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Synthesis, Characterization and Cytotoxicity of Cyclopentadienyl Ruthenium(II) Complexes Containing Carbohydrate-Derived Ligands

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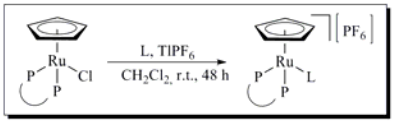
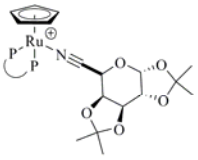
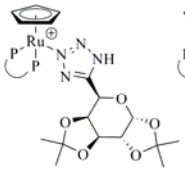
The synthesis, characterization and cytotoxic evaluation of new cyclopentadienylruthenium(II) complexes of general formula $[(\eta^5\text{-}C_5H_5)Ru(PP)L][PF_6]$, (PP = two triphenylphosphine, 1,2-diphenylphosphinoethane), L being galactose and fructose carbohydrate derivative ligands, *N*-coordinated to the metal centre by nitrile, tetrazole and 1,3,4-oxadiazole moieties, is described.

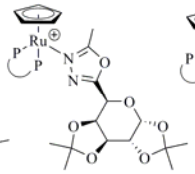




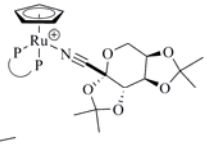
[**1**][PF_6]: PP = Dppe (81%)
[**7**][PF_6]: PP= $2PPh_3$ (77%)



[**2**][PF_6]: PP = Dppe (86%)
[**8**][PF_6]: PP= $2PPh_3$ (78%)



[**3**][PF_6]: PP = Dppe (74%)



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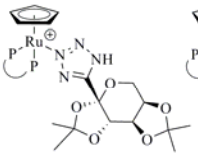

[**5**][PF_6]: PP = Dppe (82%)
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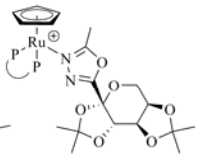

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Synthesis, Characterization and Cytotoxicity of Cyclopentadienyl Ruthenium(II) Complexes Containing Carbohydrate-Derived Ligands

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Abstract

We here report the synthesis of new cyclopentadienyl ruthenium (II) complexes of general formula $[(\eta^5\text{-}C_5H_5)Ru(PP)(\mathbf{L})]^+$(PP = two triphenylphosphine, 1,2-diphenylphosphinoethane), isolated as PF_6^- salts, with **L** being galactose and fructose carbohydrate derivative ligands, *N*-coordinated to the metal centre by nitrile, tetrazole and 1,3,4-oxadiazole moieties. The ten new organometallic compounds were fully characterized by FT-IR, ^1H, ^{13}C, and ^{31}P NMR spectroscopies, and by elemental analysis. The cytotoxicity of the ruthenium(II) compounds was tested on *HeLa* cancer cells (cervical carcinoma), unveiling IC_{50} values in the low micromolar range.

Keywords: Ruthenium(II), Cyclopentadienyl, Carbohydrates, Organometallic, Cytotoxicity

1. Introduction

Organometallic complexes containing monosaccharide ligands represent a small but challenging field in modern chemistry. Carbohydrates are the largest class of natural compounds and thereby readily available and renewable. They provide a large number of functional groups and several stereogenic centres *per* molecule, and each of the hydroxyl groups offers the opportunity of selective modification and coordination [1, 2]. They can act as monodentate as well as polydentate chelating ligands with pronounced three-dimensional characteristics [3] and their coordination capability is not limited to oxophilic metal centers: the change of donor atoms from oxygen to others, e.g., nitrogen, enables the coordination to almost every metal atom [4]. They allow also some control over the lipophilicity/aqueous solubility of the complexes, by selective modification of the carbohydrate moiety.

Since the accidental discovery of the anticancer drug cisplatin by Rosenberg and co-workers in 1965 [5], metal complexes have attracted much interest as metallopharmaceuticals. Although cisplatin is still nowadays successfully used in the treatment of many cancer types, problems such as toxicity, side effects and drug resistance lead to investigation of alternative anticancer drugs.

Among the metal atoms used in anticancer metal complexes, ruthenium is most unique. Despite being a rare noble metal, unknown to living systems, ruthenium compounds show remarkable features, such as low general toxicity, the ability to mimic iron binding to biomolecules (transferrin, albumin) and stronger affinity for cancer tissues over normal tissues [6,7]. In particular, the families of half-sandwich organometallic complexes $[(\eta^6\text{-}C_6H_6)Ru(L)_3]$ [8-17] and $[(\eta^5\text{-}C_5H_5)Ru(L)_3]$ [18-23] in which three coordination sites are occupied by the aromatic rings, have been studied for their anticancer properties, evidencing cytotoxic properties in cisplatin resistant cancer cell

lines, with IC_{50} values in nanomolar range. Apart from applications as anticancer drugs, other medical applications of ruthenium compounds have been explored. Uses include immunosupressants [24], nitric oxide scavengers [25], antimicrobial agents [26, 27], malaria [28] and Chaga's disease treatment [29].

The synthesis of ruthenium compounds bearing carbohydrate derived ligands is a relatively unexplored area: our bibliographic search revealed some examples of ruthenium carbonyl clusters containing carbohydrate moieties [30-34], ruthenium-arene complexes containing a carbohydrate phosphite derivative with anticancer properties [35-37], and a report of ruthenium cyclopentadienyl complexes with coordinated thiomonosaccharides concerning their promising anti-inflammatory effects [38].

As part of our endeavour to produce a library of carbohydrate-containing organometallic compounds, we here report the synthesis and cytotoxic evaluation against human *HeLa* cells (cervical carcinoma) of ten new η^5-cyclopentadienyl ruthenium(II) complexes of general formula $[(\eta^5\text{-}C_5H_5)Ru(PP)(\mathbf{L})]^+$, isolated as PF_6^- salts, in which **L** are galactose and fructose carbohydrate derivative ligands, functionalized with nitrile, tetrazole and 1,3,4-oxadiazole *N*-coordinating moieties. The electronic density and the stereochemichal environment of the metal centre are played by using two different phosphanes were used as co-ligands, PPh_3 and Dppe. All new compounds were characterized by IR, ^1H, ^{13}C, ^{31}P-NMR spectroscopies and by elemental analysis.

2. Results and Discussion

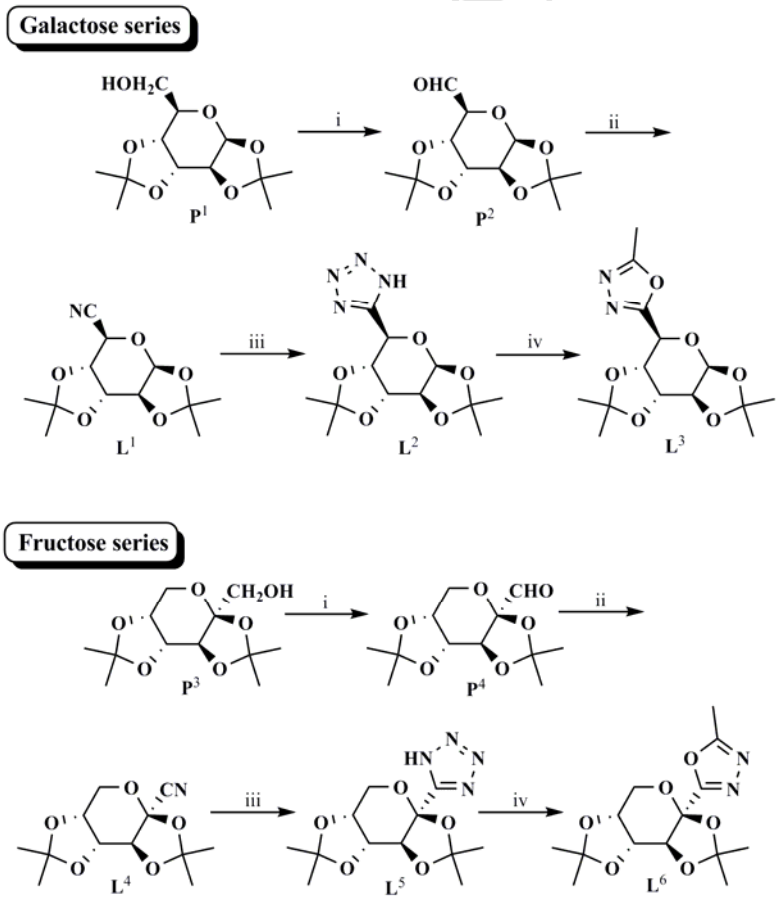
2.1. *Synthesis of the carbohydrate derivative ligands*

The aldehyde precursors $\mathbf{P^2}$ and $\mathbf{P^4}$ (Scheme 1) were obtained by oxidation of the commercially available 1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose ($\mathbf{P^1}$) and

2,3:4,5-Di-*O*-isopropylidene-β-D-fructopyranose (**P^3**), prepared as described in the literature [39], respectively. The corresponding nitrile derivatives **L^1** and **L^4** were obtained in good yields by reaction with hydroxylamine hydrochloride and subsequent dehydration of the oximes with dicyclohexylcarbodiimide (DCC).

The tetrazole derivatives **L^2** and **L^5** were obtained quantitatively by 1,3-dipolar cyclo-addition of the corresponding nitriles with sodium azide, in DMF. Finally, acylation in boiling acetic anhydride of **L^2** and **L^5** afforded the 1,3,4-oxadiazole derivatives **L^3** and **L^6**, respectively, in excellent yields.

Compounds P^2, **L^1**, **L^2**and **L^3** were fully characterized ^{1}H-, ^{13}C-NMR and FTIR spectroscopies, and by elemental analysis. Compounds **P^4**, **L^4**, **L^5** and **L^6** were obtained and its ^{1}H-, ^{13}C-NMR spectra compared with the data described in the literature [40].



Scheme 1- Synthesis of the carbohydrate-derived ligands.I) PCC, CH_2Cl_2; ii) 1- $H_2NOH{\cdot}HCl$, Pyridine; 2- $CuSO_4{\cdot}5H_2O$, Et_3N, DCC, CH_2Cl_2; iii) NaN_3, NH_4Cl, DMF, 100 ºC; iv) Ac_2O, Δ.

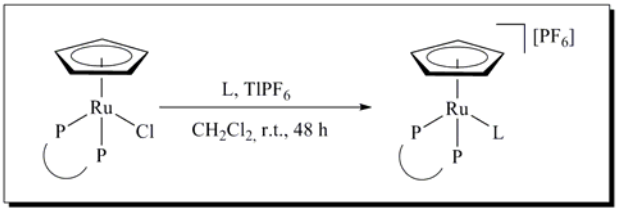
L^2 and **L^3** are derivatives of topiramate, an anticonvulsant used in epilepsy treatment, and were in this case proposed as less toxic, more efficient alternative anticonvulsant drugs.

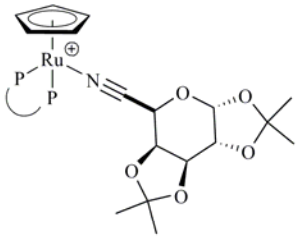
2.2. *Synthesis of the Ru(II) complexes*

The novel cationic complexes of general formula $[(\eta^5\text{-}C_5H_5)Ru(PP)(\mathbf{L})]^+$((PP) = $2PPh_3$ or Dppe), isolated as PF_6^- salts, were prepared by halide abstraction with $TlPF_6$ from the parent neutral complexes $[(\eta^5\text{-}C_5H_5)Ru(PP)Cl]$ in the presence of a slight excess of the corresponding carbohydrate-derived ligand, in dichloromethane at room temperature (Scheme 2). The compounds were recrystallized by slow diffusion of *n*-pentane or *n*-hexane in dichloromethane or acetone solutions.

The synthesis of compounds $[(\eta^5\text{-}C_5H_5)Ru(PPh_3)_2(\mathbf{L^3})][PF_6]$ and $[(\eta^5\text{-}C_5H_5)Ru(PPh_3)_2(\mathbf{L^6})][PF_6]$ was unsuccessfully attempted, resulting in product mixtures. Stereochemical hindrance, due to the methyl group in α position relatively to the coordinated nitrogen and the larger cone angle of PPh_3 over Dppe, may be the reason for the unsuccessful attempts. The same reactions were attempted in refluxing toluene, with similar results.

The ten new organometallic compounds were fully characterized by FT-IR, ^1H, ^{13}C, and ^{31}P NMR spectroscopies, and by elemental analysis, corroborating the proposed formulations and structures. The solid state FT-IR spectra of the complexes present the characteristic band of the cyclopentadienyl ring (3055-3059cm^{-1}), the hexafluorophosphate anion (~840 and 560 cm^{-1}) and the coordinated carbohydrate moieties.





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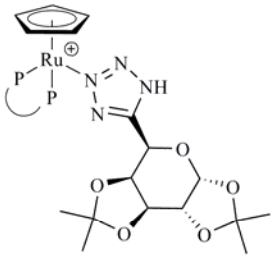

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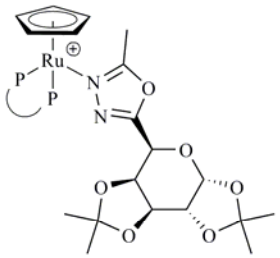

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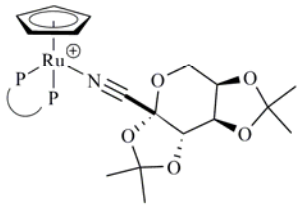

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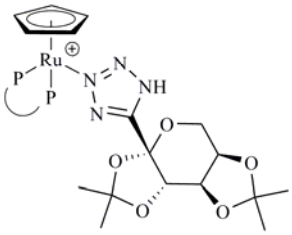

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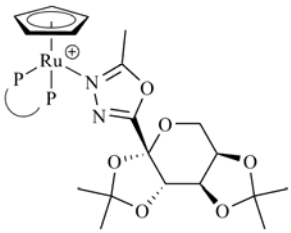
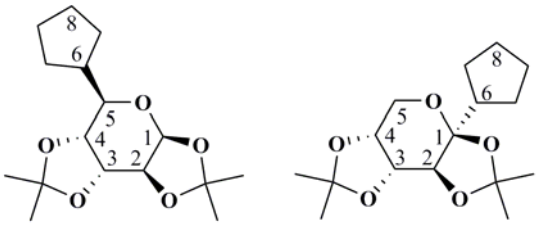


[**6**][PF_6]: PP = Dppe (80%)

Scheme 2- Synthesis of the Ru(II) organometallic complexes.

2.2.1. *NMR Spectrocopies*

Scheme 3 presents the numbering models and Tables 1 and 2 present selected ^1H NMR data for the galactose and fructose series compounds.



Scheme 3- Numbering models for NMR purposes.

Table 1- Selected ^{1}H NMR data for the galactose series compounds, in $CDCl_3$.

Compound	H1	H2	H3	H4	H5	*Cp*
L^{1}	5.53, d	4.36, dd	4.64-4.67	4.33, dd	4.64-4.67	_
	J = 5.4	*J* = 4.8, 2.8	m	*J* = 7.6, 2.4	m	
L^{2*}	5.67, d	4.53,dd	4.83, dd	4.63, dd	5.38, d	_
	J = 4.8	*J* = 4.8, 2.4	*J* = 7.8, 2.6	*J* = 8.0, 2.0	*J* = 2.0	
L^{3*}	5.65, d	4.36, dd	4.80, dd	4.58, dd	5.14, d	_
	J = 5.2	*J* = 4.8, 2.8	*J* = 7.0, 2.4	*J* = 7.6, 2.0	*J* = 2.4	
[**1**][PF_6]	5.16, d	4.11, dd	4.32, dd	3.27, d	4.37, br	4.72, s
	J = 4.8	*J* = 4.8, 2.7	*J* = 7.8, 2.7	*J* = 7.5		
[**2**][PF_6]	5.51 d	4.24, dd	4.48, dd	3.60, d	4.34, s	4.71, s
	J = 4.8	*J* = 4.8, 2.7	*J* = 7.8, 2.4	*J* = 7.5		
[**3**][PF_6]	5.44, d	4.25 - 4.28	4.36, dd	3.36, d	4.26, s	4.66, s
	J = 4.8	m	*J* = 7.6, 2.0	*J* = 8.0		
[**7**][PF_6]	5.44, d,	4.28, dd	4.62, dd	4.45 – 4.46	5.18, br	4.48, s
	J = 4.8	*J* = 4.8, 2.7	*J* = 7.8, 2.4	m		
[**8**][PF_6]	5.65, d	4.37 ,dd	4.64, dd,	4.15, dd	4.81, d	4.38, s
	J = 4.8	*J* = 5.1, 2.1	*J* = 7.8, 2.7	*J* = 7.8, 2.1	*J* = 1.8	

**In acetone* D_6

Table 2- Selected ^{1}H NMR data for the fructose series compounds, in $CDCl_3$.

Compound	H2	H3	H4	H5a	H5b	*Cp*
L^{4}	4.61, d	4.64, dd	4.26, dd,	3.82, dd	3.78, dd	_
	J = 2.0	*J* = 7.4, 2.3	*J* = 7.9, 1.3	*J* = 13.0, 1.2	*J*= 13.0, 1.8	
L^{5}	4.98, d	4.67dd,	4.34, dd	4.05, dd	3.94, d	_

	J= 2.2	*J* = 7.9, 2.2	*J* = 7.9, 1.0	*J* = 13.0, 1.0	*J* = 13.0	
L^{6}	5.03, d	4.69, dd	4.31, ddd	4.02, dd	3.91, dd	_
	J = 2.4	*J* = 8.0, 2.4	*J* = 8.0, 1.6, 0.9	*J* = 13.0, 1.6	*J* = 13.0, 0.9	
[**4**][PF_6]	3.32, d	4.33, dd	4.05, d	3.43, d	3.50, d	4.71, s
	J = 2.1	*J* = 8.1, 2.7	*J* = 7.8	*J* = 12.9	*J* = 12.9	
[**5**][PF_6]	3.66, d	4.40, dd	4.11, d	3.72, s		4.72, s
	J = 2.4	*J* = 7.8, 2.4	*J* = 7.8			
[**6**][PF_6]*	3.42, d	4.48, dd	4.20, dd	3.81, dd	3.67, d	4.89, s
	J = 2.4	*J* = 8.0, 2.4	*J* = 8.0, 1.6	*J* = 13.2, 1.6	*J* = 13.2	
[**9**][PF_6]	4.36, d	4.66, dd	4.25, d	3.78, d	3.76, dd	4.44, s
	J = 1.8	*J* = 8.1, 2.7	*J* = 7.8	*J* = 12.6	*J* = 13.2, 1.5	
[**10**][PF_6]	4.19, d	4.60, dd	4.26, d	3.92,s		4.37, s
	J = 2.4	*J* = 7.8, 2.4	*J* = 7.8			

**In acetone* D_6

The resonances of the cyclopentadienyl ring are within the characteristic range of monocationic ruthenium (II) complexes [18,41,42]. The carbohydrate-derived ligands display a general up-field shift of its protons upon coordination, with special relevance for the ones contiguous to the coordinating moiety (N≡C-, tetrazole or 1,3,4-oxadiazole), and in compounds with Dppe as co-ligand. Up-field shifts up to 1.2 ppm for H4 in compound [**2**][PF_6] and up to 1.4 for H2 in [**6**][PF_6] upon coordination (see Scheme 3 for numbering), are probably due to the anisotropic effect of the neighbour phosphine aromatic rings, since the its aliphatic nature of the ligands excludes the possibility of π-backdonation throughout the carbohydrate backbone, this considered as the major contribute to this phenomenon in other [(η^5-C_5H_5)Ru(PP)(L)]$^{+}$ derivative

complexes [18,41,42]. Also, the effect of the σ-donation upon coordination should lead to the opposite effect.

Selected ^{13}C NMR data for the organometallic complexes is presented in Table 3. The cyclopentadienyl ring chemical shifts are in the range usually observed for Ru(II) cationic derivatives. Chemical shifts of the carbohydrate-derived ligands carbon atoms remained almost unchanged upon coordination, exception made for the ones of the coordinated nitriles, with low-field shifts from 9.1 to 13.6 ppm, this further confirming the stereochemical nature of the shielding effect verified for the corresponding protons.

Table 3- Selected ^{13}C NMR data, in $CDCl_3$.

Compound	C1	C2	C3	C4	C5	C6	(η^5-C_5H_5)
L^{1}	96.3	70.0	70.5	71.0	60.3	115.3	–
L^{2*}	97.2	71.4	71.3	73.0	64.7	154.4	–
L^{3*}	97.4	71.2	71.6	73.1	65.1	163.8	–
L^{4}	93.8	74.4	69.3	69.4	61.4	116.6	–
L^{5}	100.4	74.5	68.6	70.0	61.1	156.8	–
L^{6}	97.8	73.0	69.8	70.0	61.6	164.5	–
[**1**][PF_6]	95.7	69.5	69.9	70.5	61.4	125.0	82.4
[**2**][PF_6]	96.0	70.3	70.3	71.5	62.7	154.0	82.5
[**3**][PF_6]	96.2	70.2	70.3	70.5	62.5	161,0	80.6
[**4**][PF_6]*	94.4	73.3	68.7	69.2	61.6	125.7	82.5
[**5**][PF_6]*	96.7	74.2	69.0	69.4	61.3	157.5	82.8
[**6**][PF_6]*	97.8	74.0	70.2	70.6	62.7	164.5	81.8
[**7**][PF_6]	96.3	70.3	70.0	70.9	62.5	128.9	84.3
[**8**][PF_6]	96.3	70.5	70.5	71.4	63.3	155.2	83.2

[**9**][PF_6][1]	95.2	73.6	68.9	69.3	62.5	129.2	84.5
[**10**][PF_6][1]	97.8	70.6	70.2	75.4	62.5	159.5	84.3

In acetone D_6

The intrinsic asymmetry of the chiral carbohydrate-derived ligands leads to the non-equivalency of the coordinated phosphorus atoms (see below). This effect extends to the phosphine aromatic rings, leading to an interesting multiplicity of signs in the ^{13}C-NMR spectra. Compounds with $2PPh_3$ show the non-equivalency of the coordinated phosphines, with two signs for each type of carbon (C_{ipso}, C_{ortho}, C_{meta}, C_{para}). In the case of Dppe compounds, the non-equivalency is not only between the phenyl rings bonded to different phosphorus atoms, but also between the ones bonded to the same one, this being explained by the fact that the rotation around the Ru-P axles is not possible.

^{31}P NMR spectra of the complexes showed two doublets, at ~40 ppm for compounds with the PPh_3 co-ligand and ~80 ppm for compounds with Dppe, attributed to the phosphine co-ligands, revealing the non-equivalence of the coordinated phosphorus atoms, as a result of the asymmetry induced by the chiral carbohydrate-derived ligands on the metal centre. $^2J_{PP}$ coupling constants of compound with two PPh_3 are ~36 Hz, while for compounds with Dppe as co-ligand it's within the range 22.5-25.5 Hz. This difference may be explained by the different P-Ru-P angles: PPh_3 has a larger cone angle, thus leading to a larger P-Ru-P angle and subsequently to a larger $^2J_{PP}$[43].

Table 4- Selected ^{31}P NMR data, in $CDCl_3$.

Compound	δP (ppm), $^2J_{PP}$ (Hz)
[**1**][PF_6]	78.4, 79.0 (2d, $^2J_{PP}$ = 25.5)
[**2**][PF_6]	83.3, 84.9 (2d, $^2J_{PP}$ = 25.5)
[**3**][PF_6]	84.3, 85.9 (2d, $^2J_{PP}$ = 22.7)

[**4**][PF_6]	77.3, 77.8 (2d, $^2J_{PP}$ = 25.4)
[**5**][PF_6]*	83.3, 84.1 (2d, $^2J_{PP}$ = 25.3)
[**6**][PF_6]*	82.1, 84.3 (2d, $^2J_{PP}$ = 22.5)
[**7**][PF_6]	40.2, 40.9 (2d, $^2J_{PP}$ = 35.5)
[**8**][PF_6]	40.2, 41.2 (2d, $^2J_{PP}$ = 36.3)
[**9**][PF_6]	38.9, 40.6 (2d, $^2J_{PP}$ = 35.4)
[**10**][PF_6]	39.4, 40.8 (2d, $^2J_{PP}$ = 36.3)

In acetone D_6

Furthermore, the $^2J_{PP}$ coupling constants for compounds [**3**][PF_6] and [**6**][PF_6] are approximately 3 Hz lower than for other complexes with Dppe. This difference might be explained by a larger stereochemical constraint of the coordinated 1,3,4-oxadiazole ring, due to the methyl group in α position to the coordinated nitrogen atom, which leads to lower P-Ru-P angle and $^2J_{PP}$value. This further supports the hypothesis pointed out to the unsuccessful synthesis of compounds [(η^5-C_5H_5)Ru(PPh_3)(**L**^{3})][PF_6] and [(η^5-C_5H_5)Ru(PPh_3)(**L**^{6})][PF_6].

All the compounds spectra showed also the characteristic septuplet signal of the hexafluorophosphate anion, at approximately -144.1 ppm.

2.3. Cytotoxic studies

The effect of the ruthenium complexes on human cervical carcinoma cells (*HeLa*) was assayed within the concentration range 5 to 500 μM, using the MTT assay, a colorimetric determination of cell viability during in vitro treatment with a drug, developed as an initial stage drug screening. The effects of compounds [**1**][PF_6]–[**10**][PF_6] on the growth of these cell lines were evaluated after 48 h of continuous exposure to the compounds. The IC_{50} values (final concentration ≤0.5% DMSO) were

calculated from dose–response curves obtained by nonlinear regression analysis and are shown in Table 5

Table 5- IC_{50} values (mean ± SD of three replicates each) for ruthenium compounds against *HeLa* cells.

Compound	IC_{50} (μM)
[**1**][PF_6]	3.58 ± 0.39
[**2**][PF_6]	3.92 ± 0.51
[**3**][PF_6]	6.81 ± 0.11
[**4**][PF_6]	6.07 ± 0.30
[**5**][PF_6]	10.61 ± 0.06
[**6**][PF_6]	4.64 ± 0.22
[**7**][PF_6]	2.63 ± 0.14
[**8**][PF_6]	6.39 ± 0.04
[**9**][PF_6]	9.26 ± 0.05
[**10**][PF_6]	4.49 ± 0.29
Cisplatin[a,b]	12.4±0.85

[a] Ref. [44]; [b]72 h incubation

All ruthenium complexes showed higher cytotoxic activity than cisplatin against *in vitro* growth of *HeLa* cancer cells, after 48 h incubations, with IC_{50} values ranging from 2.63 for **[7]**[PF_6] to 10.61 μM for **[3]**[PF_6]), lower than cisplatin (12.4μM), even though this value concerns data obtained from longer cell incubations with the compound (72 h vs 48 h) [44].

The best IC_{50} results were obtained for compounds [**1**][PF_6] (3.58μM) and [**7**][PF_6] (2.63μM), both with **L1** coordinated, the best result being obtained for the PPh_3 complex. Other than this, no structure–activity relationship can be disclosed.

Direct comparison of the IC_{50} values with other [(η^5-C_5H_5)Ru(PP)(L)][X] is not possible since there are no reports concerning *HeLa* cells. AnIC_{50} of 1.4 ±0.07 is reported for [(η^6-*p*-cymene)RuCl(κ^2-dppp)]Cl, slightly better than our best result, but obtained from 72 h incubation data.

3. Conclusions

A new family of Ru(II) three-legged piano stool complexes with *N*-coordinated galactose and fructose derivative ligands, was synthesized in good yields and characterized by FTIR, ^{1}H, ^{13}C and ^{31}P-NMR spectroscopies. Cytotoxic studies on *HeLa* cancer cell lines revealed very good activities, with IC_{50} values in the low micromolar range, better than cisplatin.

4. Experimental Section

4.1. *General Procedures*

All the experiments were carried out under inert atmosphere (N_2) using standard Schlenk techniques. Commercial reagents were used without further purification. All solvents were dried using standard methods [45]. Starting materials were prepared following the methods described in the literature: [(η^5-C_5H_5)Ru(Dppe)Cl] and [(η^5-C_5H_5)Ru(PPh_3)$_2$Cl] [46], 2,3:4,5-Di-*O*-isopropylidene-*β*-D-arabino-hexos-2-ulo-2,6-pyranose [47], 2,3:4,5-Di-*O*-isopropylidene-*β*-D-arabino-hex-2-ulosonitrile, 1,2:3,4-Di-*O*-isopropylidene-1-(tetrazol-5′-yl)-*β*-D-arabinopyranose and 1,2:3,4-Di-*O*-isopropylidene-1-(2′-methyl-1′,3′,4′-oxadiazol)-5′-yl-*β*-D-arabinopyranose [40]. Solid

state IR spectra were recorded on a Jasco FTIR-4100 spectrophotometer with KBr pellets; only significant bands are cited in the text. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Avance II 400 spectrometer operating at 400, 100, 162 MHz, respectively; or on a Bruker Avance II 300 spectrometer operating at 300, 75, 121 MHz, respectively, at probe temperature. The ^{1}H and ^{13}C chemical shifts are reported in parts per million (ppm) downfield from the residual solvent peak; the ^{31}P NMR spectra are reported in ppm downfield from external standard H_3PO_4 85%. Coupling constants are reported in Hz. Spectral assignments of the carbohydrate derivative ligands follow the numbering scheme shown in Scheme 3.Assignments of the ^{1}H and ^{13}C NMR spectra were confirmed with the aid of two dimensional techniques ^{1}H, ^{13}C (COSY, HSQC). Microanalyses were performed using a Fisons Instruments EA1108 system; data acquisition, integration and handling were performed using the software package Eager-200 (Carlo Erba Instruments).

4.2. *Synthesis of the carbohydrate derivatives*

4.2.1. *Precursor* ***P*^{2}**

A solution of 1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (1.30 g, 5.0mmol) in CH_2Cl_2 (10 mL) was added to a suspension of PCC (2.50 g, 11.5 mmol) and powder molecular sieves 4Å (5.00 g) in CH_2Cl_2 (20 mL). After 16 h stirring, AcOEt (40 mL) was added. The mixture was filtered through celite and the solvent removed under reduced pressure. The crude was purified by column chromatography (eluent: hexane to AcOEt:hexane 1:4), affording pure product **P^{2}** (0.72 g, 56%) as a colourless oil. FTIR (KBr, cm^{-1}): 1742 ($\nu_{C=O}$). ^{1}H NMR ($CDCl_3$, 400 MHz): 1.30, 1.33, 1.42, 1.49 (four s, 12H, -C(CH_3)$_2$), 4.17 (d, 1H, *J* =2.0, H5), 4.36 (dd, 1H, *J* =5.0, 2.6, H2), 4.58 (dd, 1H, *J* =7.6, 2.0, H4), 4.63 (dd, 1H, *J* =7.6, 2.4, H3), 5.65 (d, 1H, *J* =5.2, H1), 9.62 (s, 1H,

CHO). ^{13}C NMR ($CDCl_3$, 100 MHz): 24.3, 24.9, 25.9, 26.1 (-C($\underline{C}H_3)_2$), 70.5 (C3), 70.6 (C2), 71.8 (C4), 73.3 (C5), 96.4 (C1), 109.1, 110.1 (-$\underline{C}(CH_3)_2$), 200.4 (CHO). Anal. Calcd. for $C_{12}H_{18}O_6$: C, 55.81; H, 7.02. Found: C, 54.25; H, 7.13.

4.2.2. *Ligand* ***L*^{1}**

To a solution of **P^{2}** (517 mg, 2.00mmol) in pyridine (2 mL) was added a solution of hydroxylamine hydrochloride (167 mg, 2.40mmol) in water (1 mL). After stirring for 1 h, copper sulphate pentahydrate (1.00 g, 4.00mmol), a solution of DCC (495 mg, 2.40mmol) and Et_3N (0.56 mL, 4.0mmol) in CH_2Cl_2 (10 mL) were added to the mixture. After stirring for 2 h more, formic acid (0.40 mL) was added, the mixture was filtered, the phases separated and the aqueous phase further extracted with CH_2Cl_2 (3 x 20 mL). The organic phase was washed with HCl 10% (20 mL), dried with $MgSO_4$, filtered and pumped to dryness. The crude obtained was purified by column chromatography (eluent: AcOEt:hexane 1:9), affording the pure ligand **L^{1}** (388 mg, 76%) as a white crystalline solid. FTIR (KBr, cm^{-1}): 2262 ($\nu_{C\equiv N}$). ^{1}H NMR ($CDCl_3$, 400 MHz): 1.33, 1.38, 1.53, 1.54 (four s, 12H, -C($CH_3)_2$), 4.33 (dd, 1H, *J* =7.6, 2.4, H4), 4.36 (dd, 1H, *J* =4.8, 2.8, H2), 4.64 - 4.67 (m, 2H, H3+H5), 5.53 (d, 1H, *J* =5.4, H1). ^{13}C NMR ($CDCl_3$, 100 MHz): 24.7, 24.8, 26.0, 26.2 (-C($\underline{C}H_3)_2$), 60.3 (C5), 70.0 (C2), 70.5 (C3), 71.0 (C4), 96.3 (C1), 109.7, 111.2 (-$\underline{C}(CH_3)_2$), 115.3 (C≡N). Anal. Calcd. for $C_{12}H_{17}O_5N$: C, 56.46; H, 6.71; N, 5.49. Found: C, 56.40; H, 6.68; N, 5.48.

4.2.3.*Ligand* ***L*^{2}**

To a solution of ligand **L^{1}** (383 mg, 1.50mmol) in DMF (5 mL) were added NaN_3 (117 mg, 1.8 mmol) and NH_4Cl (120 mg, 2.20mmol), and the mixture was heated to 100 ºC. After stirring for 3 h, the solvent was removed and the crude obtained was extracted with AcOEt (3 x 20 mL), filtered and pumped to dryness. The crude was purified by column chromatography (eluent: AcOEt:hexane 1:1), affording the pure ligand **L^{2}**(425

mg, 95%) as a white crystalline solid. FTIR (KBr, cm^{-1}): 3434 (ν_{N-H}); 1559 ($\nu_{N=N}$); 1388 ($\nu_{N=C}$). ^1H NMR ($(CD_3)_2CO$, 400 MHz): 1.31, 1.35, 1.38, 1.55 (four s, 12H, -$C(CH_3)_2$), 4.53 (dd, 1H, *J* =4.8, 2.4, H2), 4.63 (dd, 1H, *J* =8.0, 2.0, H4), 4.83 (dd, 1H, *J* =7.8, 2.6, H3), 5.38 (d, 1H, *J* =2.0, H-5). 5.67 (d, 1H, *J* =4.8, H1), 15.11 (br, 1H, N-H). ^{13}C NMR ($(CD_3)_2CO$, 100 MHz): 24.2, 25.0, 26.0, 26.3 (-$C(\underline{C}H_3)_2$), 64.7 (C5), 71.3 (C3), 71.4 (C2), 73.0 (C4), 97.2 (C1), 109.8, 110.3 (-$\underline{C}(CH_3)_2$), 154.4 (C6). Anal. Calcd. for $C_{12}H_{18}O_5N_4$: C, 48.32; H, 6.08; N, 18.78. Found: C, 48.52; H, 6.22; N, 18.52.

4.2.4. *Ligand* ***L*^{3}**

A solution of ligand $\mathbf{L^2}$ (298 mg, 1.00mmol) was dissolved in Ac_2O and heated to reflux. After 3 h, the reaction was stopped by addition of EtOH and the solvent removed under reduced pressure. The remains of AcOH were removed by consecutive additions of toluene and evaporation, affording the ligand $\mathbf{L^3}$ (300 mg, 96%) as a white crystalline solid. FTIR (KBr, cm^{-1}): 1386($\nu_{N=C}$);.^1H NMR ($(CD_3)_2CO$, 400 MHz): 1.31, 1.37, 1.39, 1.53 (four s, 12H, -$C(CH_3)_2$), 2.50 (s, 3H, CH_3) 4.52 (dd, 1H, *J* =5.0, 2.6, H2), 4.58 (dd, 1H, *J* =7.6, 2.0, H4), 4.80 (dd, 1H, *J* =7.0, 2.4, H3), 5.14 (d, 1H, *J* =2.4, H-5). 5.65 (d, 1H, *J* =5.2, H1). ^{13}C NMR ($(CD_3)_2CO$, 100 MHz): 10.7 (CH_3), 24.7, 25.0, 26.2, 26.3 (-$C(\underline{C}H_3)_2$), 65.1 (C5), 71.2 (C2), 71.6 (C3), 73.1 (C4), 97.4 (C1), 109.7, 110.5 (-$\underline{C}(CH_3)_2$), 163.8, 164.8 (C6, C8). Anal. Calcd. for $C_{14}H_{20}O_6N_2$: C, 53.84; H, 6.46; N, 8.97. Found: C, 53.52; H, 6.48; N, 8.67.

4.3. *Synthesis of the complexes [(η^5-C_5H_5)Ru(P–P)(**L**)][PF_6]*

Complexes of general formula [(η^5-C_5H_5)Ru(P–P)(**L**)][PF_6] were prepared by halide abstraction from the parent neutral complexes [(η^5-C_5H_5)Ru(P–P)Cl] (0.20 mmol) with $TlPF_6$ (0.20 mmol) in dichloromethane, in the presence of a slight excess of the ligands **L** (0.22 mmol), at room temperature, under inert atmosphere for 48 h. The solutions

were double filtered to remove the TlCl formed and pumped to dryness. The compounds were washed with n-pentane and recrystallized by slow diffusion of n-pentane or n-hexane in acetone or dichloromethane solutions, affording crystalline products.

4.3.1.***[1]***$[PF_6]$

Light yellow; recrystallized from CH_2Cl_2/pentane; η = 81 %. FTIR (KBr, cm^{-1}): 3056 ($\nu_{C\text{-}H}$,η^5-C_5H_5), 2264 ($\nu_{C\equiv N}$), 840 ($\nu_{P\text{-}F}$, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.00, 1.04, 1.23, 1.48 (four s, 12H, -C(CH_3)$_2$), 2.54-2.71 (m, 4H, -CH_2CH_2-, Dppe), 3.27 (d, 1H, *J*= 7.5, H4), 4.11 (dd, 1H, *J*= 4.8, 2.7, H2), 4.32 (dd, 1H, *J*= 7.8, 2.7, H3), 4.37 (br, 1H, H5), 4.72 (s, 5H, η^5-C_5H_5), 5.16 (d, 1H, *J*= 4.8, H1), 7.13-7.84 (m, 20H, C_6H_5, Dppe). ^{13}C NMR ($CDCl_3$, 100 MHz): 24.1, 24.7, 25.5, 25.8 (-C($\underline{C}H_3$)$_2$), 27.8 (m, -CH_2CH_2-), 61.4 (C5), 69.5 (C2), 69.9 (C3), 70.5 (C4), 82.4 (η^5-C_5H_5), 95.7 (C1), 109.8, 110.0 (-$\underline{C}$(CH_3)$_2$), 125.0 (C≡N) 128.9-129.1 (m, C_{meta}), 129.3 (d, C_{meta}, $^3J_{CP}$ = 9.8), 130.3, 130.5 (two s, C_{para}), 130.6 (d, C_{ortho}, $^2J_{CP}$ = 10.5), 130.8, 131.0 (two s, C_{para}), 131.1 (d, C_{ortho}, $^2J_{CP}$ = 10.3), 133.1 (d, C_{ortho}, $^2J_{CP}$ = 10.8), 133.7 (d, C_{ortho}, $^2J_{CP}$ = 11.0), 137.7, 138.1 (two d, C_{ipso}, $^1J_{CP}$ = 45.6). ^{31}P NMR ($CDCl_3$, 121 MHz): -144.2 (quint., J_{PF} = 714, PF_6^-), 78.4 (d, $^2J_{PP}$ = 25.4, Dppe), 79.0 (d, $^2J_{PP}$ = 25.6, Dppe). Anal. Calcd. for $C_{43}H_{46}O_5NRuP_3F_6$: C, 53.53; H, 4.81; N, 1.45. Found: C, 53.45; H, 4.41; N, 1.45.

4.3.2.***[2]***$[PF_6]$

Light yellow; recrystallized from CH_2Cl_2/hexane; η = 86 %. FTIR (KBr, cm^{-1}): 3056 ($\nu_{C\text{-}H}$,η^5-C_5H_5), 839 ($\nu_{P\text{-}F}$, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.18, 1.30, 1.31, 1.46 (four s, 12H, -C(CH_3)$_2$), 2.51-3.21 (m, 4H, -CH_2CH_2-, Dppe), 3.60 (d, 1H, *J*= 7.5, H4), 4.24 (dd, 1H, *J*= 4.8, 2.7, H2), 4.34 (s, 1H, H5), 4.48 (dd, 1H, *J*= 7.8, 2.4, H3), 4.71 (s, 5H, η^5-C_5H_5), 5.51 (d, 1H, *J*= 4.8, H1), 7.16-7.46 (m, 20H, C_6H_5, Dppe). ^{13}C NMR ($CDCl_3$, 100 MHz): 24.5, 24.8, 25.6, 26.3 (-C($\underline{C}H_3$)$_2$), 28.5 (t, -CH_2CH_2-, $^1J_{CP}$ = 29.9),

62.7 (C5), 70.3 (C2, C3), 71.5 (C4), 82.5 (η^5-C_5H_5), 96.0 (C1), 108.9, 110.0 (-$\underline{C}(CH_3)_2$), 128.5, 128.8 (two d, C_{meta}, $^3J_{CP}$ = 9.5), 128.9 (d, C_{meta}, $^3J_{CP}$ = 9.9), 129.9, 130.0, 130.2, 130.3 (four s, C_{para}), 130.5, 130.7 (two d, C_{ortho}, $^2J_{CP}$ = 10.2), 132.2 (d, C_{ortho}, $^2J_{CP}$ = 10.7), 132.7 (d, C_{ortho}, $^2J_{CP}$ = 10.9), 139.5 (d, C_{ipso}, $^1J_{CP}$ = 45.1), 140.2 (d, C_{ipso}, $^1J_{CP}$ = 44.8), 154.0 (C6). ^{31}P NMR ($CDCl_3$, 121 MHz): -144.2 (quint., J_{PF} = 712, PF_6^-), 83.3 (d, $^2J_{PP}$ = 25.4, Dppe), 84.9 (d, $^2J_{PP}$ = 25.6, Dppe). Anal. Calcd. for $C_{43}H_{47}O_5N_4RuP_3F_6 \cdot 0.5C_6H_{14}$: C, 52.57; H, 5.12; N, 5.33. Found: C, 52.54; H, 4.88; N, 5.66.

4.3.3.***[3]***$[PF_6]$

Yellow; recrystallized from CH_2Cl_2/hexane; η = 74 %. FTIR (KBr, cm^{-1}): 3055 ($\nu_{C\text{-}H}$,η^5-C_5H_5), 841 ($\nu_{P\text{-}F}$, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.28, 1.30, 1.38, 1.47 (four s, 12H, -$C(CH_3)_2$), 2.12 (s, 3H, -CH_3, OxD), 2.51-2.71 (m, 2H, -CH_2CH_2-, Dppe), 2.85-2.96 (m, 1H, -CH_2CH_2-, Dppe), 3.05-3.12 (m, 1H, -CH_2CH_2-, Dppe), 3.36 (d, 1H, *J*= 8.0, H4), 4.25 – 4.28(m, 2H, H2+H5), 4.46 (dd, 1H, *J*= 7.6, 2.0, H3), 4.66 (s, 5H, η^5-C_5H_5), 5.44 (d, 1H, *J*= 4.8, H1), 7.11-7.71 (m, 20H, C_6H_5, Dppe). ^{13}C NMR ($CDCl_3$, 100 MHz): 12.5 (-CH_3, OxD), 24.3, 24.8, 25.9, 26.2 (-C($\underline{C}H_3)_2$), 28.4-28.5 (m, -CH_2CH_2-), 29.6-29.7 (m, -CH_2CH_2-), 62.5 (C5), 70.3, 70.5, 70.6 (C2, C3, C4), 80.6 (η^5-C_5H_5), 96.2 (C1), 109.5, 109.9 (-$\underline{C}(CH_3)_2$), 128.6-129.1 (m, C_{meta}), 129.9, 130.3, 130.4 (three s, C_{para}), 130.7 (d, C_{ortho}, $^2J_{CP}$ = 10.0), 130.4 (s, C_{para}), 131.2 (d, C_{ortho}, $^2J_{CP}$ = 10.0), 131.6 (d, C_{ortho}, $^2J_{CP}$ = 10.7), 132.7 (d, C_{ortho}, $^2J_{CP}$ = 10.5), 138.7 (d, C_{ipso}, $^1J_{CP}$ = 45.4), 140.3 (d, C_{ipso}, $^1J_{CP}$ = 45.9), 161.0, 169.5 (C6, C8). ^{31}P NMR ($CDCl_3$, 121 MHz): -144.3 (sept., J_{PF} = 713, PF_6^-), 84.3 (d, $^2J_{PP}$= 22.2, Dppe), 85.9 (d, $^2J_{PP}$= 23.2, Dppe). Anal. Calcd. for $C_{45}H_{49}O_6N_2RuP_3F_6$: C, 52.89; H, 4.83; N, 2.74. Found: C, 52.54; H, 4.68; N, 2.59.

4.3.4. ***[4]***$[PF_6]$

Light yellow; recrystallized from CH_2Cl_2/hexane; η = 76 %. FTIR (KBr, cm^{-1}): 3056 (ν_{C-H},η^5-C_5H_5), 2248 ($\nu_{C\equiv N}$), 840 (ν_{P-F}, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 0.72, 1.28, 1.30, 1.33 (four s, 12H, -C(CH_3)$_2$), 2.48-2.72 (m, 4H, -CH_2CH_2-, Dppe), 3.32 (d, 1H, *J* = 2.1, H2), 3.43 (d, 1H, *J* = 12.9, H5b), 3.50 (d, 1H, *J* = 12.9, H5a), 4.05 (d, 1H, *J* = 7.8, H4), 4.33 (dd, 1H, *J* = 8.1, 2.7, H3), 4.71 (s, 5H, η^5-C_5H_5), 7.15-7.77 (m, 20H, C_6H_5, Dppe). ^{13}C NMR ($(CD_3)_2CO$, 100 MHz): 23.0, 23.7, 24.4, 25.7 (-C($\underline{C}H_3$)$_2$), 27.4 (t, -CH_2CH_2-, $^1J_{CP}$ = 23.8), 61.6 (C5), 68.7 (C3), 69.2 (C4), 73.3 (C2), 82.5 (η^5-C_5H_5), 94.4 (C1), 109.1, 111.5 (-$\underline{C}$(CH_3)$_2$), 125.7 (C≡N), 128.9 (d, C_{meta}, $^3J_{CP}$ = 9.8), 129.0 (d, C_{meta}, $^3J_{CP}$ = 10.1), 129.2 (d, C_{meta}, $^3J_{CP}$ = 9.8), 129.5 (d, C_{meta}, $^3J_{CP}$ = 9.9), 130.2, 130.5 (two d, C_{para}, $^4J_{CP}$ = 2.7), 130.7 (d, C_{ortho}, $^2J_{CP}$ = 10.3), 130.8 (d, C_{para}, $^4J_{CP}$ = 2.2), 130.2 (d, C_{para}, $^4J_{CP}$ = 2.2), 131.0 (d, C_{para}, $^4J_{CP}$ = 2.3), 131.3 (d, C_{ortho}, $^2J_{CP}$ = 10.9), 133.0 (d, C_{ortho}, $^2J_{CP}$ = 10.8), 133.5 (d, C_{ortho}, $^2J_{CP}$ = 10.9), 136.8 (d, C_{ipso}, $^1J_{CP}$ = 45.0), 137.9 (d, C_{ipso}, $^1J_{CP}$ = 45.5). ^{31}P NMR ($CDCl_3$, 121 MHz): -144.2 (quint., J_{PF} = 708, PF_6^-), 77.3 (d, $^2J_{PP}$ = 25.4, Dppe), 77.8 (d, $^2J_{PP}$ = 25.4, Dppe). Anal. Calcd. for $C_{43}H_{46}O_5NRuP_3F_6$: C, 53.53; H, 4.81; N, 1.45. Found: C, 53.35; H, 4.53; N, 1.46.

4.3.5. ***[5]***$[PF_6]$

Yellow; recrystallized from CH_2Cl_2/hexane; η = 82 %. FTIR (KBr, cm^{-1}): 3056 (ν_{C-H},η^5-C_5H_5), 842 (ν_{P-F}, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.16, 1.18, 1.26, 1.45 (four s, 12H, -C(CH_3)$_2$), 2.54-2.73 (m, 2H, -CH_2CH_2-, Dppe), 2.94-3.05 (m, 2H, -CH_2CH_2-, Dppe), 3.66 (d, 1H, *J* = 2.4, H2), 3.72 (s, 2H, H5), 4.11 (d, 1H, *J* = 7.8, H4), 4.40 (dd, 1H, *J* = 7.8, 2.4, H3), 4.72 (s, 5H, η^5-C_5H_5), 7.16-7.51 (m, 20H, C_6H_5, Dppe). ^{13}C NMR ($(CD_3)_2CO$, 100 MHz): 23.1, 24.1, 25.0, 25.1 (-C($\underline{C}H_3$)$_2$), 27.6-28.0 (m, -CH_2CH_2-), 61.3 (C5), 69.0 (C3), 69.4 (C4), 74.2 (C2), 82.8 (η^5-C_5H_5), 96.7 (C1), 108.4, 110.6 (-$\underline{C}$(CH_3)$_2$), 128.4 (d, C_{meta}, $^3J_{CP}$ = 9.5), 128.6 (d, C_{meta}, $^3J_{CP}$ = 9.4), 128.6, 128.7 (d, C_{meta}, $^3J_{CP}$ = 9.6), 129.6 (d, C_{para}, $^4J_{CP}$ = 2.6), 129.7, 130.1 (two d, C_{para}, $^4J_{CP}$ = 2.2), 130.2 (d,

C_{para}, $^4J_{CP}$ = 2.5), 130.4 (d, C_{ortho}, $^2J_{CP}$ = 9.8), 130.6 (d, C_{ortho}, $^2J_{CP}$ = 10.2), 132.4, 132.5 (two d, C_{ortho}, $^2J_{CP}$ = 10.8), 139.3 (d, C_{ipso}, $^1J_{CP}$ = 44.8), 139.7 (d, C_{ipso}, $^1J_{CP}$ = 45.2), 157.5 (C6). ^{31}P NMR ($(CD_3)_2CO$, 162 MHz): -144.2 (quint., J_{PF} = 706, PF_6^-), 83.3 (d,$^2J_{PP}$ = 25.1, Dppe), 84.1 (d, $^2J_{PP}$ = 25.4, Dppe). Anal. Calcd. for $C_{43}H_{47}O_5N_4RuP_3F_6 \cdot 0.2CH_2Cl_2$: C, 50.62; H, 4.66; N, 5.46. Found: C, 50.59; H, 4.68; N, 5.04.

4.3.6. ***[6]****[PF_6]*

Yellow; recrystallized from CH_2Cl_2/hexane; η = 80 %. FTIR (KBr, cm^{-1}): 3057 ($\nu_{C\text{-}H}$,η^5-C_5H_5), 842 ($\nu_{P\text{-}F}$, PF_6^-). ^{1}H NMR ($(CD_3)_2CO$, 400 MHz): 0.94, 1.35, 1.38, 1.42 (four s, 12H, -C(CH_3)$_2$), 2.30 (s, 3H, CH_3), 2.88-3.15 (m, 2H, -CH_2CH_2-, Dppe), 3.42 (d, 1H, *J* = 2.4, H2), 3.67 (d, 1H, *J* = 13.2, H5b), 3.81 (d, 1H, *J* = 13.2, 1.6, H5a), 4.20 (dd, 1H, *J* = 8.0, 1.6, H4), 4.48 (dd, 1H, *J* = 8.0, 2.4, H3), 4.89 (s, 5H, η^5-C_5H_5), 7.25-7.60 (m, 20H, C_6H_5, Dppe). ^{13}C NMR ($(CD_3)_2CO$, 100 MHz): 13.1 (CH_3), 24.4, 25.5, 26.0, 26.4 (-C($\underline{C}H_3$)$_2$), 28.6 (t, -CH_2CH_2-, $^1J_{CP}$ = 23.8), 62.7 (C5), 70.2 (C3), 70.6 (C4), 74.0 (C2), 81.8 (η^5-C_5H_5), 97.9 (C1), 109.7, 111.4 (-$\underline{C}$(CH_3)$_2$), 129.6 (d, C_{meta}, $^3J_{CP}$ = 9.5), 129.7 (d, C_{meta}, $^3J_{CP}$ = 9.9), 129.8-129.9 (m, C_{meta}), 130.9 (d, C_{para}, $^4J_{CP}$ = 2.2), 131.0 (d, C_{para}, $^4J_{CP}$ = 2.1), 131.2, 131.5 (two s, C_{para}), 132.2 (d, C_{ortho}, $^2J_{CP}$ = 10.1), 132.4, 132.9 (two d, C_{ortho}, $^2J_{CP}$ = 10.2), 133.5 (d, C_{ortho}, $^2J_{CP}$ = 10.7), 140.2 (d, C_{ipso}, $^1J_{CP}$ = 47.0), 140.5 (d, C_{ipso}, $^1J_{CP}$ = 46.0), 164.5, 170.9 (C6, C8). ^{31}P NMR ($(CD_3)_2CO$, 162 MHz): -144.2 (sept., J_{PF} = 708, PF_6^-), 82.1 (d, $^2J_{PP}$ = 23.3, Dppe), 84.3 (d, $^2J_{PP}$ = 21.7, Dppe). Anal. Calcd. for $C_{45}H_{49}O_6N_2RuP_3F_6$: C, 52.89; H, 4.83; N, 2.74. Found: C, 52.59; H, 4.89; N, 2.22.

4.3.7. ***[7]****[PF_6]*

Yellow; recrystallized from acetone/hexane; η = 77 %. FTIR (KBr, cm^{-1}): 3058 ($\nu_{C\text{-}H}$,η^5-C_5H_5), 2260 ($\nu_{C\equiv N}$), 841 ($\nu_{P\text{-}F}$, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 0.99, 1.13, 1.31, 1.63

(four s, 12H, -C(CH_3)$_2$), 4.28 (dd, 1H, *J* = 4.8, 2.7, H2), 4.46 (m, 1H, H4), 4.48 (s, 5H, η^5-C_5H_5), 4.62 (dd, 1H, *J* =7.8, 2.4, H3), 5.18 (br, 1H, H5), 5.44 (d, 1H, *J* = 4.8, H1), 7.04-7.30 (m, 30H, C_6H_5, PPh_3). ^{13}C NMR ($CDCl_3$, 100 MHz): 22.8, 24.2, 24.9, 25.8 (-C(<u>C</u>H_3)$_2$), 62.2 (C5), 70.0 (C3), 70.3 (C2), 70.9 (C4), 84.3 (η^5-C_5H_5), 96.3 (C1), 109.9, 110.1 (-<u>C</u>(CH_3)$_2$), 128.3 (d, C_{meta}, $^3J_{CP}$ = 9.5), 128.4 (d, C_{meta}, $^3J_{CP}$ = 9.4), 128.9 (C≡N), 129.9 (m, C_{para}), 133.2 (d, C_{ortho}, $^2J_{CP}$ = 10.3), 133.5 (d, C_{ortho}, $^2J_{CP}$ = 10.6), 135.9 (dd, C_{ipso}, $^1J_{CP}$ = 41.0, $^3J_{CP}$ = 2.8), 136.2 (dd, C_{ipso}, $^1J_{CP}$ = 40.7, $^3J_{CP}$ = 2.4). ^{31}P NMR ($CDCl_3$, 121 MHz): -144.1 (quint., J_{PF} = 711, PF_6^-), 40.2 (d, $^2J_{PP}$ = 35.2, PPh_3), 40.9 (d, $^2J_{PP}$ = 35.7, PPh_3). Anal. Calcd. for $C_{53}H_{52}O_5NRuP_3F_6$: C, 58.35; H, 4.80; N, 1.28. Found: C, 58.24; H, 4.55; N, 1.31.

4.3.8. ***[8]****[PF_6]*

Yellow; recrystallized from CH_2Cl_2/hexane; η = 78 %. FTIR (KBr, cm^{-1}): 3057 ($\nu_{C\text{-}H}$,η^5-C_5H_5), 841 ($\nu_{P\text{-}F}$, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.25 (s, 3H, -C(CH_3)$_2$), 1.37(s, 6H, -C(CH_3)$_2$), 1.50 (s, 3H, -C(CH_3)$_2$), 4.15 (dd, 1H, *J* = 7.8, 2.1, H4), 4.37 (dd, 1H, *J* = 5.1, 2.1, H2), 4.38 (s, 5H, η^5-C_5H_5), 4.64 (dd, 1H, *J* = 7.8, 2.7, H3), 4.81 (d, 1H, *J* = 1.8, H5), 5.65 (d, 1H, *J* = 4.8, H1), 6.96-7.33 (m, 30H, C_6H_5, PPh_3). ^{13}C NMR ($CDCl_3$, 100 MHz): 24.5, 24.9, 25.8, 26.2 (-C(<u>C</u>H_3)$_2$), 63.3 (C5), 70.5 (C2, C3), 71.4 (C4), 83.2 (η^5-C_5H_5), 96.3 (C1), 109.3, 110.4 (-<u>C</u>(CH_3)$_2$), 128.0 (d, C_{meta}, $^3J_{CP}$ = 9.4), 128.1 (d, C_{meta}, $^3J_{CP}$ = 9.3), 129.7, 129.8 (two s, C_{para}), 133.5, 133.6 (two d, C_{ortho}, $^2J_{CP}$ = 10.2), 136.3, 136.8 (two d, C_{ipso}, $^1J_{CP}$ = 40.1), 155.2 (C6). ^{31}P NMR ($CDCl_3$, 162 MHz): -144.3 (quint., J_{PF} = 712, PF_6^-), 40.2 (d, $^2J_{PP}$ = 35.6, PPh_3), 41.2 (d, $^2J_{PP}$ = 37.1, PPh_3). Anal. Calcd. for $C_{53}H_{53}O_5N_4RuP_3F_6$: C, 56.14; H, 4.71; N, 4.94. Found: C, 56.24; H, 5.06; N, 4.51.

4.3.9. ***[9]****[PF_6]*

Yellow; recrystallized from CH_2Cl_2/hexane; η = 79 %. FTIR (KBr, cm^{-1}): 3059 ($\nu_{C\text{-}H}$,η^5-C_5H_5), 2241 ($\nu_{C\equiv N}$), 840 ($\nu_{P\text{-}F}$, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 0.94, 1.29, 1.40, 1.50 (four s, 12H, -C(CH_3)$_2$), 3.76 (dd, 1H, *J* = 13.2, 1.5, H5b), 3.82 (d, 1H, *J* = 12.6, H5a), 4.25 (d, 1H, *J* = 7.8, H4), 4.36 (d, 1H, *J* = 1.8, H2), 4.44 (s, 5H, η^5-C_5H_5), 4.66 (dd, 1H, *J* = 8.1, 2.7, H3), 6.97-7.40 (m, 30H, C_6H_5, PPh_3). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): 23.2, 23.6, 24.6, 25.8 (-C(<u>C</u>H_3)$_2$), 62.1 (C5), 68.9 (C3), 69.3 (C4), 73.6 (C2), 84.5 (η^5-C_5H_5), 95.2 (C1), 109.3, 112.2 (-<u>C</u>(CH_3)$_2$), 128.5, 128.6 (two d, C_{meta}, $^3J_{CP}$ = 9.7), 129.2 (C≡N), 130.2 (d, C_{para}, $^4J_{CP}$ = 2.3), 130.3 (d, C_{para}, $^4J_{CP}$ = 2.4), 133.0 (d, C_{ortho}, $^2J_{CP}$ = 10.4), 133.3 (d, C_{ortho}, $^2J_{CP}$ = 10.6), 135.4 (dd, C_{ipso}, $^1J_{CP}$ = 42.2, $^3J_{CP}$ = 1.7), 136.1 (dd, C_{ipso}, $^1J_{CP}$ = 42.2, $^3J_{CP}$ = 1.6). ^{31}P NMR ($CDCl_3$, 121 MHz): -144.3 (quint., J_{PF} = 708, PF_6^-), 38.9 (d, $^2J_{PP}$ = 35.7, PPh_3), 40.6 (d, $^2J_{PP}$ = 35.1, PPh_3). Anal. Calcd. for $C_{53}H_{52}O_5NRuP_3F_6$: C, 58.35; H, 4.80; N, 1.28. Found: C, 58.35; H, 4.66; N, 1.34.

4.3.10. ***[10]****[PF_6]*

Yellow; recrystallized from CH_2Cl_2/hexane; η = 71 %. FTIR (KBr, cm^{-1}): 3057 ($\nu_{C\text{-}H}$,η^5-C_5H_5), 842 ($\nu_{P\text{-}F}$, PF_6^-). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.01, 1.32, 1.35, 1.50 (four s, 12H, -C(CH_3)$_2$), 3.92 (s, 2H, H5), 4.19 (d, 1H, *J* = 2.4, H2), 4.26 (d, 1H, *J* = 7.8, H4), 4.37 (s, 5H, η^5-C_5H_5), 4.60 (dd, 1H, *J* = 7.8, 2.4, H3), 6.94-7.36 (m, 30H, C_6H_5, PPh_3). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz): 24.2, 25.3, 26.2 (-C(<u>C</u>H_3)$_2$), 62.5 (C5), 70.2 (C3), 70.6 (C2), 75.4 (C4), 84.3 (η^5-C_5H_5), 97.8 (C1), 109.6, 111.8 (-<u>C</u>(CH_3)$_2$), 129.0 (d, C_{meta}, $^3J_{CP}$ = 9.4), 130.6, 130.7 (two d, C_{para}, $^4J_{CP}$ = 2.3), 134.2 (d, C_{ortho}, $^2J_{CP}$ = 10.3), 134.3 (d, C_{ortho}, $^2J_{CP}$ = 10.6), 136.9 (dd, C_{ipso}, $^1J_{CP}$ = 40.4, $^3J_{CP}$ = 2.1), 137.7 (dd, C_{ipso}, $^1J_{CP}$ = 40.0, $^3J_{CP}$ = 1.9), 159.5 (C6). ^{31}P NMR ($CDCl_3$, 162 MHz): -144.2 (quint., J_{PF} = 709,PF_6^-), 39.4 (d, $^2J_{PP}$ = 35.6, PPh_3), 40.8 (d, $^2J_{PP}$ = 36.9, PPh_3). Anal. Calcd. for $C_{53}H_{53}O_5N_4RuP_3F_6$: C, 56.14; H, 4.71; N, 4.94. Found: C, 56.54; H, 4.81; N, 4.43.

4.4 .Cell viability assays in human HeLa tumor cells

HeLa cells were maintained in Roswell Park Memorial Institute medium (RPMI) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and 1% L-glutamine, at 37 °C in a humid incubator with 5% CO_2. In order to detach cells from the bottle, trypsin solution was used.

The MTT assay was used to determine the cell viability as an indicator for the sensitivity of the cells to the complexes Ru (II). Exponentially growing cells were seeded at a density of approximately 6×10^5 cells/mL, in a 96-well flat-bottomed microplate, and 48 h later they were treated with the complexes. The complexes were dissolved in DMSO and tested in concentrations ranging from 5 to 500 μM. Cytotoxicity of test compounds was evaluated by the MTT method [48]. The optical density was measured at 570 nm using a 96-well multi-scanner auto-reader. The IC_{50} were calculated by nonlinear regression analysis using Origin.

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- Ten new ruthenium(II) complexes with carbohydrate derivative ligands were synthesized.
- The cytotoxicity of the complexes was tested on *HeLa* cancer cells (cervical carcinoma).
- The IC_{50} values are lower than cisplatin, in the low micromolar range.