Third Generation Antitumor Platinum(II) Complexes of the [1-(Fluoro/difluorophenyl)-2-phenylethylenediamine]platinum(II) Type☆

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Summary

The diastereomeric 1-(fluoro/difluorophenyl)-2-phenylethylenediamines (4-fluoro: *erythro*-1/*threo*-1; 2,4-difluoro: *erythro*-2/*threo*-2; 2,6-difluoro: *erythro*-3/*threo*-3) and the diastereomeric 1-(4-fluorophenyl)-2-(3-hydroxyphenyl)ethylenediamines (*erythro*-4/*-threo*-4) were synthesized from appropriately substituted stilbenes by reaction with IN_3 and subsequent $LiAlH_4$ reduction. Coordination of the 1,2-diphenylethylenediamines to platinum was carried out by use of K_2PtI_4. The water-soluble aquasulfatoplatinum(II) complexes (*erythro*/*threo*-1-$PtSO_4$ – *erythro*/*threo*-4-$PtSO_4$) were obtained from the diiodoplatinum(II) complexes by reaction with Ag_2SO_4. Additionally *erythro*/*threo*-1-$PtSO_4$ and *erythro*/*threo*-4-$PtSO_4$ were transformed into the dichloroplatinum(II) complexes (*erythro*/*threo*-1-$PtCl_2$, *erythro*/*threo*-4-$PtCl_2$) by treatment with KCl. In contrast to the less effective *erythro*-configurated sulfatoplatinum(II) complexes the *threo*-analogues showed comparable or even superior activities to cisplatin on the human MDA-MB-231 breast cancer cell line. On the MXT-M-3.2 breast cancer of the mouse only *erythro*- and *threo*-4-$PtSO_4$ caused similar effects like cisplatin. The strong inhibitory effect of the diastereomeric sulfatoplatinum(II) complexes on the P-388 leukemia of the mouse was equal to that of cisplatin. On the latter tumor *threo*-4-$PtCl_2$ was the most active among the less toxic dichloroplatinum(II) derivatives.

Introduction

Since the discovery of the antitumor activity of *cis*-diamminedichloroplatinum(II) (cisplatin) in 1965 by Rosenberg [1,2] extensive research has been done to establish its mode of action. It is generally accepted that the pharmacological and also the toxicological properties of cisplatin result from its ability to react with nucleophiles under physiological conditions. The bifunctional coordination of Pt to the N(7) positions of adjacent guanosines of one DNA strand (intrastrand cross-links), which leads to an arrest of DNA synthesis, is thought to be the most important reason for the antitumor activity of cisplatin [3]. The special reaction behavior of cisplatin is also responsible for its transformation into inactive products, which is achieved by irreversible reaction with nucleophilic centers in plasma proteins like albumin[4,5]. However, the high chloride concentration in blood plasma and tissue fluid (≈ 100 mM) reduces the formation of the very reactive and toxic aquachloro- and diaquaplatinum(II) species, intermediates in the reaction with nucleophiles [6]. After uptake of the free (i.e. the non-plasma protein bound) *cis*-diamminedichloroplatinum(II) by the tumor cell the hydrolysis is favored due to the low intracellular chloride concentration (≈4 mM) [6]. The highly reactive aquated species attack DNA in a nucleophilic substitution reaction with formation of intrastrand cross-links.

These findings concerning the mode of action of cisplatin were the basis for the design of the "second generation platinum complexes". Numerous laboratories attempted to synthesize more active and less toxic cisplatin analogues by exchanging chloride for more stably bound "leaving groups" (e.g. carboxylic acids). The rationale of this approach was the assumption that by influencing the mechanism and rate of the reaction of the PtX_2-moiety (X = "leaving group") with nucleophiles an optimization of the therapeutic properties could be achieved (compare ref. [7,8]).

It was supposed that, in accordance with this concept, platinum(II) complexes reacting more slowly with plasma proteins should yield higher free drug levels than cisplatin in the vicinity of the neoplastic cells and therefore should produce stronger antitumor effects. Since the structure of the neutral ligand of cisplatin analogues did not influence the reactivity of the $PtCl_2$-moiety to a significant extent [9], the structure activity studies in the class of the "second generation platinum complexes" focussed on the "leaving group" [6,7]. However, the results of these studies were disappointing. In fact, the new complexes often proved to be less toxic but in no case more antitumor active than cisplatin. An example is the clinically used, less toxic drug carboplatin, which must be administered in a much higher dosage than cisplatin to achieve comparable antitumor effects [6,7]. Presently the development of platinum complexes of the third generation – compounds which are active on tumors endowed with "acquired" or "intrinsic" resistance against cisplatin – is a main field of the experimental cancer chemotherapy. The principal goal of this field of research is that, in comparison to first and second generation platinum complexes, the new drugs should have a wider spectrum of application as well as a comparable or even better toxicological profile (compare also the paragraph "Discussion"). The predominant structural feature of the "third generation platinum complexes" is a lipophilic neutral ligand, e. g. a chelating diamine ligand like *trans*-1,2-diaminocyclohexane [7], which is responsible for their activity on cisplatin-resistant neoplasms.

It has been shown by us that [1,2-diphenylethylenediamine]platinum(II) complexes fulfill the requirements for "third generation platinum complexes", if they are substituted with appropriate functional groups, e.g. by fluorine in the 4-position of the aromatic rings [10,11]. Especially [(±)-1,2-bis(4-fluorophenyl)ethylenediamine]dichloroplatinum(II) (*rac*-4-F-$PtCl_2$, formula see Chart 1) exerts marked inhibitory effects on human breast cancer cell lines due to its capability

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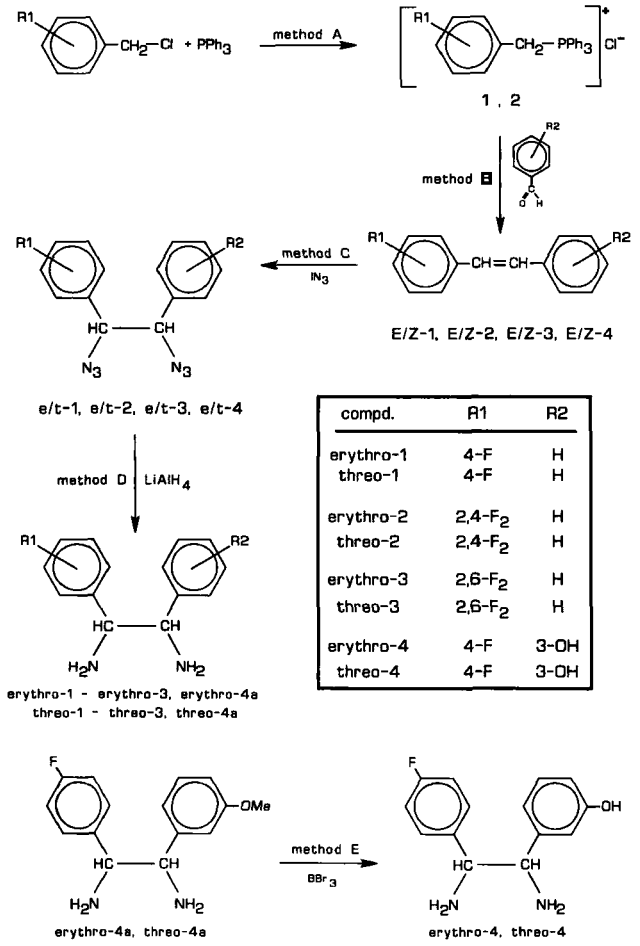
to accumulate in these cells [10–12]. This finding is of importance, since the widespread breast cancer diseases cannot be influenced by the therapeutically available platinum complexes (compare paragraph "Discussion").

In this publication we describe further fluoro-substituted [1,2-diphenylethylenediamine]platinum(II) complexes which surpass *rac*-4-F-$PtCl_2$ in its breast cancer inhibitory potency.

Results

Chemistry: 1,2-Diphenylethylenediamines with differently substituted phenyl rings are easily available from appropriately substituted stilbenes (Scheme 1) [13,14].



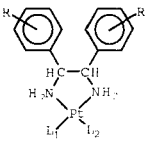
compd.	R1	R2
erythro-1	4-F	H
threo-1	4-F	H
erythro-2	2,4-F_2	H
threo-2	2,4-F_2	H
erythro-3	2,6-F_2	H
threo-3	2,6-F_2	H
erythro-4	4-F	3-OH
threo-4	4-F	3-OH

Scheme 1

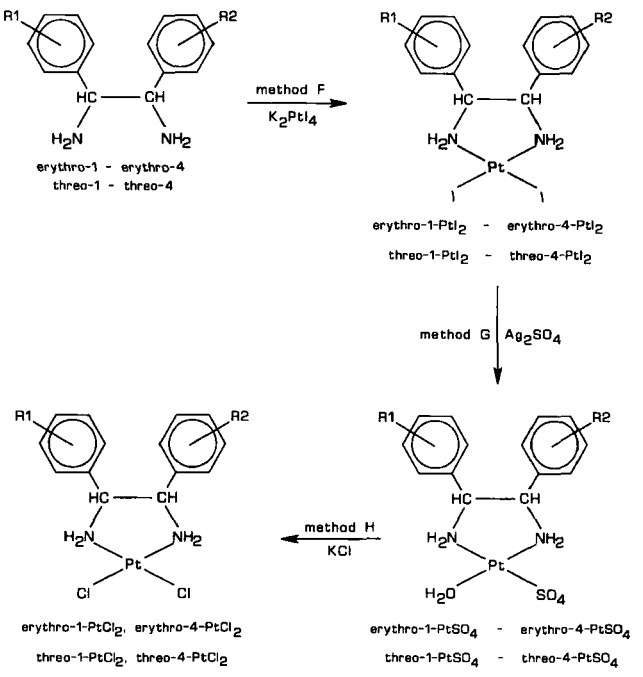
The stilbenes *E/Z*-1 – *E/Z*-4 were obtained by Wittig reaction of benzaldehydes with benzyltriphenylphosphonium chlorides, which were previously formed from benzyl chlorides and triphenylphosphine (Scheme 1, method A/B). Addition of the stilbenes *E/Z*-1 – *E/Z*-4 to a solution of IN_3* in acetonitrile at a temp. of about –60 °C gave the respective 1-azido-2-iodo-1,2-diphenylethanes. Use of an excess of IN_3 and warming to room temp. effected a nucleophilic displacement of I^- by N_3^- (Scheme 1, method C), whereupon the released I^- comproportionated with IN_3 to give I_2 and N_3^-. The resulting 1,2-diazido-1,2-diphenylethanes (*e/t*-1 – *e/t*-4) could be transformed to the 1,2-diphenylethylenediamines *erythro/threo*-1 – *erythro/threo*-3 and *erythro/threo*-4a by $LiAlH_4$-reduction (Scheme 1, method D). The isolated diastereomers mixtures of these ethylenediamines contain the *erythro*- and *threo*-isomers in a ratio of about 1:1, which was assessed by ^{1}H-NMR spectroscopy. The diastereomers were obtained in chemically pure form by fractional crystallization of their dihydrochlorides in methanol/ether. The yields of the

* IN_3 was produced in situ by reaction of ICl with an excess of NaN_3 [35].

Chart I: Reference substances.



compd.	R	L_1	L_2
rac-4-F-$PtCl_2$	4-F	Cl	Cl
meso-4-F-$PtCl_2$	4-F	Cl	Cl
rac-4-F-$PtSO_4$	4-F	H_2O	SO_4
meso-4-CH_3O-$PtCl_2$	4-CH_3O	Cl	Cl
rac-2,4-F_2-$PtCl_2$	2,4-F_2	Cl	Cl
rac-2,6-F_2-$PtCl_2$	2,6-F_2	Cl	Cl
rac-3,5-F_2-$PtCl_2$	3,5-F_2	Cl	Cl
rac-3-OH-$PtCl_2$	3-OH	Cl	Cl



Scheme 2

less soluble *erythro*-isomers amounted to 14–25%, those of the *threo*-isomers to 26–32%. The chemical purity of the isolated diastereomers was confirmed by ^{1}H-NMR spectroscopic studies (compare chapter "Spectroscopic characterization" and Table 1). Ether cleavage of *erythro*-4a and *threo*-4a was achieved by BBr_3 (Scheme 1, method E).

For coordination to platinum the 1,2-diphenylethylenediamines were reacted with K_2PtI_4 at pH 7–8 in water to yield the diiodoplatinum(II) complexes *erythro*-1-PtI_2 – *erythro*-4-PtI_2 and *threo*-1-PtI_2 – *threo*-4-PtI_2 (Scheme 2, method F). Exchange of the "leaving groups" was performed by addition of solid Ag_2SO_4 to an aqueous suspension of the diiodoplatinum(II) complexes to get the sulfatoplatinum(II) complexes *erythro*-1-$PtSO_4$ – *erythro*-4-$PtSO_4$ and *threo*-1-$PtSO_4$ – *threo*-4-$PtSO_4$ (Scheme 2, method G). Addition of an excess of solid KCl to the aqueous solution of *erythro*-1-$PtSO_4$, *erythro*-4-$PtSO_4$, *threo*-1-$PtSO_4$ and *threo*-4-$PtSO_4$ yielded the respective dichloroplatinum(II) complexes

Table 1: ^{1}H-NMR data[a] of [1-(fluoro/difluorophenyl)-2-phenylethylenediamine]platinum(II) complexes.

Compd.	–CH–	NH	Ar-H	OH
erythro-1	4.00, 4.10 (dd, *J* = 8Hz, 2H)		6.90 – 7.46 (m, 9H)	
threo-1	3.93, 4.05 (dd, *J* = 8Hz, 2H)		6.78 – 7.38 (m, 9H)	
erythro-2	5.07, 5.25 (dd, *J* = 9Hz, 2H)		7.00 – 8.23 (m, 8H)	
threo-2	5.18, 5.42 (dd, *J* = 9Hz, 2H)		6.80 – 8.23 (m, 8H)	
erythro-3	5.00, 5.30 (dd, *J* = 9Hz, 2H)		7.00 – 7.82 (m, 8H)	
threo-3	5.05, 5.43 (dd, *J* = 9Hz, 2H)		6.67 – 7.72 (m, 8H)	
erythro-4	n.d.		6.83 – 7.67 (m, 8H)	
threo-4	4.95, 5.16 (dd, *J* = 8Hz, 2H)		6.62 – 7.70 (m, 8H)	
erythro-1-PtI_2	4.42 (br, 2H)	5.47 (br, 2H) 6.07 (br, 2H)	7.03 – 7.10 (m, 2H) 7.21 – 7.28 (m, 3H) 7.51 – 7.63 (m, 4H)	
threo-1-PtI_2	4.42 (br, 2H)	5.45 (br, 2H) 6.20 (br, 2H)	7.02 – 7.08 (m, 2H) 7.16 – 7.28 (m, 3H) 7.51 – 7.62 (m, 4H)	
erythro-2-PtI_2	4.34 – 4.36 (m, br, 1H) 4.63 – 4.75 (m, br, 1H)	5.55 – 5.58 (m, br, 2H) 6.13 – 6.21 (m, br, 1H) 6.21 – 6.26 (m, br, 1H)	7.02 – 7.13 (m, 2H) 7.22 – 7.33 (m, 3H) 7.57 – 7.61 (m, 2H) 8.26 – 8.35 (m, 1H)	
threo-2-PtI_2	4.33 – 4.42 (m, br, 1H) 4.65 – 4.81 (m, br, 1H)	5.44 – 5.70 (m, br, 2H) 6.25 – 6.31 (m, br, 1H) 6.31 – 6.37 (m, br, 1H))	6.94 – 7.60 (m, 7H 8.05 – 8.11 (m, 1H)	
erythro-3-PtI_2	4.12 (d, br, 1H) 4.91 – 5.00 (m, br, 1H)	4.00 – 4.30 (m, br, 1H) 4.91 – 5.00 (m, br, 1H) 6.56 – 6.60 (m, br, 2H)	6.98 – 7.05 (m, 1H) 7.26 – 7.52 (m, 6H) 7.95 – 7.99 (m, 1H)	
threo-3-PtI_2	4.59 – 4.62 (m, br, 1H) 4.79 – 4.86 (m, br, 1H)	5.15 (t, br, 1H) 5.57 (t, br, 1H) 6.22 (d, br, 1H) 6.62 (d, br, 1H)	6.94 – 7.07 (m, 2H) 7.21 – 7.63 (m, 6H)	
erythro-4-PtI_2	4.26 – 4.45 (m, br, 2H)	5.37 – 5.50 (m, br, 2H) 5.98 – 6.22 (m, br, 2H)	6.68 – 6.80 (m, 2H) 6.95 – 7.10 (m, 3H) 7.24 (s, 1H) 7.54 – 7.63 (m, 2H)	9.65 (s,br,1H)
threo-4-PtI_2	4.23 – 4.44 (m, br, 2H)	5.32 – 5.55 (m, br, 2H) 6.15 – 6.22 (m, br, 2H)	6.64 – 6.68 (s, 1H) 6.88 – 7.10 (m, 5H) 7.55 – 7.63 (m, 2H)	9.53 (s,br,1H)
erythro-1-$PtCl_2$	4.56 (br, 2H)	5.62 (br, 2H) 6.07 (br, 2H)	7.03 – 7.10 (m, 2H) 7.25 – 7.28 (m, 3H) 7.54 – 7.68 (m, 4H)	
threo-1-$PtCl_2$	4.89 (br, 2H)	5.81 (br, 2H) 6.41 (br, 2H)	7.00 – 7.10 (m, 2H) 7.20 – 7.27 (m, 3H) 7.56 – 7.69 (m, 4H)	
erythro-4-$PtCl_2$	4.36 (m, br, 1H) 4.61 (m, br, 1H)	5.57 – 5.70 (m, br, 2H) 6.18 – 6.25 (m, br, 2H)	6.68 – 6.75 (m, 2H) 6.97 – 7.21 (m, 3H) 7.51 (s, 1H) 7.55 – 7.61 (m, 2H)	9.76 (s,br,1H)
threo-4-$PtCl_2$	5.35 (m, br, 2H)	5.98 (d, br, 2H) 6.58 (d, br, 2H)	6.65 – 6.70 (s, 1H) 7.05 – 7.12 (m, 3H) 7.17 (s, 1H) 7.31 – 7.34 (m, 1H) 7.86 – 7.91 (m, 2H)	9.57 (s,br,1H)

[a] The ligands were recorded in [D_4]methanol at 60MHz; the complexes were recorded in [D_7]DMF at 250MHz; TMS as internal standard

erythro-1-$PtCl_2$, *erythro*-4-$PtCl_2$, *threo*-1-$PtCl_2$ and *threo*-4-$PtCl_2$ (Scheme 2, method H).

Spectroscopic Characterization: Assignment of the separated diastereomeric 1,2-diphenylethylenediamines either to the *erythro*- or to the *threo*-form was accomplished by ^{1}H-NMR spectroscopy.

Due to the unequal asymmetric C-atoms the signals of the benzylic protons of both diastereomers split into an AB-system with J_{H-H} = 8–9 Hz (Table 1). Since the coupling constants follow a Karplus-type angle dependence, a predominant conformation with a dihedral angle between the protons of about 180° as realized in conformation I and V (Figure 1) can be assumed. In conformation V the aromatic rings are synclinally arranged, which leads, due to the stronger shielding compared to that of the *erythro*-configurated compounds (antiperiplanarly arranged aromatic rings, conformation I, Figure 1), to a high field shift of the aromatic protons [15,16].

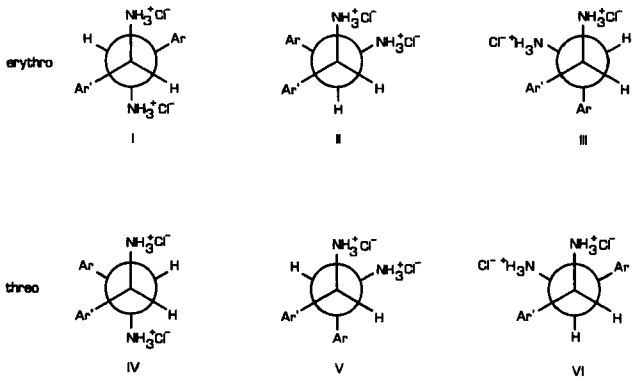


Figure 1: Stable conformations of diastereomeric 1,2-diphenylethylenediamines.

C–N axis, the NH becomes diastereotopic owing to the neighborhood of the asymmetric C atoms. This fact and the different substituents on the two benzylic C atoms normally give rise to a splitting of the NH- and CH protons into 6 signal groups [13,14]. However, if the phenyl rings do not possess substituents in the *ortho*-positions, the NH-protons located above and below the N–Pt–N plane become isochronic. The spectra of the [1-(4-fluorophenyl)-2-phenylethylenediamine]diiodoplatinum(II) complexes *erythro*-1-PtI_2 and *threo*-1-PtI_2 as well as the spectra of the [1-(4-fluorophenyl)-2-(3-hydroxyphenyl)ethylenediamine]diiodoplatinum(II) complexes *erythro*-4-PtI_2 and *threo*-4-PtI_2 show one CH- and two NH-signal groups (see Table 1), comparable to their parent compound [(±)-1,2-bis(4-fluorophenyl)ethylenediamine]diiodoplatinum(II) [12]. The broadened, less resolved signals result from the coupling between NH, CH and ^{195}Pt.

The distinction of the NH- from the CH-protons was performed on *threo*-1-PtI_2 as a model. Since the NH/ND exchange by D_2O failed, *threo*-1 was coordinated to platinum(II) in D_2O instead of H_2O. In the spectrum of the *N,N,N',N'*-tetradeuterated diiodoplatinum(II) complex the benzylic protons appear as AB-system (δ = 4.70 and 4.77) with ^3J = 12.2 Hz comparable to those of other *threo*-configurated [1,2-diphenylethylenediamine]diiodoplatinum(II) complexes [13].

Exchange of the I^--"leaving groups" by Cl^- alters the ^{1}H-NMR spectra of the complexes only marginally (Table 1), while from the ^{1}H-NMR spectra of the sulfatoplatinum(II) complexes no useful information can be obtained. Therefore, these complexes were characterized by elemental analyses and IR spectroscopy (see Tables 2 and 3).

The binding mode of the SO_4^{2-}-residue to platinum is contrarily discussed in the literature. In solid state the sulfato ion can form an unidentate, bidentate or bridged bidentate complex as well as a diaquaplatinum(II) complex with SO_4^{2-} as counter ion [17–20].

Table 2: Analytical data of aqua[1-(fluoro/difluorophenyl)-2-phenylethylenediamine]sulfatoplatinum(II) complexes.

compd.	yield %	formula	C% calcd.	C% found	H% calcd.	H% found	N% calcd.	N% found
erythro-1-$PtSO_4$	81	$C_{14}H_{17}FN_2O_5PtS$	31.2	31.1	3.15	3.57	5.2	5.0
threo-1-$PtSO_4$	88	$C_{14}H_{17}FN_2O_5PtS \times H_2O$	30.2	30.4	3.41	3.45	5.0	4.9
erythro-2-$PtSO_4$	75	$C_{14}H_{16}F_2N_2O_5PtS$	30.2	30.5	2.87	3.15	5.0	5.0
threo-2-$PtSO_4$	88	$C_{14}H_{16}F_2N_2O_5PtS$	30.2	30.4	2.87	3.31	5.0	4.8
erythro-3-$PtSO_4$	77	$C_{14}H_{16}F_2N_2O_5PtS \times 3H_2O$	27.5	27.2	3.60	3.03	4.6	4.4
threo-3-$PtSO_4$	58	$C_{14}H_{16}F_2N_2O_5PtS \times H_2O$	29.2	29.2	3.13	2.84	4.9	4.7
erythro-4-$PtSO_4$	80	$C_{14}H_{17}FN_2O_6PtS$	30.3	30.6	3.06	3.35	5.0	5.0
threo-4-$PtSO_4$	47	$C_{14}H_{17}FN_2O_6PtS \times 4H_2O$	26.8	26.6	3.99	4.00	4.5	4.1

Coordination to platinum(II) changes the ^{1}H-NMR spectra characteristically. Since the formation of the 5-membered ethylenediamine chelate ring blocks the rotation around the

In [1,2-bis(2,6-dihalo-3-hydroxyphenyl)ethylenediamine]-sulfatoplatinum(II) and in [1-(2,6-dichloro-4-hydroxyphenyl)-2-phenylethylenediamine]sulfatoplatinum(II)

Table 3: IR data[a] of aqua[1-(fluoro/difluorophenyl)-2-phenylethylenediamine]sulfatoplatinum(II) complexes.

compd.	IR-active bands in the ν (SO) region			
erythro-1-$PtSO_4$	1130cm^{-1} s,	1110cm^{-1} s,	1035cm^{-1} s,	940cm^{-1} m
threo-1-$PtSO_4$	1130cm^{-1} s,br		1040cm^{-1} s,	940cm^{-1} m
erythro-2-$PtSO_4$	1130cm^{-1} sh,	1120cm^{-1} s,	1040cm^{-1} s,	940cm^{-1} w
threo-2-$PtSO_4$	1220cm^{-1} sh,	1185cm^{-1} s,	1045cm^{-1} s	
erythro-3-$PtSO_4$	1140cm^{-1} sh,	1120cm^{-1} s,	1030cm^{-1} s,	930cm^{-1} w
threo-3-$PtSO_4$	1120cm^{-1} s,		1030cm^{-1} s,	945cm^{-1} w
erythro-4-$PtSO_4$	1220cm^{-1} s,		1030cm^{-1} s	
threo-4-$PtSO_4$	1150cm^{-1} s,	1120cm^{-1} s,	1040cm^{-1} s,	960cm^{-1} w

[a] The spectra were taken as KBr pellets

Table 4: Antitumor effect of aqua[1-(fluoro/difluorophenyl)-2-phenylethylenediamine]sulfatoplatinum(II) complexes against hormone independent MDA-MB-231 breast cancer cells.

compd.	[^{3}H]thymidine incorporation[a] T/C,% at 10^{-6}M	ED_{50}[b] × 10^{-7}M
erythro-1-$PtSO_4$	18	3.1
threo-1-$PtSO_4$	4	0.11
erythro-2-$PtSO_4$	15	2.1
threo-2-$PtSO_4$	5	0.11
erythro-3-$PtSO_4$	57	13.0
threo-3-$PtSO_4$	13	2.3
erythro-4-$PtSO_4$	7	3.4
threo-4-$PtSO_4$	3	0.36
meso-4-F-$PtSO_4$ [c]	30	5.5
rac-4-F-$PtSO_4$ [c]	21	3.3
cisplatin	15	1.3

[a] The [^{3}H]thymidine incorporation into the DNA was estimated after a 72h incubation of the breast cancer cells with the appropriate drug.
[b] ED_{50}= the effective concentration which decrease the tumor growth by 50%; mean of 2 tests

Table 5: Antitumor effect of aqua[1-(fluoro/difluorophenyl)-2-phenylethylenediamine]sulfatoplatinum(II) complexes on the growth of the hormone independent MXT-M-3.2 mammary carcinoma of the BDF1 mouse.

compd.	dose[a] [μmol/kg]	med. tumor area [mm^2]	(range)	T/C[b] %
erythro-1-$PtSO_4$	10	146	(40 – 338)	58
threo-1-$PtSO_4$	10	146	(86 – 189)	58
erythro-2-$PtSO_4$	10	166	(16 – 308)	66
threo-2-$PtSO_4$	10	120	(18 – 236)	48
erythro-3-$PtSO_4$	10	171	(35 – 286)	76
threo-3-$PtSO_4$	10	198	(4 – 290)	88
erythro-4-$PtSO_4$	10	149	(9 – 126)	20[c]
threo-4-$PtSO_4$	10	50	(4 – 153)	35[c]
cisplatin	5	36	(9 – 96)	15
control	–	249	(9 – 442)	100

[a] The compounds were administered three times a week (Monday, Wednesday, Friday), sc., as a solution in water; Cisplatin was dissolved in 0.9% NaCl-solution; duration of therapy 2 weeks.
[b] T/C = test group/control group

complexes SO_4^{2-} is predominantly bound unidentate [13,21] Lattice water molecules stabilize the complex structure by two strong hydrogen bridges from the H_2O to the sulfato ligand [22]. This binding mode, coordination of SO_4^{2-} to platinum(II) through one oxygen atom, reduces the symmetry of the free ion from T_d to C_{3v} [19] and leads to the appearance of IR active bands in the ν(SO) region at 970, 1050, 1120 and 1140 (shoulder) cm^{-1} [23].

With exception of *threo*-2-$PtSO_4$ and *erythro*-4-$PtSO_4$ the IR-spectra of the new complexes exhibit comparable bands (Table 3) allowing their assignment to the aqua[1,2-diphenylethylenediamine]sulfatoplatinum(II) complex type. *Threo*-2-$PtSO_4$ and *erythro*-4-$PtSO_4$ show only one strong band at 1220 cm^{-1}. According to Eskenazi [19] and Nakamoto [24], a structure with a bidentate SO_4^{2-} residue must be assumed. Only a chelated sulfate gives a ν(SO) band at about 1200 cm^{-1}. However, for all complexes a contamination with other coordination types cannot be excluded. Concerning the antitumor activity the coordination type is of no importance, since in aqueous solution the SO_4^{2-} is quickly replaced by water under formation of the highly reactive diaquaplatinum(II) species.

Tumor Inhibiting Properties. In the *in vitro* test on the hormone-insensitive, human MDA-MB-231 breast cancer cell line the *threo*-configurated complexes produced inhibitory effects on the [^{3}H]thymidine incorporation into DNA, which were comparable (*threo*-3-$PtSO_4$) or even superior (*threo*-1-$PtSO_4$, *threo*-2-$PtSO_4$ and *threo*-4-$PtSO_4$) to those of cisplatin and the parent compound *rac*-4-F-$PtSO_4$ (Table 4). The analogous *erythro*-configurated complexes proved to be less active than cisplatin; three out of four compounds (*erythro*-1-$PtSO_4$, *erythro*-2-$PtSO_4$ and *erythro*-4-$PtSO_4$) inhibited the [^{3}H]thymidine incorporation slightly more than their parent compound *meso*-4-F-$PtSO_4$.

However, for the confirmation of these results an extensive study of the new complexes and of their parent compounds is necessary, in which a more sensitive, the proliferate activity of the tumor cells recording kinetic method (e. g. the *"Standardized Kinetic Chemosensitivity Assay"*; compare ref. [25]) is employed instead of the single-end-point determination used so far.

The testing of the new sulfatoplatinum(II) complexes on the hormone-insensitive MXT-M-3.2 breast cancer of the mouse provided disappointing results (Table 5). Only *erythro*- and *threo*-4-$PtSO_4$ showed a similar activity like cisplatin on this tumor model. Further experiments with different dosage and schedule are required to judge the value of the new compounds.

The results, achieved with the new diastereomeric sulfatoplatinum(II) complexes on the MDA-MB-231 breast cancer cell line, did not coincide either with those of the *in vivo* experiments on the P-388 leukemia of the mouse. To obtain

Table 6: Antitumor effect of aqua[1-(fluoro/difluorophenyl)-2-phenylethylenediamine]sulfatoplatinum(II) complexes against leukemia P-388 of the CDF_1 mouse.

compd.	dose[a] [µmol/kg/d]	T–C[b] [g]	med. survival time [d]	range [d]	T/C [%]
erythro-1-$PtSO_4$	10	0.2	16	(15–20)	200
	20	–2.1	17.5	(17–19)	219
	40	–3.9	7	(5–21)	88
threo-1-$PtSO_4$	10	–2.5	16	(13–18)	200
	20	–2.7	16	(13–17)	200
	40	–4.4	7	(5–8)	88
erythro-2-$PtSO_4$	10	–0.4	17	(16–18)	213
	20	–1.9	19.5	(17–20)	244
	40	–4.0	7	(5–10)	88
threo-2-$PtSO_4$	10	0.2	15.5	(14–16)	194
	20	–1.7	16.5	(13–18)	206
	40	–4.6	5	(5–19)	63
erythro-3-$PtSO_4$	10	0.3	13	(12–18)	163
	20	0.3	15.5	(14–16)	194
	40	–0.5	16	(15–17)	200
threo-3-$PtSO_4$	10	0.1	14	(13–15)	175
	20	–0.7	15	(13–16)	188
	40	–4.2	15	(5–17)	188
erythro-4-$PtSO_4$	10	–1.0	16.5	(16–21)	206
	20	–2.0	19.5	(7–21)	244
	40	–4.0	6	(5–7)	75
threo-4-$PtSO_4$	10	–4.0	19	(15–20)	238
	20			toxic	
meso-4-F-$PtSO_4$[c]	10	1.9	15.5	(14–16)	172
	20	0.8	17	(5–19)	188
	40	–0.9	22	(18–23)	244
rac-4-F-$PtSO_4$[c]	10	–1.9	14	(6–16)	156
	20	–0.1	14.5	(13–18)	161
	40	–1.9	18	(18)	200
erythro-1-$PtCl_2$[d]	10	0.0	12.5	(13–15)	138
	20	–0.1	14	(13–15)	155
	40	–0.7	15	(13–25)	166
threo-1-$PtCl_2$[d]	10	0.5	15.5	(14–17)	172
	20	0.6	16	(15–17)	177
	40	–0.4	17.5	(17–24)	194
erythro-4-$PtCl_2$[d]	10	0.3	13.5	(13–18)	150
	20	0.2	18	(17–20)	200
	40	–0.8	18	(16–20)	200
threo-4-$PtCl_2$[d]	10	–0.8	20	(18–22)	222
	20	–1.5	21	(20–22)	233
	40	–2.4	15	(11–17)	166
control	–	2.5	8	(7–10)	100
cisplatin	5	–3.8	19	(17–21)	238

[a] The sulfatoplatinum(II) complexes were administered ip as a solution in water, the dichloroplatinum(II) complexes as a solution in polyethylene glycol 400/1.8% NaCl 1:1 on the days 1, 5, 9

[b] T = body weight difference (day 5 – day 1) of treated animals; C = body weight difference (day 5 – day 1) of control animals

[c] data from Lit. [37]

[d] control: median survival time = 9 (9–13), T–C= 2.8 g

cisplatin like effects a two- to fourfold molar dose of the new complexes was necessary (Table 6). Furthermore no significant differences in the antitumor potency of the *threo*- and *erythro*-isomers were seen. In comparison to the parent compound *meso*-4-F-$PtSO_4$ the new *erythro*-configurated complexes were equiactive (*erythro*-3-$PtSO_4$) or even superior (*erythro*-1-$PtSO_4$, *erythro*-2-$PtSO_4$ and *erythro*-4-$PtSO_4$) on the P-388 leukemia of the mouse. The same is true for the gradation in the *threo*-series. Exchange of the "leaving groups" in the diastereomeric 1-$PtSO_4$ and 4-$PtSO_4$ complexes by chloride caused a marked diminution of the toxicity, which was, however, accompanied by a drop of the antitumor activity in case of the diastereomeric 1-Pt-complexes as well as of the *erythro*-4-Pt-complex. Interestingly, *threo*-4-$PtCl_2$ showed not only the same high antitumor activity as the sulfatoplatinum(II) analogue but also an essentially better tolerability. This compound might be a candidate for a thorough preclinical study. The decline in the antitumor activity, which can be observed upon the exchange of the sulfate by the chloride "leaving group" (*erythro*-1-$PtCl_2$, *erythro*-4-$PtCl_2$ and *threo*-1-$PtCl_2$) and the lack of an exact dose-activity-relationship in the dichloroplatinum(II) series are only explainable by an insufficient bioavailability of the latter resulting from a poor water solubility. For this reason we assume that "leaving groups" or galenic formulations, which improve the water solubility of these drugs cause an increase of their antitumor potency.

Discussion

The therapeutically established drugs cisplatin and carboplatin as well as several platinum complexes of the second generation examined in the clinic show a small spectrum of activity comprising the curable testicular cancer, the readily susceptible ovarian cancer and also less sensitive tumors like small cell lung cancer, head and neck cancer and bladder cancer, while widespread cancer diseases like breast cancer cannot be influenced significantly by these drugs [6]. At present, platinum complexes are not used as a measure of routine in the therapy of the disseminated breast cancer [26], though a definite however low activity was found in single agent trials (e. g. carboplatin 400 mg/m^2: 20% PR + CR [27]) and combination chemotherapy trials (e.g. cisplatin 100 mg/m^2 + VP 16 100 mg/m^2: 63% PR + CR [28]). However, the fascinating results achieved with cisplatin and carboplatin on testicular cancer allow the assumption that systematic structural variation in the neutral ligand of these compounds will yield "third generation platinum complexes" with which therapeutically non-controllable cancer diseases like breast cancer can be cured.

In our laboratory it was shown that [1,2-diphenylethylenediamine]platinum(II) complexes fulfill the requirements for "third generation platinum complexes", if they are substituted in the aromatic rings with appropriate functional groups. An example are the 4-methoxy- and the 4-fluoro-substituted dichloro[*meso*-1,2-diphenylethylenediamine]platinum(II) complexes (*meso*-4-CH_3O-$PtCl_2$ and *meso*-4-F-$PtCl_2$). Although these compounds hydrolyzed at comparable rates to their cytotoxic metabolites, the aquachloro- and the diaquaplatinum(II) complexes[9], *meso*-4-CH_3O-$PtCl_2$ showed only weak antitumor potency, while *meso*-4-F-$PtCl_2$ was quite

active at the same molar dose in the test on the human MDA-MB-231 breast cancer cell line [12]. The diastereomer, *rac*-4-F-$PtCl_2$, was even more effective. In addition, *rac*-4-F-$PtCl_2$ proved to be also strongly active on a subline of the L-1210 leukemia which is about 100-fold resistant to cisplatin [29]. In the diastereomeric [1,2-bis(4-fluorophenyl)-ethylenediamine]platinum(II) complexes the neutral ligand exerts a carrier function, which means that this ligand facilitates or augments the uptake by the tumor cell. In fact we found a rapid accumulation of *rac*-4-F-$PtSO_4$ into human hormone-sensitive (MCF-7) and -insensitive (MDA-MB-231) breast cancer cells and also a high degree of DNA platination [10,11]. *Rac*-4-F-$PtCl_2$ was accumulated to a similar extent in MCF-7 and MDA-MB-231 breast cancer cells [10,11]. As expected, the *meso*-compounds, which caused weaker antitumor effects on these cell lines than their diastereomers, were both accumulated and bound to DNA to a lesser extent [10,11]. With cisplatin the capacity of accumulation and DNA incorporation was considerably weaker [10,11]. *Rac*-4-F-$PtCl_2$ and its sulfatoplatinum(II) derivative proved to be also highly active on a pattern of four human breast cancer cell lines (MDA-MB-231, MCF-7, ZR-75-1, T-47-D) endowed with different "intrinsic" resistance against therapeutically used drugs like 5-fluorouracil, vinblastine, melphalan and cisplatin [30]. Due to these findings *rac*-4-F-$PtCl_2$ and its sulfatoplatinum(II) derivative meet the requirements perfectly which are expected from a platinum complex designed in accordance with the drug targeting concept.

The observation that an appropriate substitution of [(±)-1,2-diphenylethylenediamine]platinum(II) can considerably enhance the extent and the specificity of the antitumor activity, prompted us to synthesize and test [(±)-1,2-diphenylethylenediamine]dichloroplatinum(II) complexes which contain two fluorine atoms in each of the phenyl rings in positions 2/3, 2/4, 2/5, 2/6, 3/4 and 3/5, respectively [31,32]. Unfortunately, the new compounds showed a weaker or – in case of *rac*-2,4-F_2-$PtCl_2$, *rac*-2,6-F_2-$PtCl_2$ and *rac*-3,5-F_2-$PtCl_2$ – a comparable activity like the parent compound *rac*-4-F-$PtCl_2$.

However, our efforts to develop "third generation platinum complexes" surpassing the parent compounds *rac*-4-F-$PtCl_2$ and *rac*-4-F-$PtSO_4$ in their mammary tumor inhibiting properties were successful, if we introduced one or two fluorine atoms into only one of the two benzene rings of [(±)-1,2-diphenylethylenediamine]platinum(II), especially in 4- and 2,4-position, respectively (*threo*-1-$PtSO_4$ and *threo*-2-$PtSO_4$). Of these compounds only a thirtieth of the molar concentration of *rac*-4-F-$PtSO_4$ was necessary for equally strong effects on the MDA-MB-231 breast cancer cell line (Parameter: (%) Inhibition of the [^{3}H]thymidine incorporation into DNA). We could also increase the mammary tumor inhibiting potency of the parent compound *rac*-4-F-$PtSO_4$ by exchange of one 4-F-substituent by the 3-OH-group (*threo*-4-$PtSO_4$). The new complex needs a tenth of the concentration of the parent compound *rac*-4-F-$PtSO_4$ to achieve the same inhibitory effect on the [^{3}H]thymidine incorporation in experiments on the MDA-MB-231 breast cancer cell line. We chose this substitution pattern, since [(±)-1,2-bis(3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) (*rac*-3-OH-$PtCl_2$) had proven to be a very interesting compound in *in vitro* as well as *in vivo* tests on several breast cancer models [33].

The most active compounds *threo*-1-$PtSO_4$, *threo*-2-$PtSO_4$ and *threo*-4-$PtSO_4$ also surpassed the parent compound *rac*-4-F-$PtSO_4$ as to its cytotoxic potency in the test on the P-388 leukemia of the mouse. However, these compounds, especially *threo*-4-$PtSO_4$, exert strong side effects apparent from the marked decrease in body weights of the test animals. The transformation of these compounds into their dichloroplatinum(II) derivatives, which was performed in case of *threo*-1-$PtSO_4$ and *threo*-4-$PtSO_4$, led to a markedly better tolerability of the drugs. With these compounds – *threo*-1-$PtCl_2$ and *threo*-4-$PtCl_2$ – we could not obtain exact dose-activity relationships in the P-388 leukemia experiments, presumably due to their poor water solubility resulting in an insufficient bioavailability. For this reason we assume that "leaving groups" or galenic formulations which improve the water solubility of these drugs can cause an increase of the antitumor potency of the latter.

In the following publication we will report on the development of a new effective galenic formulation for poorly water soluble platinum complexes.

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Experimental Section

General Procedures. Melting points (uncorrected): Büchi 530 melting point apparatus. IR spectra: Perkin Elmer Model 580 A. ^{1}H-NMR spectra of the ligands: Varian 360L 60 MHz spectrometer. ^{1}H-NMR of the platinum complexes: Bruker PFR-NMR spectrometer WM 250 at 250 MHz. Elemental analyses: Microlaboratory of the University of Regensburg.

Syntheses

Methods A–H are representative of the syntheses of the compounds reported in Table 2.

Method A: 3-Methoxybenzyltriphenylphosphonium chloride 2

3-Methoxybenzyl chloride (15.6 g, 0.1 mol) and triphenylphosphine (26.2 g, 0.1 mol) were mixed and melted for 15 min. The crude product was suspended in ether, collected by suction filtration, washed several times with ether and air dried: colorless powder (92%), mp 240–244 °C.– ^{1}H-NMR ($CDCl_3$): δ = 3.48 (s, 3H, OMe), 5.33(d, *J* = 14 Hz, 2H, CH_2), 6.47–7.13 (m, 4H, Ar-H), 7.43–8.00 (m, 15H, Ar-H)

Method B: E/Z-4-Fluoro-3'-methoxystilbene E/Z-4

Na (9.2 g, 0.4 gatom) was dissolved in 50 ml of dry ethanol under cooling in an ice bath. Compound 2 (16.8 g, 40 mmol) was added to give a yellow suspension, which was combined with a solution of 4-fluorobenzaldehyde (5.0 g, 40 mmol) in 20 ml of dry ethanol. After the reaction mixture had been stirred for 30 min, 50 ml of water was added and the stilbene was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The stilbene *E/Z*-4 was separated from triphenylphosphine oxide by dissolution in petroleum ether: colorless oil (99%).– ^{1}H-NMR ($CDCl_3$): δ = 3.65 (s, 3H, OMe), 3.82 (s, 3H, OMe), 6.52–7.60 (m, 22H, CH, Ar-H).

Method C: *erythro/threo-1,2-Diazido-1-(4-fluorophenyl)-2-(3-methoxyphenyl)ethene e/t-4*

ICl (9.7 g, 60 mmol) was added dropwise to a stirred slurry of NaN_3 (7.8 g, 120 mmol) in 70 ml of dry acetonitrile. After cooling in an isopropanol ice bath the stilbene *E/Z*-4 (6.85 g, 30 mmol) was added. The reaction mixture was allowed to warm to room temp. and was finally refluxed for 1 h. The red-brown slurry was poured into 250 ml of water and was extracted with 250 ml of ether in 3 portions. The combined organic layers were washed several times with 250 ml of water and dried over $MgSO_4$. Removal of the solvent *in vacuo* left a colorless oil (39%). –^{1}H-NMR ($CDCl_3$): δ = 3.67 (s, 3H, OMe), 3.75 (s, 3H, OMe), 4.55 (s, 2H, CH), 4.60 (s, 2H, CH), 6.50–7.33 (m, 18H, Ar-H)

Method D: *erythro- and threo-1-(4-Fluorophenyl)-2-(3-methoxyphenyl)-ethylenediamine erythro-4a, threo-4a*

At a temp. below 0 °C a solution of *e/t*-4 (3.7 g, 12 mmol) in dry ether was dropped to an ethereal suspension of $LiAlH_4$ (1,37 g, 36 mmol). After being stirred for 30 min the mixture was heated to reflux for 1 h. Subsequently 10 ml of water was added under cooling and the precipitate was filtered off. The filtrate was dried over $MgSO_4$ and evaporated to dryness. The colorless oil was dissolved in 10 ml of methanol and treated with gaseous HCl. By repeated addition of ether the less soluble *erythro*-isomer was obtained as first fraction, the *threo*-isomer as second fraction. To get a diamine dihydrochloride which is free from its diastereomer a fractional crystallization in methanol/ether was performed (yield of *erythro*-4a 23% and of *threo*-4a 29%). The chemical purity was examined by ^{1}H-NMR spectroscopy. To obtain the free diamines the dihydrochlorides were dissolved in 10 ml of water, neutralized with 0.5N NaOH and extracted with CH_2Cl_2. The organic layers were dried over $MgSO_4$ and evaporated to dryness.

erythro-1-(4-Fluorophenyl)-2-(3-methoxyphenyl)ethylenediamine erythro-4a

Colorless powder, mp: 92–94 °C.– ^{1}H-NMR ($CDCl_3$): δ = 1.38 (s, 4H, NH_2), 3.80 (s, 3H, OMe), 4.00 (s, 2H, CH), 6.72–7.55 (m, 9H, Ar-H)

threo-1-(4-Fluorophenyl)-2-(3-methoxyphenyl)ethylenediamine threo-4a

Colorless oil.– ^{1}H-NMR ($CDCl_3$): δ = 1.55 (s, 4H, NH_2), 3.71 (s, 3H, OMe), 3.76 (s, 2H, CH), 6.57–7.35 (m, 9H, Ar-H)

Method E: *erythro-1-(4-Fluorophenyl)-2-(3-hydroxyphenyl)ethylenediamine erythro-4*

A solution of *erythro*-4a (840 mg, 3 mmol) in 60 ml of dry CH_2Cl_2 was cooled to –60 °C in an isopropanol ice bath. At this temp. BBr_3 (3.0 g, 12 mmol) was added. The reaction mixture was allowed to warm and was stirred for 24 h at room temp.. Unreacted BBr_3 was decomposed with 20 ml of methanol and the solvent was evaporated. The residue was dissolved in 20 ml of water, filtered and alkalized with 2N NaOH. Unreacted *erythro*-4a was filtered off and the filtrate was brought to pH = 7–8 with 2N HCl. The precipitated *erythro*-4 was extracted with ethyl acetate and transformed into the dihydrochloride: colorless powder (49%).^{1}H-NMR: see Table 1

Method F: *[erythro-1-(4-Fluorophenyl)-2-(3-hydroxyphenyl)ethylenediamine]diiodoplatinum(II) erythro-4-PtI_2*

A solution of *erythro*-4 (320 mg, 1 mmol) in 30 ml of water was combined with KI (1.49 g, 9 mmol) and K_2PtCl_4 dissolved in 20 ml of water. The pH was adjusted to 8 and the mixture was stirred in the dark. After 24 h, 20 ml of 2N HCl was added and the precipitate was collected, washed with water, and dried over P_2O_5 *in vacuo*: yellow powder (89%). ^{1}H-NMR: see Table 1

Method G: *Aqua[erythro-1-(4-fluorophenyl)-2-(3-hydroxyphenyl)ethylenediamine]sulfatoplatinum(II) erythro-4-$PtSO_4$*

Solid Ag_2SO_4 (140 mg, 0.45 mmol) was added to a suspension of *erythro*-4-PtI_2 (347 mg, 0.5 mmol) in 40 ml of water. The mixture was stirred for 24 h in the dark. Precipitated AgI was filtered off, and the colorless filtrate was lyophilized: colorless powder (80%). IR: see Table 3

Method H: *[erythro-1-(4-Fluorophenyl)-2-(3-hydroxyphenyl)ethylenediamine]dichloroplatinum(II) erythro-4-$PtCl_2$*

Erythro-4-$PtSO_4$ (278 mg, 0.5 mmol) was dissolved in 40 ml of a 1.8% KCl solution. After stirring for 24 h at room temp., precipitated *erythro*-2-$PtCl_2$ was collected by suction filtration, washed with water and dried over P_2O_5 *in vacuo*: yellow powder (82%). ^{1}H-NMR: see Table 1.

Biological Methods

MDA-MB-231 breast cancer cell line, P-388 leukemia of the CDF_1-mouse and hormone-insensitive MXT-M-3.2 mammary carcinoma of the BDF_1-mouse: the methods applied were identical to that described by us [34].

References

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