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[^{3}H]Metyrapol and 4-[^{131}I]Iodometomidate Label Overlapping, but Not Identical, Binding Sites on Rat Adrenal Membranes

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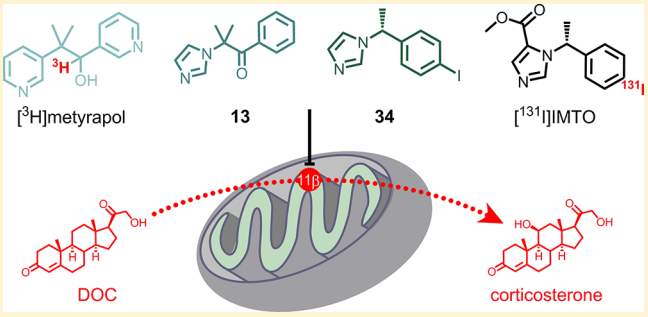
Supporting Information

ABSTRACT: Metyrapone, metyrapol, and etomidate are competitive inhibitors of 11-deoxycorticosterone hydroxylation by 11β-hydroxylase. [^{3}H]Metyrapol and 4-[^{131}I]-iodometomidate bind with high affinity to membranes prepared from bovine and rat adrenals. Here we report inhibitory potencies of several compounds structurally related to one or both of these adrenostatic drugs, against the binding of both radioligands to rat adrenal membranes. While derivatives of etomidate inhibited the binding of both radioligands with similar potencies, derivatives of metyrapone inhibited the binding of 4-[^{131}I]iodometomidate about 10 times weaker than the binding of [^{3}H]metyrapol. By X-ray structure analysis the absolute configuration of (+)-1-(2-fluorophenyl)-2-methyl-2-(pyridin-3-yl)-1-propanol [(+)-**11**, a derivative of metyrapol] was established as (*R*). We introduce 1-(2-fluorophenyl)-2-methyl-2-(pyridin-3-yl)-1-propanone (**9**; K_i = 6 nM), 2-(1-imidazolyl)-2-methyl-1-phenyl-1-propanone (**13**; 2 nM), and (*R*)-(+)-[1-(4-iodophenyl)ethyl]-1*H*-imidazole (**34**; 4 nM) as new high affinity ligands for the metyrapol binding site on 11β-hydroxylase and discuss our results in relation to a proposed active site model of 11β-hydroxylase.



KEYWORDS: *11β-hydroxylase, metyrapone, [^{3}H]metyrapol, etomidate, radioligand binding, rat adrenals*

INTRODUCTION

The adrenostatic drugs metyrapone and etomidate are active site blockers of 11β-hydroxylase (CYP11B1), a member of the mitochondrial cytochrome P450 family responsible for the synthesis of the stress hormone corticosterone from deoxycorticosterone (DOC) (for review, see Omura, 2006).[1] In contrast to drug-metabolizing P450s in microsomes, most mitochondrial P450s show high specificity to their endogenous substrate and have negligible activity toward xenobiotic compounds. In addition, etomidate acts as a sedative via the $GABA_A$ receptor,[2,3] however at concentrations about 3 orders of magnitude higher. Since 11β-hydroxylase is highly concentrated in the adrenal cortex, high affinity radioligands are of clinical diagnostic interest as imaging radiotracers to visualize adrenal tissue, especially in cases of adrenocortical cancer.[4,5] Already in 1974, [^{3}H]metyrapol was reported as a medium affinity radioligand for beef adrenal cortex mitochondria.[6] Subfractionation studies localized the binding site to the inner mitochondrial membrane, known to harbor 11β-hydroxylase. One reason for the early application of this radioligand may have been its easy synthesis (from metyrapone and [^{3}H]$NaBH_4$).[7]

Recently, we have established SARs of several etomidate derivatives at a binding site on rat adrenal membranes with high affinity for the radioligand 4-[^{131}I]iodometomidate[8] ([^{131}I]IMTO). Now, we tested the same and additional compounds at a [^{3}H]metyrapol binding site in the same preparation. Both classes of compounds appear to address the same or very similar binding sites, most likely on CYP11B1. In human adrenals, the m-RNA for this hydroxylase is 3 orders of magnitude more abundant than the m-RNA for CYP11B2,[9] another potential target for both radioligands. In rat adrenals, 65 times (Wistar rats) and 52 times (Fischer rats) more CYP11B1 than CYP11B2 mRNA is expressed[10] (we used Wistar rats). Although CYP17 is prominently expressed in

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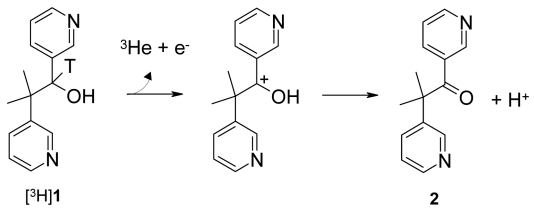
human adrenal cortex,[9] metyrapone had only weak or no effect on CYP17 (as on CYP11A1 and CYP21) in a human adrenocortical cell line.[11] In bovine adrenal mitochondria, metyrapone and A-phenyl-metyrapone inhibited CYP11A1 much more weakly than CYP11B1.[12] Some drug metabolizing cytochrome P450 enzymes in the liver are sensitive to inhibition by metyrapone (IC_{50}s from 1 to 4 μM, i.e., equivalent to CYP11B1).[13] However, these inducible enzymes are not involved in steroid biosynthesis, and their extrahepatic expression is low.[14] Thus, we assume that >90% of the binding of [^{3}H]metyrapol and of [^{131}I]IMTO was to CYP11B1 and that binding to other adrenal P450 enzymes did not significantly contribute to our results. Assessing inhibitory potencies of metyrapone-type and etomidate-type compounds at both targets, we were interested in analogues in between the molecular skeletons of metyrapone (two pyridinyl substituents connected by a two-carbon atom bridge) and etomidate (a single carbon atom bridge connecting a benzene with an imidazole ring, the latter carrying a carbonyl side chain). We were particularly interested in the question whether structural features favorable for one class might be introduced with benefit into the other class. Especially, metyrapone-type ligands with higher affinity than metyrapol may result from such a strategy, possibly allowing the visualization of other types of adrenocortical neoplasms than the established radioligands based on etomidate.

CHEMICAL SYNTHESES

Radioligands. [^{3}H]Metyrapol ([^{3}H]**1**) was custom-labeled by Amersham (12 Ci/mmol) and purified before use by TLC with $CHCl_3/CH_3OH$ (90/10), revealing another agreeable property of this radioligand (in addition to the ease of its synthesis): Upon purification, we never detected any other radioactive products than [^{3}H]**1** itself (autoradiography of the TLC plate gave a single band comigrating with unlabeled **1**). As reviewed by Morton (2001)[15] radioactive decay of a ^{3}H-labeled compound leaves a positive charge at the former position of the tritium. From this, the easiest way to form a stable product is the generation of a carbonyl group, resulting in (non-radioactive) metyrapone (**2**) (Scheme 1). Simple TLC on silica gel was sufficient to remove this radiolysis product (R_f for **1** 0.22, for **2** 0.50). The synthesis of [^{131}I]IMTO has been described previously.[16]

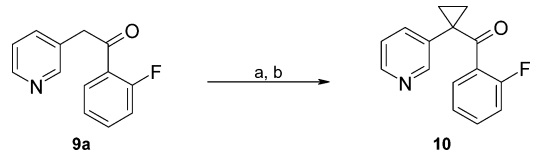
Metyrapone Derivatives. The syntheses of 4′-Br- (**3**) and 1′-O-**2** (**4**, the *N*-oxide) with substituents in the pyridinyl residue close to the quaternary carbon (ring B),[17] and of A-phenyl-metyrapone [2-methyl-1-phenyl-2-(pyridin-3-yl)-1-propanone, **5**][18] have been described. The syntheses of 2-Br- (**6**), 2-MeO- (**7**), 2-HO- (**8**), and 2-F-**5** (**9**) with substituents in the phenyl residue of **5** have been mentioned[19] but have never been described in detail. We now add a description of their syntheses as Supporting Information. Since the 2-fluoro derivative **9** turned out as the most potent compound of this series, we prepared further analogues of **9** (**10**–**12**). The cyclopropyl derivative **10**, formally derived from **9** by connecting the two methyl groups at position 2 of the aliphatic bridge, was prepared with 32% yield from ketone **9a** (precursor for **9**, see Supporting Information) by double alkylation with 1,2-dibromoethane (Scheme 2).

Scheme 1. Suggested Mechanism for Radiolysis of [^{3}H]Metyrapol, Yielding Metyrapone as the Main Product[a]



[a]The process can be interpreted as oxidation (although the electron is of nuclear origin).

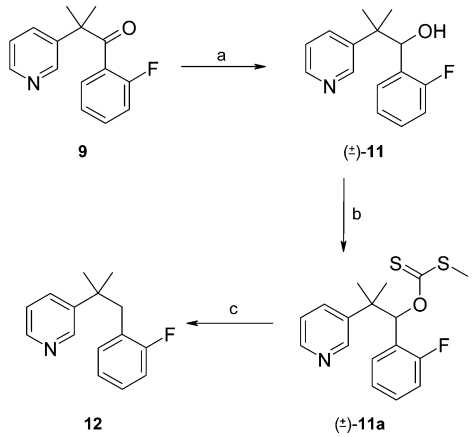
Scheme 2. Preparation of Metyrapone Analogue 10[a]



[a]Reagents and conditions: (a) *t*BuOK/DMF; (b) $BrCH_2CH_2Br$.

Reductive removal of the carbonyl group of ketone **9** in three steps furnished the 2-methylpropane derivative **12** (Scheme 3):

Scheme 3. Synthesis of the 2-Methylpropane Derivative 12 from 9[a]



[a]Reagents and conditions: (a) $NaBH_4$/EtOH; (b) *n*BuLi, CS_2, MeI; (c) $(TMS)_3SiH$, AIBN.

At first, the ketone was reduced with $NaBH_4$ to racemic alcohol (±)-**11**, which was transformed into xanthogenate (±)-**11a** by a standard procedure.[20] The lithium alkoxide formed on addition of *n*BuLi to the alcohol was added to carbon disulfide to give an *O*-alkyl dithiocarbonate, which was alkylated with MeI. Radical deoxygenation of (±)-**11a** with $(TMS)_3SiH$/AIBN in dry toluene at 90 °C furnished the desired derivate **12** in 55% yield.[20,21]

Metyrapone is biotransformed in part to metyrapol,[22−24] preferentially to the (−)-enantiomer (in the rat). At CYP11B1 both enantiomers show similar inhibitory activities.[24,25] However, the absolute configurations are not known. Here we describe optical resolution of the metyrapol derivative (±)-**11** with (+)-Noe-lactol dimer (MBF-O-MBF) (Scheme 4).[26] (+)-Camphor-10-sulfonic acid-catalyzed acetalization furnished the two preferentially formed axial diastereomers **A** and **B**, which were separated by flash chromatography in equal yields of 32% and found by ^{1}H NMR spectroscopy to be homogeneous (ee 99%). They were deblocked in MeOH/

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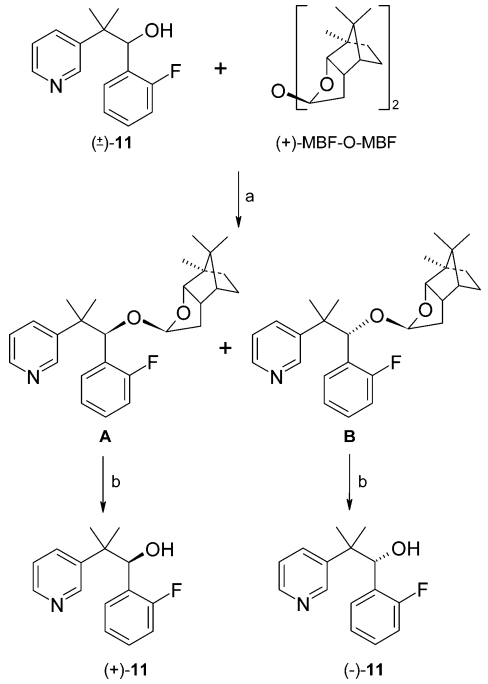
Scheme 4. Resolution of (±)-11 via Acetals A and B[a]

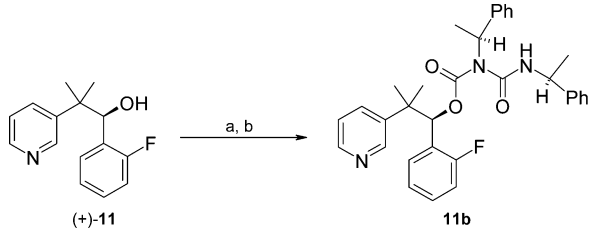


[a]Reagents and conditions: (a) DCM/H$^+$; (b) MeOH/H$^+$.

DCM/(+)-camphor-10-sulfonic acid to give (+)- and (−)-**11**, respectively. To determine the ee of the (+)-enantiomer by a second method, (±)- and (+)-**11** were converted to (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid esters [(*R*)-Mosher esters][27] and investigated by ^{1}H NMR spectroscopy. Again, the (+)-enantiomer was found to have an ee of 99%.

To assign the absolute configurations to the enantiomers, the dextrorotary alcohol was converted to the lithium alkoxide at −78 °C and reacted with excess (*S*)-1-phenylethylisocyanate (Scheme 5). Addition of two molecules of the isocyanate

Scheme 5. Preparation of Derivative 11b from (+)-11 for X-ray Structure Analysis[a]



[a]Reagents and conditions: (a) *n*BuLi/THF, −78 °C; (b) (*S*)-Ph(Me)CHNCO.

resulted in the urea derivative **11b**. The crystals obtained by slow evaporation of the solvent (DCM/hexanes) were suitable for X-ray structure analysis. The (+)-alcohol had (*R*)-configuration at the stereogenic center (C7), based on the known (*S*)-configuration of the derivatizing chiral isocyanate (Figure 1). Consequently, the (−)-alcohol has (*S*)-configuration. 2-(1-Imidazolyl)-2-methyl-1-phenyl-2−1-propanone (A-phenyl-B-imidazolyl-metyrapone, **13**), a known compound, was prepared by the literature procedure[28] in 47% yield, mp 104−105 °C (DCM/hexanes).

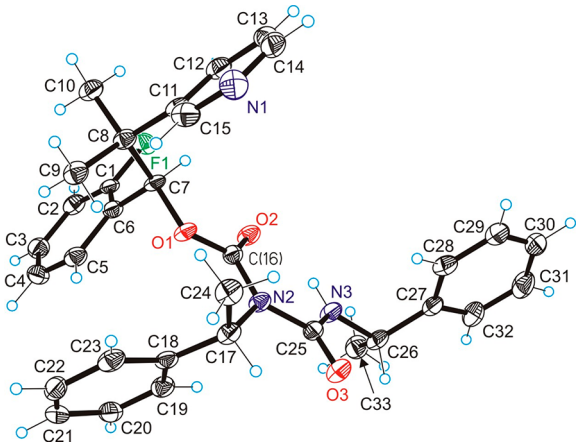


Figure 1. X-ray structure of **11b**

Etomidate Derivatives. Metomidate (**14**) was commercially available; the syntheses of its derivatives **15**−**23** (see Table 2) have been described;[8,29] only the fluoro derivative **24** was newly prepared for this study by a reported method,[29] starting from (*S*)-1-(4-fluorophenyl)ethanol of 99% ee. Etomidate (**25**) was obtained commercially; the syntheses of its derivatives **26**−**32** (see Table 2) have been described.[8,29] During a first attempt to convert **14** in one step to the *N*-methylamide **30**, unexpectedly the ester group was almost quantitatively lost, opening an easy synthetic access to etomidate analogues without ester group. Consequently, we prepared **33** and **34** from **25** and **20**, respectively (Scheme 6).

Scheme 6. Synthesis of Etomidate Derivatives without Ester Group[a]

[a]Reagents and conditions: (a) EtOH/MeNH$_2$, 180 °C.

As side products, the amides were formed in low yields, but the main products were **33** and **34**. This simple synthetic method is new, and its mechanisms should be investigated, since the decarboxylation of imidazole carboxylic acids in general requires harsher reaction conditions (e.g., refluxing in diphenyl ether at 260 °C).[30] The preparation of (*S*)-**33** from 1-phenylethylamine has been described.[30] Finally, we tested pyridin-3-yl derivatives of **14** and **16**[8,29] (**36** and **37**) and the 2,2′-bipyridin-5-yl derivatives **38**−**41**.[31,32]

EXPLORATION OF [^{3}H]METYRAPOL BINDING CONDITIONS

In our first approach to label adrenal membranes with [^{3}H]**1** we followed the protocol of Satre and Vignais (1974),[6] using mitochondrial membranes from beef adrenal cortex. Our results (K_D = 23 nM, B_M = 513 pmol/mg protein)[33] were similar to those of Satre and Vignais (K_D = 10−18 nM, B_M = 400−600 pmol/mg protein). However, material obtained from slaughterhouse bulls may be of variable quality. Therefore, we tested the possibility to switch from beef to rat adrenals. We explored possible species differences by direct comparison of [^{3}H]**1**

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affinities to 20 μm slices cut from shock-frozen beef adrenal cortex and rat whole adrenals, resulting in K_D = 60 nM for the beef and in K_D = 225 nM for the rat material.[33] Thus, we saw differences in the affinities of beef and rat adrenal binding sites for [^{3}H]**1**. For the other radioligand 4-[^{131}I]IMTO we observed similar K_D values for beef adrenocortical (9.6 ± 2.2 nM, n = 6) and for rat adrenal membranes (11.6 ± 2.5 nM, n = 8).[8] Given our previous results[8] with rat membranes and the higher affinity ligand [^{131}I]IMTO (and in light of better accessibility of rat adrenals), we decided to improve the reliability of [^{3}H]**1** binding to the rat material.

Attempts to detect specific [^{3}H]**1** binding to whole membranes from rat adrenals with the filtration technique failed. However, the centrifugation technique yielded robust specific binding (if at least 0.3 mg rat adrenal tissue per vial had been invested), with less than 10% nonspecific binding. In fact, the centrifugation technique is the method of choice for radioligands with medium affinity and fast dissociation rate.[34] Incubation on ice yielded better results than incubation at room temperature (rt) (Figure 2). Saturation analysis (on ice)

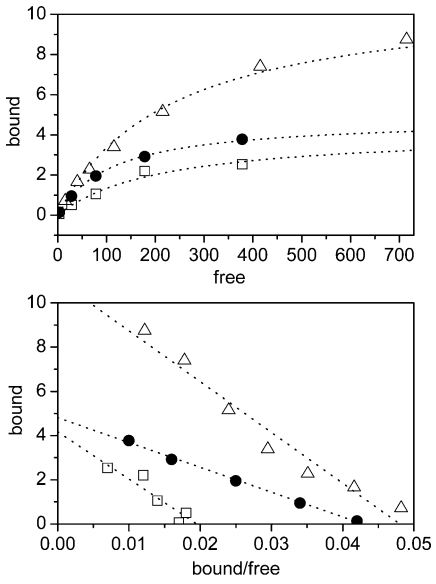


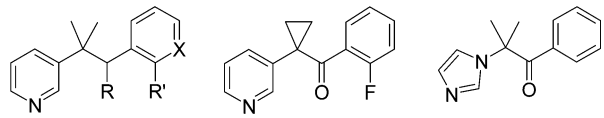
Figure 2. Representative results of experiments exploring conditions for [^{3}H]**1** binding. Increasing concentrations of the radioligand (upper panel) yielded saturation isotherms that were linearized by reciprocal transformation (lower panel). The slopes of the reciprocal plots yielded the highest affinity (112 nM in this example) for binding and centrifugation at 0 °C, if pellets were rinsed twice (filled circles). Affinities were weaker at 20 °C (213 nM; open squares), and (at 0 °C) if pellets were rinsed only once (230 nM; open triangles).

resulted in the following parameters (mean ± SD, n): K_D = 108 ± 20 nM (5); B_M = 4.9 ± 1.2 pmol/mg tissue (5). Since this standard protocol included two rinses of the pelleted membranes (some textbooks[34] even suggest three rinses), we reduced the number of rinses to one, to scrutinize the reliability of the observed B_M value. In two such experiments (one of them shown in Figure 2), we observed the following parameters: K_D = 230, 289 nM; B_M = 11.0, 14.9 pmol/mg tissue (nonspecific binding 2.6 times higher than after two washes). With the high affinity radioligand [^{131}I]IMTO, we had observed in the same membrane preparation (rat adrenals, filtration assay) a B_M = 12.1 pmol/mg tissue,[8] close to our single wash results with [^{3}H]**1**. Nevertheless, for the estimation of IC_{50} values of our test compounds against [^{3}H]**1** binding, we kept to the more reliable two-wash protocol, since IC_{50} values do not depend on absolute values.

INHIBITION OF BINDING BY METYRAPONE ANALOGUES

Both **1** and **2** blocked [^{131}I]IMTO binding to rat adrenal membranes with medium potency (K_i near 1 μM, Table 1).

Table 1. Inhibition of [^{3}H]1 and [^{131}I]IMTO Binding to Rat Adrenal Membranes by Metyrapone-Type Compounds (K_i ± SD, or Both Values if n = 2)



1-9, 11, 12 **10** **13**

				[^{3}H]**1**		[^{131}I]IMTO	
	X	R	R′	K_i (nM)	n	K_i (nM)	n
1	N	HO	H	98 ± 9	7	809 ± 315	9
2	N	O=	H	36 ± 2	3	804 ± 637	7
5	C	O=	H	13.7 ± 4.5	4	143 ± 60	9
6	C	O=	Br	11.2 ± 5.7	3	77, 85	2
7	C	O=	MeO	9.9, 11.1	2	96 ± 26	4
8	C	O=	HO	9.3 ± 2.6	3	179, 128	2
9	C	O=	F	5.84 ± 1.60	5	102, 77	2
10	C	O=	F	38.1, 22.6	2	215 ± 146	3
(*R*)-**11**	C	HO	F	235 ± 56	3	527 ± 233	3
(*S*)-**11**	C	HO	F	50 ± 8	3	154 ± 28	3
12	C	H	F	20.6 ± 2.7	3	57, 49	2
13	C	O=	H	2.05 ± 0.70	6	20 ± 6	3

However, **1** and **2** were much more potent in displacing bound [^{3}H]**1**. The SARs of metyrapone-like compounds exhibited some similarities at both sites (Table 1): (1) Replacing the pyridinyl substituent adjacent to the carbonyl group (the A-ring) by a phenyl substituent (a first step toward the molecular skeleton of etomidate) led to a considerable (more than factor 5) increase in potency (compounds **5**–**9**); (2) additional replacement of the other pyridine ring by an imidazole ring (a second step toward etomidate) increased the potency still further (by at least another factor 5; compound **13**). Structure **13** bears already remarkable resemblance to the structure of etomidate without ester group (**33**). Since **9**, the 2-F derivative of **5**, turned out as one of the most potent displacers of [^{3}H]**1**, we subjected this structure to closer exploration. Connecting the methyl substituents (**10**) was of disadvantage at both binding sites (Table 1). After reduction of the keto group and steric resolution of the secondary alcohol, we observed a slightly higher potency of (*S*)-(−)-**11** as compared to (*R*)-(+)-**11** (for **1** itself, no such steric preference has been described[24,25]). However, even the more potent isomer (*S*)-(−)-**11** was weaker than unreduced **9**. Leaving out the oxygen resulted in **12**, a compound of considerable potency at both binding sites. Already Hays et al.[18] had observed potent inhibition of 11β-hydroxylase by a derivative of A-phenyl-metyrapone without oxygen.

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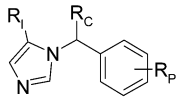
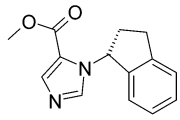
Table 2. Inhibition of [^{3}H]1 and [^{131}I]IMTO Binding to Rat Adrenal Membranes by Etomidate-Type Compounds (K_i ± SD, or both values if n = 2)



14-17, 19-35



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				[^{3}H]1		[^{131}I]IMTO	
	R_I	R_C	R_P	K_i (nM)	n	K_i (nM)	n
14	CO_2Me	(*R*)-Me		3.03 ± 1.46	4	4.02 ± 1.87	9
15	CO_2Me	(*S*)-Me		161, 157	2	502 ± 226	6
16	CO_2Me	H		10.6 ± 3.9	3	25 ± 9[a]	4
17	CO_2Me	(*R*)-Et		2.20 ± 1.52	3	5.31 ± 0.82[a]	3
18	CO_2Me	(see structure)		2.71 ± 0.93	3	2.78 ± 0.60[a]	3
19	CO_2Me	(*R*)-Me	3-I	11.1 ± 4.4	3	3.79 ± 1.52[a]	4
20	CO_2Me	(*R*)-Me	4-I	11.3 ± 1.6	3	8.7 ± 3.2	24
21	CO_2Me	(*R*)-Me	4-Br	2.83 ± 0.33	3	8.8 ± 2.4	4
22	CO_2Me	(*R*)-Me	4-Me	3.04 ± 1.32	3	3.88 ± 1.80	4
23	CO_2Me	(*R*)-Me	4-HOMe	2.49 ± 1.37	3	2.81 ± 0.77[a]	4
24	CO_2Me	(*R*)-Me	4-F	6.80 ± 2.24	3	7.3, 9.0	2
25	CO_2Et	(*R*)-Me		3.02 ± 1.82	4	0.97 ± 0.39	12
26	CO_2Et	(*R*)-Me	4-I	14.0, 18.2	2	3.73 ± 0.98[a]	5
27	$CO_2CH_2CH_2F$	(*R*)-Me		1.76, 2.12	2	2.47 ± 0.47[a]	4
28	CO_2Pr	(*R*)-Me		4.05 ± 2.12	3	2.47 ± 0.47[a]	4
29	CO_2iPr	(*R*)-Me		4.99 ± 1.38	3	1.21 ± 0.28[a]	5
30	C(O)NHMe	(*R*)-Me		47 ± 6	3	183 ± 78[a]	6
31	C(O)N(Me)OMe	(*R*)-Me		4.92, 3.84	2	14.2, 11.9[a]	2
32	C(O)Me	(*R*)-Me		7.91 ± 3.40	4	51, 53[a]	2
33	H	(*R*)-Me		57 ± 14	5	686 ± 139	3
34	H	(*R*)-Me	4-I	4.34 ± 1.92	3	31, 26	2
35	CO_2H	(*R*)-Me		38830 ± 16180	3	104910 ± 35310[a]	3

[a]From ref 8.

INHIBITION OF BINDING BY ETOMIDATE ANALOGUES

With the exception of **24**, **33**, and **34**, we have already described syntheses, properties, and potencies as inhibitors of [^{131}I]IMTO binding of the etomidate analogues **14**–**35**.[8] Comparison of the SARs at both sites (Table 2) allows the following general conclusions: (1) At both sites, (*R*)-isomers are much more potent than (*S*)-isomers (by about 2 orders of magnitude, compare **14** to **15**); (2) the free acid (**35**) is at both sites almost inactive; (3) most etomidate derivatives are highly potent inhibitors at both sites, with no obvious preference for one or the other. Apart from these more general observations, the two sites seem to follow their own SARs (see Figure 3). Interestingly, **33** and **34** without ester group displace [^{3}H]**1** more potently than [^{131}I]IMTO, possibly because of their structural resemblance to metyrapone-type compounds.

INHIBITION OF BINDING BY PYRIDINYL-CONTAINING ETOMIDATE ANALOGUES

While the replacement of one of the pyridine rings by a benzene ring took the structure of metyrapone one step closer to that of etomidate (strengthening the affinities to both binding sites), we now explored these relationships from the other side, by introducing a pyridinyl substituent into the molecular skeleton of etomidate. Remarkably, all pyridinyl analogues of etomidate acted more potently as inhibitors of

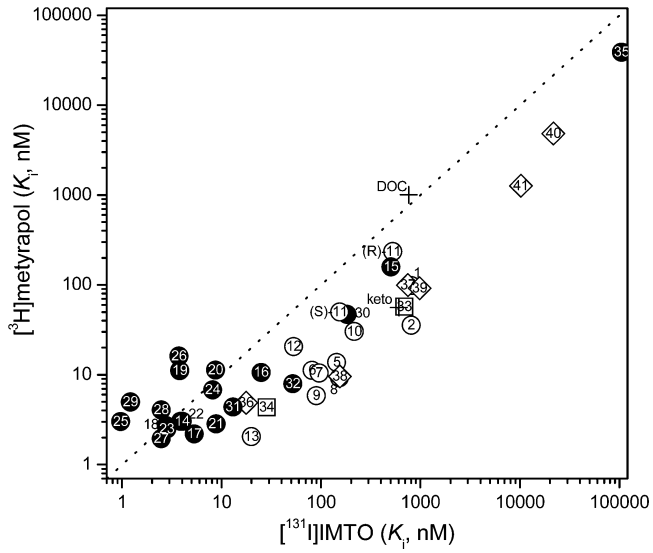

Figure 3. Comparison of inhibitory potencies at binding sites labeled with [^{3}H]metyrapol to those at sites labeled with [^{131}I]IMTO, for various structural classes; filled circles, etomidate analogues; open circles, metyrapone analogues; open diamonds, pyridinyl etomidates; open squares, etomidates without ester group; crosses, DOC and ketoconazole (keto); compound numbers inscribed to the symbols (some of them occluding each other). The dotted line indicates identical potency at both sites. All values are from Tables 1–4.

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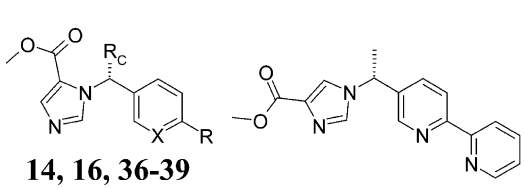
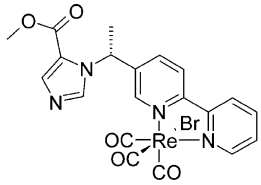
Table 3. Inhibition of [^{3}H]**1** and [^{131}I]IMTO Binding to Rat Adrenal Membranes by Pyridinyl-Metomidate and Structural Analogues (K_i ± SD, or Both Values if n = 2)



14, 16, 36-39

40



41

				[^{3}H]**1**		[^{131}I]IMTO	
	X	R_C	R	K_i (nM)	n	K_i (nM)	n
14	C	Me	H	3.03 ± 1.46	4	4.02 ± 1.87	9
36	N	Me	H	4.96, 4.81	2	17.7 ± 3.2[a]	4
16	C	H	H	10.6 ± 3.9	3	25 ± 9[a]	4
37	N	H	H	90, 109	2	742 ± 205[a]	3
38	N	Me	pyridin-2-yl	9.6 ± 3.0	3	154 ± 56	3
39	N	H	pyridin-2-yl	92 ± 22	3	981 ± 341	10
40	N	Me	pyridin-2-yl	3895, 5754	2	21580 ± 9550	3
41	N	Me	pyridin-2-yl	1268 ± 448	3	10170 ± 12860	2

[a]From ref 8.

[^{3}H]**1** binding than as inhibitors of [^{131}I]IMTO binding (Table 3; although introduction of pyridinyl always led to some loss in potency). Direct comparison was possible for the phenyl/pyridinyl pairs **36**/**14** and **37**/**16** (Table 3), with losses by factors 4.4 and 30 for inhibition of [^{131}I]IMTO binding, but only by factors 1.6 and 9.4, respectively, for the inhibition of [^{3}H]**1** binding.

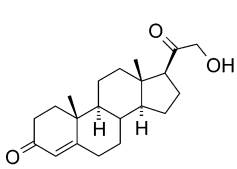
Surprising to us was the small influence of a second pyridinyl substituent attached to the first one on the inhibition of [^{3}H]**1** binding (weaker by factor 2), in contrast to the more pronounced effect of this modification on the inhibition of [^{131}I]IMTO binding (factor 9; Table 3, compare **36** to **38**). Synthesis of the 2,2-bipyridinyl derivative **38** was inspired by the option to form a complex with radioactive technetium.[35] Preliminary autoradiographic results obtained with a conjugate of the demethyl analogue **39** with a ^{99m}Tc(I)−tricarbonyl complex turned out discouraging, with no significant difference between total and nonspecific binding.[36] Nevertheless, we continued by synthesizing **41**, the complex of the 2,2-bipyridyl analogue of metomidate **38** with the Tc analogue rhenium.[31] Although this rather bulky conjugate inhibited [^{3}H]**1** binding eight times more potent than [^{131}I]IMTO binding, the observed K_i (1.27 μM) precluded any use as an in vivo imaging tracer.

In fact, application of a ^{99m}Tc(I) conjugate with **38** to mice did not result in any selective accumulation of radioactivity in the adrenals.[31] As an unintended byproduct of the synthesis of **38** we obtained its isomer **40** (with the carbonyl substituent at position 4 instead of 5). Although this modification might appear unimportant on first glance, its consequences (a reduction in potency at both radioligand binding sites by more than 2 orders of magnitude) were dramatic. For a better understanding of this effect, it would be interesting to study the 4-carbonyl analogues of other etomidate derivatives.

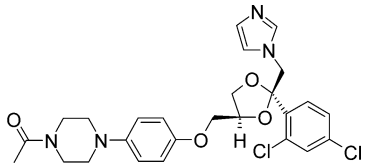
■ SPECIFIC STRUCTURAL LEADS?

Structurally unrelated substances known to interact with 11β-hydroxylase inhibited the binding to both sites. [^{131}I]IMTO and [^{3}H]**1** binding were inhibited by similar concentrations of DOC, the main substrate of the enzyme. The fungicide ketoconazole, however, was 11 times more potent at the site labeled with [^{3}H]**1** (Table 4), a preference probably related to the presence of a two-carbon bridge connecting two aromatic

Table 4. Inhibition of [^{3}H]**1** and [^{131}I]IMTO Binding to Rat Adrenal Membranes by Deoxycorticosterone (DOC) and Ketoconazole (K_i ± SD)



DOC



ketoconazole

	[^{3}H]**1**		[^{131}I]IMTO	
	K_i (nM)	n	K_i (nM)	n
DOC	1009 ± 261	3	759 ± 281[a]	5
ketoconazole	56 ± 18	3	606 ± 418[a]	7

[a]From ref 8.

nuclei, although the number of carbon atoms connecting two aromatic rings was not a general predictor of site preference.

Although all two-carbon-bridged aromatic compounds inhibited [^{3}H]**1** binding better than [^{131}I]IMTO binding (by factors 2.2−23, Table 1, open circles in Figure 3), the converse was not true. Several one-carbon-bridged compounds also exhibited preference for [^{3}H]**1**-labeled sites, especially **33** and **34** (open squares in Figure 3), and all etomidate derivatives with a pyridinyl ring (open diamonds in Figure 3), notably the 2,2-bipyridine derivative **38**. In these latter compounds, particular structural features of etomidate had been modified into the direction of closer resemblance with metyrapone-type compounds (either absence of a carbonyl group-containing substituent or replacement of a benzene with a pyridine ring). The most potent inhibitor of [^{3}H]**1** binding without a carbonyl group as substituent was the imidazole derivative **13** (K_i against [^{3}H]**1** binding 2 nM). It may be of interest to develop this new compound as a radioactive probe to study more efficiently metyrapone-type binding sites on adrenal membranes. The fluoro derivative **9** may be another new option to label with high affinity (K_i 5.8 nM) these sites, in addition to the 4-

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iodophenyl derivative **34** (K_i 4.3 nM). At least **9**, **13**, and **34** appear more appropriate for this purpose than radiolabeled **1**. In vivo studies may be warranted to further investigate these compounds as new tracers.

RELATION TO ENZYME ACTIVITY

Since metyrapone and etomidate block 11β-hydroxylase by directly binding to the active catalytic center, including interaction of an aromatic nitrogen atom with the sixth ligating position of the prosthetic group heme iron atom,[37] the affinities of these and structurally related compounds to adrenal membranes might correlate with their potencies as inhibitors of 11β-hydroxylase enzymatic activity. We have recently obtained such a correlation for several etomidate derivatives, measuring their effects on the secretion of cortisol by NCI-h295 cells and on the binding of [^{131}I]IMTO to rat adrenal membranes.[8] In the upper panel of Figure 4 we present once

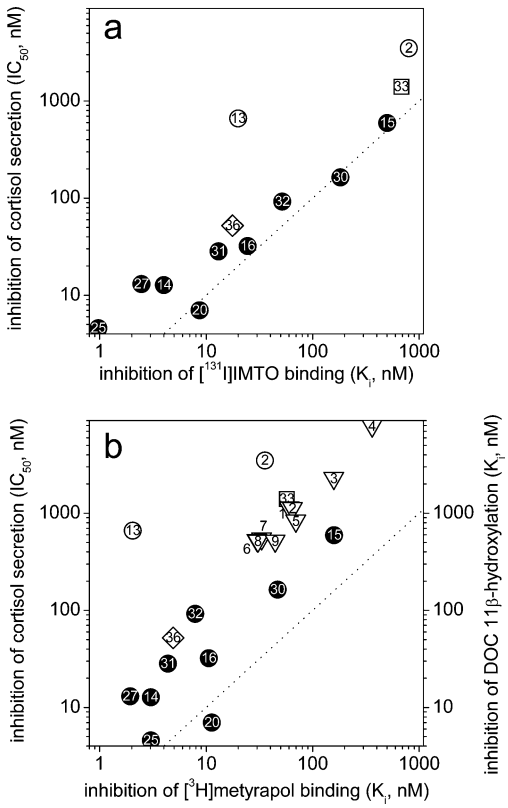

Figure 4. Comparison of inhibitory potencies against steroid 11β-hydroxylation (ordinate) with those against binding of [^{131}I]IMTO (a) and against binding of [^{3}H]**1** (b), respectively, for test compounds structurally related to etomidate (filled symbols) or to metyrapone (open symbols). The dotted line indicates identical potencies at both targets. Data on cortisol secretion by NCI-h295 cells in a and b (left ordinate) taken from ref 8; data on production of [^{14}C]corticosterone from [^{14}C]DOC by beef adrenocortical mitochondria in b (open triangles, right ordinate) taken from ref 33.

more these data, complemented with data on three further compounds: **2** (IC_{50} = 3.5 μM),[38] **13** (IC_{50} = 663 ± 139 nM; n = 3), and **32** (IC_{50} = 1220, 1590 nM; n = 2). While **2** and **32** fitted well with the other compounds, the imidazole derivative **13** had a much weaker effect on cortisol secretion than on [^{131}I]IMTO binding. Nevertheless, correlation—even including **13**—was high (R = 0.93, P < 0.001).

Much weaker correlation is seen comparing inhibition of cortisol secretion to inhibition of [^{3}H]**1** binding (Figure 4B without triangles; R = 0.65, P < 0.05). Most of the tested compounds had no recognizable effect on enzyme activity at concentrations that displaced 50% of bound [^{3}H]**1**. The triangles in Figure 4B reproduce data obtained with beef adrenocortical mitochondria[33] demonstrating, for a series of metyrapone-type compounds, that their potencies as enzyme inhibitors were much weaker (by a mean factor 16) than their potencies as inhibitors of [^{3}H]**1** binding. Binding to the "metyrapone site" may not be enough to achieve enzyme inhibition. On the other hand, binding to the "etomidate site" appears sufficient to inhibit enzyme activity (although this inhibition may be of only minor clinical relevance, since it is of short duration).

CONCLUSIONS

Our results may indicate that etomidate- and metyrapone-type compounds share overlapping binding sites at the catalytic center of 11β-hydroxylase. On the basis of our observations, we propose a hypothetical active site model illustrated schematically in Figure 5. In this model, the interaction site involved in

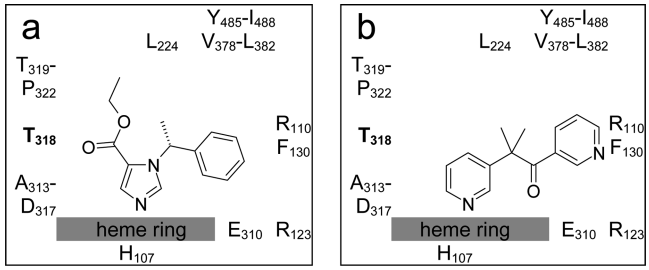

Figure 5. Tentative arrangement of etomidate (a) and metyrapone (b) in a schematic binding pocket close to the reactive center of CYP11B1 (amino acids refer to human sequence). Note that both occupy the 6th ligating position of the heme iron atom (in gray), but only etomidate reaches directly to the catalytic T_{318} (bold). Adapted from Belkina et al. (2001)[39] and Roumen et al. (2007).[37]

the binding of [^{3}H]**1** is also involved in the binding of [^{131}I]IMTO, but vice versa only a part of the site involved in the binding of [^{131}I]IMTO contributes to [^{3}H]**1** binding.

This model was chosen in analogy to the three-dimensional model proposed by Roumen et al. (2007),[37] based on docking and dynamics simulations applied to CYP11B1 and on the known crystal structure of bacterial cytochromes. In this model, metyrapone and etomidate are proposed to provide one aromatic ring for π-stacking with arg110 and phe130. A nitrogen atom of the second aromatic ring ligates the catalytic heme iron. Only etomidate forms an additional hydrogen bond with thr318, investing its carbonyl group (see Figure 7 of the reference; the model is incomplete inasmuch as the high degree of stereospecificity of etomidate remains unexplained). Radiotracers actually in use for adrenocortical tumor imaging are exclusively derived from etomidate,[4,5] including the catalytic site in their binding mode. Here we presented with **9**, **13**, and **34** three potential tracers with similar affinities, but slightly differing binding modes. The clinical value of this new type of tracer should be the subject of further studies. We would predict that the labeling achieved with the two types of tracers should be similar, but with less enzyme inhibition by metyrapone-type than with etomidate-type tracers.

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EXPERIMENTAL SECTION

General Procedures. ^{1}H NMR spectra were recorded on a Bruker DRX-400 spectrometer at 400.13 MHz, in $CDCl_3$ using the residual solvent peak as internal reference [$\delta(CHCl_3)$ = 7.24]. ^{13}C NMR spectra (mainly *J* modulated) were recorded on the same spectrometer operating at 100.61 MHz [$\delta(CDCl_3)$ = 77.00]. Chemical shifts δ are given in parts per million, coupling constants *J* in Hz. IR spectra were recorded as films on a silicon disk on a Perkin-Elmer 1600 FT-IR or a Bruker VERTEX 70 IR spectrometer. Optical rotations were measured at 20 °C on a Perkin-Elmer 341 polarimeter in a 1 dm cell. Melting points were measured on a Reichert Thermovar instrument and are uncorrected. Analytical HPLC was performed on a Jasco system (PU-980 pump, UV 975, and RI 930) using as chiral stationary phase a Chiracel OD-H column, 0.46 × 25 cm, 1 mL/min, UV (254 nm). TLC was performed on 0.25 mm Merck plates, silica gel 60 F_{254}. Flash (column) chromatography was performed with Merck silica gel 60 (230−400 mesh). Spots were visualized by UV, by exposing to iodine vapor, or by dipping the plate into a solution of 23 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 1 g of $Ce(SO_4)_2\cdot 4H_2O$ in 500 mL of 10% H_2SO_4 in water, followed by heating with a heat gun. THF was distilled from potassium, and Et_2O from $LiAlH_4$ prior to use.

Syntheses. *1-(Pyridin-3-yl)cyclopropyl 2-Fluorophenyl Ketone (**10**).* Potassium *t*-butoxide (0.317 g, 2.83 mmol, 1.2 equiv) was added to a stirred solution of ketone **9a** (0.508 g, 2.36 mmol; see Supporting Information) in dry DMF (10 mL) at 0 °C under argon, followed by 1,2-$C_2H_4Br_2$ (0.666 g, 0.31 mL, 3.54 mmol, 1.5 equiv) after 25 min. The mixture was stirred for 2.5 h at 0 °C. Then another portion of the butoxide (0.292 g, 2.60 mmol, 1.1 equiv) was added at rt, and stirring was continued for 18 h. Solvents were removed on a rotary evaporator. The residue was dissolved in EtOAc (10 mL) and extracted with water (10 mL). The aqueous phase was separated and extracted with EtOAc (2 × 10 mL). The combined organic layers were dried (Na_2SO_4) and solvents removed on a rotary evaporator. The residue was purified by flash chromatography (hexanes/EtOAc, 2/1, R_f 0.33) and bulb-to-bulb distillation (110 °C/0.3 mbar) to yield cylopropyl compound **10** (0.180 g, 32%) as a colorless oil.

^{1}H NMR: δ 8.47−8.43 (m, 1H, H_{pyr}), 8.34 (dd, *J* = 4.8, 1.5 Hz, 1H, H_{pyr}), 7.53−7.48 (m, 1H, H_{ar}), 7.35−7.29 (m, 1H, H_{pyr}), 7.28−7.20 (m, 1H, H_{ar}), 7.08 (dd, *J* = 7.7, 4.8, 1H, H_{pyr}), 7.03 (td, *J* = 7.3, 0.8, 1H, H_{ar}), 6.85−6.78 (m, 1H, H_{ar}), 1.88−1.77 (m, 2H, C_{cyclo}), 1.43−1.32 (m, 2H, H_{cyclo}). ^{13}C NMR: δ 200.55 (d, J_{FC} = 1.6, C═O), 158.45 (d, J_{FC} = 250.8, CF), 151.14 (d, *J* = 1.1, HC_{pyr}), 148.11 (HC_{pyr}), 137.03 (d, J_{FC} = 1.2, HC_{pyr}), 135.37 (C_{ar}), 132.57 (d, J_{FC} = 8.3, HC_{ar}), 129.07 (d, J_{FC} = 3.5, HC_{ar}), 127.72 (d, J_{FC} = 16.2, C_{ar}), 124.18 (d, J_{FC} = 3.2, HC_{ar}), 122.86 (HC_{pyr}), 115.76 (d, J_{FC} = 21.8, HC_{ar}), 35.69 (C_{cyclo}), 18.12 (2C, H_2C_{cyclo}). IR (Si): ν_{max} 1681, 1611, 1483, 1452, 1416, 1299, 1224, 1195, 989 cm^{-1}. Anal. Calcd for $C_{15}H_{12}FNO$ (241.26): C, 74.67; H, 5.01; N, 5.81. Found: C, 74.39; H, 4.84; N, 5.76.

*(±)-1-(2-Fluorophenyl)-2-methyl-2-(pyridin-3-yl)-1-propanol (**11**).* $NaBH_4$ (0.010 g, 0.26 mmol, 1 equiv) was added to a stirred solution of ketone **9** (0.064 g, 0.26 mmol) in ethanol (5 mL) at rt. After 30 min a drop of glacial acetic acid was added and solvents were removed on a rotary evaporator. The residue was dissolved in DCM (5 mL) and extracted with water (5 mL). The aqueous phase was separated and extracted with DCM (2 × 5 mL). The combined organic layers were washed with brine and dried (Na_2SO_4), and solvents were removed on a rotary evaporator. The residue was purified by flash chromatography (DCM/EtOAc, 3/1, R_f = 0.27) to yield alcohol (±)-**11** (0.051 g, 79%) as colorless crystals; mp 130−131 °C (DCM/hexanes).

^{1}H NMR: δ 8.51 (d, *J* = 2.1, 1H, H_{pyr}), 8.34 (dd, *J* = 4.8, 1.5, 1H, H_{pyr}), 7.64 (ddd, *J* = 7.8, 2.0, 1.5, 1H, H_{pyr}), 7.23−7.16 (m, 1H, H_{ar}), 7.16 (dd, *J* = 7.8, 4.8, 1H, H_{pyr}), 7.07−6.98 (m, 2H, H_{ar}), 6.93 (ddd, *J* = 10.5, 8.3, 1.0, 1H, H_{ar}), 5.12 (s, 1H, CHO), 2.79 (br. s, 1H, OH), 1.36 (d, *J* = 1.3, 3H, CH_3), 1.33 (s, 3H, CH_3). ^{13}C NMR: δ 159.93 (d, J_{FC} = 245.4, CF), 148.86 (HC_{pyr}), 147.22 (HC_{pyr}), 141.18 (C_{pyr}), 135.05 (HC_{pyr}), 129.22 (d, J_{FC} = 3.9, C_{ar}), 129.02 (d, J_{FC} = 8.5 Hz, C_{ar}), 128.27 (d, J_{FC} = 2.9, C_{ar}), 123.57 (d, J_{FC} = 3.4, C_{ar}), 122.61 (C_{pyr}), 114.82 (d, J_{FC} = 23.0, C_{ar}), 73.87 (CHO), 42.49 ($C(CH_3)_2$), 25.44 (d, J_{FC} = 1.2, CH_3), 22.25 (CH_3). IR (Si): ν_{max} 3.315, 2974, 1484, 1453, 1417, 1222, 1102, 1057, 913 cm^{-1}. Anal. Calcd for $C_{15}H_{16}FNO$ (245.29): C, 73.45; H, 6.57; N, 5.71. Found: C, 73.22; H, 6.72; N, 5.73.

*(±)-O-1-(2-Fluorophenyl)-2-methyl-2-(pyridin-3-yl)propyl S-Methyl Dithiocarbamate (**11a**).* *n*-BuLi (0.30 mL, 0.48 mmol, 1.5 equiv) was added dropwise to a stirred solution of alcohol (±)-**11** (0.080 g, 0.32 mmol) at −78 °C under argon, followed by CS_2 (0.251 g, 0.20 mL, 3.3 mmol, 10 equiv) at −45 °C after 20 min and MeI (0.468 g, 0.31 mL, 3.3 mmol, 10 equiv) at −30 °C 30 min later. The reaction mixture was stirred for 40 min at −30 °C, and then solvents were removed on a rotary evaporator. The residue was diluted with EtOAc (15 mL) and washed with water (15 mL). The organic phase was separated and the aqueous one extracted with EtOAc (2 × 15 mL). The combined organic layers were dried (Na_2SO_4), and solvents were removed on a rotary evaporator. The residue was purified by flash chromatography (hexanes/EtOAc, 1/1, R_f 0.56) to yield xanthogenate (±)-**11a** (0.103 g, 94%) as a colorless oil.

^{1}H NMR: δ 8.56 (m, 1H, H_{pyr}), 8.46 (dd, *J* = 4.6, 1.5, 1H, H_{pyr}), 7.62 (ddd, *J* = 8.1, 2.3, 1.5, 1H, H_{pyr}), 7.24−7.17 (m, 2H, H_{ar}, H_{pyr}), 6.98−6.91 (m, 2H, H_{ar}), 6.82 (s, 1H, CHO), 6.74 (td, *J* = 7.6, 1.5, 1H, H_{ar}), 2.45 (s, 3H, SCH_3), 1.46 (s, 6H, CH_3). ^{13}C NMR: δ 214.23 (C═S), 160.22 (d, J_{FC} = 247.7, CF), 148.84 (HC_{pyr}), 147.82 (HC_{pyr}), 139.46 (HC_{pyr}), 134.82 (HC_{pyr}), 129.81 (d, J_{FC} = 8.5, HC_{ar}), 128.76 (d, J_{FC} = 3.4, HC_{ar}), 123.63 (d, J_{FC} = 12.9, C_{ar}), 123.62 (d, J_{FC} = 3.6, HC_{ar}), 122.63 (HC_{pyr}), 115.03 (d, J_{FC} = 22.6, HC_{ar}), 83.56 (CHO), 42.00 (C_q), 25.27 (CH_3), 23.32 (CH_3), 19.00 (SCH_3). IR (Si): ν_{max} 2977, 1490, 1234, 1203, 1062 cm^{-1}. Anal. Calcd for $C_{17}H_{18}FNOS_2$ (335.46): C, 60.87; H, 5.41; N, 4.18. Found: C, 60.57; H, 5.15; N, 4.16.

*1-(2-Fluorophenyl)-2-methyl-2-(pyridin-3-yl)propane (**12**).* A solution of AIBN (0.011 g) and $(TMS)_3SiH$ (0.207 g, 0.26 mL, 0.83 mmol, 3 equiv) in dry toluene (1 mL) was added to a stirred solution of xanthogenate (±)-**11a** (0.093 g, 0.28 mmol) in dry toluene (5 mL) at 90 °C under argon. After 2 h volatile components were removed under reduced pressure (up to 80 °C, 1 mbar; bulb to bulb distillation apparatus). The residue was purified by flash chromatography (hexanes/EtOAc, 2/1, R_f = 0.29) to yield **12** (0.035 g, 55%) as a colorless oil.

^{1}H NMR: δ 8.57 (br, s, 1H, H_{pyr}), 8.43 (br. d, *J* = 4.8, 1H, H_{pyr}), 7.56 (td, *J* = 8.1, 2.0, 1H, H_{pyr}), 7.18 (dd, *J* = 8.1, 4.8, 1H, H_{pyr}), 7.15−7.07 (m, 1H, H_{ar}), 6.96−6.83 (m, 2H, H_{ar}), 6.67 (td, *J* = 7.6, 1.8, 1H, H_{ar}), 2.90 (d, *J* = 0.9, 2H, CH_2), 1.36 (d, *J* = 0.8, 6H, CH_3). ^{13}C NMR: δ 161.40 (d, J_{FC} = 244.5, CF),

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147.97 (HC_{pyr}), 147.03 (HC_{pyr}), 143.76 (C_{pyr}), 133.77 (HC_{pyr}), 132.49 (d, J_{FC} = 4.0, HC_{ar}), 128.08 (d, J_{FC} = 8.0, HC_{ar}), 124.95 (d, J_{FC} = 15.1, C_{ar}), 123.26 (d, J_{FC} = 3.0, HC_{ar}), 122.77 (HC_{pyr}), 115.12 (d, J_{FC} = 23.1, HC_{ar}), 42.90 (CH_2), 38.06 (C_q), 27.51 (2C). IR (Si): ν_{max} 2973, 2938, 1656, 1615, 1492, 1454, 1384, 1231, 986 cm^{-1}. HRMS (ESI): Calcd for $C_{15}H_{16}FNH$ (230.1345). Found: 230.1339 ± 5 ppm.

Resolution of (±)-11. *Reaction of Noe-lactol Dimer [(+)-MBF-O-MBF] with (±)-**11**.* A mixture of alcohol (±)-**11** (0.368 g, 1.5 mmol) and molecular sieves (1.5 g, 4 Å) in dry DCM (15 mL) was stirred for 15 min at rt before Noe's reagent [0.280 g, 075 mmol, 0.5 equiv, (+)-MBF-O-MBF] and (+)-camphor-10-sulfonic acid (0.492 g, 2.1 mmol, 1.4 equiv) were added at 0 °C. After 2 h the mixture was filtered and the eluate washed with a saturated aqueous solution of $NaHCO_3$ and dried (Na_2SO_4), and solvents were removed on a rotary evaporator. The residue was purified by flash chromatography (DCM/EtOAc, 7/1) to furnish diastereomer **A** (0.205 g, 32%, R_f = 0.40) as a colorless viscous oil, and diastereomer **B** (0.205 g, 32%, R_f = 0.33) as colorless crystals, mp 78−80 °C.

*Diastereomer **A**.* ^1H NMR: δ 8.65 (dd, J = 2.3, 0.5, 1H, H_{pyr}), 8.42 (dd, J = 4.8, 1.5, 1H, H_{pyr}), 7.68 (ddd, J = 8.1, 2.3, 1.5, 1H, H_{pyr}), 7.24−7.16 (m, 1H, H_{ar}), 7.19 (ddd, J = 8.1, 4.8, 0.5, 1H, H_{pyr}), 7.10−7.01 (m, 2H, H_{ar}), 6.98 (ddd, J = 11.1, 8.3, 1.0, 1H, H_{ar}), 5.06 (s, 1H, CHPh), 4.86 (dd, J = 3.3, 2.0, 1H, OCHO), 3.08 (dd, J = 9.6, 1.5, 1H, CHO), 2.71−2.61 (m, 1H, $CH(CH_2)_2$), 1.65−1.60 (m, 2H, $CH_2(CH)_2$), 1.53−1.35 (m, 3H), 1.30 (s, 3H, CH_3), 1.27 (d, J = 2.0, 3H, CH_3), 1.24−1.16 (m, 1H, CCH_2CH_2), 1.05−0.96 (m, 1H), 0.82 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.70 (s, 3H, CH_3). ^{13}C NMR: δ 161.52 (d, J_{FC} = 246.2, CF), 149.29 (HC_{pyr}), 147.04 (HC_{pyr}), 142.54 (HC_{pyr}), 134.57 (HC_{pyr}), 129.79 (d, J_{FC} = 4.1, HC_{ar}), 128.99 (d, J_{FC} = 8.4, HC_{ar}), 125.94 (d, J_{FC} = 13.2, C_{ar}), 123.47 (d, J_{FC} = 3.3, HC_{ar}), 122.45 (HC_{pyr}), 115.01 (d, J_{FC} = 23.0, HC_{ar}), 104.88 (OCHO), 88.80 (CHO), 75.48 (PhCHO), 52.67 (C_q), 48.11 (C_q), 47.08 (CH), 41.72 (C_q), 39.79 (CH), 32.08 (CH_2), 26.09 (CH_2), 25.68 (d, J_{FC} = 2.1, $PhC(CH_3)_2$), 21.37 ($PhC(CH_3)_2$), 20.91 (CH_3), 20.46 (CH_2), 18.70 (CH_3), 14.64 (CH_3). IR (Si): ν_{max} 2952, 1486, 1416, 1224, 1093, 1077, 1034, 1008, 990 cm^{-1}. $[\alpha]_D^{20}$: +143.27 (*c* 1.07, acetone).

*Diastereomer **B**.* ^1H NMR: δ 8.60 (d, J = 2.2, 1H, H_{pyr}), 8.43 (dd, J = 4.8, 1.7, 1H, H_{pyr}), 7.60 (ddd, J = 8.1, 2.3, 1.7, 1H, H_{pyr}), 7.17 (dd, J = 8.1, 4.8, 1H, H_{pyr}), 7.17−7.10 (m, 1H, H_{ar}), 6.96−6.88 (m, 3H, H_{ar}), 5.03 (br. d, J = 3.3, 1H, OCHO), 4.81 (s, 1H, PhCHO), 3.29 (dd, J = 9,6, 1.3, 1H, CHO), 2.68−2.57 (m, 1H, $CH(CH_2)_2$), 1.68−1.60 (m, 2H, $CH_2(CH)_2$), 1.50−1.35 (m, 3H), 1.35 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.27−1.18 (m, 1H), 1.02−0.93 (m, 1H), 0.77 (s, 3H, CH_3), 0.73 (s, 3H, CH_3), 0.45 (s, 3H, CH_3). ^{13}C NMR: δ 159.87 (d, J_{FC} = 244.5, CF), 149.23 (C_{pyr}), 147.11 (HC_{pyr}), 141.41 (C_{pyr}), 134.95 (HC_{pyr}), 129.68 (d, J_{FC} = 4.1, HC_{ar}), 128.37 (d, J_{FC} = 8.4, HC_{ar}), 128.12 (d, J_{FC} = 13.7, C_{ar}), 122.91 (d, J_{FC} = 3.2, HC_{ar}), 122.35 (HC_{ar}), 114.46 (d, J_{FC} = 23.0, HC_{ar}), 109.70 (OCHO), 89.36 (CHO), 78.15 (PhCHO), 52.49 (C_q), 47.86 (C_q), 47.13 (CH), 42.07 (C_q), 39.76 (CH), 32.20 (CH_2), 26.02 (CH_2), 25.34 ($ArC(CH_3)_2$), 22.55 ($ArC(CH_3)_2$), 20.79 (CH_3), 20.44 (CH_2), 18.45 (CH_3), 14.18 (CH_3). IR (Si): ν_{max} 2927, 1485, 1413, 1388, 1222, 1186, 1099, 1077, 1030, 1007, 990, 954 cm^{-1}. $[\alpha]_D^{20}$ +52.55 (*c* 1.02, acetone). Anal. Calcd for $C_{27}H_{34}FNO_2$ (423.56): C, 76.56; H, 8.11; N, 3.31. Found: C, 76.52; H, 7.90; N, 3.28.

*Deprotection of Diastereomers A and B To Get Enantiomerically Pure (+)-**11** and (−)-**11**, Respectively.* (+)-Camphor-10-sulfonic acid (0.322 g, 1.388 mmol, 3 equiv) was added to a stirring solution of diastereomer **A** in dry methanol (5 mL) at rt. After stirring for 2 h the reaction mixture was neutralized with a saturated aqueous solution of $NaHCO_3$, filtered, and concentrated under reduce pressure. Water was added and the mixture extracted with EtOAc (3 × 15 mL). The combined organic layers were dried (Na_2SO_4) and solvents removed on a rotary evaporator. The residue was purified by flash chromatography (DCM/EtOAc, 2/1, R_f = 0.41) to yield (*R*)-(+)-**11** (0.113 g, 98%) as colorless crystals; mp 78−79 °C (DCM/hexanes); $[\alpha]_D^{20}$ +30.18 (*c* 0.56, acetone). Similarly, diastereomer **B** (0.119 g, 0.281 mmol) was converted to (*S*)-(−)-**11** (0.064 g, 92%) as colorless crystals; mp 79−81 °C; $[\alpha]_D^{20}$ −34.80 (*c* 0.64, acetone). The ^1H and ^{13}C NMR spectra of (*R*)-(+)- and (*S*)-(−)-**11** were identical to those of (±)-**11**.

*Preparation of (R)-Mosher Ester of (±)- and (R)-(+)-**11**.* A mixture of alcohol (±)-**11** (0.020 g, 0.082 mmol) in dry DCM (0.5 mL), dry pyridine (1 mL), and Mosher's acid chloride (*S*)-(+)-MTPACl (0.3 mL, 0.159 mmol, 1.9 equiv, 0.53 M in dry 1,4-dioxane) was left at rt for 18 h. Water (0.5 mL) and HCl (0.5 mL, 2 M) were added. After 15 min solvents were removed on a rotary evaporator. The residue was mixed with water (5 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and solvents removed on a rotary evaporator. The residue was purified by flash chromatography (DCM/EtOAc, 7/1, R_f = 0.62) to yield a mixture of diastereomeric (*R*)-Mosher esters (*S*)-(−)- and (*R*)-(+)-**11**·MTPA-(*R*) as a colorless oil. Similarly, alcohol (*R*)-(+)-**11** (0.011 g, 0.045 mmol) was converted to (*R*)-(+)-**11**·MTPA-(*R*) as a colorless oil; ee ≥99%.

^1H NMR: diagnostic signals for (*R*)-(+)-**11**·MTPA-(*R*), δ 8.44 (dd, J = 4.8, 2.3, 1H, H_{pyr}), 8.38 (d, J = 2.3, 1H, H_{pyr}), 6.64 (td, J = 7.8, 1.5, 1H, H_{ar}), 6.39 (s, 1H, PhCHO), 3.28 (q, J = 1.0, 3H, OCH_3), 1.37 and 1.30 (two s, each 3H, CH_3); for (*S*)-(−)-**11**·MTPA-(*R*), 8.54 (d, J = 2.3, 1H, H_{pyr}), 8.48 (dd, J = 4.8, 2.3, 1H, H_{pyr}), 6.57 (td, J = 7.8, 1.5, 1H, H_{ar}), 6.32 (s, 1H, PhCHO), 3.24 (q, J = 1.0, 3H, OCH_3), 1.40 and 1.34 (two s, each 3H, CH_3).

*(R)-1-(2-Fluorophenyl)-2-methyl-2-(pyridin-3-yl)propyl N-[(S)-1-Phenylethyl]-N-[(S)-1-phenylethyl-carbamoyl]-carbamate (**11b**).* *n*-BuLi (0.060 mL, 0.096 mmol, 0.34 equiv, 1.6 M in hexanes) was added to a stirred solution of (+)-**11** (0.069 g, 0.280 mmol) in dry THF (2 mL) under argon at −78 °C. After stirring for 10 min freshly distilled (*S*)-(−)-1-phenylethyl isocyanate (0.080 mL, 0.56 mmol, 2 equiv) was added slowly, and stirring was continued for 1 h at −78 °C. Water (5 mL) was added at rt, and the mixture was stirred for 10 min before it was concentrated at reduced pressure. Water and EtOAc (5 mL each) were added. The aqueous layer was removed and extracted with EtOAc (2 × 5 mL). The three combined organic layers were dried (Na_2SO_4) and solvents removed on a rotary evaporator. The residue was flash-chromatographed (hexanes/EtOAc, 1/1, R_f = 0.51) to give carbamate **11b** (0.072 g, 48%) as colorless crystals. Crystals suitable for X-ray structure analysis were obtained by slow evaporation of solvent from a solution in hexanes/DCM at +4 °C; mp 140−143 °C; $[\alpha]_D^{20}$ −50.93 (*c* 0.49, acetone).

Further Syntheses. *(R)-(+)-Methyl 1-[1-(4-Fluorophenyl)-ethyl-1H-imidazole-5-carboxylate (**24**).* Enantioselective reduction of 4-fluorophenyl methyl ketone with (*R*)-(+)-2-methyl-oxazaborolidine/BH_3·Me_2S by a literature procedure[40] gave 60% (*S*)-(−)-1-(4-fluorophenyl)ethanol, $[\alpha]_D^{20}$ −39.86 (*c*

2.23, acetone), ee 96% by (*R*)-Mosher ester. The ee of the alcohol was increased to >99% by enantioselective esterification[41] with lipase *Candida antarctica* on acrylic resin (Sigma): (*S*)-Alcohol of 96% ee (5 mmol), vinyl acetate (20 mmol), *t*BuOMe (15 mL) and lipase (50 mg) were shaken for 18 h at rt. The enzyme was removed and the filtrate was concentrated on a rotary evaporator. The residue was purified by flash chromatography (hexanes/EtOAc, 4/1) to yield 90% (*S*)-alcohol of ee >99%, $[\alpha]_D^{20}$ −41.19 (*c* 2.18, acetone). The (*R*)-Mosher esters[27] of (±)- and (*S*)-alcohol were prepared and investigated by ^{1}H NMR spectroscopy; significant signals of (*R*)-Mosher ester of (*R*)-alcohol [δ 3.44 (q, *J* = 1.2, 3H, OCH_3), 1.55 (d, *J* = 6.5, 3H, CH_3)] and of (*S*)-alcohol [3.54 (q, *J* = 1.2, 3H, OCH_3), 1.60 (d, *J* = 6.5, 3H, CH_3)]. (*S*)-Alcohol (0.167 g, 1.19 mmol, ee >99%) was transformed into (*R*)-**24** (0.165 g, 56%) as a colorless oil by a literature procedure[29] (−30 °C to rt in 7 h) except that flash chromatography was performed with hexanes/Et_2O/iPr_2NH, 6/6/1, R_f = 0.31; ee ≥98% [by analytical HPLC on Chiracel OD-H, 1 mL/min, *i*PrOH/hexanes, 7.5/92.5; (*R*)-**24**: t_R 14.2 min, (*S*)-**24**: t_R 12.0 min]; $[\alpha]_D^{20}$ +71.92 (*c* 1.3, acetone). Similarly, (±)-**24** was prepared in 60% yield from racemic alcohol.

^{1}H NMR: δ 7.75 (s, 1H, H_{im}), 7.71 (s, 1H, H_{im}), 7.18−7.11 (m, 2H, H_{ar}), 7.03−6.96 (m, 2H, H_{ar}), 6.31 (q, *J* = 7.1, 1H, CHAr), 3.78 (s, 3H, OCH_3), 1.83 (d, *J* = 7.1, 3H, CH_3). ^{13}C NMR: δ 162.27 (d, *J* = 247.0, CF), 160.63 (CO), 139.58 (CH_{im}), 138.27 (CH_{im}), 136.93 (d, *J* = 2.1, C_{ar}), 127.99 (d, *J* = 8.4, $2CH_{ar}$), 122.26 (C_{im}), 115.75 (d, *J* = 21.4, $2CH_{ar}$), 54.72 (ArCH), 51.47 (OCH_3), 22.27 (CH_3). IR (Si): ν_{max} 2951, 1717, 1512, 1363, 1221, 1133 cm^{-1}. Anal. Calcd for $C_{13}H_{13}FN_2O_2$ (248.25): C, 62.90; H, 5.28; N, 11.28. Found: C, 63.06; H, 5.27; N, 10.99.

*(R)-(−)-[1-Phenylethyl]-1H-imidazole (**33**).* In a sealed tube under argon, a solution of **25** (0.040 g, 0.16 mmol) in 3 mL of dry ethanol with 1.5 M methylamine was heated for 18 h at 180 °C (oven temperature). After cooling, the solvents were removed on a rotary evaporator. The residue was flash chromatographed (EtOAc, *N*-substituted imidazole, R_f = 0.26; *N*-methyl amide, R_f = 0.13) to give as a side product the *N*-methyl amide **30** (7 mg, 19%) as a gum, and the desired *N*-substituted imidazole **33** (18 mg, 65%) as a liquid: $[\alpha]_D^{20}$ = −5.95 (*c* 1.66, acetone); $[\alpha]_D^{20}$ −4.62 (*c* 1.45, $CHCl_3$) lit.[29] $[\alpha]_D^{20}$ +5.20 (*c* 3.84, $CHCl_3$) for (*S*)-(+)-enantiomer. The ^{1}H NMR data were identical with those in the literature.[30]

^{13}C NMR: δ 141.46 (C_{ar}), 136.01 (CH_{het}), 129.29 (CH_{het}), 128.84 ($2\ CH_{ar}$), 128.02 (CH_{ar}), 125.92 ($2\ CH_{ar}$), 117.88 (CH_{het}), 56.51 (NCHPh), 21.95 (CH_3). IR (Si): ν_{max} 2981, 1496, 1455, 1226, 1082, 1018 cm^{-1}.

*(R)-(+)-[1-(4-Iodophenyl)ethyl]-1H-imidazole (**34**).* In a sealed tube under argon, a solution of **20** (0.178 g, 0.5 mmol) in 3 mL of dry ethanol with 1.5 M methylamine was heated for 24 h at 180 °C (oven temperature). After cooling, the solvents were removed on a rotary evaporator. The residue was flash chromatographed (EtOAc/EtOH 10/1, **34**, R_f = 0.30; *N*-methyl amide, R_f = 0.24) to give as a side product the *N*-methyl amide (11 mg, 6%) and **34** (128 mg, 86%) as an oil, which solidified after bulb to bulb distillation (bp 120 °C/0.1 mbar); mp 36−38 °C; $[\alpha]_D^{20}$ +5.91 (*c* 2.3, acetone).

^{1}H NMR (400.13 MHz, $CDCl_3$): δ 7.64 (m, 2H, H_{ar}), 7.55 (t, *J* = 1.1 Hz, 1H, H_{het}), 7.06 (t, *J* = 1.1 Hz, 1H, H_{het}), 6.87 (t, *J* = 1.1 Hz, 1H, H_{het}), 6.85 (m, 2H, H_{ar}), 5.27 (q, *J* = 7.1, 1H, NCH), 1.81 (d, *J* = 7.1, 3H, CH_3). ^{13}C NMR: δ 141.32 (C_{ar}), 137.99 ($2\ CH_{ar}$), 135.96 (CH_{het}), 129.68 (CH_{het}), 127.84 ($2\ CH_{ar}$), 117.76 (CH_{het}), 93.55 (CI), 56.03 (NCHPh), 21.84 (CH_3). IR (Si): ν_{max} 2980, 1488, 1404, 1226, 1079, 1006 cm^{-1}. Anal. Calcd for $C_{11}H_{11}IN_2$ (298.12): C, 44.32; H, 3.72; N, 9.40. Found: C, 44.34; H, 3.85; N, 9.17.

Binding Procedures. Whole membranes were prepared from rat adrenals as described.[8] Similarly, whole membranes were prepared from the adrenal cortex of slaughterhouse bulls. An aliquot of this preparation sufficient to obtain at least 0.3 mg of original tissue per vial was added to robust polypropylene vials (Biovials, Beckman Instruments) containing a solution of 12−18 nM [^{3}H]metyrapol (12 Ci/mmol, Amersham) in 10 mM K_2HPO_4/HEPES (pH 7.2), 150 mM NaCl, with or without various concentrations of test compounds, in a final volume of 0.5 mL. Nonspecific binding was estimated in the presence of 10 μM etomidate. Vials were left standing in icy water (0 °C) for 20 min and centrifuged for 15 min at 40000*g*, the supernatant was discarded, and the pellets were rinsed twice (for a few seconds) with ice cold buffer. Finally, 1.8 mL of toluene-based scintillation cocktail was added, the vials were agitated for 15 min, and radiation was quantified in a beta scintillation counter. Data were evaluated as described.[8]

Cortisol Secretion. Cortisol secretion by NCI-h295 cells was estimated as described.[8]

Inhibition of [^{14}C]Corticosterone Formation. The enzyme 11β-hydroxylase catalyzes the formation of [^{14}C]-corticosterone from [^{14}C]DOC. The influence of several metyrapone derivatives on the V_{max} of this reaction was studied as described.[33,42] In brief, 0.5 μCi of [^{14}C]DOC (NEN, 60 mCi/mmol, final concentration 13.6 μM) was incubated at rt for 1−60 min in 80 mM Tris.HCl (pH 7.4), containing (final concentrations): NADP (2.5 mM), glucose-6-phosphate (25 mM), glucose-6-phosphate dehydrogenase (Sigma; 1 unit), and mitochondria prepared from beef adrenal cortex (0.35 mg protein), in a final volume of 250 μL. For isolation of the reaction product [^{14}C]corticosterone, a 10 μL aliquot was spotted (together with carriers) on a silica gel plate and developed with benzene/Et_2O/MeOH (40/58/2). Bands were visualized under UV (R_f for corticosterone 0.17, for DOC 0.42), and radioactivity of the respective segments was quantified in a liquid scintillation counter. In the absence of inhibitors, V_{max} was 0.136 μmol/min, and the K_M was estimated to 12.4 μM.

Crystallographic Structure Determination. X-ray diffraction measurements for **11b** were performed on a Bruker X8 APEXII CCD diffractometer. A single crystal was positioned at 35 mm from the detector, and 2542 frames were measured, each for 30 s over 1° scan width. The data were processed using SAINT software.[43] The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-H atoms were refined with anisotropic displacement parameters; H atoms were inserted in calculated positions and refined with a riding model. The following programs were used: structure solution, *SHELXS-97*;[44] refinement, *SHELXL-97*;[45] molecular diagrams, *ORTEP-3*;[46] computer, Intel CoreDuo. Crystal data, data collection parameters, and structure refinement details for **11b** are given in Table S1 in the Supporting Information. Crystallographic data have been deposited at the Cambridge Crystallographic Data Center with the number CCDC 848059. A copy of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (deposit@ccdc.com.ac.uk).

■ ASSOCIATED CONTENT

Supporting Information

Syntheses of compounds **6**–**9** and of their precursors and table of crystal data and details of data collection of compound **11b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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