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Tc and Re Chelates of 8α-Amino-6-methyl-ergoline: Synthesis and Affinity to the Dopamine D_2 Receptor

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Abstract—The influence of structural changes at the 8α-amino position of 8α-amino-6-methyl-ergoline on the lipophilicity and affinity to the D_2 receptor was studied. 8α-amino-6-methyl-ergoline (**1**) was converted into the derivatives (**2a**–**f**) by mercaptoacetylation of the amino group to make it possible to prepare the rhenium and technetium complexes (**3**, **4a**,**b**). Binding tests on cloned human dopamine D_2 receptors show that the affinities of the coordination compounds (IC_{50} values between 50 and 240 nM) are less than those of the derivatives **2a**–**f** (IC_{50} = 3–50 nM) but more than those of the parent compound **1**. Biodistribution studies of the Tc complexes **4a**,**b** performed on Wistar rats show a slow blood clearance with substantial accumulation and retention in the liver and kidneys and low brain uptake.

Introduction

The ergot alkaloids,[1,2] derivatives of lysergic acid or ergoline structures exhibit a broad range of pharmacological acitivities. Many of them are dopamine D_2 receptor agonists,[3,4] but some ergoline derivatives also interact with serotonin receptors[5–7] and adrenoceptors[8,9] with very high affinities. The tetracyclic structure of the ergolines contains the essential features of the monoamine neurotransmitters norepinephrine, dopamine and serotonin. This structural relationship may contribute to the ability of many naturally occuring and synthetic ergot alkaloids to act as agonists or antagonists at receptors for these neurotransmitters.

This observation stimulated the search for partial structures and derivatives with the aim to improve pharmacological selectivity. Numerous modifications were carried out at the 8-position of ergoline. They produced, for example, the alpha-ureido derivative terguride[10] with a high D_2 activity. The high receptor affinities of the ergolines were also examined for the design of potential receptor-binding radiotracers, for example [^{123}I]2-iodolisuride, which was successfully used as a SPECT imaging agent in the study of healthy volunteers and patients.[11] [^{76}Br]bromolisuride was evaluated in baboons as a potential tool for quantitative in-vivo imaging of D_2 receptors, using positron emission tomography.[12]

Many efforts were and are being made to design receptor binding technetium tracers in view of the excellent nuclide properties and wide availability of the isotope ^{99m}Tc. To gain access to such tracers, a receptor-binding organic structure is combined with a small Tc chelate unit.[13] However, the introduction of a technetium chelate causes drastic alterations in the molecular and biological properties of the lead structure having the required receptor affinity. Studying the influence that structural modifications required for binding technetium may have on the binding to the receptor in question is therefore of basic interest.

The general pharmacological activity observed for many derivatives favours the ergoline moiety as a subject of such investigations. As deduced from terguride (Fig. 1) and a series of other *N*-substituted derivatives, the free amino group should be blocked without drastically alterating the receptor-binding ability. The 8-amino group of 8α-amino-6-methylergoline was therefore chosen as a suitable position in which to perform structural modifications. After reporting on the preparation and molecular structure of the oxorhenium(V) complex,[14] we now describe the functionalization of the ergoline moiety with a 2-mercaptoacyl group and the subsequent

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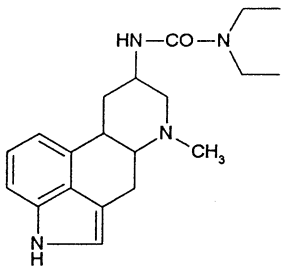

Figure 1. Terguride.

synthesis of neutral mixed-ligand oxotechnetium(V) and oxorhenium(V) complexes containing 8α-amino-6-methylergoline as a pendant group. Using terguride as a reference molecule, we investigated the effects on lipophilicity and affinities to the D_2 receptor of the derivatives that the introduction of the chelate unit in the parent 8α-amino-6-methylergoline would produce.

Results and Discussion

Chemistry

For coupling the metal to the ergoline moiety the '3 + 1' mixed-ligand concept was used.[15] In the '3 + 1' approach, a mercapto group has to be introduced into the ergoline for binding to the oxometal(V) core, while the remaining free coordination sites at the metal should have an additional tridentate chelate ligand, thus forming a neutral MO/monodentate/tridentate complex. To introduce the required SH-functionality, the parent 8α-amino-6-methylergoline (**1**) was converted into the S-benzoylated derivatives **2a** by condensation of the amino group with benzoylmercaptoacetic acid. Removal of the protective group by sodium methylate yielded the free mercaptane **2c**, from which the methylated derivative **2e** resulted. The derivatives with R = methyl (**2b**, **2d**, **2f**) were obtained, using 2-benzoylmercaptopropionic acid instead of benzoylmercaptoacetic acid (Fig. 2).

For preparation of the complexes, the benzoyl group was removed immediately before complexation. The resulting mercaptanes **2c**,**d** were used directly without purification. The complexes were prepared in solid form from the precursor molecules [ReO(SSS)Cl][16] and [TcO(SSS)Cl].[17] These species contain the preformed oxometal-chelate unit, from which the ergoline-derivatized complexes are obtained as solids after simply replacing the chlorine by the mercaptide ligand **2c** and **2d**. The synthesis of the rhenium complex **3** was described before.[14] The corresponding technetium complexes **4a**,**b** were obtained in mg amounts and their composition was confirmed by ^{1}H NMR spectroscopy. The technetium complexes **4a**,**b** were also prepared in solution by a two-step procedure, starting with the reaction of the ergolines **2c**,**d** as the monodentate ligand with Tc(V) gluconate, and subsequent addition of the tridentate thiolate ligand HS–CH_2CH_2–S–CH_2CH_2–SH (HSSSH). The complexes were separated by HPLC and identified by comparison of the Rt values and TLC data with those of the isolated ^{99}Tc complexes (Fig. 3).

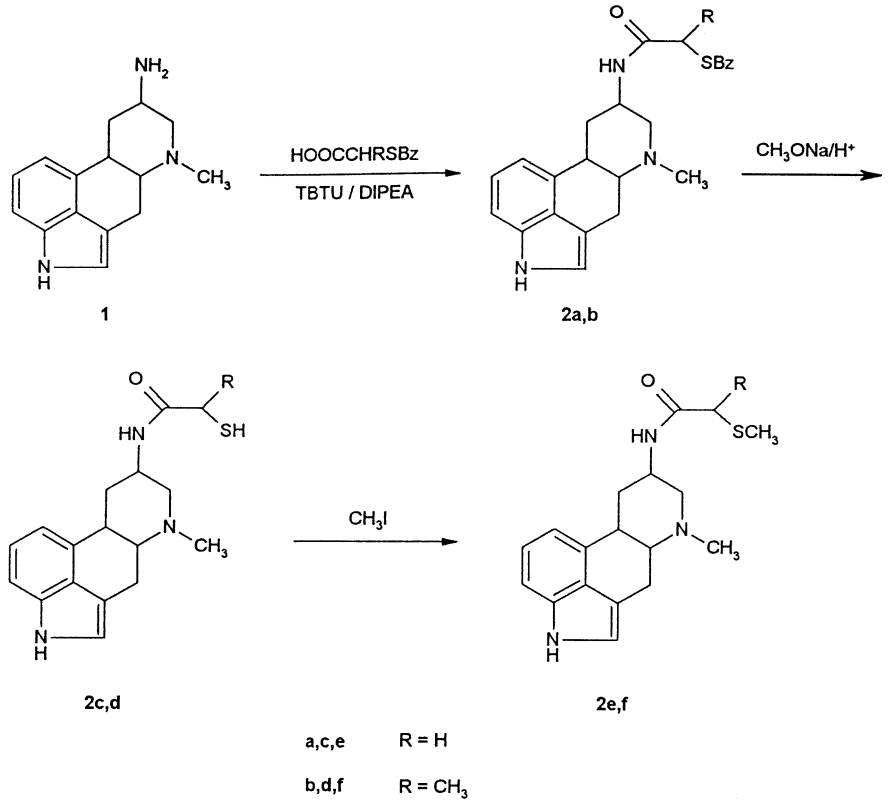

Figure 2. Reaction scheme for the preparation of derivatives **2a**–**f** of 8α-amino-6-methyl-ergoline.

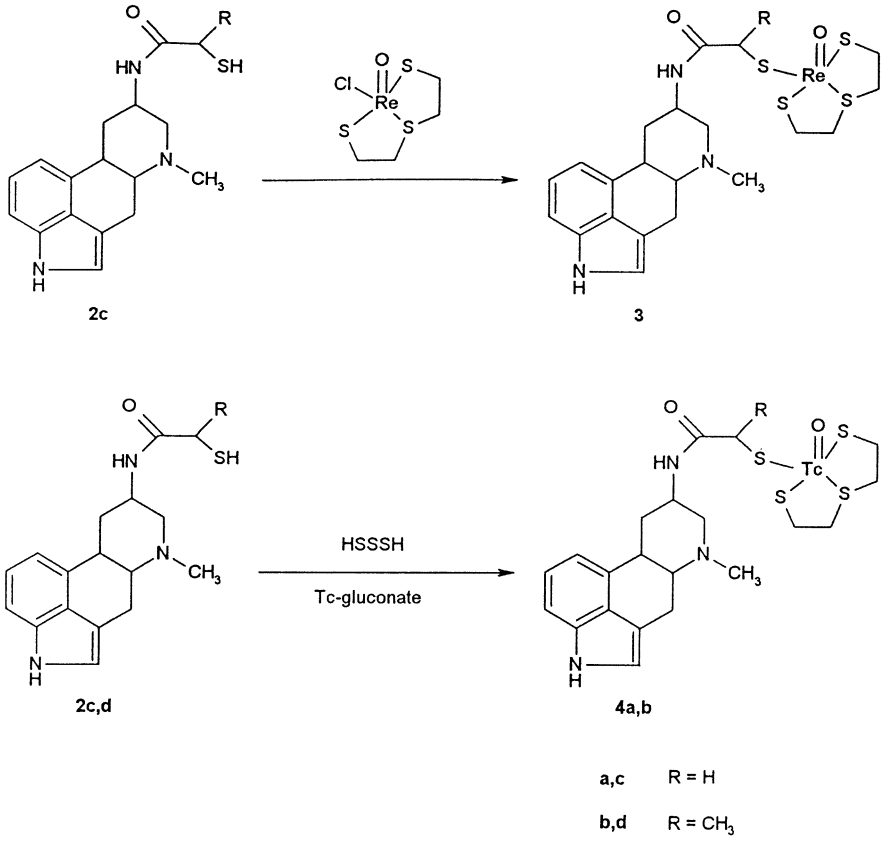

Figure 3. Reaction scheme of the preparation of Re complex (**3**) and Tc complexes (**4a,b**) derived from **1**.

Lipophilicity Data

The lipophilicity data P_{HPLC} were determined for the derivatives **2a**–**f** as well as for the chelate-bearing species **3**,**4a**,**b**. While the P_{HPLC} value of the dimethylcarbamoyl derivative, terguride, was comparable to that of 8α-amino-6-methylergoline **1**, the derived compounds (**2a**–**f**) showed P_{HPLC} values exceeding those of **1** by about one order of magnitude. The highest values were found for compounds containing the S-protecting benzoyl group **2a**,**b**, while the chelate group containing ergolines show relatively moderate lipophilicity. A remarkable enhancement of the P_{HPLC} values was due to the influence of the methyl group located at the mercapto acyl bridge.

Table 1. Lipophilicity values (P_{HPLC}) and affinity to the dopamine D_2 receptor (IC_{50} values) of ergoline derivatives

Compound	P_{HPLC}	IC_{50} (nM)
Terguride	5	4.8
1	4	1730
2a	80	10.7
2b	252	2.7
2c	n.d.	42.9
2d	n.d.	45.0
2e	8	22.1
2f	15	17.2
3	33	139
4a	45	235
4b	70	54

Affinity to the D_2 Receptor

The main point of interest was the question as to how the introduction of the chelate unit influences the affinity to the dopamine D_2 receptor. From the literature,[1,2,18] it is obvious, that bulky substituents at the 8-amino position increase the affinity. While the affinity of the parent 8α-amino-6-methylergoline is in the μmolar level (Table 1), many bulky group-bearing ergolines show high affinities.[18–20] For instance, the ureido derivative terguride, which serves as a reference molecule in our studies, shows an affinity to the D_2 receptor at the nanomolar level, as previously shown.[6,20]

Our experiments with 8α-amino-6-methylergoline modified by Tc and Re chelates reveal that the non-chelate species **2a**–**f** have moderate to high affinities to cloned human dopamine D_2 receptors (Table 1). Functionalization at the amino group in the 8α-amino-6-methylergoline drastically increases the affinity, resulting in IC_{50} values between 3 and 45 nM. The benzoyl derivative **2b** has an even better affinity than the reference molecule terguride. The introduction of a chelate into this molecule (complexes **3**, **4a**,**b**) results in a decrease of affinity. The steric requirements of the chelate unit are obviously not suited to matching the ergoline moiety and producing highly affine species, although the affinities of this coordination species are higher (IC_{50} values between 54 and 240 nM) than those of the parent compound **1**.

Another remarkable observation is the fact that both the lipophilicity and the receptor affinity are very sensitive to

Table 2. Biodistribution (% injected dose/organ) of Tc complexes **4a** and **4b** in male Wistar rats (means; $n=3$)

Complex	Min pi	Brain	Lung	Kidney	Liver	Blood
4a	10	0.17	2.5	7.7	24.9	38.7
	60	0.16	2.4	21.2	24.7	27.9
4b	10	0.13	1.8	10.1	23.2	25.2
	60	0.13	1.2	10.9	21.9	16.2

structural changes at the mercaptoacyl bridge. This can be clearly seen in the influence of a methyl group on the pairs **2a**,**b**, **2c**,**d**, **2e**,**f**, and **4a**,**b**.

Biodistribution Studies

Biodistribution studies of the technetium complexes in rats (Table 2) show a high blood pool activity and a slow blood clearance with substantial accumulation and retention in liver and kidneys. A long plasma half-life was also described for other ergoline derivatives such as transdihydrolisuride, bromerguride, terguride and cabergoline.[21–24] It was observed that the elimination of terguride occurs mainly in the urine,[23] and lisuride is also eliminated via the kidneys and liver.[25] The brain uptake of the Tc complexes **4a**,**b** is low. The brain uptake as well as the slow brain clearance of the complexes seem to indicate their capability of crossing the blood–brain barrier of rats and being trapped in the brain.

Conclusions

The experiments were aimed at studying, at a pharmacologically potent class of compounds, the influence of the structure of ergoline conjugates on their lipophilicity and their binding to the D_2 receptor. The general pharmacological activity observed for many derivatives favours the ergoline moiety for such investigations. The experience gained from studying many derivatives shows that a room-filling group is a necessary condition for dopamine D_2 affinity. It is therefore reasonable to bind the chelate to the external amino group. Step-by-step binding, first of the mercaptoacetyl groups and then of the Tc/Re chelates to amino ergoline produced derivatives whose molecular weight and steric requirements increased in this order. Compared with references **1** and terguride, the lipophilicity of all derived species was enhanced by one to two orders of magnitude. The observations concerning affinity made in our experiments with derivatives (**2a**–**f**) were consistent with our expectations. The affinity (C_{50} values) was much higher for 8α-amino-6-methylergolines functionalized with small substituents than for ergoline with the free amino group (**1**) and seemed to increase with increasing size of the derivatizing group. Surprisingly, one derivative (**2b**) showed a higher affinity than the reference molecule terguride.

The steric tolerance of the ergoline molecule seems, however, to be overtaxed by the chelate, since its introduction results in a loss of activity. The binding data of the derived complexes of rhenium (**3**) and technetium (**4**) are nevertheless higher than those of the parent compound **1**.

In general, as the ergot alkaloids and their derivatives display a broad spectrum of pharmacological action and as there is some tolerance in substitution at least at the 8α-N position, the ergoline moiety is well suited as a leading structure in the development of receptor-binding Tc complexes.

Experimental

General

All reagents are of commercial grade. ^{1}H NMR spectra were recorded on a Varian Inova-400 spectrophotometer. Elemental analyses were carried out on a LECO Elemental Analyzer CHNS-932.

Chemical synthesis

8α-Amino-6-methyl-ergoline (1). 5.15 g (0.015 mol) terguride was suspended in 500 mL of 1 N hydrochloric acid and refluxed for 20 h. The solvent was removed by rotary evaporation until a volume of 100 mL. While stirring sodium hydrogencarbonate was added to a pH of 8.5 and stirring was continued for 40 min. The precipitate was separated and dried in the vacuum. This product was dissolved in 1000 mL of hot methanol and filtered on a short column filled with silica gel. The methanol was removed until the product began to crystallize and standing overnight deliver **1**. Yield: 2.2 g (61%); mp: 292 °C, TLC (Silufol//methanol/25% ammonia: 95:5): R_f 0.4 (UV, ninhydrine). Anal. calcd for $C_{15}H_{19}N_3$: C, 74.65; H, 7.94; N, 17.41. Found: C, 74.40; H, 7.90; N, 17.21.

***N*-(2-(Benzoylmercapto)acetyl-8-α-amino-6-methylergoline (2a).** Prepared by reaction of a mixture of 98 mg (0.5 mmol) *S*-benzoylmercaptoacetic acid, 165 mg (0.5 mmol) TBTU and 170 μL (1 mmol) DIPEA in 1 mL *N*-methylpyrrolidone with a solution of 96 mg (0.4 mmol) 8α-amino-6-methyl-ergoline (**1**) in 1 mL *N*-methylpyrolidone, stirring for 4 h, and purification of the reaction product by MPLC (RP8, eluent 0.1% TFA/acetonitrile). Yield: 33.5 mg (20%); mp: 175 °C. ^{1}H NMR (400 MHz, δ DMSO-d_6 = 2.51): δ 1.87–1.90 (2H, CH_2), 2.70–2.73 (2H, 2×CH), 2.99–3.18 (3H, N–CH_3), 3.57–3.60 (2H, CH_2), 3.88–4.02 (4H, 2×CH_2), 4.27 (1H, CH), 6.88–6.90 (1H, CH), 7.09–7.20 (2H, 2×CH), 7.23–7.25 (1H, CH), 7.56–7.94 (5H, CH_{arom}), 8.67–8.68 (1H, NH_{chain}), 10.93 (1H, NH_{ring}). Anal.

calcd for $C_{24}H_{25}N_3O_2S$: C, 68.71; H, 6.01; N, 10.02, S, 7.64. Found: C, 68.57; H, 5.95; N, 9.94; S, 7.61.

***N*-(2(Benzoylmercapto)propionyl-8-α-amino-6-methylergolin (2b).** A mixture of 105 mg (0.5 mmol) *S*-benzoylmercapto propionic acid, 165 mg (0.5 mmol) TBTU and 170 μL (1 mmol) DIPEA in 1 mL *N*-methylpyrrolidone is stirred for 3 min and dropped into a solution of 96 mg (0.4 mmol) 8α-amino-6-methyl-ergoline (**1**) in 1 mL *N*-methylpyrrolidone. After stirring for another 4 h, the reaction product is purified by MPLC (RP8, eluent 0.1% TFA/acetonitrile). Evaporation of the solvent yields a light brown solid of **2b**. Yield: 43.3 mg (25%); mp: 123 °C. ^{1}H NMR: δ 1.54–1.58 (3H, CH_3), 1.84–1.90 (2H, CH_2), 2.67–2.74 (1H, CH), 2.88–2.94 (1H, CH), 3.00 (3H, N–CH_3), 3.88–4.02 (4H, 2×CH_2), 4.28 (1H, CH), 4.41–4.46 (1H, CH), 6.87–6.89 (1H, CH), 7.07–7.13 (2H, 2×CH), 7.23–7.25 (1H, CH), 7.55–7.93 (5H, CH_{arom}), 8.65 (1H, NH_{chain}), 10.91 (1H, NH_{ring}). Anal. calcd for $C_{25}H_{27}N_3O_2S$: C, 69.26; H, 6.28; N, 9.69; S, 7.39: Found: C, 68.99; H, 6.14; N, 9.43; S, 7.27.

***N*-(2-Mercapto)acetyl-8-α-amino-6-methylergoline (2c).** Sodium methylate (1 mL, 0.15 M,) was added to a stirred solution of **2a** (0.1 mmol) in 1 mL methanol. The mixture is neutralized by methanolic HCl after 1 h. After filtration and evaporation a white powder was obtained. Purification was arried out by MPLC (RP8, eluent 0.1% TFA/acetonitrile). Yield: 9.5 mg (30%); mp: 152 °C. ^{1}H NMR: δ 1.87 (t, 1H, J = 12.5 Hz, CH_2), 2.69 (d, 1H, J = 13.5 Hz, CH_2), 2.79 (t, 1H, J = 7.7 Hz, SH), 2.88–2.94 (m, 1H, CH_2), 2.98 (s, 3H, N–CH_3), 3.16–3.30 (m, 2H, CO–CH_2–S), 3.39–3.59 (m, 6H + H_2O, CH– and CH_2), 3.73 (t, 1H, J = 11.6 Hz, CH– or CH_2), 4.24 (s, 1H, >CH–N), 6.88 (d, 1H, J = 6.8 Hz, arom), 7.08–7.12 (m, 2H, arom and olefin), 7.23 (d, 1H, J = 8.2 Hz arom), 8.39 (s, 1H, NH–chain), 10.91 (s, 1H, NH–ring). Anal. calcd for $C_{17}H_{21}N_3OS$: C, 64.73; H, 6.71; N, 13.32; S, 10.16; Found C, 64.51; H, 6.42; N, 13.18; S, 10.25.

***N*-(2-Mercapto)propionyl-8-α-amino-6-methylergoline (2d).** As described for **2c**, starting from **2b**. Yield: 15.5 mg (47%); mp: 137 °C. ^{1}NMR δ 1.44 (1H, SH), 1.47 (3H, CH_3), 1.87–1.91 (1H, CH_2), 2.69 (1H, CH_2), 2.70 (2H, 2×CH), 2.96–2.97 (3H, N–CH_3), 3.00 (2H, CH_2), 3.50 (1H, CH–CH_3), 3.65–3.69 (2H, CH_2), 4.22 (1H, CH–N), 6.86–6.90 (1H, CH_{ring}), 7.08–7.13 (2H, 2×CH_{ring}), 7.22–7.24 (1H, CH_{ring}), 8.31 (1H, NH_{chain}), 10.91 (1H, NH_{ring}). Anal. calcd for $C_{18}H_{23}N_3OS$: C, 65.62; H, 7.04; N, 12.75; S, 9.73; Found C, 65.46, H, 6.99, N, 12.66: S. 9.71.

***N*-(2-Methylmercapto)acetyl-8-α-amino-6-methylergoline (2e).** A mixture of **2a** (0.3 mmol), 5% Pd/$CaCO_3$ (0.7 mg) and sodium methylate (0.42 mmol) in 5 mL methanol was stirred under hydrogen atmosphere at 67 kPa for 90 min. The filtered mixture was refluxed with methyl iodide (0.3 mmol) for 1 h. The volume of the solution was reduced to about 1 mL by evaporation and the product was purified by MPLC. Yield: 36.5 mg (37%); mp: 163 °C; ^{1}NMR: 1.83–1.89 (2H, CH_2), 2.15 (3H, S–CH_3), 2.66–2.69 (2H, 2×CH), 2.95–2.98 (3H, N-CH_3), 3.11–3.14 (2H, CH_2), 3.22–3.26 (2H, CH_2), 3.60–3.71 (2H, CH_2), 4.30 (1H, CH–N), 6.86–6.88 (1H, CH_{ring}), 7.08–7.12 (2H, 2×CH_{ring}), 7.22–7.24 (1H, CH_{ring}), 8.39–8.40 (1H, NH_{chain}), 10.92 (1H, NH_{ring}). Anal. calcd for $C_{18}H_{23}N_3OS$: C, 65.62; H, 7.04; N, 12.75; S, 9.73; Found C, 65.43; H, 7.02; N, 12.61; S, 9.68.

***N*-(2-Methylmercapto)propionyl-8-α-amino-6-methylergoline (2f).** As described for **2e**, starting from **2b**. Yield: 23.5 mg (23%); mp: 215 °C, $C_{19}H_{25}N_3OS$. ^{1}NMR: 1.37–1.39 (3H, CH_3), 1.84–1.87 (2H, CH_2), 2.09–9.10 (3H, S–CH_3), 2.66–2.69 (2H, 2×CH), 3.02 (3H, N–CH_3), 3.57–3.65 (4H, 2CH_2), 4.28 (1H, N–CH), 4.37 (1H, CH), 6.84–6.89 (1H, CH_{ring}), 7.07–7.17 (2H, 2×CH_{ring}), 7.17–7.24 (1H, CH_{ring}), 8.31 (1H, NH_{chain}), 10.91 (1H, NH_{ring}).

Rhenium complex 3. This is reported in ref 14.

Chloro(3-thiapentane-1.5-dithiolato)oxotechnetium(V). This was prepared by the reaction of the tridentate ligand HS–CH_2CH_2—S–CH_2CH_2–SH and teraphenylarsonium tetrachlorotechnetate(V) $AsPh_4[TcOCl_4]$ in acetonitrile as described in ref 17.

Tc complex 4a. The methanolic reaction mixture of the preparation of **2c** is given to a solution of 30.3 mg, (0.1 mmol) [TcO(SSS)Cl] in 5 mL acetonitrile. The stirred mixture is kept under nitrogen. After continued stirring for 2 h and standing overnight, the precipitating sodium chloride was filtered off and the solvent removed by a nitrogen stream. The residue was dissolved in DMS and purified by column chromatography [silica gel 60 (Merck) eluted with methanol (95%)–NH_3(conc)(5%)]. The brown fraction delivers after reducing the volume at 0.5 mL a brown solid substance. Yield: 12.8 mg (22%). mp: >200 °C; TLC (silica gel/95% methanol) R_f: 0.52. UV–vis: λ_{max}; 280 nm. ^{1}NMR: 1.40 (1H, C–H), 1.52 (1H, CH), 1.68 (1H, CH), 1.93 (1H, CH), 2.29 (3H, N–CH_3), 2.32 (1H, CH), 2.44 (1H, CH), 2.75 (m, 2H, CO–CH_2–S), 3.13–3.26 (3H, CH), 3.50–4.08 (6H, CH), 4.29 (2H, CH), 6.61 (1H, CH_{arom}), 6.95–7.10 (3H, $CH_{arom\ and\ olefin}$), 7.75 (1H, NH_{chain}), 10.63 (s, 1H, NH_{ring}).

Tc complex 4b. This was prepared in the same manner using **2d** instead of **2c**. Yield: 9.5 mg (16%). Mp: >200 °C; TLC R_f: 0.58. UV–vis: λ_{max}; 280 nm.

$^{99m/99}$Tc-complexes 4a,b in solution

Tc gluconate was prepared by gradual addition of 100 μL 0.01 M stannous chloride solution in 0.1 M HCl to an aqueous solution of 100 μL 0.01 M $KTcO_4$ and 0.5 mL ^{99m}Tc generator eluate in 1 mL 0.1 M sodium gluconate. 4 μmol of the *S*-benzoylic protected mercaptanes **2a** resp. **2b** were dissolved in 200 μL MeOH and saponified by adding 50 μL 1.1 M sodium methylate. After 20 min, the alkaline solution was given to Tc gluconate. After another 20 min, 2 μmol of the tridentate dithiolate ligand HSSSH, dissolved in 100 μL acetone was added. The solution was neutralized by 1 M HCl and applied to HPLC-separation [RP18 column,4×250 mm, 0.01 M phosphate buffer pH 5.8 (A) and acetonitrile

(B). 3 min 95% A/5% B; 15 min from 75% A/25% B to 10% A/90% B; 5 min 10% A/90% B; Flow rate: 1 mL/min]. **4a**: R_t 21,5 min, **4b**: R_t 22.5 min. TLC [silica gel (Merck Kieselgel60), 95% ethanol]. **4a:** R_f 0.52, **4b**: R_f 0.58.

The eluted fractions (about 40% each) were separated, the solvent removed by evaporation in a nitrogen stream and the residue dissolved in propylene glycol/water (radioactive concentration about 5–10 MBq per mL) for biodistribution studies.

Determination of lipophilicity data

Lipophilicity data lgP_{HPLC} of all species were determined by reversed-phase HPLC using a Hamilton PRP-1 column (250×4.1 mm, 10 μm) and the Perkin–Elmer HPLC Controller System Model 1022 equipped with a UV–vis spectrometer detector at 254 nm. An isocratic eluent [acetonitrile and 0.01 M phosphate buffer (ph 7.4) 3:1,v/v] was applied with a flow rate of 1.5 mL/min. The pH value of the eluent was measured by a glass electrode, which was calibrated with standard buffer daily. P_{HPLC} values were derived from the retention times according to the equation $\log P = a \log k' + b$, where $k' = (t_R - t_0)/t_0$ is the capacity factor, t_R is the retention time of the sample and t_0 the retention time of methanol as an unretained solute. Aniline (0.9), benzene (2.13) and bromobenzene (2.99) served as log P references.

Radioligand binding assay

The IC_{50} values of all species were determined for their ability in D2 receptor binding. [^{3}H]Spiperone (from NEN) was used as the radioligand. The binding assay was carried out in a final volume of 5 mL tris HCl buffer, pH 7.4, containing 10 nM $MgCl_2$, 1 nM EDTA, 0.27 nM [^{3}H]Spiperone, 1 unit of cloned D_2 receptor and various concentrations of the ergoline derivatives, dissolved in a small volume of DMSO. The samples were incubated in triplicates at 20 °C for 60 min. The incubation was terminated by rapid filtration through GF/B glass fiber filters (Whatman) using a 30-port Brandel Cell Harvester. The filters were rapidly washed with four 4-mL portions of ice-cold buffer, transferred into 10 mL scintillation fluid (Ultima-Gold, Packard) and analysed for radioactivity. In case of ^{99}Tc complexes interference by ^{99}Tc radioactivity in ^{3}H counting was eliminated by appropriate adjustment of the energy channel, except at the highest ^{99}Tc concentration used, which required correction.

Biodistribution studies

Animal experiments were carried out according to the relevant national regulations. The studies were performed on male Wistar rats (5–6 weeks old). 0.5 mL of the complex solution were injected in the tail vein and the animals were sacrified by heart puncture (2–60 min pi). The selected organs were isolated for weighing and counting and the percentage of injected dose per organ was calculated. The blood pool activity was calculated from the respective blood concentration under the assumption that the total blood volume represents 6% of the body weight of rats.

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