Synthesis of a fluorescent steroid derivative with high affinities for the glucocorticoid and progesterone receptors

Georges Teutsch, Michel Klich, Françoise Bouchoux, Evelyne Cerede, and Daniel Philibert

Centre de Recherches Roussel-Uclaf, Romainville, France

The synthesis of RU 45196, an 11β-substituted 19-norsteroid of the estra-4,9-diene series, incorporating the nitrobenzoxadiazole (NBD) fluorophore, is reported. The highly fluorescent target compound displayed remarkable affinity for both the progesterone and glucocorticoid receptors. The present work demonstrates for the first time that it is indeed possible to design fluorescent steroid conjugates which maintain very high affinities for their cognate receptors and which are potentially useful for mechanistic and diagnostic purposes.

Keywords: fluorescence; nitrobenzoxadiazole; 11β-aryl; progesterone receptor; glucocorticoid receptor.

Introduction

The design of fluorescent compounds having a high affinity for steroid hormone receptors is still considered a desirable objective, both in terms of basic endocrine research and as a tool for the quantitation of receptors in hormone dependent tumors. The main emphasis, so far, has been with fluorescent estrogens, which are potentially useful in the prognosis of breast cancer evolution and treatment. Selected examples can be found in references 1–10.

Earlier attempts at designing fluorescent conjugates of other steroids were essentially unsuccessful, due to decreased affinity for the cognate receptors.[11–13] The only success, so far, seems to be the rhodamine derivative of dexamethasone, reported by Simons and colleagues, which displayed a significant binding to the HTC cell glucocorticoid receptor in a cell-free system.[14] However, the conjugate was unable to enter whole cells, presumably due to permeability problems arising from the charged rhodamine moiety. A similar result had already been reported with estradiol-fluorescein conjugates.[15] We thought that the design of a less polar fluorescent conjugate could possibly circumvent this difficulty.

Our general synthesis of 11β-substituted 19-norsteroids has allowed us to discover that most steroid hormone receptors possess a large hydrophobic pocket, able to accommodate bulky 11β-substituents[16–20] some of which could be known molecular probes. This property has now been taken to advantage for the design of a high affinity fluorescent ligand of the progestin and glucocorticoid receptors. The nitrobenzoxadiazole fluorophore (NBD) was chosen in view of its nearly ideal fluorescence characteristics.[3,8,21]

A preliminary attempt, by coupling compound **1**, a metabolite of the antiprogestin RU 486, with NBD-chloride (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole), led to **2** which was devoid of fluorescence, due to the conjugation of the nitrogen lone pair with the aromatic ring. Intercalation of a methylene group between the amine and the phenyl ring was expected to circumvent this effect.

Experimental

Biology

Receptor binding. The relative binding affinities (RBAs) of test compounds for progestin (PR) and glucocorticoid (GR) receptors were determined in cytosol from rabbit uterus and rat thymus respectively[22]: the thymic and uterine cytosols were incubated with the suitable radioligands, 2.5 nM of dexamethasone (Dex.) and 5 nM of R5020 respectively, in the presence of increasing concentrations of unlabeled reference or test compounds. Bound radioactivity was measured by dextran-coated charcoal adsorption technique. The ratio of the concentration of the reference compound (Progesterone or Dex.) to the concentration of test compound required to displace labeled radioligand binding by 50% (RBA) was determined. The RBA's of the reference compounds, progester-

Address reprint requests to Georges Teutsch, Centre de Recherches Roussel-Uclaf, 102, route de Noisy, 93230 - Romainville, France.
Received March 27, 1993; accepted June 17, 1993.

one and Dex. for PR and GR, respectively, were taken arbitrarily equal to 100%.

Incorporation of tritiated uridine in rat thymocytes. This cellular model allows the easy evaluation of the glucocorticoid and/or antiglucocorticoid activities.[23] Thymocytes were prepared by mincing pooled thymi of adrenalectomized rats in Hank's buffer. They were washed and suspended at a final concentration of 2×10^7 cells/ml in MEM. Aliquots of 250 µl were incubated under O_2 (95%) CO_2 (5%) for 3 hours at 37 C with 5.10^{-8} M of Dex. in the presence of increasing concentrations of RU 45196 or with RU 45196 alone. [^{3}H]Uridine (0.1 µCi) was added, and incubation was continued for 1 hour. Radioactivity incorporated into trichloroacetic acid precipitable material was determined.

Chemistry

Melting points were determined on a Kofler hot plate. NMR spectra were recorded in $CDCl_3$ solution on BRUCKER WP or WH spectrometers, tetramethylsilane being used as an internal standard. UV absorption and fluorescence were measured in ethanol solution using CARY and FARRAND-Mark 1 spectrometers, respectively. Mass spectra were recorded on MAT-311A or ZAB-HFQ spectrometers. Chromatographic separations were performed using 50 parts (wt/wt) of Merck silica gel (0.04–0.063 mm). All reactions were carried out under nitrogen atmosphere. Commercial reagents were purchased from the following companies: Air Liquide, Paris, France – Aldrich, Strasbourg, France – Alfa, Karlsruhe, Germany – Fluka, Buchs, Switzerland – Janssen, Geel, Belgium – Merck, Darmstadt, Germany – Riedel de Haën, Seelze, Germany.

4 - [(*Tert* - butyldimethyl) silyloxymethyl] - bromobenzene *4*. A solution of 4-bromobenzyl alcohol (Aldrich) (46.8 g, 250 mmol) in anhydrous THF (300 ml) was cooled to 0 C. A 50% sodium hydride suspension in mineral oil (12 g, 250 mmol) was added under stirring and the reaction was left in the ice bath until the end of hydrogen evolution (30 minutes). *t*-Butyldimethyl chlorosilane (Aldrich) (37.7 g, 250 mmol) was then added in several portions, so as to maintain the temperature below 25 C. After the end of the introduction the reaction mixture was left at room temperature for 24 hours. It was then poured into a saturated aqueous ammonium chloride solution (250 ml) and extracted with ethyl ether (3 × 100 ml). The organic extracts were dried over magnesium sulfate and the solvent was evaporated. The crude oily product was purified by chromatography (*n*-hexane/CH_2Cl_2, 8:2) yielding 71.2 g (94.5%) of the desired compound **4**, ^{1}H NMR: δ = 0.08 (s, 6H, $SiMe_2$), 0.92 (s, 9H, *t*-butyl), 4.67 (s, 2H, CH_20) 7.10–7.23 (m, 2H ortho to Br), 7.38–7.52 (m, 2H, meta to Br); MS: m^+ = 301/302; Analysis calculated for $C_{13}H_{21}BrOSi$: C, 51.82; H, 7.02; Br, 26.52; Found: C, 51.7; H, 7.1; Br, 26.2. Some unreacted starting alcohol (2.6 g, 5.5%) was eluted subsequently.

3,3-Dimethoxy-17α-propynyl-estra-5(10),9(11)-dien-17β-ol *6*. A solution of dienoketal **5**[24] (50 g) in THF (240 ml) was added to a 1.0 M solution of propynylmagnesium bromide in THF (350 ml) at 20 C over 50 minutes. After a further hour the reaction mixture was poured into saturated aqueous ammonium chloride (850 ml) and extracted with ethyl ether (4 × 200 ml). Combined extracts were washed with saturated sodium bicarbonate (200 ml), dried over anhydrous sodium sulfate, and evaporated under reduced pressure, affording 62.4 g of crude **6** which was used as such for the epoxidation step. However, a small sample was purified by column chromatography (silicagel Merck H, Et_2O/Hexane, 3:1) and recrystallized from isopropyl ether to afford the analytical sample: MP = 138 C; $[\alpha]_D$ = + 187.5° (1% in $CHCl_3$); ^{1}H NMR: 0.84 (s, 3H, 18Me) 1.85 (s, 3H, ≡C-CH_3), 3.24 (s, 6H, ketal), 5.62 (m, 1H, H-11). Analysis calculated for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.3; H, 9.1.

3,3-Dimethoxy-5α,10α-epoxy-17α-propynyl-estr-9(11)-en-17β-ol *7*. To a solution of crude diene **6** (62 g, 174 mmol) in methylene chloride (280 ml) triethylamine (0.5 ml) and hexafluoroacetone trihydrate (Aldrich) (8.5 ml, 74 mmol) at 0 C was added dropwise 85% hydrogen peroxide (Air Liquide) (10 ml, ~1.5 Eq). After stirring for 40 hours at the same temperature, the reaction mixture was poured into 0.5 molar sodium thiosulfate (1.4 L) and ice (200 g) and extracted with methylene chloride (4 × 200 ml + 2 drops of pyridine). The combined extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure, affording 63.5 g of the crude epoxide which consisted of 80% of the desired α-epoxide (H-11 at 6.08 ppm) and 20% of the corresponding β-isomer (H-11 at 5.89 ppm). The pure α-isomer was obtained by chromatography on Kieselgel Merck H (Cyclohexane/AcOEt 8:2, 0.5% triethylamine) followed by recrystallization from isopropyl ether. Melting point = 166 C; $[\alpha]_D$ = −6° (0.8% in $CHCl_3$); ^{1}H NMR: 0.84 (s, 3H, 18Me), 1.83 (s, 3H, ≡C-CH_3), 3.13 (s) and 3.19 (s) (2 × 3H, ketal) 6.08 (m, 1H, H-11). Analysis calculated for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.2; H, 8.6.

11β-[4-(*Tert*-butyldimethylsilyloxymethyl)-phenyl]3,3-dimethoxy-17α-propynyl-Δ9-estren-5α,17β-diol *8*.

a) Preparation of the Grignard reagent

The reagent was prepared from 254 mg (10.4 mAt) of magnesium turnings (Fluka) and 3.013 g (10 mmol) of the protected bromobenzylalcohol **4** in 7 ml of THF.

b) Condensation

Cuprous chloride (Janssen) (100 mg, 1 mmol) was added to a cooled solution (−10 C) of the above Grignard reagent. Epoxide **7** (745 mg, 2 mmol) was added at once, leading to a rise in temperature to +5 C. After 20 minutes at −10 C, the reaction mixture was allowed to return to room temperature. A saturated aqueous solution of ammonium chloride (5 ml) was added. The reaction mixture was extracted with ethyl acetate, the combined extracts were washed with brine, dried over magnesium sulfate and the solvent was evaporated under reduced pressure. Chromatography of the crude product (CH_2Cl_2/AcOEt, 96:4) afforded 1.1 g (92%) of the desired compound **8** which was recrystallized from isopropyl ether. Melting point: 185–186 C; $[\alpha]_D$ = +73° (c = 1%, $CHCl_3$), ^{1}H NMR: δ = 0.08 (6H, $SiMe_2$), 0.93 (9H, *t*-butyl), 1.89 (3H, ≡C-Me), 0.43 (3H, 18-Me), ~3.23 (6H, OMe), 4.31 (1H, H-11), 4.72 (2H, $OC\underline{H}_2Ar$), 7.2 (4H, aromatics). Analysis calculated for $C_{36}H_{54}O_5Si$: C, 72.69; H, 9.15; Found: C, 72.5; H, 9.1.

17β-Hydroxy-11β-[(4-hydroxymethyl)-phenyl]-17α-propynyl-estra-4,9-dien-3-one *9*. To a solution of 680 mg of **8** (1.14 mmol) in methanol (12.5 ml) was added 2N hydrochloric acid (1 ml). After 1 hour at room temperature, brine was added and the reaction mixture was extracted with ethyl acetate. The combined organic extracts were washed with a saturated sodium bicarbonate solution, dried over magnesium sulfate and evaporated to dryness under reduced pressure. Chromatography of the crude product (CH_2Cl_2/Acetone, 9:1) afforded 440 mg (92.7%) of the desired compound **9**, which was re-

crystallized from isopropyl ether, yielding 365 mg of the analytically pure sample. Melting point = 208 C; $[\alpha]_D$ = + 74 ± 2.5° (c = 0.5%, $CHCl_3$); UV: λ_{max} = 223 nm (14600), 238 nm (4915) and 302 nm (21000); ^1H NMR: δ = 0.52 (3H, 18-Me), 1.92 (3H, ≡C-Me), 4.44 (1H, H-11), 4.68 (2H, $C\underline{H}_2OH$), 5.8 (1H, H-4), 7.15 and 7.32 (4H, aromatics). Analysis calculated for $C_{28}H_{32}O_3$: C, 80.73; H, 7.74. Found: C, 80.5; H, 8.0.

11β-[4-(Bromomethyl)phenyl]-17β-hydroxy-17α-propynyl-estra-4,9-dien-3-one *10*. Carbon tetrabromide (Merck) (497 mg, 1.5 mmol) was added at once to a solution of hydroxysteroid **9** (416 mg, 1 mmol) and triphenylphosphine (Fluka) (262 mg, 1 mmol) in methylene chloride (5 ml). After 1 hour at room temperature, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/AcOEt, 95:5) affording 433 mg (90%) of the desired bromide **10**. Recrystallization from isopropyl ether, afforded the analytical sample: Melting point: 198 C; $[\alpha]_D$ = +92.5 ± 2.5° (c = 0.5%, $CHCl_3$), Rf = 0.27 (SiO_2-F254, CH_2Cl_2/AcOEt, 95:5); UV: λ_{max} = 239 nm (15100) and 302 nm (19900); ^1H NMR: δ = 0.5 (3H, 18-Me), 1.94 (3H, ≡C-Me), 4.44 (1H, H-11), 4.48 (2H, CH_2Br), 5.8 (1H, H-4), 7.19 and 7.36 (4H, aromatics). Analysis calculated for $C_{28}H_{31}BrO_2$: C, 70.14; H, 6.52; Br, 16.67. Found: C, 69.9; H, 6.5; Br, 16.6.

17β-Hydroxy-17α-propynyl-11β-[4(trifluoroacetamidomethyl)-phenyl]estra-4,9-dien-3-one *11*. Trifluoroacetamide (Riedel de Haën) (113 mg, 1 mmol) was added to a suspension of sodium hydride (Alfa) (1 mmol) in THF (2 ml) at room temperature. After hydrogen evolution had ceased, 200 mg (0.4 mmol) of the bromosteroid **10** was added, followed by 1 ml of DMF. The reaction mixture was left at room temperature under nitrogen overnight, poured into water, and extracted with ethyl acetate. The organic extracts were dried over magnesium sulfate and evaporated to dryness under reduced pressure. Chromatography of the residue (CH_2Cl_2/AcOEt, 8:2) afforded the desired trifluoroacetamide derivative **11** (103 mg, 50%), MS: m^+ = 511, $[\alpha]_D$ = + 52° (c = 0.7%, $CHCl_3$). UV: λ_{max} = 222 nm (15700) and 302 nm (19500); ^1H NMR: δ = 0.5 (3H, 18-Me), 1.92 (3H, ≡C-Me), 4.44 (1H, H-11), 4.51 (2H, CH_2N), 5.79 (1H, H-4) and ~7.2 (4H, aromatics). A more polar compound, corresponding to a double steroid addition to the trifluoroacetamide, was also recovered (63 mg, 35%); MS: m^+ = 909–910; UV: λ_{max} = 223 nm (32300) and 302 nm (38300); ^1H NMR: δ 18 Me at 0.51 and 0.53.

17β-Hydroxy-11β-[4-((7-nitro-4-benzofurazanyl)amino-methyl) phenyl]-17α-propynyl-estra-4,9-dien-3-one *12*. To a solution of 51 mg (0.1 mmol) of the trifluoroacetamidosteroid **11** in dioxane (0.5 ml) at room temperature was added 1N sodium hydroxyde (0.12 ml). After 30 minutes, 21 mg (0.1 mmol) of NBD-chloride (Aldrich) was added, followed by an excess of sodium acetate. The reaction mixture was kept at room temperature for 2 hours, poured into water and extracted with methylene chloride. The organic extracts were dried over magnesium sulfate and evaporated to dryness under reduced pressure. Preparative TLC (CH_2Cl_2/AcOEt, 8:2) afforded the desired brick-red colored compound **12** which was triturated in isopropyl ether (yield: 43 mg, 75%).

Melting point = 175–176 C; $[\alpha]_D$ = + 75° (c = 0.7%, $CHCl_3$); MS: m^+ = 578; UV: λ_{max} = 222 nm (25200), 304 nm (21900), and 462 nm (20000); Fluorescence in EtOH (0.55 mg/10 ml): excitation at 480 nm, λ_{max} emission = 525 nm; ^1H NMR: δ = 0.5 (3H, 18 Me), 1.92 (3H, ≡C-Me), 4.48 (1H, H-11), 4.62–4.69 (2H, CH_2N), 5.81 (1H, H-4), 6.2–6.3 (1H, meta of NO_2), 8.44–8.54 (1H ortho of NO_2).

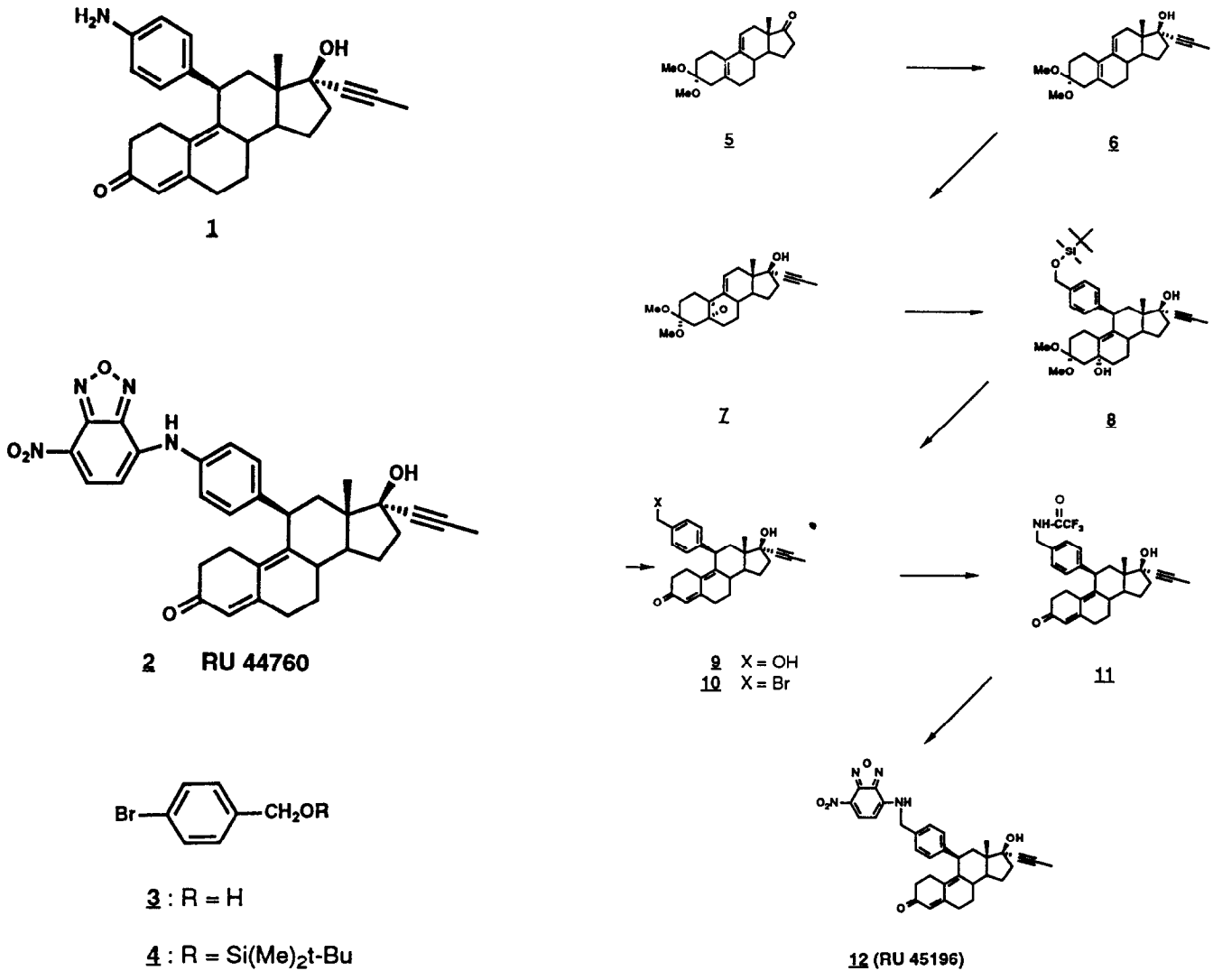
Results and discussion

The steroid-NBD conjugate **12** was prepared via the reaction sequence shown in Scheme 1. It relies on our previously described cuprate-mediated opening of Δ9(11)-5α, 10α-epoxy-19-nor steroids.[16,25] Epoxidation of the 5(10), 9(11) diene **6** using concentrated hydrogen peroxide in the presence of hexafluoroacetone sesquihydrate afforded a 4:1 mixture of the 5α, 10α, and 5β, 10β epoxides. Alternatively, the epoxidation can be achieved with 50% hydrogen peroxide in the presence of hexachloroacetone,[26] but under these conditions, the α/β ratio is less favorable (2:1). The pure α-epoxide can be separated from the unwanted β-isomer by repeated chromatography followed by recrystallization. Conjugate addition with epoxide opening of **7** by the Grignard reagent derived from *t*-butyldimethyl silyl-protected para-bromobenzyl alcohol in the presence of copper chloride proceeded uneventfully to afford **8**. This compound was transformed under aqueous acidic conditions to the dienone **9** which has also been obtained recently by Katzenellenbogen and colleagues via a slightly different pathway.[27] The bromination step using the triphenylphosphine – carbon tetrabromide system gave the best results. When CBr_4 was added at once to the mixture of **9** and triphenylphosphine, the reaction was complete in 5 minutes. Treatment of the bromo derivative **10** with the anion of trifluoroacetamide[28] afforded **11** along with some bis-adduct incorporating two steroid molecules on the nitrogen atom. Saponification of **11** and in situ alkylation with NBD chloride afforded the target compound **12** (RU 45196) which displayed very satisfactory fluorescence properties (excitation at 480 nm, emission at 525 nm) as well as high binding affinities for the glucocorticoid and progesterone receptors (see Table 1). The found RBA for progesterone receptor (PR) (336% relative to progesterone) appears to be considerably higher than the value reported earlier[29] for this compound (22% relative to R 5020). One might incriminate the difference of species (rabbit versus rat), but most likely, the discrepancy arises from the different incubation times (24 hours versus 2 hours). Indeed, it has been shown[30] that for slowly associating, slowly dissociating compounds, like R 5020, the RBA after 24 hours of incubation can be considerably higher than after 2 hours (530 versus 220). Similarly, for compound **12** (RU 45196), the RBA we found after 2 hours of incubation was only 40% relative to progesterone, which amounts

Table 1. Relative binding affinities for the glucocorticoid and progesterone receptors[a]

	GR Dex. = 100	PR Prog = 100
2	170	240
9	134	54
10	80	83
11	98	40
12	195	336

[a] Mean of at least 2 determinations.



Scheme 1 Synthesis of RU 45196

to 18% relative to R 5020, in excellent agreement with the value reported by Carlson et al.[29] This example illustrates the danger of using too short incubation times which might not allow the system to reach equilibrium. In rat thymocytes, at the concentration of 10^{-7} M, RU 45196 totally antagonized the inhibition of tritiated uridine incorporation by 5×10^{-8} M of dexamethasone, demonstrating that it readily entered into the cell and acted as a glucocorticoid antagonist (Figure 1). RU 45196 did not induce abortion when administered orally to rats at the dose of 3 mg/kg, according to a previously described protocol,[31] suggesting that it displays only poor antiprogestational activity, if any. It has been investigated more thoroughly by Carlson et al.[29] to determine its potential usefulness in progesterone receptor detection. Although the compound still fluoresced when non-specifically bound to proteins, the binding to the PR led to an as yet unexplained quenching of the fluorescence.

The present work demonstrates that it is indeed possible to design fluorescent steroid conjugates which

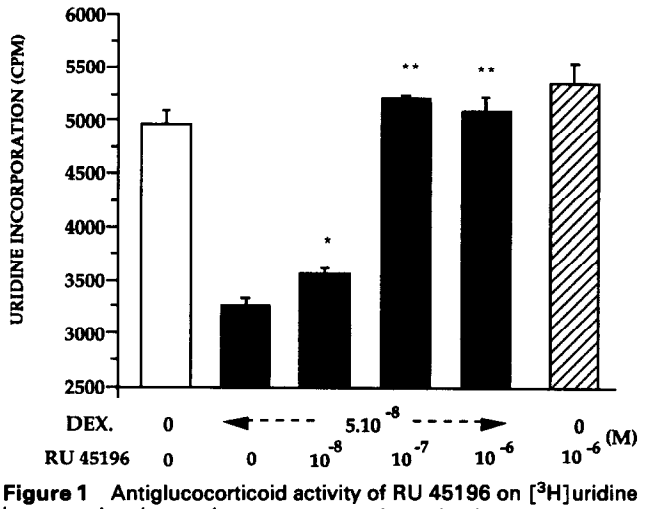


Figure 1 Antiglucocorticoid activity of RU 45196 on [^{3}H]uridine incorporation in rat thymocytes was determined according to a previously described procedure.[23] The inhibition of incorporation induced by 5×10^{-8} molar dexamethasone (Dex.) was counteracted by increasing concentrations of the test compound.

maintain very high affinities to their cognate receptors. Further investigations with this compound and similar ones will hopefully lead to a better knowledge of receptor-ligand interaction as well as to new tools for diagnostic purposes.

References

1. Levison SA, Dandliker WB, Brawn RJ, Vanderlaan WP (1976). Fluorescence polarization measurements of the hormone binding site interaction. *Endocrinology* **99**:1129–1143.
2. Dandliker WB, Brawn RJ, Hsu ML, Brawn PL, Levin J, Meyers CY, Kolb VM (1978). Investigation of hormone-receptor interactions by means of fluorescence labeling. *Cancer Res* **38**: 4212–4224.
3. Lee YJ, Notides AC, Tsay Y-G, Kende AS (1977). Coumestrol, NBD-Norhexestrol and Dansyl-Norhexestrol, fluorescent probes of estrogen-binding proteins. *Biochemistry* **16**:2896–2901.
4. Martin PM, Magdelenat HP, Benyahia B, Rigaud O, Katzenellenbogen JA (1983). New approach for visualizing estrogen receptors in target cells using inherently fluorescent ligands and image intensification. *Cancer Res* **43**:4956–4965.
5. Nelson K, Pavlik EJ, Van Nagell JR, Hanson MB, Donaldson ES, Flanigan RC (1984). Estrogenicity of coumestrol in the mouse: fluorescence detection of interaction with estrogen receptors. *Biochemistry* **23**:2565–2572.
6. Van NT, Raber M, Barrows GH, Barlogie B (1984). Estrogen receptor analysis by flow cytometry. *Science* **224**:876–879.
7. Katzenellenbogen JA, Carlson KE, Bindal RD, Neeley RL, Martin PM, Magdelenat HP (1986). Fluorescence-based assay of estrogen receptor using 12-oxo-9(11)-dehydroestradiol-17β. *Anal Biochem* **159**:336–348.
8. Fevig TL, Lloyd JE, Zablocki JA, Katzenellenbogen JA (1987). Preparation, receptor binding and fluorescence properties of hexestrol-fluorophore conjugates. *J Med Chem* **30**:156–165.
9. Salman M, Reddy BR, Delgado P, Stotter PL, Fulcher LC, Chamness GC (1991). 17α-Substituted analogs of estradiol for the development of fluorescent estrogen receptor ligands. *Steroids* **56**:375–387.
10. Hwang K-J, O'Neil JP, Katzenellenbogen JA (1992). 5,6,11,12-Tetrahydrochrysenes: synthesis of rigid stilbene systems designed to be fluorescent ligands for the estrogen receptor. *J Org Chem* **57**:1262–1271.
11. Gaetjens E, Pertschuk LP (1980). Synthesis of fluorescein-labeled steroid hormone-albumin conjugates for the fluorescent histochemical detection of hormone receptors. *J Steroid Biochem* **13**:1001–1003.
12. Berns EMJJ, Mulder E, Rommerts FFG, Blankenstein RA, De Graaf E, Van der Molen HJ (1984). Fluorescent ligands, used in histocytochemistry do not discriminate between estrogen receptor-positive and receptor-negative human tumor cell lines. *Breast Cancer Res Treat* **4**:195–204.
13. Auzou G, Nique F, Nédélec L (1988). Synthesis of new fluorescent spirolactone derivatives: determination of their affinities for aldosterone receptors. *Steroids* **51**:465–469.
14. Pons M, Robinson TEJ, Mercier L, Thompson EB, Simons SS (1985). Formation of a fluorescent glucocorticoid receptor-steroid complex in HTC cell cytosol. *J Steroid Biochem* **23**:267–273.
15. Gay G, Jozan S, Marques B, David JF (1984). Estrogen and progesterone receptors of human endometrial cells *in vivo* and *in vitro*: comparative results of radiochemical and histochemical assays. *J Recept Res* **3**:685–701.
16. Belanger A, Philibert D, Teutsch G (1981). Regio- and stereospecific synthesis of 11β-substituted 19-norsteroids. Influence of 11β-substitution on progesterone receptor affinity. *Steroids* **37**:361–382.
17. Teutsch G (1984). 11-Beta-substituted 19-norsteroids: at the crossroads between hormone agonists and antagonists. In: Agarwal MK (ed.), *Adrenal steroid antagonism*. Walter de Gruyter, Berlin/New-York, pp. 43–75.
18. Teutsch G (1985). Analogues of RU 486 for the mapping of the progestin receptor: synthesis and structural aspects. In: Baulieu EE, Segal SJ (eds.), *The Antiprogestin steroid RU 486 and human fertility control*. Plenum Publishing Corp., New York and London, pp. 27–47.
19. Teutsch G, Ojasoo T, Raynaud JP (1988). 11β-substituted steroids, an original pathway to antihormones. *J Steroid Biochem* **31**:549–565.
20. Claussner A, Nédélec L, Nique F, Philibert D, Teutsch G, Van de Velde P (1992). 11β-amidoalkyl estradiols, a new series of pure antiestrogens. *J Steroid Biochem Mol Biol* **41**:609–614.
21. Ghosh PB, Whitehouse MW (1968). 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole: a new fluorigenic reagent for amino acids and other amines. *Biochem J* **108**:155–156.
22. Ojasoo T, Raynaud JP (1978). Unique steroid congeners for receptor studies. *Cancer Res* **38**:4186–4198.
23. Dausse JP, Duval D, Meyer P, Gaignault JC, Marchandeau C, Raynaud JP (1977). The relationships between glucocorticoid structures and effects upon thymocytes. *Mol Pharmacol* **13**:948–955.
24. Pierdet A, Vignau M (1966). Noveau médicament notamment pour le traitement des troubles dûs à une insuffisance de sécrétion du corps jaune. French Patent 5183.
25. Teutsch G, Bélanger A (1979). Regio and stereospecific synthesis of 11β-substituted 19-norsteroids. *Tetrahedron Lett* 2051–2054.
26. Costerousse G, Teutsch G (1979). Noveau réactif d'époxydation et son application à l'époxydation de composés organiques acycliques, cycliques ou polycycliques comportant au moins une insaturation éthylénique. European Patent EP 5100.
27. Di Zio JP, Fiaschi R, Davison A, Jones AU, Katzenellenbogen JA (1991). Progestin- rhenium complexes: Metal-labeled steroids with high receptor-binding affinity, potential receptor-directed agents for diagnostic imaging or therapy. *Bioconjugate Chem* **2**:353–366.
28. Harland PA, Hodge P, Maughan W, Wildsmith E (1984). Synthesis of primary amines via alkylation of the sodium salt of trifluoroacetamide: an alternative to the Gabriel synthesis. *Synthesis* 941–943.
29. Carlson KE, Coppé M, Magdelenat H, Katzenellenbogen JA (1989). Receptor binding of NBD-labeled fluorescent estrogens and progestins in whole cells and cell-free preparations. *J Steroid Biochem* **32**:345–355.
30. Raynaud JP, Bouton MM, Moguilewsky M, Ojasoo T, Philibert D, Beck G, Labrie F, Mornon JP (1980). Steroid hormone receptors and pharmacology. *J Steroid Biochem* **12**:143–157.
31. Philibert D, Hardy M, Gaillard-Moguilewsky M, Nique F, Tournemine C, Nédélec L (1989). New analogues of mifepristone with more dissociated antiprogesterone activities. *J Steroid Biochem* **34**:413–417.