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# Metal-based complexes with antiplatelet properties. Antagonists of the Platelet-Activating Factor receptor (PAFR) and other aggregating agents

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Metal complexes displaying anti-inflammatory and antithrombotic properties is a promising research area. Development of new and effective anti-inflammatory and antithrombotic agents is necessary to prevent inflammatory assisted diseases, thromboembolc diseases and oxidation. In this Frontiers article we report on coordination and organometallic compounds displaying anti-inflammatory and/or antithrombotic potencies particularly through the inhibition of platelet aggregation. Non-classic targets such as the Platelet-Activating Factor (PAF) and its receptor (PAFR), a phospholipid signaling molecule of the immune system and the most potent lipid mediator of inflammation, along with collagen serve as the target moleules, besides thrombin and ADP (adenosine diphosphate). This article updates over the last 15 years in this area focusing on the great potential of the transition metal complexes as possible therapeutic agents to treat inflammatory assisted diseases along with thromboembolic diseases and oxidation. Metal-based inhibitors of inflammatory mediators could potentially constitute an interesting class of compounds as alternative to the organic analogues currently in use. The results of this study show that this class of compounds merits further research towards the preparation of new metal-based complexes with improved pharmacological profiles.

## Introduction

The serendipitous discovery of the inhibition of cell division in E. coli by *cis*-diamminedichloridoplatinum(II), *cis*-$Pt(NH_3)_2Cl_2$, also known as cisplatin, initiated intense research efforts towards the development and applications of inorganic complexes as therapeutic agents against numerous cancer malignancies.[1,2] This was the first example of a coordination compound, i.e. a Pt(II) metal ion surrounded by simple non-carbon molecules and ions ($NH_3$ and Cl) acting as ligands, which was successfully used against the compact of cancer disease. To overcome side-effects acquired with the inherent acute toxicity of cisplatin and platinum-containing analogues, a series of new metal-based drugs with other metal ions have been developed.[3] To this end, it must be noted that cancer remains responsible for the increased number of deaths worldwide.[4] Soon after, the terms metal-based drugs and medicinal inorganic chemistry were developed to highlight the high impact of coordination compounds and more specifically of the transition metal complexes, against a variety of diseases.[5]

Besides the extended research in the field, with the synthesis and evaluation of a plethora of coordination compounds, interest remains constant towards the rational design of new and more potent anticancer drugs.[6]

However, relatively less attention has been focused on the investigation of the effects of metal ions and coordination compounds in other bioactivities such as cardiovascular diseases and thrombosis, which are the leading causes of death worldwide.[7] Targeting platelets, thrombin and other aggregating agents is a realistic means to prevent acute thromboembolic artery occlusions in cardiovascular diseases and treat chronic inflammatory diseases.[8,9]

Thus far the great majority of compounds with anti-inflammatory and antithrombotic activities is of organic origin (organic small molecules). In this respect, several advantages of metal complexes over organic analogues have been well documented, highlighting their perspective for the development of new therapeutic agents.[10] Transition metal complexes display different coordination geometries ranging from square planar, tetrahedral, square pyramidal and octahedral, in contrast to the typical tetrahedral, or trigonal planar geometry of the organic congeners. Additional characteristics include their possibilities to tune thermodynamic and kinetic ligand substitution, to obtain different oxidation states and undergo redox reactions etc.[11-13] Finally it must be stated that several metal ions play a vital role for life, are involved in many natural biological processes[14-16] and in some diseases.[17]

This Frontiers article aims to report selected examples of coordination and organometallic compounds that have been developed as potent antiplatelet agents particularly via inhibition of the platelet aggregation.

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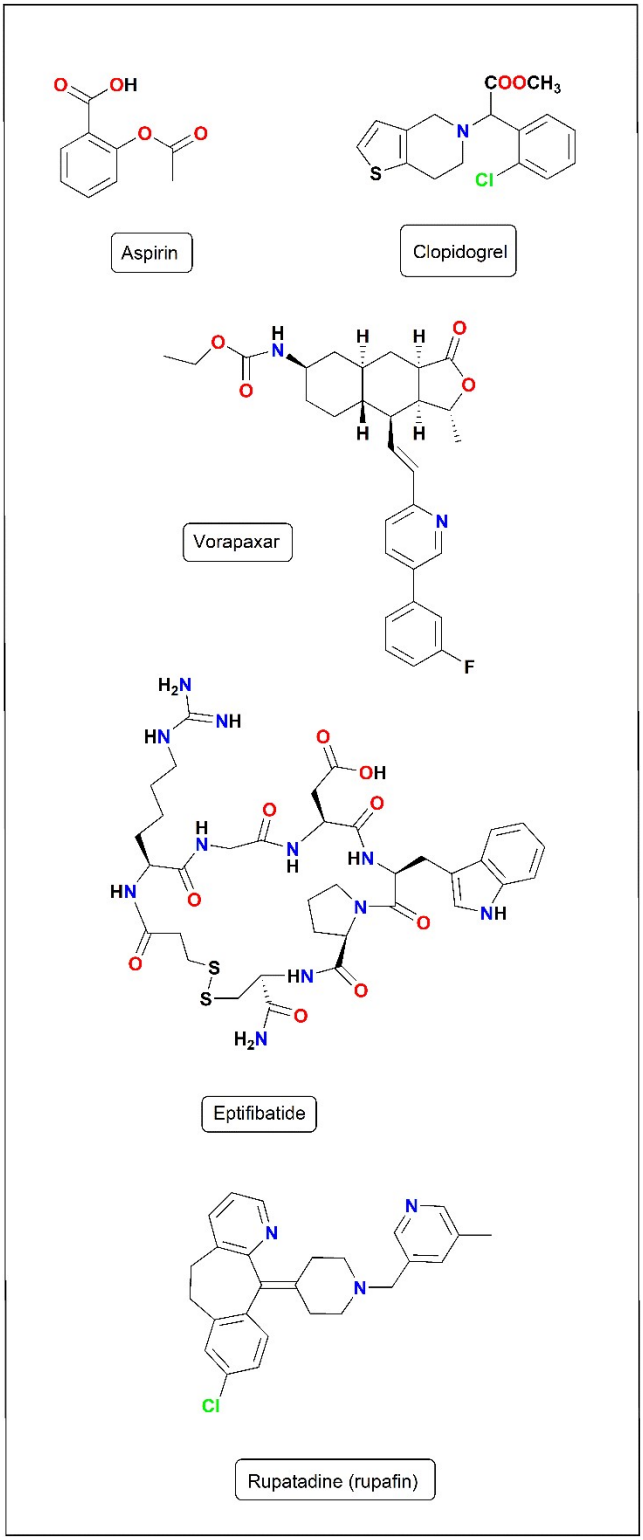


**Figure 1**. Antiplatelet drugs in clinical practice.

We will focus our study on metal-based complexes, highlighting their great potential for possible use in antiplatelet therapy, including the therapy of inflammatory assisted diseases, thromboembolic diseases and oxidation. Finally, restrictions on the application of these metal-based compounds will be also covered, along with their prospects for the future and the need for additional study to improve their efficacy and safety.

## Current antiplatelet therapies

Platelets are anucleate small circulating blood cells that play a vital role in haemostatic processes since they prevent excessive blood loss, upon vascular damage, through blood clothing.[8,18] Inappropriate activation of platelets leads to thrombosis, reducing the blood supply to the heart and brain, leading in heart attacks and strokes.[19] Pharmacologic platelet aggregation inhibitors constitute a class of diverse agents which act in a different way towards the platelet adhesion-activation-aggregation progression. In general, the primary mechanisms of actions include interruption of platelet intracellular signalling pathways and/or blockade of ligand receptors on the platelet membrane.[9,20]

Currently, there are several antiplatelet drugs in clinical practice (Fig. 1), which can be divided in five main categories: (**i**) cyclooxygenase 1 (COX 1) inhibitors, with aspirin as the typical example (**ii**) inhibitors of the adenosine diphosphate (ADP) $P2Y_{12}$ receptor (clopidogrel, cangelor, prasugrel), (**iii**) PAR1 (proteinase-activated receptor) antagonists of the thrombin-related pathways (vorapaxar) (**iv**) glycoprotein GPIIb/IIIa receptor inhibitors (abciximab, eptifibatide) of the collagen-related pathways, and (**v**) inhibitors of the receptor of Platelet-Activating Factor (PAFR), with rupatadine (rupafin) the only existing drug (Fig. 2).[21,22]
However, these agents, which are the cornerstone of therapy for acute coronary syndromes, exert undesirable side effects such as bleeding risk. This is also associated with adverse cardiovascular outcomes and mortality. As a result, several new antiplatelet drugs, focusing either new drug targets and/or not associated with bleeding, have been developed.[18]

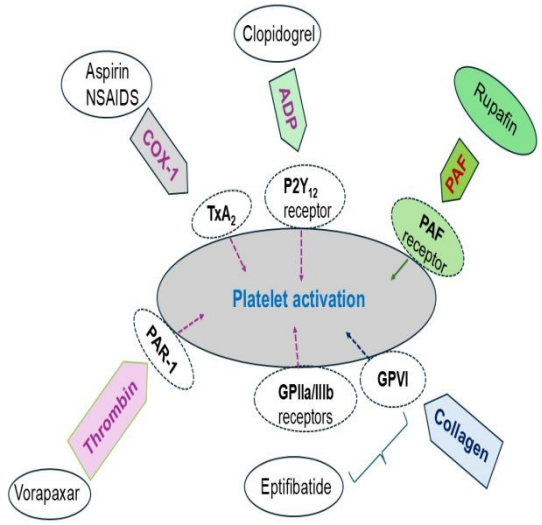


**Figure 2**. Targets of antiplatelet therapies.

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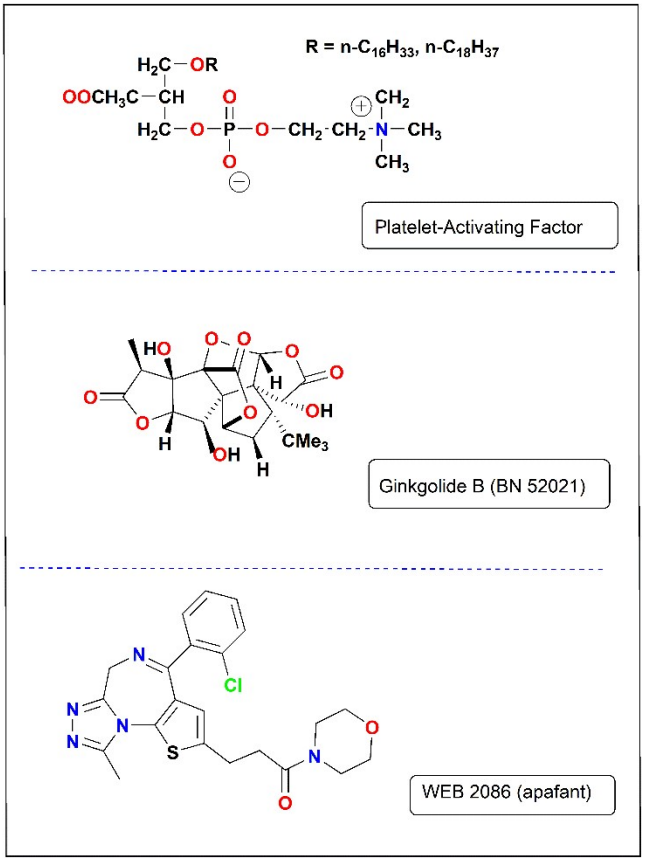


**Figure 3**. Structures of PAF and organic PAFR antagonists.

## Antiplatelet metal-based complexes

### Metal-based complexes as inhibitors of the PAF-induced aggregation

To explore the action of metal ions and their coordination compounds towards molecules of biological interest (biological probe), our recent research focused on the Platelet-Activating Factor (PAF) which is the most potent lipid mediator of inflammation and is also involved in thrombosis and oxidation. The systematic name of PAF is 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphochloline and its structure is depicted in Fig. 3.[23] This phospholipid, that was reported almost 45 years ago, is one member of a family of structurally related phospholipid signaling molecules and exerts its biological activity through binding to its receptor (PAFR), a G-protein-coupled receptor with a seven-transmembrane topology that has been a therapeutic target for many years.[24] A variety of natural and synthetic organic compounds have demonstrated an inhibitory effect on the PAF/PAF receptor acting either through direct antagonistic/competitive effects by binding to the PAFR or through indirect mechanisms.[25] In our approach, metal-based complexes have been examined, besides the classic organic molecules that act as inhibitors of PAF action. Evaluation of the antiplatelet activity expressed by these compounds may be exerted with the internationally tested method which is the inhibition of the PAF induced aggregation in washed rabbit (WRPs) and in human platelets (hWPs), via their interaction with PAFR and subsequent blocking of the PAFR activation.[26,27] Accordingly, since PAF is the most potent lipid mediator of inflammation, the anti-PAF activity exerted by a new substance can be potentially considered as of proof of its anti-inflammatory activity. This is of interest, considering that organic compounds represent well-known antagonists in the area while metal-based inhibitors have been totally ignored (Fig. 3).

Early attempts towards this goal trace back to 2009, time that we have started a systematic approach for the development of a library of coordination and organometallic compounds with anti-inflammatory along with antithrombotic activities.[28,29]

Since then, the idea of preparing metal complexes as scaffolds for the design of PAF inhibitors became more realistic and this

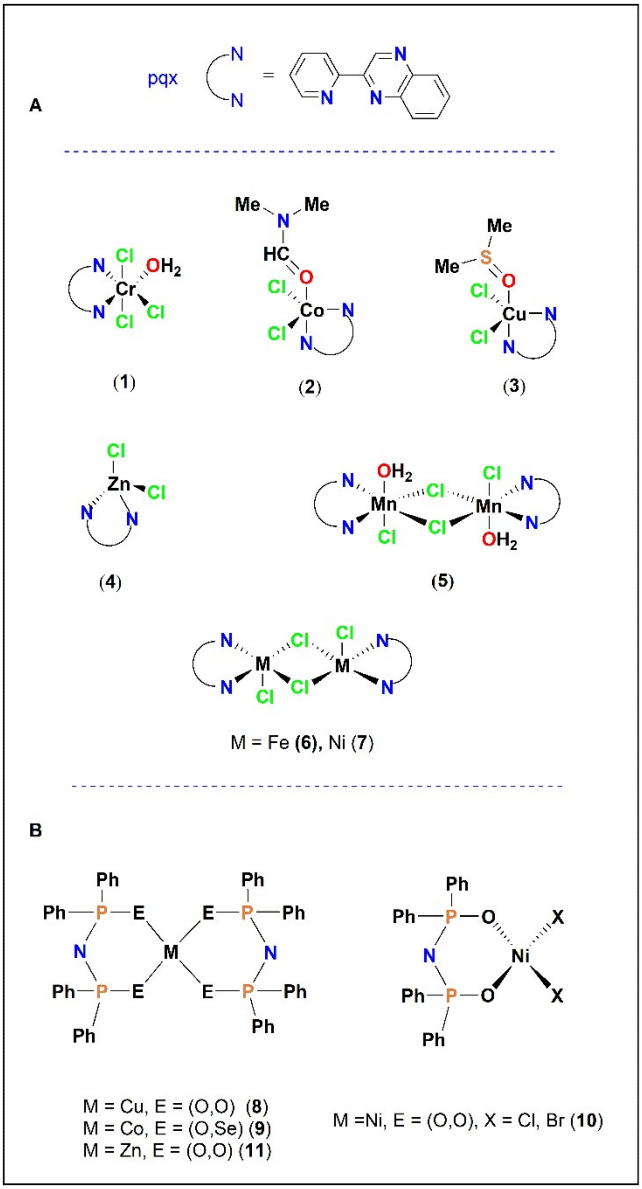


**Figure 4**. A: Structures of pqx containing metal-based PAF-inhibitors; B: structures of PNP-based PAF-inhibitors.

approach can be considered as a therapeutic opportunity using PAF-receptors pharmacological antagonists.[30] Very recently, following this strategy the mononuclear Cr(III)-pqx ($IC_{50}$ = 4.5 μM) , Co(II)-pqx ($IC_{50}$ = 4.1 μM), Cu(II)-pqx ($IC_{50}$ = 10.6 μM), Zn(II)-pqx ($IC_{50}$ = 3.3 μM) (**1-4**) and dinuclear complexes Mn(II)-pqx ($IC_{50}$ = 39 μM), Fe(II)-pqx ($IC_{50}$ = 1.79 μM) and Ni(II)-pqx ($IC_{50}$ = 6.83 μM) (**5-7**), containing the 2-(2′-pyridyl)quinoxaline ligand (pqx, $IC_{50}$ = 32 μM), were synthesized and biologically tested as inhibitors of the PAF and thrombin-induced aggregation in WRPs (Fig. 4A).[31] The coordination geometries in these complexes range from tetrahedral to trigonal pyramidal and octahedral. The Fe(II) complex **6** was the most active against PAF-induced aggregation while the bulkiest, manganese complex **5**, identified as the less active. Considering that washed rabbit platelets exhibit physiological responses to the understudied compounds—free from interference by other blood components—on PAF and other aggregating agent receptors during platelet aggregation, this model is widely regarded as one of the most internationally accepted experimental approaches for replicating findings in human platelets.[32] The antithrombotic activities of these metal complexes have been examined, revealing that the Fe-pqx complex (**6**), is the most potent inhibitor, exerting similar affinities for both the PAFR and PAR receptors.[31] Cytotoxicity studies (*in vitro*) have been performed for all relevant complexes in HEK 293T (human embryonic kidney cells) and HeLa cells (cervi-cal cancer cells) via the MTT assay. In the HEK 293T cell line, the Cu-pqx analogue (**3**) was quite toxic with potencies higher than that of cisplatin, which served as a reference compound (Table 1). Previously, a series of Cu(II)$^{(O-O)}$-PNP ($IC_{50}$ = ~1.0 μM, **8**), Co(II)$^{(O-Se)}$-PNP ($IC_{50}$ = 0.018 ± 0.005 μM) (**9**), Ni(II)$^{(O-O)}$- PNP (X = $Cl^-$, $IC_{50}$ ~ 16 μM, **10a**; X = $Br^-$, $IC_{50}$ ~ 30 μM, **10a** ) and Zn(II)$^{(O-O)}$-PNP ($IC_{50}$ = 0.54 μM) (**11**) complexes, bearing chalcogenated imidodiphosphinato ligands (PNP), have been reported (Fig. 4B).[33,34] The anti-PAF activities of the PNP-based series were in the sub-micromolar region, showing that the different ligand sphere around the metal centre has a dramatic effect on the biological activity expressed. However, these complexes could be cautiously considered as PAFR antagonists, since there are no indications of binding to the PAF receptor. In addition, cytotoxicity measurements have not been recorded, which is a drawback for further investigations. Moreover, both square planar complexes **10a**, **10b** proved unstable in DMSO, rendering them unsuitable for inhibitory action under the experimental conditions reported. As a result, the thrombin-induced WRP's aggregation by these complexes was not investigated. In this respect, docking theoretical calculations could be very helpful (vide infra in the case of Rh(I)/Rh(III) complexes).

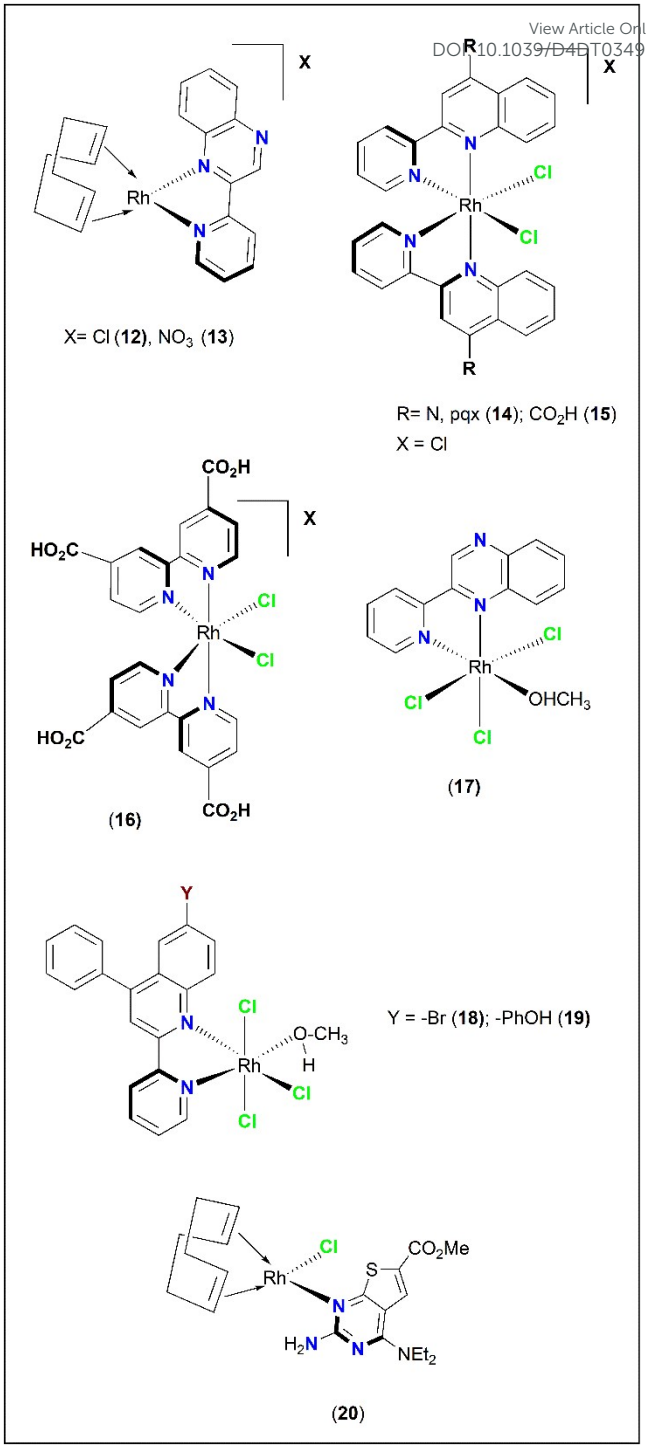


**Figure 5**. Rhodium-based PAF inhibitors.

### Metal-based inhibitors of PAF and thrombin including second and third row metal ions (heavier analogues)

Several rhodium complexes have been synthesized and subsequently evaluated as PAFR and t hrombin antagonists (anti-inflammatory and antithrombotic action) (Fig. 5). Notably, the organometallic square planar Rh(I) complexes [Rh(cod)(pqx)]X (cod = *cis*-1,5-cyclooctadiene), X = $Cl^-$ ($IC_{50}$ = 0.016 ± 0.015 μM, **12**), $NO_3^-$ ($IC_{50}$ = 0.015 ± 0.015 μM) **13**) displayed very strong antiplatelet activity in the nanomolar scale against PAF. Theoretical docking calculations suggested that these molecules can be accommodated within the ligand-binding site of PAFR (Fig. 6). The bulkier octahedral complexes *cis*-[Rh(L)$_2$Cl$_2$]Cl (L = pqx, ($IC_{50}$ = 0.51 ± 0.23 μM, **14**); cpq = 4-carboxy-2-(2′-pyridyl)quinoline, ($IC_{50}$ = 0.35 ± 0.20 μM μM, **15**); dcbpyH$_2$ =2,2′-bipyridine-4,4′-dicarboxylic acid ($IC_{50}$ = 0.12 ± 0.11 μM, **16**)) were less potent and could not fit within the PAFR binding site.

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**Table 1**. Inhibition of PAF- and thrombin-induced aggregation by complexes **1**-**20** including *in vitro* cytotoxicity data

| Complexes | $IC_{50}$ (PAF in WRPs, μM) Target PAFR | In vitro cytotoxicity (HEK293T) | $IC_{50}$ (thrombin in WRPs, μM) Targer PAR |
|---|---|---|---|
| Cr-pqx (**1**) | 4.5 ±0 .9* | 99.05 ± 6.16 | 54.6 ± 10.4 |
| Co-pqx (**2**) | 4.1 ± 0.6* | 67.03 ± 12.58 | 8.9 ± 1.3 |
| Cu(-pqx (3) | 10.6 ± 1.4 | 2.10 ± 0.52 | 3.1 ± 0.4 |
| Zn(-pqx (**4**) | 3.3 ± 0.3** | 94.92 ± 23.16 | 3.5 ± 0.3 |
| Mn-pqx (**5**) | 39 ± 6 | 28.83 ± 6.16 | 14.0 ± 2.1 |
| Fe-pqx (**6**) | 1.79±0.12** | 65.81 ± 13.17 | 0.46 ± 0.03 |
| Ni-pqx (**7**) | 6.83 ± 0.42 | 25.53 ± 8.61 | 5.60 ± 0.34 |
| pqx | 32±15 | 576 | 54.6 ± 10.4 |
| cisplatin | 0.55 ± 0.22 | 4.44 ± 1.29 | 56 ± 16 |
| Cu(O-O)-PNP (**8**) | ~1.0 | – | |
| Co(O-Se)-PNP (**9**) | 0.018±0.005 | – | 7.86 ± 4.77 |
| Ni(O-O)-PNP, X = Cl- (**10a**) | ~16 | – | - |
| Ni(O-O)- PNP, X = Br- (**10b**) | ~ 30 | – | - |
| Zn(O-O)-PNP (**11**) | 0.54 | – | 12.80 ± 5.62 |
| [Rh(cod)(pqx)]Cl (**12**) | 0.016±0.015 | – | 4.5 ± 2.3 |
| [Rh(cod)(pqx)]($NO_3$) (**13**) | 0.015±0.015 | – | 37.9 ± 6.8 |
| cis-[Rh(pqx)$_2$Cl$_2$]Cl (**14**) | 0.12±0.11 | 54% 77% [b] viability | 37.0 ± 8.4 |
| cis-[Rh(cpq)$_2$Cl$_2$]Cl (**15**) | 0.51±0.23 | – | 0.195 ± 0.098 |
| cis-[Rh( dcbpyH$_2$)$_2$Cl$_2$]Cl (**16**) | 0.35 ± 0.20 | – | 0.34 ± 0.11 |
| mer-[Rh(pqx)Cl$_3$(MeOH)] (**17**) | 2.6 ± 2.0 | – | 35 ± 12 |
| mer-[Rh(Br-Qpy)Cl$_3$(MeOH)] (**18**) | 1.0 ± 0.6 | – | - |
| mer-[Rh(OH-Ph-Qpy)Cl$_3$(MeOH)] (**19**) | 3.9 ± 0.2 | – | - |
| [Rh(cod)Cl(tpc)] (**20**) | 1.91 ± 0.50 | ~30% viability | - |
| [Rh(cod)Cl(tpc)] (**20**) | 122.6 ± 5.6 [a] | | 195 ± 8<br>353 ± 64 [c]<br>83.7 ± 11.3 [d] |

Symbols * and ** stand for statistically significant differences with $p < 0.05$ and $p < 0.005$, respectively, when these metal complexes are compared with all the other ones. [a] towards PAF in hPRPs, [b] MCF-7 cancer cell line, [c] ADP-aggregation in hPRPs, [d] collagen-induced aggregation in hPRP.

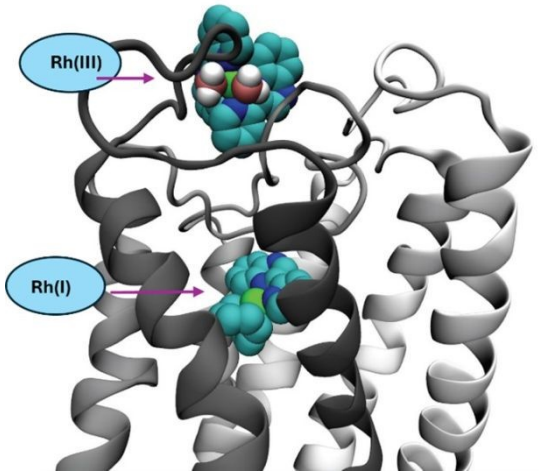


**Figure 6**. Cartoon representation of the molecular model of PAFR with two predicted binding sites of square planar Rh(I) complexes **12**-**13** and the octahedral Rh(III) complex **14**.

It has been suggested that they could bind to the extracellular domain of the receptor and therefore, antagonize the substrate's entrance to PAFR.[26] In case of **14**, the previously reported findings from theoretical calculations corroborate with the specific binding results of 3H-labelled Platelet-Activating factor ([$^3$H]-PAF) to WRPs and its inhibition exerted by this rhodium(III) complex. In this case, its inhibitory effect has been attributed only in part to the inhibition of PAF binding within the PAF receptor. It has been proposed that at low concentrations, **14** affects PAFR more effectively and probably inhibits PAF action in another pathway. Notably complexes **12**-**14** demonstrate the strongest inhibitory effect on the PAF-induced aggregation of WRPs, affecting the thrombin-related pathway of WRPs aggregation less. This implies, that these complexes may be considered as selective inhibitors of the PAF-related signalling pathways than that of thrombin. Interestingly the most potent PAFR inhibitors **12**-**13** exhibited moderate cytotoxicity against HEK 293 cell lines, which is in accord with the increased antiplatelet (anti-inflammatory) activity observed (Table 1). Cytotoxicity measurements of **15-19** are currently underway and the results will be described elsewhere. The mononuclear methanol adducts *mer*-[Br-Qpy)Cl$_3$(MeOH)] ($IC_{50}$ = 1.0 ± 0.6 μM, **18**) and *mer*-[Rh(OH-Ph-Qpy)Cl$_3$(MeOH)] ($IC_{50}$ = 3.9 ± 0.2 μM, **19**) (Br-Qpy = 6-bromo-4-phenyl-2-pyridin-2-ylquinoline ($IC_{50}$ = 3.4 ± 0.2 μM) and OH-Ph-Qpy = 4-(4-phenyl-2-(pyridin-2-yl)quinolin-6-yl)phenol ($IC_{50}$ = 4.4 ± 0.2 μM) have also been evaluated and potentially added to the library of metal-based complexes displaying antiplatelet effects in the micromolar range.[26,35] Remarkably, these metal-based inhibitors

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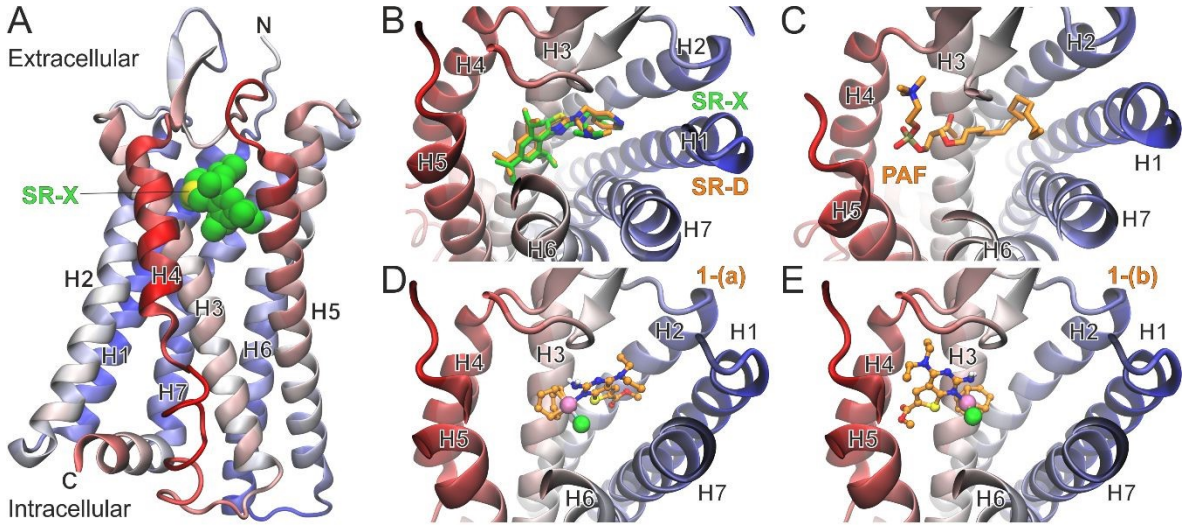


**Figure**. **7** (**a**) Ribbon representation of PAF-receptor (PAFR) in complex with the antagonist SR27417 from the X-ray structure with PDB ID: 5ZKP. The antagonist bound within the PAF-binding site is shown with green C spheres, while the seven helices and the N- and C-termini of the receptor are indicated. (**b**) Close-up view of the PAF-binding site from the extracellular (top) side of PAFR illustrating the docked pose of SR27417 (SR-D) in comparison with the crystallographic structure (SR-X). The antagonist is shown with green C sticks (X-ray) or orange C (docked), whereas N atoms are colored blue, O red, and S yellow. (**c**) Top-ranked conformation of PAF bound to PAFR, with the P atom colored brown. (**d**, **e**) The two most populated clusters of docked poses for complex **20** (**20**-(a) and **20**-(b)). Rhodium(I) center is shown as a light mauve sphere, the coordinated chloride as a green sphere, whereas cod and tpc ligands are color coded as SR-D and PAF. Selected, top-ranked conformations of the bound ligand tpc.

display comparable biological activity ($IC_{50}$ values) with those of known natural PAF antagonists from the series of Gingolides B (Fig. 3).[36] This research has also been extended to a series of substituted thieno-[2,3-*d*]-pyrimidines and their Rh(I) analogues, based on the structural similarity of thieno-[2,3-*d*]-pyrimidines with thienopyridines.[37] Generally, the latter constitute the main organic scaffold for inhibitors of the adenosine diphosphate (ADP) that bind irreversibly to the $P2Y_{12}$ receptor, while ticlopidine, clopidogrel, and prasugrel are the most representative prodrugs. In this respect, the neutral Rh(I) organometallic complex of the formula [Rh(cod)Cl(tpc)] ($IC_{50}$ = 1.91 μM, **20**) (cod = *cis*-1,5-cyclooctadiene; tpc = methyl 2-amino-4-(diethylamino)-thieno-[2,3-*d*]-pyrimidine-6-carboxylate, $IC_{50}$ = 8.12 μM) showed antiplatelet activity *in vitro*, both in human and in washed rabbit platelets (Fig. 5).[38] As expected in human platelets, and in conditions which are close to the *in vivo* ones, the inhibitory effect is dramatically reduced due to the presence of plasma proteins along with other biomolecules.[38,39] Based on molecular docking calculations performed, it has been proposed that the rhodium(I) complex **20** fit within the ligand-binding site of PAF receptor and block the activity of PAF (Fig. 7). Furthermore, the activity of this complex has been studied (in human platelet-rich plasmas), against other inflammatory and thrombotic agents such as thrombin ($IC_{50}$ = 195 ± 8 μM, along with platelet agonists like ADP ($IC_{50}$ = 353 ± 64 μM) and collagen ($IC_{50}$ = 83.7 ± 11.3 μM). This implies a higher affinity of **20** for the collagen-induced pathway. This is an interesting feature, rendering complex **20** as a very promising antiplatelet agent. Cytotoxicity studies by MTT assay for 48h of drug activity, in HEK 293 cells, reveal a dose dependent behavior, reaching ~30% viability at the concentration of 100 μM (~ 2 μM, for cis-platin).[38]

Previous studies have focused on several Ru(II) and Ru(III) heterocyclic complexes incorporating bidentate (N^N) and tridentate ligands (N^N^N) (**21**-**26**), which possess potent antiplatelet and anti-PAF activities (Fig. 8).[40,25] Interestingly, the anti-PAF activity of *cis*-[$Ru(dcbpyH_2)_2(pqx)$]$(NO_3)_2$ ($IC_{50}$ = 0.18 ± 0.01 μM, **23**) and *cis*-[$Ru(dcbpyH_2)_2(cpq)$]$(NO_3)_2$ ($IC_{50}$ = 0.24 ± 0.24 μM, **24**), which contain carboxylic acid groups in the ligand periphery, is comparable to that of rupatadine fumarate, a potent PAF receptor antagonist that is currently in clinical use with the brand name Rupafin (Fig. 1).[41] To this end, selected cytotoxicity studies in HEK-293 cells have been performed only for these two ruthenium(II) complexes which exert strong anti-PAF activities. Interestingly, the results demonstrated that the PAF inhibitors **23** and **24** are less cytotoxic compared to cisplatin, with $IC_{50}$ values of 60.2 ± 1.1 and 71.0 ± 2.8 μM respectively (Table 1). The anti-PAF potencies for all other ruthenium(II) complexes are in the micromolar range

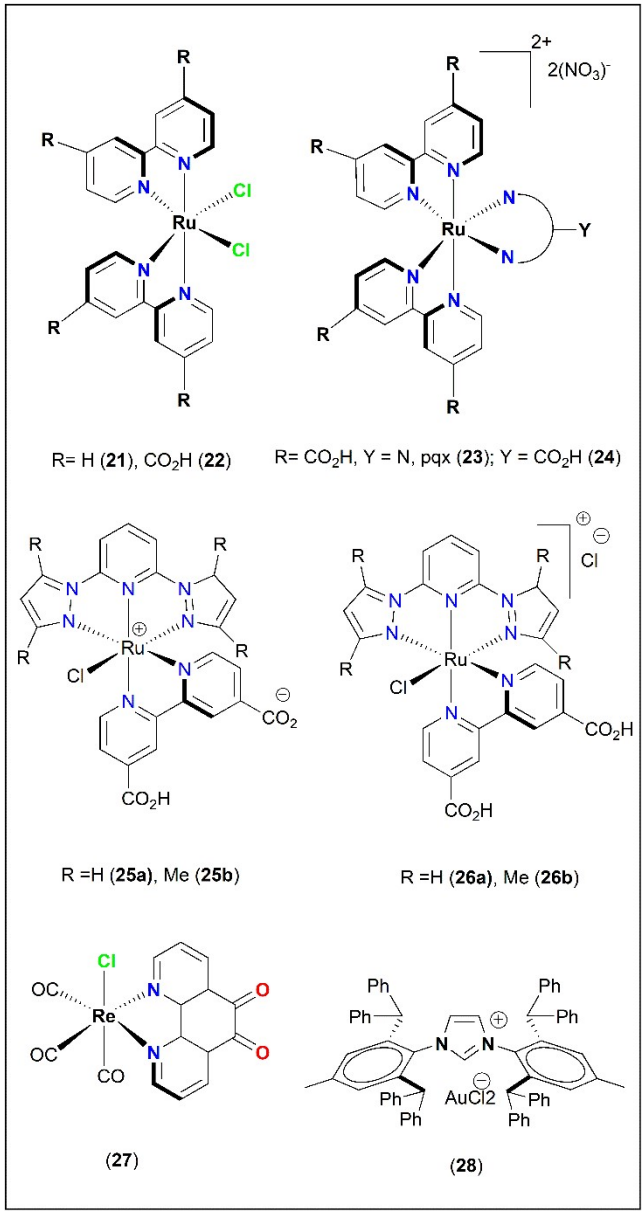


**Figure 8**. Selected Ru, Re and Au-based inhibitors of PAF

(**25a**, $IC_{50}$ = 3.1 ± 0.3 μM; **26a**, $IC_{50}$ = 11.8 ± 0.1 μM; **26b**, $IC_{50}$ = 6.4 ± 1.1 μM).

Within the series of these ruthenium complexes, the ionic ones were found to be more potent, in comparison to the neutral analogues. These were the first examples of Ru(II)/Ru(III) complexes with anti-PAF activities reported.[40] This sounds interesting, taking into consideration that in the field of medicinal chemistry, ruthenium complexes constitute an important class of compounds with a variety of potential medicinal and pharmaceutical applications such as antidiabetic, anti-HIV, anti-Alzheimer's and anti-cancer agents.[42,43] As next, the *fac*-[Re(phendione)$(CO)_3$Cl] complex (**27**) (phendione = 1,10-phenanthroline-5,6-dione), has been evaluated as another example of a heavier 3d metal complex (Fig. 8). The metal precursor [Re$(CO)_5$Cl] showed an increased inhibitory effect ($IC_{50}$ = 0.17 ± 0.09 μM) compared to phendione ( $IC_{50}$ = 0.92 ± 0.13 μM) and the Re(I) complex ($IC_{50}$ = 0.86 ± 0.15 μM) respectively.[44] This contrasts with the synergistic effect, which is a general trend that emerged upon thorough investigation of several metal-based PAF-inhibitors. This trend showcases the positive effect of ligand coordination on a metal center, that generally leads to an increase of the antiplatelet activity exerted.[25] In the absence of theoretical calculation studies or specific [$^3$H]-PAF labelled experiments, we cannot suggest if this Re(I) complex fits into the binding site of PAFR, as reported for other similar complexes. Complex **27** showed moderate cytotoxicity ~70% viability, against the breast cancer cell line (MCF-7).

Various aurate(I) salts consisting of the typical $[AuCl_2]^-$ anion and the $[NHC{\cdot}H]^+$ counter cation (NHC = N-heterocyclic carbene, $IC_{50}$ = 0.98 ± 0.15 μM, **28**) have been investigated against the PAF-induced aggregation in human platelets *in vitro* (Fig. 8).[45] It seems, that the anti-PAF activity observed, cannot be clearly attributed to the presence of the $[AuCl_2]^-$ counter anion, since all complexes studied display very similar anti-PAF activities and additionally the potency of the free ligand (NHC·HCl, $IC_{50}$ = 3.52 ± 1.39 μM) is within the same range.[46] It must be pointed out however, that further studies with thrombin or other well-established platelet agonists (i.e. ADP or collagen), to maintain the proposed antithrombotic activity, has not been performed. Nonetheless these aurates are promising PAF inhibitors and could be evaluated further, since this is the first time where the antiplatelet properties of N-heterocyclic carbene complexes, toward the PAF-induced aggregation in human platelets at concentrations comparable to those of similar metal-based PAF inhibitors, is reported. However, for further considerations cytotoxicity assays are required.

Despite the large pool of information collected from transition metal ions coordinated to different organic ligands, main group elements have been studied less. The amphiphilic oxygen tripodal Kläui ligands $[(\eta^5\text{-}C_5R_5)Co\{P(OEt)_2O\}_3]^-$, {R = H, ($NaL_{OEt}$, $IC_{50}$ = 0.88 ± 0.15 μM); Me ($NaL^*_{OEt}$, $IC_{50}$ = 0.95 ± 0.21 μM}, served as scaffolds for the coordination of Sn(II) and Sn(IV) ions. The $L_{OEt}SnCl$ ($IC_{50}$ = 10.3 ± 1.1 μM **29a**), $L^*_{OEt}SnCl$ ($IC_{50}$ = 0.5 ± 0.1 μM, **29b**) and $L_{OEt}SnPh_3$ ($IC_{50}$ = 0.5 ± 0.1 μM, **30a**), $L^*_{OEt}SnPh_3$ ($IC_{50}$ = 4.4 ± 0.6 μM, **30b**) complexes were found to be potent inhibitors of the PAF and thrombin-induced aggregation in WPRPs and in rabbit platelet rich plasma (rPRPs) aggregation assays in the micromolar range (Fig. 9).[47] For the case of thrombin, the six-coordinate organotin analogues **30a** and **30b** displayed a rather strong antithrombotic effect as expressed by the $IC_{50}$ values of 0.6 ± 0.1 μM and 0.23 ± 0.02 μM respectively. Especially for **30b** this further demonstrates a higher affinity for the PAR receptors of thrombin.
Within this series, complex **29b** was the most potent PAFR inhibitor, inducing platelet aggregation in WRPs in higher concentrations. Cross-desensitization tests performed reveal, that at high concentration levels, this complex could have a rather weak agonistic activity through the PAF/PAF-R related pathway of platelet aggregation but, at lower levels, its

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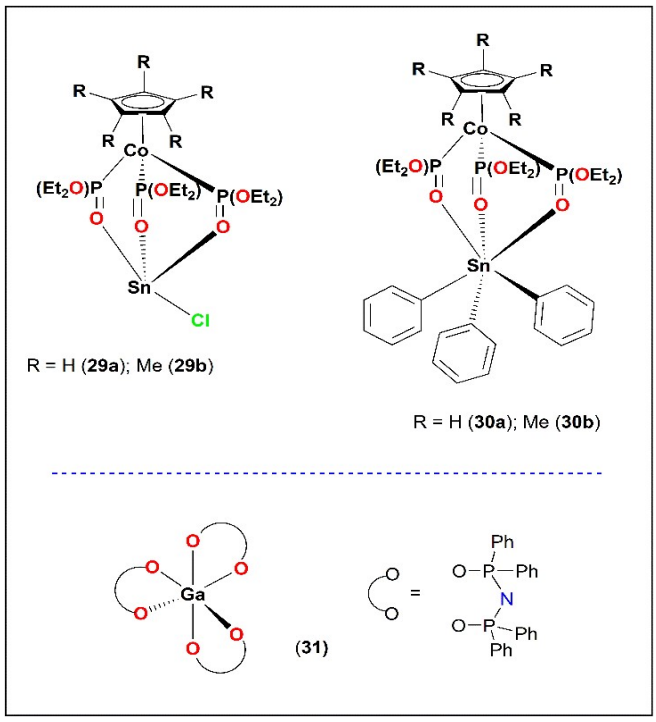


**Figure 9**. Sn(II), Sn(IV) and Ga(III) inhibitors of PAF.

inhibitory activities against PAF prevail. Cross-desensitization tests performed reveal that this complex could have the agonistic activity through the PAF-R related pathway of platelet aggregation. Tests conducted with varying concentrations of complex **29b** added to WRPs indicate that at high concentrations, the complex exhibits weak agonistic activity through the PAF/PAF- R-related pathway of platelet aggregation. However, at lower concentrations, its inhibitory effects against PAF predominate. Furthermore, these tests suggest that **29b** could also act as an agonist of the PAF/PAF-receptor pathway, in higher $EC_{50}$ (concentration effective in producing 50% of the maximum response) concentrations than its $IC_{50}$ value (inhibitory concentration that reduces the activity/binding of an inducer to its receptor and thus the associated pathway of platelet aggregation). Apparently, **29b** has the potential to substantially reduce the activation-aggregation of platelets induced by PAF. With the help of molecular docking calculations, it has been suggested that all organotin complexes that bear the sterically demanding Kläui oxygen tripodal ligand cannot fit inside the PAF-binding site of the receptor. However, they can interact with the extracellular domain of the receptor and block the entrance of PAF inside its receptor. The *in vitro* cytotoxic activities of the organotin complexes **29**-**30** were tested against the Jurkat T lymphoblastic tumour cell line. Within the series **29a** showed rather moderate activity (~50% viability) in the range of 1-20 μM (Table 2). The homoleptic Ga(III)$^{(O-O)}$-PNP ($IC_{50}$ = 0.062 μM ± 0.045 μM, **31**) consisting of three bidentate PNP ligands with O,O as the donor atoms, displayed an antiplatelet activity in the nanomolar range against the PAF-induced WRPs aggregation (Fig. 9).[34] It must be noted that this complex did not inhibit the thrombin-induced aggregation of WRPs, even at high doses, which is an indication that probably it antagonizes the platelet aggregation through the selective inhibition of the PAF-receptor pathway. Molecular modeling studies would be very helpful to assist this hypothesis.[26]

**Table 2**. Inhibition of PAF- and thrombin-induced aggregation by complexes **21**-**31** including in vitro cytotoxicity data

| Complex | $IC_{50}$ (PAF in WRPs, μM) Target- PAFR | In vitro cytotoxicity (HEK293T) | $IC_{50}$ (thrombin in WRPs, μM) Target-PAR |
|---|---|---|---|
| *cis*-[Ru(bpy)$_2$Cl$_2$] (**21**) | 7.0±0.7 | – | 56±6 |
| *cis*-[Ru(dcbpyH$_2$)$_2$Cl$_2$] (**22**) | 4.5±0.5 | – | 40±5 |
| *cis*-[Ru(dcbpyH$_2$)$_2$(pqx)](NO$_3$)$_2$ (**23**) | 0.18±0.01 | 60.2±1.1 (66.5±1.2) [a] | No inhibition |
| *cis*-[Ru(dcbpyH$_2$)$_2$(cpq)](NO$_3$)$_2$ (**24**) | 0.24±0.24 | 71.0±2.8 (81.0± 2.1) [a] | 6.1±0.7 |
| [Ru(bpp)(dcbpyH)Cl] (**25a**) | 3.1±0.3 | – | 25±3 |
| [Ru(bpp)(dcbpyH$_2$)Cl]Cl (**26a**) | 11.8±0.1 | – | No inhibition |
| [Ru(bdmpp)(dcbpyH$_2$)Cl](PF$_6$) (**26b**) | 6.4±1.1 | – | 2.1±0.3 |
| *fac*-[Re(phendione)(CO)$_3$Cl] (**27**) | 0.86 ± 0.15 | ~70% viability [a] | – |
| [Ph-NHC·H][AuCl$_2$] | 0.98±0.24 | – | – |
| Ph-NHC·HCl | 3.52±1.39 | – | – |
| L$_{OEt}$SnCl (**29a**) | 10.3± 1.1 | ~50% (range 1-20 μM) [b] | 10.3±1.3 |
| L*$_{OEt}$SnCl (**29b**) | 0.5±0.1 | Cytotoxic at > 10 μM [b] | 1.8±0.7 |
| L$_{OEt}$SnPh$_3$ (**30a**) | 0.5± 0.1 | Cytotoxic at > 5 μM [b] | 0.6±0.1 |
| L*$_{OEt}$SnPh$_3$ (**30b**) | 4.4± 0.6 | Cytotoxic at > 5 μM [b] | 0.23±0.02 |
| Ga(III)$^{(O-O)}$-PNP (**31**) | 0.062±0.045 | – | – |

[a] MCF-7 cancer cell line, [b] Jurkat T lymphoblastic tumour cell line.

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**Structure-Activity Relationship Studies**

Since the first report on this topic, the number of metal-based complexes with anti-PAF and/or antiplatelet activities against other aggregating agents that have been evaluated as PAF and/or thrombin-induced aggregation inhibitors has significantly increased. From the library of ~ 60 compounds created, and upon a thorough study, some preliminary structure-activity relationships have been established, based on simple coordination chemistry principles.[48,25]

Diverse parameters have been considered such as the coordination geometry of the metal complex (square planar *vs*. octahedral), the nature of the ligands (bidentate, tridentate), the effect of the counter anions and the total charge and size of the complex. As previously reported, the bulkier octahedral Rh(III) analogues (**14**-**19**) were less potent as opposed to the square-planar Rh(I) complexes (**12**-**13**) respectively.[25,26] This further demonstrates the effect of different coordination geometry on the biological effect exerted. Within the various Ru(II) and Ru(III) complexes which have been synthesized as possible PAF and thrombin inhibitors, it was determined an increased potency upon exchanging the chloride for a hexafluorophosphate counter anion. This behaviour could be attributed to the higher inhibitory effect towards PAF, of fluoride containing substances, such as the trifluoroacetyl analogues, compared to the trichloroacetyl ones.[25] Moreover, the activity of ionic complexes was higher than that of the neutral congeners.

Over the last years the synthesis of metal-based antagonists of PAF and thrombin and their viability as agents of pharmaceutical interest has been the subject of excellent reviews.[30,49] It must be noticed that in most cases where the antiplatelet activity was studied, control experiments, for metal and ligand precursors, were also performed. As a result, the term synergistic effect cannot be attributed to a cumulative effect of these precursor molecules, but to clear synergism of the metal ions and the coordinated organic molecules.[46] To support our findings and test our hypothesis, additional theoretical docking calculations would be advisable. Remarkable contribution to this goal may arise from the fact that the X-ray crystal structure of PAFR in complex with SR27417 antagonist has been recently elucidated.[50] Based on the outcome of the biological assays performed, the most potent PAFR inhibitors, from the small library of compounds which has been created and which probably fit within the binding site of PAFR, must be re-examined. Besides theoretical calculations, and *in vitro* assays, additional studies including *in vivo* biological experiments are required, along with a thorough investigation of the pharmacodynamics and

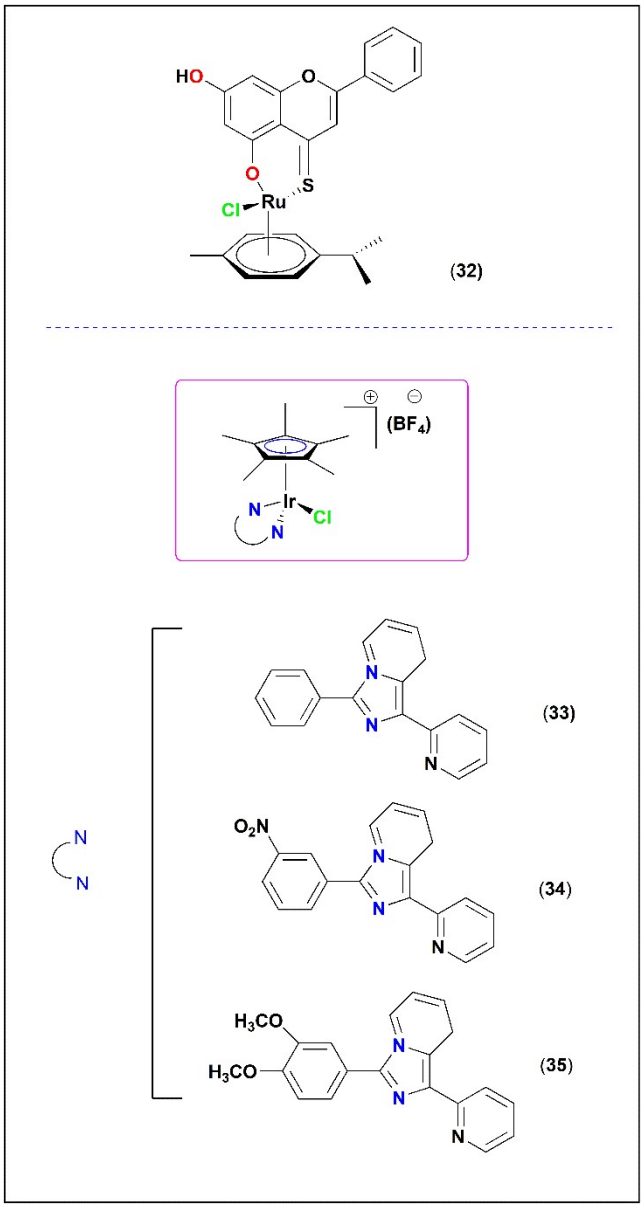


**Figure 10**. Ru(II) complexes as inhibitors of collagen-induced aggregation.

pharmacokinetics of the compounds examined thus far, before possible entering for some of them in the next step of clinical trials. This is an interesting issue considering that clinical trials against several disease pathologies with synthetic PAFR antagonists of organic origin were not very effective.[24,51]

**Metal-based complexes with anti-inflammatory and antithrombotic potencies following other pathways**

Though the field is still immature, interest in the field of metal-based anti-inflammatory, antithrombotic agents remain constant as shown by excellent contributions within recent years.[30,49] Selected examples by other research groups are included below.

Osborn and Vaiyapuri studied the use of a Ru-thio-chrysin complex (**32**), to modulate the platelet function, haemostasis and thrombosis using chrysin, a natural flavonoid. Under physiological conditions, the inhibitory effect of this complex in human platelet-rich plasma (hPRP), was enhanced in comparison to chrysin.[39] The results further showed that the active targets of thio-chrysin and its ruthenium analogue are the same, inhibiting Akt and FAK phosporylation, induced by collagen related peptide (CPR-XL), as a platelet agonist. In addition, no toxicity issues were mentioned for platelets, under the experimental conditions of this study (Fig. 10).

Chang at al. synthesized three Ir(III) complexes of the general formula [Ir(Cp*)(1-(2-pyridyl)-3-phenylimidazo[1,5-*a*]pyridine)Cl][$BF_4$] (Ir-imid, **33**), [Ir(Cp*)(1-(2-pyridyl)-3-(3-nitrophenyl)imidazo [1,5-*a*]pyridine) Cl][$BF_4$] (Ir-imid-$NO_2$, **34**) and [Ir(Cp*)(9-[4-(1-pyridin-2-yl-imidazo[1,5-*a*]pyridin-3-yl)-phenyl]-9H-carbazole) Cl][$BF_4$] (Ir-imid-$OCH_3$,, **35**) (Cp* = $C_5Me_5$), aiming to investigate the *in vitro* antiplatelet and *in vivo* antithrombotic activities (Fig. 11). Within the series, complex **33** was the only active, since it potentially inhibited adenosine triphosphate (ATP) release, calcium mobilization ([$Ca^{2+}$]i) and P-selectin expression induced by collagen-induced aggregation, without cytotoxicity.

Additionally, *in vivo* studies revealed that the iridium(III) complex **33** significantly prolonged the platelet plug formation and reduced the mortality of adenosine diphosphate (ADP)-induced acute pulmonary thromboembolism in mice. The sole activity of this iridium complex was attributed to the absence of substitution on the phenyl group, which may provide a higher rate of hydrolysis.[52]

Sheu and Chang have shown that **TQ-5** and **TQ-6**, two Ru(II)-based complexes of the formula [Ru(2-(pyridin-5-phenyl-2-yl)-quinoxaline)($\eta^6$-*p*-cymene)Cl][$BF_4$] (**36**) and [Ru(1H-benzoimidazo-2-yl)-quinoline)($\eta^6$-*p*-cymene)Cl][$BF_4$] (**37**), displayed an inhibitory effect against collagen-induced aggregation in hWPs, in a concentration-dependent way.[53,54] Their potencies are higher than that of aspirin, a well-established antithrombotic agent (Fig. 1). Moreover, both complexes inhibited thrombin induced aggregation but not in a concentration dependent manner.

It has been suggested that the new Ru(II)-*p*-cymene analogues inhibit platelet aggregation by suppressing [$Ca^{2+}$]i mobilization and ATP production with no cytotoxicity. These substances have the potential to be used as therapeutic agents for the treatment of thromboembolic disorders. Based on these interesting results, complex **37** was also investigated for its neuroprotective action against microglia activation and middle cerebral artery occlusion (MCAO)-induced embolic stroke. At the concentration of 2 μM, the administration of this ruthenium(II) complex severely influenced (diminished) the expression of inflammatory mediators (nitric oxide/inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2)), nuclear factor kappa B (NF-κB) p65 phosphorylation,

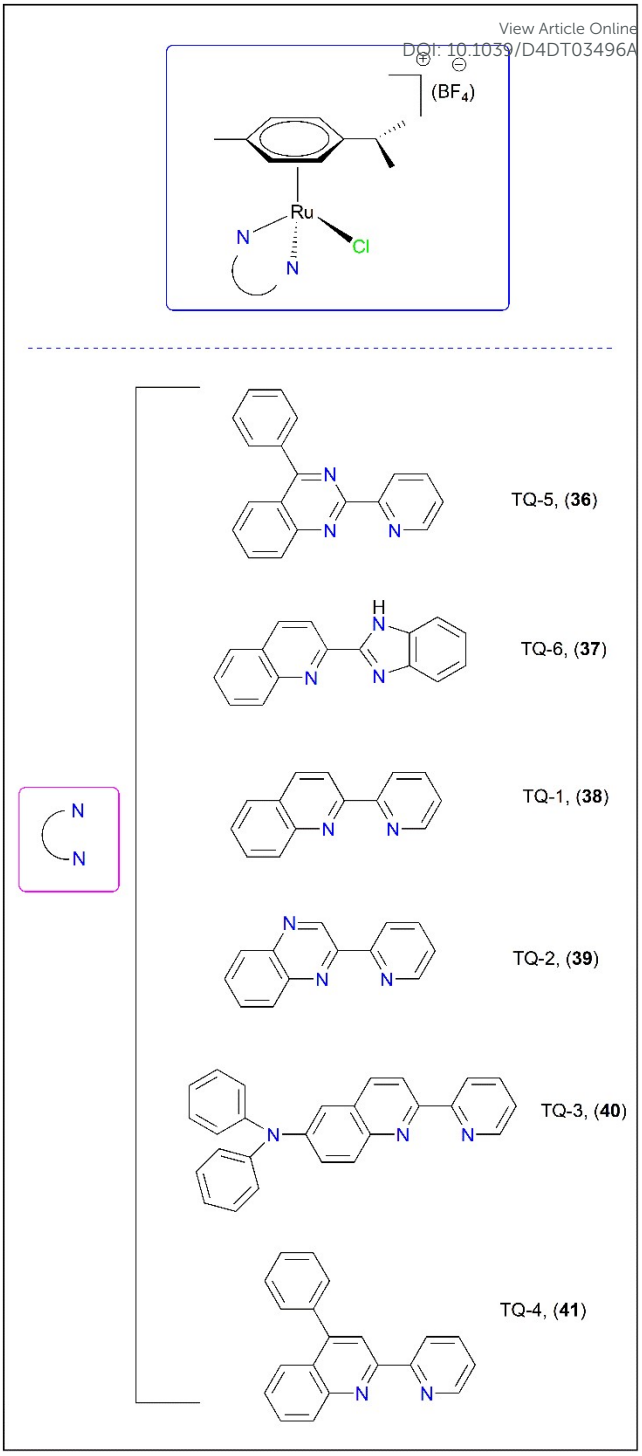


**Figure 11**. Ru(II) complexes of the TQ series with antiplatelet activities.

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**Table 3**. Inhibition of collagen- and thrombin-induced aggregation by complexes **32**-**40** including *in vitro* cytotoxicity data

| complex | In vitro $IC_{50}$ (collagen in washed hPRPs, μM) | target | In vitro $IC_{50}$ (thrombin in washed hPRPs, μM) | Cytotoxicity |
|---|---|---|---|---|
| Ru-thio-chrysin (**32**) | concentration-dependent inhibition [a] | Inhibition of Akt, FAK phosphorylation induced by CPR-XL. Inhibited ATP (dense granule secretion), $[Ca^{2+}]i$ | –[d] | No toxic effects lactate dehydrogenase (LDH) |
| Ir-imid (**33**) | 11.1 ± 3.7% (32.5 ± 2.6%) [b] | Inhibition of the Akt/PKC pathways, subsequently suppressed activation of MAPKs | –[d] | No toxic effects (LDH) |
| Ir-imid-$NO_2$ (**34**) | No effects up to 50 μM | No effects on these targets | – | No toxic effects (LDH cytotoxicity assays) |
| Ir-imid-$OCH_3$ (**35**) | No effects up to 50 μM | No effects on these targets | – | No toxic effects (LDH) |
| TQ-5 (**36**) | 1-5 | Suppression of Akt/JNK signalling cascades (phosphorylation), potentially inhibited collagen-induced (ATP) release, calcium mobilization ($[Ca^{2+}]i$) | >100 μM [Supressed thrombotic plug formation *in vivo*] | 3-10 μM (LDH) |
| TQ-6 (**37**) | ~0.3 ex vivo | Inhibition of collagen-induced (ATP) release, calcium mobilization ($[Ca^{2+}]I$), the agonist receptors-mediated inside-out signaling such as Src-Syk-PLC_2 cascade. Inhibition of the MAPKs signalling pathways [c] | ~60 | 20-100 μM (LDH activity) |
| TQ-1 (**38**) | No response even at 250 μM | Main possible targets Akt, MAPKs | up to 250 μM | up to 250 μM(LDH) |
| TQ-2 (**39**) | No response even at 250 μM | Main targets, SFK/Akt, $[Ca^{2+}]i$ mobilization and ATP production | up to 250 μM | up to 250 μM (LDH) |
| TQ-3 (**40**) | 1-5 μM | Suppression of Syk-Lyn-Fyn cascade and destruction of Akt, JNK and p38 MAPKs activation. Reduction of ATP level, surface P-selectin expression and calcium mobilization ($[Ca^{2+}]i$) | 0.63 | 5 μM |

[a] Collagen-related peptide (CPR-XL), [b] Plasma-rich platelets (PRP), [c] Not specific for GP VI receptor, [d] In vivo prolonged closure time

nuclear translocation, along with hydroxyl radical OH•) formation in LPS-stimulated microglia. Moreover, the results have shown an increase of the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1), which could be a critical therapeutic target for stroke treatment. [55]

Previously the same group presented the synthesis of three Ru(II) structurally related complexes namely [Ru(pyridin-2-yl)-quinoline)($\eta^6$-*p*-cymene)Cl][$BF_4$] (**TQ-1, 38**), [Ru(2-(pyridin-2-yl)-quinoxaline)($\eta^6$-*p*-cymene)Cl][$BF_4$] (**TQ-2, 39**), [Ru(diphenyl-2(pyridin-2-yl)-quinolin-6-yl)-amine)($\eta^6$-*p*-cymene)Cl][$BF_4$] (**TQ-3, 40**) (Fig. 9, 11). Interestingly, *in vitro* studies revealed that among them, only **40** proved a very potent inhibitor (Table 3) of the platelet aggregation induced by collagen and thrombin in washed human platelets.[56] The inhibitory effect of **40** was attributed to the inhibition of collagen-induced ATP release, calcium mobilization ($[Ca^{2+}]i$) and P-selectin expression, without cytotoxicity. A limitation of the studies reported, may be the fact that stability tests of the relevant complexes, in the appropriate media used for the biological studies, were not performed. This could be very helpful to gain further insight about the nature of the active species in solution. A recent review article has described in detail with more examples related to this topic.[57] To this end, relevant targets from these studies are included in Table 3.

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**Metal-based complexes with anti-inflammatory activities**

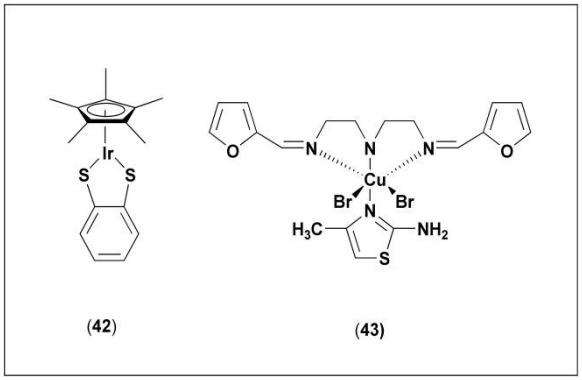
Relatively less attention has been given towards the development of metal complexes with anti-inflammatory activities, though there is great perspective owing to very promising recent studies.[58] Common strategies include coordination of bioactive ligands, mainly non-steroidal anti-inflammatory drugs (NSAIDs) affording metal complexes with improved anti-inflammatory properties. Most of them inhibit prostaglandin synthesis, by inhibition of the cyco-oxygenase (COX). As a representative example tetrakis-μ-acetylsalicylato-dicopper(II) was more potent than aspirin in rats or mice.[59]

Independently, platelets that are commonly associated with their involvement in thrombosis and haemostasis, also play significant roles in inflammation and immunity.[60] Considering that PAF is the most potent lipid mediator of inflammation, which is also involved in thrombosis and oxidation, the previously reported metal-based complexes with anti-PAF activities could be potentially examined in the treat of inflammatory assisted diseases, thromboembolic diseases and oxidation.[61]

Recent advances in the field provide encouraging results, which stem from the anti-inflammatory evaluation (*in vitro* and *in vivo* animal models) of novel synthetic ruthenium(II) complexes (namely TQ compounds) consisting of the *p*-cymene moiety and a series of substituted pyridine-quinoline based ligands.[62] It has been suggested that [Ru(4-phenyl-2-pyridin-2-yl)-quinoline)($\eta^6$-*p*-cymene)Cl][$BF_4$] (**TQ-4, 41**) (Fig. 11) presents very promising anti-inflammatory activity against mouse liver injury and RAW 264.7 macrophages by down regulating the inflammatory mediators JNK (Jun N-terminal kinases) phosphorylation and NF-kB (nuclear factor-κappa B) signalling pathways.[63]

In this respect, complex (**TQ-6, 37)** proved very potent against lipopolysaccharide (LPS)-induced *in vitro* inflammation in macrophage and *in vivo* liver injury in mice. Moreover, the results revealed that LPS-induced expression of tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) were reduced upon treatment of LPS-stimulated RAW 264.7 cells, with this complex. The authors finally suggested that NF-kB could be a promising target for protecting against LPS-induced inflammation and liver injury by the ruthenium(II) complex administrated. Accordingly, **37** has the potential to be used for treatment of inflammatory-related diseases.[64]

A series of ruthenium, osmium and iridium half-sandwich complexes incorporating 1,2-dicarba-*closo*-dodecarborane-1,2-dithiolato and benzene-1,2-dithiolatoligands, have been synthesized and their anti-inflammatory activity was evaluated




(42) (43)

**Figure 12**. Ir(III) and Cu(II) complexes with anti-inflammatory activities.

in RAW 264.7 murine macrophages and MRC-5 fibroblast cell lines, after nitric oxide production and inflammation response, induced by bacterial endotoxin lipopolysaccharide (LPS). Notably the iridium complex [Ir($\eta^5$-pentamethylcyclopentadiene)(benzene-1,2-dithiolato)] (Ir-dithiol, **42**) was found to be non-cytotoxic and triggered an anti-inflammatory response against LPS-induced NO production (Fig. 12). The authors suggested that this can be considered a new avenue for the development of non-cytotoxic anti-inflammatory drugs.[65]

Previous studies include a series of copper(II) Schiff base compounds with S,N-heterocyclic adducts, which have been assessed for their anti-inflammatory activity. In these studies, the rat carrageenan induced paw oedema assay was employed as a model for acute inflammation.[66] Among them the [Cu(dienOO)(2a-5mt)$Br_2$] complex (Cu-5mt**, 43**), where 2a-5mt stands for 2-amino-5-methyl-thiazole, was the most potent anti-inflammatory agent (Fig. 12). Analogous assays have been surveyed with different Schiff bases coordinated to diverse metal ions and their role as anti-inflammatory drugs have been examined.[67]

Limitations of the afore mentioned examples include dose-limiting side effects, resistance after several compounds use in treatment, and toxicity issues. Apparently, *in vivo* studies in different animal models may be considered, to elucidate possible mechanisms of action. Additional problems acquired with current treatment strategies include low bioavailability along with non-specific distribution against the biological targets studied.

In this respect, new challenges and perspectives of the inflammatory therapy have emerged very recently, where transition metal-based smart nanosystems (TMSNs) have been specifically engineered to block the mechanisms of initiating

inflammatory responses. These nanomaterials have been also proposed as nanocarriers to deliver anti-inflammatory drugs. Complex synthetic procedures of these nanoparticles, including controllable production and reproducibility issues constitute severe drawbacks which may limit further application. Moreover, long-term safety of TMSNs remains an obstacle for clinical use.[68]

## Conclusions and Perspectives

The results of this study have revealed the high potencies of metal-based complexes as anti-inflammatory and antithrombotic agents. Study of the effects of a variety of metal complexes on a biological target, such as the Platelet-Activating Factor and its receptor, has emerged as a versatile approach, towards this goal. The synthesis of a series of metal-based inhibitors of the PAF-induced platelet aggregation (PAFR antagonists) along with other aggregating agents (collagen, thrombin), showcases the high impact of coordination chemistry which remains a powerful tool for inorganic chemists to rational design molecules against a variety of diseases.

However, for most of the promising metal-based complexes reported, *in vivo* studies must be performed to support the aforementioned *in vitro* results. Supplementary, theoretical docking calculations (molecular modelling studies) for the most potent PAFR-inhibitors would be very informative, considering that the X-ray crystal structure of PAFR in complex with SR27417 antagonist has been reported recently. These calculations will provide the required information to predict the best properties for binding of the appropriate inhibitor and re-schedule, if necessary, our synthetic strategy towards the final aim.

Moreover, these activities would enable research in this topic to organize better and suggest more potent metal-based PAFR inhibitors in the future. Though the field is immature the recent examples reported herein, including the TQ ruthenium series and other similar complexes, clearly demonstrate the progress of therapeutic strategies for the treatment of inflammatory assisted diseases, thromboembolic diseases and oxidation. This is a cutting-edge research field with high public health impact due to the increased demands of our society for new compounds that act as potent therapeutic agents.

At this point it is worth mentioning another critical dimension related to this topic. Recent literature results concerning various diseases, have demonstrated a beneficial effect, i.e. increase of the pharmacological action, upon co-administration of PAFR inhibitors along with the required specific drugs to treat each disease. This comes from the fact that elevated levels of PAF can be measured in response to almost every type of pathology where inflammation and cell damage/death are involved.[51] Thus, for example, dual administration of PAFR inhibitors and specific anticancer agents towards certain tumor cells, leads to an improved pharmacologic profile. [69] This is an important issue considering that metal-based complexes constitute important drugs to treat inflammatory related diseases, such as cancer.

Finally further research towards the preparation of new metal-based complexes with improved pharmacological profiles is required aiming to explore them in pre-clinical or clinical trials.

## Author contributions

AP: Conceptualization; Investigation; Writing – review & editing; CAD: Investigation; Writing; Conceptualization.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

No additional data are available.

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# Metal-based complexes with antiplatelet properties. Antagonists of the Platelet-Activating Factor receptor (PAFR) and other aggregating agents

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## Data availability

No additional data are available.