

N-heterocyclic carbene complexes Ru^{II}(arene) and their application as ROMP catalysts, antioxidant and anticancer agents

Rubina Troiano,^[a] Domenico Iacopetta,^[b] Assunta D'Amato,^{*[a]} Annalisa Mariconda,^[c] Jessica Ceramella,^[b] Alessia Catalano,^[d] Maria Stefania Sinicropi^[b] and Pasquale Longo^[a]

[a] Dr. A. D'Amato, Dr. R. Troiano, Prof. P. Longo

Department of Chemistry and Biology "A. Zambelli"

University of Salerno

Via Giovanni Paolo II, 132, I-84084 Fisciano (SA), Italy

E-mail: asdamato@unisa.it

[b] Prof. A. Mariconda

Department of Science

University of Basilicata

Viale dell'Ateneo Lucano 10, I-85100 Potenza, Italy

[c] Prof. M.S. Sinicropi, Dr. D. Iacopetta, Dr. J. Ceramella

Department of Pharmacy, Health and Nutritional Sciences

University of Calabria

Via Pietro Bucci, I-87036 Arcavacata di Rende (CS), Italy

[d] Prof Alessia Catalano

Department of Pharmacy-Drug Sciences

University of Bari "Aldo Moro"

I-70126 Bari, Italy

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Abstract: N-Heterocyclic carbenes (NHCs) play an important role in metal complex catalysis and stabilisation, providing stability under non-inert conditions and a high σ -donor capacity. They are crucial ligands for the synthesis of organometallic compounds like Ru^{II}-complexes, which can operate as very active catalysts for a variety of chemical transformations and functionalizations. RA(Ruthenium-arene)NHC complexes, known for their piano-stool structure, stabilise Ru(II) oxidation state by occupying three ruthenium coordination sites with a η^6 -coordinated arene ligand. The development of dual-acting Ru^{II}-arene complexes has sparked increased interest in their catalytic and biological properties. Because of their broad spectrum, metal complexes can give distinct mechanisms of pharmacological action from organic drugs. This study aimed to explore the impact of ligand amphiphilicity and counterion on bioactivity, designing a family of compounds with the NHC ligand bearing an ester moiety and switching the wingtip substituent from methyl to benzyl units. All synthesized compounds were tested in norbornene ROMP, a benchmark reaction for Ru^{II} potential catalysts and as anticancer and antioxidant compounds.

Introduction

N-Heterocyclic carbenes (NHCs) today constitute a notable species in catalysis and in stabilization of metal complexes.^[1,2] Among the several benefits associated with the use of an NHC ligand, which include stability under non-inert conditions and strong σ -donor capacity, rely the almost endlessly possibilities to tune the steric and electronic properties of the ligand itself by modifications of the heterocycles' substituents. Specifically, they have proven to be incredibly important ligands for the

development of organometallic compounds that can be highly active catalysts for an amazing number of chemical transformations and functionalizations,^[3-6] among which olefin metathesis.^[7-9] Notably, they can frequently be effective drugs capable of acting as anticancer, antimicrobial^[10] and antioxidant agents^[11,12] as well. Among these compounds, Ru^{II}-complexes have been widely studied since the development of the first and second generations Grubbs' and Hoveyda-Grubbs' catalysts.^[13-16] Most synthetic pathways leading to NHC-Ru^{II} complexes classically involve bis(phosphane) benzyldiene intermediates;^[17,18] however, in recent years, an interesting class of complexes, RANHC, acronym for Ruthenium(II)-Arene-NHC, has been developed. These can be easily accessed either by direct reaction of a stoichiometric amount of free carbene, generated *in situ* by deprotonation of a more stable ionic precursor, and the commercially available Ru dimer [(*p*-cymene)RuCl₂]₂,^[19,20] or by transmetalation route from a parent silver complex.^[21,22] Often related to RAPTA (Ruthenium-Arene-PTA (1,3,5-triaza-7-phosphaadamantane))^[23-25] complexes, RANHC are distinguished by their piano-stool structure (Figure 1), which stabilizes the Ru(II) oxidation state by occupying three of the ruthenium coordination sites with an η^6 -coordinated arene ligand. The main ligand (ancillary ligand) occupies another coordination site, leaving two other coordination sites free to be filled by typically labile chloride ligands.

The main difference between RAPTA and RANHC relies in the substitution of the amphiphilic phosphine ligand PTA, which was originally introduced to enhance the physiological environment solubility of the resulting complex, with an N-heterocyclic ligand, which has stronger σ -donation and stereoelectronic mouldability (Figure 1).

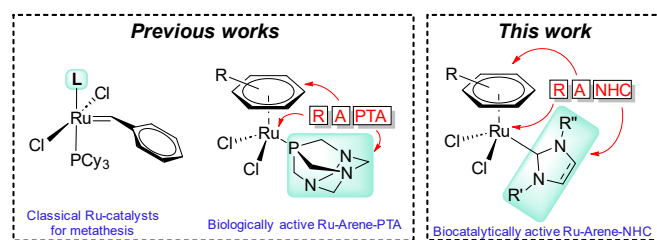


Figure 1. Left to right: Schematic structure of classical Grubbs' and Hoveyda-Grubbs' Ru^{II} pre-catalysts; schematic structure of Ru^{II}-Arene-PTA; schematic structure of Ru^{II}-Arene-NHC.

The past couple of decades have seen an increasing interest in the development of dual-acting Ru^{II}-arene complexes, displaying both catalytic^[26] and biological^[27] activity.

Possibly, the most famous class of Ru-catalysed reactions involves alkene or alkyne metathesis reactions,^[26,28] such as ring-opening metathesis polymerisation (ROMP),^[19,20,29–31] ring-closing metathesis (RCM),^[32] cross-metathesis^[33] or enyne metathesis (EYM), whose outcome is deeply influenced by the ligand constitution.^[26,34] Moreover, transformation of small molecules to obtain relevant building blocks, has recently been explored, particularly involving transfer (de)hydrogenation (reduction of double carbon-carbon and carbon-oxygen bonds)^[35,36] amine, imine and amide formation.^[37–39]

In these regards, the past few decades have been dedicated to the improvement of the catalytic performances of the metal complex by progressive modifications of the ancillary and labile ligands constitution. As far as the stability of the precatalytic complex is concerned, RANHC complexes, when compared to the parent Grubbs' and Hoveyda-Grubbs', display an increased ease of management, as they tend to be oxygen and moisture resistant. Such stability can be further enhanced by the presence of an additional hemilabile group, positioned on one of the peripheral nitrogen moieties.

For example, amine, pyridine,^[40] ether^[35] and thioether^[36] groups have been chelated to Ru^{II}-complexes, promoting stabilization of unsaturated intermediates and thereby improving catalyst stability and efficiency.

Whereas the latter transformations, in which the precatalyst is normally activated by the arene disengagement, followed by the coordination of the substrate, the former, especially ROMP, require the formation of a Ru-alkylidene specie, and are far less studied.

In fact, unlike Grubbs' ruthenium-benzylidene complexes, ruthenium-arene species lack the alkylidene moiety that is essential for enabling metathesis reactions. However, highly active species can be generated *in situ* either thermally,^[29,41] photochemically^[42] or by arene disengagement in the presence of a carbene activator.^[43–48] Noteworthy, these systems are still scarcely studied in the literature, due to unclear mechanisms involving the arene disengagement and monomer coordination.

The fundamental disadvantage of this system is the inadequate control over the initiation stage, often resulting in high molecular weights and broad polydispersities. Because few propagating species are present in solution, even minor changes in initiation efficiency can result in substantial differences in the control of molecular weights.

As far as the biological activities are concerned, both Ru^{III} and Ru^{II} complexes are able to exert cytotoxic activity against solid tumors.^[27,49]

In fact, metal complexes can provide different mechanisms of drug action compared to organic agents because of their broad range of coordination and oxidation numbers, geometries, and kinetic properties. As a result, there is a growing interest in using metal complexes as pharmaceuticals for chemotherapeutic or diagnostic purposes.

Unsurprisingly, Grubbs type prototypic ruthenium NHC complexes, akin to many organometallic bioactive compounds, exhibit considerable biological effectiveness.^[50] Lead compounds have been successfully established, and ruthenium complexes themselves have been playing a dramatically growing role in inorganic medicinal chemistry.^[51] As a result, following the revolutionary platinum cytostatic drugs, these transition metal complexes are currently regarded as the second most promising class of novel anticancer metal species. In RAPTA and RANHC prototypes, RuCl₂(arene) constitutes the organometallic pharmacophoric core,^[52–54] while the phosphine or the carbene *N*-heterocyclic ligand plays a crucial role into the stabilization of the metallic core in the biological environment; efforts have been made to design an NHC imidazol(in)ium moiety capable to infer such kinetic inertness and amphiphilicity.^[49,49,51,55] The very same complex can display anticancer, antimicrobial and antioxidant properties, furnishing a wide-spectrum bioactivity. In particular, RANHC complexes featuring an imidazolium core, endowed with a (2-methoxy-2-phenyl)ethyl hydrophobic nitrogen substituent (Figure 2, **RuA-C**), exhibit strong anticancer action, primarily against MDA-MB-231 breast cancer cells. Also, antibacterial action, especially against *S. aureus*, was observed but, most of all, their antioxidant qualities, tested in the scavenging of ABTS radical, resulted superior to commercially available products.^[56]

Building on promising preliminary data, we aimed to study here the impact of the ligand amphiphilicity and the counterion on the overall bioactivity, thus we designed a novel family of compounds bearing an ester moiety rather than an alkoxide (Figure 2, **Ru1a-c**). Moreover, further evaluations were made by switching the methyl nitrogen substituent with a benzyl unit, in order to increase the lipophilicity and steric hindrance of the metal bonded to the carbenic carbon (Figure 2, **Ru2a-c**). Secondly, given the outstanding catalytic performances of Ru^{II} complexes in olefin metathesis,^[13,16] we tested all of the synthesized compounds so far in the norbornene ROMP, a benchmark reaction for newly Ru^{II} potential catalysts.

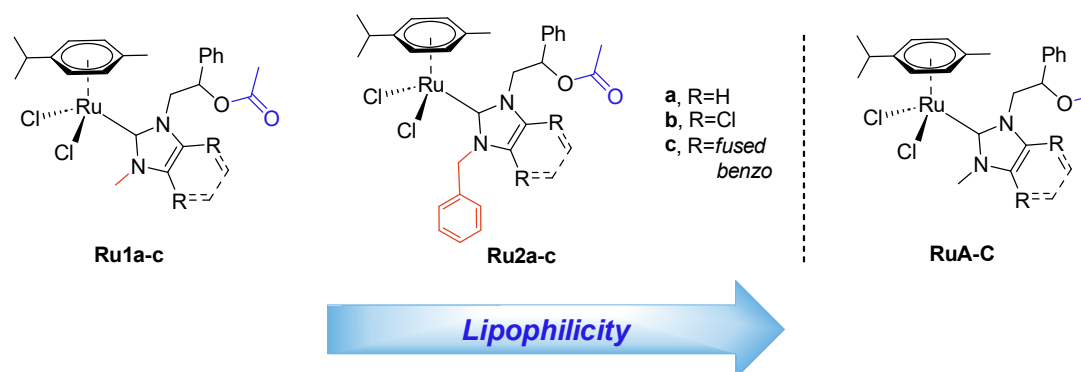


Figure 2. Left to right: Reported RANHC endowed with an ether substituent (**A-C**) and novel acetyl substituted complexes **Ru1a-c** and **Ru2a-c**.

Results and Discussion

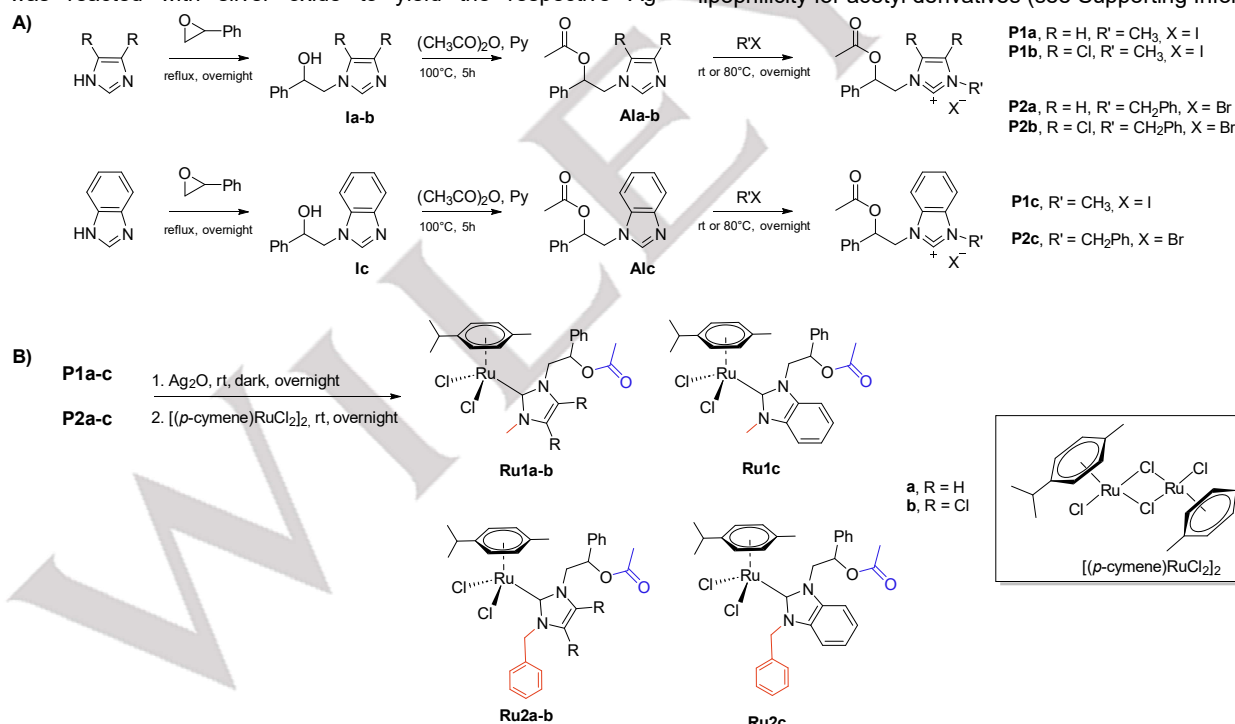
Chemistry and Norbornene Polymerization

Synthesis of acetyl endowed proligands **P1a-c** and **P2a-c** was conveniently performed starting with known intermediates **1a-c**,^[57,58] which were subjected to acylation with acetic anhydride to yield **Ala-c**.^[59] Alkylation with either iodomethane or benzyl bromide led to the respective triplet of congener imidazolium salts (Scheme 1, **A**).

Synthesis of the Ru^{II} complexes was accomplished following the transmetallation route.^[60–62] The appropriate carbene precursor was reacted with silver oxide to yield the respective Ag^I

complexes; then, metathesis of the latter with commercially available [(*p*-cymene)RuCl₂]₂ gave the desired Ru^{II} complexes, isolated as orange-brownish solids (Scheme 1, **B**). For all of the novel synthesized complexes, the NMR spectra show the disappearance of the proton on the carbocationic carbon and the notable shift towards the lower field of the NCN carbon, thus confirming the formation of carbene carbon and the desired Ru^{II} adduct, which was further validated by mass spectra.

Since lipophilicity is known to substantially affect bioproperties, logP measurements^[63,64] were conducted in order to determine the amphiphilic properties of model complexes **RuB** and **Ru1b**, which solely differ for the ether/ester switch on the wingtip substituent. 1-Octanol/water partition coefficient resulted, respectively, 0.73 and -0.11, indicating a strongly pronounced lipophilicity for acetyl derivatives (see Supporting Information).



Scheme 1. A) Synthesis of proligands **P1a-c**, **P2a-c** and B) Ru^{II} complexes **Ru1a-c**, **Ru2a-c**.

Catalytic performances of novel synthesized complexes were assessed in the benchmark ROMP reaction of norbornene, with a 3 μM catalyst loading, and a high (3000:1) monomer/catalyst ratio.^[65–68]

According to reported studies, transient Ru-alkylidene active species can be generated *in situ* by either addition in the reaction medium of alkynes,^[44,45] cyclopropenes,^[46,47] or diazocompounds.^[47,48]

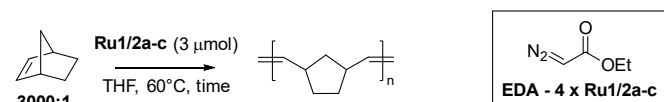
Among these, inexpensive ethyl diazoacetate (EDA) was shown to be a productive initiator both in benchmark cross metathesis and ring-opening metathesis.

Basing on these assumptions, novel Ru complexes were investigated in the catalysis both with and without ethyl diazoacetate as the initiator to gain a deeper understanding of the reaction pathway.

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Catalysis tests, performed in the optimal conditions individuated by Mauduit and colleagues,^[47] are reported in Table 1. All of the novel synthesized pre-catalysts showed good to excellent activity, as indicated by the high conversions to polynorbornene, and elevated M_n , paired with quite narrow PDI_s (polydispersity index), in the presence of EDA as initiator.

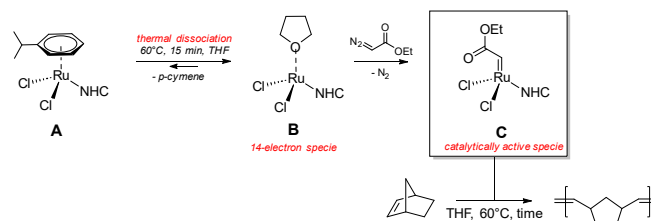
Table 1. Norbornene ROMP catalysed by complexes **Ru1a-c**, **Ru2a-c**.



Run ^a	Catalyst (+ initiator)	Time (min)	Conversion (%) ^b	E/Z ratio (%) ^c	M_n (kg/mol) ^d	PDI ^d
1	Ru1a	60	Traces	34/66	55.3	9.6
2	Ru1a + EDA	60	87	38/62	121.0	5.2
3	Ru1b	60	Traces	34/66	18.0	6.9
4	Ru1b + EDA	7 ^e	99	46/54	44.1	5.6
5	Ru1c	60	Traces	24/76	27.8	6.8
6	Ru1c + EDA	60 ^e	96	44/56	196.1	2.8
7	Ru2a	60	n.d.	-	-	-
8	Ru2a + EDA	60	91	47/53	71.5	4.0
9	Ru2b	60	6	25/75	41.8	3.4
10	Ru2b + EDA	7 ^e	99	66/34	267.3	4.2
11	Ru2c	60	Traces	24/76	182.0	5.1
12	Ru2c + EDA	10 ^e	97	38/62	155.7	4.5
13	RuB	6 ^e	95	18/81	12.2	31.0
14	RuB + EDA	15 ^e	97	16/84	16.7	18.0
15	$[(p\text{-cymene})\text{RuCl}_2]_2$	12 ^e	99	34/66	3.3	36.5
16	$[(p\text{-cymene})\text{RuCl}_2]_2$ + EDA	1 ^e	99	15/85	3.1	14.4

[a] Reaction conditions: [Ru] 3 μmol , precatalyst:monomer ratio 3000:1, 60°C, dry THF (5 mL). [b] Conversions determined by ¹H NMR (CDCl₃), using 2-bromomesitylene as internal standard. [c] Determined by ¹H NMR (CDCl₃), δ = 5.36 ppm *E* CH=CH, 5.22 ppm, *Z* CH=CH. [d] Determined by GPC (THF). [e] Gelification of the polymer observed.

Necessary thermal pre-dissociation of the *p*-cymene moiety, in order to generate the 14-electron complex **B**, was performed by 15 minutes treatment of **Ru1a-c**, **Ru2a-c** in dry THF at 60°C. This allowed the exchange with a labile THF coordinated molecule, then replaced by the formation of the active Ru-alkylidene catalyst **C**, with concomitant nitrogen evolution from ethyl diazoacetate activator. In the presence of the substrate such species gives the conversions to polynorbornene, in the indicated times, in Table 1 (Scheme 2).



Scheme 2. Mechanism of activation with ethyl diazoacetate. **A**. Precatalyst **Ru1/2a-c** is subjected to thermal *p*-cymene dissociation to intermediate **B**, coordinated by THF. Reaction with EDA yields **C**, which starts the catalytic cycle.

Crucial stabilization of the thermally generated 14-electron species **B** (Scheme 2) has been reported as the rate-determining step towards the formation of the catalytically active species.^[29,42,47,69]

The beneficial effect of the NHC ligand can be ascertained comparing novel acetyl derivatives **Ru1/2a-c** with reported ether complexes **RuA-C**.^[56] All the novel complexes conversions range from 87% to completion, while alkoxy derivatives lack efficient catalytic activity even in the presence of EDA (Table 1, entries 13-14), as stated by low molecular masses, coupled with broad polydispersity indexes. This may be due to the carbonyl-assisted stabilization of intermediate **B** by means of the acyl group (panel A, Figure 3); the same effect would not be as efficient in the presence of a methoxy group from NHC ligands in **RuA-C**. As previously mentioned, the presence of an hemilabile group on the *N*-heterocyclic carbene wingtip, such as the carboxyl residue, can enhance the stability of the catalytically active species, promoting its formation.

Interestingly, no relevant effect was observed switching the backbone NHC substituent from an electron-withdrawing (chloride, **Ru1/2b**) to an electron-donating (fused benzene, **Ru1/2c**), suggesting indeed the need for a steric encumbrance. In fact, both substituents provoke an increase of conversions and M_n , when compared with unsubstituted **Ru1/2a** (entry 2 vs. 4 and 6, and 8 vs. 10 and 12).

Further increase of lipophilicity, gained by substitution of methyl group on one of the nitrogen imidazole heteroatoms in complexes **Ru2a-c**, did not lead to conspicuous differences in catalytic performances (entries 7-12). Contrary to what was possible to hypothesize, the benzyl group did not significantly compete with the acetyl carbonyl in the stabilization of the 14-electron Ru^{II} specie (panel B, Figure 3). In fact, the yields are practically the same and the polydispersity indices are only slightly narrower. This behavior could be attributed to the over-stabilization of the complex, in the presence of the phenyl ring, which could donate up to six additional electrons to the metallic centre.

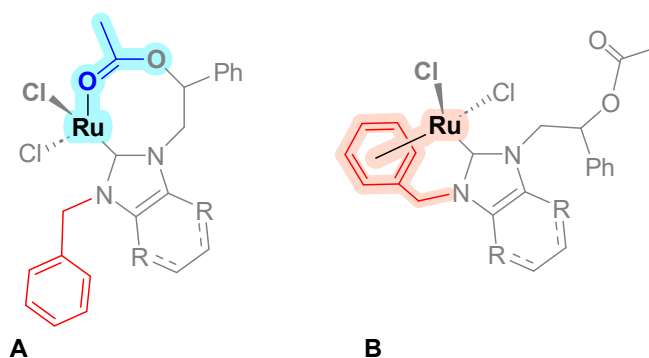


Figure 3. Hypothesized **A**. 14-electron Ru^{II} stabilization assisted by acetyl group; **B**. 14-electron Ru^{II} stabilization assisted by benzyl group.

All of the neosynthesized complexes showed superior performances when compared with commercially available [(*p*-cymene)RuCl₂]₂. The latter, in fact, in the presence of EDA gave complete conversion, but with high polydispersity (14.4) and reduced *M_n* (3.1 kg/mol), suggesting, as previously reported in the literature,^[43,69] multiple-site polymerization mechanisms and secondary metathesis reactions, including inter- and intra-molecular chain transfer.

As far as stereoselectivity is concerned, no significant *E/Z* ratios were observed, displaying a slight preference for the *Z* product, not exceeding 70%.

Bioactivity

Anticancer Activity

The six new Ru-NHC complexes were evaluated for their anticancer potential against two different breast cancer cell lines, namely the ER-positive MCF-7 and the triple negative MDA MB-231 cells, by the means of MTT assay. The obtained outcomes were expressed as IC₅₀ values, calculated after 72 hours of exposure to the studied complexes, which are reported in Table 1. As reference molecule, a well-known anticancer complex, *i.e.* Cisplatin, was used. A significant anticancer activity was recorded only for two complexes, **Ru2b** and **Ru2c**, toward both the breast cancer cells, but with a higher activity against the MDA-MB-231 cells, respectively of about four- and three-fold superior with respect to MCF-7 cells. Indeed, **Ru2b** IC₅₀ values were of 10.1±0.3 and 45.1±0.06 μM, whereas **Ru2c** values were of 14.7±0.02 and 45.8±0.07 μM, for MDA-MB-231 and MCF-7 cells, respectively. It should be noted that both the active complexes differ for the type and nature of the substituents of the carbene ring. Particularly, **Ru2b** bears two chlorine atoms at the carbene ring, whereas **Ru2c** carbene ring is fused with a benzene scaffold. The other complexes did not show any anticancer activity against both the adopted breast cancer cell lines, at least until the concentration of 50 μM and under the adopted experimental conditions. It can be deduced that the lack of the bulkier benzyl group at the carbenic nitrogen in position 5 (**Ru1a**, **Ru1b** and **Ru1c**) led to the completely drop of the anticancer activity. Furthermore, **Ru2a** possesses the benzyl substituent at N3, but does not have the chlorine atoms at C4 and C5 (**Ru2b**) or the

benzene fused to the carbenic ring (**Ru2c**) and is inactive, as well. Finally, **Ru2b** and **Ru2c** impacted MDA-MB-231 cells viability three- and two-fold better than *Cisplatin*, respectively. Moreover, proligands **P1a-c** and **P2a-c**, together with [(*p*-cymene)RuCl₂]₂, were tested against both the adopted cell lines, but no effects were evidenced, at least until the concentration of 50 μM.

Table 2. Anticancer activity of the studied Ru-NHC complexes (**Ru1a-c**, **Ru2a-c**), [(*p*-cymene)RuCl₂]₂ and *Cisplatin* used as reference, expressed as IC₅₀ values ± S.E. μM, against two breast cancer cell lines.

Compounds	IC ₅₀ ± S.E.	
	MDA-MB-231	MCF-7
Ru1a	>50	>50
Ru2a	>50	>50
Ru1b	>50	>50
Ru2b	10.1±0.3	45.1±0.06
Ru1c	>50	>50
Ru2c	14.7±0.02	45.8±0.07
[(<i>p</i> -cymene)RuCl ₂] ₂	>50	>50
<i>Cisplatin</i>	33.8±0.05	24.9±0.05

Proligands (**P1a-c** and **P2a-c**) tested against MDA-MB-231 and MCF-7 showed IC₅₀>50 μM.

Antioxidant Activity

The antioxidant effect of the synthesized Ru-NHC complexes was assessed by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the 2,20-azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid) (ABTS) radicals scavenging assays.

The DPPH scavenging activity of all the Ru-NHC complexes resulted higher compared to the Trolox, used as standard antioxidant in our assay. Indeed, they exhibited a stronger electron donating power, showing IC₅₀ values very close to each other, ranging from 1.2 to 2.3 μM (see Table 3). In particular, the best scavenging activity against DPPH radical was recorded by **Ru1b** and **Ru2b**, bearing two chlorine atoms at the carbene ring (IC₅₀ values of 1.2±0.1 and 1.3±0.09 μM, respectively), and also by **Ru1c** and **Ru2c** with the carbene ring fused with a benzene scaffold (IC₅₀ values of 1.6±0.1 and 1.2±0.1 μM, respectively). Instead, the lack of the two chlorine atoms or the fused benzene ring at the carbene produced a slight reduction of about 2 times in DPPH scavenging activity, as in **Ru1a** and **Ru2a**, which showed IC₅₀ values of 2.3±0.09 and 2.1±0.1 μM, respectively. As visible from Table 2, Trolox produced a scavenging activity towards the DPPH radical approximately 17 times lower than the more active complexes, with an IC₅₀ value of 20.8±0.04 μM.

To further investigate the Ru-NHC complexes antiradical potential, we measured their ability to interfere with the ABTS radical cation. The outcomes in terms of IC₅₀ values of Ru-NHC complexes, alongside the standard Trolox, on ABTS radical are reported in Table 3.

Likewise, in this case, all the Ru-NHC complexes exhibited a higher ABTS scavenging activity than Trolox, which showed an IC₅₀ value of 18.3±0.02 μM. All the complexes had a very good antioxidant ability, with IC₅₀ values ranging from 39±0.07 nM (**Ru2a**) to 71±0.08 nM (**Ru1a**) (see Table 3). Particularly, the ABTS scavenging activity pattern of the Ru-NHC complexes can be ranked in the following order: **Ru2a**>**Ru1b**>**Ru1c**>**Ru2c**>**Ru2b**>**Ru1a**. Anyway, the scavenging activity of the most active complexes against the ABTS radical cation is about 470 times higher than that measured for Trolox.

The obtained results allowed to conclude that the tested complexes exhibited a higher ability in inhibiting the ABTS^{•+} than DPPH, showing a better antioxidant activity than the well-known antioxidant Trolox. Additionally, proligands **P1a-c** and **P2a-c**, together with [(*p*-cymene)RuCl₂]₂, were tested for their antioxidant power against DPPH and ABTS radicals. Proligands were found inactive toward both the radicals, at least until the concentration of 30 μM, whereas the [(*p*-cymene)RuCl₂]₂ exhibited an IC₅₀ value of 0.8±0.07 against the ABTS radical, but was inactive toward DPPH.

Table 3. Radical scavenging ability against DPPH and ABTS radicals, expressed as IC₅₀ ± SE μM, of Ru-NHC complexes (**Ru1a-c**, **Ru2a-c**), [(*p*-cymene)RuCl₂]₂, and standard drug (Trolox).

Compounds	IC ₅₀ (μM) ± S.E.	
	DPPH	ABTS
Ru1a	2.3±0.09	7.1×10 ⁻² ±0.08
Ru2a	2.1±0.1	3.9×10 ⁻² ±0.07
Ru1b	1.2±0.1	4.3×10 ⁻² ±0.1
Ru2b	1.3±0.09	6.5×10 ⁻² ±0.04
Ru1c	1.6±0.1	4.5×10 ⁻² ±0.06
Ru2c	1.2±0.1	5.9×10 ⁻² ±0.06
[(<i>p</i> -cymene)RuCl ₂] ₂	>30	0.8±0.07
Trolox	20.8±0.04	18.3±0.02

Proligands (**P1a-c** and **P2a-c**) tested against DPPH and ABTS showed IC₅₀>30 μM.

Conclusions

In the plethora of Ru^{II} complexes reported in the past thirty years, very few examples of bio- and catalytically active organometallics are reported. While catalytic activity is broad and related to a number of olefin metathesis reactions and polymerizations, broad-spectrum bioactivity is not as often investigated on the same complexes. In this work, six Ru^{II}-arene-NHC complexes were reported, characterized by increasing lipophilicity granted by acyl and benzyl moieties. Their catalytic activity was assessed in the benchmark norbornene ROMP polymerization, and compared with known, akin complexes and commercial [(*p*-cymene)RuCl₂]₂, discovering a remarkable activity and interesting of M_n and polydispersities. Notably, the same complexes resulted discrete as anticancer, while outstanding antioxidant properties were found, with radical scavenging ability against DPPH and ABTS

radicals in the low μM and nM order, up to 470 times higher than the one that measured for commercial antioxidant Trolox. The Ru^{II}-arene pharmacophore's flexibility allows for further study of NHC-Ru^{II}-arene complexes. Further derivatization of the arene moiety, substitution with different chelating groups, and diversity of the NHC motif could lead to the development of new metal-based anticancer medicines, which, at the same time, could work as useful catalysts in classical double bonds functionalization.

Experimental Section

Materials and Methods

All reagents were purchased from Merck Italy (Milan, Italy) and TCI Chemicals (Zwijndrecht, Belgium) and used without any purifications. All solvents were bought by Carlo Erba Reagents srl (Milano, Italy) and were distilled over appropriate drying agents under nitrogen before use. The synthesis of Ru-complexes was carried out under a nitrogen atmosphere by using Schlenk techniques in the dark. The glassware used was dried in an oven at 120 °C overnight. Organic molecules were purified by flash column chromatography using silica gel 60 (230–400 mesh) purchased from Merck Italy. Thin layer chromatography (TLC) was performed using silica gel 60 aluminum foils with F254 fluorescent indicator. Deuterated solvents were dried on molecular sieves. ¹H and ¹³C nuclear magnetic resonance spectra (NMR) were acquired on a Bruker Avance 300 spectrometer (300 MHz for ¹H; 75 MHz for ¹³C), a Bruker AVANCE 400 spectrometer (400 MHz for ¹H; 100 MHz for ¹³C) and a Bruker AVANCE 600 spectrometer (600.13 MHz for ¹H, 150.90 MHz for ¹³C), operating at 298 K. NMR samples were prepared by dissolving about 15 mg of the compound in 0.5 mL of deuterated solvent (Eurisotop Cambridge Isotope Laboratories, Cambridge, UK). The chemical shifts of ¹H-NMR and ¹³C-NMR spectra are referenced using the residual proton impurities of the deuterated solvents. ¹H-NMR were reported relative to DMSO-*d*₆ δ 2.50 ppm, CDCl₃ δ 7.26 ppm, THF-*d*₆ δ 3.58 ppm and CD₃CN δ 1.72 ppm and δ 1.94 ppm. ¹³C-NMR were reported relative to DMSO-*d*₆ δ 39.52 ppm, CDCl₃ δ 77.16 ppm, THF-*d*₆ δ 67.21 ppm and δ 25.31 ppm, and CD₃CN δ 1.32 ppm δ 118.26 ppm. The spectra multiplicities are indicated as follows: singlet (s), doublet (d), triplet (t), multiplet (m), broad (br). MALDI-MS mass spectra were obtained by Bruker Solarix XR Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) with a 7 T refrigerated actively shielded superconducting magnet (Bruker Biospin, Wissembourg, France). MALDI ion source (Bruker Daltonik GmbH, Bremen, Germany) was used the samples in positive ion mode. The mass range was set to m/z 200–3000. The laser power was 28% and 22 laser shots were utilized for each scan. The mass spectra were calibrated externally using a mix of peptide clusters in MALDI ionization positive ion mode. ESI-MS spectra were achieved using a Waters Quattro Micro triple-quadrupole mass spectrometer equipped with an electrospray ionization source. GPC measurements were performed on a Waters 1525 binary equipped with a Waters 2414 RI detector using four Styragel columns (range 1000–1,000,000 Å) (Waters Corporation, Milford, Massachusetts, United States). Ultraviolet–visible (UV–Vis) measurements were performed with an Agilent Varian Cary 50 spectrophotometer (Agilent Technologies Inc., Santa Clara, California, USA).

Synthesis of the imidazolium salts, proligands **P1a-c**, **P2a-c**

1a-c were synthesized following the procedure reported in literature.^[58]

Synthesis of **Ala-c**

Ala-c were synthesized using a procedure reported in the literature, modified for our purposes.^[70] **1a-c** (1.00 eq) and pyridine (3.00 eq) are placed in a two-necked flask equipped with a magnetic stirrer and a condenser, under a nitrogen atmosphere. After dissolving **1a-c**, acetic anhydride (5.00 eq) was added. The reaction mixture was heated at 100 °C for 5 hours. The solution was diluted with 30 mL of diethyl ether and extracted with water (3x30mL). The organic phase was dried with MgSO₄, and filtered. The solvent was removed under reduced pressure. The product was obtained as a greenish oil. Yields: **Ala** 89%, **Alb** 78%, **Alc** 61%.

2-(1*H*-imidazol-1-yl)-1-phenylethyl acetate (**Ala**)

¹H-NMR (ppm, CD₃CN, 300 MHz) δ: 7.53 (1H, s, NCHN), 7.33-7.30 (5H, overlapping signals, Ar-H), 7.01 and 6.94 (2H, s, NCH=CHN), 5.95 (1H, t, *J*=5.89 Hz, NCH₂CH), 4.33 (2H, d, *J*=5.89 Hz, NCH₂CH), 2.01 (3H, s, COCH₃).

¹³C-NMR (ppm, CD₃CN, 75 MHz) δ: 170.62 (C=O), 138.80 (*ipso carbon of aromatic ring*), 138.41 (NCHN), 129.61 (x2), 129.47, 129.04 (x2), 127.26, 121.05 (*Ph-group* and NCHCHN), 75.34 (NCH₂CH), 52.02 (NCH₂), 21.11 (COCH₃).

MALDI-MS (CH₃CN) calcd/found (m/z): [C₁₃H₁₄N₂O₂]⁺ 231.1128/231.1129.

2-(4,5-dichloro-1*H*-imidazol-1-yl)-1-phenylethyl acetate (**Alb**)

¹H-NMR (ppm, DMSO-*d*₆, 300 MHz) δ: 7.72 (1H, s, NCHN), 7.40-7.32 (5H, overlapping signals, Ar-H), 6.00 (1H, t, *J*=5.86 Hz, NCH₂CH), 4.40 (2H, m, *J* = 5.86 Hz, NCH₂CH), 2.05 (3H, s, COCH₃).

¹³C-NMR (ppm, DMSO-*d*₆, 100 MHz): δ 169.25 (C=O), 136.70 (*ipso carbon of aromatic ring*), 136.39 (NCHN), 128.63 (x2), 126.28 (x2), 124.09 (*Ph-group* and NCCICCN), 72.89 (NCH₂CH), 49.56 (NCH₂), 20.56 (COCH₃).

MALDI-MS (CH₃CN) calcd/found (m/z): [C₁₃H₁₂Cl₂N₂O₂]⁺ 299.0349/299.0351.

2-(1*H*-benzoimidazol-1-yl)-1-phenylethyl acetate (**Alc**)

¹H-NMR (ppm, DMSO-*d*₆, 600 MHz) δ: 8.09 (1H, s, NCHN), 7.65-7.63 (2H, overlapping signals, Ar-H), 7.40-7.37 (5H, overlapping signals, Ar-H), 7.25 (1H, t, *J*_{ortho}=7.29 Hz, Ar-H), 7.19 (1H, t, *J*_{ortho}=7.29 Hz, Ar-H), 6.07 (1H, t, *J*=6.08 Hz, NH₂CH), 4.67 (2H, m, NCH₂CH), 1.92 (3H, s, COCH₃).

¹³C-NMR (ppm, DMSO-*d*₆, 150 MHz) δ: 169.26 (C=O), 144.37 (NCHN), 143.06, 137.21, 134.04, 128.52, 128.41 (x2), 126.46 (x2), 122.30, 121.48, 119.34, 110.65 (*Ph-groups*), 73.66 (NCH₂CH), 48.49 (NCH₂), 20.59 (COCH₃).

MALDI-MS (CH₃CN) calcd/found (m/z): [C₁₇H₁₆N₂O₂]⁺ 281.1284/281.1288.

Synthesis of **P1a-c**

To a solution of **Ala-c** (1.00 eq) in acetonitrile (0.5 M) was added methyl iodide (7.00 eq) and the resultant solution was stirred at room temperature for 18h. After the solvent was removed under reduced pressure, the residual powder was washed with acetone (3x10mL) and dried by reduced pressure to obtain the product (**P1a-c**) as a white powder. Yields: **P1a** 85%, **P1b** 66%, **P1c** 52%.
N-Methyl, *N'*-(2-acethoxy-2-phenyl)ethyl imidazolium iodide (**P1a**)
¹H-NMR (ppm, CD₃CN, 400 MHz) δ: 8.76 (1H, s, NCHN), 7.41-7.35 (7H, overlapping signals, Ar-H and NCH=CHN), 6.01 (1H,

m, NCH₂CH), 4.56 (2H, m, NCH₂CH), 3.83 (3H, s, NCH₃), 2.05 (3H, s, COCH₃).

¹³C-NMR (ppm, CD₃CN, 100 MHz) δ: 170.49 (C=O), 137.70 (*ipso carbon of aromatic ring*), 137.15 (NCHN), 129.88, 129.81, 127.22 (x2), 124.40, 124.21 (*Ph-group*), 74.12 (NCH₂CH), 54.23 (NCH₂), 37.08 (NCH₃), 21.15 (COCH₃).

MALDI-MS (CH₃CN) calcd/found (m/z): [C₁₄H₁₆N₂O₂]⁺ 245.1284/245.1282.

N-Methyl, *N'*-(2-acethoxy-2-phenyl)ethyl-4,5-dichloroimidazolium iodide (**P1b**)

¹H-NMR (ppm, DMSO-*d*₆, 400 MHz) δ: 9.43 (1H, s, NCHN), 7.43-7.39 (5H, overlapping, Ar-H), 5.98 (1H, m, NCH₂CH), 4.69 (2H, m, NCH₂CH), 3.87 (3H, s, NCH₃), 2.08 (3H, s, COCH₃).

¹³C-NMR (ppm, DMSO-*d*₆, 75 MHz) δ: 169.39 (C=O), 137.21 (NCHN), 135.77 (*ipso carbon of aromatic ring*), 128.98, 128.85, 126.33 (x2), 119.43, 118.87 (*Ph-group* and NCCICCN), 72.46 (NCH₂CH), 51.63 (NCH₂), 35.23 (NCH₃), 20.81 (COCH₃).

MALDI-MS (CH₃CN) calcd/found (m/z): [C₁₄H₁₅Cl₂N₂O₂]⁺ 313.0505/313.0506.

N-Methyl, *N'*-(2-acethoxy-2-phenyl)ethyl-benzoimidazolium iodide (**P1c**)

¹H-NMR (ppm, DMSO-*d*₆, 300 MHz) δ: 9.73 (1H, s, NCHN), 8.07-8.00 (2H, overlapping signals, Ar-H), 7.70-7.67 (2H, overlapping signals, Ar-H), 7.49-7.38 (5H, overlapping signals, Ar-H), 6.13 (1H, m, NCH₂CH), 4.99 (2H, m, NCH₂CH), 4.12 (3H, s, NCH₃), 2.07 (3H, s, COCH₃).

¹³C-NMR (ppm, DMSO-*d*₆, 75 MHz): δ 169.43 (C=O), 143.24 (NCHN), 136.29, 131.50, 131.36, 128.76, 126.51, 113.82, 113.53 (*Ph-groups*), 73.20 (NCH₂CH), 50.42 (NCH₂), 33.47 (NCH₃), 20.69 (COCH₃).

MALDI-MS (CH₃CN) calcd/found (m/z): [C₁₈H₁₈N₂O₂]⁺ 295.1441/295.1448.

Synthesis of **P2a-c**

Ala-c (1.00 eq) in acetonitrile (0.5 M) were introduced into a two-necked flask, equipped with a magnetic stirrer and condenser. Subsequently, benzyl bromide (1.50 eq) was added and the reaction mixture was stirred overnight at 80 °C in a nitrogen atmosphere. After the solvent was removed under reduced pressure. The residue obtained was purified by column chromatography on silica gel using eluent hexane: ethyl acetate (9:1) to obtain the product as a white solid. Yields: **P2a** 66%, **P2b** 88%, **P2c** 34%.

N-Benzyl, *N'*-(2-acethoxy-2-phenyl)ethyl-imidazolium bromide (**P2a**)

¹H-NMR (ppm, CD₃CN, 400 MHz) δ: 9.31 (1H, s, NCHN), 7.50-7.32 (12H overlapping signals, Ar-H and NCH=CHN), 6.06 (1H, m, NCH₂CH), 5.45 (2H, s, NCH₂Ph), 4.59 (2H, m, NCH₂CH), 2.02 (3H, s, COCH₃).

¹³C-NMR (ppm, CD₃CN, 62.5 MHz) δ: 170.43 (C=O), 137.61 (NCHN), 137.00, 135.04 (*ipso carbon of aromatic rings*), 130.03 (x2), 129.95, 129.67 (x3), 129.44, 129.25, 127.05 (x2), 124.53, 123.00 (*Ph-groups* and NCHCHN), 73.91 (NCH₂CH), 54.19 (NCH₂Ph), 53.40 (NCH₂CH), 21.11 (COCH₃).

MALDI-MS (CH₃CN) calcd/found (m/z): [C₂₀H₂₁N₂O₂]⁺ 321.1597/321.1615.

N-Benzyl, *N'*-(2-acethoxy-2-phenyl)ethyl-4,5-dichloroimidazolium bromide (**P2b**)

¹H-NMR (ppm, CDCl₃, 600 MHz) δ: 11.27 (1H, s, NCHN), 7.46-7.35 (10H, overlapping, Ar-H), 6.12 (1H, m, NCH₂CH), 5.63 (1H, d, *J*=18.0 Hz, NCHHPh), 5.59 (1H, d, *J*=18.0 Hz, NCHHPh), 4.92

(1H, dd, NCHHCH, $J_{gem}=5.84$ Hz, $J_{vicinal}=4.70$ Hz), 4.76 (1H, dd, NCHHCH, $J_{gem}=5.84$ Hz, $J_{vicinal}=14.70$ Hz), 2.08 (3H, s, COCH₃).
¹³C-NMR (ppm, CDCl₃, 150 MHz): δ 170.68 (C=O), 139.18 (NCHN), 135.00 and 132.35 (ipso carbon of aromatic rings), 130.51, 130.36, 130.28, 130.02, 129.76 (x2), 129.51 (x2) and 127.25 (x2) (Ph-groups), 120.31 and 119.82 (NCCICCN), 73.12 (NCH₂CH), 53.40 (NCH₂CH), 53.18 (NCH₂Ph), 21.76 (COCH₃).
 MALDI-MS (CH₃CN) calcd/found (m/z): [C₂₀H₁₉Cl₂N₂O₂]⁺ 389.0818/389.0818.

N-Benzyl, *N'*-(2-acethoxy-2-phenyl)ethyl-4,5-benzoimidazolium bromide (**P2c**)

¹H-NMR (ppm, DMSO-*d*₆, 300 MHz) δ: 9.85 (1H, s, NCHN), 8.12 (1H, d, $J_{ortho}=7.81$ Hz, Ar-H), 7.96 (1H, d, $J_{ortho}=7.81$ Hz, Ar-H), 7.66 (2H, m, Ar-H), 7.43-7.38 (10H, overlapping signals, Ar-H), 6.21 (1H, m, NCH₂CH), 5.79 (2H, br s, NCH₂Ph), 5.02 (2H, br s, NCH₂CH), 1.95 (3H, s, COCH₃).

¹³C-NMR (ppm, DMSO-*d*₆, 75 MHz) δ: 169.28 (C=O), 142.99 (NCHN), 136.15, 133.86, 131.64, 130.52, 129.06 (x2), 128.82 (x2), 128.73 (x2), 128.19 (x2), 126.79 (x2), 126.43 (x2), 114.24, 113.81 (Ph-groups), 72.82 (NCH₂CH), 50.55 (NCH₂CH), 49.88 (NCH₂Ph), 20.64 (COCH₃).

MALDI-MS (CH₃CN) calcd/found (m/z): [C₂₄H₂₃N₂O₂]⁺ 371.1754/371.1765.

Synthesis of Ru1a-c and Ru2a-c complexes

Ruthenium complexes **Ru1a-c** and **Ru2a-c** were synthesized by *trans*-metalation from silver complexes, following the procedure reported in our previous study.^[56] The imidazolium salt, **P1a-c** or **P2a-c** (1.00 eq), and Ag₂O (0.50 eq) were stirred in dichloromethane (0.02 M) in darkness at room temperature overnight. Dichloro(*p*-cymene)ruthenium(II) dimer (0.5 eq) was added, and the mixture was stirred for 12h at room temperature. After filtration the solvent was removed by reduced vacuum and the brownish-orange powder obtained was washed with hexane (3x10mL). Yields: **Ru1a** 76%, **Ru1b** 51%, **Ru1c** 94%, **Ru2a** 27%, **Ru2b** 88%, **Ru2c** 91%.

Ru1a

¹H-NMR (ppm, CDCl₃, 600 MHz) δ: 7.48-7.04 (7H, overlapping signals, Ar-H and NCH=CHN) 6.13 (1H, br m, NCH₂CH), 5.34 (2H, br m, *p*-cymene Ar-H), 5.04 (2H, m, NCH₂CH), 4.80-4.50 (2H, br, *p*-cymene Ar-H), 3.96 (3H, s, Ar-CH₃), 2.86 (1H, m, CH(CH₃)₂), 2.05 (3H, s, NCH₃), 1.94 (3H, s, COCH₃), 1.21-1.19 (6H, overlapping, CH(CH₃)₂).

¹³C-NMR (ppm, CDCl₃, 150 MHz) δ: 175.30 (C=O), 169.55 (NCN), 137.75 (ipso-carbon of aromatic ring) 128.89, 128.79 (x2), 128.49, 126.75 (x2), 126.47, 124.09, 123.06 (Ph groups and NCH=CHN), 109.28, 98.82, 85.79, 81.93 (aromatic carbon *p*-cymene), 73.54 (NCH₂CH), 55.31 (NCH₂CH), 39.79 (NCH₃), 30.74 (*p*-cymene, CHC₂H₆), 22.30 (COCH₃), 21.24 (*p*-cymene, CHC₂H₆), 18.62 (*p*-cymene, CCH₃).

ESI-MS (CH₂Cl₂) calcd/found (m/z): [C₂₄H₃₀ClN₂O₂Ru]⁺ 515.1037/515.1054

Ru1b

¹H-NMR (ppm, THF-*d*₈, 300 MHz) δ: 7.57 (2H, d, Ar-H), 7.40-7.30 (3H, overlapping signals, Ar-H), 6.40 (1H, m, NCH₂CH), 5.58 (1H, br s, *p*-cymene Ar-H), 5.50 (2H, s, NCH₂CH), 5.28 (3H, broad, *p*-cymene Ar-H), 4.06 (3H, s, Ar-CH₃), 3.01 (1H, m, CH(CH₃)₂), 2.01 (3H, s, NCH₃), 1.99 (3H, s, COCH₃), 1.30-1.26 (6H, overlapping, CH(CH₃)₂).

¹³C-NMR (ppm, THF-*d*₈, 75 MHz) δ: 179.06 (C=O), 168.07 (NCN), 137.04 (ipso-carbon of aromatic ring), 127.52, 127.26, 125.70, 117.24, 116.69 (Ph groups and NCCI=CCIN), 109.28, 98.82,

85.79, 81.93 (aromatic carbon *p*-cymene) 73.78 (NCH₂CH), 53.18 (NCH₂CH), 37.53 (NCH₃), 29.70 (*p*-cymene, CH(C₂H₆), 22.43 (COCH₃), 19.14 (*p*-cymene, CH(C₂H₆), 16.97 (*p*-cymene, CCH₃).
 ESI-MS (CH₂Cl₂) calcd/found (m/z): [C₂₄H₂₈Cl₃N₂O₂Ru]⁺ 583.0254/583.0728

Ru1c

¹H-NMR (ppm, THF-*d*₈, 300 MHz) δ: 7.52-7.29 (9H overlapping signals, Ar-H), 6.51 (1H, br s, NCH₂CH), 5.59-5.42 (3H, overlapping signals, Ar-H *p*-cymene), 5.18 (2H, br s, NCH₂CH), 4.75 (1H, br s, Ar-H *p*-cymene) 4.27 (3H, s, Ar-CH₃), 3.06 (1H, m, CH(CH₃)₂), 2.48 (3H, s, NCH₃), 2.44 (3H, s, COCH₃), 1.30 and 1.28 (6H, overlapping signals, CH(CH₃)₂).

¹³C-NMR (ppm, THF-*d*₈, 75 MHz) δ: 169.92 (C=O), 162.99 (NCN), 136.83 (ipso-carbon of aromatic ring), 129.87, 129.42, 129.37, 128.99, 128.63, 127.67, 127.28, 126.69, 123.26, 122.86 and 117.15 (aromatic carbons), 110.49, 98.90, 82.07, 79.30, 79.09, 78.87 (aromatic carbon *p*-cymene), 74.27 (NCH₂CH), 48.70 (NCH₂CH), 37.28 (NCH₃), 31.42 (*p*-cymene, CHC₂H₆), 20.09 (COCH₃), 18.46 (*p*-cymene, CHC₂H₆), 15.48 (*p*-cymene, CCH₃).
 ESI-MS (CH₂Cl₂) calcd/found (m/z): [C₂₈H₃₂Cl₂N₂O₂Ru]⁺ 565.1190/565.1210

Ru2a

¹H-NMR (ppm, CDCl₃, 600 MHz) δ: 7.53-7.26 (11H, o, Ph-groups), 6.85 (1H, bs, Ph-groups), 6.21(1H, bs, NCH₂CH), 5.85 (1H, o, Ph-group of *p*-cymene), 5.63 (1H, o, Ph-group of *p*-cymene), 5.37-5.30 (2H, o, NCH₂CH), 5.01 (2H, bs, NCH₂Ph), 4.50 (1H, m, Ph-group of *p*-cymene), 2.81 (1H, m, CH of *p*-cymene), 2.08 (3H, s, CH₃ of *p*-cymene), 1.97 (3H, s, COCH₃), 1.21 (6H, o, CH(CH₃)₂ of *p*-cymene).

¹³C-NMR (ppm, CDCl₃, 150 MHz) δ: 176.90 (C=O), 168.91 (NCN), 137.39 (ipso-carbon of aromatic ring), 137.39, 129.27, 128.95, 128.80, 128.11, 127.89, 126.98, 123.19, 122.99 (aromatic carbons), 108.42, 98.27 (aromatic carbon *p*-cymene), 85.91 (NCH₂CH), 85.41, 83.72, 82.25, 81.79 (aromatic carbon *p*-cymene), 55.49 (NCH₂Ph), 55.21 (NCH₂CH), 30.85 (*p*-cymene, CHC₂H₆), 23.10 (COCH₃), 22.17 and 21.32 (*p*-cymene, CHC₂H₆), 18.70 (*p*-cymene, CCH₃).

ESI-MS (CH₂Cl₂) calcd/found (m/z): [C₃₀H₃₄ClN₂O₂Ru]⁺ 591.1347/591.1414

Ru2b

¹H-NMR (ppm, THF-*d*₈, 600 MHz) δ: 7.62 (2H, d, Ph-group), 7.40 (2H, t, $J=8.84$ Hz, Ar-H), 7.34 (3H, t, $J=7.52$ Hz, Ar-H), 7.25 (1H, t, $J=7.61$ Hz, Ar-H), 7.11 (2H, d, $J=7.61$ Hz, Ar-H) 6.55 (1H, br s, 2H, Ar-H), 5.95 (1H, br s, NCH₂CH), 5.51 (3H, overlapping signals, NCH₂CH and *p*-cymene Ar-H), 5.19 (2H, br s NCH₂Ph), 4.96 (1H, br ss, *p*-cymene Ar-H), 2.77 (1H, m, CH(CH₃)₂), 2.08 (3H, s, Ar-CH₃), 1.87 (3H, s, COCH₃), 1.23-1.20 (6H, overlapping signals, CH(CH₃)₂).

¹³C-NMR (ppm, THF-*d*₈, 150 MHz) δ: 182.60 (C=O), 170.06 (NCN), 139.74 (ipso-carbon of aromatic ring), 129.24, 129.14, 129.08, 127.78, 127.56, 126.83, 126.78, 119.49, 119.35 (aromatic carbons and NCCICCN), 107.96, 98.08, 87.39, 87.18, 87.01, 84.43 (aromatic carbons *p*-cymene), 74.96 (NCH₂CH), 55.43 (NCH₂Ph), 54.81 (NCH₂CH), 31.39 (*p*-cymene, CHC₂H₆), 22.73 (COCH₃), 22.53 and 20.83 (*p*-cymene, CHC₂H₆), 18.29 (*p*-cymene, CCH₃).

ESI-MS (CH₂Cl₂) calcd/found (m/z): [C₃₀H₃₂Cl₃N₂O₂Ru]⁺ 695.03315/695.3949.

Ru2c

¹H-NMR (ppm, THF-*d*₈, 300 MHz) δ: 7.91 (1H, br s, Ar-H), 7.73 (2H, br s, Ar-H), 7.42-7.04 (11H, overlapping signals, Ar-H), 6.63

(1H, br s, NCH₂CH), 6.31 (1H, d, *J*=17.20 Hz, *p*-cymene Ar-H), 6.13 (1H, d, *J*=17.20 Hz, *p*-cymene Ar-H) 5.55-5.43 (2H, overlapping signals, NCH₂CH), 5.12 (2H, br s, NCH₂Ph), 4.86 (1H, m, *p*-cymene Ar-H), 2.89 (1H, m, CH(CH₃)₂), 1.73 (3H, s, Ar-CH₃), 1.70 (3H, s, COCH₃), 1.23 (6H, overlapping signals, CH(CH₃)₂).
¹³C-NMR (ppm, THF-*d*₈, 75 MHz): δ 194.73 (C=O), 176.83 (NCN), 139.18 (*ipso*-carbon of aromatic ring), 137.47, 136.39, 129.13, 127.96, 127.71, 123.22, 122.94, (aromatic carbons), 112.34, 109.47, 98.93, 89.53, 88.64, 83.77 (aromatic carbons *p*-cymene), 54.55 (NCH₂Ph), 54.43 (NCH₂CH), 31.63 (*p*-cymene, CHC₂H₆), 23.27 (COCH₃), 22.22 and 20.73 (*p*-cymene, CHC₂H₆), 18.54 (*p*-cymene, CCH₃).
 ESI-MS (CH₂Cl₂) calcd/found (m/z): [C₃₄H₃₆ClN₂O₂Ru]⁺ 641.1509/641.1535.

General Polymerization Procedure

In a general ROMP experiment, 3.0 μmol of catalyst were introduced in 0.15 mL of dry THF, and the solution was stirred for 15 minutes at 60 °C. A second solution was prepared introducing NBE (9.0 mmol) and EDA (12 μmol) into 5 mL of THF. The first solution containing the catalyst was then injected into the second solution. The polymerization was quenched using ethyl vinyl ether and the polymer was coagulated in methanol, filtered and dried under vacuum.

Determination of the logarithm of the partition coefficient (LogP)

The logarithm of the partition coefficient (LogP) was obtained by following the procedure reported in the literature with a slight modification.^[63,71] 100 mL of water (distilled after milli-Q purification) and 100 mL of 1-octanol were stirred together for 72h to allow saturation of both phases. Five standard solutions of complexes were prepared in the 1-octanol phase and other five in the water phase (20, 40, 60, 80 and 100 μM), and a calibration curve (absorbance vs concentration) was obtained using UV/vis spectroscopy. A further solution of both complexes was prepared in a 1-octanol phase (50 μM) and an equal volume of water was added. The biphasic solutions were mixed for 20 minutes and then centrifuged for 1h at 6500 rpm to allow separation. Concentration in each phase was determined by UV/VIS. LogP was calculated using the following formula $\log[\text{complex}]_{\text{oct}}/[\text{complex}]_{\text{H}_2\text{O}}$. **RuB**, logP 0.73; **Ru1b**, logP -0.11.

Cell Cultures

The two breast cancer cells used in this assay, the human estrogen receptor positive [(ER(+)) MCF-7 and the triple negative MDA-MB-231 breast cancer cells, were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured as already indicated.^[72] The cell cultures were maintained at the temperature of 37 °C in a humidified atmosphere containing 5% CO₂.

MTT Assay

MTT assays [Sigma Aldrich (St. Louis, MO, USA)] were employed to evaluate the *in vitro* anticancer activities of all the studied compounds, as already defined.^[73] The compounds were tested at different concentrations (0.1-1-10-25-50 μM) for 72 h. The IC₅₀ values were calculated from the percent (%) of control using GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The radical scavenging properties of the Ru-NHC complexes on the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical were evaluated as previously described.^[56]

All the compounds were used at different concentrations (0.1, 1, 10, 20 and 30 μM) and the DPPH radical scavenging activity,

measured at 515 nm using a microplate reader, was expressed as inhibition percentages (%I_{DPPH}) compared to the initial concentration of DPPH (control) according to the following expression:

$$I_{\text{DPPH}} (\%) = [(ABS_{\text{CTRL}} - ABS_{\text{sample}}) / ABS_{\text{CTRL}}] * 100$$

where ABS_{CTRL} and ABS_{sample} are the absorbance at 515 nm in the control and in the presence of samples, respectively. The obtained I_{DPPH} percentages allowed us to calculate the IC₅₀ values using Graph-Pad Prism 9 software (GraphPad Inc., San Diego, CA, USA). Trolox was employed as a positive control.

2,20-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Assay

The radical scavenging properties of the complexes on a 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS⁺) were evaluated as previously described.^[56]

All the compounds were used at different concentrations (0.001, 0.01, 0.1, 1, 10, 20 and 30 μM) and the ABTS radical scavenging activity, measured at 730 nm using a microplate reader, was expressed as inhibition percentages (%I_{ABTS}) compared to the control, according to the following expression:

$$I_{\text{ABTS}} (\%) = [(ABS_{\text{CTRL}} - ABS_{\text{sample}}) / ABS_{\text{CTRL}}] * 100$$

where ABS_{CTRL} and ABS_{sample} are the absorbance at 730 nm in the control and in the presence of samples, respectively.

IC₅₀ values were calculated from the %I_{ABTS} using GraphPad Prism 9 software (Graph-Pad Inc., San Diego, CA, USA). Trolox was employed as a positive control.

Acknowledgements

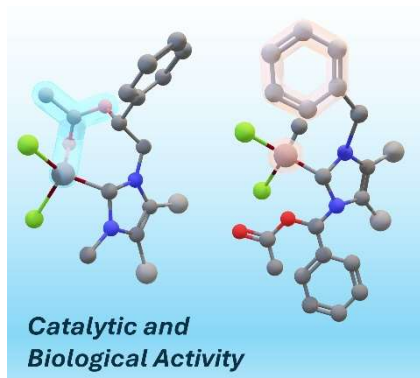
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- [1] D. Bourissou, O. Guerret, F. P. Gabbaï, G. Bertrand, *Chem. Rev.* **2000**, *100*, 39–92.
- [2] M. N. Hopkinson, C. Richter, M. Schedler, F. Glorius, *Nature* **2014**, *510*, 485–496.
- [3] K.-S. Feichtner, V. H. Gessner, *Chem. Commun.* **2018**, *54*, 6540–6553.
- [4] R. F. R. Jazzar, S. A. Macgregor, M. F. Mahon, S. P. Richards, M. K. Whittlesey, *J. Am. Chem. Soc.* **2002**, *124*, 4944–4945.
- [5] S. Gaillard, C. S. J. Cazin, S. P. Nolan, *Acc. Chem. Res.* **2012**, *45*, 778–787.
- [6] C. M. Crudden, D. P. Allen, *Coord. Chem. Rev.* **2004**, *248*, 2247–2273.
- [7] M. J. Benedikter, F. Ziegler, J. Groos, P. M. Hauser, R. Schowner, M. R. Buchmeiser, *Coord. Chem. Rev.* **2020**, *415*, 213315.
- [8] C. Samojłowicz, M. Bieniek, K. Grela, *Chem. Rev.* **2009**, *109*, 3708–3742.
- [9] G. C. Vougioukalakis, R. H. Grubbs, *Chem. – Eur. J.* **2008**, *14*, 7545–7556.
- [10] F. Prencipe, A. Zanfardino, M. Di Napoli, F. Rossi, S. D'Errico, G. Piccialli, G. F. Mangiatordi, M. Saviano, L. Ronga, M. Varcamonti, D. Tesaro, *Int. J. Mol. Sci.* **2021**, *22*, 2497.
- [11] Q. Zhao, B. Han, C. Peng, N. Zhang, W. Huang, G. He, J.-L. Li, *Med. Res. Rev.* **n.d.**, *n/a*, DOI 10.1002/med.22039.
- [12] I. Ott, in *Adv. Inorg. Chem.* (Eds.: P.J. Sadler, R. van Eldik), Academic Press, **2020**, pp. 121–148.
- [13] H. Clavier, K. Grela, A. Kirschning, M. Mauduit, S. P. Nolan, *Angew. Chem. Int. Ed.* **2007**, *46*, 6786–6801.
- [14] M. S. Sanford, J. A. Love, R. H. Grubbs, *J. Am. Chem. Soc.* **2001**, *123*, 6543–6554.

- [15] D. J. Nelson, S. Manzini, C. A. Urbina-Blanco, S. P. Nolan, *Chem. Commun.* **2014**, 50, 10355–10375.
- [16] S. P. Nolan, H. Clavier, *Chem. Soc. Rev.* **2010**, 39, 3305–3316.
- [17] M. Scholl, T. M. Trnka, J. P. Morgan, R. H. Grubbs, *Tetrahedron Lett.* **1999**, 40, 2247–2250.
- [18] J. Huang, E. D. Stevens, S. P. Nolan, J. L. Petersen, *J. Am. Chem. Soc.* **1999**, 121, 2674–2678.
- [19] N. Ledoux, B. Allaert, F. Verpoort, *Eur. J. Inorg. Chem.* **2007**, 2007, 5578–5583.
- [20] A. M. Maj, L. Delaude, A. Demonceau, A. F. Noels, *J. Organomet. Chem.* **2007**, 692, 3048–3056.
- [21] P. Csabai, F. Joó, *Organometallics* **2004**, 23, 5640–5643.
- [22] S. P. Shan, X. Xiaoke, B. Gnanaprakasam, T. T. Dang, B. Ramalingam, H. V. Huynh, A. M. Seayad, *RSC Adv.* **2014**, 5, 4434–4442.
- [23] B. S. Murray, M. V. Babak, C. G. Hartinger, P. J. Dyson, *Coord. Chem. Rev.* **2016**, 306, 86–114.
- [24] C. S. Allardyce, P. J. Dyson, D. J. Ellis, S. L. Heath, *Chem. Commun.* **2001**, 0, 1396–1397.
- [25] A. Weiss, R. H. Berndsen, M. Dubois, C. Müller, R. Schibli, A. W. Griffioen, P. J. Dyson, P. Nowak-Sliwinska, *Chem. Sci.* **2014**, 5, 4742–4748.
- [26] G. C. Vougiouklakis, R. H. Grubbs, *Chem. Rev.* **2010**, 110, 1746–1787.
- [27] K. M. Hindi, M. J. Panzner, C. A. Tessier, C. L. Cannon, W. J. Youngs, *Chem. Rev.* **2009**, 109, 3859–3884.
- [28] L. Delaude, A. Demonceau, *Dalton Trans.* **2012**, 41, 9257–9268.
- [29] Y. Kong, S. Xu, H. Song, B. Wang, *Organometallics* **2012**, 31, 5527–5532.
- [30] R. Troiano, C. Costabile, F. Grisi, *Catalysts* **2023**, 13, 34.
- [31] A. Peretto, C. Costabile, P. Longo, F. Grisi, *Organometallics* **2014**, 33, 2747–2759.
- [32] I. C. Stewart, T. Ung, A. A. Pletnev, J. M. Berlin, R. H. Grubbs, Y. Schrodi, *Org. Lett.* **2007**, 9, 1589–1592.
- [33] C. K. Chung, R. H. Grubbs, *Org. Lett.* **2008**, 10, 2693–2696.
- [34] V. Paradiso, C. Costabile, F. Grisi, *Molecules* **2016**, 21, 117.
- [35] J. DePasquale, M. Kumar, M. Zeller, E. T. Papish, *Organometallics* **2013**, 32, 966–979.
- [36] W. Chen, J. Egly, A. I. Poblador-Bahamonde, A. Maise-Francois, S. Bellemin-Lapponnaz, T. Achard, *Dalton Trans.* **2020**, 49, 3243–3252.
- [37] J. Egly, W. Chen, A. Maise-François, S. Bellemin-Lapponnaz, T. Achard, *Eur. J. Inorg. Chem.* **2022**, 2022, e202101033.
- [38] V. Mechrouk, A. Maise-François, S. Bellemin-Lapponnaz, T. Achard, *Eur. J. Inorg. Chem.* **2023**, 26, e202300188.
- [39] N. Kaloğlu, M. Achard, C. Bruneau, İ. Özdemir, *Eur. J. Inorg. Chem.* **2019**, 2019, 2598–2606.
- [40] M. Yiğit, B. Yiğit, İ. Özdemir, E. Çetinkaya, B. Çetinkaya, *Appl. Organomet. Chem.* **2006**, 20, 322–327.
- [41] D. Yang, Y. Tang, H. Song, B. Wang, *Organometallics* **2015**, 34, 2012–2017.
- [42] L. Delaude, M. Szypa, A. Demonceau, A. F. Noels, *Adv. Synth. Catal.* **2002**, 344, 749–756.
- [43] T. Opstal, K. Couchez, F. Verpoort, *Adv. Synth. Catal.* **2003**, 345, 393–401.
- [44] X. Sauvage, Y. Borguet, A. F. Noels, L. Delaude, A. Demonceau, *Adv. Synth. Catal.* **2007**, 349, 255–265.
- [45] C. Lo, R. Cariou, C. Fischmeister, P. H. Dixneuf, *Adv. Synth. Catal.* **2007**, 349, 546–550.
- [46] T. E. Zehnder, D. J. Nasrallah, J. L. Stanley, J. J. Kiernicki, N. K. Szymczak, C. S. Schindler, *Organometallics* **2023**, 42, 479–485.
- [47] D. S. Müller, Y. Raoul, J. Le Nôtre, O. Baslé, M. Mauduit, *ACS Catal.* **2019**, 9, 3511–3518.
- [48] D. P. Oliveira, T. R. Cruz, D. M. Martins, P. I. S. Maia, A. E. H. Machado, A. L. Bogado, B. E. Goi, B. S. Lima-Neto, V. P. Carvalho-Jr, *Catal. Today* **2021**, 381, 34–41.
- [49] A. D'Amato, A. Mariconda, D. Iacopetta, J. Ceramella, A. Catalano, M. S. Sinicropi, P. Longo, *Pharmaceuticals* **2023**, 16, 1729.
- [50] L. Oehninger, H. Alborzina, S. Ludewig, K. Baumann, S. Wölfl, I. Ott, *ChemMedChem* **2011**, 6, 2142–2145.
- [51] W. Liu, R. Gust, *Chem. Soc. Rev.* **2013**, 42, 755–773.
- [52] A. Casini, C. Gabbiani, F. Sorrentino, M. P. Rigobello, A. Bindoli, T. J. Geldbach, A. Marrone, N. Re, C. G. Hartinger, P. J. Dyson, L. Messori, *J. Med. Chem.* **2008**, 51, 6773–6781.
- [53] R. E. Morris, R. E. Aird, P. del Socorro Murdoch, H. Chen, J. Cummings, N. D. Hughes, S. Parsons, A. Parkin, G. Boyd, D. I. Jodrell, P. J. Sadler, *J. Med. Chem.* **2001**, 44, 3616–3621.
- [54] P. Nowak-Sliwinska, J. R. van Beijnum, A. Casini, A. A. Nazarov, G. Wagnières, H. van den Bergh, P. J. Dyson, A. W. Griffioen, *J. Med. Chem.* **2011**, 54, 3895–3902.
- [55] L. Oehninger, M. Stefanopoulou, H. Alborzina, J. Schur, S. Ludewig, K. Namikawa, A. Muñoz-Castro, R. W. Köster, K. Baumann, S. Wölfl, W. S. Sheldrick, I. Ott, *Dalton Trans.* **2013**, 42, 1657–1666.
- [56] J. Ceramella, R. Troiano, D. Iacopetta, A. Mariconda, M. Pellegrino, A. Catalano, C. Saturnino, S. Aquaro, M. S. Sinicropi, P. Longo, *Antibiotics* **2023**, 12, 693.
- [57] A. Mariconda, M. Sirignano, C. Costabile, P. Longo, *Mol. Catal.* **2020**, 480, 110570.
- [58] M. Sirignano, A. D'Amato, C. Costabile, A. Mariconda, A. Crispini, F. Scarpelli, P. Longo, *Front. Chem.* **2023**, 11.
- [59] M. Picq, A. F. Prigent, G. Nemoz, A. C. Andre, H. Pacheco, *J. Med. Chem.* **1982**, 25, 1192–1198.
- [60] G. Lv, L. Guo, L. Qiu, H. Yang, T. Wang, H. Liu, J. Lin, *Dalton Trans.* **2015**, 44, 7324–7331.
- [61] W.-Q. Wang, Y. Yuan, Y. Miao, B.-Y. Yu, H.-J. Wang, Z.-Q. Wang, W. Sang, C. Chen, F. Verpoort, *Appl. Organomet. Chem.* **2020**, 34, e5323.
- [62] H. Cheng, M.-Q. Xiong, C.-X. Cheng, H.-J. Wang, Q. Lu, H.-F. Liu, F.-B. Yao, C. Chen, F. Verpoort, *Chem. – Asian J.* **2018**, 13, 440–448.
- [63] J. Ceramella, A. Mariconda, M. Sirignano, D. Iacopetta, C. Rosano, A. Catalano, C. Saturnino, M. S. Sinicropi, P. Longo, *Pharmaceuticals* **2022**, 15, 507.
- [64] D. Cirri, S. Pillozzi, C. Gabbiani, J. Tricomi, G. Bartoli, M. Stefanini, E. Michelucci, A. Arcangeli, L. Messori, T. Marzo, *Dalton Trans.* **2017**, 46, 3311–3317.
- [65] A. Demonceau, A. F. Noels, E. Saive, A. J. Hubert, *J. Mol. Catal.* **1992**, 76, 123–132.
- [66] L. Delaude, A. Demonceau, A. F. Noels, *Curr. Org. Chem.* **2006**, 10, 203–215.
- [67] J. Baran, I. Bogdanska, D. Jan, L. Delaude, A. Demonceau, A. F. Noels, *J. Mol. Catal. Chem.* **2002**, 190, 109–116.
- [68] F. Quintin, J. Pinaud, F. Lamaty, X. Bantreil, *Organometallics* **2020**, 39, 636–639.
- [69] J. Chen, X. Chen, C. Zhu, J. Zhu, *J. Mol. Catal. Chem.* **2014**, 394, 198–204.
- [70] M. Picq, A. F. Prigent, G. Nemoz, A. C. Andre, H. Pacheco, *J. Med. Chem.* **1982**, 25, 1192–1198.
- [71] D. Cirri, S. Pillozzi, C. Gabbiani, J. Tricomi, G. Bartoli, M. Stefanini, E. Michelucci, A. Arcangeli, L. Messori, T. Marzo, *Dalton Trans.* **2017**, 46, 3311–3317.
- [72] J. Ceramella, A. Mariconda, C. Rosano, D. Iacopetta, A. Caruso, P. Longo, M. S. Sinicropi, C. Saturnino, *ChemMedChem* **2020**, 15, 2306–2316.
- [73] D. Iacopetta, C. Rosano, M. Sirignano, A. Mariconda, J. Ceramella, M. Ponassi, C. Saturnino, M. S. Sinicropi, P. Longo, *Pharmaceuticals* **2020**, 13, 91.

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Ruthenium-arene-N-heterocyclic carbene (RANHC) complexes piqued researchers' interest since they exhibit broad catalytic and biological activities. In this work, NHC characteristics were studied in order to conduct a thorough examination into the impact of ancillary ligand lipophilicity on overall catalytic performance in benchmark norbornene ROMP, as well as the bioactivity of the resultant complexes as anticancer and antioxidants.