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Original paper

Tumor inhibiting [1,2-bis(fluorophenylethylenediamine]platinum-(II) complexes. Part I: synthesis

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Summary — The synthesis of the diastereomeric 1,2-bis(2-,3- and 4-fluorophenyl)ethylenediamines **4–6, 10–12** from *meso*-1,2-bis(2-hydroxyphenyl)ethylenediamine and 2-, 3- and 4-fluorobenzaldehyde by a diaza-Cope-rearrangement and their conversion into the [1,2-bis(2-, 3- and 4-fluorophenyl)ethylenediamine]dihaloplatinum (II) complexes (Hal = Cl, **13–18**; Hal = I, **19–24**) with K_2PtHal_4 is described. From the diiodoplatinum(II) complexes (**19–24**) the better water soluble diaqua[1,2-bis(2-, 3- and 4-fluorophenyl)ethylenediamine]platinum(II) sulfates (**25–30**) and [1,2-bis(4-fluorophenyl)ethylenediamine]dinitratoplatinum (II) complexes (**31** and **32**) are obtained by reacting with Ag_2SO_4 or $AgNO_3$. On the $P388D_1$ leukemia cell line the racemic platinum (II) complexes are more active than their *meso*-analogues and equiactive with *cis*-platin. The position of the fluorine atom and the kind of the leaving group do not influence the activity.

Résumé — Synthèse des diastéréoisomères 1,2-bis(2-,3- et 4-fluorophényl)éthylènediamines 4–6, 10–12 à partir de *méso* 1,2-bis(2-hydroxyphényl)éthylènediamine et de 2-,3- et 4-fluorobenzaldéhyde par la transposition de diaza-Cope et leur transformation en complexes [1,2-bis(2-,3- et 4-fluorophényl)éthylènediamine]dihaloplatine (II) (Hal = Cl: 13–18; Hal = I: 19–24) à l'aide de K_2PtHal_4. *A partir des complexes de diiodoplatine (II) (**19–24**), on obtient des composés plus hydrosolubles tels des sulfates de diaquaplatine (II) [1,2-bis(fluoro-2, -3 et 4 phényl)éthylènediamine] (**25–30**) et des [1,2-bis(4-fluorophényl) éthylènediamine]dinitratoplatine (II) complexes (**31** et **32**) par réaction avec Ag_2SO_4 et $AgNO_3$ respectivement. Dans les cellules en lignées de leucémie $P388D_1$, les complexes racémiques de platine (II) sont plus actifs que leurs analogues* méso *et aussi actifs que le* cis*-platine. La position du fluor et la nature du groupe partant n'influencent pas l'activité.*

diastereomeric 1,2-bis(2-,3- and 4-fluorophenyl)ethylenediamines / diaza-Cope-rearrangement / [1,2-bis(2-,3- and 4-fluorophenyl)ethylenediamine] dihaloplatinum(II) complexes / diaqua[1,2-bis(2-,3- and 4-fluorophenyl)ethylenediamine] platinum(II) salts / $P388D_1$ leukemia cell line / structure activity–relationship

Introduction

Strong antitumor activities of stereoisomeric (1,2-diphenylethylenediamine)platinum (II) complexes have been reported [1–5]. Whereas Gulotti *et al.* [1] and Noji *et al.* [2] found that there is no clear-cut preference for one stereoisomer, we generally observed in our studies [3–5] with stereoisomeric 4-substituted (1,2-diphenylethylenediamine) platinum (II) complexes on several *in vitro* and *in vivo* tumor models significantly better effects of the racemates compared with the *meso*-forms. Among these derivatives [D,L-1,2-bis(4-fluorophenyl)ethylenediamine]-dichloroplatinum (II) was the most active on the human MDA-MB 231 breast cancer cell line and the P 388 leukemia of the mouse [4].

Dichloro(1,2-diphenylethylenediamine)platinum(II) complexes are characterised by extremely low solubilities in water. This disadvantage can be overcome by their transformation into the better water soluble diaqua-(1,2-diphenylethylenediamine)platinum(II) sulfates and (1,2-diphenylethylenediamine)dinitratoplatinum(II) complexes, respectively.

However, the diaqua complexes are charged species, and it is generally accepted that a charged complex will exhibit no antitumor activity because ionized compounds are unable to diffuse freely across the membrane barrier. Nevertheless, in several studies antitumor activity of ionic platinum(II) complexes has been observed [1, 6, 7]. Bernasetti *et al.* [6] found that potassium *tert*-butylaminetrichloroplatinate(II) was active against murine P 388 and

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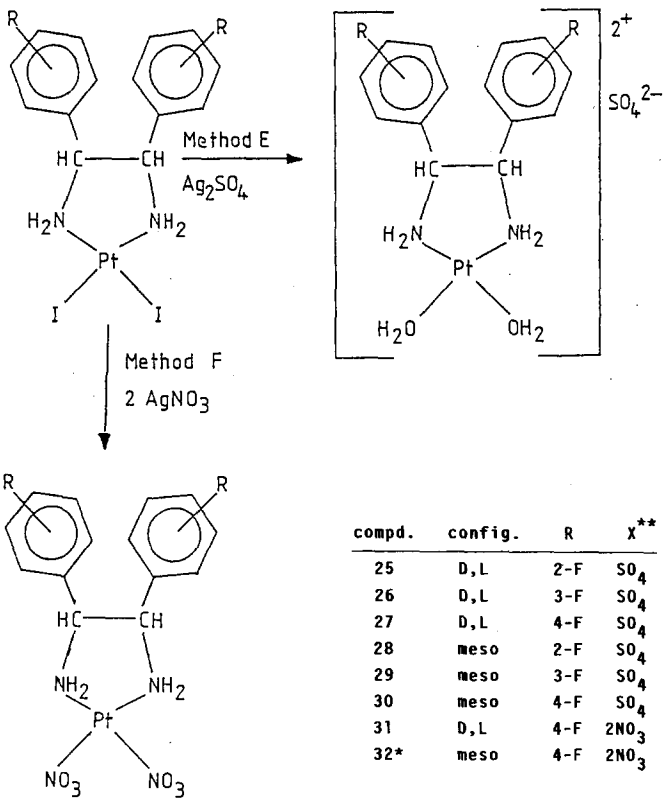
L 1210 leukemia models. Gulotti *et al.* [1], who compared a series of C-substituted dichloro(ethylenediamine)platinum(II) complexes with the analogous diaqua(ethylenediamine)platinum(II) sulfates, reported similar results for the nonionic and the ionic complexes. With *cis*-(diammine)diaquaplatinum(II)nitrate and -sulfate, Macquet and Butour [7] saw no tumor-inhibiting effect in the L 1210 leukemia of the mouse. However, a very high toxicity was observed which was attributed to the high reactivity of the diaqua complex. On the other hand, by replacing the NH_3-ligands with 1,2-diaminocyclohexane they observed a marked antitumor effect and less toxicity. This suggests that even charged complexes enter the cell if the diamine ligands are lipophilic. However, it cannot be excluded that charged complexes are partially transformed into neutral species by reaction with Cl^- or other nucleophiles in the plasma, then entering the tumor cell more quickly.

The aim of this work was to investigate the influence of: (1) the position of the fluorine atom; (2) the configuration at C_1 and C_2 of the ethylenediamine moiety; (3) the nature of leaving group (Cl^-, NO_3^- or H_2O) on the antitumor activity of diastereomeric [1,2-bis(fluorophenyl)ethylenediamine]platinum(II) complexes.

Chemistry

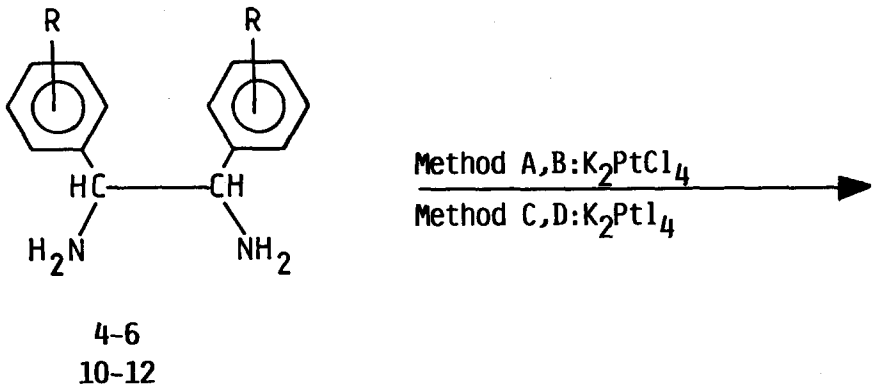
The (1,2-diphenylethylenediamine)dihaloplatinum(II) complexes **13–24** were synthesized by reacting K_2PtCl_4 or K_2PtI_4 with the corresponding diamines **4–6** and **10–12** in water or *t*-butanol/water solution at pH 5.5–6.5 and a temperature of 50°C (Scheme 1, *Methods A–D*). The analytical and NMR data are listed in Table Ia and Ib.



compd.	config.	R	X**
25	D,L	2-F	SO_4
26	D,L	3-F	SO_4
27	D,L	4-F	SO_4
28	meso	2-F	SO_4
29	meso	3-F	SO_4
30	meso	4-F	SO_4
31	D,L	4-F	$2NO_3$
32*	meso	4-F	$2NO_3$

Scheme 2.

*This compound was obtained by the reaction of the dichloroplatinum(II)-complex with $AgNO_3$.
**X = leaving group.



4-6
10-12

compd.	config.	R
4	meso	2-F
5	meso	3-F
6	meso	4-F
10	D,L	2-F
11	D,L	3-F
12	D,L	4-F

13-24

compd.	config.	R	Hal
13/19	D,L	2-F	Cl/I
14/20	D,L	3-F	Cl/I
15/21	D,L	4-F	Cl/I
16/22	meso	2-F	Cl/I
17/23	meso	3-F	Cl/I
18/24	meso	4-F	Cl/I

Scheme 1.

Table Ia. Dihalo(1,2-diphenylethylenediamine)platinum(II) complexes.

Compd.	Substituent	Yield	Formula	Halogen ligand	C %		H %		N %	
		(%)			Calcd.	Found	Calcd.	Found	Calcd.	Found
D,L-**13**	2-F	100	$C_{14}H_{14}F_2Cl_2N_2Pt$	Cl	32.70	33.09	2.74	2.80	5.45	5.46
D,L-**14**	3-F	87	"	"	"	32.87	"	2.77	"	5.38
D,L-**15**	4-F	78	"	"	"	32.83	"	2.71	"	5.42
meso-**16**	2-F	97	"	"	"	32.73	"	2.85	"	5.57
meso-**17**	3-F	83	"	"	"	32.84	"	2.84	"	5.23
meso-**18**	4-F	75	"	"	"	32.85	"	2.83	"	5.48
D,L-**19**	2-F	99	$C_{14}H_{14}F_2I_2N_2Pt$	I	24.12	24.48	2.02	2.15	4.02	4.12
D,L-**20**	3-F	94	"	"	"	24.18	"	2.05	"	4.05
D,L-**21**	4-F	92	"	"	"	24.14	"	2.05	"	3.91
meso-**22**	2-F	92	"	"	"	24.36	"	2.11	"	3.90
meso-**23**	3-F	85	"	"	"	24.34	"	2.07	"	3.90
meso-**24**	4-F	88	"	"	"	24.21	"	2.28	"	3.80

Table Ib. ^{1}H NMR data for dihalo(1,2-diphenylethylenediamine)platinum(II) complexes.

Comp.	Substituent	Halogen	Arom. H	CH	*N*H	
D,L-**13**	2F	Cl	6.94-7.97 (m,8H)	4.69 (s,2H)	5.68 (m^b*,2H)	6.45 (m^b,2H)
D,L-**14**	3-F	"	6.99-7.64 (m,8H)	5.10 (s,2H)	5.98 (m^b,2H)	6.57 (m^b,2H)
D,L-**15**	4-F	"	7.05-7.12 (t,4H; 3.5-Pos.) 7,82-7,88 (q,4H; 2.6-Pos.)	5.44 (s,2H)	6.06 (m^b,2H)	6.62 (m^b,2H)
meso-**16**	2-F	"	6.99-8.48 (m,8H)	4.75 (s,2H)	5.73 (m^b,2H)	6.38 (m^b,2H)
meso-**17**	3-F	"	7.03-7.61 (m,8H)	4.65 (s,2H)	5.82 (m^b,2H)	6.29 (m^b,2H)
meso-**18**	4-F	"	7.07-7.14 (t,4H; 3.5-Pos.) 7.63-7.69 (q,4H; 2.6-Pos.)	4.67 (s,2H)	5.76 (m^b,2H)	6.26 (m^b,2H)
D,L-**19**	2-F	I	6.94-8.08 (m,8H)	4.75 (s,2H)	5.50 (m^b,2H)	6.40 (m^b,2H)
D,L-**20**	3-F	"	7.01-7.53 (m,8H)	4.47 (s,2H)	5.58 (m^b,2H)	6.32 (m^b,2H)
D,L-**21**	4-F	"	7.02-7.24 (t,4H; 3.5-Pos.) 7.58-7.63 (q,4H; 2.6-Pos.)	4.44 (s,2H)	5.50 (m^b,2H)	6.23 (m^b,2H)
meso-**22**	2-F	"	6.99-8.41 (m,8H)	4.72 (s,2H)	5.61 (m^b,2H)	6.29 (m^b,2H)
meso-**23**	3-F	"	7.01-7.58 (m,8H)	4.51 (s,2H)	5.64 (m^b,2H)	6.15 (m^b,2H)
meso-**24**	4-F	"	7.07-7.14 (t,4H; 3.5-Pos.) 7.63-7.69 (q,4H; 2.6-Pos.)	4.43 (s,2H)	5.76 (m^b,2H)	6.26 (m^b,2H)

250 MHz, δ(ppm), solvent: DMF-d_7, standard: TMS_{INT}
*^{b} = broad.

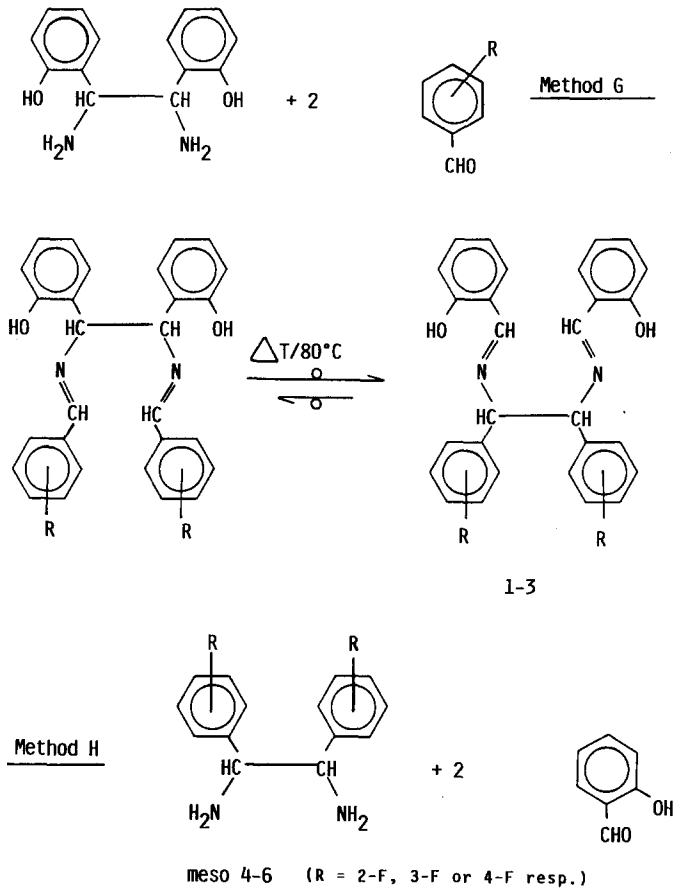
Table II. Diaqua(1,2-diphenylethylenediamine)platinum(II) complexes and (1,2-diphenylethylenediamine)dinitratoplatinum(II) complexes.

Compd.	Substituent	Yield	Formula	Leaving groups	C%		H%		N%	
		(%)			Calcd.	Found	Calcd.	Found	Calcd.	Found
D,L-**25**	2-F	79	$C_{14}H_{14}F_2N_2O_4PtS\cdot 2H_2O$	SO_4^-	29.23	29.67	3.15	3.17	4.87	4.48
D,L-**26**	3-F	74	"	"	"	29.52	"	3.22	"	4.85
D,L-**27**	4-F	88	"	"	"	29.20	"	3.29	"	4.63
meso-**28**	2-F	94	"	"	"	29.32	"	3.12	"	4.84
meso-**29**	3-F	81	"	"	"	28.98	"	3.17	"	4.97
meso-**30**	4-F	85	"	"	"	29.54	"	3.15	"	4.87
D,L-**31**	4-F	78	$C_{14}H_{14}F_2N_4O_6Pt$	$2NO_3^-$	29.64	30.04	2.49	2.76	9.88	9.68
meso-**32***	4-F	17	"	"	"	30.07	"	2.89	"	9.68

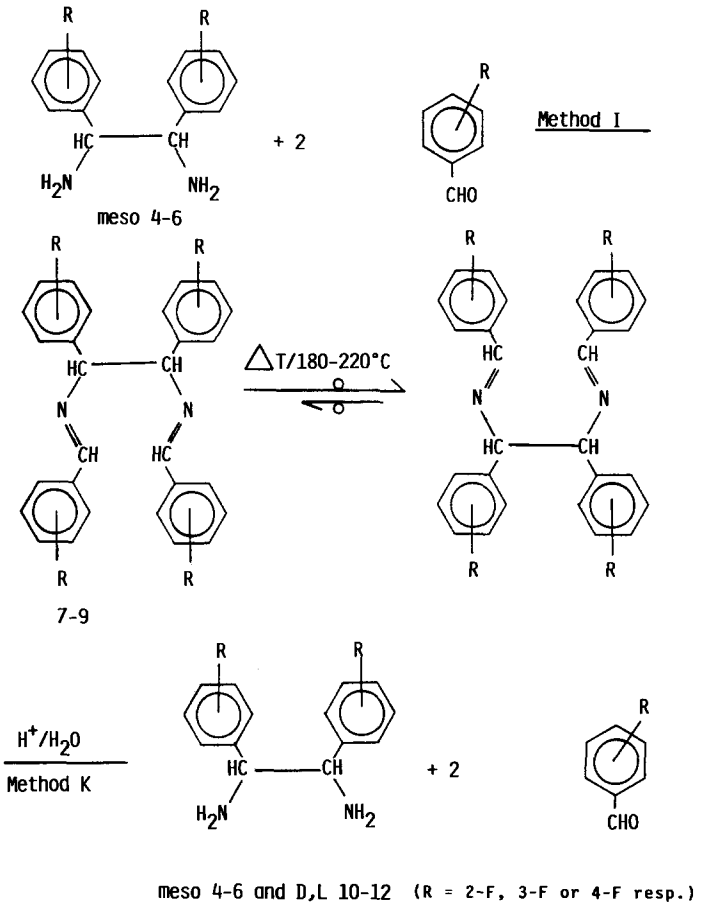
*This compound was obtained by the reaction of the dichloroplatinum(II) complex instead of the diiodoplatinum(II) complex with $AgNO_3$. The lower reactivity of the dichloroplatinum(II) complex is responsible for the small yield.

The diaqua(1,2-diphenylethylenediamine)platinum(II) sulfates **25**–**30** and (1,2-diphenylethylediamine)dinitratoplatinum(II) complexes **31**–**32** are obtained by the reaction of the (1,2-diphenylethylenediamine)diiodoplatinum(II) compounds **19**–**24** with Ag_2SO_4 or $AgNO_3$ in water (Scheme 2, *Method E,F*; Table II).

The *meso*-1,2-diphenylethylenediamines **4**–**6** were synthesized by the [3.3] sigmatropic diaza-Cope-rearrangement reaction as described by Vögtle and Goldschmitt [8] (Scheme 3). At a temperature below 120°C *meso*-1,2-bis(2-hydroxyphenyl)ethylenediamine and the respective aldehyde react to form quantitatively the corresponding *meso-N,N'*-disalicylidene-diphenylethylenediamines **1**–**3** (*Method G*, Table III). Subsequent acidic hydrolysis yielded the *meso*-1,2-diphenylethylenediamines **4**–**6** (*Method H*). The D,L-1,2-diphenylethylenediamines **10**–**12** were synthesized by the *meso*-D,L-stereoisomerisation of the *meso-N,N'*-dibenzylidene-1,2-diphenylethylenediamines **7**–**9** (Table IV), which are identically substituted in all 4 phenyl rings, at a temperature >200°C and followed by a sulfuric acid hydrolysis (Scheme 4, *Method K*). The diastereomers were separated by fractional crystallisation of the dihydrosulfates or the free bases (see experimental section, *Methods* K_a and K_b). The analytical data of the 1,2-diphenylethylenediamines are listed in Table V.



Scheme 3.



Scheme 4.

Table III. *Meso-N,N'*-disalicylidene-1,2-diphenylethylenediamines.

Compd.	Substituent	mp (°C)	Yield (%)	^{1}H NMR data, 60 MHz, standard: TMS^{INT} solvent: $CDCl_3$		
				H-benzyl.	H-arom.	N=C–H
1	2-F	215-218	85	a ^{1}H NMR spectrum was not recorded		
2	3-F	213-214	73	4.73 (s,2H)	6.73-7.38 (m,16H)	8.11 (s,2H)
3	4-F	178-180	72	4.63 (s,2H)	6.62-7.44 (m,16H)	8.10 (s,2H)

Table IV. *Meso-N,N'*-dibenzylidene-1,2-diphenylethylenediamines.

Compd.	Substituent	mp (°C)	Yield (%)	^{1}H NMR data, 60 MHz, standard: TMS^{INT} solvent:$CDCl_3$		
				H benzyl.	H arom.	N=C−H
7	2-F	186-188	85	a ^{1}H NMR spectrum was not recorded		
8	3-F	148-149	87	4.64 (s,2H)	6.79-7.63 (m,16H)	7.91 (s,2H)
9	4-F	166-167	88	4.62 (s,2H)	6.72-7.84 (m,16H)	7.93 (s,2H)

Table Va. 1,2-diphenylethylenediamines.

Compd.	Substituent	Yield (%)	mp (°C)	C		H		N	
				Calcd.	Found	Calcd.	Found	Calcd.	Found
meso-**4**	2-F	74	91-92	67.72	67.72	5.68	5.70	11.28	11.28
meso-**5**	3-F	92	60-61	"	67.63	"	5.71	"	11.03
meso-**6**	4-F	44	99-101	"	67.74	"	5.75	"	11.27
D,L-**10***2HCl	2-F	34	226-228	52.35	52.31	5.02	4.97	8.72	8.67
D,L-**11***2HCl	3-F	22	237-238	"	52.23	"	5.16	"	8.56
D,L-**12***2HCl	4-F	66	237-238	"	52.37	"	4.94	"	8.65

Table Vb. ^{1}H NMR data of the 1,2-diphenylethylenediamines.

Compd.	Substituent	H arom.	H benzyl.	N-H
meso-**4**	2-F	6.82-7.68 (m,8H)	4.48 (s,2H)	1.50 (s,4H)
meso-**5**	3-F	6.74-7.74 (m,8H)	3.99 (s,2H)	1.73 (s,4H)
meso-**6**	4-F	6.83-7.46 (m,8H)	3.98 (s,2H)	1.39 (s,4H)
D,L-**10**	2-F	6.76-7.60 (m,8H)	4.47 (s,2H)	1.73 (s,4H)
D,L-**11**	3-F	6.68-7.34 (m,8H)	4.02 (s,2H)	1.59 (s,4H)
D,L-**12**	4-F	6.69-7.28 (m,8H)	3.97 (s,2H)	1.57 (s,4H)

60 MHz, standard: TMS_{INT}, solvent: $CDCl_3$.

^{1}H NMR spectroscopy

The classification of the new 1,2-diphenylethylenediamines **4**−**6** and **10**−**12** as the D,L- or *meso*-form results from the stereoselective course of the diaza-Cope-rearrangement reaction. The aromatic protons of the D,L compounds are shifted upfield relative to the respective protons of the *meso*-compounds. By the formation of the dichloroplatinum(II) complexes all resonances of the 1,2-diphenylethylenediamines, particularly those of the amine and benzylic protons are shifted to lower field. Since the complexation blocks rotation around the C−N axis, both N-bound protons become diastereotopic owing to the neighborhood of the asymmetric C-atoms. This leads to the appearance of separate signals for the axially and for the equatorially orientated N-H atoms. Due to a coupling between NH_2, benzylic CH and ^{195}Pt, the NH and CH signals are broadened. The distinction of the 2 NH signals from the CH (benzylic) signals was performed using the 4-fluorosubstituted complex D,L-15 as a model. The lack of free electron pairs at the N of the complex prevents an exchange of the N-standing H atoms by deuterium. Therefore the H−D exchange was effected with the free ligand, which was then transformed into the dichloroplatinum(II) complex. The NH signals appeared downfield from the CH signal.

The resonances of the amine and benzylic protons of the dichloroplatinum(II) compounds are shifted downfield compared with the diiodoplatinum(II) complexes. Evaluable ^{1}H NMR spectra of the diaquaplatinum(II) and nitratoplatinum(II) complexes could not be obtained in available solvents like trifluoroacetic acid, DMF-d_7 or CD_3OD because of the insufficient solubility or reactions with the solvent. Due to the formation of various coordination species the resonance signals are broadened.

The solubility of these diaquaplatinum(II) complexes in water is sufficient to get clear solutions for the cell culture and animal experiments but the solubility is too low for ^{1}H NMR spectroscopy.

IR spectroscopy

The IR spectra (KBr) of the dichloroplatinum(II) complexes (**13**−**18**) reveal that the absorption band for the N−H stretching vibration is shifted by ≈ 100 cm^{-1} to

Table VI. Dihalo(1,2-diphenylethylenediamine)platinum(II) complexes.

Compd.	Substituent	Formula	Halogen ligand	νN-H			νPt-Cl
D,L-**13**	2F	$C_{14}H_{14}F_2Cl_2N_2Pt$	Cl	3270 s,	3200 s		320 m
D,L-**14**	3F	"	"	"	"		"
D,L-**15**	4F	"	"	3280 m,	"		"
meso-**16**	2F	"	"	3260 m,	3210 s		"
meso-**17**	3F	"	"	"	"		"
meso-**18**	4F	"	"	"	"		"
D,L-**19**	2F	$C_{14}H_{14}F_2I_2N_2Pt$	I	3240 s,	3180 m,		
D,L-**20**	3F	"	"	"	"		
D,L-**21**	4F	"	"	3230 m,	3200 m,		
meso-**22**	2F	"	"	3250 s,	3200 s,	3160 s	
meso-**23**	3F	"	"	3265 s,	3220 s,	3195 s	
meso-**24**	4F	"	"	3260 s,	3200 m,	3145 m	

lower wave numbers (3200–3280 cm^{-1}) upon formation of the platinum-nitrogen bonds. The absorption band in the far infrared close to 320 cm^{-1} indicates a *cis*-$PtCl_2$ structure [9] and is absent in the diiodoplatinum(II) complexes (Table VI).

The sulfatoplatinum(II) complexes (KBr) (**25–30**) show a strong absorption band at 1130 cm^{-1} and a weaker band at 620 cm^{-1}, which are indicative for a ionic sulfate residue, and a broad absorption band for the OH stretching vibration at 3440 cm^{-1}. On the basis of these results and of elemental analyses we suppose a structure in which 2 H_2O molecules are bound to the platinum atom with sulfate as counter ion. In case of coordination to platinum the high T_d symmetry of the sulfate ion is lowered resulting in further absorption bands [10]. Additional weak bands at 900–1000 cm^{-1} and at 1180–1220 cm^{-1} in the spectra of **25–30** suggest a contamination with other types of coordination complexes.

The IR spectra of the nitratoplatinum(II) complexes (KBr) (**31** and **32**) show, when compared with the corresponding diiodoplatinum(II) complexes 4 new absorption bands: 1500 cm^{-1} (m), 1390 cm^{-1} (s) and 1270 cm^{-1} (m) and 990 cm^{-1} (m). The absorption bands at 1500 cm^{-1} and 1270 cm^{-1} originate from an asymmetric and a symmetric N-O_2 stretching vibration, while the N-O stretching vibration produces an absorption band at 990 cm^{-1} [11]. The absorption band at 1390 cm^{-1} (NO_2 asym. str.) can be attibuted to the free nitrate ion [12].

However, this band is not present when the spectrum is taken as a Nujol mull. The other absorptions remain unchanged. This phenomenon can be explained by a reaction of the nitratoplatinum(II) complex with the KBr; a bromoplatinum(II) complex is formed and free nitrate is liberated. From this observation and the fact that nitratoplatinum(II) complexes (**31** and **32**) contain no additional H_2O molecules it can be concluded that 2 nitrate ions are coordinated unidentate at one platinum atom. For the sulfatoplatinum(II) complexes the IR spectra were identical in KBr and Nujol.

Pharmacological results

The experiments on the $P388D_1$ leukemia cell line show that identically configurated [1,2-bis(fluorophenyl)ethylenediamine]dichloroplatinum(II) complexes (**13–18**) and their diaquaplatinum(II) sulfates and dinitratoplatinum(II) complexes (**25–32**) are comparably active in inhibiting cell proliferation and ^{3}H-thymidine incorporation regardless of the position of the fluorine atoms. Generally compared with the *R,S*-compounds a stronger activity of the *R,R / S,S* diastereomers was observed, the latter compounds being equiactive with *cis*-platin (Table VII).

Experimental protocols

Syntheses

*Method A: Dichloro-D,L-(1,2-diphenylethylenediamine)platinum(II) (D,L-**13** to D,L-**15**; Table Ia, Ib)*

D,L-1,2-Diphenylethylenediamine– 2HCl (1 mmol) is dissolved in 10 ml water and a solution of K_2PtCl_4 (1 mmol) in 8 ml water is added slowly. The pH is adjusted to 5.5–6.5 with 1N NaOH. The solution is stirred at 50°C for 24–30 h in the dark. During this time the pH is adjusted several times until pH stabilizes. The yellow precipitate is collected by suction filtration using a no. 3 fritted glass filter, washed with 1N HCl, water and dried in vacuum over silica gel / $CaCl_2$.

*Method B: Dichloro-*meso*-(1,2-diphenylethylenediamine)platinum(II) (*meso*-**16** to* meso*-**18**; Table Ia, Ib)*

Meso-1,2-diphenylethylenediamine (1 mmol) is suspended in 10–15 ml water and dissolved by heating (*ca.* 50°C) and addition of 1 N HCl. The solution is diluted with 25 ml *t*-butanol. The subsequent reaction is conducted in analogy to Method A.

*Method C: D,L-(1,2-Diphenylethylenediamine)diiodoplatinum(II) (D,L-**19** to D,L-**21**; Table Ia, Ib)*

K_2PtCl_4 (1 mmol), D,L-1,2-diphenylethylenediamine–2 HCl (1 mmol) and KI (20 mmol) are each dissolved in 10 ml water. The KI solution is added to the K_2PtCl_4 solution. The mixture is stirred for 30 min at 50°C. The color changes from red to black. This mixture is added slowly to the solution of the ligand and the complex precipitates immediately. The pH is adjusted once with 1 N NaOH to 5.5–6.5. After stirring for 30 min at 50°C in the dark the precipitate is collected by suction filtration using a no. 3 fritted glass filter, washed with water and dried in vacuum over silica gel / $CaCl_2$.

Method D: meso*-(1,2-Diphenylethylenediamine)diidoplatinum(II) (*meso*-**22** to* meso*-**24**; Table Ia, Ib)*

Meso-1,2-diphenylethylenediamine (1 mmol) is suspended in 10–15 ml water and dissolved by heating (*ca.* 50°C) and addition of 1 N HCl. The solution is diluted with 25 ml-*t*-butanol. The subsequent reaction is carried out as in Method C.

Table VII. Effect of diastereomeric [1,2-bis(fluorophenyl)ethylenediamine]dichloroplatinum(II) complexes and diaqua[1,2-bis(fluorophenyl)ethylenediamine]platinum(II) sulfates and [1,2-bis(4-fluorophenyl)ethylenediamine]dinitratoplatinum(II) complexes on ^{3}H-thymidine incorporation and cell proliferation of P388D$_1$ leukemia cells, 48 h drug incubation.

Compound (No.)	Cell No.		^{3}H-Thymidine incorporation	
	% T/C at $1 \cdot 10^{-6}$M	ED_{50}* (M)	% T/C at $1 \cdot 10^{-6}$M	ED_{50} (M)
meso-2F-$PtSO_4$ (**28**)	93	$2.6 \cdot 10^{-6}$	92	$2.6 \cdot 10^{-6}$
meso-3F-$PtSO_4$ (**29**)	77	$1.8 \cdot 10^{-6}$	77	$2.3 \cdot 10^{-6}$
meso-4F-$PtSO_4$ (**30**)	88	$3.8 \cdot 10^{-6}$	84	$4.0 \cdot 10^{-6}$
meso-4F-$Pt(NO_3)_2$ (**32**)	86	$3.8 \cdot 10^{-6}$	92	$3.4 \cdot 10^{-6}$
meso-2F-$PtCl_2$ (**16**)	69	$2.3 \cdot 10^{-6}$	75	$2.2 \cdot 10^{-6}$
meso-3F-$PtCl_2$ (**17**)	83	$4.1 \cdot 10^{-6}$	93	$4.8 \cdot 10^{-6}$
meso-4F-$PtCl_2$ (**18**)	90	$2.6 \cdot 10^{-6}$	91	$3.4 \cdot 10^{-6}$
D,L-2F-$PtSO_4$ (**25**)	34	$4.3 \cdot 10^{-7}$	8	$3.5 \cdot 10^{-7}$
D,L-3F-$PtSO_4$ (**26**)	32	$5.1 \cdot 10^{-7}$	26	$6.5 \cdot 10^{-7}$
D,L-4F-$PtSO_4$ (**27**)	31	$5.4 \cdot 10^{-7}$	24	$7.2 \cdot 10^{-7}$
D,L-4F-$Pt(NO_3)_2$ (**31**)	–	$4.6 \cdot 10^{-7}$	–	$5.9 \cdot 10^{-7}$
D,L-2F-$PtCl_2$ (**13**)	23	$2.0 \cdot 10^{-7}$	8	$4.8 \cdot 10^{-7}$
D,L-3F-$PtCl_2$ (**14**)	30	$4.4 \cdot 10^{-7}$	14	$4.9 \cdot 10^{-7}$
D,L-4F-$PtCl_2$ (**15**)	31	$4.3 \cdot 10^{-7}$	23	$5.5 \cdot 10^{-7}$
Cis-platin	29	$4.8 \cdot 10^{-7}$	19	$4.5 \cdot 10^{-7}$

*ED_{50} = the effective dose, which decreases the tumor growth by 50 %.

*Method E: Diaqua-(1,2-diphenylethylenediamine)platinum(II) sulfate (D,L-**25** to D,L-**27**, meso-**28** to meso-**30**; Table II)*
Diiodoplatinum(II) complex (1 mmol) and Ag_2SO_4 (0.95 mmol) are suspended in 150 ml water. The mixture is stirred for 24 h at 50°C in the dark. The precipitated AgI is filtered off using a no. 4 fritted glass filter. The filtrate is freeze-dried. An excess of water can be removed by drying in vacuum over silica gel / $CaCl_2$.

*Method F: (1,2-diphenylethylenediamine)dinitratoplatinum(II) (D,L-**31** and meso-**32**; Table II)*
The reaction is conducted in the same manner as described in method E, but instead of 0.95 mmol Ag_2SO_4 1.90 mmol $AgNO_3$ are used.

Method G: meso-N,N'-*disalicylidene-1,2-diphenylethylenediamine (meso-**1** to* meso-*3; Table III)*
Meso-1,2-bis(2-hydroxyphenyl)ethylenediamine (0.1 mol) and the related isomeric fluorobenzaldehyde (0.2 mol) are heated under reflux in 100 ml acetonitrile for 4 h. The solution is concentrated until the product begins to precipitate. After standing in the refrigerator for 1 h the precipitate is collected in a Buchner funnel, washed with small amounts of ice-cold acetonitrile and dried over P_2O_5.

Method H: meso-*1,2-diphenylethylenediamine (meso-**4** to* meso-*6; Table Va and Vb)*
Meso-N,N'-disalicylidene-1,2-diphenylethylenediamine (30 g) is hydrolyzed with 300 ml 5 N H_2SO_4 and the salicylic aldehyde formed is removed by steam distillation. The hot solution is filtered and made alkaline with 20% NaOH under vigorous stirring until the free base is precipitated completely. The diamine is extracted with CH_2Cl_2 and the organic layer dried over $MgSO_4$. The solvent is removed in vacuum and the residue is dried over P_2O_5.

Method I: meso-N,N'-*dibenzylidene-1,2-diphenylethylenediamine (meso-**7** to* meso-*9; Table IV)*
Meso-1,2-diphenylethylenediamine (0.1 mol) and the aldehyde (0.2 mol) are heated under reflux in 100 ml acetonitrile for 5 h. The mixture is concentrated until the product begins to precipitate. After standing in the refrigerator for 1 h the product is collected in a Buchner funnel, washed with small amounts of ice-cold acetonitrile and dried over P_2O_5.

*Method K: D,L-1,2-diphenylethylenediamine (D,L-**10** to D,L-**12**; Table Va and Vb)*
Meso-N,N'-dibenzylidene-1,2-diphenylethylenediamine (25 mmol) is melted under vigorous stirring. After 10 min the liquid is allowed to cool. The glassy mass is hydrolized with 150 ml 5 N H_2SO_4 and the salicylic aldehyde formed is removed by steam distillation. The hot solution is filtered.

Isolation method K_a. The sulfate salt of D,L-1,2-diphenylethylenediamine crystallizes at room temperature. The salt is collected in a Buchner funnel. In a separatory funnel the sulfate salt is treated with 1 N NaOH, extracted with CH_2Cl_2 and the organic layer is dried over $MgSO_4$. After removal of the solvent the free base is obtained as an oil (D,L-**12**) or as crystals (D,L-**10**: mp: 79–81°C). To gain the *meso*-1,2-diphenylethylenediamine the mother liquor is treated as described in Method H.

Isolation method K_b. The diastereomeric 1,2-diphenylethylenediamines are precipitated with 20% NaOH, extracted with CH_2Cl_2 and the organic layer is dried over $MgSO_4$. After removal of the solvent the diastereomers are separated by fractional crystallisation in ether / petrolether 5:1. The *meso*-1,2-diphenylethylenediamine crystallizes and the D,L isomer remains in the mother liquor. The *meso*-compound is separated by suction filtration using a fritted glass filter, The D,L-compound is received from the filtrate by removing the solvent.(D,L-**11**: oil) The transformation of the 1,2-diphenylethylenediamines into their dihydrochlorides was achieved by dissolving in small amount of methanol and addition of a saturated solution of anhydrous HCl in Et_2O. The crystalline precipitate is isolated by suction filtration using a fritted glass filter.

Biological methods

P388D$_1$ leukemia cell structure experiments: standard conditions. The P388D$_1$ cells (ATCC CCL 46) were grown in static suspension culture in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated horse serum (Boehringer), 10 mM HEPES buffer (Biochrom), 2 mM glutamine (Biochrom) and $NaHCO_3$ (0.85 g/l) in a H_2O saturated atmosphere containing 5% CO_2 at 37°C. Stock cultures were grown in 75 cm^2 culture flasks (Falcon). The diaqua and nitratoplatinum(II) complexes were dissolved in bidistilled water or methanol, the dichloroplati-

num(II) complexes in DMF. The test compounds were added as 500- or 1000-fold concentrated stock solutions.

Determination of ED_{50}. 2 ml of cell suspension ($7-8 \cdot 10^4$ cells / ml) were placed in glass centrifuge tubes sealed with aluminium caps and incubated under standard conditions. After 4 h incubation cells were counted with a Coulter Counter (Coulter Electronics Ltd.) and the test compounds were added. 2 h prior to the end of the experiment the cells were labeled with 0.3 μCi [^{3}H]thymidine (40–60 Ci / mmol, New England Nuclear) per tube. After 48 h 0.5 ml of culture were used to determine the cell number, the remaining portion was centrifuged and washed twice with ice-cold PBS. (Phosphate buffered saline: NaCl (8g), KCl (0.2 g), $Na_2PO_4 \times 2\ H_2O$ (1.0 g), $NaH_2PO_4 \times 1H_2O$ (0.15 g) and KH_2PO_4 (0.2 g) in 1 l of H_2O. The pellet was resuspended in 1 ml water and the cells were destroyed by sonication using a Branson sonifier; 4 ml 10% TCA were added and the precipitate was filtered with 0.45 μm filter (Sartorius). The ^{3}H-thymidine incorporation was determined in 10 ml Quickszint 212 (Zinsser Analytik) in a Beckmann LS 1801 liquid scintillation counter. Percentage T / C-values based on cell number were calculated according to $[(T\text{-}Z) / (C\text{-}Z)] \cdot 100$ (T: cell number of treated cell culture at the end of incubation, C: cell number of untreated cell culture at the end of incubation, Z: cell number at the beginning of incubation).

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