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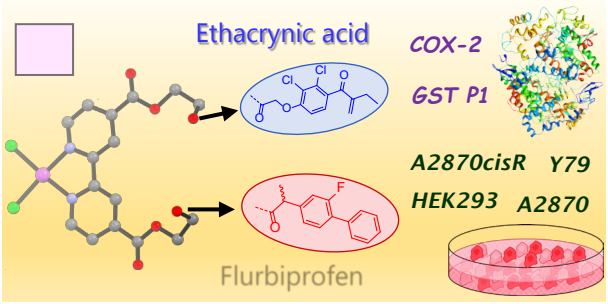


Derivatization of a bipyridine ligand allows pairs of bioactive fragments to be tethered to Pt(II) complexes, the ethacrynic acid/flurbiprofen combination leads to potent cytotoxicity and cancer cell selectivity.

# Bis-Conjugation of Bioactive Molecules to Cisplatin-Like Complexes via (2,2'-Bipyridine)-4,4'-Dicarboxylic Acid with Optimal Cytotoxicity Profile Provided by Ethacrynic Acid/Flurbiprofen Combination

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**Abstract**

A facile route to Pt(II) complexes doubly functionalized with bioactive moleculesviaabipyridine-type ligandis described.Initially,ligands $\mathbf{L}^{EE}$ (containing two ethacrynic acid units), $\mathbf{L}^{EF}$ (ethacrynic acid + flurbiprofen) and $\mathbf{L}^{EB}$ (ethacrynic acid + biotin) were obtained in moderate to good yields from(2,2'-bipyridine)-4,4'-dicarboxylic acid. Subsequent reaction of the ligands with $PtCl_2(DMSO)_2$ afforded complexes [$PtCl_2(\mathbf{L}^{EE})$] (**2**), [$PtCl_2(\mathbf{L}^{EF})$] (**3**) and [$PtCl_2(\mathbf{L}^{EB})$] (**4**) in high yields. All the compounds were fully characterized by analytical and spectroscopic methods. Complexes **2-4**are highly stable in water/DMSO solution at 37 °C after 72 hours, whereas a progressive release of the bioactive fragments was detected under the same conditions in a cell culture medium. The compounds were assessed for their *in vitro* antiproliferative activity towards tumorigenic A2780, A2780cisR and Y79 cells and non-tumorigenic HEK293 cells. In particular, the combination of ethacrynic acid and flurbiprofen in **3** overcomes cisplatin-based resistance and provides strong cancer cell selectivity. Enzyme inhibition assays on human GST P1 and human COX-2 and cross experimentsusing complex **1**, analogous to **2-4** but lacking bio-groups, reveal a clear synergy between the Pt(II) frame and the bioactive organic components.

**Keywords**: bioinorganic chemistry; metal-based drugs; anticancer platinum complexes;enzyme inhibition;multitargeted activity.

## Introduction

Cisplatin, oxaliplatin and carboplatin are extensively employed in chemotherapy to treat different kinds of tumours, usually in combination with other drugs (Figure 1).[1] Despite their potency and broad applicability, these platinum compounds manifest severe side effects and both intrinsic and acquired resistance remain problematic.[2]

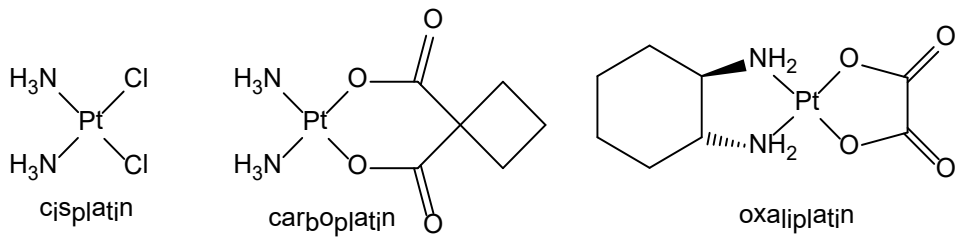
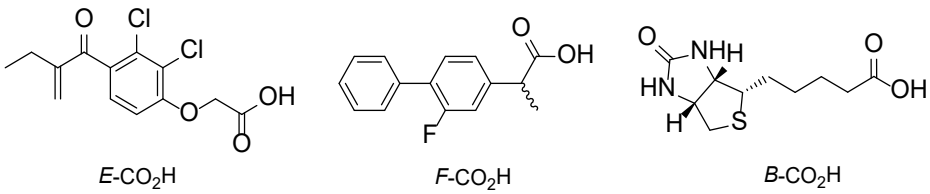



**Figure 1**. Structures of platinum-based drugs used worldwide in the treatment of cancer.

Extensive efforts have been made to overcome these issues,[3] and in particular one widely investigated approach is to tether a fragment with a targeted mechanism of action to the metal structure.[4] Ideally, the incorporation of bioactive molecules within an appropriate metal unit should reasonably provide synergic effects from the different components.[5] Indeed, the conjugation of a single biologically active fragment to Pt(II) complexes can result in a clearlyfavourable effect on the anticancer activity,[4,6]and the introduction of two different bioactive ligands is potentially a more promising strategy (multi-targeted compounds).[7]The design of Pt(IV) compounds, in which one or two "axial" sites are occupied by bioactive molecules able to intervene in cellular processes, is routinely practicable and has been intensively investigated.[8] However, the assembly of two different bioactive units within simple Pt(II) structures is synthetically more challenging and currently limited.[8a,9]

Herein, we describe the straightforward synthesis of new Pt(II) compounds containing a series of bipyridine ligands doubly modified with bioactive species, including unusual examples of hetero-functionalization ("triple action" compounds). It has to be mentioned that various substituted bipyridine ligands have been employed as a robust scaffold for the conjugation of bioactive molecules to other metal complexes.[14a,10] In our case, we selected the commercially available 2,2'-bipyridine-4,4'-dicarboxylic acid as a suitable platform for functionalization reactions via sequential Steglich esterifications (Scheme 1). Three carboxylic acids were selected to demonstrate our approach (Figure 2), *i.e.* ethacrynic acid (***E*-$CO_2$H**), an inhibitor of glutathione transferase enzymes (GST), which is involved in the cellular detoxification of platinum drugs,[11] flurbiprofen (***F*-$CO_2$H**), belonging to the family of nonsteroidal anti-inflammatory drugs (NSAIDs), able to inhibit cyclooxygenase

enzymes (COX),[12] and biotin (***B*-CO$_2$H**), which is expected to favour the delivery of the drug to cancer cells.[13] In a number of cases, the conjugation of these organic compounds to various transition metal species has been shown to enhance the anticancer behaviour of the resulting complexes.[14,15,16]




*E*-$CO_2$H  *F*-$CO_2$H  *B*-$CO_2$H

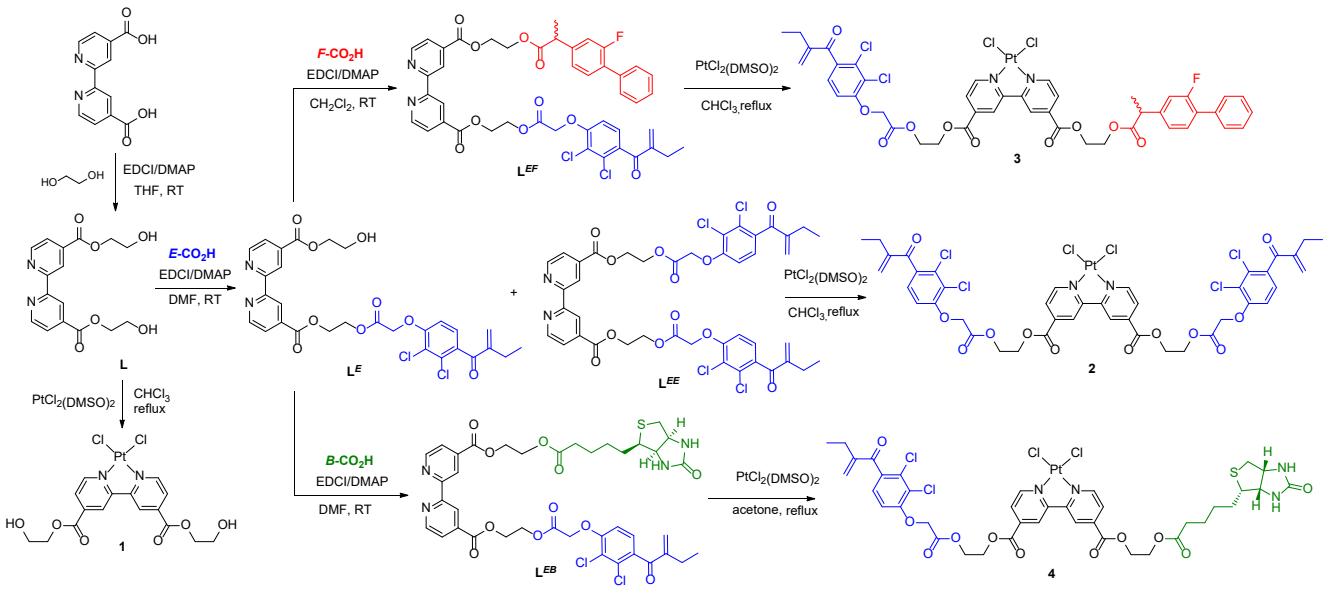
**Figure 2**. Bioactive carboxylic acids discussed in this work: ethacrynic acid (*E*-$CO_2$H), flurbiprofen (*F*-$CO_2$H; racemic mixture), biotin (*B*-$CO_2$H).

## Results and discussion

### Synthesis and characterization of compounds and stability studies

For the synthesis of ligands, all the esterification reactions were conducted using the EDCI·HCl/DMPA protocol (Scheme 1), and the products were efficiently purified by means of silica chromatography. The preliminary derivatization of the commercial 2,2′-bipyridine-4,4′-dicarboxylic acidwith ethylene glycol is needed to produce a soluble, reactive di-alcohol (**L**, 67% yield) from the otherwise untreatable diacid. Then, the reaction of **L** with a two-fold excess of ***E*-CO$_2$H** led to the homo-bis-functionalized ligand **L$^{EE}$** in 63% yield. On the other hand, the formation of the mono-esterified product**L$^{E}$**is a key step in order to subsequently obtain the hetero-functionalized ligands. Thus, **L$^{E}$**was prepared from **L** and ***E*-CO$_2$H** upon regulation of the reagents ratio, and separated from a less amount of **L$^{EE}$**and unreacted **L**by a careful chromatography. Starting from **L$^{E}$**, straightforward derivatization with flurbiprofen or biotin afforded**L$^{EF}$** and **L$^{EB}$**, respectively, in approximately 70% yields. Subsequently, complexes **1**-**3** were prepared in 72-94% yields by reactions of the appropriate bipyridine ligand with *cis*-[$PtCl_2(DMSO)_2$]in refluxing chloroform. The synthesis of **4** was more tricky, presumably due to the competing coordination of the sulphur atom in the biotinyl fragment of**L$^{EB}$** (see Experimental), and after several attempts the desired product was obtained in 73% yieldfrom thereaction of *cis*-[$PtCl_2(DMSO)_2$] with **L$^{EB}$** in refluxing acetone during a carefully controlled time.All compounds (ligands and

complexes) were fully characterized by elemental analysis, IR and multinuclear NMR spectroscopy. Moreover, the structure of **1** was confirmed by a single crystal X-ray diffraction study (see Figure S1 and Table S1 in the ESI).The IR spectra of **2-4** (solid state) exhibit the absorptions related to the ester moieties adjacent to the bipyridine scaffold in the range 1730-1740 $cm^{-1}$, i.e. slightly shifted respect to the values detected for the corresponding ligands and for **L** (1721 $cm^{-1}$). The $^{1}H$-decoupled $^{195}Pt$ NMR spectra of **1-4** display a single resonance around -2280 ppm, in alignment with previously reported $PtCl_2$(bipy) complexes.[17]




**Scheme1**. Synthesis of ligands and related complexes reported in this work: [$PtCl_2$(**L**)] (**1**), [$PtCl_2$($\mathbf{L}^{EE}$)] (**2**), [$PtCl_2$($\mathbf{L}^{EF}$)] (**3**) and [$PtCl_2$($\mathbf{L}^{EB}$)] (**4**). Blue: ethacrynic acid fragment (*E*-$CO_2$); red: flurbiprofen (*F*-$CO_2$); green: biotin (*B*-$CO_2$).

In view of the biological studies, we investigated the behavior of **1**-**4**in aqueous media. Since the complexes are insoluble in pure water, they were assessed for their stability in DMSO-$d_6$/$D_2O$ mixtures (see ESI for details). According to $^{1}H$ NMR, the complexes did not modify over 72 hours at 37 °C, and 97-99% of the starting material was recovered at the end of each experiment.This contrasts with the feasible hydrolytic cleavage of the Pt-Cl bonds of cisplatin and analogous di-amino compounds,[18] which is responsible for the activation of the drug and its subsequent covalent binding with DNA.[1] The superior resistance to hydrolysis of 1-4 is ascribable to the electron withdrawing effect exerted by the bipyridine moiety;[19]in order to promotethe cleavage of one Pt-Cl bond in **1**, the addition of a silver salt

(silver triflate) was required (Scheme S1).The absence of the classical activation mechanism in previously investigated dihalido complexes [$PtX_2$(bipy*)], containing bipyridine ligands with small substituents (i.e., Me and/or $CO_2H$), does not prevent an efficient interaction with DNA, which preferentially occurs via intercalative mode.[20,21]

The behaviour of **2-4** was then investigated in RPMI cell culture medium,wherethe stability of the [$PtCl_2$(bipyridine)] core was confirmed, but a progressive release of the bioactive carboxylic acids and the respective 2-hydroxyethyl esters was recognized by mass spectrometry over 48 hours at 37 °C (Figures S2-S5).The tendency of the bipyridine ligands and Pt complexes to undergo breaking of the ester linkages was clearly observed in $CD_3OD$ solution, where alcoholysis of the ester bonds occurred withvariable kinetics (for details, see Figures S6-S12 and Schemes S2-S8).In particular, the Pt(II) complexes **2-4**are more reactive than their ligands (Table 1), and while the former release ethacrynic acid 2-hydroxyethyl ester instead of the methyl ester, the opposite tendency was observed for the bipyridines. Overall, the collected data indicate that compounds **2**-**4**are based on a robust scaffold, but areable to release their bioactive payload in a physiological environment, thus acting as pro-drugs.

**Table 1**. Half-time ($t_{1/2}$) and total time ($t_{tot}$) for consumption of thestarting material due to trans-esterification in $CD_3OD$ solution at 25 °C.

| Starting material | $t_{1/2}$ / hours | $t_{tot}$ / hours |
|---|---|---|
| $L^{EE}$ | 18 | *ca*. 160 |
| $L^{EF}$ | 18 | *ca*. 118 |
| $L^{EB}$ | 45 | >100 |
| **1** | 35 | > 115 |
| **2** | minutes | 24 |
| **3** | minutes | 24 |
| **4** | 25 | > 160 |

**Cytotoxicity studies**

The cytotoxicity of the compounds was assessed on cisplatin sensitive and cisplatin resistant human ovarian cells (A2780 and A2780cisR) and non-tumorigenic human embryonic kidney (HEK-293) cells (see Table 2 and Experimental for details). Complexes **2** and **3** display strong cytotoxicity against both the sensitive and the resistant ovarian cancer cell lines, substantially superior to that exhibited by the respective bipyridine ligands $\mathbf{L}^{EE}$ and $\mathbf{L}^{EF}$. Notably, **2** and **3** are highly cancer cell selective, with **3**exhibiting a HEK293/A2780cisR selectivity index ($SI^R$) exceeding 40. In contrast, the performance of **4** is comparable to that of its bipyridine ligand $\mathbf{L}^{EB}$.

**Table 2**. $IC_{50}$ values (μM) determined for complexes, ligands and ligand/complex combinations on human ovarian (A2780 and A2780cisR) cancer cells and human embryonic kidney (HEK-293) cells after 72 h incubation. Values are given as the mean ± SD. SI = $IC_{50}$(HEK-293)/$IC_{50}$(A2780), $SI^R$ = $IC_{50}$(HEK-293)/$IC_{50}$(A2780cisR).

| Compound | A2780 | SI | A2780cisR | $SI^R$ | HEK293 |
|---|---|---|---|---|---|
| **2** | 5.7 ± 0.5 | 6 | 2.4 ± 0.5 | 15 | 36 ± 2 |
| **3** | 3.0 ± 0.2 | 12 | 0.8 ± 0.1 | 44 | 35 ± 3 |
| **4** | 11.4 ± 1.3 | 2.5 | 10.4 ± 0.8 | 3 | 29 ± 3 |
| $\mathbf{L}^{EE}$ | 34 ± 3 | 2 | 34 ± 2 | 2 | 60 ± 4 |
| $\mathbf{L}^{EF}$ | >200 | - | >200 | - | >200 |
| $\mathbf{L}^{EB}$ | 11.5 ± 1.3 | 2 | 16.0 ± 1.4 | 1.6 | 25 ± 2 |
| ***E*-$CO_2$H** [14a] | 40 ± 3 | 1 | 53 ± 5 | <1 | 39 ± 1 |
| ***F*-$CO_2$H** | >200 | - | >200 | - | >200 |
| **1 + *E*-$CO_2$H + *F*-$CO_2$H** | 25 ± 2 | 1 | 33 ± 2 | <1 | 22 ± 2 |
| **cisplatin** | 2.0 ± 0.2 | 4 | 18 ± 2 | <1 | 8 ± 1 |
| **RAPTA-C** | >200 | - | >200 | - | >200 |

A number of [$PtCl_2$(bipy*)] complexes, bearing variably substituted bipyridine ligands, was investigated by other groups for the in vitro cytotoxicity towards different cell lines, and a moderate activity was generally recognized.[17a,20,22] This set of data strongly suggests that the higher potency of **2** and **3** is ascribable to the presence of the conjugated bioactive groups. Furthermore, complex **1**, lacking the bioactive moieties, was

incubated in combination with ***E*-$CO_2H$** and ***F*-$CO_2H$**, and both the cytotoxicity against the A2780 and A2780cisR cells and the selectivity towards HEK293 decreased, compared to **3** in which all three components are covalently linked (see Table 1). This difference evidences that conjugation of ethacrynic acid and flurbiprofen to the Pt(II)-bipy scaffold via covalent bonding is required to provide a synergistic activity. Note that the combined administration of cisplatin andflurbiprofen was previously documentedto suppress cytotoxicity on HeLa tumoral cells.[23]

The best performing complexes, *i.e.***2** and **3**, were investigated (at a fixed concentration of10 μM) in the retinoblastoma (Y79) cell line due to the role of GSTs in this disease.[24] Both compounds, but especially **3**, are more efficacious than topotecan which iswidely used to treat retinoblastoma (Figure 3).[25] Notably, **2** and**3**are faster acting than topotecan, which is potentially advantageous when associated with thermotherapy as the tumour is heated shortly after injection of the drug. Thus, **2** and **3** were also evaluated at elevated temperature (42 °C), to mimic the current treatment for retinoblastoma that combines chemotherapy with hyperthermia.[26]In combination with heat, the efficacy of complexes **2** and **3** is maintained and is superior to that of topotecan.

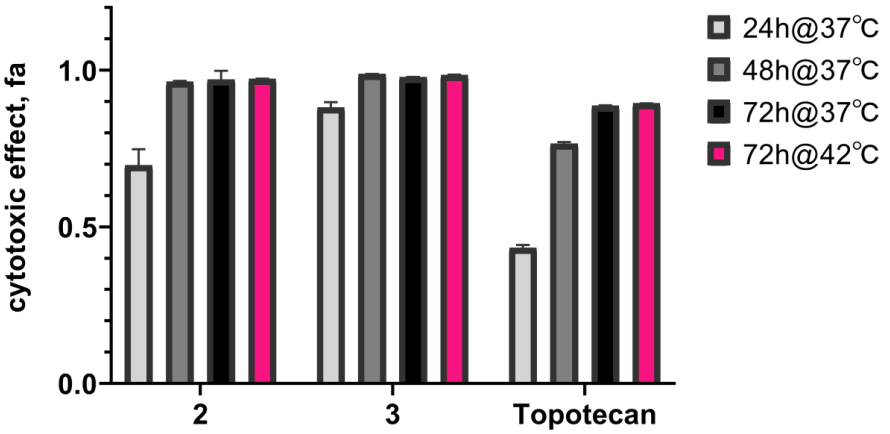



**Figure 3**. The cytotoxic effect of **2**, **3** and topotecan (at a fixed concentration of 10 μM) following incubation for 24, 48 and 72 hours in Y79 retinoblastoma cells at 37 °C and at 42 °C for 1 hour, with a total incubation time of 72 hours. fa is the fraction of cell death induced by drug treatment and ranges from 0, meaning no cell death, to 1, representing 100% cell death.

## Enzyme inhibition assays

In order to give insight into the action of the complexes, the effect of **2** and **3** on the activity of human GST P1 (expressed from E. coli) and human COX-2 (expressed in baculovirus infected Sf21 cells) enzymes was spectrophotometricallyevaluated (see Experimental for details); ***E*-$CO_2$H** and ***F*-$CO_2$H** were also analysed as reference inhibitors. Thus, enzymes were treated with the compounds and the residual enzyme activity was recorded to determine the $IC_{50}$ concentrations (Table 3). The results clearly indicate that both complexes **2** and **3**are better GST P1 inhibitors compared to ethacrynic acid, while**3** is a ca. 10-fold more active COX-2 inhibitor than flurbiprofen. This data is in alignment with the cytotoxicity outcomes obtained with the mixture **1**/***E*-$CO_2$H**/***F*-$CO_2$H** (see above), supporting a synergistic mode of action of the Pt(II) complex core and the bioactive molecules.

**Table 3**. $IC_{50}$ values (μM) determined for complexes **2** and **3**, ethacrynic acid and flurbiprofen in the inhibition of GST P1 and COX-2 enzymes. Values are given as the mean ± SD.

| Compound. | GST P1 | COX-2 |
|---|---|---|
| **2** | 4.2 ± 0.1 | - |
| **3** | 4.4 ± 0.8 | 77 ± 3 |
| ***E*-$CO_2$H** | 13.58 ± 0.02 | - |
| ***F*-$CO_2$H** | - | 719 ± 135 |

## Conclusions

Although the introduction of two bioactive groups is relatively straightforward from a synthetic point of view on Pt(IV) complexes, and thus has been largely investigated to optimize the anticancer properties of the resulting drug candidates, it is much less straightforward for Pt(II) compounds. Herein, we have described a facile synthetic strategy to tether two different bioactive molecules to the[$PtCl_2$] fragment via modified bipyridine-type ligands. The bipyridine-platinum(II)unit exhibits high stability in aqueous media, and,compared to related compounds that lack bioactive units,the

antiproliferative activity is considerably higher. Indeed, the biological studies on the new complexes are promising compared to the data available forthe reference drug cisplatin, and suggest that this approach can be further exploited in the search for new effective Pt(II) chemotherapics. The*in vitro* cytotoxicity assaysindicate that the ethacrynic acid moiety helps to overcome drug resistance and further conjugation with flurbiprofen leads to the best cytotoxicity/selectivity profile. Enzyme inhibition assays reveal a clear synergy between the Pt(II) frame and the bioactive organic components, which might be related to the progressive release of the latter via ester bond cleavage. Notably, the activity of the compound containing the different active parts covalently linked, i.e. **3**, is considerable superior to that of a mixture (combination) of the three constituent components.

## Experimental

### 1. General experimental details

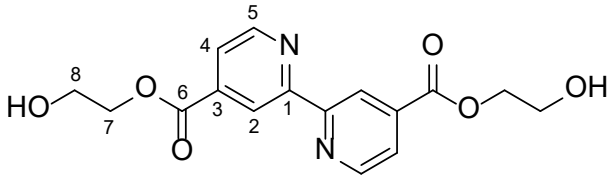
$K_2[PtCl_4]$ was purchased from Alfa Aesar, while other reactants and solvents were obtained from Alfa Aesar, Merck, Apollo Scientific or TCI Chemicals and were of the highest purity available. Ethacrynic acid (***E*-$CO_2$H**),[27] flurbiprofen (***F*-$CO_2$H**),[27] biotin (***B*-$CO_2$H**),[27] 4-dimethylaminopyridine (DMAP), 2,2′-bipyridine-4,4′-dicarboxylic acid and ethylene glycol were dried under vacuum over $P_2O_5$and then stored under $N_2$; ethyl(diisopropylamino)carboxydiimide hydrochloride (EDCI·HCl) was stored under $N_2$ at –20 °C. Silica gel (70-230 mesh) was used for column chromatography. Compound *cis*-$[PtCl_2(\kappa S\text{-DMSO})_2]$ was prepared according to the literature.[28] Steglich esterifications[29] were performed under $N_2$ using standard Schlenk techniques and solvents distilled from appropriate drying agents (DMF from BaO, THF from $CaH_2$, $CH_2Cl_2$ and $CHCl_3$ from $P_2O_5$). All the other operations were carried out in air with common laboratory glassware. Once isolated, organic bipyridine esters were stored at 4 °C; all Pt compounds are air- and moisture- stable in the solid state. NMR spectra were recorded at 25 °C on a Bruker Avance II DRX400 instrument equipped with a BBFO broadband probe. Chemical shifts (expressed in parts per million) are referenced to the residual solvent peaks[30] ($^1$H, $^{13}$C) or

to external standards[31] ($^{19}$F to $CFCl_3$, $^{35}$Cl to 1 M NaCl in $D_2O$, $^{195}$Pt to 1.2 M $Na_2PtCl_6$ in $D_2O$). $^1$H and $^{13}$C spectra were assigned with the assistance of $^{13}$C DEPT 135, $^1$H-$^1$H COSY, $^1$H-$^{13}$C *gs*-HSQC and $^1$H-$^{13}$C *gs*-HMBC experiments.[32] $CDCl_3$ stored in the dark over $Na_2CO_3$ was used for NMR analysis. IR spectra (650-4000 $cm^{-1}$) were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer, equipped with a UATR sampling accessory and processed with Spectragryph software.[33] Carbon, hydrogen and nitrogen analyses were performed on a Vario MICRO cube instrument (Elementar). MS analyses were performed with a API3000 instrument (SCIEX) equipped with ESI(+) source.

**2. Synthesis and characterization of bipyridine ligands**

## Bis(2-hydroxyethyl)[2,2'-bipyridine]-4,4'-dicarboxylate, L (Chart 1).

**Chart 1**. Structure of **L** (numbering refers to C atoms).

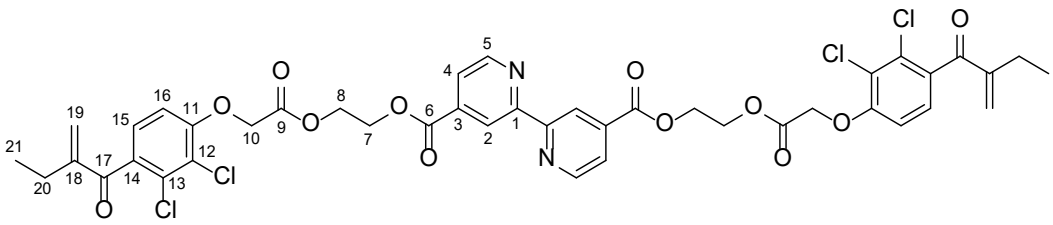



In a 100-mL Schlenk tube, 2,2'-bipyridine-4,4'-dicarboxylic acid(513 mg, 2.10 mmol), DMAP (51.4 mg, 0.42 mmol), ethylene glycol (1.50 mL, 27 mmol), EDCI·HCl (1.20 g, 6.26 mmol) and THF (15 mL) were introduced in this order. The suspension was stirred at room temperature for 15 h, affording a colourless solution layered over a light-pink milky suspension. The mixture was transferred on top of an alumina pad (70-230 mesh, activity I, h = 2.0 cm, d = 3 cm), followed by elution with THF (ca. 200 mL). Volatiles were removed under vacuum (40 °C) and the resulting colourless wet solid was suspended in water (10 mL). The milky suspension was stirred at room temperature for 30 minutes then filtered. The resulting colourless solid was washed with $H_2O$, acetone (- 20 °C, 2 mL), $Et_2O$ then dried under vacuum (50 °C, then RT over $P_2O_5$). Yield: 527 mg, 75 %. *1 g scale*: the reaction was performed by doubling the amounts of all reagents, under otherwise identical conditions. Filtration: alumina pad (h = 4.0 cm, d = 3 cm), THF (300 mL). Yield: 916 mg, 67 %. Compound **L** is soluble in DMSO, DMF, poorly soluble in water, acetone, MeOH, MeCN, THF, $CH_2Cl_2$, insoluble in $Et_2O$. Anal. Calcd. for $C_{16}H_{16}N_2O_6$: C, 57.83; H, 4.85; N, 8.43. Found: C, 57.90; H, 4.90; N, 8.37. IR (solid state): ũ/$cm^{-1}$ =

3246m-br, 3202m-sh ($\nu_{OH}$); 3095w-sh, 2971w, 2947w, 2874w, 1721s ($\nu_{C=O}$), 1596w, 1557m, 1505w, 1463w, 1450w, 1373m-sh, 1363s, 1286s, 1255s, 1247s, 1137s, 1096m-sh, 1080s, 1073s-sh, 1061s, 1021s, 991w-sh, 904m, 873m, 866m, 838m, 761s, 722m, 690s, 667w. $^{1}$H NMR (DMSO-$d_6$): δ/ppm = 8.98 (d, $^{3}J_{HH}$ = 4.9 Hz, 2H, C5-H), 8.89 (s, 2H, C2-H), 8.00 (dd, $^{3}J_{HH}$ = 4.9 Hz, $^{4}J_{HH}$ = 1.5 Hz, 2H, C4-H), 5.01 (t, $^{3}J_{HH}$ = 5.6 Hz, 2H, OH), 4.39 (t, $^{3}J_{HH}$ = 4.8 Hz, 4H, C7-H), 3.75 (app. q, $^{3}J_{HH}$ = 4.9, 5.4 Hz, 4H, C8-H). $^{13}$C{$^{1}$H} NMR (DMSO-$d_6$): δ/ppm = 164.6 (C6), 155.5 (C1), 150.8 (C5), 138.6 (C3), 123.5 (C4), 119.4 (C2), 67.5 (C7), 58.9 (C8).

## Bis(2-ethacrynyloxyethyl)[2,2'-bipyridine]-4,4'-dicarboxylate, $L^{EE}$ (Chart 2).

**Chart 2**. Structure of $\mathbf{L}^{EE}$ (numbering refers to C atoms).

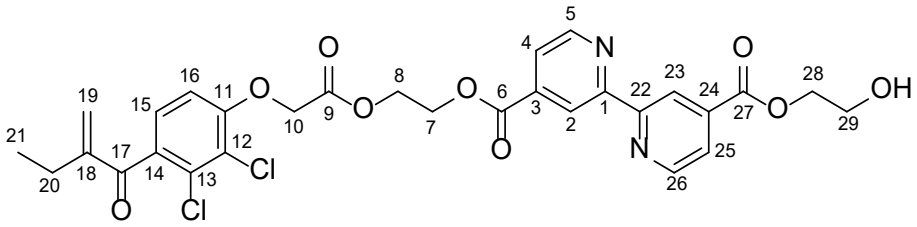



In a 50-mL Schlenk tube, bis(2-hydroxyethyl)[2,2'-bipyridine]-4,4'-dicarboxylate **L**(112 mg, 0.337 mmol), ***E*-$CO_2$H** (225 mg, 0.743 mmol), DMAP (7.2 mg, 0.059 mmol), EDCI·HCl (147 mg, 0.764 mmol) and DMF (10 mL) were introduced in this order. The colourless solution was stirred at room temperature for 14 h then volatiles were removed under vacuum. The residue was dissolved in $CH_2Cl_2$ and moved on top of a silica column (h 7 cm, d 2.5 cm). Impurities were eluted with $CH_2Cl_2$, then the title compound was eluted with $CH_2Cl_2$/$Et_2O$ 8:1 *v*/*v*. Fractions containing $\mathbf{L}^{EE}$ were dried under vacuum (40 °C), affording an oily residue. A colourless foamy solid was obtained upon dissolution of the residue in few mL of $Et_2O$ followed by volatiles removal without heating.[34] The solid was dried under vacuum at room temperature then stored at 4 °C. Yield: 192 mg, 63 %. Alternatively, $\mathbf{L}^{EE}$ was obtained by EDCI/DMAP-mediated esterification of 2,2'-bipyridine-4,4'-dicarboxylic acid with ***E*-$CO_2(CH_2)_2OH$** in $CH_2Cl_2$; however complete conversion of the alcohol required 5 days at room temperature. Compound $\mathbf{L}^{EE}$is soluble in acetone, $CH_2Cl_2$, $CHCl_3$, $Et_2O$, insoluble in hexane and water. Anal. Calcd. for $C_{42}H_{36}Cl_4N_2O_{12}$: C, 55.89; H, 4.02; N, 3.10. Found: C, 55.94; H, 4.10; N, 3.06. IR (solid state): $\tilde{\nu}/cm^{-1}$ = 3096w, 3080w, 2968w, 2938w, 2879w, 1762m-sh ($\nu_{C9=O}$), 1731s ($\nu_{C6=O}$), 1663m ($\nu_{C17=O}$), 1585m ($\nu_{C18=C19}$), 1557m,

1469m, 1458m-sh, 1438m, 1384m, 1362m, 1340w, 1286s, 1255s, 1243s-sh, 1190s, 1134m-sh, 1121m, 1080s, 1066s-sh, 1046m-sh, 1001m, 943m, 915w, 895w, 862w, 819w-sh, 802m, 761s, 724m, 692m, 666w. $^{1}$H NMR ($CDCl_3$): δ/ppm = 8.96 (s, 2H, C2-H), 8.87 (d, $^{3}J_{HH}$ = 4.5 Hz, 2H, C5-H), 7.88 (d, $^{3}J_{HH}$ = 4.5 Hz, 2H, C4-H), 7.09 (d, $^{3}J_{HH}$ = 8.5 Hz, 2H, C15-H), 6.82 (d, $^{3}J_{HH}$ = 8.5 Hz, 2H, C16-H), 5.90 (s, 2H, C19-H), 5.54 (s, 2H, C19-H'), 4.82 (s, 4H, C10-H), 4.67−4.60 (m, 8H, C7-H + C8-H), 2.43 (q, $^{3}J_{HH}$ = 7.3 Hz, 4H, C20-H), 1.12 (t, $^{3}J_{HH}$ = 7.4 Hz, 6H, C21-H). $^{13}$C{$^{1}$H} NMR ($CDCl_3$): δ/ppm = 195.8 (C17), 167.8 (C9), 165.0 (C6), 156.6 (C1), 155.5 (C11), 150.4 (C5), 150.3 (C18), 138.2 (C3), 134.1 (C12), 131.6 (C13), 128.7 (C19), 126.9 (C15), 123.5 (C4 + C14), 120.7 (C2), 111.0 (C16), 66.2 (C10), 63.3 (C7 + C8), 23.5 (C20), 12.5 (C21).

## 4-(2-Ethacrynyloxyethyl)-4'-(2-hydroxyethyl)[2,2'-bipyridine]-4,4'-dicarboxylate, L$^{E}$ (Chart 3).

**Chart 3**. Structure of **L$^{E}$** (numbering refers to C atoms).

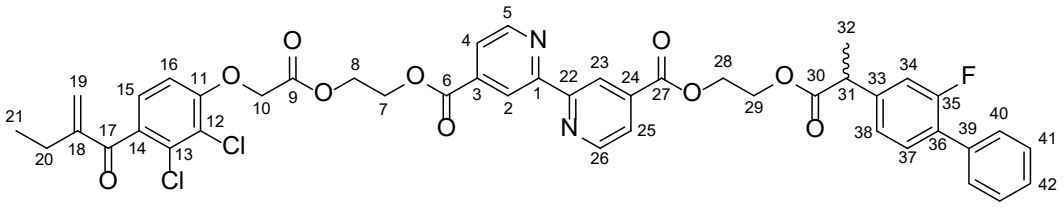



In a 50-mL Schlenk tube, bis(2-hydroxyethyl)[2,2'-bipyridine]-4,4'-dicarboxylate **L**(505 mg, 1.52 mmol), DMAP (74 mg, 0.61 mmol), ***E*-$CO_2$H** (606 mg, 2.00 mmol, 1.3 equivalents), EDCI·HCl (400 mg, 2.09 mmol) and DMF (10 mL) were introduced in this order. The colourless solution was stirred at room temperature for 42 h then volatiles were removed under vacuum. The pale pink oily residue was dissolved in $CH_2Cl_2$ and moved on top of a silica column (h 6 cm, d 3.3 cm). Impurities were eluted with $CH_2Cl_2$ then **L$^{E}$** was eluted with $CH_2Cl_2$:$Et_2O$ 9:1.5 *v*/*v* (*ca.* 210 mL), **L$^{E}$**was eluted with $CH_2Cl_2$:acetone 5.5:1 *v*/*v* (*ca.* 400 mL) and last, unreacted **L** was eluted with acetone (ca. 100 mL). Fractions containing **L$^{EE}$**and **L$^{E}$** were dried under vacuum (40 °C), affording oily residues. The two compounds were obtained as colourless foamy solids upon volatiles removal without heating from their $Et_2O$ or $CH_2Cl_2$:acetone solutions, respectively.[34] The solids were dried under vacuum at room temperature then stored at 4 °C. Fractions containing **L**were taken to dryness under vacuum; the resulting colourless solid was washed with $Et_2O$ and dried under vacuum (40 °C). Yield: **L$^{EE}$**, 242 mg, 18 %;**L$^{E}$**,363 mg, 39

%; **L**, 112 mg, 22 %; total: 96 %. Compound $\mathbf{L}^{E}$is soluble in acetone, $CH_2Cl_2$, $CHCl_3$, $Et_2O$, insoluble in hexane. Anal. Calcd. for $C_{29}H_{26}Cl_2N_2O_9$: C, 56.41; H, 4.24; N, 4.54. Found: C, 56.31; H, 4.19; N, 4.48. IR (solid state): ũ/$cm^{-1}$ = 3524w-br, 3450w-br ($\nu_{OH}$); 3079w, 2966w, 2938w-sh, 2879w-sh, 1762m-sh ($\nu_{C9=O}$), 1727s ($\nu_{C6=O}$ + $\nu_{C27=O}$), 1663m ($\nu_{C17=O}$), 1586m ($\nu_{C18=C19}$), 1557m, 1463m, 1439m, 1384m-sh, 1362m, 1340w, 1285s, 1255s, 1245s-sh, 1191s, 1134s, 1124s, 1079s, 1068s, 1002m, 944w, 915w, 895w, 863w, 803w, 761s, 724m, 692m, 666m. $^1$H NMR ($CDCl_3$): δ/ppm = 8.97, 8.95 (s, 1H/1H, C2-H + C23-H); 8.87, 8.85 (d, $^2J_{HH}$ = 5.6 Hz, 1H/1H, C5-H + C26-H), 7.93 (dd, $^3J_{HH}$ = 4.9 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C25-H), 7.88 (dd, $^3J_{HH}$ = 4.9 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, C4-H), 7.06 (d, $^3J_{HH}$ = 8.5 Hz, 1H, C15-H), 6.81 (d, $^3J_{HH}$ = 8.0 Hz, 1H, C16-H), 5.89 (s, 1H, C19-H), 5.53 (s, 1H, C19-H'), 4.82 (s, 2H, C10-H), 4.67–4.60 (m, 4H, C7-H + C8-H), 4.57–4.52 (m, 2H, C28-H), 4.05–3.98 (m, 2H, C29-H), 2.43 (q, $^3J_{HH}$ = 7.4 Hz, 2H, C20-H), 2.36–2.25 (m, 1H, OH), 1.12 (t, $^3J_{HH}$ = 7.4 Hz, 3H, C21-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 195.9 (C17), 167.8 (C9); 165.4, 165.0 (C6 + C27); 156.6, 156.4 (C1 + C22), 155.5 (C11); 150.*32*, 150.*28*, 150.*23* (C5 + C18 + C26); 138.6, 138.2 (C3 + C24), 134.1 (C12), 131.6 (C13), 128.8 (C19), 126.9 (C15); 123.5, 123.4 (C4 + C25); 120.7 (C2 + C23), 110.9 (C16), 67.6 (C28), 66.2 (C10); 63.3, 63.2 (C7 + C8), 61.1 (C29), 23.5 (C20), 12.5 (C21).

## 4-(2-Ethacrynyloxyethyl)-4'-(2-flurbiprofenoxyethyl)[2,2'-bipyridine]-4,4'-dicarboxylate, $\mathbf{L}^{EF}$ (Chart 4).

**Chart 4**. Structure of $\mathbf{L}^{EF}$ (numbering refers to C atoms).

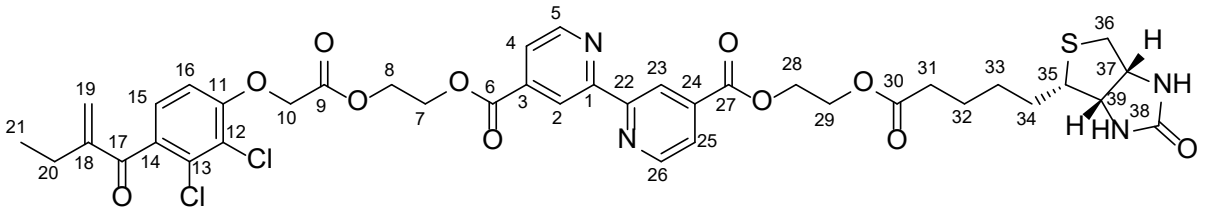



In a 50-mL Schlenk tube under $N_2$, $\mathbf{L}^{E}$(278 mg, 0.450 mmol), DMAP (18 mg, 0.15 mmol), ***F*-$\mathbf{CO_2H}$** (115 mg, 0.515 mmol), EDCI·HCl (109 mg, 0.568 mmol) and $CH_2Cl_2$ (15 mL) were introduced in this order and the colourless solution was stirred at room temperature. After 5 h, conversion was checked by $^1$H NMR ($CDCl_3$) then volatiles were removed under vacuum. The pale yellow residue was dissolved in $CH_2Cl_2$ and moved on top of a silica column (h 6 cm, d 2.5 cm). Impurities were eluted with $CH_2Cl_2$ (50 mL) then $\mathbf{L}^{EF}$ was eluted with

$CH_2Cl_2$:$Et_2O$:hexane mixtures (200 mL 7:1:2 *v*/*v* then 100 mL 7:2:2 *v*/*v*). Fractions containing **L**$^{EF}$were dried under vacuum (40 °C), affording an oily residue. A colourless foamy solid was obtained upon dissolution of the residue in few mL of $CH_2Cl_2$ followed by volatiles removal without heating.[34] The solid was dried under vacuum at room temperature then stored at 4 °C. Yield: 279 mg, 74 %. Compound **L**$^{EF}$is soluble in $Et_2O$, $CH_2Cl_2$, poorly soluble in MeOH,[35] insoluble in hexane, water. Anal. Calcd. for $C_{44}H_{37}Cl_2FN_2O_{10}$: C, 62.64; H, 4.42; N, 3.32. Found: C, 62.75; H, 4.47; N, 3.28. IR (solid state): ũ/cm$^{-1}$ = 3075w, 3034w, 2964w, 2940w-sh, 2878w-sh, 1764m-sh ($\nu_{C9=O}$), 1729s ($\nu_{C6=O}$ + $\nu_{C27=O}$ + $\nu_{C30=O}$), 1665m ($\nu_{C17=O}$), 1625w, 1585m ($\nu_{C18=C19}$), 1558m, 1516w, 1484w-sh, 1461m, 1434m-sh, 1417w, 1383m-sh, 1362m, 1336w, 1284s, 1255s, 1244s-sh, 1222m, 1189s, 1131s, 1122s, 1081s, 1066s, 1043m-sh, 1010m, 938w, 916w, 895w, 872w, 863w, 801m, 761s, 724m, 693s, 666m. $^1$H NMR ($CDCl_3$): δ/ppm = 8.92 (s, 1H/1H, C2-H + C23-H); 8.85, 8.80 (d, $^3J_{HH}$ = 4.9 Hz, 1H/1H, C5-H + C26-H); 7.86, 7.80 (d, $^3J_{HH}$ = 4.9 Hz, 1H/1H, C4-H + C25-H), 7.43–7.29 (m, 6H, C37-H + $C_6H_5$), 7.15–7.09 (m, 2H, C34-H + C38-H), 7.10 (d, $^3J_{HH}$ = 8.3 Hz, 1H, C15-H), 6.82 (d, $^3J_{HH}$ = 8.5 Hz, 1H, C16-H), 5.90 (s, 1H, C19-H), 5.54 (s, 1H, C19-H'), 4.82 (s, 2H, C10-H), 4.68–4.56 (m, 6H, C7-H + C8-H + C28-H), 4.50 (t, $^3J_{HH}$ = 4.2 Hz, 2H, C29-H), 3.81 (q, $^3J_{HH}$ = 7.2 Hz, 1H, C31-H), 2.43 (q, $^3J_{HH}$ = 7.2 Hz, 2H, C20-H), 1.56 (d, $^3J_{HH}$ = 7.4 Hz, 3H, C32-H), 1.12 (t, $^3J_{HH}$ = 7.4 Hz, 3H, C21-H). $^{13}$C{$^1$H} NMR ($CDCl_3$): δ/ppm = 195.8 (C17), 173.9 (C30), 167.8 (C9); 165.1, 164.9 (C6 + C27), 159.8 (d, $^1J_{CF}$ = 249 Hz, C35); 156.7, 156.6 (C1 + C22), 155.5 (C11); 150.4, 150.3 (C5 + C26 + C18), 141.5 (d, $^3J_{CF}$ = 8 Hz, C33); 138.3, 138.1 (C3 + C24), 135.4 (C39), 134.2 (C12), 131.7 (C13), 130.9 (d, $^3J_{CF}$ = 4 Hz, C37), 129.0 (d, $^4J_{CF}$ = 3 Hz, C40), 128.7 (C19), 128.5 (C41), 128.0 (d, $^2J_{CF}$ = 13 Hz, C36), 127.8 (C42), 126.9 (C15), 123.7 (d, $^4J_{CF}$ = 3 Hz, C38), 123.6 (C14); 123.*42*, 123.*38* (C4 + C25); 120.8, 120.7 (C2 + C23), 115.4 (d, $^2J_{CF}$ = 23.8 Hz, C34), 111.0 (C16), 66.3 (C10); 63.5, 63.3, 63.2 (C7 + C8 + C28), 62.5 (C29), 45.1 (C31), 23.5 (C20), 18.3 (C32), 12.5 (C21). $^{19}$F{$^1$H} NMR ($CDCl_3$): δ/ppm = – 117.4.

## 4-(2-Ethacrynyloxyethyl)-4'-(2-biotinyloxyethyl)[2,2'-bipyridine]-4,4'-dicarboxylate, L$^{EB}$ (Chart 5).

**Chart 5**. Structure of **L**$^{EB}$ (numbering refers to C atoms).



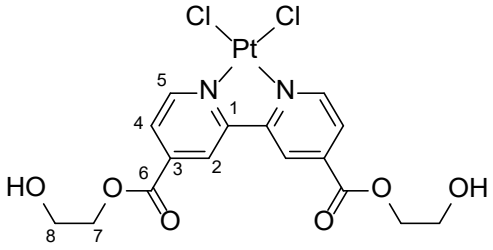
In a 50-mL Schlenk tube, $\mathbf{L}^{E}$(363 mg, 0.588 mmol), DMAP (23 mg, 0.19 mmol), ***B*-$\mathbf{CO_2H}$** (155 mg, 0.636 mmol), EDCI·HCl (144 mg, 0.75 mmol) and DMF (10 mL) were introduced in this order and the suspension (colourless solution + solid) was stirred at room temperature. After 19 h, conversion was checked by $^{1}$H NMR ($CDCl_3$) then volatiles were removed under vacuum. The colourless oily residue was dissolved in $CH_2Cl_2$ and moved on top of a silica column (h = 5 cm, d = 2.5 cm). Impurities were eluted with $CH_2Cl_2$ (50 ml) and $CH_2Cl_2$:acetone mixtures (80 mL 3:5 *v*/*v* then 80 mL 1:1 *v*/*v*) then $\mathbf{L}^{EB}$ was eluted with acetone (250 mL). Volatiles were removed under vacuum without heating,34 affording the title compound as a colourless solid. The solid was dried under vacuum at room temperature then stored at 4 °C. Yield: 304 mg, 67 %. Compound $\mathbf{L}^{EB}$is soluble in acetone, $CH_2Cl_2$, $CHCl_3$, EtOAc, MeOH,[35] almost insoluble in $Et_2O$, toluene; insoluble in hexane, water. Anal. Calcd. for $C_{39}H_{40}Cl_2N_4O_{11}S$: C, 55.52; H, 4.78; N, 6.64. Found: C, 55.61; H, 4.70; N, 6.58. IR (solid state): ũ/cm$^{-1}$ = 3410w-br, 3240w-br ($\nu_{NH}$); 2964w-sh, 2930w, 2875w-sh, 1764m-sh ($\nu_{C9=O}$), 1729s ($\nu_{C6=O}$ + $\nu_{C27=O}$ + $\nu_{C30=O}$); 1704s, 1699s ($\nu_{C38=O}$), 1667s-sh ($\nu_{C17=O}$), 1586m ($\nu_{C18=C19}$), 1557w, 1464w, 1456w, 1440w, 1383w-sh, 1362w, 1337w, 1286s, 1255s, 1245s-sh, 1192s, 1135s, 1122s, 1081s, 1066s-sh, 1002w, 945w, 915w, 895w, 862w, 823w, 805w, 761s, 724w, 692s, 666w. $^{1}$H NMR ($CDCl_3$): δ/ppm = 8.95 (s, 2H, C2-H + C23-H); 8.88, 8.86 (d, $^{3}J_{HH}$ = 5.0 Hz, 1H/1H, C5-H + C26-H); 7.91, 7.88 (dd, $^{3}J_{HH}$ = 5.0 Hz, $^{4}J_{HH}$ = 1.5 Hz, 1H/1H, C4-H + C25-H), 7.09 (d, $^{3}J_{HH}$ = 8.5 Hz, 1H, C15-H), 6.82 (d, $^{3}J_{HH}$ = 8.5 Hz, 1H, C16-H), 5.89 (s, 1H, C19-H), 5.54 (s, 1H, C19-H'); 5.50, 5.10 (s-br, 1H/1H, NH + NH'), 4.82 (s, 2H, C10-H), 4.66–4.59 (m, 6H, C7-H + C8-H + C28-H), 4.48–4.43 (m, 3H, C29-H + C37-H), 4.27–4.23 (m, 1H, C39-H), 3.12–3.06 (m, 1H, C35-H), 2.86 (dd, $^{2}J_{HH}$ = 12.8 Hz, $^{3}J_{HH}$ = 5.0 Hz, 1H, C36-H), 2.69 (d, $^{2}J_{HH}$ = 12.7 Hz, 1H, C36-H'), 2.46–2.35 (m, 4H, C20-H + C31-H), 1.71–1.60 (m, 4H, C32-H + C34-H), 1.48–1.40 (m, 2H, C33-H), 1.11 (t, $^{3}J_{HH}$ = 7.4 Hz, 3H, C21-H). $^{13}$C{$^{1}$H} NMR ($CDCl_3$): δ/ppm = 195.9 (C17), 173.5 (C30), 167.8 (C9), 165.1 (C6 + C27), 163.5 (C38); 156.6, 156.5 (C1 + C22), 155.5 (C11), 150.*44*, 150.*37*, 150.2 (C5 + C26 + C18); 138.5, 138.2 (C3 + C24), 134.1 (C12), 131.6 (C13), 128.8 (C19), 126.9 (C15); 123.*53*, 123.*44* (C4 + C25); 123.*48* (C14), 120.8, 120.7

(C2 + C23), 111.0 (C16), 66.2 (C10); 63.7, 63.3, 63.2 (C7 + C8 + C28), 62.0 (C29 + C39), 60.2 (C37), 55.4 (C35), 40.6 (C36), 33.8 (C31); 28.4, 28.3 (C33 + C34), 24.8 (C32), 23.5 (C20), 12.5 (C21).

**3. Synthesis and characterization of Pt compounds**

**$[PtCl_2(\kappa^2N\text{-L})]$, 1 (Chart 6).**

**Chart 6**. Structure of **1** (numbering refers to C atoms).

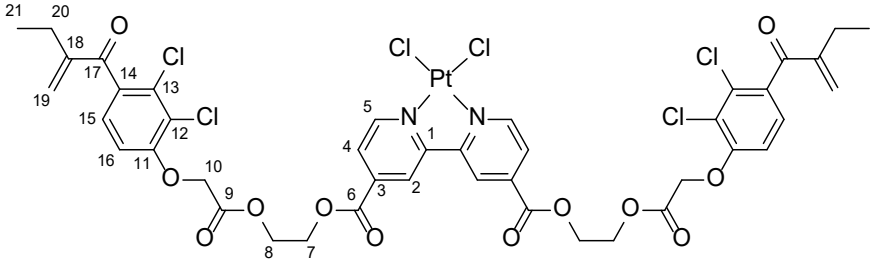



In a 50-mL round bottom flask, a suspension of *cis*-$[PtCl_2(\kappa S\text{-DMSO})_2]$ (61 mg, 0.14 mmol) and **L** (50 mg, 0.15 mmol) in $CHCl_3$ (15 mL) was stirred at reflux. After 5 h, the reaction mixture was cooled to room temperature and filtered. The bright orange[36] solid was washed with $CH_2Cl_2$, $Et_2O$ and dried under vacuum (50 °C). Yield: 77 mg, 90 %. *Alternative procedure*: A suspension of $K_2[PtCl_4]$ (48 mg, 0.11 mmol) and **L** in $H_2O$ (15 mL) was stirred at reflux. After 6 h, the reaction mixture was cooled to room temperature and filtered. The red-orange[36] solid was washed with $H_2O$, acetone and dried under vacuum. Yield: 79 mg, 72 %. Compound **1** is soluble in DMSO and hot DMF, insoluble in water, $Et_2O$, $CH_2Cl_2$, $CHCl_3$, MeCN, MeOH,[35] THF, acetone. Yellow, needle-like crystals suitable for X-ray diffraction were obtained from a DMSO solution of **1** layered with toluene and settled aside at room temperature (Table S1 / Figure S1). Anal. Calcd. for $C_{16}H_{16}Cl_2N_2O_6Pt$: C, 32.12; H, 2.70; N, 4.68. Found: C, 32.01; H, 2.72; N, 4.59. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3430w-br, 3350w-br ($\nu_{OH}$); 3118w, 3076w, 2955w, 2932w, 2879w, 1727s ($\nu_{C=O}$), 1622w, 1557w, 1484w, 1446w, 1412m, 1373m, 1322s, 1280s, 1254s, 1235s, 1137m, 1111m, 1073m, 1013m, 938w, 911w, 891w, 870m, 847w, 788w, 763s, 752w-sh, 706m. $^1$H NMR (DMSO-$d_6$): δ/ppm = 9.75 (d, $^3J_{HH}$ = 6.0 Hz, 2H, C5-H), 9.05 (s, 2H, C2-H), 8.30 (d, $^3J_{HH}$ = 6.3 Hz, 2H, C4-H), 5.07 (t, $^3J_{HH}$ = 5.7 Hz, 2H, OH), 4.43 (app. t, $^3J_{HH} \approx$ 5 Hz, 4H, C7-H), 3.78

(app. q, $^{3}J_{HH} \approx 5$ Hz, 4H, C8-H). $^{13}C\{^{1}H\}$ NMR (DMSO-$d_6$): δ/ppm = 163.3 (C6), 157.4 (C1), 149.6 (C5), 140.2 (C3), 127.3 (C4), 123.9 (C2), 68.2 (C7), 58.8 (C8). $^{195}Pt\{^{1}H\}$ (DMSO-$d_6$): δ/ppm = – 2282.

**[$PtCl_2$(κ$^2$*N*-$L^{EE}$)], 2 (Chart 7).**

**Chart 7**. Structure of **2** (numbering refers to C atoms).

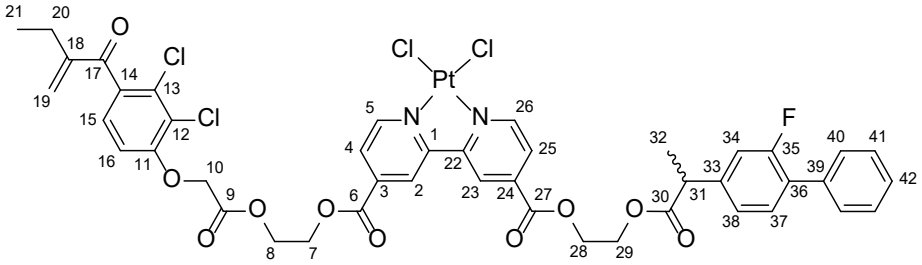



In a 50-mL round bottom flask, a suspension of *cis*-[$PtCl_2$(κ*S*-DMSO)$_2$] (72 mg, 0.17 mmol) and **$L^{EE}$** (167 mg, 0.19 mmol) in $CHCl_3$ (15 mL) was stirred at reflux for 5 hours. The yellow-orangesolution was cooled to room temperature and extracted with $H_2O$ (3 x 15 mL). Volatiles were removed under vacuum from the organic phase and the residue was triturated in $Et_2O$. The suspension was filtered; the resulting yellow-orange solid was washed with $Et_2O$ and dried under vacuum (room T). Yield: 177 mg, 89 %. Conversely, the reaction of **$L^{EE}$** and $K_2[PtCl_4]$ in refluxing MeOH:$H_2O$ 1:1 *v*/*v* was not successful (thermal degradation of the ligand). Compound **2** is soluble in DMSO, acetone, $CH_2Cl_2$ and $CHCl_3$, insoluble in $Et_2O$ and water. Anal. Calcd. for $C_{42}H_{36}Cl_6N_2O_{12}Pt$: C, 43.17; H, 3.10; N, 2.40; found: C 43.31; H 3.21; N; 2.42. IR (solid state): ũ/cm$^{-1}$ = 3116w-sh, 3077w, 2968w, 2936w-sh, 2877w-sh, 1760m-sh ($\nu_{C9=O}$), 1738s, 1733s ($\nu_{C6=O}$); 1662s ($\nu_{C17=O}$), 1623w-sh, 1584s ($\nu_{C18=C19}$), 1557w, 1469m, 1436m, 1414m, 1384m, 1339w, 1318m-sh, 1294s-sh, 1253s, 1235s, 1192s, 1143m, 1120s, 1079s, 1050m-sh, 1001m, 942m, 896w, 866w, 821w-sh, 803m, 761s, 730w, 706m. $^{1}H$ NMR ($CDCl_3$): δ/ppm = 9.52 (d, $^{3}J_{HH}$ = 6.0 Hz, 2H, C5-H), 8.60 (d, $^{4}J_{HH}$ = 1.3 Hz, 2H, C2-H), 8.00 (dd, $^{3}J_{HH}$ = 6.0 Hz, $^{4}J_{HH}$ = 1.6 Hz, 2H, C4-H), 7.04 (d, $^{3}J_{HH}$ = 8.5 Hz, 2H, C15-H), 6.84 (d, $^{3}J_{HH}$ = 8.6 Hz, 2H, C16-H), 5.89 (s, 2H, C19-H), 5.51 (s, 2H, C19-H'), 4.91 (s, 4H, C10-H), 4.69–4.64 (m, 8H, C8-H + C9-H), 2.40 (q, $^{3}J_{HH}$ = 7.4 Hz, 4H, C20-H), 1.09 (t, $^{3}J_{HH}$ = 7.4 Hz, 6H, C21-H). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$): δ/ppm = 195.7 (C17), 168.1 (C9), 162.8 (C6), 157.3 (C1), 155.4 (C11), 150.2 (C18), 149.9

(C5), 139.7 (C3), 133.9 (C12), 131.5 (C13), 128.8 (C19), 127.1 (C15), 124.2 (C2), 123.1 (C14), 111.0 (C16), 66.3 (C10); 64.7, 62.7 (C7 + C8), 23.5 (C20), 12.5 (C21). $^{195}Pt\{^{1}H\}$ ($CDCl_3$): δ/ppm = – 2275.

## $[PtCl_2(\kappa^2\textit{N}\text{-}L^{\textit{EF}})]$, 3 (Chart 8).

**Chart 8**. Structure of **3** (numbering refers to C atoms).

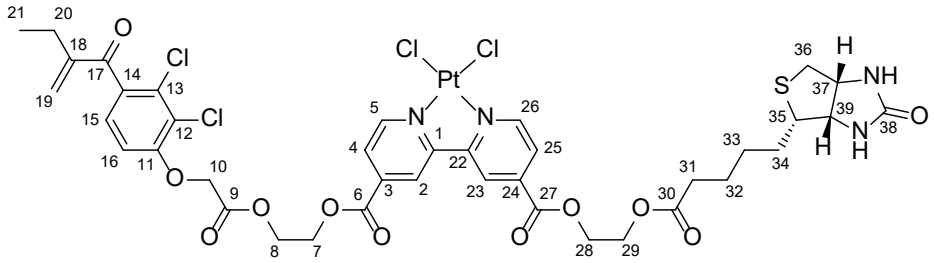



In a 50-mL round bottom flask, a suspension of *cis*-$[PtCl_2(\kappa\textit{S}\text{-DMSO})_2]$ (110 mg, 0.13 mmol) and $\mathbf{L}^{\textit{EF}}$ (53 mg, 0.13 mmol) in $CHCl_3$ (15 mL) was stirred at reflux for 13 hours. The orange solution was cooled to room temperature and extracted with $H_2O$ (3 x 15 mL). Volatiles were removed under vacuum from the organic phase and the residue was triturated in $Et_2O$. The suspension was filtered; the resulting orange solid was washed with $Et_2O$ and dried under vacuum (room T over $P_2O_5$). Yield: 130 mg, 94 %. Compound **3** is soluble in DMSO, MeCN, $CH_2Cl_2$ and $CHCl_3$, barely soluble in $Et_2O$ and MeOH,35 insoluble in hexane and water. Anal. Calcd. for $C_{44}H_{37}Cl_4FN_2O_{10}Pt$: C, 47.62; H, 3.36; N; 2.52. Found: C 46.60; H 3.39; N, 2.45. IR (solid state): $\tilde{u}/cm^{-1}$ = 3115w, 3070w, 3034w, 2970w, 2937w, 2878w, 1761m-sh ($\nu_{C9=O}$); 1738s, 1731s ($\nu_{C6=O} + \nu_{C27=O} + \nu_{C30=O}$), 1662m ($\nu_{C17=O}$), 1623w, 1584m ($\nu_{C18=C19}$), 1558w, 1517w, 1485w, 1469m, 1445-1436m, 1415s, 1384m, 1338w-sh, 1317m-sh, 1295s-sh, 1253s, 1234s, 1192s, 1144s, 1131s, 1121s, 1077s, 1068s, 1043m-sh, 1010m-sh, 1002m-sh, 940-928w, 896w, 870m, 839w, 803w, 762s, 724w, 700s, 663w. $^{1}H$ NMR ($CDCl_3$): δ/ppm = 9.73, 9.71 (d, $^{3}J_{HH}$ = 6.1 Hz, 1H/1H, C5-H + C26-H); 8.48, 8.44 (d, $^{4}J_{HH}$ = 1.1 Hz, 1H/1H, C2-H + C23-H); 7.99, 7.90 (dd, $^{3}J_{HH}$ = 6.0 Hz, $^{4}J_{HH}$ = 1.6 Hz, 1H/1H, C4-H + C25-H), 7.37–7.32 (m, 4H, C40-H + C41-H), 7.25–7.21 (m, 2H, C37-H + C42-H), 7.10–7.04 (m, 3H, C15-H + C34-H + C38-H), 6.83 (d, $^{3}J_{HH}$ = 8.5 Hz, 1H, C16-H), 5.89 (s, 1H, C19-H), 5.52 (s, 1H, C19-H'), 4.88 (s, 2H, C10-H), 4.70–4.66 (m, 4H, C7-H + C8-H), 4.65–4.56 (m, 3H, C28-H + C29-H), 4.55–4.48 (m, 1H, C29-H'), 3.82 (q, $^{3}J_{HH}$ = 7.2 Hz, 1H, C31-H), 2.42 (q, $^{3}J_{HH}$ = 7.3 Hz, 2H, C20-H), 1.54 (d, $^{3}J_{HH}$ = 7.3 Hz, 3H, C32-H), 1.10 (t, $^{3}J_{HH}$ =

7.4 Hz, 3H, C21-H). No change in the $^{1}$H NMR spectrum was observed after 14 h at room temperature. $^{13}C\{^{1}H\}$ NMR ($CDCl_3$): δ/ppm = 195.7 (C17), 173.9 (C30), 168.0 (C9); 162.8, 162.6 (C6 + C27), 159.6 (d, $^{1}J_{CF}$ = 249 Hz, C35); 157.3, 157.2 (C1 + C22), 155.4 (C11), 150.3 (C5 + C18 + C26), 141.5 (d, $^{3}J_{CF}$ = 8 Hz, C33), 139.5 (C3 + C24), 134.8 (C39), 134.1 (C12), 131.6 (C13), 130.8 (C37), 128.9–128.7 (m, C19 + C36 + C40 + C41), 128.0 (C42); 127.1, 126.9 (C4 + C25), 127.0 (C15), 123.8 (C38), 123.6–123.3 (m, C2 + C14 + C23), 115.4 (d, $^{2}J_{CF}$ = 24 Hz, C34), 111.0 (C16), 66.3 (C10); 64.8, 64.6 (C7 + C8), 62.7 (C28), 61.9 (C29), 45.0 (C31), 23.5 (C20), 18.0 (C32), 12.5 (C21). $^{19}F\{^{1}H\}$ NMR ($CDCl_3$): δ/ppm = – 117.2 ppm. $^{195}Pt\{^{1}H\}$ ($CDCl_3$): δ/ppm = – 2274.

## [$PtCl_2(\kappa^2$*N*-$L^{EB})$], 4 (Chart 9).

**Chart 9**. Structure of **4** (numbering refers to C atoms).



In a 25-mL round bottom flask, a suspension of *cis*-[$PtCl_2(\kappa$*S*-$DMSO)_2$] (27 mg, 0.063 mmol) and $\mathbf{L}^{EB}$ (55 mg, 0.063 mmol) in acetone (8 mL) was stirred at reflux for 13 hours, affording an orange solid and a yellow solution. The mixture was filtered; the solid was washed with acetone (2 mL) and $Et_2O$, and then dried under vacuum (room T over $P_2O_5$). Yield: 47 mg, 73 %. Shorter reaction times lead to incomplete conversion, while longer reaction times lowers the yield of the precipitate (23 h: 70%; 47 h: 11%). The productis soluble in DMSO, DMF and generally insoluble in other solvents.

Inan alternative procedure, a solution of *cis*-[$PtCl_2(\kappa$*S*-$DMSO)_2$] (15 mg, 0.036 mmol) and $\mathbf{L}^{EB}$ (31 mg, 0.037 mmol) in DMF (8 mL) was stirred at 80 °C for 24 hours; when complete conversion was achieved ($^{195}$Pt NMR), the volatiles were removed under vacuum. The residue was suspended in a $CHCl_3/Et_2O$ mixture under vigorous stirring and then filtered. The resulting yellow-orange solid was washed with $Et_2O$ then dried under vacuum. Yield: 24 mg, 59%. The isolated product does not apparently differ from that obtained using acetone as solvent (see above), based on analytical and spectroscopic analyses, but is soluble in $CH_2Cl_2$ and $CHCl_3$ other than

DMSO and DMF. Other procedures, i.e. the reactions of $\mathbf{L}^{EB}$ with $[PtCl_2(COD)]$[37] and $[Bu_4N]_2[PtCl_4]$[38] were not satisfactory.

Anal. Calcd. for $C_{39}H_{40}Cl_4N_4O_{11}PtS$: C, 41.21; H, 3.63; N, 5.05. Found: C, 41.09, H, 3.59; N, 4.99. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3340w-br, 3213w ($\nu_{NH}$); 3115w-sh, 3075w, 2966w, 2936w, 2877w-sh, 1762m-sh ($\nu_{C9=O}$), 1731s ($\nu_{C6=O}$ + $\nu_{C27=O}$ + $\nu_{C30=O}$), 1715s-sh, 1706s ($\nu_{C38=O}$), 1667m-sh ($\nu_{C17=O}$), 1659m-sh, 1624w-sh, 1585m ($\nu_{C18=C19}$), 1557w-sh, 1469-1436m, 1415m, 1384m, 1337w-sh, 1318m-sh, 1300m-sh, 1277s-sh, 1253s, 1235s, 1196s, 1143m, 1122m, 1109m, 1080-1070s, 1003m, 949w, 895w, 866w, 806w, 762s, 706m. $^1$H NMR (DMSO-$d_6$): δ/ppm = 9.76 (dd, $^3J_{HH}$ = 6.0 Hz, $^4J_{HH}$ = 1.9 Hz, 2H, C5-H + C26-H), 8.91 (d, $^3J_{HH}$ = 7.6 Hz, 2H, C2-H + C23-H), 8.25 (d, $^3J_{HH}$ = 6.0 Hz, 2H, C4-H + C25-H), 7.15 (s, 2H, C15-H + C16-H), 6.36 (s, 1H, NH), 6.32 (s, 1H, N'H), 6.01 (s, 1H, C19-H), 5.45 (s, 1H, C19-H'), 5.11 (s, 2H, C10-H), 4.66–4.57 (m, 6H, C7-H + C8-H + C28-H), 4.47–4.43 (m, 2H, C29-H), 4.28 (dd, $^3J_{HH}$ = 7.2, 5.5 Hz, 1H, C37-H), 4.09–4.04 (m, 1H, C39-H), 3.01–2.95 (m, 1H, C35-H), 2.78 (dd, $^2J_{HH}$ = 12.4 Hz, $^3J_{HH}$ = 5.1 Hz, 1H, C36-H), 2.54* (d, $^2J_{HH}$ = 12.7 Hz, 1H, C36-H'), 2.38–2.30 (m, 4H, C20-H + C31-H), 1.54 (d, $^3J_{HH}$ = 6.1 Hz, 3H, C32-H + C34-H), 1.46–1.36 (m, 1H, C34-H'), 1.34–1.22 (m, 2H, C33-H), 1.05 (t, $^3J_{HH}$ = 7.4 Hz, 3H, C21-H). *Partially superimposed on solvent residual peak. No changes in the $^1$H NMR spectrum were observed after 3 days at room temperature. $^{13}C\{^1H\}$ NMR (DMSO-$d_6$): δ/ppm = 194.8 (C17), 172.9 (C30), 168.1 (C9), 162.9 (C27), 162.6 (C38), 157.2 (C1 + C22), 155.1 (C11); 149.8, 149.7 (C5 + C26), 149.3 (C18), 139.7 (C3 + C24), 132.5 (C12), 129.5 (C13), 129.1 (C19), 127.4 (C15); 127.3, 127.2 (C4 + C25); 123.8, 123.6 (C2 + C23), 121.0 (C14), 111.7 (C16), 65.6 (C10); 64.*43*, 64.*39*, 62.4 (C7 + C8 + C28), 61.4 (C29), 61.0 (C39), 59.1 (C37), 55.3 (C35), 33.2 (C31); 28.0, 27.9 (C33 + C34), 24.4 (C32), 22.9 (C20), 12.3 (C21). $^{195}Pt\{^1H\}$ NMR (DMSO-$d_6$): δ/ppm = – 2277. $^1$H NMR ($CD_3CN$): δ/ppm = 9.76–9.69 (m, 2H, C5-H + C26-H); 8.62, 8.59 (d, $^4J_{HH}$ = 1.1 Hz) (2H; C2-H + C23-H), 8.10–8.04 (m, 2H, C4-H + C25-H), 6.99 (d, $^3J_{HH}$ = 8.6 Hz, 1H, C15-H), 6.93 (d, $^3J_{HH}$ = 8.6 Hz, 1H, C16-H), 5.92 (s, 1H, C19-H)*; 5.02, 4.97 (s, 2H, NH + N'H), 4.93 (s, 2H, C10-H), 4.65–4.57 (m, 6H, C7-H + C8-H + C28-H), 4.49–4.44 (m, 2H, C29-H),

4.39–4.33 (m, 1H, C37-H), 4.14 (s, 1H, C39-H), 3.05–2.99 (m, 1H, C35-H), 2.81 (dd, $^{2}J_{HH}$ = 12.8 Hz, $^{3}J_{HH}$ = 5.0 Hz, 1H, C36-H), 2.58 (d, $^{2}J_{HH}$ = 12.8 Hz, 1H, C36-H'), 2.39–2.31 (m, 4H, C20-H + C31-H), 1.65–1.26 (m, 6H, C32-H + C33-H + C34-H), 1.06 (t, $^{3}J_{HH}$ = 7.4 Hz, 3H, C21-H). *Signal for C19-H' hidden by $H_2O$. $^{1}$H NMR (acetone-$d_6$): δ/ppm 9.85 (pseudo-t, $^{3}J_{HH}$ = 6.0 Hz, 2H, C5-H + C26-H); 8.92, 8.88 (d, $^{4}J_{HH}$ = 1.2 Hz, 2H, C2-H + C23-H), 8.30–8.25 (m, 2H, C4-H + C25-H), 7.15 (m/AB system, $^{3}J_{HH}$ = 8.5 Hz, 2H, C15-H + C16-H), 5.98 (s, 1H, C19-H); 5.62, 5.60 (s-br, 1.4H*, NH + N'H), 5.51 (s, 1H, C19-H'), 5.10 (s, 2H, C10-H), 4.73 (s, 4H, C7-H + C8-H), 4.70–4.66 (m, 2H, C28-H), 4.58–4.53 (m, 2H, C29-H), 4.49–4.44 (m, 1H, C37-H), 4.27–4.23 (m, 1H, C39-H), 3.13–3.07 (m, 1H, C35-H), 2.89 (dd, $^{2}J_{HH}$ = 12.8 Hz, $^{3}J_{HH}$ = 5.3 Hz, C36-H)**, 2.67 (d, $^{2}J_{HH}$ = 12.6 Hz, 1H, C36-H'), 2.44–2.36 (m, 4H, C20-H + C31-H), 1.74–1.37 (m, 6H, C32-H + C33-H + C34-H), 1.09 (t, $^{3}J_{HH}$ = 7.5 Hz, 3H, C21-H). *Lower integral due to H/D exchange. **Partially superimposed on HDO signal. $^{1}$H (CDCl3): δ/ppm = 9.67 (2H), 8.72 (2H), 8.06 (2H), 7.10 (d, 1H), 6.88 (d, 1H), 5.91 (1H), 5.55 (1H), 4.91 (2H), 4.70-4.40 (m, 10H), 3.06 (1H), 2.87-2.74 (m, 2H), 2.42 (4H), 1.11 (t, 3H). All resonances are considerably broadened in $CDCl_3$.

**4. Biological studies**

Human ovarian carcinoma (A2780 and A2780cisR) cell lines were obtained from the European Collection of Cell Cultures. The human embryonic kidney (HEK-293) and human retinoblastoma (Y79) cell line were obtained from ATCC (Merck, Buchs, Switzerland). Penicillin streptomycin, RPMI 1640 GlutaMAX (where RPMI = Roswell Park Memorial Institute), and DMEM GlutaMAX media (where DMEM = Dulbecco's modified Eagle medium) were obtained from Life Technologies, and fetal bovine serum (FBS) was obtained from Merck. The cells were cultured in RPMI 1640 GlutaMAX (A2780, A2780cisR and Y79) and DMEM GlutaMAX (HEK-293) media containing 10% heat-inactivated FBS and 1% penicillin streptomycin at 37 °C and CO2 (5%). The A2780cisR cell line was routinely treated with cisplatin (2 μM) in the media to maintain cisplatin resistance. The cytotoxicity was determined using the 3-(4,5-dimethyl 2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT)

assay.[39] Cells were seeded in flat-bottomed 96-well plates as a suspension in a prepared medium (100 µL aliquots and approximately 4300 cells/well) and preincubated for 24 h. Stock solutions of compounds were prepared in DMSO and were diluted in medium. The solutions were sequentially diluted to give a final DMSO concentration of 0.5% and a final compound concentration range (0−200 µM). Cisplatin and RAPTA-C were tested as a positive (0−100 µM) and negative (200 µM) controls respectively. The compounds were added to the preincubated 96-well plates in 100 µL aliquots, and the plates were incubated for a further 72 h. MTT (20 µL, 5 mg/mL in Dulbecco's phosphate buffered saline) was added to the cells, and the plates were incubated for a further 4 h. The culture medium was aspirated and the purple formazan crystals, formed by the mitochondrial dehydrogenase activity of vital cells, were dissolved in DMSO (100 µL/well). The absorbance of the resulting solutions, directly proportional to the number of surviving cells, was quantified at 590 nm using a SpectroMax M5e multimode microplate reader (using SoftMax Pro software, version 6.2.2). The percentage of surviving cells was calculated from the absorbance of wells corresponding to the untreated control cells. The reported $IC_{50}$ values are based on the means from two independent experiments, each comprising four tests per concentration level.The compoundswere further estimated in Y79 cells and compared to topotecan – obtained from Prestwick Chemicals (Washington, DC). The compounds were tested at a fixed concentration of 10 µM with gambogic acid (GA) taken as a positive control and an equivalent volume of DMSO used as a negative control. The Y79 cells were seeded at an optimized density of 4.0 - 4.5 × $10^5$ cells per well after plating the drugs. Each plate was incubated at 37 °C in different timeline (i.e. 24, 48 and 72 h), and moreover, the hyperthermia stimulation were incubated at 42 °C for 1 h in the start and then at 37 °C for a total 72 h. The PrestoBlue fluorescent-based cell viability assay (Thermo Fisher Scientific, Switzerland) was selected as a readout. To estimate the cytotoxicity effect, the results were normalized to the controls for every plate and presented as scores, where a score of 1 corresponds to the average fluorescence intensity of the positive control wells, and indicates very efficacious compounds, which means high cytotoxicity to the Y79 cells. The scores are based on the mean from the independent experiments, each performed in duplicate.

**5. Enzyme activity assays**

The enzymatic activity of GST P1 (20 nM) was spectrophotometrically assayed at 340 nm at 37 ºC by measuring the CDNB – GSH (1-chloro-2,4-dinitrobenzene−glutathione) rate conjugation as a function of time, using a protocol reported earlier.[14a] The assay mixture contained 1 mM CDNB (1-chloro-2,4-dinitrobenzene) and 2 mM GSH (glutathione) of 0.1 M of potassium phosphate buffer (pH 6.5). The inhibitory efficacy of ethacrynic acid, **2** and **3** (dissolved in DMSO) was determined by recording the residual activity of GST P1 in the presence of variable concentrations of the analyzed compound (1 – 1000 µM). The enzymatic activity of COX-2 (0.25 UN) was fluorimetrically assayed at 576nm/586nm and at 25 ºC by measuring the rate of arachidonic acid (ARA) conjugation with COX-2 as a function of time (COX-2 assay kit from Cayman Chemical Company, Ann Arbor, MI, USA). The assay mixture contained 25 µM ADHP, 5 µM haemin and 37.5 µM ARA in 0.1 M of tris-HCl buffer (pH = 8). The inhibitory efficacy of flurbiprofen and **3** was determined by recording the residual activity of COX-2 in the presence of variable concentrations of the analyzed compound (35 – 3200 µM). The $IC_{50}$ value for each compound was obtained using GraphPad Prism 7 software.All the inhibitor assays were performed at least in triplicate, for both enzymes.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## Supporting Information Available

X-ray crystallography, solution stability studies, NMR/IR spectra. CCDC reference number 1999305 (**1**) contains the supplementary crystallographic data. These data can be obtained free of charge from the Cambridge Crystallographic Data centre via www.ccdc.cam.ac.uk/data_request/cif..

## References

1 (a) I. A. Riddell and S. J. Lippard, Met. Ions Life Sci. 2018, 18, 1–42. (b) F. Arnesano, M. I. Nardella, G. Natile, Coord. Chem. Rev. 2018, 374, 254–260. (c) M. G. Apps, E. H. Y. Choi and N. J. Wheate, Endocrine-Related Cancer 2015, 22, R219–R233. (d) D. Gibson, Dalton Trans., 2009, 10681–10689.

2 (a) R. Oun, Y. E. Moussa, N. J. Wheate, Dalton Trans., 2018, 47 , 6645–6653. (b) (d) Z. H. Siddik, Oncogene 2003, 22, 7265–7279.

3 (a) T. C. Johnstone, K. Suntharalingam, and S. J. Lippard, Chem. Rev. 2016, 116, 3436 − 3486. (b) Y. Min, C.-Q. Mao, S. Chen, G. Ma, J. Wang, Y. Liu, Angew. Chem. 2012, 124, 6846 –6851.

4 (a) A. Khoury, K. M. Deo, J. R. Aldrich-Wright, J. Inorg. Biochem. 2020, 207, 111070. (b) M. Hanif, C. G. Hartinger, Fut. Med. Chem. 2018, 10, 615-617.

5 (a) F. Caruso, M. Rossi, A. Benson, C. Opazo, D. Freedman, E. Monti, M. B. Gariboldi, J. Shaulky, F. Marchetti, R. Pettinari, and C. Pettinari, J. Med. Chem. 2012, 55, 1072-1081. (b) L. Biancalana, L. K. Batchelor, A. De Palo, S. Zacchini, G. Pampaloni, P. J. Dyson and F. Marchetti, Dalton Trans., 2017, 46, 12001–12004. (c) W. Kandioller, E. Balsano, S. M. Meier, U. Jungwirth, S. Göschl, A. Roller, M. A. Jakupec, W. Berger, B. K. Keppler, and C. G. Hartinger, Chem. Commun., 2013, 49, 3348-3350. (d) M. Patra and G. Gasser, Nat. Chem. Rev. 2017, 1, 1-12. (e) A. Pettenuzzo, D. Montagner, P. McArdle and L. Ronconi, Dalton Trans. 2018, 47, 10721-10736.

6 (a) M. Patra, T. C. Johnstone, K. Suntharalingam, S. J. Lippard, Angew. Chem. Int. Ed. 2016, 55, 2550 –2554. (b) M. E. Cucciolito, F. De Luca Bossa, R. Esposito, G. Ferraro, A. Iadonisi, G. Petruk, L. D' Elia, C. Romanetti, S. Traboni, A. Tuzi, D. M. Monti, A. Merlin F. Ruffo, Inorg. Chem. Front., 2018, 5, 2921–2933. (c) F. P. Intini, J. Zajac, V. Novohradsky, T. Saltarella, C. Pacifico, V. Brabec, G. Natile, and J. Kasparkova, Inorg. Chem. 2017, 56, 1483−1497. (d) N. Margiotta, N. Denora, R. Ostuni, V. Laquintana, A. Anderson, S. W. Johnson, G. Trapani, and G. Natile, J. Med. Chem. 2010, 53, 5144–5154. (e) A. I. Matesanz, J. Perles and P. Souza, Dalton Trans., 2012,41, 12538-12547. (f) A. Upadhyay, S. Gautam, V. Ramu, P. Kondaiah, A. R. Chakravarty, Dalton Trans., 2019, 48, 17556–17565.

7 Selected references: (a) D. Gibson, J. Inorg. Biochem. 2019, 191, 77–84. (b) E. Petruzzella, J. P. Braude, J. R. Aldrich-Wright, V. Gandin and D. Gibson, Angew. Chem. Int. Ed. 2017, 56, 11539 – 11544. (c) M. Ravera, E. Gabano, M. J. McGlinchey, D. Osella, Inorg. Chim. Acta 2019, 492, 32–47. (d) T. Yempala, T. Babu, S. Karmakar, A. Nemirovski, M. Ishan, V. Gandin and D. Gibson, Angew. Chem. Int. Ed. 2019, 58, 18218 –18223. (e) S. Jin, Y. Guo, D. Song, Z. Zhu, Z. Zhang, Y. Sun, T. Yang, Z. Guo and X. Wang, Inorg. Chem. 2019, 58, 6507−6516.

8 Selected references: (a) R. G. Kenny and C. J. Marmion, Chem. Rev. 2019, 119, 1058- 1137. (b) H. Chen, F. Chen, W. Hu, S. Gou, J. Inorg. Biochem. 2018, 180, 119–128. (c) R. Zhang, X.-Q. Song, R.-P. Liu, Z.-Y. Ma and J.-Y. Xu, J. Med. Chem. 2019, 62, 4543-4554. (d) W. H. Ang, I. Khalaila, C. S. Allardyce, L. Juillerat-Jeanneret and P. J. Dyson, J. Am. Chem. Soc. 2005, 127, 1382-1383. (e) N. Muhammad, N. Sadia, C. Zhu, C. Luo, Z. Guo and X. Wang, Chem. Commun. 2017, 53, 9971-9974. (f) X. Qin, L. Fang, F. Chen and S. Gou, Eur. J. Med. Chem. 2017, 137, 167-175. (g) S. Dhar and S. J. Lippard, PNAS 2009, 106, 22199–22204.

9 S. Ding, X. Qiao, G. L. Kucera and U. Bierbach, Chem. Commun., 2013, 49, 2415-2417.
10 (a) D. Pucci, A. Bellusci, S. Bernardini, R. Bloise, A. Crispini, G. Federici, P. Liguori, M. F. Lucas, N. Russo, A. Valentini, Dalton Trans., 2008, 5897–5904. (b) E. Paunescu, S. McArthur, M. Soudani, R. Scopelliti, P. J. Dyson, Inorg. Chem. 2016, 55, 1788−1808.
11 (a) J. D. Hayes, J. U. Flanagan, I. R. Jowsey, Ann. Rev. Pharmacol. Toxicol. 2005, 45, 51–88. (b) W. H. Ang, L. J. Parker, A. De Luca, L. Juillerat-Jeanneret, C. J. Morton, M. Lo Bello, M. W. Parker and P. J. Dyson, Angew. Chem. Int. Ed. 2009, 48, 3854-3857.
12 (a) K. M. Knights, A. A. Mangoni, J. O. Miners, Expert Rev. Clin. Pharmacol. 2010, 3, 769-776. (b) A. A. M. Abdel-Aziz, A. A. Al-Badr, G. Abdel Hafez, Profiles of Drug Substances, Excipients, and Related Methodology 2012, 37, 113-181.
13 (a) H. P Lesch, M. U. Kaikkonen, J. T. Pikkarainen, S. Ylä-Herttuala, Expert Opin. Drug Deliv. 2010, 7 (5). (b) S. Maiti, P. Paira, Eur. J. Med. Chem. 2018, 145, 206-223.
14 Ethacrynic acid: (a) E. Păunescu, M. Soudani, P. Martin, R. Scopelliti, M. Lo Bello, P.J. Dyson, Organometallics 2017, 36, 3313-3321. (b) E. Păunescu, M. Soudani, C. M. Clavel, P. J. Dyson, J. Inorg. Biochem. 2017, 175, 198–207. See also reference [8d].
15 NSAIDs, selected references: (a) Q. Cheng, H. Shi, H. Wang, Y. Min, J. Wang and Y. Liu, Chem. Commun., 2014, 50, 7427-7430. (b) I. Ott, B. Kircher, C. P. Bagowski, D. H. W. Vlecken, E. B. Ott, J. Will, K. Bensdorf, W. S. Sheldrick and R. Gust, Angew. Chem. Int. Ed. 2009, 48, 1160 –1163. (c) P. Mandal, B. K. Kundu, K. Vyas, V. Sabu, A. Helen, S. S. Dhankhar, C. M. Nagaraja, D. Bhattacherjee, K. P. Bhabak and S. Mukhopadhyay, Dalton Trans., 2018, 47, 517–527. (d) J. Tan, C. Li, Q. Wang, S. Li, S. Chen, J. Zhang, P. C. Wang, L. Ren and X.-J. Liang, Mol. Pharmaceutics 2018, 15, 1724−1728 (flurbiprofen).
16 Biotin: (a) B. Bertrand, M. A. O'Connell, Z. A. E. Waller, M. Bochmann, Chem. Eur. J. 2018, 24, 3613–3622 (b) L. Côrte-Real, B. Karas, A. R. Bras, A. Pilon, F. Avecilla, F. Marques, A. Preto, B. T. Buckley, K. R. Cooper, C. Doherty, M. H. Garcia and A. Valente, Inorg. Chem. 2019, 58, 9135−9149. (c) W. Hu, L. Fang, W. Hua, S. Gou, J. Inorg. Biochem. 2017, 175, 47–57. (d) D. Plazuk, J. Zakrzewski, M. Salmain, A. Błauz, B. Rychlik, P. Strzelczyk, A. Bujacz, G. Bujacz, Organometallics 2013, 32, 5774−5783.
17 (a) J. Yoo, J.-H. Kim, Y. S. Sohn, Y. Do, Inorg. Chim. Acta, 1997, 263, 53-60. (b) M. Benedetti, D. Antonucci, S. A. De Pascali, C. R. Girelli, F. P. Fanizzi, J. Organomet. Chem. 714, 2012, 60-66.
18 J. A. Platts, M. Ravera, E. Gabano, M. Sardi, S. Bianco, D. Osella, Eur. J. Inorg. Chem. 2012, 5625–5631.
19 (a) G. Ma, F. Huang, X. Pu, L. Jia, T. Jiang, L. Li, Y. Liu, Chem Eur J 2011, 17, 11657-11666 (b) L. Biancalana, L. K. Batchelor, P. J. Dyson, S. Zacchini, S. Schoch, G. Pampaloni, F. Marchetti, New J. Chem., 2018, 42, 17453-17463 (c) S. Wimmer, P. Castan, F. L. Wimmer, N. P. Johnson, Inorg. Chim. Acta 1988, 142, 13-15.
20 M. Ángeles Martínez, M. Pilar Carranza, A. Massaguer, L. Santos, J. A. Organero, C. Aliende, R. de Llorens, I. Ng-Choi, L. Feliu, M. Planas, A. M. Rodríguez, B. R. Manzano, G. Espino, F. A. Jalòn, Inorg. Chem. 2017, 56, 13679-13696.
21 (a) Y.-g. Sun, D. Sun, W. Yu, M.-c. Zhu, F. Ding, Y.-n. Liu, E.-j. Gao, S.-j. Wang, G. Xiong, I. Dragutan, V. Dragutan, Dalton Trans., 2013, 42, 3957-3967. (b) R. Křikavová, J. Vančo, T. Šilha, J. Marek, Z. Trávníček, J. Coord. Chem. 2016, 69, 2422–2436.

[22] (a) J. Yoo, Y. Soo Sohn, Y. Do, J. Inorg. Biochem., 1999, 73, 187-193. (b) C. Perez-Arnaiz, J. Leal, N. Busto, M. C. Carrion, A. R. Rubio, I. Ortiz, G. Barone, B. Díaz de Greñu, J. Santolaya, J. M. Leal, M. Vaquero, F. A. Jalon, B. R. Manzano, B. García, Inorg. Chem. 2018, 57, 6124−6134.

[23] B. He, X. Tong, L. Wang, Q. Wang, H. Ye, B. Liu, X. Hong, L. Tao and A. L. Harris, Clin Cancer Res 2009, 15, 5803-5810.

[24] N. A. Krucher, A. Zygmunt, N. Mazloum, S. Tamrakar, J. W. Ludlow, M. Y. Lee, Oncogene 2000, 19, 5464-5670.

[25] H. E. Grossniklaus, Am. J. Ophthalmol. 2014, 158, 875–891.

[26] F. L. Muniera, M. Beck-Popovic, G. L.Chantada, D. Cobrinik, T. T. Kiveläh, D. Lohmann, P. Maeder, A. C. Moll, A. Montero Carcaboso, A. Moulina, P. Schaiquevich, C. Bergin, P. J. Dyson, S. Houghton, F. Puccinelli, Y. Vial, M.-C. Gaillard, C. Stathopoulos, Prog. Retin. Eye Res., 2019, 73, 100764.

[27] Ethacrynic acid (CAS 58-54-8): 2,3-dichloro-4-(2-methylene-1-oxobutyl)phenoxyacetic acid. Flurbiprofen (CAS 5104-49-4): (±)-2-fluoro-α-methyl-(1,1'-biphenyl)-4-acetic acid (racemic mixture). Biotin (CAS 58-85-5): 5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid.

[28] a) J. H. Price, A. N. Williamson, R. F. Schramm and B. B. Wayland, *Inorg. Chem*., 1972, **11**, 1280–1284; b) see note to the procedure in ref. 19b.

[29] B. Neises and W. Steglich, Angew. Chem. Int. Ed. 1978, 17, 522-524.

[30] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, Organometallics 2010, 29, 2176–2179.

[31] R. K. Harris, E. D. Becker, S. M. Cabral De Menezes, R. Goodfellow and P. Granger, Pure Appl. Chem. 2001, 73, 1795–1818.

[32] W. Willker, D. Leibfritz, R. Kerssebaum and W. Bermel, Magn. Reson. Chem. 1993, 31, 287-292.

[33] F. Menges, "Spectragryph - optical spectroscopy software", Version 1.2.9, @ 2018, http://www.effemm2.de/spectragryph.

[34] A colourless oily residue was always obtained when solutions of the title compound were taken to dryness above room temperature.

[35] The compound undergoes trans-esterification in methanol ($CH_3OH$ or $CD_3OD$), *vide infra*.

[36] Despite a different colour in the solid state, IR and $^1H$ NMR (DMSO-$d_6$) spectra of products obtained by the two procedures (*cis*-[$PtCl_2$(κ*S*-DMSO)$_2$]/L and $K_2[PtCl_4]$/L) were identical. They both gave yellow solutions in DMSO.

[37] E. Gabano, E. Perin, C. Fielden, J. A. Platts, A. Gallina, B. Rangone and M. Ravera, Dalton Trans. 2017, 46, 10246-10254.

[38] F. Bagnoli, D. Belli Dell'Amico, F. Calderazzo, U. Englert, F. Marchetti, A. Merigo and S. Romello, J. Organomet. Chem. 2001, 622, 180-189.

[39] T. Mosmann, J. Immunol. Methods 1983, 65, 55–63.