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[$^{123/131}$I]Iodometomidate as a radioligand for functional diagnosis of adrenal disease: synthesis, structural requirements and biodistribution

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Dedicated to the memory of Prof. Dr. Dr. h.c. Gerhard L. Stöcklin

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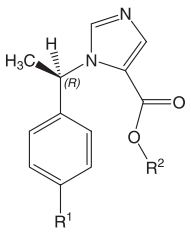
Metomidate / [^{131}I]IMTO / Inhibitor of 11β-hydroxylase / Adrenal scintigraphy / SPECT

Summary. Metomidate [(R)-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylic acid methyl ester] (MTO, **1**, Fig. 1) is a potent and selective inhibitor of the cytochrome P-450 enzyme system in the adrenal cortex. Labelled in the 4-position with radioiodine, (R)-4-[^{131}I]iodometomidate, **2**, [^{131}I]IMTO has been evaluated by *in-vitro* studies and also *ex-vivo* in rats. [^{131}I]IMTO was synthesized by oxidative radioiododestannylation using a suitable precursor which was prepared by a new stereoselective synthesis. Optimization of the labelling reaction was performed by systematic variation of the most important reaction parameters. Under optimum reaction conditions, a labelling yield of 95% was obtained. *In-vitro*-stability of the tracer was studied over 8 days, indicating slow deiodination (0.27%/h). Displacement studies using [^{131}I]IMTO and rat adrenal membranes revealed the structural requirements for high affinity binding, namely an intact ester group and (R)-configuration of the radioligand. Pharmacokinetic studies in rats showed fast accumulation of [^{131}I]IMTO in the adrenals (approx. 10% ID/g tissue) with an activity plateau for 2 hours. Metabolic degradation was indicated by a steady increase of renal activity up to 4 hours post injection. Based on target to non-target ratios the highest contrast for imaging of the adrenals was observed between 30 and 60 min post injection of [^{131}I]IMTO. We conclude that SPECT using [^{123}I]IMTO will be a promising method for the characterization of adrenal incidentalomas.



No.	R^1	R^2	Compound
1	H	Me	Metomidate, MTO
2	I-131	Me	[^{131}I]IMTO
3	H	Et	Etomidate, ETO
4	I	Me	IMTO
5	$SnMe_3$	Me	Precursor
6	H	H	MTO-acid

Fig. 1. Derivates of metomidate.

Introduction

Clinically silent masses at the site of the adrenals, so-called incidentalomas, are detected accidentally in approximately 2% of abdominal CT-scans [1, 2]. The majority of incidentalomas consist of benign hormonally inactive adrenocortical adenoma, or of metastasis from other tumours, cysts, lipomas and pheochromocytomas. Adrenocortical carcinoma (ACC) is a rare but highly malignant neoplasm with poor prognosis. The incidence is approximately 1–2 per million population per year [3] leading to 0.2% of cancer deaths [4].

The available radiocholesterol analogs for adrenal scintigraphy, [^{131}I]iodomethyl-norcholesterol (NP-59) [5] and [^{75}Se]selenomethyl-norcholesterol (Scintadren™) [6] have been especially useful for functional evaluation of benign adrenal disease [1]. However, the very slow accumulation of these tracers requires labelling with a long-lived isotope that leads to a high radiation exposure, and needs patient compliance for several days.

Adrenocortical enzyme inhibitors achieved wide application for the treatment of hypercortisolism [7, 8]. Reports indicated, that inhibitors of the cytochrome P-450 linked 11β-hydroxylase enzyme system bind with high affinity to adrenocortical tissue at the site of enzyme production [9, 10]. As a consequence, several radiolabelled derivatives have been evaluated for medical application, radiolabelled metyrapone being the most thoroughly investigated ligand [11–21]. Since a few years, attention has been focused on etomidate (ETO, **3**), which was described as the most potent inhibitor of steroid 11β-hydroxylation [22]. ETO is a short-acting hypnotic drug used clinically as an anesthetic, which reportedly produced low levels of plasma cortisol in patients receiving long-term infusion to sustain hypnotic activity [23–25]. In-

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deed, it was demonstrated that ETO interacts selectively with the mitochondrial cytochrome P-450 species resulting in an inhibition of steroid 11β-hydroxylase, responsible for the synthesis of cortisol and aldosterone in the adrenal cortex [26].

The first labelled derivative, [^{11}C]MTO was introduced by Bergström and co-workers [27] showing excellent selectivity and high adrenal uptake in Rhesus monkey with PET. Clinical data on the differential diagnosis of lesions of the adrenals with PET have also been reported [28]. [^{11}C]MTO visualizes non-necrotic adrenocortical carcinoma and their metastases with high contrast, but due to the limitations posed by C-11, labelling with longer-lived isotopes was suggested [29]. Consequently, 2-[^{18}F]fluoro-ETO ([^{18}F]FETO) was recently introduced as a radiotracer for PET [30, 31].

In order to extend the application of the highly specific ligand MTO, it was the aim of this study to develop (R)-4-[^{123}I]iodometomidate ([^{123}I]IMTO) as a tracer for SPECT. For characterization of the radiotracer we used the longer-lived and inexpensive I-131 as a label.

Experimental

Reagents and materials

Solvents, reagents, ETO, metyrapone, ketoconazole, and deoxycorticosterone were purchased from commercial sources in the highest purity available. ^{1}H-NMR spectra were recorded on a Bruker AM 400 spectrometer in $CDCl_3$ using the residual solvent peak as internal reference [$\delta(CHCl_3) = 7.24$]. Chemical shifts, δ, are given in parts per million. 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, 0.46×25 cm. Optical rotations were measured at 20 °C on a Perkin–Elmer 341 polarimeter in a 1 dm cell. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

N.c.a. [^{123}I]NaI and [^{131}I]NaI in 0.02 N NaOH were purchased from Research Center Karlsruhe, Germany and Nycomed Amersham Buchler, Germany, respectively.

GF 52 glass fiber filters were obtained from Schleicher & Schuell, Germany.

Chemistry

For the synthesis of a suitable precursor **5** and standard **4** a new stereoselective synthesis was developed, which is based on coupling of two building blocks (Fig. 2). The racemic alcohol **7** was chloroacetylated and the corresponding racemic ester **8** resolved enzymatically in a biphasic system using lipase SAM II [32]. Alcohol (R)-**7** and the ester (S)-**8** were separated and the latter saponified yielding the desired alcohol (S)-**7**. The most crucial step of the synthesis was the coupling of the alcohol (S)-**7** with methyl 1*H*-imidazole-5-carboxylate using the Mitsunobu-reaction [33, 34] at low temperature yielding IMTO **4**. Similar, (S)-MTO was prepared from (R)-1-phenylethanol. Finally, the exchange of iodine for the trimethylstannyl substituent was catalysed by a palladium complex and furnished the precursor **5**.

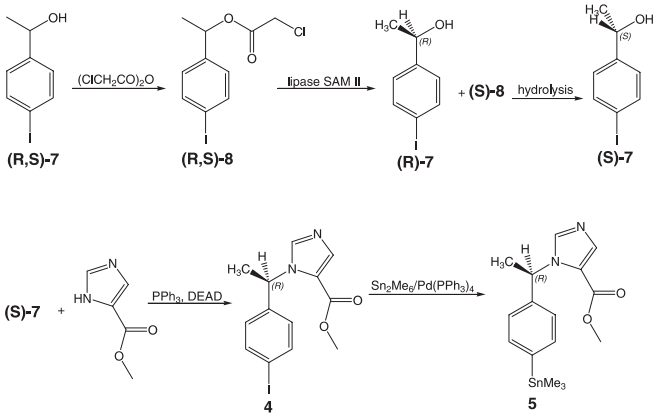

Fig. 2. Synthetic route to IMTO **4** and the stannylated precursor **5**.

(R,S)-1-(4-Iodophenyl)ethyl chloroacetate (R,S)-8

Dry pyridine (6.0 mL) and chloroacetic anhydride (6.2 g, 36.26 mmol) were added to a stirred solution of (R,S)-1-(4-iodophenyl)ethanol (5.95 g, 24.0 mmol) in dry dichloromethane (100 mL) at 0 °C under an atmosphere of argon. When the reaction was finished (2 h), water (40 mL) and concentrated hydrochloric acid (3.6 mL) were added. After stirring for 10 min, the organic phase was separated and the aqueous phase was extracted with dichloromethane (3×15 mL). The combined organic phases were washed with water (50 mL) and a saturated solution of sodium hydrogen carbonate (25 mL), dried (sodium sulfate) and evaporated under reduced pressure. The residue was purified by flash chromatography (hexane-dichloromethane 3 : 2) on silica gel and bulb to bulb distillation (0.1 mm Hg/105 °C) to give (R,S)-1-(4-iodophenyl)ethyl chloroacetate (7.09 g, 91%) as a colourless liquid, which crystallised spontaneously; mp 50–51 °C. ^{1}H-NMR 1.54 (3H, d, CH_3CH), 4.03 (2H, d, CH_2Cl), 5.90 (1H, q, CH_3CH), 7.08 (2H, d, $2 \times H_{arom}$), 7.68 (2H, d, $2 \times H_{arom}$).

Enzymatic hydrolysis of (R,S)-1-(4-Iodophenyl)ethyl chloroacetate (R,S)-8

Racemic 1-(4-iodophenyl)ethyl chloroacetate (0.835 g, 2.57 mmol), *t*-butyl methyl ether (4 mL), phosphate buffer (50 mmol, sterile, 17 mL) and lipase SAM II (96 mg) were stirred vigorously at room temperature. The pH was kept constant at 7.0 by addition of 0.5 N sodium hydroxide using an autotitrator.

98% of the calculated amount of base were consumed in 2.6 h. The reaction was stopped after another 14 h by adjusting the pH to about 2.0 using 2 N hydrochloric acid. Water (100 mL) was added and ester and alcohol were extracted with dichloromethane (3×200 mL). The combined organic layers were washed with water and a saturated solution of sodium hydrogen carbonate (50 mL each), dried (sodium sulfate), and evaporated under reduced pressure to leave a residue which was purified by flash chromatography (hexane-dichloromethane 3 : 2 for chloroacetate, hexane-dichloromethane 1 : 2 for alcohol) on silica gel to give (S)-1-(4-iodophenyl)ethyl chloroacetate (0.367 g, 44%, $[\alpha]_D^{20} = -82.57$ (c 2.57 in acetone), ee = 98% and $[\alpha]_D^{20} = -35.68$ (c 2.04 in acetone) after chemical hydrolysis as a liquid

and (R)-alcohol (0.393 g, 43%, ee 98%; $[\alpha]_D^{20} = +35.92$ (c 1.96 in acetone) before crystallisation from petroleum ether (40–60 °C)-dichloromethane, afterwards $[\alpha]_D^{20} = +35.95$ (c 2.05 in acetone); mp 48–49 °C as a crystalline solid.

(S)-1-(4-Iodophenyl)ethanol (S)-7

(S)-1-(4-iodophenyl)ethyl chloroacetate **(S)-8** (0.340 g, 1.05 mmol) was dissolved in methanol/sodium methoxide (17 mL, obtained by dissolving 69 mg of sodium in 30 cm^3 of dry methanol). After 1 h, water (few drops) was added and the solution was concentrated under reduced pressure. Water (30 mL) and dichloromethane (15 mL) were added. The organic layer was separated and the aqueous one was extracted with dichloromethane (2×15 mL). The combined organic layers were dried (sodium sulfate) and evaporated to leave a residue, which was purified by flash chromatography (hexane-dichloromethane 1 : 2) to give **(S)-7** (0.240 g, 92%, ee $\geq$ 98%) as a crystalline solid.

(R)-(+)-Methyl 1-[1-(4-iodophenyl)ethyl]-1*H*-imidazole-5-carboxylate 4

A solution of (S)-alcohol **(S)-7** (1.98 g, 7.98 mmol, ee > 98%) in dry THF (14.5 mL) was added dropwise to a stirred solution of methyl 1*H*-imidazole-5-carboxylate (1.008 g, 7.98 mmol) and triphenylphosphane (2.503 g, 9.43 mmol) in dry THF (22.0 mL) in an atmosphere of argon at −30 °C. Then, a solution of di-*t*-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 °C to 0 °C within 2.5 hr. No alcohol could by 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 (triphenylphosphaneoxide and hydrazo ester) were collected and washed with diethyl ether (3×15 mL). The filtrate was evaporated under reduced pressure to leave a residue, which was purified by flash chromatography (hexane-diethyl ether-diisopropylamine 50 : 30 : 1) on silica gel to give IMTO **4** (1.91 g, 67%, ee 99%); $[\alpha]_D^{20} = +76.0$ (c 1.09 in acetone). ^{1}H-NMR 1.81 (3H, d, CH_3CH), 3.77 (3H, s, OCH_3), 6.26 (1H, q, CH_3CH), 6.88 (2H, d, $2 \times H_{arom}$), 7.63 (2H, d, $2 \times H_{arom}$), 7.73 (1H, s, $H_{hetarom}$), 7.75 (1H, d, $H_{hetarom}$).

(R)-(+)-Methyl 1-[1-(4-trimethylstannylphenyl)ethyl]-1*H*-imidazole-5-carboxylate 5

Hexamethylditin (0.645 g. 3.2 mmol, 6.5 mL of a solution of 1.0 g hexamethylditin in 10 mL of dry toluene), tetrakis(triphenylphosphane)palladium (58 mg, 5 mol %) and triethylamine (1.6 mL, 11.6 mmol) were added to a stirred solution of IMTO **4** (0.368 g, 1.03 mmol, ee > 98%) in an atmosphere of argon and refluxed (bath temperature 135 °C) for 17 hr. The cooled solution was concentrated under reduced pressure and the residue was purified by flash chromatography (hexane-diethyl ether-diisopropylamine 60 : 30 : 1) on silica gel to give **5** (0.377 g, 96%) as a crystalline solid mp 77–79 °C (from hexane); $[\alpha]_D^{20} = +82.09$ (c 2.06 in acetone).

^{1}H-NMR 0.25 (9 H, s, $(CH_3)_3Sn$, $^{117/119}$Sn satellites), 1.82 (3H, d, CH_3CH), 3.77 (3H, s, OCH_3), 6.30 (1H, q, CH_3CH), 7.13 (2H, d, $2 \times H_{arom}$, $^{117/119}$Sn satellites), 7.43 (2H, d, $2 \times H_{arom}$, $^{117/119}$Sn satellites), 7.71 (1H, s, $H_{hetarom}$), 7.74 (1H s, $H_{hetarom}$).

(R)-(+)-Methyl 1-[1-phenylethyl]-1*H*-imidazole-5-carboxylate (MTO, 1)

A solution of ETO (0.10 g, 0.405 mmol) in dry methanol/sodium methoxide (5 mL, 1 M) was kept for 5 h at room temperature. After neutralisation with acetic acid, the solution was concentrated under reduced pressure, diluted with water (2 mL) and extracted three times with ethyl acetate. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to leave a residue, which was purified by flash chromatography (diethylether/diisopropylamine 10/1) on silica gel to give MTO (0.066 g, 71%) as a viscous oil; $[\alpha]_D^{20} = +77.09$ (c 1.1, acetone), spectroscopically identical with an authentic sample of **1**.

(S)-(-)-Methyl 1-[1-phenylethyl]-1*H*-imidazole-5-carboxylate (S-MTO, S-1)

(R)-(+)-1-Phenylethanol (0.244 g, 2 mmol, ee 99% by HPLC) was transformed into (S)-MTO using the Mitsunobu reaction under the conditions described above; flash chromatography (hexane/diethylether/diisopropylamine 60/30/1) gave 0.237 g (51%) of viscous product; $[\alpha]_D^{20} = -78.36$ (c 2.075, acetone); ee 99%, spectroscopically identical with an authentic sample of the (R)-(+) enantiomer of MTO.

(R)-1-(1-Phenylethyl)-1*H*-imidazole-5-carboxylic acid (MTO-acid, 6)

6 was obtained by hydrolysis of ETO in approx. 10 N NaOH solution as previously described [23]: mp 156.3 °C; $[\alpha]_D^{20} = +62.8$ (c 1.0 in water).

Radiosynthesis of [$^{123/131}$I]IMTO

Labelling is performed in a sealed conical vial containing 30 μg of the stannylated precursor in 30 μL ethanol and [$^{123/131}$I]NaI in 0.02 N NaOH. To initiate the reaction 6 μL 1 N HCl and 10 μL chloramine-T (1.5 mg/mL) were added. The reaction was allowed to proceed at room temperature for 1 min and quenched by the addition of 6 μL 1 N NaOH.

For HPLC-determination of the specific activity of radiolabelled IMTO a calibration curve (linear regression analysis of mass from solutions of known IMTO-concentrations versus peak area of UV-absorption) was plotted. Due to the low detection limit (1.5 ng, 4 pmol) of IMTO at 254 nm this wavelength was chosen without recording a UV spectrum.

HPLC system

Radio-HPLC was performed on a system consisting of a Shimadzu pump (LC-10AT) and a Shimadzu UV/vis

detector (SPD-10A) with a wavelength of 254 nm. Sample injection was accomplished by a Rheodyne-Injector-block (7725i). For measurement of radioactivity the outlet of the UV detector was connected to a NaI(Tl) scintillation detector (Berthold LB 1200) and the recorded data was processed by a software system (Shimadzu Class-VP). Purification and quality control of [^{131}I]IMTO was performed using a C18 phase (Nucleosil 100-7 250×4.6 mm, CS Chromatographie Service, Langerwehe, Germany), eluent: CH_3OH/H_2O/diethylamine 60/40/0.2 v/v/v, flow: 1.5 mL/min. Capacity factor of [^{131}I]IMTO: $k' = 6.8$.

Formulation

The [^{131}I]IMTO containing HPLC fraction was evaporated to dryness at room temperature under reduced pressure. Heating during this step should be strictly avoided in order to suppress deiodination and to maintain high radiochemical purity. For i.v.-injection, the residue was redissolved in a suitable volume of saline and passed through a sterile 0.22 μm Millipore filter into a sterile vial.

In-vitro stability

To the HPLC eluate containing [^{131}I]IMTO different amounts of ascorbic acid were added, because for this antioxidant a stabilizing effect on [^{131}I]MIBG-solutions was described [35]. The product was formulated as before and stored for one week at +4 °C. Aliquots (100 μL) were examined at daily intervals for free iodide using HPLC-analysis.

Binding assay

Whole adrenals from adult male Wistar rats were homogenized in 100 parts of homogenisation buffer (10 mM K_2HPO_4/10 mM HEPES, pH 7.1, 4 °C) with a glass/teflon homogenizer (Potter-type). The homogenate was centrifuged at $35\,000 \times$ g for 10 min at 4 °C, the pellet was resuspended in 100 parts fresh buffer and centrifuged again. Membranes were washed twice (the first washing step included a 10 min warming to 37 °C) and stored as aliquots at −80 °C. For binding experiments, a sufficient quantity of suspension was thawed, diluted in incubation buffer (10 mM K_2HPO_4/10 mM HEPES, 150 mM NaCl, pH 7.1) and centrifuged once more.

Glass vials containing 0.5 ml of incubation buffer, 20 000–40 000 cpm [^{131}I]IMTO together with 2 nM IMTO as carrier (*i.e.* 1 pmol/vial, resulting in a specific activity of 330–660 GBq/mmol), and the adrenal membrane suspension corresponding to 0.06 mg tissue/vial, were immersed in a 23 °C water bath for 20–30 minutes. After incubation, membranes with bound radioligand were isolated by filtration through glass fibre filters (presoaked in buffer), followed by 2×4 mL washings with buffer, then filters were measured in a gamma-counter. Compounds to be evaluated as competitive inhibitors of [^{131}I]IMTO binding were incubated at 0.01–100 nM concentrations. ETO carrier (10 μM) was used to determine non-specific binding. Inhibition data were fitted by non-linear, least squares regression analysis (Origin, Microcal, Inc.) to the formula $B(x) = B_o \times IC_{50}^{n_H} / (IC_{50}^{n_H} + x^{n_H}) + \mathrm{NB}$, where B_o is total binding in the absence of inhibitor, n_H the Hill coefficient, x the inhibitor concentration, and NB is non-specific binding. From these IC_{50} values, K_i values were obtained by the equation: $K_i = IC_{50}/(1 + L/K_D)$, where L is 2 nM (the concentration of the radioligand) and K_D is 7.4 nM.

Biodistribution of [^{131}I]IMTO

Biodistribution studies were performed according to the National Institutes of Health principles of laboratory animal care [36] and Austrian national law, approved by local government (Permit No. VI/4-TVG-41).

[^{131}I]IMTO with a radiochemical purity > 99% and a specific activity of 1.0 GBq/μmol was used. The radiotracer (0.5–1.1 MBq) was injected into the tail vein of Wistar rats (female, 180–220 gram). Groups of four rats were bled and sacrificed under ether anesthesia at specified times (10, 30, 60, 120 minutes, 4, 6, 18 and 24 hours) post injection. Blood and twelve organs, namely heart, lung, liver, jejunum, caecum, large intestine, stomach, spleen, kidneys, adrenals, ovaries, and thyroid were excised and weighed. The radioactivity was measured at constant geometry using a gamma-counter with a NaI(Tl)-crystal. Results were expressed as percentage of injected dose per organ (%ID/organ) and per gram of tissue (%ID/g) [37].

Toxicology

The toxicology study of IMTO was performed by BSL Bioservice, Planegg, Germany and followed internationally accepted guidelines and recommendations. 5 male and 5 female mice were treated in a single exposition with 2.0 μg/kg bw of IMTO by intravenous injection. This dosing regime ensured a dosage per animal which is the 100-fold of the expected dosage in clinical use. A careful examination was made once a day. At the end of the observation period the animals were sacrificed and necroscopy was carried out to record gross pathological changes.

Additionally, a reverse mutation assay using bacteria (Ames-test) was performed with a maximum of 100 μg/plate, corresponding to the 100-fold clinical dose.

Results and discussion

Radiosynthesis

The radiosynthesis of [^{131}I]IMTO *via* an oxidative radioiododestannylation reaction was optimized with regard to the most important reaction parameters. Highest radiochemical yields of 95% were obtained with very small amounts of chloramine-T (> 1.0 μg) and the stannylated precursor (Fig. 3) and enabling a clean HPLC-separation using analytical columns. Even 1.0 μg of the stannylated precursor were sufficient for obtaining high RCY; optimal RCY were obtained by the use of 30 μg of the tin compound. The labelling should be performed at a pH of 0.5–2.0 (Table 1) within a very short reaction time of > 30 s at room temperature. Most investigated oxidizing agents provided high radiochemical yields, except for a mixture of the enzymes lactoperoxidase and glucose oxidase (25%–30% RCY). Chloramine-T and potassium iodate

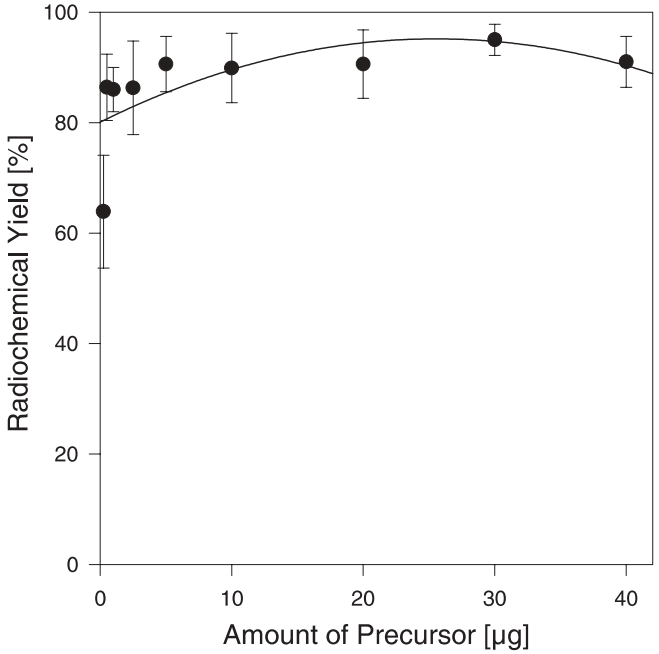

Fig. 3. Effect of the amount of precursor on the RCY of [131I]IMTO; pH = 1, 15 µg Chloramine-T, 3 min at RT, $n = 3$.

Table 1. Effect of pH on RCY of [131I]IMTO; 30 µg precursor, 15 µg Chloramine-T, 1 min RT, $n = 3$.

pH	RCY [%]
0.0	43.8 ± 8.4
0.5	94.7 ± 3.3
1.0	95.1 ± 2.8
1.5	95.3 ± 2.4
2.0	92.7 ± 2.9
3.0	51.7 ± 13.1
4.0	1.9 ± 0.8

(95% RCY) were slightly superior compared to Iodogen and peracetic acid (85%–90% RCY). Finally we chose chloramine-T for labelling because potassium iodate is a potential source of iodide carrier. The observed specific activities of 50 GBq/µmol ([123I]IMTO) and 1.0 GBq/µmol ([131I]IMTO) and the radiochemical purity > 97% were sufficiently high for the performance of *in-vitro* and *in-vivo* studies.

In-vitro stability

HPLC-analysis of formulated [131I]IMTO ($n = 2$) demonstrated a linear and considerable loss of radioiodine with time (0.27% per hour). Addition of 2 mg of ascorbic acid ($n = 2$) had no significant stabilizing effect, while the addition of 10 mg of ascorbic acid ($n = 1$) surprisingly increased deiodination considerably. However, *in-vitro* studies with [131I]IMTO showed only a slight increase of background activity over time.

Radioligand binding

Typical displacement curves are presented in Fig. 4. The calculated IC_{50} values are listed in Table 2 and indicate the

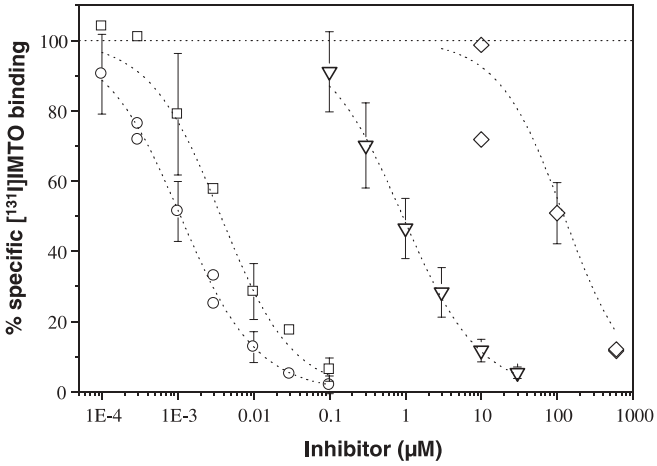

Fig. 4. Inhibition of specific [131I]IMTO binding by ETO (circles), MTO (squares), 11-deoxycorticosterone (triangles), and free acid (diamonds), data pooled from 4–11 experiments.

Table 2. Displacement of [131I]IMTO by test compounds.

Ligand	IC_{50}-values (nM)	(n)
(R)-Metomidate (MTO, **1**)	3.69 ± 1.92	(6)
(S)-MTO, **S-1**	492 ± 2.81	(4)
(R)-IMTO, **4**	8.98 ± 3.72	(15)
(R)-Etomidate (ETO, **3**)	1.08 ± 0.42	(11)
MTO-acid **6**	123 000 ± 41 000	(3)
Metyrapone	1160 ± 790	(5)
Ketoconazole	710 ± 490	(6)
Deoxycorticosterone	890 ± 330	(5)

structural requirements for high affinity binding. The intact ester function was essential for binding, cleavage of the ester resulted in deactivation, the free acid (MTO-acid) was practically inactive ($IC_{50} = 123$ µM). (R)-configuration of the chiral C-atom increased specifity, (S)-configuration resulted in a much lower affinity ($IC_{50} = 492$ nM). Limited modification of the ester was well tolerated (MTO: $IC_{50} = 3.7$ nM; ETO: $IC_{50} = 1.1$ nM). Substitution with iodine resulted in a slight loss of affinity (IMTO: $IC_{50} = 9.0$ nM). Metyrapone and ketoconazole, two clinically used inhibitors of hypercortisolism, showed micromolar activity ($IC_{50} = 1.2$ µM and 0.71 µM, respectively) when tested with this assay. The IC_{50} value of deoxycorticosterone, the natural substrate, indicated similar inhibitory potency.

Biodistribution of [131I]IMTO

[131I]IMTO showed high specific uptake in the adrenals, approx. 10% ID/g tissue, with a radioactivity plateau lasting for at least two hours (Fig. 5). The radiotracer is eliminated by hepatobiliary and renal excretion, indicated by increasing radioactivity in the intestinal tract and in the kidneys. Renal activity is attributed to [131I]IMTO-acid, which results from enzymatic cleavage of the methyl ester. The renal activity reached a maximum 4 hours post injection, namely 11% of the injected dose. Uptake values two hours post injection expressed as a percentage of the administered dose are: liver 3.7 ± 1.8%, jejunum 12.3 ± 2.9%, stomach 1.1 ± 0.2%, kidneys 5.3 ± 4.8%, blood 15.2 ± 4.5%, and lung

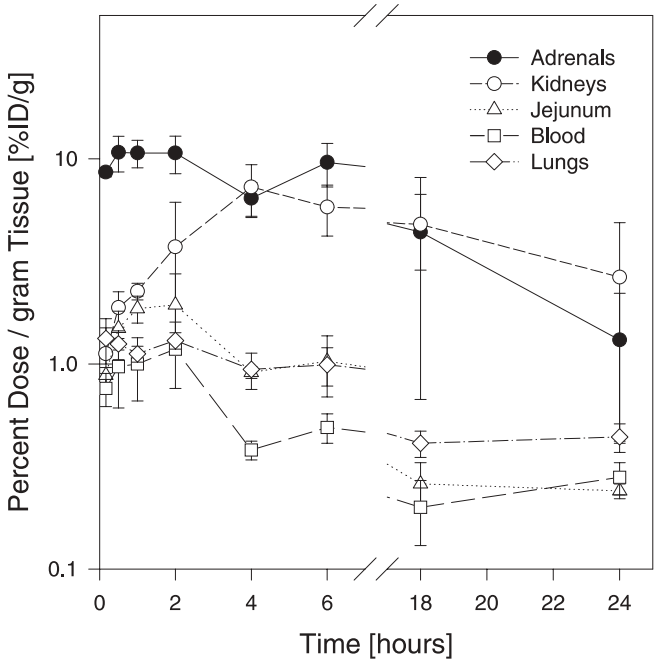

Fig. 5. Distribution of [^{131}I]IMTO in rats.

Table 3. Target/non-target ratios obtained with [^{131}I]IMTO.

Organ	10	30	60	120 (min)
Adrenal/Kidney	7.7	5.7	4.7	2.9
Adrenal/Liver	8.4	13.9	19.7	15.5
Adrenal/Jejunum	9.8	7.2	5.7	5.5
Adrenal/Blood	11.3	11.1	10.7	9.0

$1.5 \pm 0.3\%$. Organs such as heart, caecum, large intestine, spleen, ovaries, and the thyroid, accumulated less than 1 percent of the injected dose. The elimination of the radiotracer from organs and whole body is described by an effective half-time of 19.9 hours. 36.2% of the radioactive dose is retained in the body 24 hours after injection. Whole-body elimination of radioactivity indicated a slow release caused mainly by metabolization and deiodination of the radiotracer.

Based on calculations of the target-to-non-target concentration ratios the highest contrast for imaging of the adrenals is observed up to 2 hours post injection (Table 3).

Toxicology

The IMTO-dosages of 2 μg/kg bw caused no compound-related mortality either in the five female or in the five male animals within 14 days post-dose. All animals survived throughout the test period showing normal food intake and weight gain. No weight loss was recorded. At necroscopy no evidence of gross pathology of organs was found. In the Ames-tests no signs of mutagenicity were observed.

Conclusions

The selective P-450c11 inhibitor MTO was labelled with I-131 and I-123 in high yield and purity. The required precursor and standard compounds were synthesized by a new stereoselective synthesis.

[^{131}I]IMTO served as a selective radioligand for the characterization of structurally modified MTO derivatives and related compounds. The high binding potency of MTO was shown to reside exclusively in the R-isomer, the ester group being essential for binding. Compounds used clinically to block the adrenal biosynthesis of cortisol were shown to displace [^{131}I]IMTO binding. Evaluation of the *in-vivo* kinetics of [^{131}I]IMTO revealed specific adrenal uptake offering highest contrast for adrenal imaging within two hours after administration. During this period there is no interference of renal activity; on the contrary, renal activity might facilitate adrenal localization. The results of this study indicate that rapid and selective adrenal uptake of [^{131}I]IMTO is based on specific binding to adrenocortical tissue. The excellent characteristics of the radiotracer encourage us to evaluate [^{123}I]IMTO for adrenal scintigraphy in a patient study in the near future.

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