

New Selective Inhibitors of Steroid 11 β -Hydroxylation in the Adrenal Cortex. Synthesis and Structure–Activity Relationship of Potent Etomidate Analogues

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Derivatives of etomidate were evaluated as inhibitors of adrenal steroid 11 β -hydroxylations. Stereoselective coupling by Mitsunobu produced chirally pure analogues to study the effect of configuration, modification of the ester, and substitution in the phenyl ring, with the aim to probe specific sites for introducing a radionuclide. Iodophenyl metomidate (IMTO) labeled with iodine-131 served as radioligand for structure–affinity relationship studies. We have characterized the kinetic parameters of specific ¹³¹I-IMTO binding on rat adrenal membranes and used the displacement of ¹³¹I-IMTO binding to evaluate functionalized MTO analogues. Our results indicated that (1) (*R*)-configuration is essential for high affinity, (2) highest potency resides in the ethyl, 2-propyl, and 2-fluoroethyl esters, and (3) substitution of the phenyl ring is well tolerated. The clinically used inhibitors metyrapone and ketoconazole inhibited ¹³¹I-IMTO binding with low affinity. Incubation of selected analogues with human adrenocortical NCI-h295 cells demonstrated a high correlation with the inhibitory effect on cortisol secretion.

Introduction

Etomidate (*R*)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid ethyl ester (ETO^a) (**1**) and the methyl ester metomidate (**2**) are short-acting anesthetic drugs with an adrenostatic side effect, producing low levels of plasma cortisol after long-term infusion.^{1,2} Investigations of the effects of **1** on the cytochrome P-450 species and on steroid biosynthesis in adrenal cortex mitochondria revealed that **1** interacts selectively with the mitochondrial cytochrome P-450 species affecting mitochondrial 11 β -hydroxylations exclusively.³ Metomidate is listed in the European Pharmacopeia as an inherent impurity of **1**.⁴

Another inhibitor of cytochrome P-450 (P-450c11) is metyrapone (2-methyl-1,2-bis-(3-pyridyl)-1-propanone), which binds specifically to the inner mitochondrial membrane.⁵ Several investigators have shown that the steroid 11 β -hydroxylation system and the cytochrome P-450 are located in the inner mitochondrial membrane of adrenal cortex mitochondria.^{6–8} The fact that deoxycorticosterone (DOC) hydroxylation is sensitive to metyrapone was another strong argument that the high-affinity binding site for metyrapone is located on the P-450 specific for 11 β -hydroxylations.^{9,10} Clinically, specific binding at the site of steroid 11 β -hydroxylation is associated with the inhibition of cortisol secretion. Metyrapone and etomidate are highly selective drugs for the treatment of hypercortisolemia.^{2,11}

Clinical application of adrenocortical enzyme inhibitors has stimulated their investigation as radiotracers. Several derivatives

of metyrapone were evaluated by structure–activity relationship (SAR) studies^{12–15} as precursors for labeling with radionuclides.^{16–22} The reduced derivative ³H-metyrapol has been used as a radiotracer for the evaluation of metyrapone derivatives.^{18,23,24}

High-affinity binding of P-450c11 inhibitors to adrenal membranes permits the application of *in vitro* binding procedures. Recently, we have introduced ¹³¹I-IMTO to characterize high-affinity binding sites on crude membranes prepared from whole rat adrenals.^{25,26} Derivatives of etomidate and of metyrapone were investigated as displacers of specific radioligand binding using ¹³¹I-IMTO and ³H-metyrapol, respectively.²⁴ Analogues of etomidate displaced both radioligands with similar potencies, whereas metyrapol and structurally related compounds inhibited ¹³¹I-IMTO binding with low affinity (IC₅₀ in micromolar range). ETO (**1**) and MTO (**2**) were identified as considerably more potent inhibitors of P-450c11 than metyrapone and metyrapol.²⁴ Selected correlates were also tested as inhibitors of cortisol secretion in human adrenocortical NCI-h295 cells supporting the validity of SAR data.²⁴

¹³¹I-IMTO was used as a radioligand to characterize high-affinity binding sites on crude membranes prepared from whole rat adrenals. A comparison of IC₅₀ values obtained by the displacement of specifically bound ¹³¹I-IMTO by structurally related compounds offered insight into the structural requirements for high-affinity binding *in vivo*.

The superior binding characteristics of ETO (**1**) and MTO (**2**) as adrenocortical enzyme inhibitors have promoted the development of radiolabeled derivatives. ¹¹C-metomidate was introduced as the first carbon-11 labeled radiotracer for PET investigations of the adrenal cortex and its tumors.^{27,28} Subsequent developments include ¹⁸F-FETO²⁹ for PET and ¹²³I-IMTO²⁶ for SPECT investigations.

Here, we describe the synthesis and biochemical evaluation of a series of analogues of ETO (**1**) and MTO (**2**) and present quantitative data on SAR studies. Derivatives reported previously had been obtained as racemic mixtures.³⁰ The adoption of the Mitsunobu reaction,³¹ which is based on coupling a chiral precursor alcohol with commercially available methyl 1*H*-

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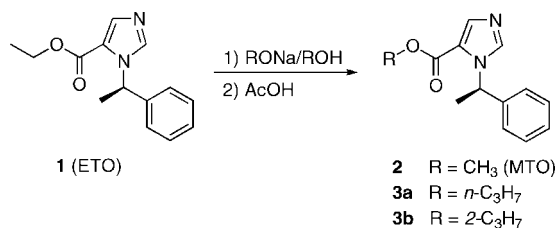
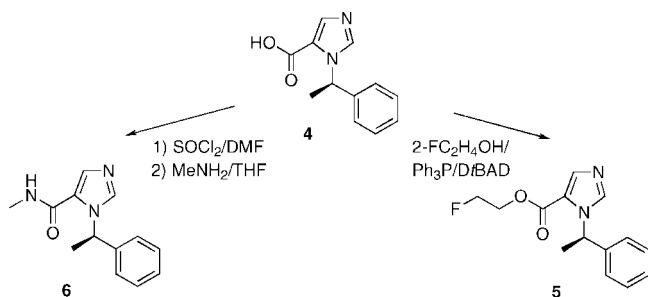
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^a Abbreviations: ETO, etomidate; MTO, metomidate; IMTO, 4-iodophenyl metomidate; FETO, fluoroetomidate; ETO acid, etomidate free acid; DOC, deoxycorticosterone; SPECT, single photon emission computed tomography; PET, positron emission tomography; P-450c11, cytochrome P-450 11 β -hydroxylase; NCI-h295, human adrenocortical cancer cell line.

Scheme 1. Modification of the Ester Function (**2**, **3a**, **3b**)**Scheme 2.** Synthesis of the 2-Fluoroethyl Ester (**5**) and the *N*-Methylamide (**6**)

imidazole-5-carboxylate, offered access to chiral derivatives modified at the active center and substituted in the phenyl ring. Modified esters were generally obtained by transesterification of commercially available etomidate.

Chemistry

Synthesis of MTO Analogues. Ester derivatives included the methyl ester (**2**) and the *n*-propyl and 2-propyl esters. Transesterification of commercially available (*R*)-enantiomer **1** at ambient temperature in dry methanol, *n*-propanol, or 2-propanol in the presence of the corresponding sodium alkoxide yielded **2** and the *n*-propyl³² and 2-propyl esters (**3a** and **3b**), in yields of 71–80% (Scheme 1).

The 2-fluoroethyl ester (**5**) was prepared from ETO acid (**4**) (derived from **1**) and 2-fluoroethanol using triphenylphosphine (Ph₃P) and di-*tert*-butyl azodicarboxylate (*Dt*BAD) in toluene (Scheme 2).^{31,33}

In an attempt to increase the *in vivo* stability of MTO analogues, the *N*-methylamide (**6**) and its *N*-methoxy derivative (**7**) were synthesized. The methyl ketone (**8**) was also considered as a suitable replacement.

Synthesis of **6** started from ETO acid (**4**), which was converted to the acid chloride³⁰ and then reacted with excess methylamine in THF (Scheme 2). Reaction of the acid chloride with *N,O*-dimethylhydroxylamine in pyridine yielded crystalline Weinreb amide (**7**), formed in 57% yield, which was transformed to the methyl ketone (**8**) by adding CH₃Li at low temperature.³⁴ The reaction sequence is shown in Scheme 3.

Modifications Affecting Chirality and the Substitution of the Phenyl Ring. Derivatives with different chirality (**10a–f**) and also with various substituents in the phenyl ring (**10g–k**) were produced by enantioselective synthesis (Scheme 4). The Mitsunobu reaction is based on coupling a chiral benzylic alcohol (**9a–k**) with methyl 1*H*-imidazole-5-carboxylate.^{26,31,33,35,36} Two of these (**9a** and **9d**) are achiral. The chiral precursor alcohols were obtained separately with high enantiomeric excesses (95–99%) by lipase-catalyzed enantioselective hydrolysis of their racemic esters^{37,38} and by preparative HPLC on a chiral stationary phase in the case of (±)-**9h**. Mitsunobu coupling of chiral alcohols with commercially available methyl 1*H*-imidazole-5-carboxylate offered a direct and stereoselective

approach, with yields varying between 25% and 67%. However, under strictly controlled reaction conditions, racemization was prevented and alkylation occurred selectively at N-1 with inversion of configuration. Secondary alcohols with (*S*)-configuration yielded derivatives of physiologically more active (*R*)-MTO, whereas for the preparation of (*S*)-MTO (**10b**), the (*R*)-configured alcohol (**9b**) was reacted. Enantiomeric excesses for the substituted imidazoles agreed with those of the alcohols used as starting materials, documenting inversion of configuration.³⁶

For the synthesis of derivatives of **1** and **2** substituted in the phenyl ring in position 3 or 4 (**10g–k**), synthesis started from the 3- and 4-substituted phenylethanol, respectively, to produce (*S*)-alcohols³⁶ (**9g–k**), followed by Mitsunobu coupling, shown in Scheme 5. 4-Br-MTO (**10k**) was obtained by halodestannylation of the 4-trimethylstannyl-MTO precursor with bromine.³⁶ The polar hydroxymethyl substituent of **11** was introduced by palladium-catalyzed formylation of 4-iodo-MTO^{26,36} (**10j**) with carbon monoxide and tributyltin hydride (yield 64%), followed by reduction of the aldehyde with NaBH₄ in 85% yield.³⁹

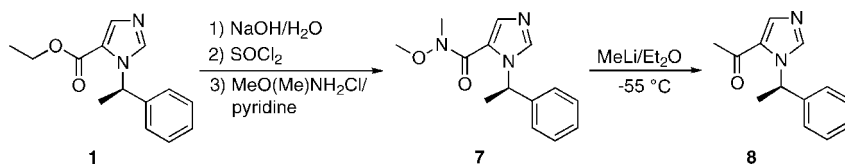
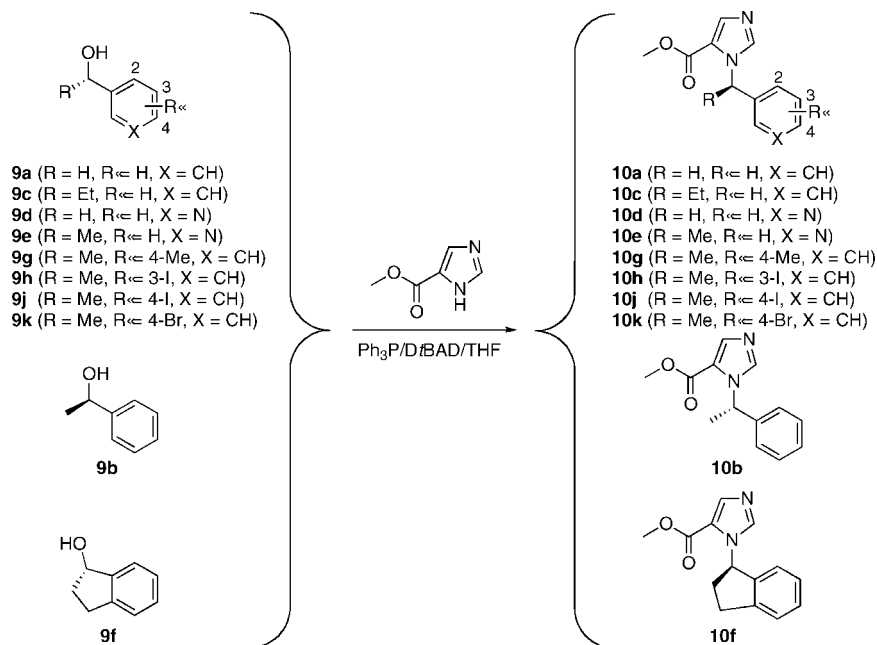
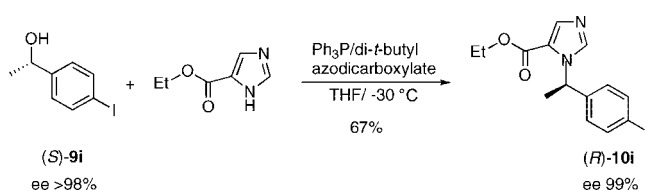
The synthesis of I-ETO (**10i**) may serve as an example for coupling (*S*)-configured 1-(4-iodophenyl)ethanol (**9i**) with ethyl 1*H*-imidazole-5-carboxylate by the Mitsunobu reaction (see Experimental Procedures). For this purpose, racemic 4-iodophenylethanol was transformed to a racemic ester (1-(4-iodophenyl)ethyl chloroacetate),³⁶ which was resolved by lipase SAM II, an enzyme known to hydrolyze (*R*)-esters of secondary benzylic alcohols.³⁷ Transesterification of the isolated (*S*)-ester yielded (**9i**).³⁶

Synthesis of Radioligand. The radioligand ¹³¹I-IMTO was derived from (*R*)-IMTO (**10j**), which was converted to the 4-trimethylstannyl-MTO precursor.^{26,36} The precursor facilitates labeling at room temperature, producing ¹³¹I-IMTO with high specific activity. The product was obtained with high RCY, high radiochemical purity, and high *in vitro* stability (Table 1). ¹³¹I-IMTO was formulated in 1 mL of saline, and *in vitro* stability was analyzed by HPLC, showing a slow linear loss of radioiodine with time.

Results and Discussion

Binding of MTO Analogues. Kinetics of ¹³¹I-IMTO Binding. In pilot studies the association of ¹³¹I-IMTO to adrenal membranes was followed for 75 min, using the original radioligand solution without addition of carrier (final concentration 0.1 nM). Specific binding reached a high maximum after 10–20 min that decreased steadily at later time points; i.e., no stable saturation was reached. If the radioligand concentration was increased by addition of carrier IMTO (**10j**) to a total concentration of 2 nM, a stable plateau was reached within 10 min (Figure 1a). Thus, a small amount of ¹³¹I-IMTO was progressively lost during the incubation process, presumably because of degradation, uptake, or nonspecific binding to surfaces. This small loss could be neglected if the total concentration was chosen to be sufficiently high.

The association time course of ¹³¹I-IMTO to rat adrenal membranes is given in Figure 1a (data pooled from seven experiments). Computerized curve fitting to the association function $B(t) = B_0[1 - \exp(-\nu t)]$ resulted in an observable association rate constant ν of $0.65 \pm 0.06 \text{ min}^{-1}$ (\pm SD) at a ligand concentration $L = 2 \text{ nM}$, corresponding to an association half-time of 1.1 min. For dissociation experiments, membranes were first fully equilibrated with 2 nM radioligand, and dissociation was initiated by the addition of excess unlabeled IMTO and stepwise filtration at 15 s intervals. Data pooled from four experiments (Figure 1b) demonstrated fast reversibility of

Scheme 3. Synthesis of the *N*-Methoxyamide (7) and the Methyl Ketone (8)**Scheme 4.** Enantioselective Synthesis of *N*-1 Derivatives Affecting the 1-Phenylethyl Moiety (**10a–k**)^a^a Synthesis of **10i** shown in Scheme 5.**Scheme 5.** Enantioselective Synthesis of (*R*)-4-Iodophenyl Etomidate (**10i**)

binding and resulted in a dissociation constant $b = 0.36 \pm 0.03 \text{ min}^{-1}$ (\pm SD) and a derived dissociation half-time of 1.93 min. Once b is known, the true association rate constant (a) can be calculated from the relationship $v = La + b$, giving $a = 0.10 \text{ min}^{-1} \text{ nM}^{-1}$. Thus, a first rough estimate for the dissociation equilibrium constant K_D was obtained from $K_D = b/a = (0.36 \text{ min}^{-1})/(0.10 \text{ min}^{-1} \text{ nM}^{-1}) = 3.6 \text{ nM}$. A more reliable K_D value should be accessible by saturation analysis at equilibrium conditions.

For saturation analysis, adrenal membranes were incubated with five concentrations of ¹³¹I-IMTO ranging from 2 to 200 nM for 20–30 min. At the lowest concentration, 0.06 mg of tissue bound approximately 10% of the free ligand ($\sim 0.1 \text{ pmol}$). Figure 2 gives representative examples for saturation isotherms

Table 1. Characteristics of ¹³¹I-IMTO

radiochemical yield	93.7 \pm 1.2% (15)
radiochemical purity	97.3 \pm 1.3% (15)
specific activity	50 GBq/ μ mol
concentration	30–160 MBq/mL
in vitro stability (dec rate)	0.27%/h

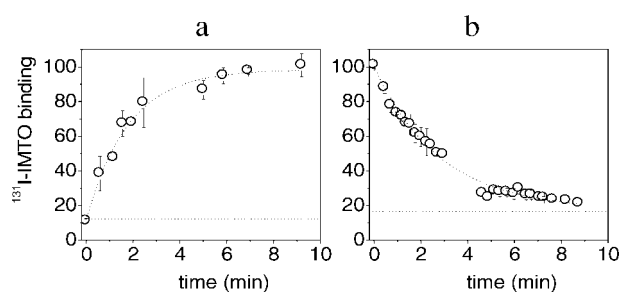


Figure 1. Association (a) and dissociation kinetics (b) of total ¹³¹I-IMTO binding to rat adrenal membranes. Data are the mean \pm SD pooled from four experiments performed in duplicate. Dotted lines follow the best fit functions, and dotted horizontal lines indicate nonspecific binding.

(a) and reciprocal plots (b), the linearity of the latter suggesting a single class of binding sites. As a mean of eight analyses, we obtained $K_D = 11.6 \pm 2.5 \text{ nM}$ and $B_{\text{max}} = 12.1 \pm 0.4 \text{ pmol/mg}$ tissue. This K_D value appears to be more reliable than the estimate from the rate constants.

Displacement of ¹³¹I-IMTO Binding by MTO Analogues. SAR studies of MTO analogues are based on the high affinity of the radiotracer ¹³¹I-IMTO. Test compounds were evaluated by the displacement of specific ¹³¹I-IMTO binding on rat adrenal membranes; the concentration of the radioligand was 2 nM. All effective inhibitors of ¹³¹I-IMTO binding yielded monophasic displacement curves, with steepness close to unity ($n_H \sim 1.0$). Figure 3 shows displacement curves for potent P-450c11 inhibitors ETO (**1**), I-ETO (**10i**), and FETO (**5**), in comparison with metyrapol.

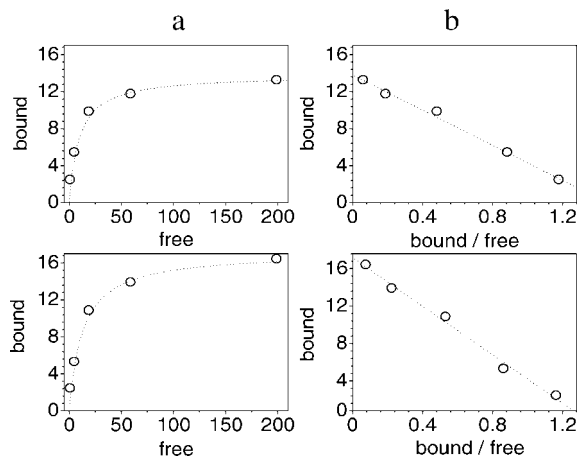


Figure 2. Dependence of specific ^{131}I -IMTO binding ("bound") on free ^{131}I -IMTO concentration ("free"), with two representative examples of eight experiments performed in duplicate: (a) saturation isotherms; (b) reciprocal transformations (Eadie-Hofstee plots). In most experiments, reciprocal transformation resulted in linear correlations.

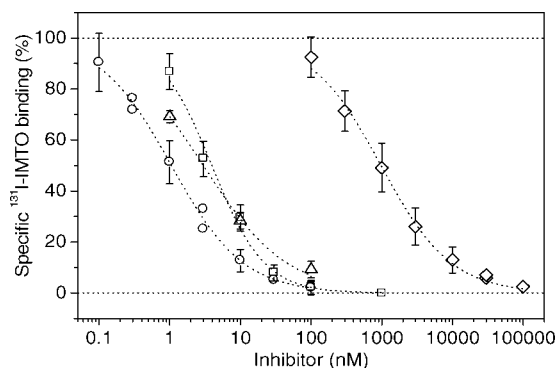


Figure 3. Inhibition of specific ^{131}I -IMTO binding by **1** (ETO, circles), **10i** (4-I-ETO, squares), **5** (FETO, triangles), and metyrapol (diamonds). Data were pooled from 4 to 11 experiments. Dotted graphs were constructed from the parameters given in Tables 2, 3, and 5.

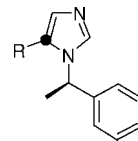
Table 2. Inhibition of Specific ^{131}I -IMTO Binding to Rat Adrenal Membranes by ETO (**1**) and MTO (**2**) Analogues with Modifications of the Phenyl Ring^a

compd	R	R'	X	IC ₅₀ (nM)	±SD	n	n _H	±SD	n
1	C ₂ H ₅		CH	1.08*	±0.42	11	0.86	±0.16	6
2	CH ₃		CH	3.69	±1.92	6	0.92	±0.24	4
10e	CH ₃		N	20.7**	±3.81	4	0.80	±0.16	4
10g	CH ₃	4-CH ₃	CH	3.58	±1.03	3	0.73	±0.05	3
10h	CH ₃	3-I	CH	4.44	±1.78	4	0.84	±0.04	3
10i	C ₂ H ₅	4-I	CH	4.37	±1.15	5	1.17	±0.21	4
10j	CH ₃	4-I	CH	8.98	±3.72	15	1.04	±0.26	13
10k	CH ₃	4-Br	CH	8.95	±0.48	3	0.90	±0.11	3
11	CH ₃	4-CH ₂ OH	CH	3.29	±0.90	4	0.74	±0.18	4

^a IC₅₀ and Hill coefficients (n_H) expressed as mean values ± SD (n, number of experiments). (*) Significantly more potent than all other compounds in the table, *p* < 0.01. (**) Significantly less potent than other compounds shown, *p* < 0.001.

The effect of phenyl substitution was studied in detail in order to facilitate regioselective labeling with radiohalogen (Table 2, Figure 4). The inhibitory potencies of halogenated derivatives 3-I-MTO (**10h**), 4-I-MTO (**10j**), and 4-Br-MTO (**10k**) were compared with MTO (**2**). Statistical tests based on several

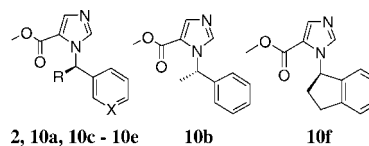
Table 3. Inhibition of Specific ^{131}I -IMTO Binding on Rat Adrenal Membranes by Analogues with Modifications of the Ester^a



Compd.	R	IC ₅₀ (nM)	±SD	n	n _H	±SD	n
1		1.08*	± 0.42	11	0.86	± 0.16	6
3b		1.42*	± 0.33	5	0.99	± 0.05	5
3a		2.89	± 0.55	4	0.92	± 0.20	4
5		2.89	± 0.55	4	0.74	± 0.06	4
2		3.69	± 1.92	6	0.92	± 0.24	4
6		215**	± 91	6	0.81	± 0.29	4
7		16.7, 14.0	-	2	n.d.	-	-
8		59.8, 62.1	-	2	0.80, 0.71	-	2
4		122 670*** ± 41 360	-	3	n.d.	-	-

^a IC₅₀ and Hill coefficients (n_H) expressed as mean values ± SD (n, number of experiments). If *n* = 2, both values are indicated. (*) Significantly more potent than other compounds shown, *p* < 0.05. (**) Significantly less potent than other compounds shown, *p* < 0.001. (***) Practically inactive. n.d.: not determined (n_H was fixed to 1.0 for computer analysis).

Table 4. Inhibition of Specific ^{131}I -IMTO Binding to Rat Adrenal Membranes by MTO Analogues with Modifications at the Chiral Center^a



compd	R	X	IC ₅₀ (nM)	±SD	n	n _H	±SD	n
2	(<i>R</i>)-CH ₃	CH	3.69	±1.92	6	0.92	±0.24	4
10a	H	CH	28.8**	±10.9	4	0.90, 0.85		2
10b	(<i>S</i>)-CH ₃	CH	492***	±281	4	0.88	±0.18	4
10c	(<i>R</i>)-C ₂ H ₅	CH	6.23*	±0.96	3	0.83, 0.67		2
10d	H	N	870***	±240	3	1.07, 1.01		2
10e	(<i>R</i>)-CH ₃	N	20.7**	±3.81	4	0.80	±0.16	4
10f	1-indanyl		3.26	±0.70	3	0.91	±0.17	3

^a IC₅₀ and Hill coefficients (n_H) expressed as mean values ± SD (n, number of experiments). If *n* = 2, both values are indicated. (*) Significantly less potent than **2**, *p* < 0.05. (**) Significantly less potent than **2**, *p* < 0.01. (***) Significantly less potent than all other compounds shown.

experiments indicated that substitution with iodine in position 4 (para) resulted in a slight decrease in potency, whereas iodine in position 3 (meta) was tolerated (compare **2** and **10h**). In contrast, 4-methyl-MTO (**10g**), and 4-hydroxymethyl-MTO (**11**) showed no negative effect of substitution. Thus, the slight shift in potency of 4-I-MTO (**10j**), also observed in case of (**10i**), is unlikely to be due to steric hindrance. Replacing the phenyl ring by pyridine yielded 3-pyridyl-MTO (**10e**), with slightly reduced potency.

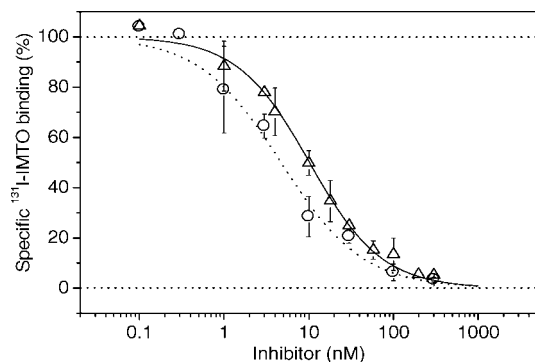


Figure 4. Displacement of specific ^{131}I -IMTO binding by **2** (MTO, dotted line; $n = 6$) and **10j** (I-MTO, full line; $n = 13$). Lines follow best fit functions.

Table 5. Inhibition of Specific ^{131}I -IMTO Binding on Rat Adrenal Membranes by Compounds Known To Interact with Steroid Hydroxylation^a

inhibitor	IC ₅₀ (μM)	±SD	<i>n</i>	<i>n</i> _H	±SD	<i>n</i>
ketoconazole	0.71	±0.49	7	1.19	±0.22	6
11-deoxycorticosterone	0.89	±0.33	5	0.86	±0.16	4
metyrapol	1.02	±0.39	7	0.88	±0.13	5
metyrapone	1.16	±0.79	5	0.85	±0.12	3
aminoglutethimide	82.9		1	n.d.		
proadifen	137, 142		2	n.d.		

^a IC₅₀ and Hill coefficients (*n*_H) expressed as mean values ± SD (*n*, number of experiments). If *n* = 2, both values are indicated. n.d.: not determined (*n*_H was fixed to 1.0 for computer analysis).

Studies of the effect of ester size on the binding potency demonstrated that the ethyl and 2-propyl esters (**1** and **3b**), showed the highest potency, followed by the *n*-propyl (**3a**), 2-fluoroethyl (**5**), and methyl ester (**2**) (Table 3, Figure 3). The methyl ester was chosen as a radioligand. Further structural modifications at position 5 of the imidazole ring were performed to control enzymatic hydrolysis in vivo. The ester was replaced by amide (**6**) and by methyl ketone (**8**), resulting in a considerable loss of affinity. Additional substitution of **6** with *N*-methoxy partly restored affinity (**7**). However, amide nitrogen in this position of the molecule was considered unfavorable. Thus, attempts to increase the in vivo stability by replacing the ester had a negative effect on the binding potency. Hydrolysis of the ester resulted in a total loss of inhibitory potency (**4**) (Table 3).

Derivatives listed in Table 4 differ in their geometries at their chiral centers. The potency of (*S*)-MTO (**10b**) was found to be 2 orders of magnitude weaker than that of (*R*)-MTO (**2**). If the methyl substituent at the center of chirality was omitted (**10a**), the effect was moderate and additional replacement of phenyl by pyridyl caused a drop in potency (**10d** and **10e**). Replacing methyl by ethyl did not affect potency (**10c**). Integrating ethyl into a bicyclic indanyl system⁴⁰ was well tolerated, on the basis of IC₅₀ values (**2** and **10f**).

The importance of a one-carbon bridge with alkyl branching between N-1 of imidazole and the phenyl ring had been emphasized for sustaining the hypnotic activity of etomidate derivatives.³⁰ Lengthening of the side chain to include two carbon atoms, with or without branching, or omission of branching or direct attachment of the aryl group to the nitrogen resulted in a total loss of hypnotic properties.³⁰ Omission of the alkyl substituent described as compound **10a** in this study showed reduced potency. These early investigations of the structural requirements of ETO analogues as hypnotic agents led to the conclusion that the nature of the N-substituent was critical, anticipating the influence of chirality, although results

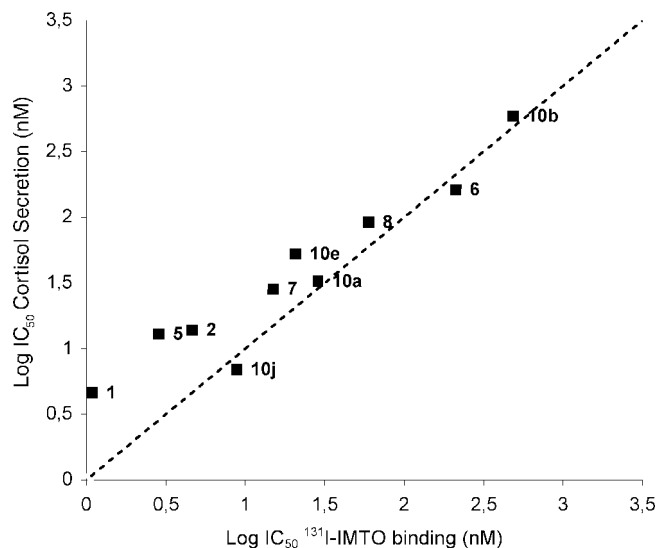


Figure 5. Correlation of log IC₅₀ values obtained by the inhibition of cortisol secretion and by ^{131}I -IMTO displacement. The dotted line indicates identity ($R = 0.954$, $p < 0.001$).

were derived from racemic mixtures.³⁰ Differences in hypnotic potency among various esters were minor, and yet the presence of the ester moiety was essential.

It is of considerable interest that the structural requirements of ETO analogues postulated for sustaining hypnotic activity correlated well with our quantitative SAR data based on their inhibitory potencies as competitive displacers of ^{131}I -IMTO binding. Ester modification had almost no effect, yet hydrolysis of the ester abolished inhibitory potency (Table 3). Chirality determined the potency of selected substituents at the carbon bridge (Table 4). Studies with intact adrenocortical mitochondria reported previously have also demonstrated that the interaction of etomidate with cytochrome P-450c11 was highly selective and stereospecific.³ Both biological functions of etomidate (inhibition of P-450c11 and hypnotic activity) require the (*R*)-configuration.

Besides the structurally derived analogues of MTO, other compounds known to suppress P-450c11 activity were also tested (Table 5.) These included metyrapone, metyrapol, and ketoconazole, with IC₅₀ values in the micromolar range (Figure 4).⁴¹ The natural substrate 11-deoxycorticosterone displaced specific ^{131}I -IMTO binding at low micromolar concentrations. Very weak inhibition of ^{131}I -IMTO binding was observed with 4-aminoglutethimide (an inhibitor of 20 α -hydroxylation of cholesterol) and with proadifen (an inhibitor of oxidative drug metabolism). Despite the fact that metyrapone is known to bind to cytochrome P-450 enzymes in adrenocortical mitochondria and in liver microsomes³ and that ketoconazole is an inhibitor of many cytochrome P-450 enzyme reactions⁴² including steroid 11 β -hydroxylations,⁴³ displacement of specific ^{131}I -IMTO binding by these inhibitors and by DOC strongly suggested that P-450c11 is the site of interaction.

Direct Measurement of Cortisol Secretion. A comparison of the inhibitory potencies obtained by radioligand displacement with measurements of the inhibitory effect of etomidate and selected derivatives on cortisol secretion is shown in Figure 5. The corresponding IC₅₀ values subjected to regression analysis are presented in Table 6. Direct measurements of cortisol secretion in a living cell culture correlated well with the inhibitory potencies of analogues observed by ^{131}I -IMTO displacement.

Table 6. Comparison of the Inhibitory Potency of ETO Derivatives Obtained by Displacement of ^{131}I -IMTO Binding and by Inhibition of Cortisol Secretion

compd	inhibitor	displacement of ^{131}I -IMTO		inhibition of cortisol secretion ^a	
		IC ₅₀ (nM)	log IC ₅₀	IC ₅₀ (nM)	log IC ₅₀
1	(<i>R</i>)-etomidate	1.08 ± 0.42	0.033	4.57 ± 1.41	0.66
2	(<i>R</i>)-metomidate	3.69 ± 1.92	0.57	13.9 ± 10.2	1.14
10b	(<i>S</i>)-metomidate	492 ± 281	2.69	592.9	2.77
10a	demethyl-MTO	28.8 ± 10.9	1.46	32.1	1.51
10e	3-pyridyl-MTO	20.7 ± 3.81	1.32	52.2	1.72
10j	4-iodo-MTO	9.0 ± 3.72	0.954	6.95	0.84
5	2-fluoroethyl ester	2.9 ± 0.55	0.46	8.92, 17.1	1.11
6	<i>N</i> -methanamide	215 ± 91	2.33	135.3, 191	2.21
7	<i>N</i> -methoxyamide	16.7; 14.0	1.18	19.5, 37.0	1.45
8	methyl ketone	59.8; 62.1	1.78	59.0, 125	1.96

^a Maximum inhibition exceeded 90%.

Clinical Application. Etomidate is known as a short-acting hypnotic used parenterally for the induction and maintenance of anesthesia and for sedation. Prolonged sedation, however, revealed a potent side effect of this drug as an inhibitor of adrenal steroidogenesis.^{44,45} The intravenous induction dose for anesthesia in adults was reported as 0.3 mg/kg, whereas adrenal suppression has been observed with doses of 0.04 mg/kg.⁴⁶ Etomidate potentiates the action of GABA at the GABA_A receptor complex at concentrations around 1 μM,^{47,48} whereas its affinity for ^{131}I -IMTO binding sites on adrenal membranes lies around 1 nM (this study). Very weak uptake of radioactivity in the brain was observed after injection of ^{131}I -IMTO for imaging, indicating that binding of trace amounts of IMTO to GABA receptors was insignificant.⁴⁹

Labeled etomidate derivatives have shown excellent characteristics as adrenal imaging agents.^{26,28,50} These properties are based on high-affinity binding on tissue rich in P-450c11 enzyme activity and on excellent selectivity, restricting specific uptake to adrenocortical tissue.

Ample experience with ^{11}C -metomidate has been documented in patients with adrenal pathology,^{51–54} and preliminary results with ^{18}F -FETO were obtained in healthy subjects.⁵⁵ Since safe application devoid of any pharmacological effect is dose-related, trace amounts of the radiotracer warrant a wide safety margin (SF). This is accomplished by carrier-free labeling of PET and SPECT radiotracers with high specific activities so that a negligible mass of an ETO derivative will provide an adequate amount of radioactivity in a single intravenous dose. Actually, the PET tracer ^{11}C -MTO was generally produced with a specific activity of 60 GBq/μmol, and the SPECT tracer ^{123}I -IMTO is now available with 1000 GBq/μmol (27 Ci/μmol). External scintigraphy thus is performed with a microdose (0.06–5 μg) of the PET or SPECT tracer avoiding a hypnotic effect (SF ≥ 21.000) as well as cortisol suppression (SF ≥ 2.800).

Substitution with a radiohalogen in the phenyl ring offered access to diagnostic as well as therapeutic MTO derivatives, all derived from a stannylated precursor. Radionuclide therapy is based on β- and α-emitting isotopes, e.g., ^{131}I , ^{82}Br , and ^{211}At , while ^{123}I -IMTO is being evaluated for diagnostic purposes.

Conclusions

Derivatization of ETO (**1**) and MTO (**2**) at three distinct sites provided information on the structural requirements for high-affinity binding. The choice of substituents gave an indication of which labeling approach would produce suitable radioligands. Structural modifications concerned the ester moiety, the chiral center, and the phenyl ring.

The radioligand ^{131}I -IMTO displayed fast on- and off-kinetics and bound with high affinity to a single class of binding sites on rat adrenal membranes. IC₅₀ values obtained by the displace-

ment of specific ^{131}I -IMTO binding demonstrated that the inhibitory potency resided exclusively in the (*R*)-configured analogues and that the intact ester was essential for binding. Detailed SAR studies revealed that none of the esters or substituents in the phenyl ring affected binding affinity.

Compounds known to suppress adrenocortical P-450c11, e.g., metyrapone and ketoconazole, were also tested. Since specific binding of ^{131}I -IMTO was inhibited by concentrations of metyrapone and metyrapol known to block P-450c11 (more specifically so than ketoconazole) and also by low micromolar concentrations of the natural substrate DOC, the most likely target of specific ^{131}I -IMTO binding was the enzyme P-450c11. Additionally, measurements based on steroid secretion in a living cell culture indicated a dose-dependent inhibition of 11β-hydroxylations. The inhibitory potency of selected etomidate derivatives measured by cortisol secretion and by the displacement of ^{131}I -IMTO binding showed a high correlation and provided further evidence that specific binding of inhibitors occurred at the site of cortisol synthesis. In fact, analogues of ETO (**1**) and MTO (**2**) showing high binding affinity, have been identified as potent inhibitors of cortisol secretion.^{1–3,24,45,46,49}

Derivatization offered valuable guidance for precursor synthesis. New synthetic methods have been developed to label MTO analogues with high specific activity,^{26,27,29} which is a prerequisite for receptor imaging. Results further indicated that halogenation of the phenyl ring may offer considerable versatility for labeling with SPECT and PET radionuclides (i.e., ^{123}I , ^{124}I , ^{76}Br , ^{18}F). Some (*R*)-configured substituents might fulfill the strict steric requirements for introducing a radioactive label at the chiral center (see Table 4). Labeled derivatives may also gain importance for in depth evaluation of the pharmacology of this versatile molecule as a potent P-450c11 enzyme inhibitor and as a valued anesthetic.

Experimental Procedures

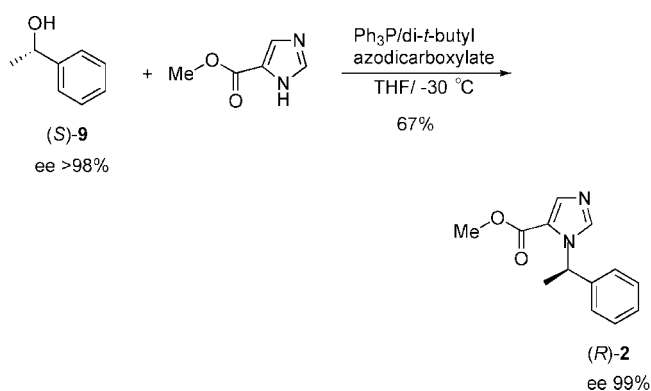
Chemical Synthesis. Chemicals were obtained from Aldrich, Lancaster, and Sigma and were generally used without further purification. Lipase SAM II (Amano Enzyme Europe Ltd., England) was stored at +4 °C and used as supplied.

^1H NMR spectra were recorded on a Bruker DRX400 spectrometer in CDCl₃ using the residual solvent peak as internal reference [$\delta(\text{CHCl}_3) = 7.24$] at 400.13 MHz. ^{13}C NMR spectra (in part *J*-modulated) were recorded on the same spectrometer operating at 100.61 MHz (internal reference CDCl₃; $\delta = 77.00$). Chemical shifts, δ , are given in parts per million. Coupling constants, *J*, are given in Hz. IR spectra were recorded as films on a silicon disk using a Perkin-Elmer 1600 FT-IR spectrometer.⁵⁶ Optical rotations were measured at 20 °C on a Perkin-Elmer 341 polarimeter in a 1 dm (10 cm) cell, and concentration is expressed as g/100 mL. TLC was performed on 0.25 mm thick Merck plates, silica gel 60 F₂₅₄. Flash chromatography was performed with Merck silica gel 60

(230–400 mesh). Spots were visualized by UV and/or with iodine or dipping of the plate into a solution of 23 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ and 1 g of $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$ in 500 mL of 10% H_2SO_4 in water, followed by heating with a heat gun. A Metrohm 702 SM Titrino instrument was used as an autotitrator.

Analytical HPLC was performed on a Jasco System (PU-980 pump, UV 975 and RI 930) using a Chiracel OD-H column, 0.46 cm \times 25 cm. Preparative HPLC was performed on a Rainin system (Dynamax SD-1 pump, equipped with a model UV-1 absorbance detector) using a Chiracel OD column, 5 cm \times 50 cm. Retention times (t_R) were measured on an analytical column using 5% 2-propanol–hexane, 1 mL/min, UV detection (254 nm). Tetrahydrofuran (THF) was distilled from potassium, and Et_2O was distilled from LiAlH_4 .

Mitsunobu Reaction of Benzylic Alcohols **9** with Methyl *1H*-Imidazole-5-carboxylate.



General Procedure.³⁶ A solution of (*S*)-alcohol (1.1 mmol, >98% ee) in dry THF (2 mL) was added dropwise to a stirred solution of methyl *1H*-imidazole-5-carboxylate (0.139 g, 1.1 mmol) and triphenylphosphine (0.345 g, 1.3 mmol) in dry THF (3.0 mL) in an atmosphere of argon at $-30\text{ }^\circ\text{C}$. Then a solution of di-*tert*-butyl azodicarboxylate (0.304 g, 1.32 mmol) in dry THF (2 mL) was added, and the reaction mixture (while stirring) was allowed to warm up from -30 to $0\text{ }^\circ\text{C}$ within 2.5 h. No alcohol could be detected by TLC (diethyl ether/diisopropylamine, 10:1). The reaction mixture was concentrated under reduced pressure. The residue was mixed with diethyl ether (5 mL) and stirred for 2 h. The crystals (Ph_3PO and hydrazo ester) were collected and washed with diethyl ether (3 \times 2 mL). The filtrate was evaporated under reduced pressure to yield a residue, which was purified by flash chromatography (*n*-hexane/diethyl ether/diisopropylamine, 60:30:1) on silica gel to give a chirally pure product.

The enantiomeric excesses of (*R*)- and (*S*)-alcohols were determined by HPLC on a chiral stationary phase (Chiracel OD-H, column size 5 cm \times 50 cm). (*R*)-Configuration was assigned to the alcohol formed by enzymatic saponification, since lipase SAM II is known to hydrolyze preferentially the (*R*)-esters of secondary benzylic alcohols.

(R)-(+)-Methyl 1-[1-(4-Iodophenyl)ethyl]-1H-imidazole-5-carboxylate (10j).²⁶ The (*S*)-alcohol **9j** (1.98 g, 7.98 mmol, 98% ee) in dry THF (14.5 mL) was added to a stirred solution of methyl *1H*-imidazole-5-carboxylate (1.008 g, 7.98 mmol) and triphenylphosphine (2.503 g, 9.43 mmol) in dry THF (22.0 mL) in an atmosphere of argon at $-30\text{ }^\circ\text{C}$. Then a solution of di-*tert*-butyl azodicarboxylate (2.204 g, 9.57 mmol) in dry THF (14.5 mL) was added and the stirred reaction mixture was allowed to warm up from -30 to $0\text{ }^\circ\text{C}$ within 2.5 h. No alcohol could be detected by TLC (diethyl ether/diisopropylamine, 10:1). The reaction mixture was concentrated under reduced pressure. The residue was mixed with diethyl ether (36 mL) and stirred for 2 h. The crystals (Ph_3PO and hydrazo ester) were collected and washed with diethyl ether (3 \times 15 mL). The filtrate was evaporated under reduced pressure to yield a residue, which was purified by flash chromatography (*n*-hexane/diethyl ether/diisopropylamine, 50:30:1) on silica gel to give

IMTO **10j** (1.91 g, 67%, 99% ee); $[\alpha]_D^{20} +76.0$ (*c* 1.09, acetone). Anal. ($\text{C}_{13}\text{H}_{13}\text{IN}_2\text{O}_2$) C, H, N.

General Transesterification Procedure. A solution of **1** (0.10 g, 0.405 mmol) in dry MeOH, *n*-PrOH or *i*PrOH (5 mL) and the respective sodium alkoxide (1 M) was kept for 5 h at room temperature. After neutralization with glacial acetic acid, the solution was concentrated under reduced pressure, diluted with water (2 mL), and extracted three times with EtOAc. The combined organic layers were dried (MgSO_4) and concentrated under reduced pressure to leave a residue, which was purified by flash chromatography on silica gel to give the desired ester.

(R)-(+)-Methyl 1-[1-Phenylethyl]-1H-imidazole-5-carboxylate (2). $R_f = 0.65$ ($\text{Et}_2\text{O}/\text{Pr}_2\text{NH}$ 10/1); yield 71% as a viscous oil; $[\alpha]_D^{20} +77.09$ (*c* 1.1, acetone). Infrared spectrum was superimposable with an authentic sample and with the (*S*)-isomer **10b**.²⁶

(R)-(+)-Propyl 1-[1-Phenylethyl]-1H-imidazole-5-carboxylate (3a). $R_f = 0.36$ (hexane/ $\text{Et}_2\text{O}/\text{Pr}_2\text{NH}$, 5/10/1); yield 78% as a viscous oil; $[\alpha]_D^{20} +60.06$ (*c* 1.57, acetone). The racemate³⁰ and (*R*)-**3a**³² are known compounds. Anal. ($\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_2$) C, H, N.

(R)-(+)-2-Propyl 1-[1-Phenylethyl]-1H-imidazole-5-carboxylate (3b). $R_f = 0.36$ (hexane/ $\text{Et}_2\text{O}/\text{Pr}_2\text{NH}$, 5/10/1); yield 80% as a crystalline solid; mp $56\text{ }^\circ\text{C}$ (hexane); $[\alpha]_D^{20} +44.52$ (*c* 0.93, acetone). The racemate is a known compound.³⁰ Anal. ($\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_2$) C, H, N.

(R)-(+)-2-Fluoroethyl 1-[1-Phenylethyl]-1H-imidazole-5-carboxylate (5). A solution of *DtBAD* (0.128 g, 0.554 mmol) in dry toluene (2 mL) was added to a stirred mixture of Ph_3P (0.145 g, 0.554 mmol), methyl *1H*-imidazole-5-carboxylate (0.100 g, 0.462 mmol) and 2-fluoroethanol (44 mg, 0.040 mL, 0.681 mmol; handle with care!) in dry toluene (2 mL) under an atmosphere of argon. After 18 h, water (two drops) was added and the mixture was concentrated under reduced pressure to give a residue, which was purified by flash chromatography (first column, 60 g of silica gel, hexane/ $\text{Et}_2\text{O}/\text{Pr}_2\text{NH}$, 5/10/1, $R_f = 0.25$, 98 mg of mixture of 2-fluoroethyl ester and hydrazo ester; second flash chromatography, 40 g of silica gel, Et_2O as eluent, $R_f = 0.30$) to give **5** (38 mg, 31%) as a crystalline solid, mp $51\text{ }^\circ\text{C}$ (hexane); $[\alpha]_D^{20} +106.29$ (*c* 0.72, acetone). Anal. ($\text{C}_{14}\text{H}_{15}\text{FN}_2\text{O}_2$) C, H, N.

(R)-(+)-*N*-Methyl-1-[1-phenylethyl]-1H-imidazole-5-carboxamide (6). A solution of **4** (27 mg, 0.125 mmol) derived from **1** and DMF (1 drop) in thionyl chloride³⁰ (3 mL) was refluxed for 1 h. After cooling, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in a solution (2 M, 4 mL) of methylamine in THF and stirred for 18 h. The mixture was concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc , $R_f = 0.09$) to give the amide **6** (20 mg, 70%) as a gum; $[\alpha]_D^{20} +85.40$ (*c* 2.52, acetone). Anal. Calcd for $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}$ (229.12): C, 68.10; H, 6.59. Found: C 68.13; H 6.70.

(R)-(+)-*N*-Methoxy-*N*-methyl-1-[1-phenylethyl]-1H-imidazole-5-carboxamide (7). A solution of **1** (0.247 g, 1.01 mmol) in ethanol (2.5 mL) and a solution of NaOH (0.048 g, 1.2 mmol) in water (5.4 mL) were combined and refluxed for 30 min (TLC). The cooled mixture was concentrated under reduced pressure and dried in a vacuum desiccator over KOH. Thionyl chloride (5 mL) was added to the residue. The mixture was stirred vigorously and refluxed for 30 min. Removal of SOCl_2 under reduced pressure gave a residue, which was dissolved in dry CH_2Cl_2 (10 mL). *N,O*-Dimethylhydroxylamine hydrochloride³⁴ (0.11 g, 1.21 mmol) was added, followed by dry pyridine (0.32 g, 0.33 mL, 4.04 mmol) after cooling to $0\text{ }^\circ\text{C}$. Stirring was continued for 2.5 h, and then the reaction mixture was concentrated under reduced pressure. Brine (30 mL) was added to the residue, and the product was extracted with ethyl acetate (3 \times 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (EtOAc/EtOH , 10/1, TLC $R_f = 0.52$) to give **7** (0.148 g, 57%) as a crystalline product; mp $120\text{--}122\text{ }^\circ\text{C}$ ($\text{CH}_2\text{Cl}_2/\text{hexane}$); $[\alpha]_D^{20} +107.38$ (*c* 0.75, acetone). Anal. ($\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_2$) C, H, N.

(R)-(+)-5-Acetyl-[1-phenylethyl]-1H-imidazole (8). A solution of MeLi³⁴ (0.34 mL, 0.54 mmol, 1.6 M in Et₂O, 0.4% LiCl) was added to a solution of **7** (0.108 g, 0.42 mmol) in dry THF (7.6 mL) at -55 °C under argon. Stirring was continued until the starting material was consumed (TLC, 2.5 h). Water (5 mL) was added, and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried (Na₂SO₄) and concentrated under reduced pressure. The residue was flash-chromatographed (EtOAc, TLC *R_f* = 0.49) to yield **8** (0.066 g, 74%) as an oil; [α]²⁰_D +71.98 (*c* 0.94, acetone). Anal. (C₁₃H₁₄N₂O) C, H, N.

Methyl 1-Phenylmethyl-1H-imidazole-5-carboxylate (10a). Phenylmethanol (0.216 g, 2 mmol, 0.21 mL) was transformed into **10a** according to the general procedure for the Mitsunobu reaction; *R_f* = 0.78 (Et₂O/Pr₂NH, 10/1); yield 47% as a viscous oil. Crystallization from hexane furnished colorless needles, mp 58 °C. Anal. Calcd for C₁₂H₁₂N₂O₂ (216.24): C, 66.65; H, 5.59; N, 12.96. Found: C, 66.84; H, 5.76; N, 13.02.

(S)-(-)-Methyl 1-[1-Phenylethyl]-1H-imidazole-5-carboxylate (10b). (*R*)-(+)-1-Phenylethanol³⁷ (0.244 g, 2 mmol, 99% ee by HPLC) was transformed into **10b** according to the general procedure for the Mitsunobu reaction; *R_f* = 0.61 (Et₂O/Pr₂NH, 9/1); yield 51% as viscous product; [α]²⁰_D -78.36 (*c* 2.08, acetone). Infrared spectrum was superimposable with an authentic sample and with the (*R*)-enantiomer **2**.

(R)-(+)-Methyl 1-[1-Phenylpropyl]-1H-imidazole-5-carboxylate (10c). (*S*)-(-)-1-Phenylpropanol³⁷ (0.272 g, 2 mmol, 97% ee by HPLC) was transformed into **10c** according to the general procedure for the Mitsunobu reaction; *R_f* = 0.78 (Et₂O/Pr₂NH, 10/1); yield 61% as viscous product; [α]²⁰_D +93.47 (*c* 0.95, acetone). The hydrochloride of the racemate is a known compound.³⁰ Anal. (C₁₄H₁₆N₂O₂) C, H, N.

Methyl 1-[3-Pyridylmethyl]-1H-imidazole-5-carboxylate (10d). At 0 °C and under argon a solution of 3-pyridylmethanol (0.164 g, 1.5 mmol) in dry THF (3 mL) was added to a stirred suspension of Ph₃P (0.465 g, 1.77 mmol, 1.18 equiv) and methyl 1H-imidazole-5-carboxylate (0.189 g, 1.5 mmol, 1.0 equiv) in dry THF (5 mL). After the addition of *D*/BAD (0.414 g, 1.8 mmol, 1.2 equiv) the reaction mixture was stirred for 3 h at 0 °C. The solvent was removed under reduced pressure. HCl (2 M) was added to the viscous oil, and the acidic solution was extracted twice with CH₂Cl₂. Then 2 M NaOH was added to the aqueous phase, and the basic solution was extracted three times with CH₂Cl₂. The combined organic solutions were dried (MgSO₄) and concentrated under reduced pressure to leave a residue, which was purified by flash chromatography (MeOH/Et₂O, 1/5, TLC *R_f* = 0.38) to yield **10d** as a crystalline solid (0.196 g, 60%); mp 52–53 °C (hexane). Anal. (C₁₁H₁₁N₃O₂) C, H, N.

(R)-(+)-Methyl 1-[1-(3-Pyridyl)ethyl]-1H-imidazole-5-carboxylate (10e). (*S*)-(-)-1-(3-Pyridyl)ethanol [0.096 g, 0.78 mmol, prepared by a literature procedure,³⁸ 96% ee (by ¹H NMR of Mosher ester); [α]²⁰_D -33.21 (*c* 1.33, acetone), [α]²⁰_D -18.13 (*c* 1.24, CHCl₃), [α]²⁰_D -101.9 (*c* 1.21, CHCl₃), methyl 1H-imidazole-5-carboxylate, triphenylphosphane, and di-*tert*-butyl azodicarboxylate were reacted according to the general procedure for the Mitsunobu reaction. The reaction mixture was concentrated in vacuo. The residue was taken up in 2 N HCl. The mixture was extracted twice with CH₂Cl₂. The pH of the aqueous phase was brought to 7–8 by adding solid K₂CO₃. The product was extracted three times with CH₂Cl₂. These three organic phases were combined, dried (MgSO₄), and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/Et₂O, 1/5, TLC *R_f* = 0.38) to give **10e** (0.045 g, 25%) as a viscous oil; [α]²⁰_D +70.70 (*c* 1.14, acetone). Anal. (C₁₂H₁₃N₃O₂) C, H, N.

(R)-(-)-Methyl 1-[Indan-1-yl]-1H-imidazole-5-carboxylate (10f). (*S*)-(+)-1-Indanol³⁷ (0.134 g, 1.0 mmol, >95% ee by HPLC, [α]²⁰_D +29.57 (*c* 1.15, distilled CHCl₃)) was transformed into **10f** according to the general procedure for the Mitsunobu reaction. Flash chromatography (Et₂O/Pr₂NH, 10/1; TLC *R_f* = 0.77 in Et₂O/Pr₂NH, 10/1) gave **10f** (0.077 g, 32%); mp 93 °C (hexane), ([α]²⁰_D -67.86 (*c* 1.54, acetone)). The racemate

is a known compound.⁴⁰ Anal. (C₁₄H₁₄N₂O₂) (242.27) C, H, N: calcd, 69.41; found, 68.95.

(R)-(+)-Methyl 1-[1-(4-Methylphenyl)ethyl]-1H-imidazole-5-carboxylate (10g). (*S*)-(-)-1-(4-Methylphenyl)ethanol³⁷ [0.136 g, 1.0 mmol, 95% ee by ¹H NMR of Mosher ester, [α]²⁰_D -29.74 (*c* 1.14, MeOH)⁵⁷ [α]²⁵_D -37.30 (*c* 1.02, MeOH)] was transformed into **10g** according to the general procedure for the Mitsunobu reaction. Flash chromatography (Et₂O/Pr₂NH, 10/1; TLC *R_f* = 0.66 in Et₂O/Pr₂NH, 10/1) gave **10g** (0.104 g, 44%) as a viscous oil; [α]²⁰_D +82.53 (*c* 1.47, acetone). The hydrochloride of the racemate is a known compound. Anal. Calcd for C₁₄H₁₆N₂O₂ (244.29): C, 68.83; H, 6.60. Found: C, 69.11; H, 6.93.

(R)-(+)-Methyl 1-[1-(3-Iodophenyl)ethyl]-1H-imidazole-5-carboxylate (10h). (*S*)-(-)-1-(3-Iodophenyl)ethanol (0.248 g, 1 mmol, 98% ee by HPLC; obtained by preparative HPLC on chiral stationary phase (Chiracel OD-H, 5 cm × 50 cm, 5% 2-propanol/hexane, 240 mL/min, 10 °C) of racemic mixture, (*S*)-alcohol *t_R* = 7.7 min, (*R*)-alcohol *t_R* = 9.1 min, (*S*)-alcohol [α]²⁰_D -12.92 (*c* 1.18, MeOH); (*R*)-alcohol [α]²⁰_D +48.4 (*c* 1.25, acetone)) was transformed into **10h** according to the general procedure for the Mitsunobu reaction. Flash chromatography (Et₂O/Pr₂NH 10/1; TLC *R_f* = 0.64, Et₂O/Pr₂NH, 10/1) gave **10h** (0.136 g, 38%) as a viscous oil; mp 64–66 °C (hexane/trace of CH₂Cl₂) ([α]²⁰_D +44.31 (*c* 0.77, acetone). Anal. (C₁₃H₁₃IN₂O₂) C, H, N.

(R)-(+)-Methyl 1-[1-(4-Hydroxymethyl)phenylethyl]-1H-imidazole-5-carboxylate (11). Bu₃SnH (2.358 g, 2.18 mL, 8.1 mmol, 2.1 equiv, dissolved in 40 mL of dry toluene) was added to a degassed and stirred solution of **10j** (1.425 g, 4.0 mmol) and Pd(Ph₃P)₄ (0.184 g, 0.16 mmol) in dry toluene (20 mL) slowly (5 h, syringe pump) at 50 °C under an atmosphere of carbon monoxide.³⁹ After the addition of the Bu₃SnH, the reaction mixture was stirred for another 16 h, then cooled and concentrated under reduced pressure. The residue was flash-chromatographed (Et₂O/MeOH, 100/1, TLC *R_f* = 0.28 for aldehyde) to give starting material (0.328 g, 23%) and the aldehyde (0.781 g, 75%) containing a small amount of an impurity. Immediately after recording the ¹H NMR spectrum the compound was reduced. A solution of the aldehyde (0.746 g, 2.89 mmol) dissolved in dry MeOH (6 mL) was added to a stirred suspension of NaBH₄ (0.055 g, 1.45 mmol) in dry MeOH (3 mL) at room temperature under argon. When the reduction was finished (15 min, TLC Et₂O/MeOH, 50/1), the solvent was removed under reduced pressure. Water (10 mL) was added to the residue, and the mixture was extracted with CH₂Cl₂ (2 × 20 mL). The combined organic layers were dried (MgSO₄) and concentrated. The residue was flash-chromatographed (first Et₂O/MeOH, 100/1, then 25:1, TLC *R_f* = 0.28 for 25/1) to give **11** (0.639 g, 85% from aldehyde, 64% from **10j**) as a colorless gum; [α]²⁰_D +96.24 (*c* 1.25, acetone). Anal. (C₁₄H₁₆N₂O₃) C, H, N.

Radiochemistry. ¹³¹I-NaI was obtained from GE Healthcare Buchler, Braunschweig, Germany. Analytical HPLC was performed on a C18 column, Nucleosil 100-7 250 mm × 4.6 mm, CS Chromatographie Service, Langerwehe, Germany.

To a sealed conical glass vial containing 30 μg of stannylated precursor in 30 μL of ethanol, radioiodine corresponding to 37–185 MBq nca in 1–10 μL of 0.02 N NaOH and 6 μL of 1 N HCl were added. A solution of 15 μg of chloramine-T trihydrate in 10 μL of water (1.5 mg/mL) was added last to initiate labeling. The sample was mixed and allowed to proceed for 3 min at room temperature before adding 7 μL of 1 N NaOH. ¹³¹I-IMTO **10j** was isolated from the reaction mixture by analytical HPLC using a C18 phase and CH₃OH/H₂O/diethylamine 60/40/0.2 as eluent with a flow rate of 1.5 mL/min. After evaporation of the solvent under reduced pressure the tracer was formulated in 1 mL of saline. In vitro stability in this vehicle was analyzed by HPLC in daily intervals for 1 week, demonstrating a slow linear loss of radioiodine with time. The specific activity of radiolabeled IMTO was determined by HPLC using a calibration curve generated by linear regression analysis of recorded mass from solutions of known concentrations of IMTO versus peak area of UV absorption at 254 nm.

Biochemistry. Whole adrenals from adult male Wistar rats were stored at -80°C . Membranes obtained by homogenization were stored as aliquots at -80°C .

Membrane Preparation. For preparation of total membranes, adrenals were thawed and homogenized in 100 parts of homogenization buffer (10 mM K_2HPO_4 /10 mM HEPES, pH 7.1) with a glass/Teflon homogenizer (Potter-type). The homogenate was centrifuged at 35,000g for 10 min. The pellet was resuspended in fresh buffer and centrifuged. Membranes were washed two more times, including a 10 min warming period in a 37°C water bath, and stored as aliquots at -80°C . On the day of the experiment, an aliquot was thawed, diluted with incubation buffer (containing NaCl; see below), and centrifuged and the pellet resuspended in incubation buffer.

^{131}I -MTO Binding Procedure. Glass vials containing a final volume of 0.5 mL of incubation buffer (10 mM K_2HPO_4 /10 mM HEPES, 150 mM NaCl, pH 7.1), 20,000–40,000 cpm ^{131}I -IMTO together with IMTO carrier (2 nM, i.e., 1 pmol/vial, having a specific activity of 9–18 Ci/mmol) and the adrenal membrane suspension corresponding to 0.06–0.1 mg original tissue/vial were immersed in a 23°C water bath for 20–30 min. Membranes with bound radioligand were isolated by filtration over glass fiber filters presoaked in incubation buffer (conventional presoaking in polyethylene imine resulted in elevated background values). Filters were washed twice with buffer, transferred into vials, and measured in a γ counter.

Inhibition Studies Assay. Compounds to be evaluated as competitive inhibitors of ^{131}I -IMTO binding were incubated at concentrations between 0.1 nM and 300 μM . Constant ligand concentration (2 nM) was maintained in all experiments by the addition of IMTO carrier to the ^{131}I -IMTO radiotracer solution immediately before use. Glass vials contained a final volume of 0.5 mL of the incubation buffer (10 mM K_2HPO_4 /10 mM HEPES, 150 mM NaCl, pH 7.1), 20,000–40,000 cpm ^{131}I -IMTO radiotracer solution (2 nM), and the test compounds competing with specific radioligand binding. ETO carrier (10 μM) was used to determine nonspecific binding. The adrenal membrane suspension corresponding to 0.06–0.1 mg of the original tissue per vial was added last. The reaction vials were immersed in a 23°C water bath for 20–30 min. After incubation, membranes with bound radioligand were separated by filtration and filters were washed twice and measured in a γ counter. The cpm (total binding) values were corrected by subtraction of cpm for nonspecific binding. Binding potency was expressed as the IC_{50} value.

Mathematical Analysis. Data from association and dissociation experiments were computer-fitted to the appropriate monoexponential functions. Data from saturation experiments were reciprocally transformed to Eadie–Hofstee plots and subjected to linear correlation analysis. Data from inhibition experiments were computer-fitted to the function $B(x) = (B_0)/(\text{IC}_{50}^{n_H}/(\text{IC}_{50}^{n_H} + x^{n_H}))$, where B_0 is specific binding in the absence of inhibitor, x the inhibitor concentration (nM), and n_H the Hill coefficient. Specific binding (B_0) was obtained by subtracting nonspecific from total binding. K_i values, as an independent measure of the ligand concentration L , can be obtained with the equation of Cheng and Prusoff: $K_i = \text{IC}_{50}/(1 + L/K_D)$; thus, IC_{50} values have to be divided by $1 + 2/11.6 = 1.17$. As a measure of reproducibility, mean values of repeated measurements are given with standard deviations (SD). Statistical comparison between two mean values is done by the Student's t test and between more than two mean values by ANOVA and post hoc Newman–Keuls test.

Cortisol Assay. The human adrenocortical cancer cell line NCI-h295⁵⁸ was obtained from the American type Culture Collection (ATCC, Rockville, MD). Cell medium RPMI 1640 and fetal calf serum (FCS) were supplied by Invitrogen (Eggenstein, Germany). Insulin, transferrin, and selenium were obtained from Sigma (Deisenhofen, Germany). Cortisol radioimmunoassay kits were obtained from DPC Biermann, Bad Nauheim, Germany.

Cells were grown in RPMI 1640 medium supplemented with transferrin, insulin, selenium, and 5% fetal calf serum (FCS) at 37°C under an atmosphere of 5% CO_2 /95% air. For experiments, FCS

was reduced to 2%. Test compounds were added to cell suspensions of 1 million cells in 1 mL at concentrations ranging from 10 nM to 100 μM and incubated for 48 h. Experiments were performed in triplicate. At the end of the incubation period, cells were centrifuged and aliquots (50 μL) of the supernatant were analyzed using a cortisol RIA kit. Cortisol values ($\mu\text{g}/\text{dL}$) were expressed as percent of controls. The control value was based on six determinations without addition of inhibitor. IC_{50} values were calculated using PRISM Graph Pad, version 3.0, San Diego, CA.

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Supporting Information Available: Elemental analysis results, spectroscopic data, and cortisol inhibition data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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