brooks of the Merck Institute, Rahway, N.J., for these determinations.) The topical antiinflammatory corticoids fluocinolone 16,17-acetonide and bethamethasone 17-valerate are reported[6] to be progestagens 50.3 and 7.2 times as active as progesterone, respectively.

which a 0.1% concentration of compound 21 in an alcoholic gel vehicle was shown to be active in the treatment of psoriasis.

Experimental Section

Except where otherwise stated, uv spectra were measured in methanol, and rotations as approximately 1% solutions in chloroform. Where analyses are indicated only by symbols of the elements, results were within ±0.4% of the theoretical values.

Pyrazole Formation. Two sets of standard conditions were used.

17α,20:20,21-Bis(methylenedioxy)-11β-hydroxy-6,16α-dimethylpregna-4,6-dieno[3,2-c]-2′-(4-pyridyl)pyrazole (Method A). A solution of 4-pyridylhydrazine hydrochloride (0.380 g, 2.62 mmol) and KOAc (2.57 g, 2.62 mmol) in EtOH (10 ml)–H_2O (5 ml) was added to a solution of 17α,20:20,21-bis(methylenedioxy)-2-hydroxymethylene-11β-hydroxy-6,16α-dimethylpregna-4,6-dien-3-one[1c] (1.00 g, 2.18 mmol) in EtOH (25 ml) and the solution boiled under reflux for 4 hr. Distillation of part of the solvent (10 ml) induced crystallization which was completed by slow addition of water (20 ml) to the hot slurry. The crude product was filtered off and crystallized from EtOH to give 700 mg, mp 279–285°. Chromatography of the mother liquors on silica gel yielded an additional 176 mg. The analytical sample melted at 279–285°: λ_{max} 228, 282.5, 331 nm (ε 8,600, 24,300, 16,800); λ_{max} (MeOH + H^+) 223, 310, 375 nm (ε 10,200, 44,800, 6,300); $[\alpha]^{25}D$ −114.2°. *Anal.* ($C_{31}H_{37}N_3O_5$) C, H, N.

17α,20:20,21-Bis(methylenedioxy)-11β-hydroxy-16α-methylpregn-4-eno[3,2-c]-2′-(n-propyl)pyrazole (Method B). A suspension of 17α,20:2,21-bis(methylenedioxy)-2-hydroxymethylene-11β-hydroxy-16α-methylpregn-4-en-3-one[1c] (446 mg, 1.0 mmol), n-propylhydrazine oxalate (828 mg, 2.0 mmol), and KOAc (196 mg, 2.0 mmol) in HOAc (15 ml) was stirred at room temperature for 3 hr. The addition of H_2O (50 ml) precipitated an oil which was extracted by $CHCl_3$, purified by preparative tlc on silica ($CHCl_3$–4% MeOH), and crystallized from EtOH to give 188 mg: mp 183–186°; λ_{max} 214, 220, 248, 258, 277 nm (ε 11,830, 12,300, 6,900, 7,700, 11,600); $[\alpha]^{25}D$ +34.5°. *Anal.* ($C_{28}M_{40}N_2O_5$) C, H, N.

11β,17α,21-Trihydroxy-6,16α-dimethyl-20-oxopregna-4,6-dieno[3,2-c]-2′-(4-pyridyl)pyrazole (21) (BMD Reversal Conditions). A solution of 0.365 g of 17α,20:20,21-bis(methylenedioxy)-11β-hydroxy-6,16α-dimethylpregna-4,6-dieno[3,2-c]-2′-(4-pyridyl)-pyrazole in 7 ml of aqueous 60% formic acid was heated on the steam bath for 25 min. The mixture was then cooled in ice and made alkaline with aqueous sodium hydroxide. The suspension containing product was extracted with ethyl acetate. The crude product (0.375 g) after crystallization from methylene chloride displayed mp 209–213°: $[\alpha]^{25}D$ −27.5°; λ_{max} 232.5, 282.5, 330 nm (ε 9,300, 24,400, 15,765). *Anal.* ($C_{29}H_{39}N_3O_4$) C, H, N.

16α,17α,21-Trihydroxy-20-oxopregn-4-eno[3,2-c]-2′-(4-fluorophenyl)pyrazole 16,17-Acetonide (2). To a stirred suspension of 1.289 g of 16α,17α,21-trihydroxypregn-4-ene-3,20-dione 16,17-acetonide in 2.68 g of dihydropyran at room temperature was added 64 mg of p-toluenesulfonic acid monohydrate in 1 ml of ether. After 15 min 0.2 ml of pyridine and 50 ml of ether was added and the combined solvents were washed with water, dried, and removed to leave 1.25 g of crude tetrapyranyl ether. This was dissolved in 20 ml of dry benzene, and 1.18 g of 52.7% NaH oil dispersion and 0.1 ml of MeOH were added to the stirred mixture. Two additions of ethyl formate (1.05 ml) were made at 15 and 45 min. Work-up after 18 hr was by aqueous extraction and then back extraction into ethyl acetate from saturated NaH_2PO_4. The yield of 2-hydroxymethylene ketone was 985 mg of yellow foam: λ_{max} 250 nm ($E_{1\ cm}^{1\%}$ 206), 307 nm ($E_{1\ cm}^{1\%}$ 90) in methanol shifted with base to λ_{max} 242 nm ($E_{1\ cm}^{1\%}$ 259), 368 nm ($E_{1\ cm}^{1\%}$ 163).

Pyrazole formation was according to method A and the dihydropyran removal with concentrated HCl in MeOH under reflux for 15 min. The product displayed mp 218–221° (EtOAc): $[\alpha]^{25}D$ +135°; λ_{max} 263 nm (ε 16,660). *Anal.* ($C_{31}H_{37}FN_2O_4$) C, H, N.

The following compounds were prepared by the same process starting with 11β,16α,17α,21-tetrahydroxy-9α-fluoropregn-4-ene-3,20-dione 16,17-acetonide.

(a) 11β,16α,17α,21-Tetrahydroxy-9α-fluoro-20-oxopregn-4-eno[3,2-c]-2′-(4-fluorophenyl)pyrazole 16,17-acetonide (3): mp 244–258° dec (MeOH–CH_2Cl_2); $[\alpha]^{27}D$ +109.2°; ε 262 nm (ε 16,250). *Anal.* ($C_{31}H_{36}F_2N_2O_5$) C, H, F, N.

(b) 11β,16α,17α,21-Tetrahydroxy-9α-fluoro-20-oxopregn-4-eno[3,2-c]-2′-(4-pyridyl)pyrazole 16,17-acetonide (22): mp 285–290° (MeOH–CH_2Cl_2); λ_{max} 212, 269 nm (ε 15,180, 22,160); λ_{max} (MeOH + H^+) 223, 268, 301 nm (ε 10,800, 12,380, 32,400). *Anal.* ($C_{30}H_{36}FN_3O_5$) C, H, N.

(c) 11β,16α,17α,21-Tetrahydroxy-9α-fluoro-20-oxopregn-4-eno[3,2-c]-2′-(4-acetamidophenyl)pyrazole 16,17-acetonide (23): mp 200–205° (MeOH–H_2O); $[\alpha]^{25}D$ +86.4°. *Anal.* ($C_{33}H_{40}FN_3O_6$) C, H, N.

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Preparation, Hydrolysis, and Oral Absorption of α-Carboxy Esters of Carbenicillin

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Twelve α-carboxy esters of carbenicillin, a parenteral broad spectrum semisynthetic penicillin, were synthesized and examined as potential oral carbenicillin derivatives. The rates at which the esters were hydrolyzed *in vitro* to carbenicillin by animal and human tissues were compared and the carbenicillin serum levels arising after oral administration of the esters were measured in squirrel monkeys and human volunteer subjects. The α-carboxyphenyl ester of carbenicillin [carfecillin (British Pharmacopoeia approved name), BRL 2475] was selected for further study and is presently undergoing clinical trial.

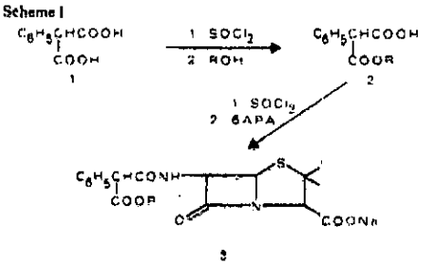
Carbenicillin (α-carboxybenzylpenicillin) has a broad spectrum of antibacterial activity and has been shown to be effective in the treatment of serious infections caused by *Pseudomonas aeruginosa* and other gram-negative bacteria. However, the compound is poorly absorbed from the gastrointestinal tract after administration by the oral route and its use is limited to parenteral administration. It has been found that certain α-carboxy esters of carbenicillin are absorbed by the oral route and undergo hydrolysis in the body to liberate carbenicillin.[1] One such ester (carbenicillin indanyl sodium) has been described in detail.[2]

Carbenicillin α-carboxy esters (that is, the ester group is in the side chain) possess a free thiazolidine carboxyl group and, hence, can be expected to demonstrate antibacterial activity *per se*. In general, we and others have found that the unhydrolyzed ester is more active than carbenicillin against gram-positive bacteria but shows a lesser degree of activity against most gram-negative bacilli.[3] However, the antibacterial spectrum shown by this class of compound will depend upon the extent of hydrolysis to carbenicillin that occurs in the course of the antibacterial test. Esters that are hydrolyzed rapidly to carbenicillin show an antibacterial spectrum similar to that of carbenicillin while esters with a slow rate of hydrolysis demonstrate activity primarily against gram-positive bacteria. In practice carbenicillin esters are of clinical interest only as a means of providing carbenicillin activity in the body after oral administration and in this context their intrinsic activity may be disregarded.

This report describes the preparation, *in vitro* hydrolysis rates, and oral absorption characteristics of a group of 12 α-carboxy esters of carbenicillin.

Chemistry. Scheme I illustrates the general route used to prepare α-carboxy esters of carbenicillin.[4] Phenylmalonic acid (1) was converted to its monoacid chloride by treatment with 1 equiv of thionyl chloride. The crude product was allowed to react with 1 equiv of alcohol or phenol (ROH) to afford the half-esters 2 described in Table I. The crystalline half-esters 2 were converted to their acid chlorides with excess thionyl chloride at 70° (higher temperatures caused degradation) and coupled directly to 6-aminopenicillanic acid (6-APA) in aqueous acetone. The penicillins 3 were isolated as their sodium salts and are described in Table II. A variation of this procedure has also been used to prepare penicillins of this type.[5]

Scheme I



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The α-carboxy esters, listed in Table II, as in the case of carbenicillin, contain an asymmetric center in their side chains and in solution their nmr spectra (Table III) suggested an approximately 1:1 mixture of epimers. However, in a number of instances (Table II) it was possible to obtain the penicillin sodium salts crystalline and these compounds were considered on nmr evidence to be single epimers in the solid state. Thus the nmr spectrum for the crystalline phenyl ester showed initially a singlet for the thiazolidine C-3 proton which rapidly changed to two singlets of equal intensity, consistent with racemization at the side-chain chiral center.†

Biological Properties. The 12 α-carboxy esters of carbenicillin, described in Table II, were examined for rates of hydrolysis to carbenicillin in aqueous solution and in the presence of isolated tissue homogenates. The 12 esters were also orally administered to both squirrel monkeys and human volunteers and levels of carbenicillin in blood and urine were measured. Details of these procedures are described in the Experimental Section. The results are discussed below.

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Synthesis of novel arylpyrazolo corticosteroids as potential ligands for imaging brain glucocorticoid receptors

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Abstract

Corticosteroids regulate a variety of essential physiological functions, such as mineral balance and stress. The great interest in these steroids, especially the glucocorticoids, stems from roles they are thought to play in neuropsychiatric disorders, such as severe depression and anxiety.

The development of glucocorticoid receptor (GR) ligands which are appropriately labeled with short-lived positron-emitting radioisotopes would allow the non-invasive in-vivo imaging and mapping of brain GRs by means of positron emission tomography (PET). In this context we have synthesized a series of novel arylpyrazolo steroids exhibiting different substitution patterns at the D-ring of the steroid skeleton, as ligands for brain GRs. Special attention was given to 4-fluorophenyl pyrazolo steroids, which are known to display high binding affinity toward the GR. The compounds were evaluated in a competitive radiometric receptor binding assay to determine their relative binding affinities (RBA) to the GR. Some compounds show good binding affinities of up to 56% in comparison to dexamethasone (100%). In initial experiments, selected candidates were labeled with the positron emitter fluorine-18 and in one case with the γ-emitter iodine-131.

Keywords: Arylpyrazolo corticosteroids; Glucocorticoid receptor binding; Radiolabeling; Positron emission tomography (PET)

1. Introduction

Endogenous corticosteroids exert a wide range of physiologic, biochemical, and behavioral effects in the central nervous system where the hormonal response is organized [1–3]. Corticosteroid hormones are produced in the adrenal gland, and their production is controlled via the hypothalamo-pituitary-adrenocortical (HPA) axis, a classical closed-loop endocrine system. They are released into the circulation particularly in high amounts after periods of stress. Due to their lipophilic nature, corticosteroids not only act on peripheral organs but also readily enter the brain. In neurons, they bind to two different intracellular receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). MRs and GRs are co-located in brain regions that are involved in the regulation of fear and anxiety, such as hippocampus, septum, and amygdala. GRs are also distributed in other parts of the brain, and the highest concentrations are found in regions involved in the feedback regulation of the hormonal stress response, such as the paraventricular hypothalamus, hippocampus and pituitary [4,5]. There is accumulating evidence that corticosteroids, in addition to noradrenaline, corticotropin releasing factor and serotonin, play an important role in stress and the pathophysiology of anxiety disorders and depression. It has been suggested that a disturbed HPA axis regulation may be a primary causal factor in depression. Since feedback inhibition of glucocorticoids is decreased in many depressive patients, it has been hypothesized that disturbances in the MR/GR balance may have deleterious consequences for regulation of the stress response, and it may increase vulnerability to depression.

The development of GR ligands which are appropriately labeled with short-lived positron-emitting radioisotopes would allow the non-invasive in vivo imaging and mapping of brain GRs by means of positron emission tomography (PET). In this context, PET studies of brain GRs would provide important information on the neurobiological

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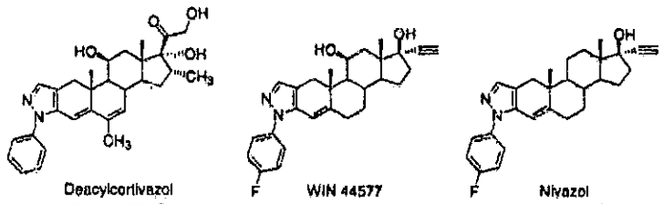

Fig. 1. High-affinity arylpyrazolo glucocorticoids (a) K.E. Carlson and J.A. Katzenellenbogen, unpublished results; (b) Rat liver cytosol, [14].

basis of GR-mediated functional abnormalities of HPA axis function in depression [6,7].

Several attempts have been made to synthesize glucocorticoids labeled with the short-lived positron emitter fluorine-18 ($t_{1/2}$ = 109.6 min) to image brain GRs [8–13]. Some of the compounds exhibited excellent in vitro GR binding, in some cases even significantly higher than the high-affinity glucocorticoid dexamethasone. However, none of the investigated compounds were suitable for in vivo visualization of brain GRs with PET. Rapid in vivo defluorination and the inability to cross the blood–brain-barrier are possible reasons for the lack of any accumulation of these compounds in the brain.

A unique set of synthetic arylpyrazolo steroids, exemplified by deacylcortivazol, nivazol and WIN 44577 (Fig. 1), have been shown to be very high-affinity ligands for the GR [14,15]. The strong GR binding affinity of compounds like WIN 44577, as well as their stable aryl-bound fluorine and their higher lipophilicity (for an anticipated enhanced brain uptake), make this class of compounds attractive for the development of novel fluorine-18 labeled ligands for PET studies of brain GR.

Intrigued by the high GR binding affinity of several arylpyrazolo steroids, we have synthesized a series of novel pyrazolo steroids exhibiting different substitution patterns at the D-ring of the steroid skeleton. The binding affinity of these compounds for the GR was determined in a competitive radiometric receptor binding assay, and in initial experiments potential candidates were labeled with the positron emitter fluorine-18 and in one case with the γ-emitter, iodine-131.

2. Experimental

2.1. General

Melting points were determined on a BOËTIUS melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on Merck silica-gel F-254 plastic plates, with visualization under UV (254 nm). Flash chromatography was performed as described by Still et al. [16] using Merck silica-gel (0.040–0.063 mm). ^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR spectra were recorded on a Varian Inova-400 at 400, 100 and 376 MHz, respectively, with $CDCl_3$ and $DMSO_{d6}$. Low resolution mass spectra were recorded on a Micromass tandem quadrupole mass spectrometer system (1100 series Hewlett-Packard) and on Mariner mass spectrometer system (Applied Biosystems). Analytical HPLC was conducted using LaChrom systems from Merck–Hitachi. The chemical purity was determined with a PHENOMENEX LUNA C18 column (250 mm × 4.6 mm, 5 μm). The mobile phase was MeOH/water (90/10) (A) and acetonitrile/water (70/30) (B). The system was run with a flow rate of 1.0 ml/min.

Solvents and reagents were purchased from Sigma, Fluka, or Aldrich. THF was distilled from sodium/benzophenone ketyl prior to use. Other reagents were used as received. Iodophenylhydrazine [17] and 4-hydroxy(tosyloxy)iodotoluene [18] were prepared according to literature procedures.

2.2. Organic chemistry

2.2.1. 11β-Hydroxy-17α,20:20,21-bis-(methylenedioxy)-pregn-4-ene-3-one (2)

To a suspension of hydrocortisone 1 (2.54 g, 7 mmol) in CH_2Cl_2 (100 ml) was added formalin (40 ml, 40%) and concentrated HCl (40 ml). The bi-layer system was vigorous stirred at room temperature for 3 h. The organic layer was separated, washed with saturated $NaHCO_3$ solution and flushed through a silica-gel plug. After evaporation of the solvent under reduced pressure, 2.9 g (102%) of 2, contaminated with the 11β-MOM-ether (20%), was obtained as a wax. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.11 (s, 3H; 18-CH_3), 1.43 (s, 3H; 19-CH_3), 3.98 (m, 2H; 21-CH_2), 4.47 (m, 1H; 11α-H), 4.98 (m, 4H; O–CH_2–O from bis(methylenedioxy)), 5.66 (s, 1H; 4-H), LRMS (ESI positive) 405 [M + H].

2.2.2. 11β-Hydroxy-2-hydroxymethylene-17,20:20,21-bis-(methylenedioxy)-pregn-4-ene-3-one (3)

Steroid 2 (2.8 g, contaminated with 11β-MOM-ether) was dissolved in dry toluene (15 ml) and methyl formate (2 ml). Then, NaH (600 mg, 15 mmol, 60% dispersion in mineral

oil) was added. After stirring at room temperature for 4 h 1N HCl (100 ml) was added and the mixture was extracted several times with methylene chloride. The methylene chloride was extracted several times with 1N NaOH. After acidification of the NaOH extract with HCl and subsequent re-extraction with methylene chloride the solvent was removed to give 1.8 g (60%) of 3 as yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 3H; 18-CH_3), 1.33 (s, 3H; 19-CH_3), 4.00 (m, 2H; 21-CH_2), 4.40 (m, 1H; 11α-H), 5.03 (m, 4H; O–CH_2–O from bis(methylenedioxy)), 5.73 (d, J = 1.8 Hz, 1H, 4-H), 7.35 (s, 1H=CHOH). LRMS (ESI positive) 433 [M + H].

2.2.3. 2′-(4-Fluorophenyl)-11β-hydroxy-2-hydroxymethylene-17,20:20,21-bis-(methylenedioxy)-pregn-4-eno[3,2-c]pyrazole (4)

Compound 3 (1.04 g, 2.4 mmol) in HOAc (20 ml) was added to a solution of NaOAc (197 mg, 2.4 mmol) and 4-fluorophenylhydraziniumchloride (390 mg, 2.4 mmol) in HOAc (10 ml) and water (5 ml). The reaction mixture was stirred at room temperature overnight. 1N HCl (100 ml) was added followed by extraction with methylene chloride. The organic layer was washed with $NaHCO_3$-solution followed by water. Drying (Na_2SO_4) and evaporation of the solvent gave 1.06 g (84%) of steroid 4 as a brown foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.14 (s, 3H; 18-CH_3), 1.31 (s, 3H; 19-CH_3), 2.83 (AB quartet, $\Delta\nu$ = 128.7 Hz, J = 15.46 Hz, 2H; 1α/β-H), 4.00 (m, 2H; 21-CH_2), 4.47 (m, 1H; 11α-H), 5.03 (m, 4H; O–CH_2–O), 6.06 (d, J = 2.2 Hz, 1H, 4-H), 7.12–7.17 (m, 2H; Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 524 [M + H].

2.2.4. 2′-(4-Fluorophenyl)-11β,17α,21-trihydroxy-20-oxo-pregn-4-eno[3,2-c]pyrazole (5)

Steroid 4 (1.06 g, 2.02 mmol) was refluxed in 50% formic acid (100 ml) for 45 min. The formic acid was evaporated and the residue was re-dissolved in MeOH (75 ml) and 1N NaOH (15 ml). After stirring for 1 h at room temperature the solution was acidified with 1N HCl (150 ml). Extraction with methylene chloride, evaporation of the solvent and subsequent purification by flash chromatography (80% EtOAc–hexane) gave 780 mg (80%) of the desired product 5. HPLC-analysis mobile phase B: t_R = 5.61 min, 98% chemical purity, m.p. 187–189 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.96 (s, 3H; 18-CH_3), 1.30 (s, 3H; 19-CH_3), 2.82 (AB quartet, $\Delta\nu$ = 123.8 Hz, J = 15.2 Hz, 2H; 1α/β-H), 4.48 (AB quartet, $\Delta\nu$ = 137.1, J = 19.9 Hz, 2H; CH_2–OH), 4.51 (m, 1H; 11α-H), 6.05 (d, J = 2.2 Hz, 1H, 4-H), 7.13–7.17 (m, 2H; Ar–H), 7.40 (s, 1H; H-pyrazole), 7.43–7.46 (m, 2H; Ar–H). LRMS (ESI positive) 481 [M + H].

2.2.5. 2′-(4-Fluorophenyl)-11β-hydroxy-androst-4-ene-17-one[3,2-c]pyrazole (6)

To a solution of steroid 5 (220 mg, 0.457 μmol) in EtOH–CH_2Cl_2 (10 ml, 1:1) was added $NaBH_4$ (9 mg, 0.23 mmol). After 1 h, acetone (1 ml) and water (3 ml) was added followed by $NaIO_4$ (244 mg, 1.14 mmol) and the mixture was stirred at room temperature overnight. Water (50 ml) was added and the mixture was extracted with EtOAc. The EtOAc layer was flushed through a silica-gel plug and the solvent was evaporated to give 177 mg (92%) of 6 as a solid. HPLC-analysis mobile phase B: t_R = 10.15 min, 97% chemical purity, m.p. 223–226 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.17 (s, 3H; 18-CH_3), 1.33 (s, 3H; 19-CH_3), 2.82 (AB quartet, $\Delta\nu$ = 123.8 Hz, J = 15.2 Hz, 2H; 1α/β-H), 4.50 (m, 1H; 11α-H), 6.07 (d, J = 2.2 Hz, ^{1}H, 4-H), 7.13–7.17 (m, 2H; Ar–H), 7.41 (s, ^{1}H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 421.

2.2.6. 2′-(4-Fluorophenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (7)

To a solution of ketone 6 (193 mg, 0.46 mmol) in dry THF (10 ml) was added 1-propynylmagnesium bromide (9.14 ml, 4.57 mmol, 0.5 M in THF). The solution was stirred at room temperature under an nitrogen atmosphere overnight. Then, saturated NH_4Cl-solution (100 ml) was added and the mixture was thoroughly extracted with EtOAc. The solvent was removed under reduced pressure and subsequent purification by flash chromatography (50% EtOAc/hexane) afforded 212 mg (57%) of steroid 7 as a solid. HPLC-analysis mobile phase A: t_R = 5.56 min, 95% chemical purity, m.p. 95–98 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.85 (s, 1H; C≡CCH_3), 2.85 (AB quartet, $\Delta\nu$ = 115.5 Hz, J = 15.2 Hz, 2H; 1α/β-H), 4.49 (bs, 1H; 11α-H), 6.05 (d, J = 2.2 Hz, 1H, 4-H), 7.13–7.17 (m, 2H; Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 461.

2.2.7. 2′-(4-Fluorophenyl)-17α[(3-hydroxy)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (8)

Tetrahydro-2-(2-propynyloxy)-2H-pyran (1.05 g, 7.5 mmol) in THF (15 ml) was treated with n-BuLi (4.68 ml, 7.5 mmol, 1.6 M in hexane) at 0 °C. After 1 h at 0 °C, ketone 6 (315 mg, 0.75 mmol) in THF (8 ml) was added and the mixture was stirred at room temperature overnight. 1N HCl (1 ml) was added and stirring was continued for 10 min at 60 °C. After the addition of water (50 ml) the solution was extracted with EtOAc. The EtOAc was washed with $NaHCO_3$-solution, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (10% MeOH–CH_2Cl_2) to afford steroid 8 (222 mg, 62%) as a solid. HPLC-analysis mobile phase B: t_R = 5.21 min, 98.5% chemical purity, m.p. 147–150 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.14 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 2.85 (AB quartet, $\Delta\nu$ = 115.3 Hz, J = 15.4 Hz, 2H; 1α/β-H), 4.32 (s, 2H; CH_2–OH), 4.49 (m, 1H; 11α-H), 6.05 (d, J = 2.1 Hz, 1H, 4-H), 7.13–7.17 (m, 2H; Ar–H), 7.42 (s, 1H; H-pyrazole), 7.44–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 477.

2.2.8. 2'-(4-Fluorophenyl)-17α[(3-methansulfonyl)oxy-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (9)

To a solution of alcohol **8** (47.8 mg, 0.1 mmol) in THF (5 ml) and Et_3N (30.4 μl, 0.22 mmol) was added MsCl (15.7 μl, 0.2 mmol) at 0 °C. After stirring overnight the solvent was partially removed and the residue was purified by flash chromatography (80% EtOAc–hexane) to give 48 mg (86.5%) of mesylate **9**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.16 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 2.86 (AB quartet, Δν = 112.4 Hz, *J* = 15.3 Hz, 2H; 1α/β-H), 3.08 (s, 3H; OSO_2CH_3), 4.51 (m, 1H; 11α-H), 4.89 (s, 2H; CH_2-OMs), 6.06 (d, *J* = 1.8 Hz, 1H, 4-H), 7.13–7.18 (m, 2H: Ar–H), 7.42 (s, 1H; H-pyrazole), 7.44–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 555.

2.2.9. 2'-(4-Fluorophenyl)-17α[(3-fluoro)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (10)

To a solution of mesylate **9** in THF (1.5 ml) was added TBAF (150 μl, 150 μmol, 1 M in THF) and the resulting mixture was heated to 60 °C for 30 min. The solvent was evaporated and the residue was purified by flash chromatography (60% EtOAc–hexane) to give 7.5 mg (38%) of fluoride **10**. HPLC-analysis mobile phase A: t_R = 4.85 min, 95.5% chemical purity, m.p. 125–127 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.16 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 2.86 (AB quartet, Δν = 113, *J* = 15.4 Hz, 2H; 1α/β-H), 4.50 (bs, 1H; 11α-H), 5.00 (d, J_{HF} = 45.9 Hz, 2H; CH_2-F), 6.06 (bs, 1H, 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.44–7.47 (m, 2H; Ar–H). ^{19}F-NMR (376 MHz, $CDCl_3$): δ −214.24 (*t*, J_{HF} = 47 Hz), −115.33 (m). LRMS (ESI positive) 479 [M + H].

2.2.10. General procedure for the preparation of amine-containing steroids 11, 12 and 13

To a solution of Na_2CO_3 (50 mg, 0.47 mmol) in water (250 μl) was added the amine (1.0 mmol) followed by mesylate **9** (45 mg, 81 μmol) in acetone (1.5 ml). The solution was stirred for 2 h at room temperature. Saturated $NaHCO_3$-solution (20 ml) was added and EtOAc was used for extraction. After drying (Na_2SO_4) and evaporation of the solvent the residue was purified by flash chromatography (10% MeOH–CH_2Cl_2) to yield the corresponding amine.

2.2.11. 2'-(4-Fluorophenyl)-17α[(3-N-pyrrolidino)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (11)

Yield: 47%. HPLC-analysis mobile phase A: t_R = 5.85 min, 98.5% chemical purity, m.p. 196–198 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.82 (m, 4H; pyrrolidine), 2.67 (m, 4H; pyrrolidine), 2.84 (AB quartet, Δν = 127.9, *J* = 15.2 Hz, 2H; 1α/β-H), 3.48 (s, 2H; C≡CCH_2-pyrrolidine), 4.50 (bs, 1H; 11α-H), 6.05 (d, *J* = 1.8 Hz, 1H; 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 530 [M + H].

2.2.12. 2'-(4-Fluorophenyl)-17α[(3-N-piperidino)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (12)

Yield: 77%. HPLC-analysis mobile phase A: t_R = 6.24 min, 96.5% chemical purity, m.p. 200–203 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.61 (quint., *J* = 5.4 Hz, 2H; piperidine), 1.94 (m, 4H; piperidine), 2.49 (m, 6H; piperidine), 2.84 (AB quartet, Δν = 129.8, *J* = 15.2 Hz, 2H; 1α/β-H), 3.31 (s, 2H; C≡CCH_2-piperidine), 4.50 (bs, 1H; 11α-H), 6.06 (bs, 1H; 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.48 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 544 [M + H].

2.2.13. 2'-(4-Fluorophenyl)-17α[(3-N-morpholino)-1-propyn-1-yl]-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (13)

Yield: 70%. HPLC-analysis mobile phase B: t_R = 6.29 min, 96.5% chemical purity, m.p. 96–99 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 2.55 (m, 4H; morpholine), 2.82 (AB quartet, Δν = 126.4, *J* = 15.2 Hz, 2H; 1α/β-H), 3.34 (s, 2H; C≡CCH_2-morpholine), 3.74 (m, 4H; morpholine), 4.50 (bs, 1H; 11α-H), 6.05 (d, *J* = 1.9 Hz, 1H; 4-H), 7.13–7.17 (m, 2H: Ar–H), 7.40 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 546 [M + H].

2.2.14. 2'-(4-Fluorophenyl)-21-(methansulfonyl)oxy-20-oxo-11β,17α-dihydroxy-pregn-4-eno[3,2-c]pyrazole (14)

A solution of steroid **5** (200 mg, 0.416 mmol) in dry pyridine (4 ml) was cooled to 0 °C and MsCl (65.4 μl, 0.832 mmol) was slowly added. After stirring at 0 °C for 3 h, the solution was poured into 1N HCl (100 ml) followed by extraction with EtOAc. After drying (Na_2SO_4) the solvent was evaporated and the residue was purified by flash chromatography (80% EtOAc/hexane) to yield 182 mg (78%) of mesylate **14** as a pale yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.97 (s, 3H; 18-CH_3), 1.30 (s, 3H; 19-CH_3), 2.82 (AB quartet, Δν = 128, *J* = 15.3 Hz, 2H; 1α/β-H), 3.24 (s, 3H; OSO_2CH_3), 4.52 (m, 1H; 11α-H), 5.16 (AB quartet, Δν = 108.6, *J* = 17.8 Hz, 2H; CH_2-OMs), 6.05 (d, *J* = 1.7 Hz, 1H, 4-H), 7.13–7.18 (m, 2H: Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.46 (m, 2H; Ar–H). LRMS (ESI positive) 559 [M + H].

2.2.15. Oxetan-3'-one (15)

Mesylate **14** (31 mg, 55.5 μmol) was dissolved in dry THF (750 μl) and potassium *tert*-butoxide (9.34 mg, 83.2 μmol) in THF (400 μl) was added at room temperature. A clear yellow solution occurs. After 5 min, 1N HCl (100 μl) was added to quench the reaction followed by water (10 ml). Extraction with $CHCl_3$, drying (Na_2SO_4), evaporation of the solvent and subsequent flash chromatography (30%

EtOAc/hexane) gave 4 mg (16%) of oxetanone **15** as a white foam. HPLC-analysis mobile phase B: t_R = 15.13 min, 96% chemical purity, m.p. 217–220 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H; 18-CH_3), 1.31 (s, 3H; 19-CH_3), 2.83 (AB quartet, $\Delta\nu$ = 129.3, J = 15.1 Hz, 2H; 1α/β-H), 4.55 (m, 1H; 11α-H), 4.98 (AB quartet, $\Delta\nu$ = 51.4, J = 14.5 Hz, 2H; oxetanone), 6.05 (d, J = 2.1 Hz, 1H, 4-H), 7.13–7.18 (m, 2H; Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.46 (m, 2H; Ar–H). LRMS (ESI positive) 463 [M + H].

2.2.16. 2'-(4-Fluorophenyl)-21-iodo-20-oxo-11β,17α-dihydroxy-pregn-4-eno[3,2-c]pyrazole (16)

To a solution of mesylate **14** (129 mg, 86 μmol) in acetone (1 ml) was added NaI (129 mg, 860 μmol) in acetone (5 ml). After being stirred overnight while protected from light the solution was poured into water (20 ml) and 0.1N $Na_2S_2O_3$-solution (20 ml). Extraction with EtOAc, drying (Na_2SO_4), evaporation of the solvent and subsequent flash chromatography (40% EtOAc/hexane) afforded 43 mg (85%) of iodide **16** as a pale yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.00 (s, 3H; 18-CH_3), 1.31 (s, 3H; 19-CH_3), 2.83 (AB quartet, $\Delta\nu$ = 121.8, J = 15.3 Hz, 2H; 1α/β-H), 4.10 (AB quartet, $\Delta\nu$ = 75.4, J = 11.3 Hz, 2H; CH_2-I), 4.52 (m, 1H; 11α-H), 6.05 (d, J = 2.1 Hz, 1H, 4-H), 7.13–7.17 (m, 2H; Ar–H), 7.41 (s, 1H; H-pyrazole), 7.43–7.47 (m, 2H; Ar–H). LRMS (ESI positive) 591 [M + H].

2.2.17. 2'-(4-Fluorophenyl)-21-fluoro-20-oxo-11β,17α-dihydroxy-pregn-4-eno[3,2-c]pyrazole (17)

To a solution of mesylate **14** (182 mg, 0.325 mmol) in acetonitrile (10 ml) was added TBAF (450 μl, 450 mmol, 1 M in THF). After stirring for 15 min, at room temperature the solution was heated to 60 °C and an additional 450 μl of TBAF was added after 40 min. Addition of saturated $NaHCO_3$-solution, extraction (EtOAc), drying (Na_2SO_4), evaporation of the solvent and subsequent preparative TLC (60% EtOAc–hexane) gave 9 mg (5.8%) of fluoride **17** as a white solid. HPLC-analysis mobile phase B: t_R = 9.04 min, 93% chemical purity, m.p. 165–168 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.99 (s, 3H; 18-CH_3), 1.31 (s, 3H; 19-CH_3), 2.82 (AB quartet, $\Delta\nu$ = 125, J = 13.9 Hz, 2H; 1α/β-H), 4.53 (m, 1H; 11α-H), 5.20 (AB quartet, $\Delta\nu$ = 77, J_{HH} = 16.5 Hz, with additional doublet splitting due to J_{HF} = 47.5 Hz, 2H; CH_2-F), 6.06 (s, 1H, 4-H), 7.13–7.17 (m, 2H; Ar–H), 7.40 (s, 1H; H-pyrazole), 7.43–7.46 (m, 2H; Ar–H). ^{19}F-NMR (376 MHz, $CDCl_3$): δ −231.13 (t, J_{HF} = 48 Hz), −115.28 (m). LRMS (ESI positive) 483 [M + H].

2.2.18. 11β-Hydroxy-androst-4-ene-3,17-dione (18)

A solution of cortisol **1** (1.06 g, 2.92 mmol) in EtOH–CH_2Cl_2 (20 ml, 1:1) was treated with $NaBH_4$ (44.2 mg, 1.17 mmol). After 2 h acetone (5 ml) was added followed by water (5 ml) and $NaIO_4$ (1.56 g, 7.3 mmol). The solution was stirred at room temperature overnight followed by the addition of water (100 ml), extraction with $CHCl_3$ and flushing through a silica-gel plug. After evaporation of the solvent 877 mg (100%) of ketone **18** was isolated a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.11 (s, 3H; 18-CH_3), 1.42 (s, 3H; 19-CH_3), 4.49 (bs, 1H; 11α-H), 5.64 (s, 1H, 4-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 219.71, 199.82, 172.29, 122.26, 67.36, 56.39, 52.10, 46.57, 40.46, 39.08, 35.05, 34.57, 33.49, 31.88, 31.21, 30.67, 21.35, 20.68, 15.49. LRMS (ESI positive) 303 [M + H].

2.2.19. 3-(N-Pyrrolidinyl)-3,5-androstadien-11β-ol-17-one (19)

Ketone **18** (877 mg, 2.9 mmol) in toluene (70 ml) was refluxed with pyrrolidine (2.5 ml) and $TsOH \cdot H_2O$ (45 mg) while removing the formed water. After 4 h the solvent was evaporated and ether was added. Further evaporation of the solvent gave 1 g (97%) of **19** as a yellowish-brown foam which was efficiently pure. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.15 (s, 3H; 18-CH_3), 1.25 (s, 3H; 19-CH_3), 3.14 (m, 4H; CH_2), 4.47 (m, 1H; 11α-H), 4.74 (s, 1H; 4-H), 4.96 (m, 1H; 6-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 220.15, 144.03, 142.82, 111.01, 95.32, 68.15, 53.63, 52.37, 46.96, 46.86, 40.52, 35.25, 34.92, 33.47, 30.47, 27.75, 24.79, 24.25, 21.94, 21.52, 15.60. LRMS (ESI positive) 356 [M + H].

2.2.20. 17α(1-Propyn-1-yl)-androst-4-ene-11β,17β-diol-3-one (20)

To a solution of enamine **19** (194 mg, 0.54 mmol) in dry THF (2 ml) was added 1-propynylmagnesium bromide (5.7 ml, 2.86 mmol, 0.5 M in THF). The solution was stirred at room temperature under an nitrogen atmosphere overnight. Saturated NH_4Cl-solution (150 ml) was added and the mixture was thoroughly extracted with EtOAc. The EtOAc was removed under reduced pressure and the residue was redissolved in a solution of NaOAc (3 g) in water (3 ml), HOAc (2 ml) and MeOH (20 ml). The resulting mixture was refluxed for 1 h. Afterwards, water (200 ml) was added followed by extraction with EtOAc. Evaporation of the solvent and subsequent purification by flash chromatography (75% EtOAc/hexane) gave 96 mg (52%) of steroid **20** as a pale yellow foam. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.12 (s, 3H; 18-CH_3), 1.45 (s, 3H; 19-CH_3), 1.84 (s, 3H; CH_3), 4.44 (m, 1H; 11α-H), 5.68 (s, 1H, 4-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 200.05, 172.84, 122.24, 82.46, 82.24, 80.05, 68.09, 55.88, 51.20, 45.83, 42.31, 39.15, 38.70, 34.72, 33.67, 32.03, 31.92, 31.82, 22.99, 20.77, 15.22, 3.53. LRMS (ESI positive) 343 [M + H].

2.2.21. General procedure for the synthesis of steroids 21 and 22

To a solution of steroid **20** (103 mg, 0.3 mmol) in toluene (5 ml), pyridine (1.5 ml) and methyl formate (300 μl) was

added 60% NaH (72 mg, 1.8 mmol) followed by methanol (150 μl). The solution was stirred overnight while the color changed to dark red. Addition of 1N HCl (50 ml) and extraction with methylene chloride gave a yellow solution. The methylene chloride was extracted several times with 1N NaOH. After acidification of the NaOH extract with HCl and subsequent re-extraction with methylene chloride the solvent was removed to give the corresponding 2-hydroxymethylene compound of **20** as a yellow foam. The foam was dissolved in HOAc (10 ml) and a solution of 4-bromophenylhydraziniumchloride or 4-iodophenylhydrazine (0.3 mmol) [17] and NaOAc (0.3 mmol) in HOAc (5 ml) and water (2 ml) was added. The dark red colored solution was stirred at room temperature overnight. Addition of water, extraction with $CHCl_3$ and subsequent purification by flash chromatography (60% EtOAc/hexane) gave compounds **21** or **22**, respectively.

2.2.22. 2'-(4-Bromophenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (21)

Yield: 48%. HPLC-analysis mobile phase A: t_R = 8.12 min, 97% chemical purity, m.p. 130–132 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.85 (s, 3H; C C≡CCH_3), 2.85 (AB quartet, Δν = 114.9, J = 15.3 Hz, 2H; 1α/β-H), 4.49 (bs, 1H; 11α-H), 6.08 (d, J = 2.2 Hz, 1H, 4-H), 7.43 (s, 1H; H-pyrazole), 7.38 and 7.58 (2d of AA'BB' system, J = 8.8 Hz, 4H; Ar–H). LRMS (ESI positive) 522 [M + H].

2.2.23. 2'-(4-Iodophenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (22)

Yield: 50%. HPLC-analysis mobile phase A: t_R = 8.79 min, 96.5% chemical purity, m.p. 104–106 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.85 (s, 3H; C C≡CCH_3), 2.85 (AB quartet, Δν = 115, J = 15.2 Hz, 2H; 1α/β-H), 4.49 (bs, 1H; 11α-H), 6.08 (d, J = 1.8 Hz, 1H, 4-H), 7.43 (s, 1H; H-pyrazole), 7.25 and 7.78 (2d of AA'BB' system, J = 8.7 Hz, 4H; Ar–H). LRMS (ESI positive) 569 [M + H].

2.2.24. 2'-(4-Trimethylstannylphenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole (23)

A solution of steroid **22** (160 mg, 0.28 mmol) in toluene (3 ml) was refluxed with Sn_2Me_6 (250 mg, 0.76 mmol) and $Pd[PPh_3]_4$ (4.8 mg, 4.15 μmol) for 3 h. After removal of the solvent the residue was purified by flash chromatography (60% EtOAc/hexane) to afford 100 mg (59%) of steroid **23**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.32 (s, 9H; $Sn(CH_3)_3$), 1.13 (s, 3H; 18-CH_3), 1.32 (s, 3H; 19-CH_3), 1.85 (s, 3H; C C≡CCH_3), 2.86 (AB quartet, Δν = 108.8, J = 15.2 Hz, 2H; 1α/β-H), 4.50 (bs, 1H; 11α-H), 6.14 (d, J = 1.9 Hz, 1H, 4-H), 7.43 (s, 1H; H-pyrazole), 7.46 (AA'BB', J_{AB} = 8.2 Hz, 2H, Ar–H), 7.57 (AA'BB', J_{AB} = 8.2 Hz, 2H, Ar–H). LRMS (ESI positive) 607 [M + H].

2.2.25. General procedure for the synthesis of Iodonium salts 24 and 25

A solution of steroid **23** (115 mg, 0.19 mmol) and [hydroxy(tosyloxy)iodo]benzene or [hydroxy(tosyloxy)iodo] toluene (0.19 mmol) [18] in methylene chloride (1 ml) was stirred at room temperature for 2 h. The solvent was evaporated by means of a nitrogen stream. The residue was treated with ether resulting in the formation of a white solid. The solid was filtered off, washed with ether and dried under vacuo to yield the iodonium salt **24** and **25**.

2.2.26. Diaryliodonium tosylate (24)

Yield: 28%. ^{1}H-NMR (400 MHz, $DMSO_{d6}$): δ 0.29 (s, 3H; CH_3), 0.50 (s, 3H; CH_3), 1.04 (s, 3H; CH_3), 1.53 (s, 3H; CH_3), 2.05 (AB quartet, Δν = 150, J = 15.5 Hz, 2H; 1α/β-H), 3.60 (bs, 1H; 11α-H), 5.35 (d, J = 1.8 Hz, 1H, 4-H), 6.42 and 6.89 (2d of AA'BB' system, J_{AB} = 8.3 Hz, 4H; Ar–H), 6.75 (m, 3H; Ar–H), 6.68 (s, 1H; H-pyrazole), 7.64 and 7.13 (2d of AA'BB' system, J_{AB} = 9 Hz, 4H, Ar–H), 7.4–7.5 (m, 2H; Ar–H). LRMS (ESI positive) 645 [M-OTs].

2.2.27. Diaryliodonium tosylate (25)

Yield: 57%. ^{1}H-NMR (400 MHz, $DMSO_{d6}$): δ 0.29 (s, 3H; CH_3), 0.50 (s, 3H; CH_3), 1.00 (s, 3H; CH_3), 1.55 (s, 3H; CH_3), 1.60 (s, 3H; CH_3), 2.04 (AB quartet, Δν = 150.4, J = 15.5 Hz, 2H; 1α/β-H), 3.59 (bs, 1H; 11α-H), 5.34 (d, J = 1.8 Hz, 1H, 4-H), 6.41 and 6.56 (2d of AA'BB' system, J_{AB} = 7.9 Hz, 4H, Ar–H), 6.67 (s, 1H; H-pyrazole), 6.82 and 7.45 (2d of AA'BB' system, J_{AB} = 9.0 Hz, 4H, Ar–H), 6.88 and 7.4 (2d of AA'BB' system, J_{AB} = 8.3 Hz, 4H, Ar–H). LRMS (ESI positive) 659 [M-OTs].

2.3. Radiochemistry

2.3.1. General

^{18}F was produced by the ^{18}O(p, n)^{18}F reaction on an enriched water target using an IBA CYCLONE 18/9 cyclotron. The [^{18}O] water containing [^{18}F]fluoride (ca. 5 mCi, 185 MBq) was transferred to a 5 ml Vacutainer® containing a solution of Kryptofix [2.2.2] (6.0 mg, 16.2 μmol) and K_2CO_3 (1.5 mg, 10.9 μmol). Water was azeotropically evaporated using HPLC grade acetonitrile (3× 0.5 ml) in a 100 °C oil bath under a stream of nitrogen. Potassium [^{18}F]fluoride was resolubilized into 500–1000 μl of anhydrous solvent (acetonitrile or DMF) and transferred to a glass vial pre-charged with the labeling precursor (2–10 mg). Progress of the reaction was monitored by means of radio-TLC. A pre-purification was performed by passing the diluted reaction mixture through a silica-gel plug (a Pasteur pipet with ca. 150 mg silica-gel). Radioiodination with iodine-131 was performed using a solution of [^{131}I]NaI (Nycomed-Amersham) in NaOH.

2.3.2. 2'-([^{18}F]-4-Fluorophenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole ([^{18}F]-7)

The reaction was employed using 10 mg of iodonium salts **24** or **25**. After resolubilization the radioactivity (ca. 3–4 mCi, 110–150 MBq) was transferred to the labeling precursor **24** or **25** in DMF (500 μl). The vial was sealed and heated for 40 min at 120 °C. Radio-TLC (R_f = 0.38; 80% EtOAc/hexane) showed the formation of 0.2% (with iodonium salt **24**) and 2% (with iodonium salt **25**) of the desired compound [^{18}F]-**7** together with large amounts of non-reacted [^{18}F]fluoride and [^{18}F]fluorobenzene and [^{18}F]fluorotoluene, respectively.

2.3.3. 2'-(4-Fluorophenyl)-17α-(3-[^{18}F]fluoro)-1-propyn-1-yl)]-11β,17β-dihydroxy-androst-4-eno [3,2-c]-pyrazole ([^{18}F]-10)

The reaction was employed using 2–3 mg of mesylate **9**. After resolubilization the radioactivity (4.0 mCi, 150 MBq) was transferred to mesylate **9** in acetonitrile (500 μl). The vial was sealed and heated for 20 min at 65 °C. After cooling and addition of ethyl acetate (2 ml) the solution was passed through a silica-gel plug. The eluate (11 MBq, 9%, decay-corrected) was analyzed by radio-TLC (R_f = 0.43; 80% EtOAc/hexane) indicating a radiochemical purity of >93%.

2.3.4. 2'-(4-Fluorophenyl)-21-[^{18}F]fluoro-20-oxo-11β,17α-dihydroxy-pregn-4-eno [3,2-c]pyrazole ([^{18}F]-17)

The reaction was employed using 2–4 mg of mesylate **14** or iodo precursor **16**. After resolubilization the radioactivity (3–5.4 mCi, 110–200 MBq) was transferred to the labeling precursor **14** or **16** in acetonitril (500 μl). The vial was sealed and heated for 20 min at 65 °C. After cooling and addition of ethyl acetate (2 ml) the solution was passed through a silica-gel plug. The eluate (3.1 MBq, 4%, decay-corrected for precursor **14** and 21.8 MBq, 14%, decay-corrected for precursor **16**) was analyzed by radio-TLC (R_f = 0.55; 80% EtOAc/hexane) indicating a radiochemical purity of >95%.

2.3.5. 2'-([^{131}I]-4-Iodophenyl)-17α(1-propyn-1-yl)-11β,17β-dihydroxy-androst-4-eno[3,2-c]-pyrazole ([^{131}I]-22)

The reaction was employed using 1.5 mg of organostannane **23**. [^{131}I]NaI (2 μCi, 74 kBq) was added to a solution consisting of 50 μl buffer solution (5% NaOAc in HOAc) and 50 μl oxidizing solution (30% H_2O_2/HOAc 2:1). Tin precursor **23** in 200 μl MeOH was added and the solution was stirred at room temperature for 30 min. Then, 70 μl of a 0.1 M $Na_2S_2O_3$-solution was added to stop the reaction. Radio-TLC (R_f = 0.47; 80% EtOAc/hexane) revealed the formation of 78% of the desired product [^{131}I]-**22** besides non-reacted [^{131}I]iodide. After the addition of ethyl acetate (2 ml) and silica-gel purification the radiochemical purity of the eluate was >95%.

2.4. Biological methods

2.4.1. Determination of the receptor binding affinity

Compounds tested in the GR-binding assay were analyzed by HPLC indicating a chemical purity of 93% for compound **17** and a chemical purity ≥ 95% for compounds **5–8**, **10–13**, **15** and **21–22**. The binding affinity of the arylpyrazolo steroids for the GR was determined by a modification of the method reported for the estrogen receptor [8,19]. The radiotracer was [^{3}H]dexamethasone and adrenalectomized male rat liver cytosol was used as the protein. The cytosol was incubated with buffer or several concentrations of unlabeled competitor together with [^{3}H]dexamethasone at 0 °C for 18–24 h. The unlabeled arylpyrazolo steroid competitor was prepared in 1:1 dimethylformamide-buffer to ensure solubility. The free steroid was adsorbed on dextran-coated charcoal as described by Katzenellenbogen et al. [20]. [^{3}H]Dexamethasone was [1,2,4,-^{3}H]dexamethasone (Amersham Biosciences, Piscataway, NJ).

3. Results

3.1. Synthesis of 4-fluorophenylpyrazolo steroids containing 17α-alkynyl side chains

Natural corticosteroids possess the characteristic 17α,21-dihydroxy-20-keto substitution pattern of the pregnane side chain, as exemplified by cortisol **1**. However, several reports on glucocorticoids have shown that the D-ring is tolerant toward different substitution patterns at position C17 of the steroid skeleton while retaining high binding affinity to the GR [21]. Moreover, D-ring modified derivatives often possess greatly reduced binding affinities for the mineralocorticoid receptor, thus constituting steroids called "pure glucocorticoids" [21,22]. Since 17α-alkynyl substituted steroids are easily accessible by 1,2 addition of lithium or magnesium based organometallic acetylides to 17-keto steroids [23,24], we undertook the synthesis of a series of novel 4-fluorophenylpyrazole steroids in which the characteristic corticosteroid 1,3-dihydroxyacetone side chain was replaced by a 17α-alkynyl-17β-hydroxy moiety. The resulting steroids (**7**, **8**, **10–13**) combine the beneficial effects of the 17α-alkynyl-17β-hydroxy and 4-fluorophenyl pyrazole moieties for high and selective binding to the GR. Furthermore, the modification of the 17α-alkynyl side chain by either neutral (Me, OH, F) or basic (cyclic aliphatic amines) substituents results in interesting effects on biologically important parameters like receptor binding and lipophilicity (vide infra).

The synthesis of 17-keto steroid **6** as prerequisite starting material for 17α-alkynyl substituted steroids via organometallic 1,2 addition is shown in Fig. 2.

Starting from commercially available cortisol **1**, the synthesis commenced by protecting the 1,3-dihydroxyacetone side chain of **1** by treatment with formaldehyde and

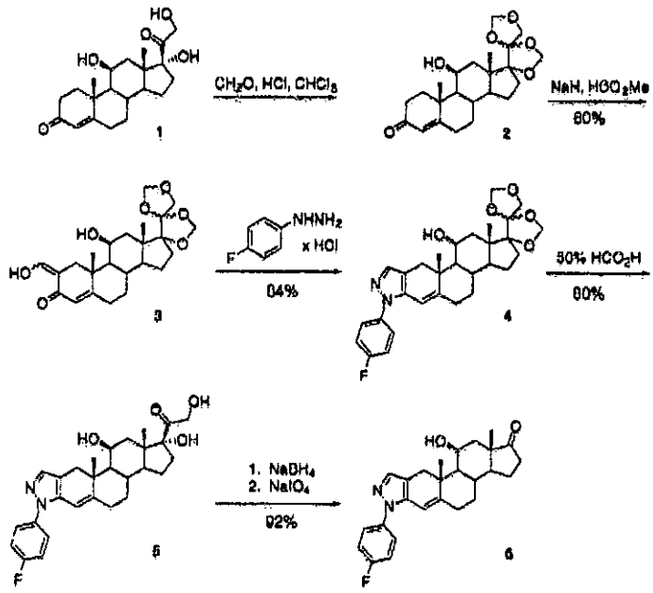

Fig. 3. Synthesis of 2′ (4-fluorophenyl)-11β-hydroxy-androst-4-eno-17-one[3,2-c]pyrazole 6.

concentrated HCl in chloroform to afford cortisol bis(methylenedioxy)ether 2. It was observed that a portion of the product was also converted to the 11β-MOM ether of 2 under these reaction conditions.

The amount of the 11β-MOM ether contamination was determined to be 20% by means of ^{1}H-NMR using the 11α-H signals at 4.47 ppm (OH) and 4.25 ppm (MOM). However, the following steps are not affected by the 11β-MOM ether group, and 2 could be used as a mixture of the 11β-OH and 11β-MOM compound. The introduction of the 2-hydroxymethylene functionality in 3 was achieved by reacting the sodium enolate of 2 with methyl formate in toluene. Subsequent condensation of 3 with 4-fluorophenylhydrazine hydrochloride in the presence of NaOAc in acetic acid gave 4-fluorophenyl pyrazole 4 in 84% yield. By using these reaction conditions, the desired 2′-fluorophenylpyrazole steroid 4 was formed exclusively, and the corresponding 1′-fluorophenylpyrazole isomer was not observed. The bis(methylenedioxy) ether protecting group and the partially present 11β-MOM ether were removed in boiling 50% formic acid. The resulting 1,3-dihydroxyacetone side chain in 5 was transformed into the 17-keto steroid 6 by reduction of the 20-keto group in 5 with $NaBH_4$ and subsequent oxidative cleavage of the intermediate 17,20,21-trihydroxy side chain with $NaIO_4$. The total yield of 6 was 37%, based on cortisol 1. The reaction pathway for the introduction of 17α-alkynyl side chains and their further functionalization is depicted in Fig. 3.

Treatment of ketone 6 with 1-propynylmagnesium bromide in THF at room temperature gave steroid 7 in 57% yield. The 1,2 addition of the lithium derivative of tetrahydro-2-(2-propynyloxy)-2*H*-pyran to ketone 6 afforded alcohol 8 in 62% yield after removal of the THP-ether protecting group. Alcohol 8 was used as a suitable starting material for further modifications of the 17α-alkynyl side chain. For this purpose the primary hydroxyl group in 8 was first converted into the corresponding mesylate to obtain a good leaving group. This approach allows the convenient and flexible terminal functionalization of the alkynyl side chain with a variety of substituents by displacement of the mesylate with several nucleophiles. Thus, treatment of mesylate 9 with TBAF in THF afforded the fluoride-substituted steroid 10 in 38% yield. In another set of reactions, mesylate 9 was treated with several aliphatic cyclic amines in the system acetone-Na_2CO_3 to provide steroids 11–13 in 47, 77 and 70% yield, respectively.

The steroids obtained according to the reaction scheme in Fig. 3 can be subdivided into two classes: one set of compounds (7, 8, and 10) possess neutral substituents (Me in 7, OH in 8, F in 10) in the 17α-alkynyl side chain, whereas compounds 11–13 contain pyrrolidino, piperidino or morpholino as basic amino substituents.

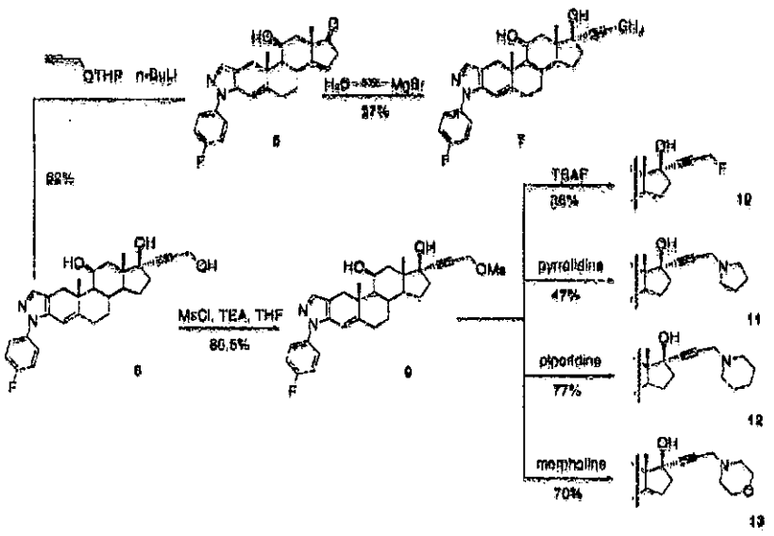

Fig. 3. Synthesis of 17α-alkynyl-17β-hydroxy steroids.

3.2. Synthesis of 4-fluorophenylpyrazolo steroids containing an oxetan-3′-one moiety and modified 1,3-dihydroxyacetone side chains

As another set of compounds, we have synthesized several 4-fluorophenyl pyrazolo steroids containing an intact and modified 1,3-dihydroxyacetone side chain, as well as a spiro-oxetanone. Steroid **5** possessing the 1,3-dihydroxyacetone side chain was obtained as an intermediate in the reaction sequence for the synthesis of ketone **6** (vide supra, Fig. 2). Steroid **5** was used to convert the 1,3-dihydroxyacetone side chain into the oxetan-3′-one **15** and to form iodide **16** and fluoride **17** (Fig. 4).

Although several methods are known for preparing oxetan-3-ones (e.g. by oxidation [25], by acid-catalyzed decomposition of diazo ketones [26], by [2 + 2] cycloaddition [27]), only intramolecular displacement reactions [28] have been used to prepare steroidal oxetane-3′-ones. In the case of C17 spiro-oxetan-3′-ones, the reaction seems especially challenging, since forcing reaction conditions are required, and the reaction only proceeds in poor yields. However, Pons and Simons [29] have described a general method to overcome these problems and to provide the desired C17 spiro-oxetan-3′-ones in good yields. Following this procedure, first mesylate **14** was prepared from triol **5** using MsCl in pyridine in 78% yield. An intramolecular oxetan-3′-one ring closure reaction of mesylate **14** using potassium *tert*-butoxide in THF at room temperature provided the desired spiro-oxetan-3′-one **15** in 16% yield. It is noteworthy that this intramolecular displacement reaction was also observed as a competitive reaction in the synthesis of several fluorine-18 containing corticosteroids starting from the corresponding α-keto mesylates [8,11].

The formation of 21-fluoro steroid **17** proved to be challenging, and α-keto mesylate **14** could be converted into 21-fluoro steroid **17** using TBAF in acetonitrile only in very a low yield (5.8%). Oxetanone **15** was always inevitably formed as a side product, because of the strong basic character of the fluoride anion under these conditions. Adaptation of this procedure for the formation of the corresponding F-18 labeled compound also gave disappointing results. Better results in the F-18 fluorination could be achieved using the 21-iodo compound **16** rather than the α-keto mesylate **14**. The required 21-iodo steroid **16** was easily prepared by the reaction of mesylate **14** with NaI in acetone in 85% yield.

3.3. Synthesis of 4-bromophenylpyrazolo steroid 21 and 4-iodophenylpyrazolo steroid 22

Within the series of 17α-alkynyl-17β-hydroxy steroids, we also wanted to study the influence of 4-bromophenyl and 4-iodophenyl derivatives in terms of their GR binding, since bromine and iodine also have radioisotopes (Br-76, I-123 or I-124, respectively) suitable for in vivo imaging. Therefore, we set up the synthesis of two additional 17α-(1-propyn-1-yl)-17β-hydroxy steroids **21** and **22**, containing bromine or iodine in the arylpyrazolo moiety (Fig. 5).

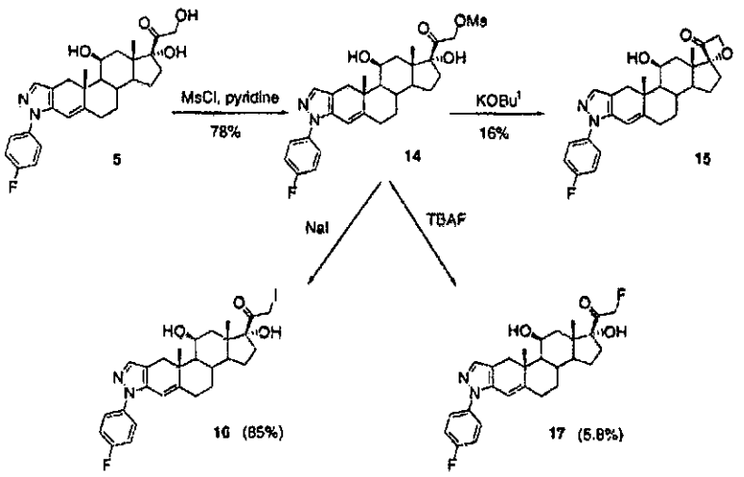

Fig. 4. Synthesis of steroids containing an oxetan-3'-one moiety and modified 1,3-dihydroxyacetone side chains.

Employing the same oxidative cleavage reaction as exemplified by the transformation of steroid **5** to 17-keto steroid **6**, cortisol **1** was converted into steroid **18** in quantitative yield. Further reaction of compound **18** requires the selective protection of the C3 keto group in the presence of the C17 keto group. Such a selective protection of the 3-keto group in **18** could conveniently be achieved through formation of the corresponding 3-enamine by refluxing 3,17-dienone **18** in benzene with pyrrolidine and a trace of TsOH, while removing the water generated by this reaction. Subsequent 1,2-addition of 1-propynylmagnesium bromide to the 17-keto group in **19**, followed by restoration of the 3-enone structure by deprotection with NaOAc/HOAc, gave compound **20** in a total yield of 50% based on cortisol **1**. Pyrazole formation was performed according to the described procedure (vide supra, Fig. 2), using the condensation of the corresponding 1,3-dicarbonyl compound (derived from **20** by treatment with NaH and methyl formate in toluene) with 4-bromo- and 4-iodophenylhydrazine [17] in acetic acid. This procedure afforded the desired arylpyrazolo steroids **21** and **22** in 48% and 50% yield, respectively.

3.4. Synthesis of diaryliodonium salts 24 and 25

The use of [^{18}F]fluoride in nucleophilic aromatic substitution reactions represents a very important and widely applied reaction in fluorine-18 chemistry [30,31]. Nucleophilic aromatic substitutions with [^{18}F]fluoride proceed efficiently when a suitable good leaving group (nitro, trialkylammonium, etc.) is attached ortho- or para to an

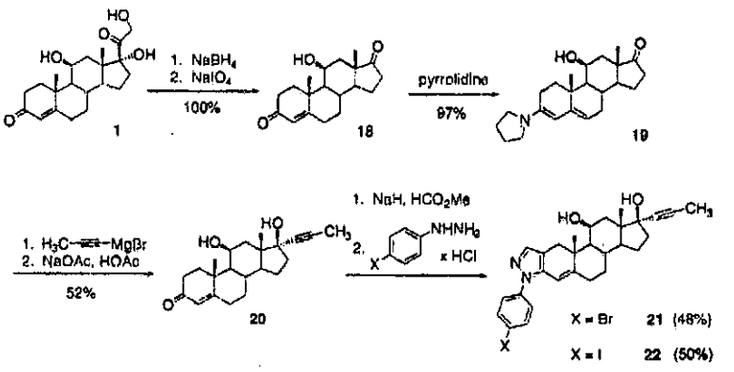

Fig. 5. Synthesis of 4-bromo- and 4-iodophenylpyrazolo steroids **21** and **22**.

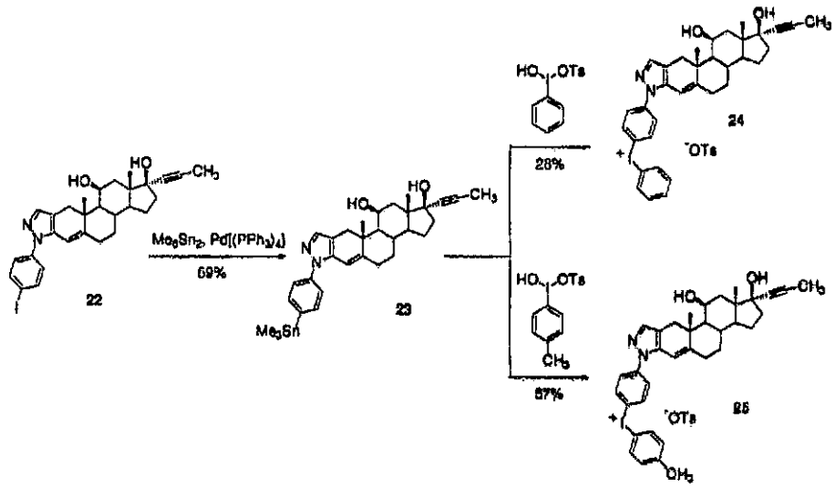

Fig. 6. Synthesis of diaryliodonium salts 24 and 25.

electron-withdrawing group (nitro, CHO, CN, etc.) essential to activate the aromatic ring. In the case of arylpyrazolo steroids, the pyrazole moiety is not well suited to activate the aromatic ring efficiently for a nucleophilic aromatic substitution with [^{18}F]fluoride. However, several diaryliodonium salts have been shown to react with [^{18}F]fluoride to generate the corresponding aryl fluorides in reasonable yield, even from non-activated aromatic rings [32]. Therefore, we have synthesized diaryliodonium tosylates **24** and **25** as precursors for the introduction of [^{18}F]fluoride into the aromatic ring. The synthesis of aryliodonium salts **24** and **25** is given in Fig. 6.

Several methods have been reported for the preparation of diaryliodonium salts that comprise the reaction of either aryltrialkylsilanes [33], aryltrialkylstannanes [34] or arylboronic acids [35] with hydroxy(tosyloxy)iodobenzenes, as well as electrochemical methods [36]. We choose the reaction employing aryltrialkylstannanes. For this purpose, the required trimethylstannyl-containing steroid **23** was prepared via a Pd-catalyzed cross-coupling reaction between iodoaryl steroid **22** and hexamethyldistannane. Treatment of steroid **23** with hydroxy(tosyloxy)iodobenzene (Koser's reagent) or hydroxy(tosyloxy)-iodotoluene [18] resulted in an electrophilic aromatic substitution (ipso-destannylation) reaction that provided the desired diaryliodonium tosylates **24** and **25** in 28 and 57% yield, respectively.

3.5. Radiochemistry I—^{18}F-labeling on aliphatic side chains

The synthesis of ^{18}F-labeled steroids [^{18}F]-**17** and [^{18}F]-**10** is depicted in Fig. 7. The incorporation of fluorine-18 proceeded by [^{18}F]fluoride ion displacement of mesylate precursors **14** and **9** or iodo precursor **16**. For the synthesis of [^{18}F]-**17**, resolubilized [^{18}F]fluoride (Kryptofix®/potassium carbonate, [37]) in acetonitrile was transferred to a glass vial containing the appropriate precursor **14** or **16**, and the reaction mixture was heated at 65 °C for 20 min. To remove non-reacted [^{18}F]fluoride, the reaction was diluted with ethyl acetate (2000 μl) and applied to a silica "plug" (a Pasteur pipet with ca. 150 mg silica-gel) and eluted into a flask. Analysis of the product by radio-TLC (R_f = 0.55; 80% EtOAc/hexane) indicated a radiochemical purity >95%. The average radiochemical yield (decay-corrected) was 4% starting from mesylate precursor **14**. However, the radiochemical yield could be increased up to 14% (decay-corrected) when iodo precursor **16** was used.

Employing the same protocol, steroid [^{18}F]-**10** was obtained in 9% decay-corrected radiochemical yield after the reaction of mesylate **9** with resolubilized [^{18}F]fluoride. Radio-TLC (R_f = 0.43; 80% EtOAc/hexane) indicated a radiochemical purity of >93% after purification by silica-gel filtration.

3.6. Radiochemistry II—^{18}F-labeling using diaryliodonium salts 24 and 25

In order to incorporate the F-18 label into the metabolically stable position of the aromatic ring, we made use of diaryliodonium salts **24** and **25** as suitable labeling precursors (Fig. 8). Diaryliodonium salts **24** and **25** differ in the functionalization of the aryl ring, being phenyl and tolyl, respectively. Radiochemistry was performed using the powerful nucleophilic radiofluorinating agent K[^{18}F]F

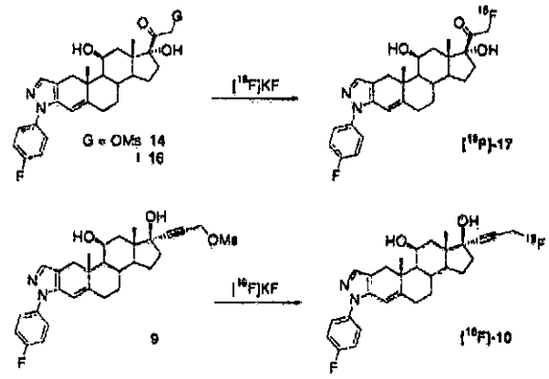

Fig. 7. Radiosynthesis of ^{18}F-labeled steroids [^{18}F]-17 and [^{18}F]-10.

(generated by treatment of cyclotron-produced [^{18}F]fluoride with Kryptofix/potassium carbonate) in DMF as the solvent at 120 °C for 40 min. The use of phenyl-functionalized iodonium salt **24** gave the desired product [^{18}F]-7 in only 0.2% yield. Radio-TLC analysis after silica-gel purification (vide supra) showed the desired product [^{18}F]-7 (R_f = 0.38; 80% EtOAc/hexane) together with [^{18}F]fluorobenzene. Because of its volatility, the amount of [^{18}F]fluorobenzene could not be quantitated.

The observation of [^{18}F]fluorobenzene formation is consistent with the findings in the literature when non-symmetric phenyl-functionalized iodonium salts were used for fluorinations with [^{18}F]fluoride [32]. Since the attack of the fluoride ion preferentially occurs on the more electron-deficient aromatic ring, the use of the more electron-donating tolyl-fuctionalized iodonium salt **25** should favor the formation of F-18 labeled steroid [^{18}F]-7. This assumption was confirmed, because using this precursor, the radiochemical yield of [^{18}F]-7 could be increased up to 2%. However, radio-TLC analysis also indicated the formation of large amounts of 4-[^{18}F]fluoro-toluene as a by-product.

3.7. Radiochemistry III—^{131}I-labeling with trimethylstannyl steroid 23

Several iodine isotopes such as iodine-120, -123, -124 possess decay properties suitable for in vivo imaging by positron or single-photon emission tomography (PET or

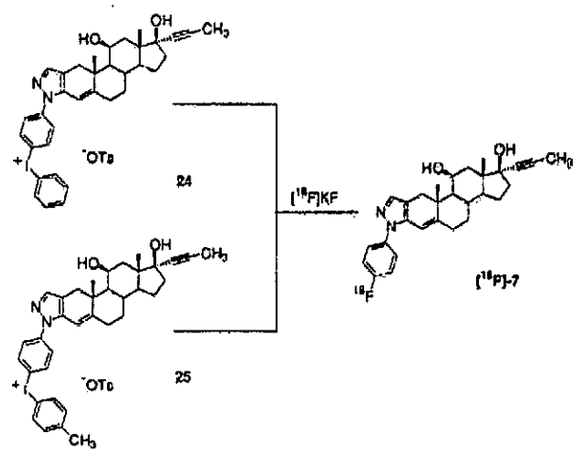

Fig. 8. Radiosynthesis of ^{18}F-labeled steroid [^{18}F]-7 via diaryliodonium salts **24** and **25**.

Comparison of the RBA values within a third series of compounds reveal a strong influence of the halogen attached to the phenyl ring on the GR binding affinity. By far the best binding affinity is observed with 4-fluorophenyl pyrazole **7**, being 56%. With bromine in compound **21** or iodine in compound **22**, the RBA drops to 6.5 and 1.5%, respectively. The low RBA values for bromine- and iodine-containing steroids **21** and **22** in comparison to fluorine-containing steroid **7** confirm the beneficial effect of the 4-fluorophenyl moiety in terms of high binding affinity to the GR.

4. Discussion

In our effort to develop imaging agents for brain GRs, we have prepared several novel arylpyrazolo steroids. The compounds that we have synthesized can be divided into three classes: 4-fluorophenyl pyrazoles possessing a 17α-alkynyl side chain, 4-fluorophenyl pyrazoles containing a selection of different substitution patterns in the D-ring, and compounds which differ with respect to the halogen attached to the arylpyrazole moiety. All steroids were tested for binding to the GR, and their lipophilicity ($\log P_{o/w}$ values) was calculated.

We found that all steroids containing a 4-fluorophenyl pyrazole moiety possess at least the same binding capability to the GR as the natural stress-induced hormone cortisol **1**. Relatively high binding of 50 and 56%, relative to dexamethasone, was observed for compounds **5** and **7**, which contain the intact 1,3-dihydroxyacetone side chain or the relatively small and neutral 17α-propynyl side chain, respectively. These binding affinities are 6–7 times better than the GR binding of cortisol **1**. This finding also shows that the 1,3-dihydroxyacetone side chain is not necessary for high GR binding and can be replaced by neutral 17α-alkynyl side chains. By comparing the measured RBA values, it also became evident that basic substituents like morpholine and to a greater extent pyrrolidine and piperidine diminish GR binding significantly. The GR seems to tolerate neutral 17α-alkynyl side chains rather than basic side chains. Apparently, the basic character of the cyclic amines seems to have more influence on the GR binding than the steric bulk when the RBA values of the strong basic pyrrolidine **11** (RBA = 7.5%) and piperidine **12** (RBA = 8.5%) are compared with the less basic morpholine **13** (RBA = 17.5%).

Further evidence for the GR tolerance toward modifications of the steroidal D-ring was revealed by the relatively good binding affinities of ketone **6** (RBA = 18%) and oxetanone **15** (RBA = 22%). However, only moderate binding affinity of 11% was found for 21-fluoro steroid **17**, which represents a compound with a modified 1,3-dihydroxyacetone side chain. The beneficial effect of 4-fluorophenyl pyrazoles on the GR was confirmed by comparison of the RBA values of fluorine **7** (RBA = 56%), bromine **21** (RBA = 6.5%), and iodine **22** (RBA = 1.5%). By introduction of the halogens bromine and iodine into the aromatic ring the electronic and steric characteristics apparently prevent any sufficient binding to the GR. This effect is especially distinct when iodine is used.

Selected candidates were chosen to investigate the labelling of arylpyrazolo steroids with the short-lived positron emitter fluorine-18. The fluorine-18 label could be easily introduced into aliphatic side chains to afford radiolabeled steroids [^{18}F]-**17** and [^{18}F]-**10** in radiochemical yields of 14 and 9%, respectively. It is noteworthy that the incorporation of fluorine-18 proceeds well despite the free OH-groups present in precursors **9**, **14** or **16**.

In an effort to label arylpyrazolo steroids in a metabolically stable position, we attempted the synthesis of fluorine-18 labeled steroid [^{18}F]-**7**. Since the aromatic ring is not sufficiently activated by a strong electron-withdrawing group, we used diaryliodonium salts **24** and **25** as precursors for the incorporation of fluorine-18. The experiments show the influence of the structure of the iodonium salt used on the yield and product distribution. Iodonium salt **25** contains a more electron releasing tolyl substituent compared to the phenyl ring in **24**. This change causes an improved yield in the attack of the [^{18}F]fluoride to the steroidal part of the iodonium salt. Despite the low decay-corrected radiochemical yields (0.2 and 2.0%, respectively), the metabolically stable fluorine-18 labeled glucocorticoid [^{18}F]-**7** represents an interesting agent to investigate further for the study of brain GR by means of PET.

Acknowledgments

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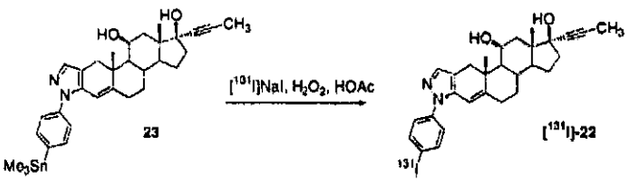

Fig. 9. Radiosynthesis of ^{131}I-labeled steroid [^{18}F]-22.

SPECT). The convenient half-life of iodine-131 ($t_{1/2}$ = 8.04 days) makes this isotope ideal for the elaboration of labeling protocols using iodine isotopes. Several radioiodination reactions using organometallic intermediates have been shown to be very effective, rapid and site-specific methods for the incorporation of iodine isotopes into small molecules like steroids [38]. In this context, the use of organostannanes has provided especially good results for the radioiodination of aromatic rings via iodo-destannylation reactions. Thus, we used organostannane **23** as a precursor for radiolabeling with iodine-131 (Fig. 9). For this purpose, [^{131}I]NaI (ca. 2 μCi, 75 kBq) in a buffer solution (5% NaOAc in HOAc) was oxidized with H_2O_2 in HOAc. The progress of the reaction with tin precursor **23** was monitored by radio-TLC. The reaction was completed after 30 min, at which point a solution of $Na_2S_2O_3$ was added to stop the reaction. After silica-gel purification (vide supra), the eluate was analyzed by radio-TLC (R_f = 0.47; 80% EtOAc/hexane), which indicated a radiochemical purity of >95% for [^{131}I]-**22**. Radio-TLC analysis revealed the formation of 78% of [^{131}I]-**22** besides non-reacted [^{131}I]iodide.

3.8. Glucocorticoid receptor binding affinities and log $P_{o/w}$ values of arylpyrazolo corticosteroids

The GR binding affinities of all arylpyrazolo steroids and reference compounds dexamethasone and cortisol **1** are listed, along with calculated log $P_{o/w}$, values in Table 1.

The GR binding affinities were assayed using liver cytosol from adrenalectomized rats, with [^{3}H]dexamethasone as radiotracer and unlabeled dexamethasone as standard (RBA = 100%). All steroids tested in this work show lower GR binding in comparison to the synthetic and high-affinity ligand dexamethasone. However, some compounds still exhibit appreciable binding, up to 56% that of dexamethasone, which is considerably better than the binding affinity of the natural hormone cortisol **1** (RBA = 8.4%). Moreover, all synthesized arylpyrazolo steroids show an increased lipophilicity (expressed as calculated log $P_{o/w}$ values) by at least one order of magnitude compared to dexamethasone or cortisol **1**. The enhanced lipophilicity might be a benefit for improved blood–brain-barrier penetration when appropriately radiolabeled compounds are used for brain imaging studies.

Within the series of 17α-alkynyl substituted steroids, the character of the terminal substitutent shows an interesting

Table 1
Relative binding affinities (RBAs) of arylpyrazolo steroids for the GR

Compound	RBA (0 °C) (%)	Log $P_{o/w}$[a]
Dexamethasone	100	2.06
Cortisol **1**	8.4[b]	1.43, (1.61[c])
5	50	3.46
6	18	3.30
7	56	4.50
8	31	3.34
10	37	4.31
11	7.5	4.82
12	8.5	5.38
13	17.5	3.91
15	22	3.08
17	11	3.87
21	6.5	5.22
22	1.5	5.48

Each value represents the mean of two determinations, with a coefficient of variance of 0.3.

[a] Log $P_{o/w}$ values have been calculated based on ACDLabs predictions.

[b] Literature value [8].

[c] Literature value [39].

effect on the RBA value. In the case of the neutral terminal substituents F (**10**), OH (**8**) and Me (**7**), the RBA values vary between 37, 31 and 56%, respectively. These values represent relatively good binding to the GR, and the binding is significantly higher compared to steroids **11**–**13** containing basic groups. Thus, the morpholine-containing steroid **13** reaches an RBA of about 17.5%, whereas the RBA drops markedly when more basic cyclic amines are used, being 7.5% for pyrrolidine **11** and 8.5% for piperidine **12**. These findings indicate that neutral side chains support GR binding better than basic side chains. Nevertheless, all measured RBA values within the series of 17α-alkynyl substituted steroids display at least the same (compounds **11** and **12**) or better GR binding (compounds **7**, **8**, **10** and **13**) compared to the natural occurring steroid cortisol **1**.

In the second set of compounds, the 1,3-dihydroxyacetone side chain containing steroid **5** shows the highest RBA value, 50% relative to dexamethasone. In contrast, the corresponding 21-fluoro compound **17** displays a reduced binding affinity of 11%, which is comparable to that of cortisol **1**. On the other hand, structurally very different ketone **6** and oxetanone **15** exhibit moderate to good binding of 18 and 22%, respectively.

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