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(54) Title: POLYELECTROPHILIC METALATED HETEROCYCLES DERIVED FROM 2-(PYRIDIN-2-YL)IMIDAZO[1,2-A]PYRIDINE AND USE THEREOF AS CHEMOTHERAPEUTIC AGENTS

(57) Abstract: The present invention is directed to new compounds based on polyelectrophilic metalated heterocycles derived from 2-(pyridin-2-yl)imidazo[1,2-a]pyridine, to their obtaining procedure, as well as to their use as chemotherapeutic agents, in particular against tumours sensitive or resistant to other chemotherapeutic agents, such as cisplatin.

## DESCRIPTION

POLYELECTROPHILIC METALATED HETEROCYCLES DERIVED FROM 2-(PYRIDIN-2-  
YL)IMIDAZO[1,2-A]PYRIDINE AND USE THEREOF AS CHEMOTHERAPEUTIC AGENTS

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### FIELD OF THE INVENTION

The present invention is comprised among therapeutic treatments intended for various cancer types, with a special emphasis on tumors resistant to cisplatin or analogs in clinical use. In particular, the invention relates to aromatic heterocycles capable of incorporating a doubly electrophilic square planar metal center and at least one  $sp^3$ -hybridized carbon center-based electrophile capable of undergoing second-order nucleophilic substitution reactions. The synthesized molecules incorporate at least three electrophilic centers, such that lesions generated by nucleophilic substitution on bases located in two DNA strands (interstrand cross linking), as well as lesions in one and the same strand (intrastrand cross linking), give rise to heavily deformed adducts, such that the lesions thus generated are more abundant and harder to repair.

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### BACKGROUND

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Cancer is one of the leading causes of death in developed countries. According to the World Health Organization (WHO), 10 million people died in the year 2020 from malignant tumors. Likewise, in the year 2020, approximately 20 million new cases of cancer were diagnosed, and by the year 2040 this figure is expected to exceed 30 million [Ferlay, J. *et al.* Global Cancer Observatory: Cancer Today. International Agency for Research on Cancer; 2020 (<https://gco.iarc.fr/today>, accessed on 10 September 2021)]. Despite a 1.3% reduction in cancer mortality in recent years, tumors continue to be the leading cause of death in men and the second leading cause of death in women. This data clearly demonstrates the need to develop new therapeutic strategies that are more effective in the fight against cancer.

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The use of agents which damage cellular DNA, predominantly radiation or genotoxic molecules, is one of the most widely used strategies in the fight against malignant tumors. This therapeutic strategy has been used with some success for several decades and continues to be key in the treatment of various cancer types. Electrophilic molecules based on  $sp^3$ -hybridized carbon atoms stand out among small molecules capable of generating covalent adducts with DNA, permanently deforming it (at least in the first instance) [Larrañaga, O. *et al.*,

*ChemPhysChem.*, 2017, 18, 3390-3401] (Figure 1A) or in square planar metal compounds which incorporate a platinum atom in oxidation state (+2) [De Cózar, A. *et al.*, *ChemPhysChem.*, 2016, 17, 3932-3947] (Figure 1B). In both cases, the chemical process consists of second-order nucleophilic substitutions ( $S_N2$ ) with retention (square planar metal electrophiles) or inversion (electrophiles centered on  $sp^3$  carbons) of configuration. In both cases, the nucleophiles are the DNA bases guanine (G) and, to a lesser extent, adenine (A).

The first group includes nitrogen mustards [More, G. S. *et al.*, *Anticancer Agents Med. Chem.*, 2019, 19, 1080-1102], among which drugs such as chlorambucil, cyclophosphamide, ifosfamide, melphalan, or bendamustine stand out.

In the case of the second group, at present, it includes various drugs based on platinum complexes in oxidation state (+2) the clinical use of which is approved in various countries [Dilruba, S. *et al.*, *Cancer Chemother. Pharmacol.*, 2016, 77, 1103-1124]. Therefore, cisplatin, carboplatin, and oxaliplatin are in clinical use worldwide, whereas nedaplatin and miriplatin are used in Japan, heptaplatin in South Korea, and lobaplatin (isomer mixture) in China. Several prodrugs in which platinum has an oxidation state (+4), which transitions to the (+2) state *in vivo*, are also in clinical trials [Mi, Q. *et al.*, *Int. J. Med. Phys. Clin. Eng. Rad. Oncol.*, 2018, 7, 231-247]. Pt(+2)-based drugs, which have been very successful in chemotherapy [Kelland, L. *et al.*, *Nat. Rev. Cancer.*, 2007, 7, 573-584], have been selected from among thousands of potential candidates [Kerpel-Fronius S., *Cisplatin and its Analogues for Cancer Chemotherapy*, in *Analogue-based Drug Discovery*, Fischer J, Ganellin CR (eds.), Wiley-VCH: Weinheim., 2006, pp 385-394]. Among all these compounds, cisplatin [Thomas, K. *et al.*, *Nature*, 1965, 205, 698-699] (CisPt,  $\text{cis-[Pt(NH}_3\text{)}_2\text{Cl}_2\text{]}$ ) was the first metal compound of this type discovered and approved by the Food and Drug Administration (FDA) in 1978, being active in solid tumors such as ovarian, testicular, bladder, colorectal, and head and neck cancers [Lebwohl, D. *et al.*, *Eur. J. Cancer.*, 1998, 34, 1522-1534]. However, CisPt presents a series of problems among which its nephrotoxicity, neuropathy, and ototoxicity, as well as its capacity to cause nausea and vomiting, should be highlighted. Furthermore, many tumors are either intrinsically resistant to CisPt or may develop this resistance gradually after an initially favorable response [Galluzzi, L. *et al.*, *Oncogene*, 2012, 31, 1869-1883]. In fact, the other platinum derivatives mentioned above were developed in order to at least partially reduce these drawbacks. Papers on platinum derivatives containing aromatic mono- and poly-aza-heterocycles have also been published [Park, G. Y. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2012, 109, 11987-11992. Tu, C. *et al.*, *Inorg. Chim. Acta.*, 2004, 357, 95-102. Fachetti, G. *et al.*, *Bioorg. Med. Chem. Lett.*, 2019, 29, 1257-1263].

However, most tumors develop adaptive mechanisms for repairing the DNA lesions thus generated such as non-homologous and/or homologous recombination, base excision repair, and alkyl transferase, topoisomerase, endo- or exonuclease activation, among others, generating resistance to Pt-derived molecules [Lebwohl, D. *et al.*, Eur. J. Cancer., 1998, 34, 1522-1534., Galluzzi, L. *et al.*, Oncogene, 2012, 31, 1869-1883]. Moreover, these chemotherapeutic agents present a high capacity for off-target binding to sulfur-rich molecules and/or proteins, such as glutathione in its reduced form (GSH), or to amino acids such as cysteine, methionine, or metallothioneins, inhibiting their action on DNA [Moore, J. K. *et al.*, Mol. Cell. Biol. 1996, 16, 2164-2173. Davis, J. A. *et al.*, Transl. Cancer Res., 2013, 2, 130 - 143. Pegg, E. A. *et al.*, Chem. Res. Toxicol., 2011, 24, 618 -639. Bjoras, M. *et al.*, Cold Spring Harb. Perspect. Biol. 2013, 5 a0125B3. Lindahl, T. *et al.*, Science, 1999, 286, 1897-1905. Karanam, K. *et al.*, Mol. Cell., 2012, 47, 320-329].

CisPt and related drugs work by similar mechanisms of action and cause cancer cell death by irreversible damage to nuclear DNA [Thomas, K. *et al.*, Nature 1965, 205, 698-699]. This process consists of several steps or chemical reactions including (i) cellular uptake by passive diffusion and active transport (for example, by means of copper transporter CTR1 or by means of organic cation transporters OCT1-3); (ii) intracellular activation by reaction with water molecules in the presence of low concentrations (10-20 mM) of chloride ions, such as cis-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl(OH<sub>2</sub>)]<sup>+</sup> and cis-Pt(NH<sub>3</sub>)<sub>2</sub>(OH<sub>2</sub>)<sub>2</sub><sup>2+</sup>: electrophilic compounds that bind to DNA); and, finally, (iii) nuclear uptake and interaction with the N7 nitrogen atom of the DNA nitrogenous bases G and A, preferably forming bonds between two guanine residues [Thomson, A. J. *et al.*, Prog. Nucleic Acid Res. Mol. Biol. 1979, 22, 71-133. Lippard, S. J., Science, 1982, 218, 1075-1082]. Thus, it has been determined in *in vitro* assays [Fichtinger-Schepman, A. M. J. *et al.*, Biochemistry, 1985, 24, 707-713] that CisPt generates 47-50% adducts on a single strand (intrastrand) comprising two contiguous cis-[Pt(NH<sub>3</sub>)<sub>2</sub>{d(GpG)}] (or cis-GG) type G units, together with 23-28% of intrastrand adducts which incorporate two consecutive cis-[Pt(NH<sub>3</sub>)<sub>2</sub>{d(ApG)}] (or cis-AG) type A and G units, as well as 8-10% of intrastrand adducts formed by two Pt-G bonds separated by another base X in the form of cis-[Pt(NH<sub>3</sub>)<sub>2</sub>{d(GpXpG)}] (or cis-GXG); finally, approximately 2-3% of Cis-Pt DNA adducts consist of bonds to a single G base. An *in vitro* study carried out with a CisPt analog [Eastman, A., Biochemistry, 1986, 25, 3912-3915] showed similar results. Moreover, studies performed with *ex vivo* samples from cancer patients [Fichtinger-Schepman, A. M. *et al.*, Cancer Res., 1987, 47, 3000-3004] yielded similar results with cis-GG, cis-AG, and cis-GXG proportions of 65, 22, and 13%, respectively. These results indicate that lesions involving two DNA strands

(interstrand crosslinks), which are much more difficult for the cancer cell to repair [Deans, A. J. *et al.*, Nat. Rev. Cancer, 2011, 11, 467-480. McHugh, P. J. *et al.*, Lancet Oncol., 2001, 2, 483-490.], account for a very low percentage of the total number of CisPt-DNA adducts, being lower than 5% [Lippard, S. J., *Science*, 1982, 218, 1075-1082] (Figure 2A). In the case of nitrogen mustards and natural agents, such as mitomycin C, this percentage is 5-10% [Gargiulo, D., *Nucleic Acids Symp. Ser.* 1995, 169-170].

There is a limited number of hybrid chemotherapeutic agents [Chen, Y. *et al.*, Front. Pharmacol. 2018, 9, 1453. Qin, X., Eur. J. Med. Chem., 2017, 137, 167-175] that combine the characteristics of a nitrogen mustard and a CisPt analog. An example is the hybrid of chlorambucil and platinum complexes in which the metal center is in oxidation state (+4), which has shown interesting properties, with increased *in vitro* activity with respect to its two components [Qin, X., Eur. J. Med. Chem., 2017, 137, 167-175], particularly with CisPt-resistant cell lines.

Likewise, in recent years there has been a growing interest in metallodrugs incorporating various metal centers in addition to Pt(II, IV), such as Ti (titanocenyl dichloride, budotitane), Cu (cassiopeins, elesclomol), Ru (NAMI-A, KP1339/IT139), As (arsenic trioxide), Ga (KP46, Ga(III) nitrate, Ga(III) trichloride), and Au (auranofin, sodium aurothiomalate), all of which are in various stages of clinical trials [Peña, Q. *et al.*, Chem. Soc. Rev. 2022, 51, 2544-2582; Anthony, E. J. *et al.*, Chem. Sci. 2020, 11, 12888-12917; Mjos, K. D.; Orvig, C. Chem. Rev. 2014, 114, 4540-4563]. It should also be noted that these compounds with metal centers are not without problems such as low solubility (budotitane), low activity (NAMI-A), cardiotoxicity (cassiopeins), hepatotoxicity and nephrotoxicity (titanocenyl dichloride), and low biodistribution (Ga(III) trichloride).

Based on the foregoing, it should be noted that the behavior of neutral molecules or S<sub>N</sub>2-type polyelectrophilic salts incorporating an electrophilic square planar metal center coordinated with respect to fused aromatic nitrogenous heterocycles and at least one benzyl-type electrophile as chemotherapeutic agents is unknown.

## BRIEF DESCRIPTION OF THE INVENTION

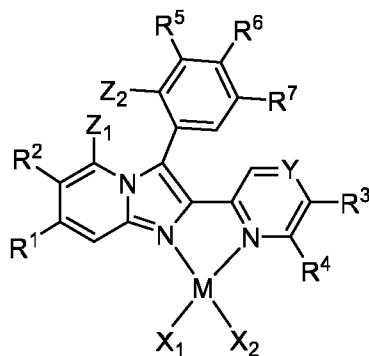
The authors of the present invention have developed a series of compounds the structure of which is based on aromatic heterocycles which incorporate a doubly electrophilic square

planar metal center and at least one  $sp^3$ -hybridized carbon center-based electrophile capable of undergoing second-order nucleophilic substitution reactions.

The criteria to be followed when designing the compounds of the invention are:

- 5        a) Increase of electrophilic positions  $S_N2$  above two, with the presence of metal electrophiles such as Pt(II) and chloromethyl groups. The viability of these second-order nucleophilic substitution processes is shown in Figure 3, in which computational studies of the transition state geometries, as well as the activation energies, have been included;
- 10       b) inclusion of intercalating heterocycles capable of interacting with DNA bases by means of pi stacking;
- c) inclusion of basic nitrogenous centers susceptible to *in vivo* transport through organic cation transporters (OCTs);
- 15       d) inclusion of fluorophores that allow (i) *in vivo* tracking of the intracellular distribution of the chemotherapeutic agents; (ii) diagnosis of the permeability of the analyzed (tumor or healthy) cells to these agents, in line with one of the areas of emergent healthcare technologies identified by the European Union [European Innovation Council. *Identification of emergent technologies and breakthrough innovations*. EIC Working paper 1/2022, DOI: 10.2826/06288].
- 20       Based on the experimental data provided herein, it has been observed that the compounds of the invention, compared to cisplatin, significantly reduce cell viability in all the tested tumor lines, such as extra- and intrahepatic cholangiocarcinoma, ovarian cancer, and breast cancer lines, without affecting the viability of normal cells, making these compounds powerful, in addition to being selective, with respect to tumor cells.
- 25       Furthermore, it has been demonstrated that these compounds increase the rate of cell death, in addition to causing greater DNA damage, in said tumor lines compared to the data obtained with cisplatin.
- 30       Moreover, a decrease in the volume and weight of tumors treated with the compounds of the invention has been observed in *in vivo* assays compared to tumors treated with cisplatin.

Therefore, a first aspect of the invention relates to a compound of formula (1):



(1)

or a salt, solvate, or stereoisomer thereof;

wherein:

M is a metal center selected from Pt(II), Cu(II), and Au(III), with the proviso that when M is Au(III), formula (I) incorporates an anion selected from Cl<sup>-</sup>, PF<sub>6</sub><sup>-</sup>, and R<sub>8</sub>C(O)O<sup>-</sup>, wherein R<sub>8</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group;

X<sub>1</sub> and X<sub>2</sub> are both chloride or together form a group selected from (·O-C(O)-C(O)-O<sup>-</sup>), (·O-C(O)-C(R<sub>9</sub>)(R<sub>10</sub>)-C(O)O<sup>-</sup>), and (·O-C(O)-CH(R<sub>11</sub>)O<sup>-</sup>), wherein the oxygen atoms (O<sup>-</sup>) are attached to the metal center, and wherein R<sub>9</sub> and R<sub>10</sub> are independently a C<sub>1</sub>-C<sub>6</sub> alkyl group or both form a C<sub>4</sub>-C<sub>6</sub> cycloalkyl group together with the carbon to which they are linked, and R<sub>11</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group;

Z<sub>1</sub> is hydrogen or can form a single covalent bond together with Z<sub>2</sub>;

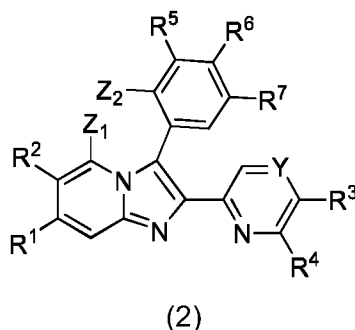
Z<sub>2</sub> is hydrogen, halogen, or can form a single covalent bond together with Z<sub>1</sub>, or can be attached to R<sub>5</sub> forming an aryl group optionally substituted by at least one C<sub>1</sub>-C<sub>6</sub> alkyl group, halogen, or OH;

Y is N or CR<sub>12</sub>, wherein R<sub>12</sub> is H or a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen, a hydroxyl, or a leaving functional group;

R<sub>1</sub>-R<sub>4</sub> are selected independently from H; a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen, a hydroxyl, or a leaving functional group;

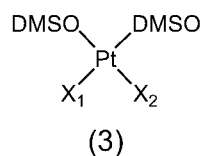
R<sub>5</sub>-R<sub>7</sub> are all H, or grouped in R<sub>5</sub>-R<sub>6</sub> pairs, or R<sub>6</sub>-R<sub>7</sub> can form a C<sub>6</sub> aryl group optionally substituted by at least one C<sub>1</sub>-C<sub>6</sub> alkyl group, halogen, or hydroxyl.

A second aspect of the invention relates to a method for the preparation of a compound of formula (1) as defined above, which comprises reacting a compound of formula (2):



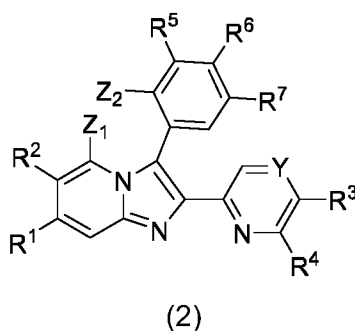
wherein Y, Z<sub>1</sub>, Z<sub>2</sub>, R<sub>1</sub>-R<sub>7</sub> have the meaning defined above,

with a metal salt selected from gold trichloride (AuCl<sub>3</sub>), copper dichloride (CuCl<sub>2</sub>), and platinum salts of formula (3):



wherein X<sub>1</sub> and X<sub>2</sub> have the meaning defined above and DMSO represents a dimethyl sulfoxide ligand.

An additional aspect of the invention relates to the compound of formula (2), precursor of the compound of formula (1):



or a salt, solvate, or stereoisomer thereof;

wherein,

$Z_1$  is hydrogen or can form a single covalent bond together with  $Z_2$ ;

$Z_2$  is hydrogen, halogen, or can form a single covalent bond together with  $Z_1$ , or can be attached to  $R_5$  forming an aryl group optionally substituted by at least one  $C_1$ - $C_6$  alkyl group, halogen, or OH;

$Y$  is N or  $CR_{12}$ , wherein  $R_{12}$  is H or a  $C_1$ - $C_6$  alkyl group, optionally substituted at the end of the chain by a halogen, a hydroxyl, or a leaving functional group;

$R_1$ - $R_4$  are selected independently from H and a  $C_1$ - $C_6$  alkyl group, optionally substituted at the end of the chain by a halogen, a hydroxyl, a  $C(O)OMe$  group, or a leaving functional group;

$R_5$ - $R_7$  are all H, or grouped in  $R_5$ - $R_6$  pairs, or  $R_6$ - $R_7$  can form a  $C_6$  aryl group optionally substituted by at least one  $C_1$ - $C_6$  alkyl group, halogen, or hydroxyl;

with the proviso that the compound for which  $Z_1=Z_2=R^1=R^2=R^3=R^4=R_5=R_6=R^7=H$  and  $Y=CR^{12}=CH$ , is not included.

Another aspect of the invention relates to a pharmaceutical composition comprising a compound of formula (1) as defined above, or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, and a pharmaceutically acceptable excipient or vehicle thereof.

An additional aspect of the invention relates to a compound of general formula (1) as defined above, or a pharmaceutically acceptable salt, or stereoisomer, or solvate, or prodrug thereof, or a pharmaceutical composition as defined above, for use as a medicinal product.

A last aspect of the invention relates to a compound of formula (1) as defined above, or a pharmaceutically acceptable salt, or stereoisomer, or solvate, or prodrug thereof, or a pharmaceutical composition as defined above, for use in the treatment of a cancerous condition, hematological and hepatic malignancy, proliferative diseases, autoimmune diseases, and viral infections.

## DESCRIPTION OF THE FIGURES

**Figure 1.** S<sub>N</sub>2 reactions between DNA bases and electrophilic chemotherapeutic agents. (A) Sp<sup>3</sup>-hybridized carbon atom-based electrophiles (nitrogen mustards). (B) Square planar metal center-based electrophiles (generally Pt(II)-based. Lg and Lg' denote leaving groups). The DNA bases can be G and, to a lesser extent, A.

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**Figure 2.** Possible adducts formed by the DNA and chemotherapeutic agents from the S<sub>N</sub>2 attack of two (A) or more than two (B) DNA bases on polyelectrophiles, denoted as In. Note that in case (B) the formation of adducts between two DNA strands is much more likely. Although the bases presented in the diagram are guanine (G) units, the participation of one or two adenine units is also possible.

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**Figure 3.** Computational simulations (theoretical level: B3LYP-D3BJ/6-31+G\*) of the S<sub>N</sub>2 reactivity of a compound of formula (1) with a guanine (G) unit, in which a chloride ion or aqua ligand act as a leaving group, on a metal center (TS1-Pt and TS2-Pt, *b* pathways) or on a carbon atom (TS1-C and TS2-C, *a* pathways). Note that the lower internal energy (ΔE) and Gibbs activation energy (ΔG) (higher reactivity) correspond to the reaction on Pt(II) and with an aqua ligand as a leaving group. Bond distances are given in Å and energies in kcal/mol.

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**Figure 4.** AFM (Atomic Force Microscopy) images of DNA samples (methylated λ-phage expressed by means of *E. coli* W3110, 103 kDa, 48 kb, panel A), in the presence of the compound (1o). Images were obtained after two minutes (B) and ten minutes (C) of incubation. Damage generated by the compound (1g), particularly in panel C, is shown.

20

**Figure 5.** TEM micrographs of the lyophilized DNA of methylated λ-phage in *E. coli* (host strain W3110. Mw. 31.5103 kDa, 48 kb, Sigma-Aldrich). (A) Baseline conditions (0.167 mg/ml). (B) Incubated with the compound (1g) for 3 minutes. (C) Incubated with the compound (1g) for 10 minutes. Damage generated by the compound (1g), particularly in panel C, is shown.

25

**Figure 6.** Cell viability in the presence of CisPt or the new family of compounds of formula (1). Effect of CisPt (10 μM) and of the new chemical entities of formula (1) (10 μM) on the viability of CCA (EGI-1 and HUCCT1), ovarian cancer (A2780Cis), breast cancer (MDA-453 and MDA-231), and normal human cholangiocyte (NHC) cell lines after a 48-hour incubation. Statistics of the results are calculated with respect to the control vehicle (DMF) and CisPt.

30

**Figure 7.** Cell viability in the presence of CisPt or the new family of compounds (1). Effect of CisPt (20 μM) and of the new chemical entities of formula (1) (20 μM) on the viability of CCA

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(EGI-1 and HUCCT1), ovarian cancer (A2780Cis), breast cancer (MDA-453 and MDA-231), and normal human cholangiocyte (NHC) cell lines after a 48-hour incubation. Statistics of the results are calculated with respect to the control vehicle (DMF) and CisPt.

5 **Figure 8.** Early cell death in the presence of CisPt or of the new family of compounds (**1**). Effect of CisPt (10  $\mu$ M and 20  $\mu$ M) and of the new chemical entities of formula (**1**) (10  $\mu$ M and 20  $\mu$ M) on early cell death (caspase-3) evaluated by means of flow cytometry on CCA cell lines sensitive to CisPt (EGI-1 and HUCCT1) and resistant to CisPt (EGI-1R), normal human cholangiocyte (NHC) cell line, ovarian cancer (A2780Cis) cell line, and breast cancer (MDA-  
10 453 and MDA-231) cell lines after 48 hours of incubation. Statistics of the results are calculated with respect to the control vehicle (DMF) and CisPt.

**Figure 9.** Late cell death in the presence of CisPt or of the new family of compounds (**1**). Effect of CisPt (10  $\mu$ M and 20  $\mu$ M) and of the new chemical entities of formula (**1**) (10  $\mu$ M and 20  $\mu$ M)  
15 on early cell death (annexin-V/TO-PRO-3) evaluated by means of flow cytometry on CCA cell lines sensitive to CisPt (EGI-1 and HUCCT1) and resistant to CisPt (EGI-1R), normal human cholangiocyte (NHC) cell line, ovarian cancer (A2780Cis) cell line, and breast cancer (MDA-453 and MDA-231) cell lines after 48 hours of incubation. Statistics of the results are calculated with respect to the control vehicle (DMF) and CisPt

20 **Figure 10.** DNA damage in the presence of CisPt or of the new family of compounds (**1**), evaluated by means of the COMET assay. Images representative of the COMET assay and quantification of DNA damage in CCA (EGI-1 and HUCCT1), normal human cholangiocyte (NHC), and ovarian cancer (A2780Cis) cells incubated for 48 hours with compounds (**1a**), (**1h**),  
25 (**1g**), and (**1d**), as representative examples. By way of comparison, results obtained with DMF and CisPt in identical conditions are also provided.

**Figure 11.** Therapeutic effect of compound (**1g**) on a subcutaneous xenograft model using CCA cells implanted from immunodeficient mice. (A) Diagram representative of the  
30 subcutaneous CCA model. (B) Tumor growth of CCA cells injected subcutaneously in immunodeficient mice in the presence or absence of intraperitoneal administration of compound (**1g**) (0.5 mg/kg). (C) Images representative of tumors. (D) Tumor weight of the xenografts after sacrifice. #  $p < 0.05$  compared to the DMF control, \*  $p < 0.05$  compared to CisPt, used as a comparative reference.

**Figure 12.** Spectra for absorption (UV-vis) **A**, excitation and emission (fluorescent) **B**, and for lifetime measurement, **C**, for compound **1a** ( $1\text{E}^{-5}$  M in N,N-dimethylformamide). Spectra for absorption (UV-vis) **D**, excitation and emission (fluorescent) **E**, and for lifetime measurement, **F**, for compound **1a** ( $1\text{E}^{-5}$  M in DMEM/F12 basal medium).

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**Figure 13.** Effect of compounds (**1a**), (**1d**), and (**1g**) on OCT1-, OCT3-, OATP1A2-, OATP1B3-, and OATP2B1-mediated transport. Cells with or without overexpression of the entry transporter were incubated with a specific fluorescent substrate in the presence or absence of the typical inhibitor of the transporter (OCT1/3: quinine; 1A2: Rif, rifampicin; 1B3: TC, taurocholate; 2B1: Mif, mifepristone) or compounds (**1**)  $10\text{ }\mu\text{M}$ , for 15 min at  $37^{\circ}\text{C}$ . Substrate content was determined by means of flow cytometry and net transporter-mediated uptake was calculated. Values are mean  $\pm$  SEM of 4 experiments in duplicate. \*,  $p<0.05$ ; \*\*,  $p<0.01$ ; \*\*\*,  $p<0.001$  compared to control (one-way ANOVA + Dukey's test for multiple comparisons).

15

**Figure 14.** Comparison of the effect of the compounds of formula (1) with cisplatin on OCT1-, OCT3-, and OATP1A2-mediated transport. Cells with or without overexpression of the entry transporter were incubated with a specific fluorescent substrate in the presence or absence of the typical inhibitor of the transporter (OCT1/3: quinine; 1A2: Rif, rifampicin), the corresponding compound (**1**)  $10\text{ }\mu\text{M}$ , or cisplatin (CDDP)  $10\text{ }\mu\text{M}$ , for 15 min at  $37^{\circ}\text{C}$ . Substrate content was determined by means of flow cytometry and net transporter-mediated uptake was calculated. Values are mean  $\pm$  SEM of 4 experiments in duplicate. \*,  $p<0.05$ ; \*\*,  $p<0.01$ ; \*\*\*,  $p<0.001$  compared to control (one-way ANOVA + Dukey's test for multiple comparisons).

20

**Figure 15.** Aurki-Pt16 uptake by entry transporters. Cells with or without overexpression of the entry transporter were incubated with  $10\text{ }\mu\text{M}$  compound (**1g**), for 60 min at  $37^{\circ}\text{C}$ . Intracellular content of the compound was determined by means of HPLC-MS/MS. Data is represented as percentage of control cells (Mock). Values are mean  $\pm$  SEM of at least 3 experiments in triplicate. \*,  $p<0.05$ ; \*\*,  $p<0.01$ ; compared to mock (t-Student, unpaired data).

25

**Figure 16.** Compound (**1d**) uptake by entry transporters. Cells with or without overexpression of the entry transporter were incubated with Aurki-Pt18  $10\text{ }\mu\text{M}$  for 60 min at  $37^{\circ}\text{C}$ . Intracellular content of the compound was determined by means of HPLC-MS/MS. Data is represented as percentage of control cells (Mock). Values are mean  $\pm$  SEM of at least 3 experiments in triplicate. \*,  $p<0.05$ ; \*\*,  $p<0.01$ ; compared to mock (t-Student, unpaired data).

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## DETAILED DESCRIPTION OF THE INVENTION

In the context of the present invention, the following terms have the meaning described in detail below.

5

The term “C<sub>1</sub>-C<sub>6</sub> alkyl” refers to a linear or branched hydrocarbon chain radical, said chain consisting of 1 to 6 carbon atoms, preferably 1 to 3 carbon atoms, which does not contain any unsaturation and is attached to the rest of the molecule by means of a single bond, for example, methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, n-pentyl, etc.

10

The term “aryl” refers to an aromatic ring radical comprising 6 to 10 carbon atoms, more preferably 6 carbon atoms. According to a particular embodiment, aryl is a phenyl radical. The aryl radical can be optionally substituted by one or more substituents, particularly it can be substituted by C<sub>1</sub>-C<sub>6</sub> alkyl, halogen, or hydroxyl.

15

The term “C<sub>4</sub>-C<sub>6</sub> cycloalkyl” refers to a saturated ring radical comprising between 4 and 6 carbon atoms, for example cyclobutyl, cyclopentyl, or cyclohexyl.

The terms “halogen” or halide are used interchangeably and include fluorine, chlorine, bromine, and iodine, preferably chlorine or bromine.

20

The term “leaving group” refers to any functional group with leaving group properties in second-order nucleophilic substitution reactions, such as tosylate (*p*-CH<sub>3</sub>(C<sub>6</sub>H<sub>4</sub>)SO<sub>3</sub><sup>-</sup>), mesylate (CH<sub>3</sub>SO<sub>3</sub><sup>-</sup>), or triflate (CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>).

25

The term “salt” should be understood to mean any form of a compound of formula (I) according to the present invention in which said compound is in ionic form, or in ionic form and coupled to a counterion (a cation or anion). Preferably, the salt is a pharmaceutically acceptable salt, i.e., a physiologically tolerable salt, which means that it is not toxic, particularly as a result of the counterion, when used in a suitable manner (i.e., in reasonable medical doses) for a treatment according to the present invention.

30

The salts can be prepared by methods known in the art. Generally, said salts are prepared by reacting the free base forms of the compounds of the invention with the suitable base or acid in water or in an organic solvent or in a mixture of both. Non-aqueous media such as ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are generally preferred.

35

The term “solvate” according to this invention should be understood to mean any compound of formula (I) according to the present invention in which said compound is attached by means of a non-covalent bond to another molecule (usually a polar solvent), including particularly hydrates and alcoholates such as methanolate. A preferred solvate is hydrate. Preferably, the solvate is a pharmaceutically acceptable solvate, i.e., a physiologically tolerable solvate, which means that it is not toxic, particularly as a result of the solvating molecule when used in a suitable manner (i.e., in reasonable medical doses) for a treatment according to the present invention.

As it is used herein, the term “stereoisomer” refers to the enantiomers, diastereomers, or mixtures thereof, such as racemates, of the compounds of formula (I) according to the present invention. Likewise, the term also encompasses geometric isomers with respect to any double bond present in the compound of formula (I), i.e., isomers (E) and isomers (Z) (trans and cis isomers). Furthermore, the term also encompasses rotamers of the compounds of formula (I).

Any compound of formula (I) according to the present invention can exist in different tautomeric forms. Specifically, the term “tautomer” refers to one of the two or more structural isomers of a compound of formula (I) that exist in equilibrium and are readily converted into an isomeric form with respect to the other.

The term “prodrug” refers to the derivatives of the compounds of formula (I) which are converted *in vivo* into the compounds of formula (I) such as, for example, by means of enzymatic hydrolysis.

As mentioned above, the first aspect of the invention relates to a compound of formula (1), or a salt, solvate, or stereoisomer thereof.

In a particular embodiment, in the compound of formula (1) M is Pt(II).

In another particular embodiment, in the compound of formula (1),  $X_1$  and  $X_2$  are both chloride, or together form a group selected from  $(^-\text{O}-\text{C}(\text{O})-\text{C}(\text{O})-\text{O}^-)$  and  $(^-\text{O}-\text{C}(\text{O})-\text{C}(\text{R}_9)(\text{R}_{10})-\text{C}(\text{O})-\text{O}^-)$ , wherein the oxygen atoms ( $\text{O}^-$ ) are attached to the metal center, and wherein  $\text{R}_9$  and  $\text{R}_{10}$  form a  $\text{C}_4$ - $\text{C}_6$  cycloalkyl group together with the carbon to which they are linked.

In another particular embodiment, in the compound of formula (1), Y is N or CR<sub>12</sub>, wherein R<sub>12</sub> is H or a C<sub>1</sub>-C<sub>6</sub> alkyl group. Preferably, Y is N or -CH-, more preferably Y is -CH-.

In a preferred embodiment of the compound of formula (1), Z<sub>1</sub> is hydrogen or can form a single  
5 covalent bond together with Z<sub>2</sub>.

In another preferred embodiment of the compound of formula (1), Z<sub>2</sub> is hydrogen, halogen, or can form a single covalent bond together with Z<sub>1</sub>, or can be attached to R<sub>5</sub> forming a C<sub>6</sub> aryl group. When Z<sub>2</sub> is a halogen, it is preferably chloride or bromide, more preferably bromide.

10

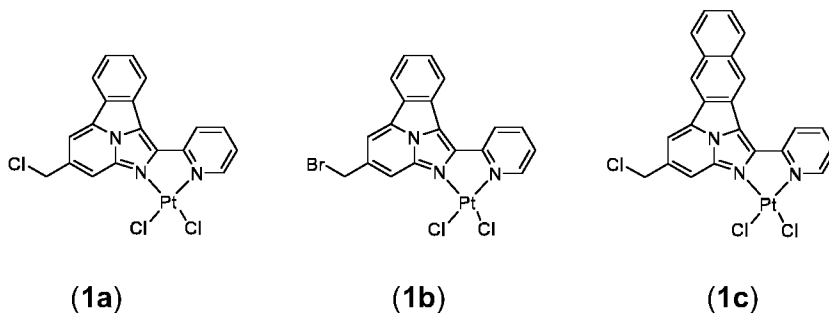
In another particular embodiment, in the compound of formula (1), R<sub>1</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group substituted at the end of the chain by a halogen or by a leaving functional group. Preferably, R<sub>1</sub> is a methyl group substituted at the end of the chain by a halogen, more preferably by a chloride.

15

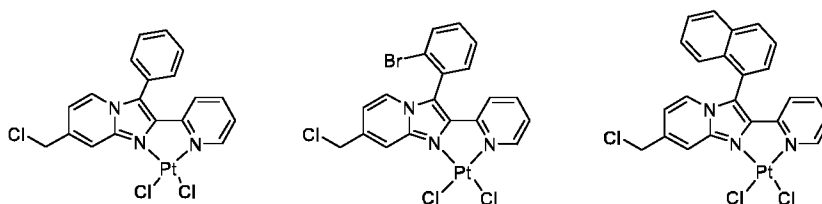
In another particular embodiment, in the compound of formula (1), R<sub>2</sub>-R<sub>4</sub> are independently a hydrogen or a C<sub>1</sub>-C<sub>6</sub> alkyl group, preferably H or an alkyl group, more preferably they are all H.

20 In another particular embodiment, in the compound of formula (1), R<sub>5</sub>-R<sub>7</sub> are all H, or grouped in R<sub>5</sub>-R<sub>6</sub> pairs, or R<sub>6</sub>-R<sub>7</sub> can form a C<sub>6</sub> aryl group.

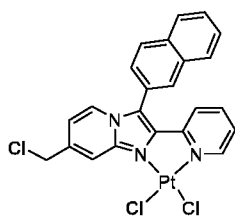
Preferred compounds of formula (1) are selected from the following:



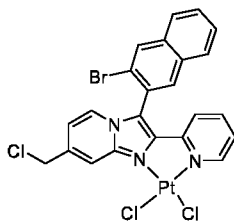
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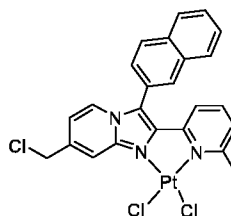
(1d)



(1e)

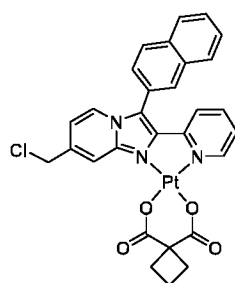


(1f)

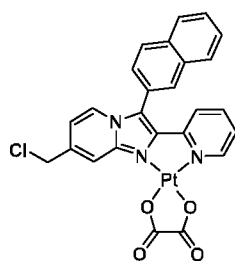


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(1g)

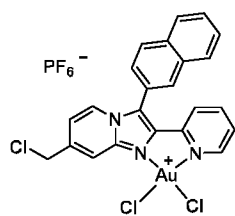


(1h)

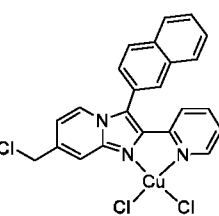


(1i)

(1k)



(1l)



10

(1m)

(1n)

or a salt, solvate, or stereoisomer thereof.

15 An additional aspect of the present invention relates to a method for the preparation of a compound of formula (1), which comprises reacting a compound of formula (2) as defined above, with a metal salt selected from gold trichloride ( $\text{AuCl}_3$ ), copper dichloride ( $\text{CuCl}_2$ ), and platinum salts of formula (3) as defined above as well.

20 Said reaction allows the incorporation of the square planar electrophilic metal center and the coordination thereof with respect to the corresponding nitrogenous heterocycle of formula (2).

Preferably, the reaction is carried out in an organic solvent such as an alcohol, preferably methanol or ethanol, or in acetonitrile.

In a preferred embodiment, when in the compound of formula (1) the metal is Pt(II) and  $X_1$  and  $X_2$  are both chlorides, the compound of formula (3) which is used in this reaction is platinum salt  $Pt(DMSO)_2Cl_2$ . Said salt can be obtained from  $K_2PtCl_4$  through the previously described methodology [Bar-Nahum, I. *et al.*, *J. Am. Chem. Soc.*, 2004, 126, 10236-10237]. Preferably, in this particular embodiment, the organic solvent used in the reaction between the compound of formula (2) and the metal salt is methanol or ethanol, more preferably methanol.

In another preferred embodiment, when in the compound of formula (1) the metal is Cu(II) and  $X_1$  and  $X_2$  are both chloride, the compound of formula (3) which is used in this reaction is copper salt  $CuCl_2$ . In this preferred embodiment, the organic solvent used in the reaction between the compound of formula (2) and the metal salt is preferably ethanol.

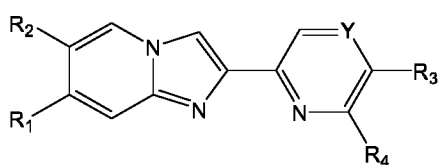
In another preferred embodiment, when in the compound of formula (1) the metal is Au(III) and  $X_1$  and  $X_2$  are both chloride, the compound of formula (3) which is used in this reaction is gold salt  $AuCl_3$ . In this preferred embodiment, the organic solvent used in the reaction between the compound of formula (2) and the metal salt is preferably acetonitrile.

Particularly, as expressed above, when in the compound of formula (1) the metal is Au(III), said compound is a cation which incorporates its corresponding anionic counterion selected from  $Cl^-$ ,  $PF_6^-$ , and  $R_8C(O)O^-$ , wherein  $R_8$  is a  $C_1$ - $C_6$  alkyl group. To that end, in the process of preparing said compound of formula (1), in addition to reacting the corresponding compound of formula (2) and the gold salt in an organic solvent, a compound providing the corresponding anion such as, for example,  $AgCl$ ,  $AgPF_6$ , and  $CH_3C(O)Ag$ , respectively, must be added to the reaction mixture.

In a particular embodiment, when in the compound of formula (1)  $X_1$  and  $X_2$  together form a  $(-O-C(O)-C(O)-O^-)$  group, the method of the invention may comprise a prior step of forming complex  $[Pt(O-C(O)-C(O)-O^-)(DMSO)_2]$ , based on the reaction of the compound  $cis-[PtCl_2(DMSO)_2]$  with a salt that allows removing chloride ions such as, for example, silver nitrate, and causing it to subsequently react with oxalic acid. In this preferred embodiment, the organic solvent used in the reaction between the compound of formula (2) and the complex is preferably methanol.

In another particular embodiment, when in the compound of formula (1)  $X_1$  and  $X_2$  together form a  $(^-\text{O}-\text{C}(\text{O})-\text{C}(\text{R}_9)(\text{R}_{10})-\text{C}(\text{O})-\text{O}^-)$  or  $(^-\text{O}-\text{C}(\text{O})-\text{CH}(\text{R}_{11})-\text{O}^-)$  group, the method of the invention may comprise a prior step of forming complex  $[\text{Pt}(^-\text{O}-\text{C}(\text{O})-\text{C}(\text{R}_9)(\text{R}_{10})-\text{C}(\text{O})-\text{O}^-)/(\text{DMSO})_2]$  or  $[\text{Pt}(^-\text{O}-\text{C}(\text{O})-\text{CH}(\text{R}_{11})-\text{O}^-)/(\text{DMSO})_2]$ , respectively, by means of reacting the compound cis-  
 5  $[\text{PtCl}_2(\text{DMSO})_2]$  in the presence of a neutralizing agent, such as  $\text{Ag}_2\text{O}$ , with the corresponding dicarboxylic acid  $\text{HO}-\text{C}(\text{O})-\text{C}(\text{R}_9)(\text{R}_{10})-\text{C}(\text{O})-\text{OH}$  or  $\alpha$ -hydroxycarboxylic acid  $\text{HO}-\text{C}(\text{O})-\text{CH}(\text{R}_{11})\text{OH}$ , respectively. In this preferred embodiment, the organic solvent used in the reaction between the compound of formula (2) and the corresponding complex is preferably methanol.

In a particular embodiment, the method of the invention further comprises a prior step of preparing the compound of formula (2) by means of a method comprising the coupling or cycloaddition reaction between a compound of formula (4):



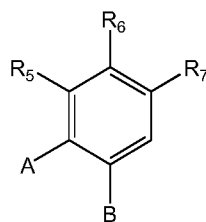
(4)

wherein,

$\text{Y}$  is  $\text{N}$ ;  $\text{CR}_{12}$ , wherein  $\text{R}_{12}$  is  $\text{H}$  or a  $\text{C}_1\text{-C}_6$  alkyl group optionally substituted at the end of the chain by a halogen or hydroxyl; a leaving functional group; or  $\text{C}(\text{O})\text{OMe}$ ;

$\text{R}_1\text{-R}_4$  are selected independently from  $\text{H}$ ; a  $\text{C}_1\text{-C}_6$  alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; a leaving functional group; and a  $\text{C}(\text{O})\text{OMe}$  group;

and a compound of formula (5):



(5)

wherein

R<sub>5</sub>-R<sub>7</sub> have the meaning defined in claim 1;

A is H, halogen, a trifluoromethylsulfonyl group, or a trialkylsilyl group, or is attached to R<sub>5</sub> forming an optionally substituted aryl group;

5

B is a halogen, a trifluoromethylsulfonyl group, or a trialkylsilyl group;

with the proviso that when A is a trifluoromethylsulfonyl group, B is a trialkylsilyl group or vice versa.

10 In a particular embodiment, R<sub>1</sub>-R<sub>4</sub> are selected independently from H; a methyl group optionally substituted by a halogen, preferably chlorine, or a hydroxyl; and a C(O)OMe group.

In another preferred embodiment, Y is N; CR<sub>12</sub>, wherein R<sub>12</sub> is H or a methyl group optionally substituted by a halogen or hydroxyl; or C(O)OMe.

15

In another particular embodiment, when in the compound of formula (2), Z<sub>1</sub> and Z<sub>2</sub> together form a single covalent bond, the compound of formula (5) has, as substituent A, a trifluoromethylsulfonyl group and, as substituent B, a trialkylsilyl group, or vice versa.

20 In said particular embodiment, cycloaddition (8+2) takes place based on 2-(pyridin-2-yl)imidazo[1,2-a]pyridine (compound of formula (2)), preferably in the presence of a suitable additive which takes up the cation Cs(+), such as, for example, 18-crown-6, as described in detail in method E of the examples herein.

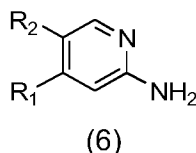
25 In another particular embodiment, when in the compound of formula (2) Z<sub>1</sub> is hydrogen and Z<sub>2</sub> is hydrogen, halogen, or is attached to R<sub>5</sub> forming an optionally substituted aryl group, the compound of formula (5) has, as substituent A, a hydrogen, a halogen, or is attached to R<sub>5</sub> forming an aryl group, and, as substituent B, a halogen.

30 In another particular embodiment, once the compound of formula (2) is formed, the method may further comprise, when any of R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, or R<sub>4</sub> is a C(O)OMe ester group, the substitution of said ester by a methyl group substituted by a halogen or a hydroxyl; or by a leaving functional group.

For example, said ester group can be reduced to the corresponding alcohol (methyl group substituted by a hydroxyl group) in the presence of a reducing agent such as lithium-aluminum hydride ( $\text{LiAlH}_4$ ) as described in detail in method C described in the examples herein.

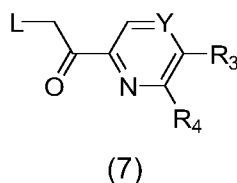
- 5 In turn, the alcohol obtained according to the preceding paragraph can be chlorinated in the presence of a chlorinating agent such as, for example, thionyl chloride ( $\text{SOCl}_2$ ), as described in method D of the examples herein.

10 In another particular embodiment, the method of the invention further comprises a prior step of preparing the compound of formula (4) by means of a method comprising the condensation reaction between a compound of formula (6):



- 15 wherein  $R_1$  and  $R_2$  are selected independently from H; a  $\text{C}_1\text{-C}_6$  alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; a leaving functional group; and a  $\text{C(O)OMe}$  group;

and a compound of formula (7):



20

or a hydrobromide salt thereof;  
wherein,

- 25 Y is N;  $\text{CR}_{12}$ , wherein  $R_{12}$  is H; a  $\text{C}_1\text{-C}_6$  alkyl group optionally substituted at the end of the chain by a halogen or hydroxyl; a leaving functional group; or  $\text{C(O)OMe}$ ;

$R_3$  and  $R_4$  are selected independently from H; a  $\text{C}_1\text{-C}_6$  alkyl group optionally substituted at the end of the chain by a halogen or a hydroxyl; a leaving functional group; and a  $\text{C(O)OMe}$  group;

30

L represents a halogen, a methylsulfonyl group, or a p-toluenesulfonyl group.

Said step can be carried out as described, for example, in method B of the examples herein.

In a particular embodiment, in the compound of formula (4),  $R_1$ - $R_2$  are selected independently from H; a methyl group substituted by a hydroxyl group or a halogen and a C(O)OMe group.

5

In a particular embodiment, in the compound of formula (4),  $R_3$ - $R_4$  are selected independently from H and a methyl group.

10 In a particular embodiment, in the compound of formula (4), Y is selected from N and  $CR_{12}$ , wherein  $R_{12}$  is H, a methyl group substituted by a hydroxyl group or a halogen; or a C(O)OMe group.

In a particular embodiment in the step of obtaining the compound of formula (4), in the compound of formula (6),  $R_1$ - $R_2$  are selected independently from H and a C(O)OMe group.

15

In another particular embodiment in the step of obtaining the compound of formula (4), in the compound of formula (7),  $R_3$  and  $R_4$  are selected independently from H; and a  $C_1$ - $C_6$  alkyl group, preferably methyl.

20 In a preferred embodiment in the step of obtaining the compound of formula (4), in the compound of formula (7), Y is N or  $CR_{12}$ , wherein  $R_{12}$  is H or C(O)OMe.

In another preferred embodiment, L is a halogen, preferably Br.

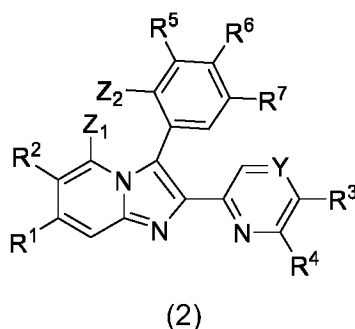
25 In a particular embodiment, once the compound of formula (4) is formed, the method may further comprise, when any of  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ , or  $R_{12}$  is a C(O)OMe ester group, the substitution of said ester by a methyl group substituted by a halogen or a hydroxyl; or by a leaving functional group.

30 For example, said ester group can be reduced to the corresponding alcohol (methyl group substituted by a hydroxyl group) in the presence of a reducing agent such as lithium-aluminum hydride ( $LiAlH_4$ ) as described in detail in method C described in the examples herein.

35 In turn, the alcohol obtained according to the preceding paragraph can be chlorinated in the presence of a chlorinating agent such as, for example, thionyl chloride ( $SOCl_2$ ), as described in method D of the examples herein.

The compounds of formula (7) can be obtained from methyl 2-acetylisonicotinate, from the corresponding 1-(pyridin-2-yl)ethan-1-one, or from 1-(pyrazin-2-yl)ethan-1-one, depending on whether Y is C-CO<sub>2</sub>Me, C-CH, or N, respectively. These methods are described, for example,  
 5 in *J. Med. Chem.* 2016, 59, 1388-1409 or in *Med. Chem. Lett.* 2007, 17, 4374-4377.

An additional aspect of the present invention relates to a compound of formula (2):



or a salt, solvate, or stereoisomer thereof;  
 wherein,

Z<sub>1</sub> is hydrogen or can form a single covalent bond together with Z<sub>2</sub>;

Z<sub>2</sub> is hydrogen, halogen, or can form a single covalent bond together with Z<sub>1</sub>, or can be attached to R<sub>5</sub> forming an aryl group optionally substituted by at least one C<sub>1</sub>-C<sub>6</sub> alkyl group, halogen, or OH;

Y is N or CR<sub>12</sub>, wherein R<sub>12</sub> is H; a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; a C(O)OMe group; or a leaving functional group.

R<sub>1</sub>-R<sub>4</sub> are selected independently from H; a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; a C(O)OMe group; and a leaving functional group;

R<sub>5</sub>-R<sub>7</sub> are all H, or grouped in R<sub>5</sub>-R<sub>6</sub> pairs, or R<sub>6</sub>-R<sub>7</sub> can form a C<sub>6</sub> aryl group optionally substituted by at least one C<sub>1</sub>-C<sub>6</sub> alkyl group, halogen, or hydroxyl;

with the proviso that the compound for which  $Z_1=Z_2=R^1=R^2=R^3=R^4=R_5=R_6=R^7=H$  and  $Y=CR^{12}=CH$ , is not included.

In a particular embodiment, in the compound of formula (2),  $Z_1$  is H or forms a single covalent bond together with  $Z_2$ .

In a particular embodiment, in the compound of formula (2),  $Z_2$  is H, halogen, or can be attached to  $R_5$  forming a  $C_6$  aryl group, or forms a single covalent bond together with  $Z_1$ .

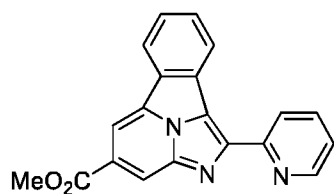
In another particular embodiment, in the compound of formula (2),  $R_1$  is a  $C_1$ - $C_6$  alkyl group, preferably methyl, optionally substituted by hydroxyl or halogen; or a  $C(O)OMe$  group.

In another particular embodiment, in the compound of formula (2),  $R_2$  is H.

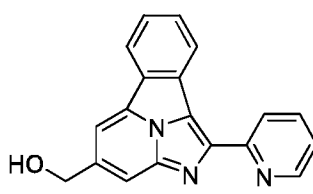
In another particular embodiment, in the compound of formula (2),  $R_3$  and  $R_4$  are independently H or a methyl group.

In another particular embodiment, in the compound of formula (2),  $R_5$ - $R_7$  are H or grouped in  $R_5$ - $R_6$  pairs, or  $R_6$ - $R_7$  form a  $C_6$  aryl group.

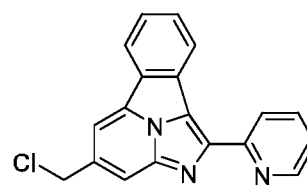
Preferably, the compound of formula (2) is selected from the following:



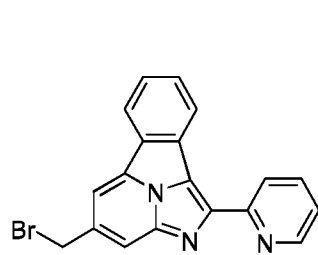
(2a)



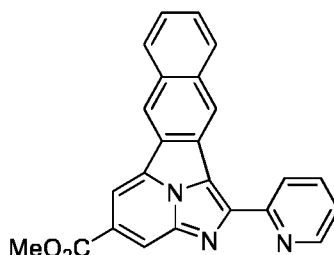
(2b)



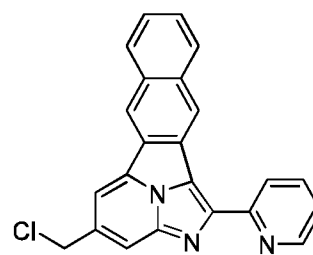
(2c)



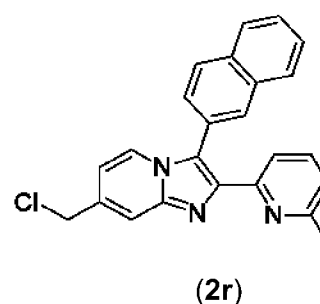
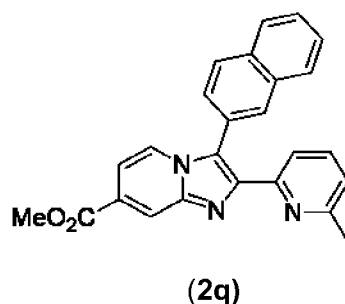
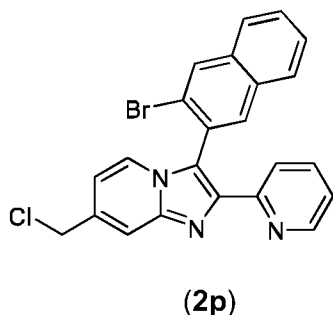
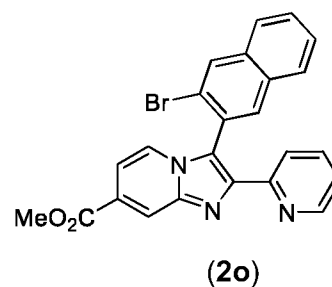
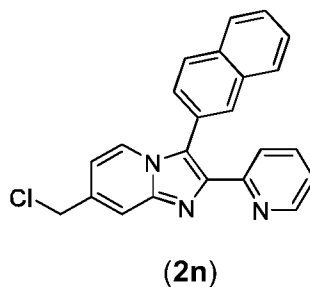
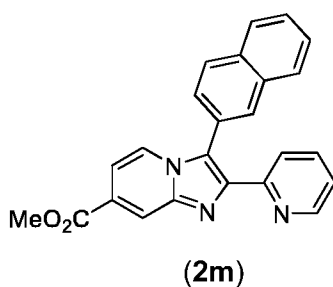
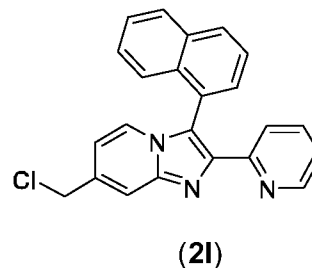
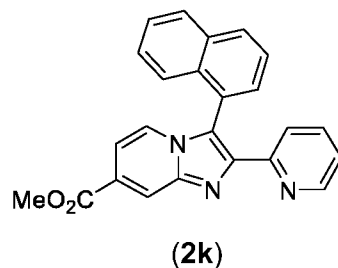
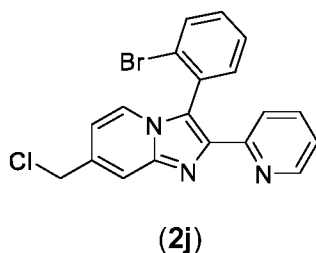
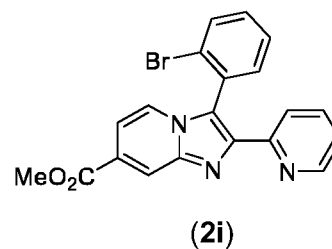
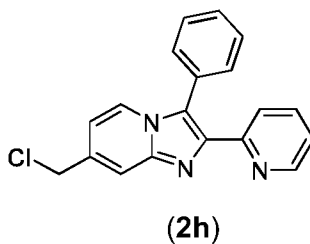
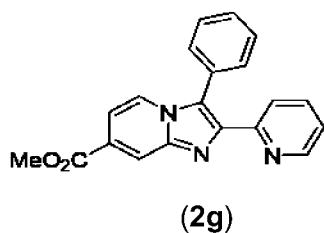
(2d)



(2e)



(2f)



or a salt, solvate, or stereoisomer thereof.

15 An additional aspect of the invention relates to a pharmaceutical composition comprising a compound of formula (I) as defined above, or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, and at least one pharmaceutically acceptable excipient thereof.

20 The term "excipient" refers to the components of a pharmaceutical compound other than the active ingredient (according to the definition of the European Medicines Agency - EMA). It preferably includes a "carrier, adjuvant, and/or vehicle". Carriers are forms in which substances

are incorporated to improve drug administration and efficacy. Drug carriers are used in drug administration systems, such as controlled release technology, to prolong the action of the drug *in vivo*, reduce its metabolism, or reduce its toxicity. Carriers are also used to increase drug administration efficacy at sites of pharmacological action. Adjuvant is a substance added to the formulation of a medicinal product which affects the action of the active ingredient in a predictable manner. Vehicle is an excipient or a substance, preferably without therapeutic action, which is used as a means to provide volume for medicinal product administration (Stedman's Medical Spellchecker© 2006 Lippincott Williams & Wilkins). Said pharmaceutical carriers, adjuvants, or vehicles can be sterile liquids such as water and oils, including those of petroleum, animal, plant, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil, and the like. Suitable pharmaceutical supports are described in "Remington's Pharmaceutical Sciences" of E.W. Martin. The selection of these excipients and the amounts to be used will depend on the form of application of the pharmaceutical composition.

The pharmaceutical composition according to the present invention can be in any suitable form for application in human beings and/or animals, preferably human beings, including infants, children, and adults, and can be produced by means of conventional methods known by those skilled in the art, for example the methods described or mentioned in the Spanish and United States pharmacopeias and similar reference texts.

The pharmaceutical composition of the present invention can be presented in any form suitable for drug administration such as, for example, intraperitoneal, intramuscular, intraarticular, intravenous, intraarterial, intravesical, intraosseous, intracavernous, pulmonary, buccal, sublingual, ocular, intravitreal, intranasal, percutaneous, rectal, vaginal, oral, epidural, intrathecal, intraventricular, intracerebral, intracerebroventricular, intracisternal, intraspinal, perispinal, intracranial, or topical.

Pharmaceutical forms suitable for oral administration can be tablets and capsules and can contain conventional excipients known in the art, such as binders, for example syrup, gum arabic, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, cornstarch, calcium phosphate, sorbitol, or glycine; lubricants for tablet preparation, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone, sodium starch glycolate, or microcrystalline cellulose; or pharmaceutically acceptable wetting agents, such as sodium lauryl sulphate. Pharmaceutical forms suitable for parenteral administration can be sterile solutions, suspensions, or lyophilized products. Suitable excipients, such as filling agents, buffering agents, or surfactants can be used.

The compounds of the present invention can be used with at least another drug to provide a combination therapy. The at least another drug can be part of the same composition or can be provided as a separate composition for administration at the same time or at different times.

5

In an additional aspect, the invention also relates to a compound of general formula (1) as defined above, or a pharmaceutically acceptable salt, or stereoisomer, or solvate, or prodrug thereof, or a pharmaceutical composition as defined above, for use as a medicinal product.

10 Another aspect of the invention relates to a compound of formula (1) as defined above, or a pharmaceutically acceptable salt, or stereoisomer, or solvate, or prodrug thereof, or a pharmaceutical composition as defined in the above claim, for use in the treatment of a disease selected from proliferative diseases, autoimmune diseases, and viral infections.

15 In a preferred embodiment, the compounds according to the invention are suitable for the prophylaxis and therapy of proliferative diseases or disorders.

A “proliferative diseases or disorder” refers to a disease that occurs due to an abnormal growth or expansion of cells as a result of multiplication. A proliferative disease can be associated with: 1) pathological proliferation of normally quiescent cells; 2) pathological migration of cells from their normal location (for example, cancer cells metastases); 3) pathological expression of proteolytic enzymes such as matrix metalloproteinases (for example, collagenases, gelatinases, and elastases); or 4) pathological angiogenesis such as in proliferative retinopathy and tumor metastasis. Examples of proliferative diseases include cancer (i.e., “malignant neoplasms”), benign neoplasms, angiogenesis, and inflammatory diseases.

25

In a particular embodiment, the proliferative disease is cancer.

The term “cancer” refers to a class of disease characterized by the development of abnormal cells which proliferate in an uncontrolled manner and have the capacity to infiltrate and destroy normal tissues of the body. Examples of cancer include, among others, solid tumors and hematological tumors.

30

Solid tumors which can be treated according to the invention are, for example, breast, respiratory tract, brain, reproductive organs, gastrointestinal tract, urogenital tract, eye, liver, skin, head and neck, thyroid gland, parathyroid gland, bone, connective tissue tumors, and the

35

metastasis of these tumors. In a particular embodiment, the solid tumor to be treated is a breast tumor, a reproductive organ tumor, or a liver tumor.

5 Liver tumors which can be treated are, for example, hepatocellular carcinomas and cholangiocellular carcinomas.

Hematological tumors which can be treated are, for example, multiple myelomas, lymphomas, or leukemias.

10 In a preferred embodiment, the cancer to be treated is selected from intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, ovarian cancer, and breast cancer.

More particularly, the cancer or cancerous condition is produced in the A2780Cis ovarian cancer cell line, in the MDA-453 and MDA-231 breast cancer cell lines, or in the EGI-1 and  
15 HUCCT1 extra- and intrahepatic cholangiocarcinoma cell lines.

More preferably, the cancer to be treated is ovarian cancer or breast cancer.

More particularly, the cancer or cancerous condition is produced in the A2780Cis ovarian  
20 cancer cell line or in the MDA-453 and MDA-231 breast cancer cell lines.

Also more particularly, the cancer or cancerous condition to be treated is that which has developed resistance to other anticancer agents as a result of previous chemotherapy cycles. Said anticancer agents include, for example, cisplatin, carboplatin, oxaliplatin, nedaplatin,  
25 miriplatin, and lobaplatin. More preferably, the cancer or cancerous condition to be treated is that which has developed resistance to cisplatin as a result of previous chemotherapy cycles with this compound.

Autoimmune diseases include, among others, autoimmune diseases of the eye (such as autoimmune uveitis); autoimmune diseases of the blood (such as autoimmune hemolytic anemia, pernicious anemia, and autoimmune thrombocytopenia), autoimmune diseases of the central and peripheral nervous system (such as multiple sclerosis, myasthenia gravis, myasthenic Eaton-Lambert syndrome), autoimmune neuropathies (such as Guillain-Barre), autoimmune diseases of the vascular system (such as antiphospholipid syndrome, vasculitis, temporal arteritis, and Behcet's disease); autoimmune diseases of the skin (such as dermatitis herpetiformis, pemphigus vulgaris, bullous pemphigoid, alopecia areata, psoriasis, and

vitiligo); autoimmune diseases of the gastrointestinal tract (such as Crohn's disease, ulcerative colitis, celiac disease, primary biliary cirrhosis, and autoimmune hepatitis); autoimmune disorders of the adrenal gland (such as Addisons disease), and multisystem autoimmune diseases including diseases of the musculoskeletal system and connective tissue (such as systemic lupus erythematosus, scleroderma, rheumatoid arthritis, polymyositis, dermatomyositis), autoimmune disorders of the endocrine glands (such as type I diabetes mellitus, autoimmune thyroiditis, Grave's disease, Hashimoto's thyroiditis, autoimmune oophoritis and orchitis), autoimmune-mediated glomerulonephritis, inflammatory bowel disease, and type I autoimmune diabetes.

Viral infections or disorders include, for example, infections caused by papilloma virus, herpes virus, influenza virus, Epstein-Barr virus, hepatitis B or C virus, and human immunodeficiency virus.

5

In the present invention, a method for the treatment of a disease selected from proliferative diseases, autoimmune diseases, and viral infections, which comprises administering a compound of formula (I) or a stereoisomer, or a salt, solvate, or prodrug thereof to a patient in need of same, is also described.

10

The term "treatment" or "treating", in the context of this specification, means the administration of a compound or a pharmaceutical composition according to the invention to prevent, improve, or eliminate the disease or one or more symptoms associated with said disease. "Treatment" also encompasses preventing, improving, or eliminating the physiological sequela of the disease.

15

The invention also describes the use of a compound of formula (I) or a stereoisomer, or a salt, solvate, or prodrug thereof, for the preparation of a medicinal product intended for the treatment of a disease selected from proliferative diseases, autoimmune diseases, and viral infections.

20

## Examples

### ***Chemical synthesis of compounds of formula (1) and of their precursors (2), (4), and (7).***

25

The following methods **A-G** describe the methods for obtaining the compounds referred to in the invention. These methods are indicated by way of illustration, where they can be modified

by means of changes in the substituents according to the aforementioned variations, and in the solvents, catalysts, treatments, and temperatures used.

Likewise, it should be noted that the decimal notation used in the following examples is based on the English language, in which dots substitute commas.

#### Method A:

Method A represents a method for preparing platinum complexes of general formula (1) by means of reaction between different 2,2'-bipyridine or 2-(pyridin-2-yl)imidazo[1,2-a]pyridine derivatives of general formula (2) and Pt(DMSO)<sub>2</sub>Cl<sub>2</sub> crystals (3a), which are obtained from K<sub>2</sub>PtCl<sub>4</sub> through the previously described methodology [Bar-Nahum, I. *et al.*, *J. Am. Chem. Soc.*, 2004, 126, 10236-10237]. After dissolving both reagents in methanol (MeOH), the resulting mixture is left under stirring at room temperature for 16 h. Once the reaction has completed, the mixture is introduced in an ultrasound bath for 2 h, for subsequent purification by means of washing with ethanol (EtOH) and ethyl ether (Et<sub>2</sub>O). The end product, a yellow solid, is purified by means of vacuum filtration.

#### Method B

Method B represents a method for preparing 2-(pyridin-2-yl)imidazo[1,2-a]pyridine derivatives of formula (4). The corresponding 2-bromoacetylpyridine (7) and 2-aminopyridine (6) are dissolved together with sodium bicarbonate in ethanol and left under reflux for 16 h. Once the reaction has ended, the solvent is removed under vacuum, and after adding water the product is extracted using dichloromethane. The organic phase is dried with magnesium sulfate and the solvent is again evaporated. Finally, the resulting mixture is purified by means of silica gel column chromatography using a 1:2 (v/v) ethyl acetate:n-hexane mixture as the mobile phase.

#### Method C

Method C represents a method for reducing methyl esters of general formula (4a), (4d), (4g), (2a) to the corresponding alcohols (4b), (4e), (4h), and 2(b), respectively. The different 2-(pyridin-2-yl)imidazo[1,2-a]pyridine derivatives containing methyl ester-type substituents are dissolved in dry tetrahydrofuran (THF). The solution is introduced in an ice bath for the slow addition of a 1 M lithium-aluminum hydride (LiAlH<sub>4</sub>) solution. The reaction is stirred for 2 h at room temperature for subsequent cooling in an ice bath with a saturated sodium bicarbonate solution. After separating the organic phase and evaporating tetrahydrofuran, the resulting residue is again dissolved in dichloromethane and washed with a saturated aqueous sodium bicarbonate solution. The organic phase is dried with magnesium sulfate and the solvent is

again evaporated. The resulting mixture is purified by means of silica gel column chromatography using a 1:10 (v/v) methanol:dichloromethane mixture as the mobile phase. [Selwood, D. L. *et al.*, *J. Med. Chem.*, 2001, 44, 78-93].

#### 5 Method D

Method D represents a method for chlorinating (4b)-, (4e)-, (4h)-, (2b)-type alcohols in order to obtain the corresponding methylchlorides (4c), (4f), (4i), (2c). Thionyl chloride is added dropwise to a solution of the different alcohols in dichloromethane in an ice bath. The mixture is heated under reflux with dichloromethane for 90 min. Then, the mixture is neutralized with a saturated sodium bicarbonate solution and extracted with dichloromethane. Finally, the oil obtained is purified by means of silica gel column chromatography using a 1:1 (v/v) ethyl acetate:n-hexane mixture as the mobile phase. [Abdelfattah, M. A. O. *et al.*, *Bioorg. Med. Chem. Lett.*, 2013, 23, 5077-5081].

#### 15 Method E

Method E represents a method for cycloaddition (8+2) based on 2-(pyridin-2-yl)imidazo[1,2-a]pyridines. The selected heterocycle, the different 2-(trimethylsilyl)aryl trifluoromethanesulfonate benzyne precursors, 18-crown-6, and cesium fluoride are mixed in a microwave vial and the resulting mixture is heated in the sealed vial for 15 min at 180°C setting 250 W and 100 psi as limits for reactor power and pressure. Once the reaction is completed, the mixture obtained is dissolved in dichloromethane, filtered, and the solvent is vacuum evaporated. The end product is purified by means of silica gel column chromatography using a 1:2 (v/v) ethyl acetate:n-hexane mixture as the mobile phase [Aginagalde, M. *et al.*, *J. Org. Chem.*, 2010, 75, 2776-2784].

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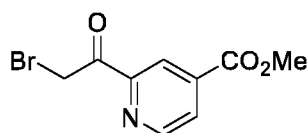
#### Method G

Method G represents a method for arylating position C<sub>3</sub> of methyl 2-(pyridin-2-yl)imidazo[1,2-a]pyridines. In a sealed microwave vial, the corresponding imidazo[1,2-a]pyridine, potassium carbonate, and Pd(PPh<sub>3</sub>)<sub>4</sub> are mixed under argon in N,N-dimethylformamide. Then, the selected aryl bromide is added and the reaction mixture is heated for 16 h at 160°C. Once the reaction has ended, the solvent is evaporated and the organic reaction product is dissolved again in dichloromethane to be filtered subsequently. The filtrate is evaporated and the end product is obtained by means of purification using silica gel column chromatography with ethyl acetate as the eluent.

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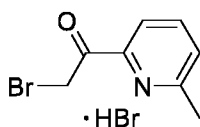
Several examples for the synthesis of compounds (1), (2), (4), and (7) are described in detail below. These examples are for purposes of illustration and do not limit the structural possibilities of the corresponding heterocycle families.

5 **Example 1.** Synthesis of methyl 2-(2-bromoacetyl)isonicotinate (**7a**):



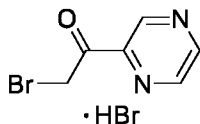
10 A reaction mixture containing methyl 2-acetylisonicotinate (8 g, 44.64 mmol) and acetic acid (56 mL) is cooled, and to this mixture a suitable volume of a 48% aqueous HBr solution (44.64 mmol) is added in an ice bath. Finally, Br<sub>2</sub> (8.16 g, 51.04 mmol) is added. The resulting mixture is stirred for one hour at room temperature and then for another 1.5 h at 75°C under argon atmosphere. After this time, the reaction mixture is left to cool at room temperature and diluted with THF (90 mL), leaving the resulting reaction mixture under stirring for 16 h. When the  
15 reaction ends, the solution obtained is diluted with ethyl acetate (AcOEt) and washed successively with NaHCO<sub>3</sub> (saturated solution) and H<sub>2</sub>O. The resulting organic phase is dried over MgSO<sub>4</sub>, filtered, and the solvent is removed at reduced pressure. Finally, product **7a** is obtained after purification by means of silica gel column chromatography using a 1:8 (v/v) ethyl acetate:n-hexane mixture as the eluent [Bavetsias, V. *et al.*, *J. Med. Chem.* 2016, 59, 1388-  
20 1409]. Methyl 2-(2-bromoacetyl)isonicotinate (**7a**). Red colored oil. Yield: 33%; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.84 (dd, J = 0.7, 5.0 Hz, 1H), 8.59 (dd, J = 0.9, 1.7 Hz, 1H), 8.07 (dd, J = 1.7, 5.0 Hz, 1H), 4.84 (s, 2H), 4.00 (s, 3H).

25 **Example 2.** Synthesis of 2-bromo-1-(6-methylpyridin-2-yl)ethan-1-one hydrobromide (**7b**):



1-(6-methylpyridin-2-yl)ethan-1-one (2.5 g, 18.5 mmol) is dissolved in an HBr (48%)/acetic acid mixture (1.3/2 mL) and Br<sub>2</sub> (0.948 mL, 18.5 mmol) is added in an ice bath. The mixture is left  
30 under stirring for 1 h at room temperature. Toluene is added and the resulting solution is evaporated, obtaining a yellowish-orange solid which is used without subsequent purification in the next step. Yield: 75%.

**Example 3.** Synthesis of 2-bromo-1-(pyrazin-2-yl)ethan-1-one hydrobromide (**7c**).



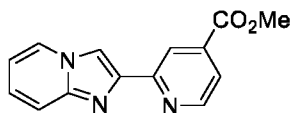
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1-(pyrazin-2-yl)ethan-1-one (4.0 g, 32.76 mmol) is dissolved in an HBr (48%)/acetic acid mixture (3.7/40 mL) and Br<sub>2</sub> (1.68 mL, 32.76 mmol) is added in an ice bath. The mixture is left under stirring for 2 h at 60°C. Toluene is added and the mixture is evaporated, obtaining product (**7c**) as a black solid which is used without further purification in the next step. Keith, J. M.; Gomez, L.A.; Barbier, A.J.; Wilson, S.J.; Boggs, J.D.; Lord, B.; Mazur, C.; Aluisio, L.; Lovenberg, T.W.; Curruthers, N.I., *Bioorg. Med. Chem. Lett.* 2007, 17, 4374-4377. Black solid. Yield: 55%;

10

**Example 4.** Synthesis of methyl 2-(2-(imidazo[1,2-a]pyridin-2-yl)pyridin-4-yl)acetate (**4a**).

15



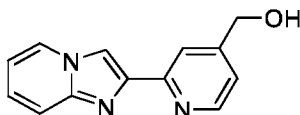
The method described as Method B was followed. Compound (**7a**) (1.6 g, 6.20 mmol), 2-aminopyridine (729.4 mg, 7.75 mmol), and NaHCO<sub>3</sub> (812.4 mg, 9.67 mmol) are dissolved in EtOH (25 mL). The reaction mixture is kept under reflux with EtOH for 16 h. Once cooled to room temperature, the reaction mixture obtained is filtered and the resulting solid is dried, obtaining (**4a**) as a brown solid. Yield: 72%; m.p.: 158-159°C; IR 1721, 1604, 1355, 1297, 1115, 754 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.79 (dd, J = 5.0, 0.9 Hz, 1H), 8.73 (s, 1H), 8.30 (s, 1H), 8.19 (dt, J = 6.9, 1.2 Hz, 1H), 7.80 (dd, J = 5.0, 1.7 Hz, 1H), 7.70 (dt, J = 9.1, 1.0 Hz, 1H), 7.24 (ddd, J = 9.1, 6.7, 1.3 Hz, 1H), 6.85 (td, J = 6.8, 1.2 Hz, 1H), 4.00 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 156.78, 155.69, 149.83, 149.34, 147.07, 137.20, 124.19, 123.08, 121.43, 120.44, 44.49; HRMS (ESI) calculated for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 254.0851, obtained: 254.0929.

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25

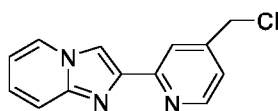
**Example 5.** Synthesis of 2-(imidazo[1,2-a]pyridin-2-yl)pyridin-4-yl)methanol (**4b**).

30



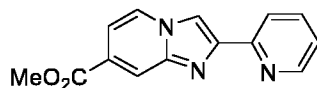
The method described as Method C was followed, starting from derivative **4a** (405.2 mg, 1.60 mmol),  $\text{LiAlH}_4$  (1M) (3.68 mmol), and THF (20 mL). The title product was obtained as a white solid. Yield: 52%; m.p.: 196-197°C; IR 3139, 2884, 1607, 1364, 1043, 826, 762  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.61 (d,  $J$  = 5.0 Hz, 1H), 8.29 (s, 1H), 8.24–8.15 (m, 2H), 7.67 (dd,  $J$  = 9.1, 1.1 Hz, 1H), 7.39 (m, 1H), 7.24 (ddd,  $J$  = 9.1, 6.7, 1.3 Hz, 1H), 6.85 (td,  $J$  = 6.7, 1.2 Hz, 1H), 4.85 (s, 2H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  152.82, 151.19, 149.49, 145.57, 145.41, 126.03, 125.18, 120.16, 117.90, 117.68, 112.86, 111.03, 63.49; HRMS (ESI) calculated for  $\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}$   $[\text{M}+\text{H}]^+$ : 226.0902, obtained: 226.0978.

**Example 6.** Synthesis of 2-(4-(chloromethyl)pyridin-2-yl)imidazo[1,2-a]pyridine (**4c**).



The method described as Method D was followed, starting from compound (**4b**) (180.2 mg, 0.8 mmol),  $\text{SOCl}_2$  (190.4 mg, 1.6 mmol), and dichloromethane (10 mL). The title product was obtained as a light brown solid. Yield: 82%; m.p.: 129-130°C; IR 3239, 2970, 1738, 1366, 1216, 1091, 677  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.65 (d,  $J$  = 5.0 Hz, 1H), 8.30 (s, 1H), 8.25 (d,  $J$  = 1.7 Hz, 1H), 8.19 (dt,  $J$  = 6.8, 1.2 Hz, 1H), 7.69 (d,  $J$  = 9.1 Hz, 1H), 7.32 (dd,  $J$  = 5.1, 1.7 Hz, 1H), 7.26–7.20 (m, 1H), 6.86 (td,  $J$  = 6.8, 1.1 Hz, 1H), 4.65 (s, 2H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  153.34, 149.90, 146.81, 145.60, 144.98, 126.06, 125.29, 121.87, 119.69, 117.81, 112.96, 111.19, 44.23; HRMS (ESI) calculated for  $\text{C}_{13}\text{H}_{10}\text{ClN}_3$   $[\text{M}+\text{H}]^+$ : 244.0563, obtained: 244.0641.

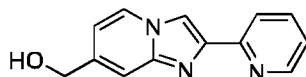
**Example 7.** Synthesis of methyl 2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-carboxylate (**4d**)



The method described as Method B was followed, starting from 2-(bromoacetyl)pyridine hydrobromide (1.74 g, 6.20 mmol), methyl 2-aminoisonicotinate (1.18 g, 7.75 mmol), and  $\text{NaHCO}_3$  (812.4 mg, 9.67 mmol) dissolved in EtOH (25 mL). The reaction mixture was kept under reflux with EtOH for 16 h. Once cooled to room temperature, the reaction mixture is filtered and the resulting solid is dried. The title product is obtained as a reddish white solid. Yield: 74%; m.p.: 225-226°C; IR 1715, 1593, 1327, 1228, 1117, 761, 740  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400

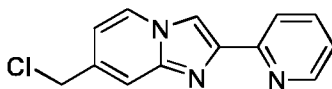
MHz, CDCl<sub>3</sub>)  $\delta$  8.65 (dd,  $J$  = 4.8, 0.9 Hz, 1H), 8.40 (s, 1H), 8.37 (s, 1H), 8.26–8.19 (m, 2H), 7.83 (td,  $J$  = 7.7, 1.8 Hz, 1H), 7.43 (dd,  $J$  = 7.1, 1.7 Hz, 1H), 7.31–7.26 (m, 1H), 3.99 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  165.55, 152.30, 149.45, 147.94, 144.48, 136.89, 126.32, 125.45, 123.09, 120.67, 120.46, 112.26, 112.04, 52.58; HRMS (ESI) calculated for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 254.0851, obtained: 254.0932.

**Example 8.** Synthesis of (2-(pyridin-2-yl)imidazo[1,2-*a*]pyridin-7-yl)methanol (**4e**).



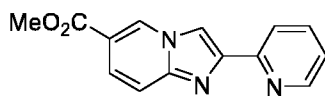
The method described as Method C was followed, starting from compound (**4b**) (405.2 mg, 1.60 mmol), LiAlH<sub>4</sub> (1M) (3.68 mmol), and THF (20 mL). The title compound was obtained as a white solid. Yield: 56%; m.p.: 227-228°C; IR 3167, 2583, 1739, 1493, 1375, 1066, 787, 718 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.60 (d,  $J$  = 4.7 Hz, 1H), 8.52 (d,  $J$  = 6.9 Hz, 1H), 8.43 (s, 1H), 8.10 (d,  $J$  = 7.8 Hz, 1H), 7.88 (td,  $J$  = 7.7, 1.8 Hz, 1H), 7.48 (s, 1H), 7.32 (ddd,  $J$  = 7.5, 4.8, 1.2 Hz, 1H), 6.87 (dd,  $J$  = 7.0, 1.6 Hz, 1H), 4.56 (s, 2H); <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  152.87, 149.44, 145.07, 144.62, 144.48, 140.76, 137.00, 126.70, 126.66, 122.70, 119.78, 112.46, 111.96, 111.80, 111.08, 111.03, 62.04; HRMS (ESI) calculated for C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O [M+H]<sup>+</sup>: 226.0902, obtained: 226.0978.

**Example 9.** Synthesis of 7-(chloromethyl)-2-(pyridin-2-yl)imidazo[1,2-*a*]pyridine (**4f**).



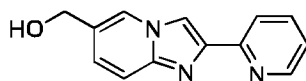
The method described as Method D was followed, starting from compound (**4e**) (180.2 mg, 0.8 mmol), SOCl<sub>2</sub> (190.4 mg, 1.6 mmol), and dichloromethane (10 mL). The title compound was obtained as a grayish solid. Yield: 78%; m.p.: 292-293°C; IR 3157, 3034, 1739, 1594, 1373, 1257, 1229, 1091, 806, 742 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.67–8.55 (m, 2H), 8.52 (s, 1H), 8.12 (d,  $J$  = 8.0 Hz, 1H), 7.90 (t,  $J$  = 7.7 Hz, 1H), 7.72 (s, 1H), 7.35 (t,  $J$  = 6.2 Hz, 1H), 7.00 (d,  $J$  = 7.0 Hz, 1H), 4.86 (s, 2H); <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  152.32, 149.47, 145.03, 144.15, 137.11, 135.36, 127.53, 122.98, 119.92, 116.05, 113.47, 111.84, 45.47. HRMS (ESI) calculated for C<sub>13</sub>H<sub>10</sub>ClN<sub>3</sub> [M+H]<sup>+</sup>: 244.0563, obtained: 244.0641

**Example 10:** Synthesis of methyl 2-(pyridin-2-yl)imidazo[1,2-*a*]pyridine-6-carboxylate (**4g**).



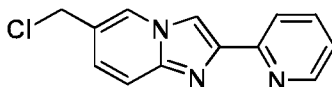
The method described as Method B was followed, starting from 2-(bromoacetyl)pyridine hydrobromide (1.74 g, 6.20 mmol), methyl 6-aminopyridine-3-carboxylate (1.18 mg, 7.75 mmol), NaHCO<sub>3</sub> (812.4 mg, 9.67 mmol), and EtOH (30 mL). The title product was obtained as a grayish solid. Yield: 63%; m.p.: 233-234°C; IR 3080, 1720, 1594, 1427, 1301, 1197, 1095, 767 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.94 (s, 1H), 8.65 (d, *J* = 4.9 Hz, 1H), 8.43 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.84 (t, *J* = 7.8 Hz, 1H), 7.77 (d, *J* = 9.5 Hz, 1H), 7.66 (d, *J* = 9.5 Hz, 1H), 7.33–7.24 (m, 1H), 3.97 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.10, 151.71, 148.96, 146.45, 145.95, 137.50, 130.25, 124.96, 123.24, 121.03, 117.08, 116.98, 112.22, 52.53; HRMS (ESI) calculated for C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 254.0851, obtained: 254.0932.

**Example 11.** Synthesis of (2-(pyridin-2-yl)imidazo[1,2-a]pyridin-6-yl)methanol (**4h**).



The method described as Method B was followed, starting from 2-(bromoacetyl)pyridine hydrobromide (1.74 g, 6.20 mmol), (2-aminopyridin-4-yl)methanol (962.1 mg, 7.75 mmol), NaHCO<sub>3</sub> (812.4 mg, 9.67 mmol), and EtOH (30 mL). The title product was obtained as a white solid. Yield: 52%; m.p.: 161-162°C; IR 3222, 2970, 1739, 1370, 1217, 1032, 861, 824, 721 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.60 (d, *J* = 4.7 Hz, 1H), 8.51 (s, 1H), 8.49 (s, 1H), 8.10 (d, *J* = 8.0 Hz, 1H), 7.88 (td, *J* = 7.7, 1.8 Hz, 1H), 7.58 (d, *J* = 9.2 Hz, 1H), 7.32 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 1H), 7.24 (dd, *J* = 9.3, 1.6 Hz, 1H), 4.53 (s, 2H); <sup>13</sup>C NMR (101 MHz, DMSO-d<sub>6</sub>) δ 152.87, 149.39, 144.66, 144.37, 136.98, 127.15, 125.70, 123.96, 122.69, 119.68, 116.47, 111.57, 60.46; HRMS (ESI) calculated for C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O [M+H]<sup>+</sup>: 226.0902, obtained: 226.0979.

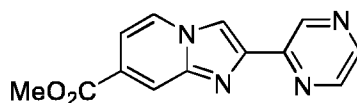
**Example 12:** Synthesis of 6-(chloromethyl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine (**4i**).



The method described as Method D was followed, starting from compound (**4h**) (180.2 mg, 0.8 mmol), SOCl<sub>2</sub> (190.4 mg, 1.6 mmol), dichloromethane (10 mL). The title product was obtained as a grayish solid. Yield: 65%; m.p.: 298-299°C; IR 3034, 1737, 1594, 1375 1347, 1258, 1214,

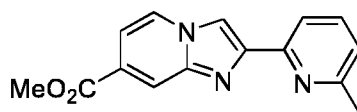
741 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.73 (s, 1H), 8.61 (dd, J = 4.8, 0.9 Hz, 1H), 8.50 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.89 (td, J = 7.7, 1.8 Hz, 1H), 7.65 (d, J = 9.3 Hz, 1H), 7.37–7.31 (m, 2H), 4.85 (s, 2H); <sup>13</sup>C NMR (101 MHz, DMSO-d<sub>6</sub>) δ 152.35, 149.50, 145.04, 144.11, 137.19, 126.95, 126.76, 123.05, 119.96, 117.12, 112.00, 43.95; HRMS (ESI) calculated for C<sub>13</sub>H<sub>10</sub>ClN<sub>3</sub> [M+H]<sup>+</sup>: 244.0563, obtained: 244.0640.

**Example 13:** Synthesis of methyl 2-(pyrazin-2-yl)imidazo[1,2-a]pyridine-7-carboxylate (**4j**).



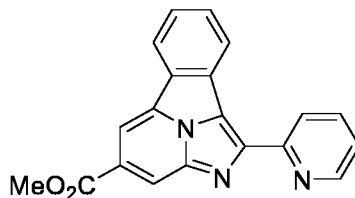
The method described as Method B was followed, starting from compound (**7c**) (3.2 g, 8.52 mmol), methyl 2-aminoisonicotinate (1.14 g, 7.5 mmol), NaHCO<sub>3</sub> (0.95 g, 11.24 mmol), and EtOH (70 mL). The title compound was obtained as a dark brown solid. Yield: 48%; m.p.: 243–244°C; IR 3052, 1712, 1469, 1396, 1231, 1119, 745, 436, 404 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.52 (d, J = 1.7 Hz, 1H), 8.68–8.58 (m, 2H), 8.45 (m, 2H), 8.27 (dd, J = 7.0, 1.1 Hz, 1H), 7.51 (dd, J = 7.1, 1.7 Hz, 1H), 4.05 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.66, 148.17, 145.68, 145.14, 144.29, 144.13, 142.89, 127.21, 125.82, 121.02, 113.36, 112.70, 52.94; HRMS (ESI) calculated for C<sub>13</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub> [M+H]<sup>+</sup>: calculated: 255.2490, obtained: 255.0804.

**Example 14:** Synthesis of methyl 2-(6-methylpyridin-2-yl)imidazo[1,2-a]pyridine-7-carboxylate (**4k**).



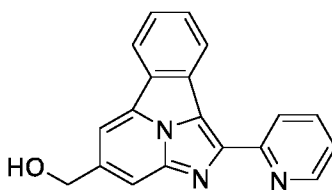
The method described as Method B was followed, starting from compound (**7b**) (4.9 g, 16.61 mmol), methyl 2-aminoisonicotinate (2.11 g, 13.84 mmol), NaHCO<sub>3</sub> (1.76 g, 20.76 mmol), and EtOH (80 mL). The title compound was obtained as a light brown solid. Yield: 68%; m.p.: 268–269°C; IR 2953, 1713, 1571, 1440, 1328, 1275, 1238, 1125, 1086, 817, 767, 740 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.44–8.35 (m, 2H), 8.19 (dd, J=7.0, 1.0 Hz, 1H), 8.02 (d, J=7.7 Hz, 1H), 7.71 (t, J=7.7 Hz, 1H), 7.42 (dd, J=7.0, 1.7 Hz, 1H), 7.14 (d, J=7.6 Hz, 1H), 3.99 (s, 3H), 2.64 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) 165.88, 158.57, 151.96, 148.58, 144.74, 137.30, 126.46, 125.59, 123.00, 120.76, 118.01, 112.56, 112.26, 52.82, 24.86; HRMS (ESI) calculated for C<sub>15</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 268.2880, obtained: 268.1088.

**Example 15:** Synthesis of methyl 1-(pyridin-2-yl)benzo[*a*]imidazo[5,1,2-*cd*]indolizine-4-carboxylate (**2a**)



The method described as Method E was followed, starting from compound (**4d**) (150 mg, 0.59 mmol), 2-(trimethylsilyl)phenyl trifluoromethanesulfonate (176 mg, 0.59 mmol), 18-crown-6 (156.8 mg, 0.59 mmol), and CsF (89.6 mg, 0.59 mmol). The title product was obtained as a yellow solid. Yield: 23%; m.p.: 214-215°C; IR 1710, 1455, 1272, 1246, 1081, 767, 731 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.07 (d, J = 8.1 Hz, 1H), 8.95 (d, J = 4.8 Hz, 1H), 8.79 (s, 1H), 8.70 (s, 1H), 8.57 (d, J = 7.8 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H), 7.94 (td, J = 7.7, 1.8 Hz, 1H), 7.86 (td, J = 7.9, 7.4, 1.0 Hz, 1H), 7.68 (td, J = 7.9, 7.3, 1.1 Hz, 1H), 7.40 (ddd, J = 7.4, 4.7, 1.2 Hz, 1H), 4.12 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 166.65, 152.92, 149.95, 148.13, 138.22, 136.81, 131.96, 130.29, 130.12, 129.70, 128.29, 125.98, 124.46, 124.20, 123.51, 122.71, 121.91, 115.51, 109.52, 52.86; HRMS (ESI) calculated for C<sub>20</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 328.1008, obtained: 328.1090.

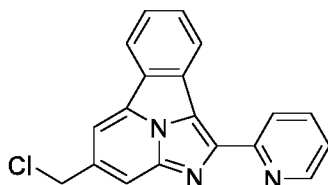
**Example 16:** Synthesis of (1-(pyridin-2-yl)benzo[*a*]imidazo[5,1,2-*cd*]indolizine-4-yl)methanol (**2b**).



The method described as Method C was followed, starting from compound (**2a**) (327.4 mg, 1 mmol), LiAlH<sub>4</sub> (1M) (2.3 mmol), and THF (10 mL). The title product was obtained as a yellow solid. Yield: 49%; m.p.: 222-223°C; IR 3049, 2839, 1532, 1402, 1341, 1191, 1120, 1082, 1055, 767, 735 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.07 (d, J = 8.0 Hz, 1H), 8.97 (d, J = 4.1 Hz, 1H), 8.55 (d, J = 7.9 Hz, 1H), 8.28 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 2.5 Hz, 2H), 7.94 (td, J = 7.7, 1.8 Hz, 1H), 7.84 (t, J = 7.6 Hz, 1H), 7.63 (t, J = 7.6 Hz, 1H), 7.39 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 5.12 (s, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 153.42, 149.95, 145.79, 141.52, 141.37, 139.04,

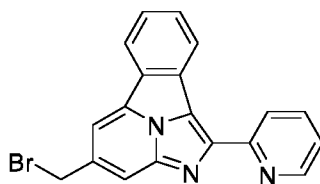
136.83, 131.47, 130.82, 130.37, 129.47, 125.46, 123.91, 123.14, 122.68, 121.66, 111.30, 108.21, 65.50; HRMS (ESI) calculated for  $C_{20}H_{13}N_3O_2$   $[M+H]^+$ : 300.1059, obtained: 300.1140.

**Example 17:** Synthesis of 4-(chloromethyl)-1-(pyridin-2-yl)benzo[a]imidazo[5,1,2-*cd*]indolizine (**2c**).



The method described as Method D was followed, starting from compound (**2b**) (120 mg, 0.4 mmol),  $SOCl_2$  (95.2 mg, 0.8 mmol), dichloromethane (10 mL). The title product was obtained as a yellow solid. Yield: 72%; m.p.: 231-232°C; IR 3039, 1528, 1400, 1265, 1194, 1083, 765, 735, 703  $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  9.11 (d,  $J$  = 8.0 Hz, 1H), 8.97 (dt,  $J$  = 4.5, 1.4 Hz, 1H), 8.59 (d,  $J$  = 7.9 Hz, 1H), 8.39 (d,  $J$  = 7.9 Hz, 1H), 8.14 (s, 1H), 8.09 (s, 1H), 7.95 (td,  $J$  = 8.1, 1.9 Hz, 1H), 7.88 (t,  $J$  = 7.6 Hz, 1H), 7.69 (d,  $J$  = 7.9 Hz, 1H), 7.40 (ddd,  $J$  = 7.5, 4.8, 1.2 Hz, 1H), 5.07 (s, 2H);  $^{13}C$  NMR (101 MHz,  $CDCl_3$ )  $\delta$  150.05, 137.06, 131.57, 131.33, 130.49, 130.10, 126.45, 126.24, 124.53, 124.36, 123.65, 122.92, 122.18, 122.03, 118.04, 113.01, 110.33, 107.13, 46.50; HRMS (ESI) calculated for  $C_{19}H_{12}ClN_3$   $[M+H]^+$ : 318.0720, obtained: 318.0804.

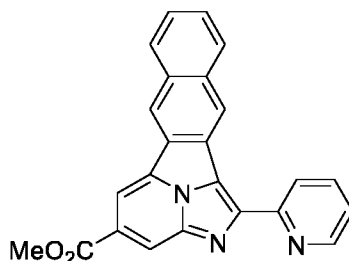
**Example 18:** Synthesis of 4-(bromomethyl)-1-(pyridin-2-yl)benzo[a]imidazo[5,1,2-*cd*]indolizine (**2d**).



Compound (**2a**) (101.7 mg, 0.34 mmol) is dissolved in dichloromethane (10 mL) and the resulting solution is cooled in an ice bath.  $PBr_3$  (184.1 mg, 0.68 mmol) is slowly added at 0-5°C. The resulting mixture is stirred for 1 h at room temperature. Once the reaction has ended, the pH of the reaction is adjusted using a saturated aqueous  $Na_2CO_3$  solution. The resulting mixture is extracted with dichloromethane and water and the organic phase is dried with  $MgSO_4$ , filtered, and evaporated. The end product is purified by means of silica gel column chromatography using a 1:2 (v/v) ethyl acetate:n-hexane mixture as the mobile phase. The

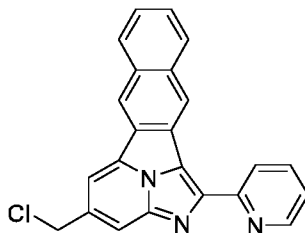
title compound is obtained as a yellow solid. Yield: 74%; m.p.: 249-250°C; IR  $\text{cm}^{-1}$  3039, 1494, 1398, 1264, 1192, 1082, 765, 735, 664;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.10 (dt,  $J$  = 8.0, 1.0 Hz, 1H), 8.97 (ddd,  $J$  = 4.8, 1.8, 0.9 Hz, 1H), 8.56 (dt,  $J$  = 7.9, 1.1 Hz, 1H), 8.38 (dt,  $J$  = 8.0, 1.0 Hz, 1H), 8.11 (s, 1H), 8.07 (s, 1H), 7.94 (td,  $J$  = 7.7, 1.8 Hz, 1H), 7.87 (td,  $J$  = 7.4, 1.0 Hz, 1H), 7.68 (td,  $J$  = 7.7, 1.1 Hz, 1H), 7.39 (ddd,  $J$  = 7.6, 4.8, 1.2 Hz, 1H), 4.98 (s, 2H);  $^{13}\text{C}$ -NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  153.21, 149.97, 138.72, 137.36, 136.83, 131.51, 131.04, 130.56, 129.77, 125.73, 124.10, 123.30, 122.81, 121.77, 113.73, 110.29, 33.84; HRMS (ESI) calculated for  $\text{C}_{19}\text{H}_{12}\text{BrN}_3$   $[\text{M}+\text{H}]^+$ : 362.0215, obtained: 362.0289.

- 10 **Example 19:** Synthesis of methyl 1-(pyridin-2-yl)imidazo[5,1,2-*cd*]naphtho[2,3-*a*]indolizine-4-carboxylate (**2e**).



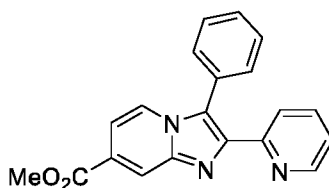
The method described as Method E was followed, starting from compound (**4d**) (150 mg, 0.59 mmol), 3-(trimethylsilyl)-2-naphthyl trifluoromethanesulfonate (205.6 mg, 0.59 mmol), 18-corona-6 (156.8 mg, 0.59 mmol), and  $\text{CsF}$  (89.6 mg, 0.59 mmol). The title compound was obtained as a dark yellow solid. Yield: 19%; m.p.: 263-264°C; IR 1713, 1456, 1293, 1233, 1220, 1070, 893, 761, 730  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.49 (s, 1H), 9.05 (d,  $J$  = 4.8 Hz, 1H), 8.87 (s, 1H), 8.74 (s, 1H), 8.63 (s, 1H), 8.57 (d,  $J$  = 7.9 Hz, 1H), 8.28 (d,  $J$  = 8.2 Hz, 1H), 8.17 (d,  $J$  = 8.1 Hz, 1H), 7.96 (m, 1H), 7.75–7.62 (m, 3H), 7.43 (dd,  $J$  = 7.5, 4.9 Hz, 1H), 4.13 (s, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.58, 153.15, 149.98, 138.30, 136.85, 134.05, 131.64, 131.38, 129.20, 129.02, 128.74, 127.78, 127.04, 126.05, 123.30, 122.98, 122.81, 121.76, 116.40, 107.93, 52.88; HRMS (ESI) calculated for  $\text{C}_{24}\text{H}_{15}\text{N}_3\text{O}_2$   $[\text{M}+\text{H}]^+$ : 378.1164, obtained: 378.1243.

- 25 **Example 20:** Synthesis of 4-(chloromethyl)-1-(pyridin-2-yl)imidazo[5,1,2-*cd*]naphthol[2,3-*a*]indolizine (**2f**).



First, the method described as Method C was followed, starting from compound (**2e**) (301.7 mg, 0.8 mmol), LiAlH<sub>4</sub> (1M) (2 mmol), and THF (10 mL). The corresponding hydroxylated derivative was obtained, which was used without purification in the next reaction step according to Method D, starting from (1-pyridin-2-yl)imidazo[5,1,2-*cd*]naphtho[2,3-*a*]indolizine-4-yl)methanol (104.8 mg, 0.3 mmol), SOCl<sub>2</sub> (71.4 mg, 0.6 mmol), dichloromethane (8 mL). The title product (**2f**) was obtained as a yellow solid. Yield: 70%; m.p.: 232-233°C; FTIR 3031, 1587, 1427, 1412, 1291, 1180, 1118, 883, 793, 743, 702 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.48 (s, 1H), 9.05 (d, J = 4.8 Hz, 1H), 8.84 (s, 1H), 8.54 (dt, J = 7.8, 1.1 Hz, 1H), 8.28 (d, J = 8.1 Hz, 1H), 8.17 (d, J = 8.2 Hz, 1H), 8.04 (s, 1H), 8.00–7.92 (m, 2H), 7.74–7.62 (m, 2H), 7.41 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 5.03 (s, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  153.33, 149.97, 138.69, 137.52, 136.84, 134.12, 132.09, 131.36, 131.25, 129.17, 129.00, 127.92, 127.03, 125.92, 123.05, 122.88, 122.71, 121.58, 113.77, 108.47, 46.77; HRMS (ESI) calculated for C<sub>23</sub>H<sub>14</sub>ClN<sub>3</sub> [M+H]<sup>+</sup>: 368.0876, obtained: 368.0956.

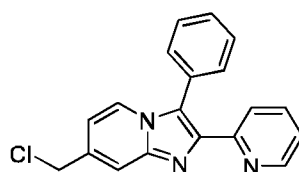
**Example 21:** Synthesis of methyl 3-phenyl-2-(pyridin-2-yl)imidazo[1,2-*a*]pyridin-7-carboxylate (**2g**).



The method described as Method G was followed, starting from compound (**4d**) (501.5 mg, 1.98 mmol), bromobenzene (464.7 mg, 2.96 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (160.6 mg, 0.14 mmol), K<sub>2</sub>CO<sub>3</sub> (819.6 mg, 5.93 mmol), and DMF (15 mL). The title product was obtained as a white solid. Yield: 54%; m.p.: 142-143°C; IR 1716, 1347, 1268, 1225, 1144, 1084, 752, 733, 713 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.61 (d, J = 4.9 Hz, 1H), 8.47–8.43 (m, 1H), 8.04 (dd, J = 7.2, 1.0 Hz, 1H), 7.70 (dt, J = 8.0, 1.2 Hz, 1H), 7.63 (td, J = 7.6, 1.8 Hz, 1H), 7.60–7.50 (m, 5H), 7.37 (dd, J = 7.2, 1.7 Hz, 1H), 7.19 (ddd, J = 7.4, 4.8, 1.3 Hz, 1H), 3.99 (s, 3H); <sup>13</sup>C NMR (101 MHz,

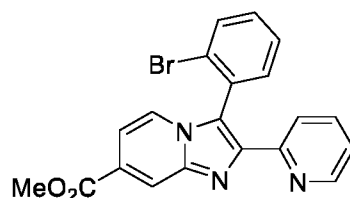
$\text{CDCl}_3$ )  $\delta$  165.63, 152.64, 149.69, 144.13, 143.71, 136.07, 130.57, 129.25, 129.18, 128.97, 126.29, 124.63, 123.08, 122.84, 122.44, 120.77, 111.84, 52.55; HRMS (ESI) calculated for  $\text{C}_{20}\text{H}_{15}\text{N}_3\text{O}_2$   $[\text{M}+\text{H}]^+$ : 330.1164, obtained: 330.1245.

- 5 **Example 22:** Synthesis of 7-(chloromethyl)-3-phenyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridine (**2h**).



First, the method described as Method C was followed, starting from compound (**2g**) (329.4 mg, 1 mmol),  $\text{LiAlH}_4$  (1M) (2.3 mmol), and THF (15 mL). The corresponding hydroxylated derivative was obtained, which was used without purification in the next reaction step according to Method D, starting from (3-phenyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-yl)methanol (120.5 mg, 0.4 mmol),  $\text{SOCl}_2$  (95.2 mg, 0.8 mmol), dichloromethane (10 mL). The title product was obtained as a yellowish oil. Yield: 68%; IR 3053, 1589, 1508, 1365, 1217, 794, 720, 699  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.61 (d,  $J$  = 4.7 Hz, 1H), 8.01 (d,  $J$  = 7.1 Hz, 1H), 7.72–7.68 (m, 1H), 7.67–7.61 (m, 2H), 7.60–7.51 (m, 5H), 7.17 (ddd,  $J$  = 7.2, 4.8, 1.5 Hz, 1H), 6.84 (dd,  $J$  = 7.2, 1.8 Hz, 1H), 4.67 (s, 2H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  153.03, 149.65, 144.23, 142.54, 136.00, 134.64, 130.72, 129.41, 129.21, 128.97, 123.89, 123.54, 122.71, 122.19, 117.08, 113.07, 45.42; HRMS (ESI) calculated for  $\text{C}_{20}\text{H}_{14}\text{ClN}_3$   $[\text{M}+\text{H}]^+$ : 320.0876, obtained: 320.0958.

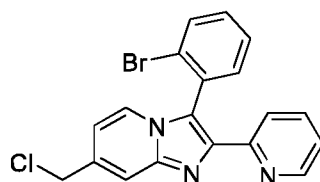
**Example 23:** Synthesis of methyl 3-(2-bromophenyl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-carboxylate (**2i**)



The method described as Method G was followed, starting from compound (**4d**) (501.5 mg, 1.98 mmol), 1,2-dibromobenzene (698.3 mg, 2.96 mmol),  $\text{Pd}(\text{PPh}_3)_4$  (160.6 mg, 0.14 mmol),  $\text{K}_2\text{CO}_3$  (819.6 mg, 5.93 mmol), and DMF (15 mL). The title product was obtained as a white

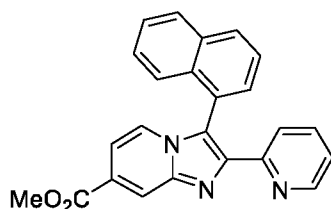
solid. Yield: 49%; m.p.: 162-163°C; IR 1712, 1333, 1244, 1231, 1144, 1085, 754, 736 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.55 (d, J = 4.9 Hz, 1H), 8.50 (s, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.71–7.62 (m, 2H), 7.54–7.39 (m, 4H), 7.18 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 4.00 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 165.66, 152.45, 149.70, 144.61, 143.76, 136.17, 133.68, 133.34, 131.08, 130.78, 127.99, 126.54, 125.66, 123.60, 123.52, 122.48, 122.16, 120.67, 111.87, 52.60; HRMS (ESI) calculated for C<sub>20</sub>H<sub>14</sub>BrN<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 408.0269, obtained 408.0392.

**Example 24:** 3-(2-bromophenyl)-7-(chloromethyl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine (**2j**).



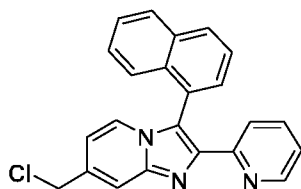
First, the method described as Method C was followed, starting from compound (**2i**) (408.3 mg, 1 mmol), LiAlH<sub>4</sub> (1M) (2.3 mmol), and THF (15 mL). The corresponding hydroxylated derivative was obtained, which was used without purification in the next reaction step according to Method D, starting from 3-(2-bromophenyl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-yl)methanol (152 mg, 0.4 mmol), SOCl<sub>2</sub> (95.2 mg, 0.8 mmol), and dichloromethane (10 mL). The title product was obtained as a yellowish oil. Yield: 73%; IR 3026, 1738, 1424, 1336, 1229, 1217, 794, 744 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.55 (d, J = 4.8 Hz, 1H), 7.83 (d, J = 7.9 Hz, 1H), 7.78–7.67 (m, 2H), 7.70–7.58 (m, 2H), 7.55–7.37 (m, 4H), 7.16 (ddd, J = 7.5, 4.8, 1.3 Hz, 1H), 6.88 (dd, J = 7.1, 1.8 Hz, 1H), 4.68 (s, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 152.58, 149.65, 144.14, 142.85, 136.11, 134.96, 133.83, 133.73, 133.32, 131.03, 130.94, 127.98, 125.85, 124.33, 122.39, 122.26, 122.04, 116.91, 113.13, 45.38; HRMS (ESI) calculated for C<sub>19</sub>H<sub>13</sub>BrClN<sub>3</sub> [M+H]<sup>+</sup>: 399.9952, obtained 400.0044.

**Example 25:** Synthesis of methyl 3-(naphthalen-1-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-carboxylate (**2k**).



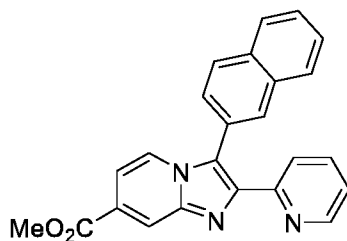
The method described as Method G was followed, starting from compound (**4d**) (501.5 mg, 1.98 mmol), 1-bromonaphthalene (612.9 mg, 2.96 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (160.6 mg, 0.14 mmol), K<sub>2</sub>CO<sub>3</sub> (819.6 mg, 5.93 mmol), and DMF (15 mL). The title product was obtained as a white solid. Yield: 51%; m.p.: 80-81°C; IR 1715, 1331, 1226, 1145, 1088, 756, 739 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.58 (d, J = 4.6 Hz, 1H), 8.55–8.52 (m, 1H), 8.11 (dd, J = 7.6, 2.0 Hz, 1H), 8.04 (d, J = 8.2 Hz, 1H), 7.74–7.65 (m, 2H), 7.60–7.46 (m, 4H), 7.42–7.33 (m, 2H), 7.32–7.29 (m, 1H), 7.11 (ddd, J = 6.7, 4.8, 1.9 Hz, 1H), 4.00 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  165.71, 152.27, 149.93, 144.14, 136.03, 134.03, 132.13, 132.03, 131.92, 130.28, 130.04, 128.82, 128.54, 128.42, 127.32, 126.62, 126.47, 125.94, 124.82, 123.70, 122.38.

**Example 26.** Synthesis of 7-(chloromethyl)-3-(naphthalen-1-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine (**2l**).



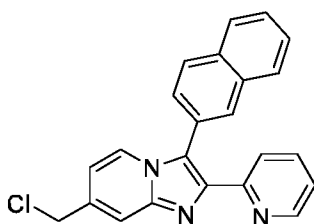
First, the method described as Method C was followed, starting from compound (**2k**) (379.4 mg, 1 mmol), LiAlH<sub>4</sub> (1M) (2.3 mmol), and THF (15 mL). The corresponding hydroxylated derivative was obtained, which was used without purification in the next reaction step according to Method D, starting from 3-(naphthalen-1-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-yl)methanol (140.5 mg, 0.4 mmol), SOCl<sub>2</sub> (95.2 mg, 0.8 mmol), dichloromethane (10 mL). The title product was obtained as a yellow solid. Yield: 73%; m.p.: 153-154°C ; IR 3051, 1587, 1375, 1348 1247, 1147, 775, 744 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.56 (d, J = 4.8 Hz, 1H), 8.08 (dd, J = 7.8, 1.8 Hz, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.78 (s, 1H), 7.68–7.60 (m, 2H), 7.59–7.53 (m, 1H), 7.50–7.36 (m, 5H), 7.07 (ddd, J = 7.3, 4.8, 1.5 Hz, 1H), 6.75 (dd, J = 7.1, 1.8 Hz, 1H), 4.66 (s, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  152.48, 149.81, 144.52, 143.59, 135.91, 134.71, 133.99, 132.17, 130.09, 130.05, 128.71, 127.20, 126.82, 126.51, 125.91, 124.94, 124.37, 122.18, 122.05, 121.50, 117.04, 113.03, 45.41; HRMS (ESI) calculated for C<sub>23</sub>H<sub>16</sub>ClN<sub>3</sub> [M+H]<sup>+</sup>: 370.1033, obtained: 370.1112.

**Example 27.** Synthesis of methyl 3-(naphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine-7-carboxylate (**2m**)



The method described as Method G was followed, starting from compound (**4d**) (501.5 mg, 1.98 mmol), 2-bromonaphthalene (612.9 mg, 2.96 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (160.6 mg, 0.14 mmol), K<sub>2</sub>CO<sub>3</sub> (819.6 mg, 5.93 mmol), and DMF (15 mL). The title product was obtained as a light brown solid. Yield: 58%; m.p.: 172-173°C: IR 1713, 1331, 1225, 1141, 1088, 816, 755, 737 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.59 (ddd, J = 4.8, 1.8, 0.9 Hz, 1H), 8.49 (dd, J = 1.7, 1.0 Hz, 1H), 8.11 (dd, J = 7.2, 1.0 Hz, 1H), 8.10–8.03 (m, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.97 (dd, J = 7.5, 2.6 Hz, 1H), 7.92 (d, J = 7.4 Hz, 1H), 7.75 (dt, J = 8.0, 1.1 Hz, 1H), 7.66–7.53 (m, 4H), 7.39 (dd, J = 7.4, 1.8 Hz, 1H), 7.19 (ddd, J = 7.5, 4.9, 1.2 Hz, 1H), 4.01 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  165.65, 152.68, 149.71, 144.41, 143.86, 136.15, 136.09, 133.46, 133.36, 130.60, 129.86, 129.27, 129.01, 128.24, 127.97, 127.88, 127.07, 126.66, 126.47, 126.41, 124.63, 123.14, 122.86, 122.51, 122.46, 120.84, 111.96, 111.87, 77.31, 77.20, 76.99, 76.68, 52.59; HRMS (ESI) calculated for C<sub>24</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 380.1321, obtained: 380.1401.

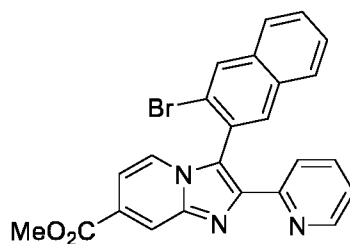
**Example 28.** Synthesis of 7-(chloromethyl)-3-(naphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine (**2n**).



First, the method described as Method C was followed, starting from compound (**2m**) (379.4 mg, 1 mmol), LiAlH<sub>4</sub> (1M) (2.3 mmol), and THF (15 mL). The corresponding hydroxylated derivative was obtained, which was used without purification in the next reaction step according to Method D, starting from 3-(naphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-yl)methanol (140.5 mg, 0.4 mmol), SOCl<sub>2</sub> (95.2 mg, 0.8 mmol), dichloromethane (10 mL). The title product was obtained as a yellow oil. Yield: 69%; IR 3051, 1738, 13655, 1229, 1217, 794, 748 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.58 (ddd, J = 4.9, 1.8, 1.0 Hz, 1H), 8.11–7.95 (m, 4H), 7.91 (dd, J = 7.4, 1.8 Hz, 1H), 7.79–7.69 (m, 2H), 7.67–7.53 (m, 4H), 7.17 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 6.87 (dd, J = 7.2, 1.8 Hz, 1H), 4.68 (s, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$

152.66, 149.67, 144.15, 136.13, 135.08, 133.50, 133.31, 129.90, 128.97, 128.21, 128.16, 127.87, 126.98, 126.62, 123.97, 122.72, 122.34, 116.97, 113.36, 45.35; HRMS (ESI) calculated for  $C_{23}H_{16}ClN_3$   $[M+H]^+$ : 370.1033, obtained: 369.1968.

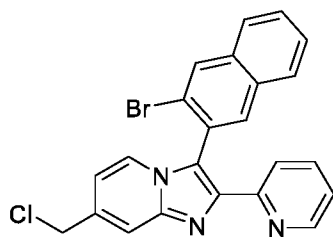
- 5 **Example 29:** Synthesis of methyl 3-(3-bromonaphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-carboxylate (**2o**).



The method described as Method G was followed, starting from compound (**4d**) (501.5 mg, 1.98 mmol), 2,3-dibromonaphthalene (846.4 mg, 2.96 mmol),  $Pd(PPh_3)_4$  (160.6 mg, 0.14 mmol),  $K_2CO_3$  (819.6 mg, 5.93 mmol), and DMF (15 mL). The title product was obtained as a light yellow solid. Yield: 55%; m.p.: 118-120°C; IR 1716, 1331, 1268, 1228, 1143, 1087, 751, 739  $cm^{-1}$ ;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.54–8.49 (m, 2H), 8.36 (s, 1H), 8.00 (s, 1H), 7.92 (d,  $J = 7.7$  Hz, 1H), 7.87 (d,  $J = 7.7$  Hz, 1H), 7.79 (d,  $J = 7.9$  Hz, 1H), 7.72 (dd,  $J = 7.2, 1.0$  Hz, 1H), 7.69–7.58 (m, 3H), 7.41 (dd,  $J = 7.2, 1.7$  Hz, 1H), 7.15 (ddd,  $J = 7.5, 4.8, 1.2$  Hz, 1H), 4.01 (s, 3H);  $^{13}C$  NMR (101 MHz,  $CDCl_3$ )  $\delta$  165.69, 152.51, 149.73, 145.05, 143.79, 136.20, 134.69, 133.14, 132.27, 132.01, 128.67, 128.26, 128.06, 127.00, 126.93, 126.59, 123.57, 122.48, 122.41, 122.20, 120.69, 111.97, 52.61; HRMS (ESI) calculated for  $C_{24}H_{16}BrN_3O_2$   $[M+H]^+$ : 460.0405, obtained: 460.0492.

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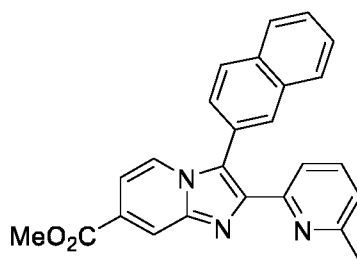
- Example 30:** Synthesis of 3-(3-bromonaphthalen-2-yl)-7-(chloromethyl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine (**2p**).



- 25 First, the method described as Method C was followed, starting from compound (**2o**) (458.3 mg, 1 mmol),  $LiAlH_4$  (1M) (2.3 mmol), and THF (15 mL). The corresponding hydroxylated

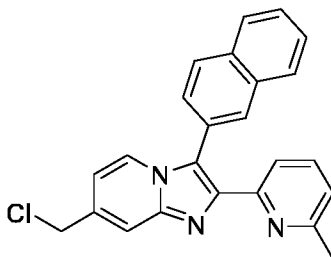
derivative was obtained, which was used without purification in the next reaction step according to Method D, starting from 3-(3-bromonaphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-yl)methanol (172.1 mg, 0.4 mmol), SOCl<sub>2</sub> (95.2 mg, 0.8 mmol), dichloromethane (10 mL). The title product was obtained as a yellow oil. Yield: 70%; IR 3049, 1736, 1420, 1385, 1350, 1230, 1217, 783, 743, 713 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.49 (d, J = 4.9 Hz, 1H), 8.35 (s, 1H), 7.99 (s, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.87–7.78 (m, 2H), 7.75 (dt, J = 7.9, 1.1 Hz, 1H), 7.71–7.55 (m, 4H), 7.13 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 6.89 (dd, J = 7.2, 1.8 Hz, 1H), 4.68 (s, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  152.36, 149.64, 143.98, 142.94, 136.18, 135.23, 134.60, 133.23, 132.25, 131.95, 128.20, 128.08, 127.98, 126.93, 126.87, 124.28, 122.59, 122.30, 122.07, 116.77, 113.35, 45.31; HRMS (ESI) calculated for C<sub>23</sub>H<sub>15</sub>BrClN<sub>3</sub> [M+H]<sup>+</sup>: 450.0117, obtained: 450.0200.

**Example 31.** Synthesis of methyl 3-(3-bromonaphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridin-7-carboxylate (**2q**).



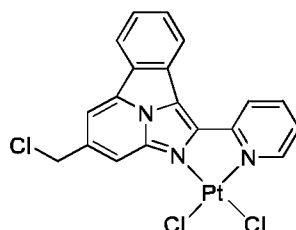
The method described as Method G was followed, starting from compound (**4k**) (1.6 mg, 6.25 mmol), 1-bromonaphthalene (1.9 mg, 9.36 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (489 mg, 0.44 mmol), K<sub>2</sub>CO<sub>3</sub> (2.59 mg, 18.73 mmol), and DMF (15 mL). The title product was obtained as a yellowish solid. Yield: 44%; m.p.: 163–164°C; IR 2963, 1797, 1589, 1573, 1431, 1329, 1273, 1247, 1084, 797, 751, 734 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.50 (s, 1H), 8.12 (dd, J = 7.2, 0.9 Hz, 1H), 8.10 (d, J = 1.6 Hz, 1H), 8.00 (d, J = 8.5 Hz, 1H), 7.97–7.95 (m, 1H), 7.91 (dd, J = 7.7, 1.5 Hz, 1H), 7.65–7.55 (m, 3H), 7.48 (d, J = 4.9 Hz, 1H), 7.38 (dd, J = 7.2, 1.7 Hz, 1H), 7.05 (t, J = 4.3 Hz, 1H), 4.00 (s, 3H), 2.48 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  165.95, 158.73, 152.12, 149.96, 145.01, 144.11, 136.59, 133.65, 133.58, 130.29, 128.99, 128.44, 128.35, 128.10, 127.25, 126.87, 126.76, 126.53, 123.36, 122.40, 121.15, 120.34, 112.16, 52.83, 24.73; HRMS (ESI) calculated for C<sub>25</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 394.1321, obtained: 394.1553.

**Example 32.** Synthesis of 3-(3-bromonaphthalen-2-yl)-7-(chloromethyl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine (**2r**).



First, the method described as Method C was followed, starting from compound (**2q**) (700 mg, 1.78 mmol), LiAlH<sub>4</sub> (1M) (4.10 mmol), and THF (20 mL). The corresponding hydroxylated derivative was obtained, which was used without purification in the next reaction step according to Method D, starting from 2-(6-methylpyridin-2-yl)-3-(naphthalen-2-yl)imidazo[1,2-a]pyridin-7-yl)methanol (400 mg, 1.09 mmol), SOCl<sub>2</sub> (391 mg, 3.28 mmol), dichloromethane (30 mL). The title product was obtained as a yellowish oil. Yield: 53%; IR 3048, 1560, 1375, 1349, 1252, 1147, 1050, 780, 742 cm<sup>-1</sup> <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.10–8.07 (m, 2H), 7.98 (d, J=8.4 Hz, 1H), 7.95 (dd, J=7.8, 1.5 Hz, 1H), 7.89 (dd, J=7.5, 1.6 Hz, 1H), 7.73 (s, 1H), 7.62–7.55 (m, 3H), 7.45 (d, J=5.6 Hz, 2H), 7.02 (dd, J=5.3, 3.3 Hz, 1H), 6.84 (dd, J=7.2, 1.8 Hz, 1H), 4.66 (s, 2H), 2.48 (s, 3H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  158.59, 152.42, 144.56, 143.33, 136.48, 134.82, 133.67, 133.46, 130.23, 128.87, 128.57, 128.38, 128.06, 127.12, 127.08, 126.76, 124.12, 123.67, 122.09, 120.16, 117.41, 113.32, 45.70, 24.74; HRMS (ESI) calculated for C<sub>24</sub>H<sub>18</sub>ClN<sub>3</sub> [M+H]<sup>+</sup>: 384.1189, obtained: 384.1244.

**Example 33.** Synthesis of dichloro(4-(chloromethyl)-1-(pyridin-2-yl)benzo[a]imidazo[5,1,2-cd]indolizine)platinum(II) (**1a**).

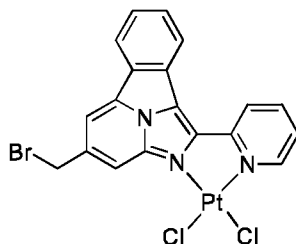


The method described as Method A was followed, starting from compound (**2c**) (63.6 mg, 0.2 mmol), Pt(DMSO)<sub>2</sub>Cl<sub>2</sub> (75 mg, 0.18 mmol), and MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 70%; m.p.: > 410°C; IR 3038, 1529, 1400, 1265, 1194, 1138, 1083, 765, 734, 703 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMF-d<sub>7</sub>)  $\delta$  9.29 (dt, J = 8.1, 1.0 Hz, 1H), 9.23 (dt, J = 4.7, 1.2 Hz, 1H), 8.90 (d, J = 7.8 Hz, 1H), 8.79–8.74 (m, 2H), 8.56 (s, 1H), 8.29 (td, J = 7.7, 1.8 Hz, 1H), 8.13 (t, J = 7.0 Hz, 1H), 7.96 (td, J = 7.7, 1.1 Hz, 1H), 7.73 (ddd, J = 7.5, 4.7, 1.2 Hz,

1H);  $^{13}\text{C}$  NMR (126 MHz, DMF- $d_7$ )  $\delta$  153.57, 150.51, 146.60, 138.99, 138.63, 137.52, 131.81, 131.00, 130.31, 129.90, 126.11, 123.97, 123.95, 123.83, 121.76, 114.31, 111.40, 46.99;  $^{195}\text{Pt}$  NMR (107 MHz, DMF- $d_7$ ) -2204.00; HRMS (ESI) calculated for  $\text{C}_{19}\text{H}_{12}\text{Cl}_3\text{N}_3\text{Pt}$   $[\text{M}+\text{Na}]^+$ : 605.9642, obtained: 605.9611.

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**Example 34.** Synthesis of dichloro(4-(bromomethyl)-1-(pyridin-2-yl)benzo[*a*]imidazo[5,1,2-*cd*]indolizine)platinum(II) (**1b**).

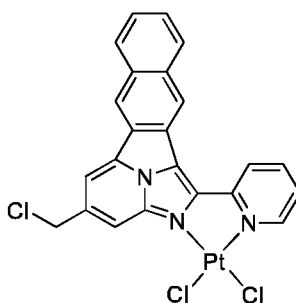


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The method described as Method A was followed, starting from compound (**2d**) (54.3 mg, 0.15 mmol),  $\text{Pt}(\text{DMSO})_2\text{Cl}_2$  (58.3 mg, 0.14 mmol), MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 76%; m.p. > 410°C; IR 3057, 1610, 1491, 1462, 1402, 1211, 1133, 788, 762, 732, 664  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR 500 MHz, DMF- $d_7$ )  $\delta$  9.69 (d,  $J$  = 6.2 Hz, 1H), 9.16 (s, 1H), 8.86–8.78 (m, 5H), 8.56 (td,  $J$  = 7.7, 1.6 Hz, 1H), 7.94 (t,  $J$  = 7.6 Hz, 1H), 7.90–7.83 (m, 1H), 5.41 (d,  $J$  = 2.2 Hz, 2H);  $^{13}\text{C}$  NMR (126 MHz, DMF- $d_7$ )  $\delta$  149.37, 143.59, 140.64, 132.69, 131.16, 128.50, 126.34, 124.69, 124.37, 123.46, 114.06, 113.42, 45.99;  $^{195}\text{Pt}$  NMR (107 MHz, DMF- $d_7$ ) -2207.29; HRMS (ESI) calculated for  $\text{C}_{19}\text{H}_{12}\text{BrCl}_2\text{N}_3\text{Pt}$   $[\text{M}+\text{K}]^+$ : 667.8856, obtained: 667.8838.

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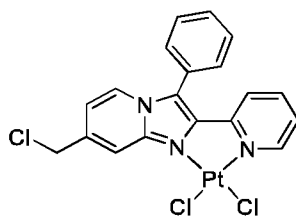
**Example 35:** Synthesis of dichloro(4-(chloromethyl)-1-(pyridin-2-yl)imidazo[5,1,2-*cd*]naphtho[2,3-*a*]indolizine)platinum(II) (**1c**).



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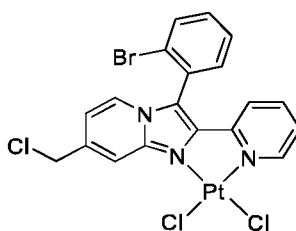
The method described as Method A was followed, starting from compound (**2f**) (55.2 mg, 0.15 mmol), Pt(DMSO)<sub>2</sub>Cl<sub>2</sub> (58.3 mg, 0.14 mmol), and MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 73%; m.p.: > 410°C; IR 3058, 1633, 1488, 1456, 1257, 1148, 1075, 878, 770, 734 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMF-d<sub>7</sub>) δ 9.90 (d, J = 6.0 Hz, 1H), 9.58 (s, 1H), 9.56 (s, 1H), 9.27 (s, 1H), 9.08 (d, J = 8.0 Hz, 1H), 8.87 (s, 1H), 8.69 (td, J = 7.8, 1.5 Hz, 1H), 8.62 (d, J = 8.0 Hz, 1H), 8.55 (d, J = 8.0 Hz, 1H), 8.07–7.97 (m, 4H), 5.51 (s, 2H); <sup>13</sup>C NMR 126 MHz, DMF-d<sub>7</sub>) δ 149.58, 140.44, 129.58, 128.50, 127.92, 126.01, 125.25, 124.01, 123.24, 113.98, 111.78, 46.07; HRMS (ESI) calculated for C<sub>23</sub>H<sub>14</sub>Cl<sub>3</sub>N<sub>3</sub>Pt [M+H]<sup>+</sup>: 655.9799, obtained: 655.9790.

**Example 36:** Synthesis of dichloro(7-(chloromethyl)-3-phenyl-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)platinum(II) (**1d**).



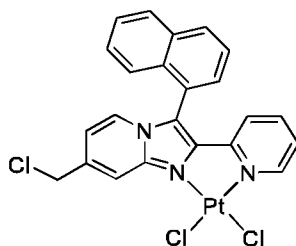
The method described as Method A was followed, starting from compound (**2h**) (48 mg, 0.15 mmol), Pt(DMSO)<sub>2</sub>Cl<sub>2</sub> (58.3 mg, 0.14 mmol), and MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 77%; m.p.: > 410°C; IR 3066, 1620, 1510, 1252, 778, 695 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMF-d<sub>7</sub>) δ 9.62 (d, J = 5.6 Hz, 1H), 9.26 (s, 1H), 8.20 (dd, J = 7.2, 1.0 Hz, 1H), 8.12 (td, J = 7.8, 1.5 Hz, 1H), 7.92–7.86 (m, 2H), 7.84–7.80 (m, 2H), 7.68–7.58 (m, 1H), 7.39–7.31 (m, 2H), 4.98 (d, J = 0.8 Hz, 2H); <sup>13</sup>C NMR (126 MHz, DMF-d<sub>7</sub>) δ 153.27, 148.92, 145.67, 141.17, 139.64, 131.44, 131.35, 130.37, 126.26, 125.31, 125.06, 121.56, 116.01, 114.65, 44.86; <sup>195</sup>Pt NMR (107 MHz, DMF-d<sub>7</sub>) -2178.24; HRMS (ESI) calculated for C<sub>19</sub>H<sub>14</sub>Cl<sub>3</sub>N<sub>3</sub>Pt [M+Na]<sup>+</sup>: 607.9799, obtained: 607.9785.

**Example 37:** Synthesis of dichloro(3-(2-bromophenyl)-7-(chloromethyl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)platinum(II) (**1e**).



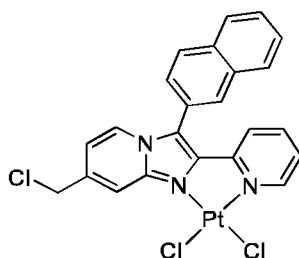
The method described as Method A was followed, starting from compound (**2j**) (59.5 mg, 0.15 mmol), Pt(DMSO)<sub>2</sub>Cl<sub>2</sub> (58.3 mg, 0.14 mmol), and MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 76%; m.p.: > 410°C; IR 065, 1618, 1509, 1252, 778, 696 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMF-d<sub>7</sub>) δ 9.60 (d, J = 5.9 Hz, 1H), 9.23 (s, 1H), 8.20 (d, J = 7.1 Hz, 1H), 8.11 (td, J = 7.9, 1.5 Hz, 1H), 7.94–7.87 (m, 1H), 7.83–7.80 (m, 3H), 7.62 (d, J = 7.6 Hz, 1H), 7.35 (dd, J = 7.3, 1.8 Hz, 2H), 4.97 (s, 2H); <sup>13</sup>C NMR (126 MHz, DMF-d<sub>7</sub>) δ 153.49, 149.06, 145.83, 141.24, 139.73, 131.58, 131.56, 130.49, 128.35, 127.39, 126.46, 126.22, 125.41, 125.32, 121.75, 116.13, 114.86, 45.04; <sup>195</sup>Pt NMR (107 MHz, DMF-d<sub>7</sub>) -2177.53; HRMS (ESI) calculated for C<sub>19</sub>H<sub>13</sub>BrCl<sub>3</sub>N<sub>3</sub>Pt [M+Na]<sup>+</sup>: 685.8904, obtained: 685.8892.

**Example 38:** Synthesis of dichloro(7-(chloromethyl)-3-(naphthalen-1-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)platinum(II) (**1f**).



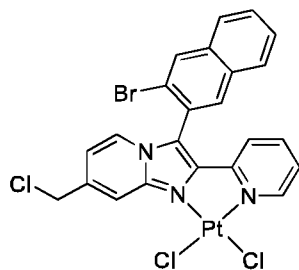
The method described as Method A was followed, starting from compound (**2i**) (55.5 mg, 0.15 mmol), Pt(DMSO)<sub>2</sub>Cl<sub>2</sub> (58.3 mg, 0.14 mmol), and MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 74%; m.p.: > 410°C; IR 3054, 1628, 1506, 1247, 1138, 792, 779, 769 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMF-d<sub>7</sub>) δ 9.63–9.56 (m, 1H), 9.29 (dd, J = 1.8, 0.9 Hz, 1H), 8.43 (dd, J = 8.4, 1.2 Hz, 1H), 8.28–8.23 (m, 1H), 8.15 (dd, J = 7.0, 1.2 Hz, 1H), 8.01–7.95 (m, 2H), 7.91 (dd, J = 8.3, 7.0 Hz, 1H), 7.72 (dd, J = 7.9, 6.5 Hz, 2H), 7.65–7.53 (m, 2H), 7.31 (dd, J = 7.2, 1.8 Hz, 1H), 6.96 (dd, J = 7.7, 1.2 Hz, 1H), 5.02 (s, 2H); <sup>13</sup>C NMR (126 MHz, DMF-d<sub>7</sub>) 153.07, 148.83, 146.14, 142.56, 141.21, 139.77, 134.26, 132.15, 131.82, 131.48, 129.21, 128.33, 127.35, 126.62, 126.51, 125.37, 124.44, 123.75, 121.91, 121.76, 116.11, 114.75, 44.90; <sup>195</sup>Pt NMR (107 MHz, DMF-d<sub>7</sub>) -2175.67; HRMS (ESI) calculated for C<sub>23</sub>H<sub>16</sub>Cl<sub>3</sub>N<sub>3</sub>Pt [M+Na]<sup>+</sup>: 657.9955, obtained: 657.9953.

**Example 39:** Synthesis of dichloro(7-(chloromethyl)-3-(naphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)platinum(II) (**1g**).



The method described as Method A was followed, starting from compound (**2n**) (55.5 mg, 0.15 mmol), Pt(DMSO)<sub>2</sub>Cl<sub>2</sub> (58.3 mg, 0.14 mmol), and MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 71%; m.p.: > 410°C; IR 3032, 1639, 1509, 1245, 1017, 774, 744, 698 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMF-d<sub>7</sub>) δ 9.81 (d, J = 5.2 Hz, 1H), 9.46 (s, 1H), 8.75 (s, 1H), 8.58 (d, J = 7.2 Hz, 1H), 8.54 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 8.0 Hz, 1H), 8.32 (d, J = 8.1 Hz, 1H), 8.27 (td, J = 7.9, 1.5 Hz, 1H), 8.15 (dd, J = 8.4, 1.7 Hz, 1H), 8.01–7.89 (m, 2H), 7.82 (td, J = 6.1, 1.5 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H), 7.54 (dd, J = 7.2, 1.8 Hz, 1H), 5.22 (s, 2H); <sup>13</sup>C NMR (126 MHz, DMF-d<sub>7</sub>) δ 153.26, 148.86, 145.65, 141.56, 141.24, 139.76, 134.37, 133.76, 131.99, 130.22, 128.82, 128.23, 128.15, 127.41, 127.28, 126.75, 125.85, 125.34, 122.46, 121.82, 115.98, 114.58, 44.92; <sup>195</sup>Pt NMR (107 MHz, DMF-d<sub>7</sub>) -2177.42; HRMS (ESI) calculated for C<sub>23</sub>H<sub>16</sub>Cl<sub>3</sub>N<sub>3</sub>Pt [M+Na]<sup>+</sup>: 657.9955, obtained: 657.9954.

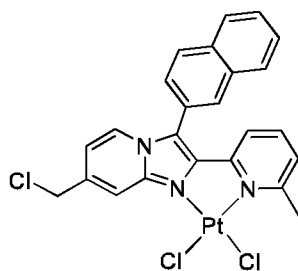
**Example 40:** Synthesis of dichloro(3-(3-bromonaphthalen-2-yl)-7-(chloromethyl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)platinum(II) (**1h**).



The method described as Method A was followed, starting from compound (**2p**) (67 mg, 0.15 mmol), Pt(DMSO)<sub>2</sub>Cl<sub>2</sub> (58.3 mg, 0.14 mmol), and MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 70%; m.p.: > 410°C; IR 3052, 1622, 1505, 1395, 1129, 1029, 949, 765, 752, 694 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMF-d<sub>7</sub>) δ 9.64 (d, J = 6.0 Hz, 1H), 9.33 (s, 1H), 8.73 (s, 1H), 8.70 (s, 1H), 8.38 (d, J = 7.1 Hz, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.17–8.09 (m, 2H), 7.87 (ddd, J = 8.3, 6.8, 1.3 Hz, 1H), 7.82–7.78 (m, 1H), 7.69 (ddd, J = 7.6, 5.9, 1.5 Hz, 1H), 7.44 (dd, J = 7.2, 1.7 Hz, 1H), 7.32–7.27 (m, 1H), 5.06 (s, 2H); <sup>13</sup>C NMR (126 MHz, DMF-d<sub>7</sub>) δ 152.72, 148.98, 141.76, 140.20, 135.75, 135.17, 132.91, 132.63, 129.33, 128.88, 127.90,

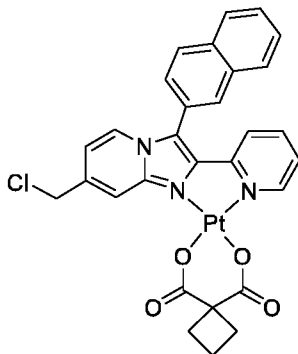
127.47, 126.98, 125.80, 122.91, 122.12, 120.59, 116.41, 114.61, 44.82;  $^{195}\text{Pt}$  NMR (107 MHz, DMF-d<sub>7</sub>) -2179.79; HRMS (ESI) calculated for  $\text{C}_{23}\text{H}_{15}\text{BrCl}_3\text{N}_3\text{Pt}$   $[\text{M}+\text{Na}]^+$ : 735.9060, obtained: 735.9053.

- 5 **Example 41:** Synthesis of dichloro(7-(chloromethyl)-2-(6-methylpyridin-2-yl) 3-(naphthalen-2-yl)imidazo[1,2-a]pyridine)platinum(II) (**1i**).



- 10 The method described as Method A was followed, starting from compound (**2r**) (48 mg, 0.13 mmol),  $\text{Pt}(\text{DMSO})_2\text{Cl}_2$  (52 mg, 0.13 mmol), and MeOH (5 mL). The title product was obtained as a yellow solid. Yield: 52%; m.p.: > 410°C; IR 3044, 1711, 591, 1505, 1418, 1299, 1195, 1126, 750, 478  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz, DMF-d<sub>7</sub>)  $\delta$  8.57 (s, 1H), 8.13 (s, 1H), 7.96–7.92 (m, 2H), 7.80 (d, J = 8.3 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.47–7.43 (m, 1H), 7.40–7.36 (m, 1H), 7.34–7.30 (m, 1H), 7.06 (d, J = 7.7 Hz, 1H), 6.93 (dd, J = 7.2, 1.6 Hz, 1H), 6.85 (d, J = 8.0 Hz, 2H), 4.62 (s, 2H), 2.93 (s, 3H);  $^{13}\text{C}$  NMR (126 MHz, DMF) 153.07, 148.83, 146.14, 142.56, 141.21, 139.77, 134.26, 132.15, 131.82, 131.48, 129.21, 128.33, 127.35, 126.62, 126.51, 125.37, 124.44, 123.75, 121.91, 121.76, 116.11, 114.75, 44.90;  $^{195}\text{Pt}$  NMR (107 MHz, DMF-d<sub>7</sub>) -1931.50; HRMS (ESI) calculated for  $\text{C}_{24}\text{H}_{18}\text{Cl}_3\text{N}_3\text{Pt}$   $[\text{M}+\text{Na}]^+$ : 671.0112, obtained: 671.0150.
- 15  
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- Example 42:** Synthesis of cyclobutane-1,1-dicarboxylate(7-(chloromethyl)-3-(naphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)platinum(II) (**1k**).

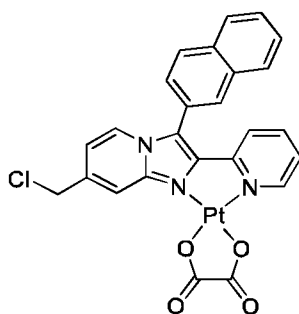


The title product was prepared in two steps, which are described in detail below.

Cyclobutane-1,1-dicarboxylic acid (CBDCA) (138 mg, 0.96 mmol) is dissolved in H<sub>2</sub>O (20 mL) and neutralized with Ag<sub>2</sub>O (223 mg, 0.96 mmol). The solution is stirred in the dark for 20 minutes at room temperature and cis-[PtCl<sub>2</sub>(DMSO)<sub>2</sub>] (400 mg, 0.99 mmol) is added. This mixture is left for 10 minutes at 70°C and then for 24 h at room temperature. A suspension is formed, from which the AgCl precipitate is prepared by means of filtration in order to concentrate the solution to about 5 mL. Finally, this mixture is left at 0°C until forming a white precipitate which is washed with cold water and vacuum dried. The complex [Pt(CBDCA)(DMSO)<sub>2</sub>] is obtained as a whitish solid [Ranaldo, R. *et al.*, *Inorg. Chem.* 2008, 47, 2820-2830].

[Pt(CBDCA)(DMSO)<sub>2</sub>] (72 mg, 0.15 mmol) is dissolved in MeOH (10 mL) together with compound (**2n**) (55 mg, 0.15 mmol) and the resulting mixture is stirred under reflux (65°C) overnight. Methanol is evaporated and the yellow solid obtained is washed successively with H<sub>2</sub>O and diethyl ether, to finally redissolve same in DCM and obtain the title product as a yellow solid. Yield: 75%; m.p.: 310-311°C; IR 2944, 1736, 1649, 1618, 1519, 1348, 1218, 1113, 778, 697, 477 cm<sup>-1</sup> <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 9.09 (d, J=5.6 Hz, 1H), 8.91 (s, 1H), 8.74 (m, 2H), 8.60–8.52 (m, 1H), 8.50 (td, J=7.9, 1.6 Hz, 1H), 8.33 (d, J = 1.4 Hz, 1H), 8.28 (dd, J = 8.4, 1.8 Hz, 1H), 8.21–8.10 (m, 3H), 8.02 (ddd, J=7.4, 5.8, 1.5 Hz, 1H), 7.81 (d, J=8.3 Hz, 1H), 7.67 (dd, J=7.2, 1.8 Hz, 1H), 5.38 (s, 2H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 178.74, 153.06, 147.88, 140.98, 138.53, 134.27, 132.09, 131.53, 130.62, 128.86, 128.51, 128.24, 128.04, 127.73, 126.94, 126.33, 125.17, 124.40, 121.36, 115.98, 114.02, 44.58, 31.47, 30.35, 15.57; <sup>195</sup>Pt NMR (107 MHz, CDCl<sub>3</sub>) -1942.63; HRMS (ESI) calculated for C<sub>25</sub>H<sub>16</sub>ClN<sub>3</sub>Pt [M+H]<sup>+</sup>: 708.0947, obtained: 708.0999.

**Example 43:** Synthesis of oxalate(7-(chloromethyl)-3-(naphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)platinum(II) (**1I**).

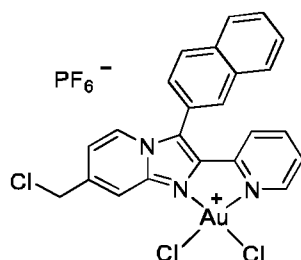


The title product was prepared in two steps, which are described in detail below.

- 5 Cis-[PtCl<sub>2</sub>(DMSO)<sub>2</sub>] (250 mg, 0.59 mmol) is dissolved in H<sub>2</sub>O (125 mL) and AgNO<sub>3</sub> (196 mg, 1.15 mmol) is added. The resulting mixture is heated in the dark for 6 h at 50°C and then at room temperature overnight. The suspension formed is filtered by means of celite and the filtrate is treated with an aqueous solution (1.5 mL) of oxalic acid (74.6 mg, 0.59 mmol) neutralized with KOH (66.4 mg, 1.18 mmol). The resulting mixture is stirred for 24 h until  
10 forming a precipitate. The solvent is evaporated at reduced pressure and washed with 1 mL of cold water to be vacuum dried. [Pt(OXA)(DMSO)<sub>2</sub>] is obtained as a white solid [Papadia, P. *et al.*, *RSC Adv.* 2019, 9, 32448-32452]

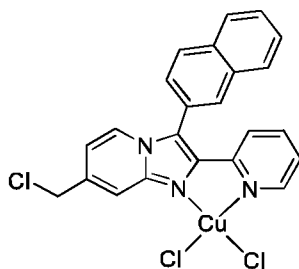
- [Pt(OXA)(DMSO)<sub>2</sub>] (58 mg, 0.13 mmol) is dissolved in MeOH (10 mL) together with compound  
15 (**2n**) (50 mg, 0.13 mmol). The resulting mixture is stirred under reflux (65°C) overnight. The solvent is evaporated and the yellow solid is washed successively with H<sub>2</sub>O and diethyl ether to obtain the title product as a yellow solid. Yield: 68%; m.p.: 333-334°C; IR 3487, 3054, 1699, 1615, 1520, 1350, 1218, 776, 703, 478 cm<sup>-1</sup> <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.75 (d, J=5.7 Hz, 1H), 8.47 (s, 1H), 8.28 (s, 1H), 8.21 (d, J=8.4 Hz, 1H), 8.17 (d, J=7.2 Hz, 1H), 8.04 (d, J=7.7  
20 Hz, 1H), 7.97 (d, J=7.2 Hz, 1H), 7.77–7.68 (m, 2H), 7.58 (dd, J=7.2, 1.6 Hz, 1H), 7.53–7.47 (m, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.22 (ddd, J=7.2, 5.6, 1.4 Hz, 1H), 7.08 (dd, J=7.3, 1.8 Hz, 1H), 4.68 (s, 2H), 2.83 (m, 4H), 1.88 (m, 2H).); <sup>195</sup>Pt NMR (107 MHz, DMF-d<sub>7</sub>) -1821.61; HRMS (ESI) calculated for C<sub>29</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>4</sub>Pt [M+H]<sup>+</sup>: 654.0477, obtained: 654.0522.

- 25 **Example 44:** Synthesis of [dichloro(7-(chloromethyl)-3-(naphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)gold(III)]PF<sub>6</sub> (**1m**).



Compound (**2n**) (50 mg, 0.13 mmol) is dissolved in MeCN (3 ml) and a solution in MeCN (5 mL) of AuCl<sub>3</sub> (40 mg, 0.13 mmol) and AgPF<sub>6</sub> (37 mg, 0.14 mmol) is added. The resulting solution is stirred at room temperature for 15 minutes. Then, the mixture thus obtained is filtered to remove AgCl and the solvent is removed at reduced pressure. Finally, the product is obtained as a reddish solid by means of precipitation in n-hexane from a DCM solution. Yield: 82%; m.p.: 182-183°C; IR 2970, 1703, 1648, 1613, 1502, 1352, 128, 834, 775, 557, 480 cm<sup>-1</sup> <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN) δ 9.56 (d, J=6.0 Hz, 1H), 9.18 (s, 1H), 8.49–8.41 (m, 3H), 8.32–8.23 (m, 3H), 7.98–7.86 (m, 3H), 7.82 (dd, J=8.4, 1.8 Hz, 1H), 7.72 (d, J=8.0 Hz, 1H), 7.57 (dd, J=7.2, 1.7 Hz, 1H), 5.06 (s, 2H); <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>CN) δ 151.00, 147.87, 146.63, 145.62, 145.02, 138.94, 135.30, 134.22, 132.56, 131.44, 129.40, 129.35, 128.75, 128.26, 128.00, 127.97, 127.84, 126.89, 124.89, 120.51, 118.51, 113.26, 44.71.

**Example 45:** Preparation of dichloro(7-(chloromethyl)-3-(naphthalen-2-yl)-2-(pyridin-2-yl)imidazo[1,2-a]pyridine)copper(II) (**1n**).



CuCl<sub>2</sub> (24 mg, 0.17 mmol) is dissolved in EtOH (2 mL) and a solution of compound (**2n**) (33 mg, 0.09 mmol) in EtOH (1.5 mL) is added. The resulting mixture is left under stirring at room temperature. A light gray precipitate is formed after 15-20 minutes, washed with EtOH and dried to obtain the end product, which is isolated as a light gray solid. Yield: 46%; m.p.: 170-171°C; IR 2970, 1737, 1609, 1505, 1458, 1354, 1217, 1093, 783, 748, 706, 478 cm<sup>-1</sup> The paramagnetic character of this compound did not allow recording NMR spectra.

**Characterization of the attachment of the compounds of the invention to DNA.**

The interaction between the molecules of formula (1) and DNA with broken strands was clearly shown by means of AFM (atomic force microscopy) and TEM (transmission electron microscopy).

5 DNA of *Escherichia coli* infected with  $\lambda$  phage (0.167 mg/ml) was incubated in the presence of Pt(II) compounds of formula (1) (10  $\mu$ M) between 2 and 10 minutes, and they were then analyzed by means of AFM (Figure 4) and TEM (Figure 5). The behavior of compound (1g) is shown below as an example. Similar results, with variable times, were observed in other tested compounds.

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The results of the present invention showed extremely striking differences between unincubated DNA (Figures 4A and 5A) and DNA after incubation in the presence of compound (1g), particularly after 10 minutes of exposure (Figures 4C and 5C). In the case of AFM records, complete degradation is observed after exposure to the metal complex, with a double  
15 breakage of the DNA strands. In the case of TEM images, a significant decrease in DNA density was observed in the sample after incubation with (1g), indicating the existence of mass lesions in the DNA strands.

These results indicate that, in the absence of cell barriers, the compounds of formula (1) react  
20 with DNA within minutes and cause, in addition to covalent distortions, irreversible lesions in the two complementary strands.

#### **Effect of the compounds of formula (1) on cell viability.**

25 Cell viability of different (tumor or normal) cell cultures was analyzed in the absence or presence of the compounds of formula (1) at two different concentrations (10  $\mu$ M and 20  $\mu$ M) in a quiescent culture medium for 48 hours (Figure 6). The assayed cell cultures were extra- and intrahepatic cholangiocarcinoma CCA (EGI-1 and HUCCT1), ovarian cancer (A2780Cis), breast cancer (MDA-453 and MDA-231), and normal human cholangiocyte (NHC) cell cultures.  
30 Cell viability was analyzed using the WST-1 cell proliferation assay (Roche) according to the manufacturer's instructions. Cells ( $2.5 \times 10^4$ ) were seeded in collagen-coated 96-well plates in their corresponding culture media and incubated overnight at 37°C. Treatments with compounds of formula (1) and with cisplatin (CisPt), for purposes of comparison, were then added. Cells incubated with a vehicle solution were considered the control group. Finally, 10  
35  $\mu$ L of WST-1 were added to each well, incubated at 37°C for 1 hour, and the signal was

measured at 450 nm on a Halo LED 96® plate reader (Dynamic Scientific Ltd., United Kingdom).

The data indicates that compounds (1d), (1e), (1f), (1g), and (1h) significantly reduce cell viability in all tumor cell lines (CCA, breast, and ovarian) compared to CisPt or untreated cells (control). Moreover, compound (1a) also significantly reduced viability in breast and ovarian tumor cells compared to CisPt.

It is important to note that the tested compounds did not have any significant effect on the viability of normal cholangiocytes. These results were obtained both at a concentration of 10  $\mu$ M (Figure 6) and at a concentration of 20  $\mu$ M (Figure 7) for each molecular entity. It can thus be concluded that the new synthesized chemotherapeutic compounds are highly potent and selective with respect to tumor cells, but do not affect the viability of normal cells.

#### **Effect of the compounds of formula (1) on cell death (early apoptosis) in different cell cultures.**

Caspase-3 activation is a cellular event associated with early cell death, so its detection by means of flow cytometry (PhiPhiLux®-G2D2, Oncolmmunin, Inc) is a very useful assay for analyzing one of the first biochemical events associated with apoptosis.

Cells ( $1-3 \times 10^4$ , depending on the cell line) were seeded in 24-well plates in their corresponding culture media. Once the cells were adhered, they were incubated with chemotherapeutic agents (at concentrations of 10  $\mu$ M and 20  $\mu$ M) or fresh medium (according to the assay). After 48 hours, supernatants were collected and cells were trypsinized and centrifuged at 1500 rpm for 5 minutes at room temperature. The cell pellets were then washed once with cold DPBS 1X (Gibco-Thermo Fisher Scientific). The cells were then labeled with the reactive substrate Caspase-3 (Catalog # A304R2G) at a concentration of 75  $\mu$ L/ $10^6$  cells and incubated for 30-60 min at 37°C in the dark. After incubation, the cells were washed with a flow cytometry dilution buffer at a concentration of  $10^6$  cells/mL and centrifuged at 1500 rpm for 5 minutes at room temperature. Lastly, the cell pellet was resuspended in the flow cytometry dilution buffer at the recommended concentration according to the manufacturer's instructions (400 cells/ $\mu$ L). Finally, differentially labeled cell populations were distinguished by placing suitable filters (532, 543, 561, or 568 nm) in the Guava Easycyte 8HT flow cytometer (Merck Millipore). Labeled but untreated samples were used to establish fluorescence compensation levels. Results are shown in relation to cells treated with vehicle or control.

The data, shown in Figure 8, indicates that examples (1d), (1g), and (1h) caused significantly more early death than CisPt in extrahepatic (EGI-1) and intrahepatic (HUCCT1) CCA cells, as well as in cisplatin-resistant ovarian cancer cells (both at 10  $\mu$ M and at 20  $\mu$ M). Moreover, compound (1a) also induced more death in ovarian tumor cells compared to CisPt. In turn, cell death in breast cancer lines was also increased in the case of compound (1h) compared to CisPt. It is important to note that none of these four compounds induced cell death in normal human cholangiocytes.

#### 10 **Effect of the compounds of formula (1) on cell death (late apoptosis) in different cell cultures.**

Late apoptosis was determined by means of flow cytometry using a dual fluorescent staining method with FITC annexin V (BioLegend) and TO-PROTM-3 iodide (Invitrogen-Thermo Fisher Scientific). The FITC-labeled annexin V fluorescent dye (Ex 494 nm/Em 520 nm; green) is a  $\text{Ca}^{2+}$ -dependent phospholipid-binding protein with a high affinity for the phospholipid, phosphatidylserine, translocated to the outer part of the plasma membrane during early apoptosis, whereas the TO-PROTM-3 iodide fluorescent dye (Ex 642 nm/Em 661 nm; red) has a very high affinity for dsDNA after loss of the membrane and nuclear integrity, indicating late apoptosis.

Cells ( $1-3 \times 10^4$ , depending on the cell line) were seeded in 24-well plates in their corresponding culture media. Once the cells were adhered, they were incubated with chemotherapeutic agents (at concentrations of 10  $\mu$ M and 20  $\mu$ M) or fresh medium (according to the assay). In parallel, a positive cell death control was included by adding 2  $\mu$ g/mL of puromycin dihydrochloride (Sigma-Aldrich). After 48 hours, supernatants were collected and cells adhered to the plate were trypsinized and centrifuged at 1500 rpm for 5 minutes at room temperature. The cell pellets were then washed once with cold DPBS 1X (Gibco-Thermo Fisher Scientific) and resuspended in annexin V binding buffer diluted in deionized water ( $\text{dH}_2\text{O}$ ) 1:10 (BioLegend) at a concentration of  $10^6$  cells/mL. The cells were then placed in a 96-well plate with a U-shaped bottom (Falcon) and labeled with 100  $\mu$ g/mL of FITC Annexin V fluorescent dye (5  $\mu$ L/well) for 15 minutes at room temperature in the dark. The cells were then incubated in the dark for 15 minutes on ice with TO-PROTM-3 iodide fluorescent dye 1  $\mu$ M (5  $\mu$ L/well) and then diluted with annexin V binding buffer at the recommended concentration, according to the manufacturer's instructions. Finally, differentially labeled cell populations were distinguished by placing suitable filters (525/30 nm and 661/15 nm) in the Guava EasyCyte

8HT flow cytometer (Merck Millipore). The rate of apoptosis was analyzed with the Incyte™ 3.1 software (Merck Millipore). Single staining samples were used to establish fluorescence compensation levels. Results are shown in Figure 9 compared to cells incubated with vehicle (control).

5

The data of the present invention revealed that compounds (1d) and (1g) significantly increased the rate of cell death compared to CisPt in extrahepatic CCA cells (both cisplatin-sensitive and resistant), cisplatin-resistant ovarian cancer cells, and cells from the CisPt-resistant breast cancer cell line. Moreover, compound (1h) had a significant effect on cell death in the intrahepatic CCA line, the CisPt-resistant ovarian cancer line, and the partly CisPt-resistant breast cancer line compared to CisPt. In terms of compound (1a), it induced significantly more death than CisPt in CisPt-resistant ovarian cells. In a relevant manner, none of these four compounds induced apoptosis in normal human cholangiocytes, as shown in the second right panel of Figure 9.

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#### **Effect of the compounds of formula (1) on DNA damage in different cell cultures.**

Cellular DNA damage in different cell cultures caused by the new family of chemical entities was evaluated using a COMET assay (Comet Assay Kit-ab238544). Said assay, also called single cell gel electrophoresis (SCGE), is a quick and sensitive technique for quantifying and analyzing DNA damage in individual cells. As such, it is one of the techniques used in the area of cancer research for evaluating the genotoxicity and efficacy of chemotherapeutic agents.

Cells ( $1-3 \times 10^5$ , depending on the cell line) were seeded in 6-well plates in their corresponding culture media. Once the cells were adhered, chemotherapeutic agents were added at a concentration of 10  $\mu$ M or fresh medium was added (depending on the assay). After 48 hours, supernatants were discarded and cells adhered to the plate were trypsinized and centrifuged at 1500 rpm for 5 minutes. The cell pellets were then washed once with cold 1X DPBS (Gibco-Thermo Fisher Scientific), centrifuged at 1500 rpm for 5 minutes, and the supernatants were discarded. The pellets were then resuspended in cold 1X DPBS (Gibco - Thermo Fisher Scientific) at a concentration of  $1 \times 10^5$  cells/mL.

In parallel, agarose was heated at 90-95°C in the bath for 20 minutes or until the agarose became liquid, and then cooled in a bath at 37°C for another 20 minutes. Next, 75  $\mu$ L of agarose per well were added to the slide to create a base. Complete coverage of the well was

35

ensured by spreading the agarose solution with the pipette tip. The slide was stored at 4°C for 15 minutes, keeping it horizontal.

5 Once the slide was prepared with the agarose and the cells raised up, the cells were combined with agarose at a 1:10 ratio and 75 µL/well of the dilution were transferred to the top part of the previously created agarose layer. Complete coverage of the well was ensured by spreading the suspension carefully with the pipette tip, without disturbing the base layer. The slide was stored at 4°C for 15 minutes in the dark, keeping it horizontal.

10 The slide was carefully immersed in a small container with previously cooled lysis buffer (~ 25 mL/slide) for 30-60 minutes at 4°C in the dark. The lysis buffer was carefully aspirated from the container and replaced with previously cooled alkaline solution (~ 25 mL/slide) for 30 minutes at 4°C in the dark. This was performed for the purpose of obtaining the “nucleoids” containing the supercoiled DNA attached to the nuclear matrix.

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Then, keeping the slide horizontal, it was transferred to an electrophoresis chamber filled with a cold alkaline solution. Electrophoresis was performed at a voltage of 1 volt/cm and 300 mA for 15-30 minutes. In this way, those cells with broken DNA would lose their supercoiling and have a greater capacity to migrate towards the anode, giving rise to the formation of a dispersion similar to a comet tail, hence the name COMET for this type of experiment (Figure 10). The comet head reflects undamaged DNA, whereas the comet tail reflects damaged cellular DNA. Said comet-type distributions were visualized by means of confocal fluorescence microscopy. Therefore, the relative fluorescence of the comet tail in relation to the head provides a measure of DNA damage.

Once electrophoresis ended, the slide was washed with deionized water (dH<sub>2</sub>O, ~ 25 mL/slide) for 2 minutes. After 3 washes, the slide was incubated with cold 70% ethanol (~ 25 mL/slide) for 5 minutes and then allowed to air dry. Once the agarose and slide were completely dry, 100 µL/well of a 1:10000 dilution of view Green DNA Dye in tris-EDTA (TE) buffer was added and incubated for 15 minutes at room temperature. Finally, the slides were visualized under an LS900 ZEISS confocal microscope.

Approximately 50-100 comets were visualized and quantified for each condition in each cell type. To quantify DNA damage, the displacement between the genetic material in the nucleus (comet head) and the resulting tail was measured. The two most common parameters for

analyzing comet assay results are Tail Moment and Tail DNA%, according to the following expressions:

$$\text{Tail DNA\%} = 100 \times \text{Tail DNA Intensity} / \text{Cell DNA Intensity}$$

$$\text{Tail Moment} = \text{Tail DNA\%} \times \text{Tail Moment Length}$$

The data of the present invention demonstrated greater DNA damage in CCA cells treated with the new compounds of formula (1) compared to CisPt, as measured by an increase in the relative amount of DNA in the comet tail, as well as by an increased migration of genetic material. This result can be observed both in the confocal microscopy images in which a larger amount of genetic material is observed in the comet tail, and the quantification thereof (Figure 10). The two parameters used, both Tail Moment and Tail DNA%, are higher in CCA cells incubated with the new compounds (1) compared to CisPt and DMF control (Figure 10a). In turn, in ovarian cancer cells, a dispersion of genetic material is also observed in microscopy images (Figure 10b). At the quantitative level, both Tail DNA% and Tail Moment also increased in all four examples, although this increase was more significant in cells treated with compound (1a) compared to CisPt and control. This result is in line with previous results, which seem to suggest that (1a) has a more specific effect on CisPt-resistant ovarian cancer cells. In a relevant manner, none of these four compounds (1a), (1d), (1g), and (1h) induced cellular DNA damage in normal human cholangiocytes (Figure 10b).

5

**Therapeutic effect of the compounds of formula (1) on a subcutaneous xenograft model of immunodeficient mice using CCA cells.**

The therapeutic effect of the most promising new compounds was evaluated on a subcutaneous xenograft model of CCA. To that end, subcutaneous tumors were generated by injecting human CCA cells (EGI-1,  $1 \times 10^6$  tumor cells in each hind flank of mice) in immunodeficient mice (CD1-Foxn1 un). Immunodeficient mice were used to avoid immunological rejection of the mouse to human tumor cells. The size of the tumors was then evaluated using caliper measurements every 2 days. Once the tumors reached a minimum size of 0.5 cm in diameter, the compounds were administered in monotherapy for 4-6 weeks. The compounds were administered by intraperitoneal injection at a concentration of 0.5 mg/kg once a week.

In this assay, stabilization of the tumor volume of mice treated with the compound (1g) is observed (Figure 11) from day 35, whereas at that concentration the tumor volume of mice

treated with CisPt increased similarly to the control group (DMF). Likewise, it is observed that both the tumor volume and the weight of tumors treated with compound (**1g**) were significantly lower than those of tumors treated with DMF and CisPt, once extracted after sacrifice.

### Fluorescent properties of the compounds of formula (1)

By way of example, solutions were prepared at a concentration of  $1 \cdot 10^{-3}$  M in DMF of compound (**1a**) and  $1 \cdot 10^{-5}$  M in DMEM/F12 (Modified Eagle Medium: Nutrient Mixture). The latter was prepared from a solution of compound (**1a**)  $1.8 \cdot 10^{-3}$  M in DMF. The most relevant photophysical properties of this compound, measured in both media, are shown in the following table:

| Compound                | $\lambda(\text{exc})$ | $\lambda(\text{em})$ | $\phi$ | $\Delta\lambda$ | $\tau(1/2)$   | $\varepsilon$     | $\sigma$ | $B(\lambda)$ |
|-------------------------|-----------------------|----------------------|--------|-----------------|---------------|-------------------|----------|--------------|
| <b>(1a)</b><br>DMF      | 366                   | 490                  | 0.13   | 124             | 1.06          | 18528.57<br>(267) | 0.71     | 2408.71      |
|                         | 390                   |                      |        | 100             | (60%)<br>5.03 | 9594.10<br>(329)  | 0.37     | 1247.23      |
|                         | 442                   |                      |        | 48              | (40%)         | 5373.55<br>(405)  | 0.20     | 698.56       |
| <b>(1a)</b><br>DMEM/F12 | 303                   | 432<br>454           | 0.11   | 129<br>151      | 1.41          | 11710.31<br>(264) | 0.45     | 1288.13      |
|                         | 395                   |                      |        | 37<br>59        | (50%)<br>5.02 | 3702.92<br>(395)  | 0.14     | 407.32       |
|                         | 415                   |                      |        | 17<br>59        | (50%)         | 3849.10<br>(415)  | 0.15     | 423.39       |

5

In said table,  $\lambda(\text{exc})$  and  $\lambda(\text{em})$  are the excitation and emission wavelengths, respectively, expressed in nm;  $\phi$  is the quantum yield of the fluorescent emission corresponding to  $\lambda(\text{em})$ ;  $\tau(1/2)$  is the half-life of said emission, expressed in ns a  $\lambda(\text{exc}) = 340$  nm;  $\varepsilon$  is the molar extinction coefficient expressed in  $\text{M}^{-1} \cdot \text{cm}^{-1}$ ;  $\sigma$  is the effective absorption section corresponding to  $\varepsilon$ , expressed in  $\text{\AA}^2$  and, finally,  $B(\lambda)$  is the brightness of the fluorescent emission, expressed as  $B(\lambda) = \phi \times \varepsilon$ , expressed in  $\text{M}^{-1} \cdot \text{cm}^{-1}$ .

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As can be seen in said table, compound (**1a**) showed fluorescence in the visible range (Figure 12), with useful photophysical properties for monitoring by means of confocal microscopy.

### **Elucidation of the mechanisms for the uptake and discharge of the compounds of formula (1)**

In order to determine the molecular mechanisms for the uptake and discharge of the new family of chemical entities, tools consisting of monoclonal or polyclonal cell sublines with homologous or heterologous expression of human proteins involved in hepatic drug transport were obtained by lentiviral transduction. In most cases, cells derived from induced Chinese hamster ovarian cancer (CHO) were used as starting cell lines, due to their homogeneity, easy manipulation in transport studies, and good plasma membrane expression of the transduced constructs. In some cases, cell sublines derived from human liver cancers (HepG2 and PLC/PRF/5) including CCA (TFK1) were also obtained. The transporters were selected due to being involved in the uptake of various antitumor drugs by liver cells, and include the following: OATP1B1, OATP1B3, OATP1A2, OATP2B1, OCT1, OCT3, and CTR1. Transporter expression was validated by RT-qPCR, WB, IF and by means of transport assays with typical substrates.

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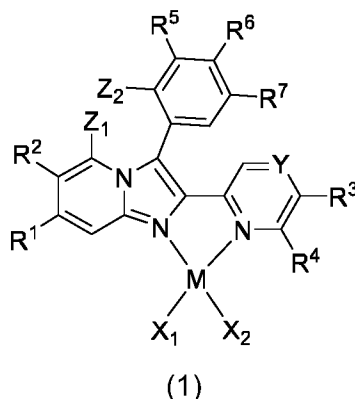
To elucidate the involvement of each transporter in the uptake of these platinized compounds, competition and accumulation experiments were carried out. In the competition experiment, control and transporter-expressing cells were exposed to a known fluorescent substrate of the transporter in the presence or absence of the typical inhibitor of the transporter or compounds of formula (**1**), and the intracellular load of the substrate was determined by end-time flow cytometry. In the second type of experiment, cells were exposed to the corresponding compound (**1**) for a given time, after which the cells were processed to determine the intracellular content of the compound (**1**) under study by means of HPLC-MS/MS.

The results obtained allowed ruling out the involvement of OATP1B3 and OATP2B1, from the organic anion transporting polypeptide (OATP) family, in the transport of compounds of formula (**1**). Despite the fact that the presence of said compounds stimulates transport mediated by both proteins (Figure 13), which could reflect some kind of interaction of the compounds of formula (**1**) with the transporter protein, no increased accumulation of said compounds has been observed in OATP1B3- or OATP2B1-expressing cells compared to cells present in controls (pWPI or Mock; Figures 15 and 16). However, OCT1 and OCT3, organic cation transporters, as well as OATP1A2, mediate the uptake of compounds (**1d**) and (**1g**). Co-incubation with the new compounds (**1**) partially inhibits transport mediated by OCT1, OCT3

(Figure 13), and OATP1A2 (Figure 13), and particularly compound (**1d**) in the case of OATP1A2. Furthermore, CHO-OCT1, CHO-OCT3, and CHO-OATP1A2 cells took up significantly more (**1d**) and (**1g**) than control cells (Figures 15 and 16). It is important to note that OCT1-, OCT3-, and OATP1A2-mediated transport was not inhibited by cisplatin (Figure 14). This result, together with previously described evidence (PMID: 16914559), suggests that probably none of the transporters of the new compounds of formula (**1**) transports cisplatin.

## CLAIMS

1. A compound of formula (1):



5 wherein:

M is a metal center selected from Pt(II), Cu(II), and Au(III), with the proviso that when M is Au(III), formula (I) incorporates an anion selected from Cl<sup>-</sup>, PF<sub>6</sub><sup>-</sup>, and R<sub>8</sub>C(O)O<sup>-</sup>, wherein R<sub>8</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group;

10 X<sub>1</sub> and X<sub>2</sub> are both chloride or together form a group selected from (·O-C(O)-C(O)-O<sup>-</sup>), (·O-C(O)-C(R<sub>9</sub>)(R<sub>10</sub>)-C(O)-O<sup>-</sup>), and (·O-C(O)-CH(R<sub>11</sub>)-O<sup>-</sup>), wherein the oxygen atoms (O<sup>-</sup>) are attached to the metal center, and wherein R<sub>9</sub> and R<sub>10</sub> are independently a C<sub>1</sub>-C<sub>6</sub> alkyl group or both form a C<sub>4</sub>-C<sub>6</sub> cycloalkyl group together with the carbon to which they are linked, and R<sub>11</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group;

Z<sub>1</sub> is hydrogen or can form a single covalent bond together with Z<sub>2</sub>;

15 Z<sub>2</sub> is hydrogen, halogen, or can form a single covalent bond together with Z<sub>1</sub>, or can be attached to R<sub>5</sub> forming an aryl group optionally substituted by at least one C<sub>1</sub>-C<sub>6</sub> alkyl group, halogen, or OH;

Y is N or CR<sub>12</sub>, wherein R<sub>12</sub> is H or a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; or a leaving functional group;

20 R<sub>1</sub>-R<sub>4</sub> are selected independently from H; a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; and a leaving functional group;

R<sub>5</sub>-R<sub>7</sub> are all H, or grouped in R<sub>5</sub>-R<sub>6</sub> or R<sub>6</sub>-R<sub>7</sub> pairs, can form a C<sub>6</sub> aryl group optionally substituted by at least one C<sub>1</sub>-C<sub>6</sub> alkyl group, halogen, or hydroxyl.

2. The compound of formula (1) according to claim 1, wherein M is Pt(II).

25

3. The compound according to claim 1 or 2, wherein X<sub>1</sub> and X<sub>2</sub> are both chloride, or together form a group selected from (·O-C(O)-C(O)-O<sup>-</sup>) and (·O-C(O)-C(R<sub>9</sub>)(R<sub>10</sub>)-C(O)-

O<sup>-</sup>), wherein the oxygen atoms (O<sup>-</sup>) are attached to the metal center, and wherein R<sub>9</sub> and R<sub>10</sub> form a C<sub>4</sub>-C<sub>6</sub> cycloalkyl group together with the carbon to which they are linked.

4. The compound according to any one of claims 1 to 3, wherein Y is N or CR<sub>12</sub>, wherein R<sub>12</sub> is H or a C<sub>1</sub>-C<sub>6</sub> alkyl group.

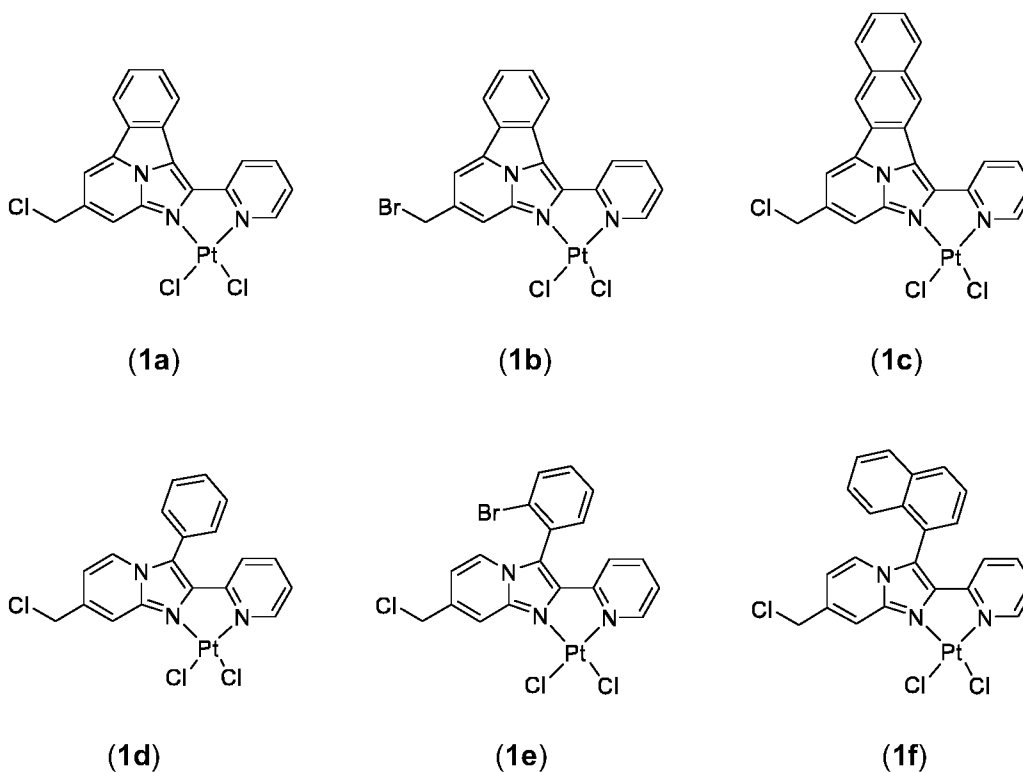
5. The compound according to claim 4, wherein Y is -CH-.

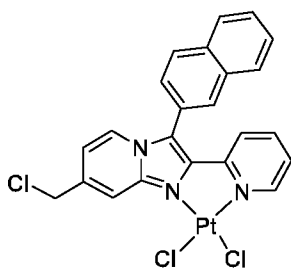
6. The compound according to any one of claims 1 to 5, wherein Z<sub>2</sub> is hydrogen, halogen, or can form a single covalent bond together with Z<sub>1</sub>, or can be attached to R<sub>5</sub> forming a C<sub>6</sub> aryl group.

7. The compound according to any one of claims 1 to 6, wherein R<sub>1</sub> is a methyl substituted at the end of the chain by a halogen or is a leaving functional group.

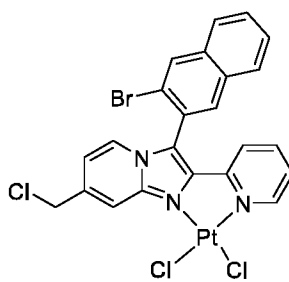
8. The compound according to any one of claims 1 to 7, wherein R<sub>5</sub>-R<sub>7</sub> are all H, or grouped in R<sub>5</sub>-R<sub>6</sub> or R<sub>6</sub>-R<sub>7</sub> pairs, can form a C<sub>6</sub> aryl group.

9. A compound of formula (1) selected from the following:

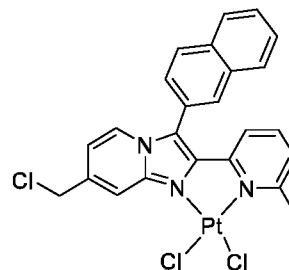




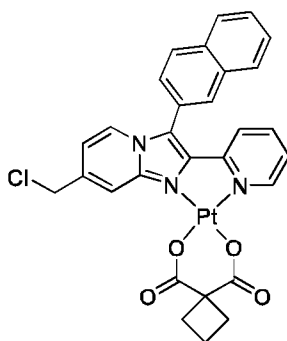
(1g)



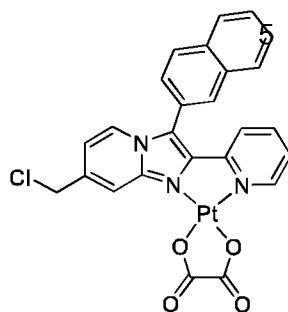
(1h)



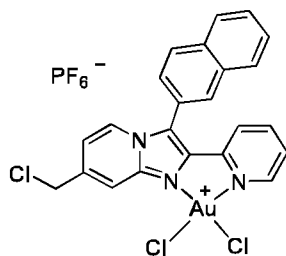
(1i)



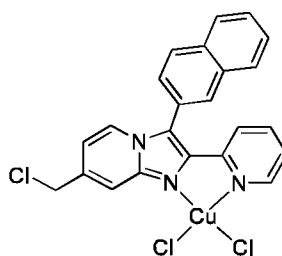
(1k)



(1l)



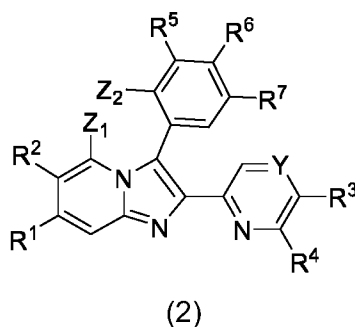
(1m)



(1n)

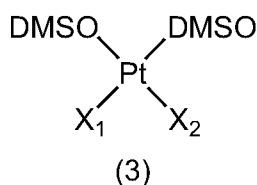
or a salt, solvate, or stereoisomer thereof.

10. A method for the preparation of a compound of formula (1), which comprises reacting a compound of formula (2):



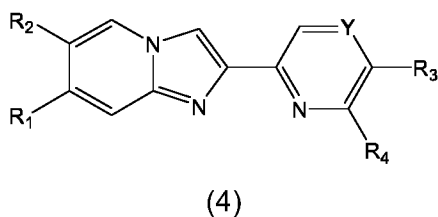
wherein Y, Z<sub>1</sub>, Z<sub>2</sub>, R<sub>1</sub>-R<sub>7</sub> have the meaning defined in claim 1,

- 5 with a metal salt selected from gold trichloride (AuCl<sub>3</sub>), copper dichloride (CuCl<sub>2</sub>), and platinum salts of formula (3):



10 wherein X<sub>1</sub> and X<sub>2</sub> have the meaning defined in claim 1 and DMSO represents a dimethyl sulfoxide ligand.

11. The method according to claim 10, which further comprises the prior step of preparing the compound of formula (2) by means of a method comprising the coupling or cycloaddition reaction between a compound of formula (4):



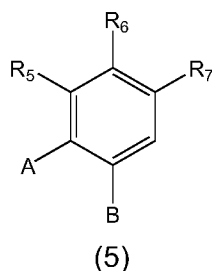
20 wherein,

Y is N; CR<sub>12</sub>, wherein R<sub>12</sub> is H or a C<sub>1</sub>-C<sub>6</sub> alkyl group optionally substituted at the end of the chain by a halogen or hydroxyl; a leaving functional group; or C(O)OMe;

R<sub>1</sub>-R<sub>4</sub> are selected independently from H; a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; a leaving functional group; and a C(O)OMe group;

25 and a compound of formula (5):

68



wherein

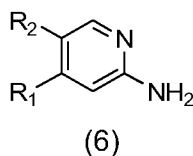
R<sub>5</sub>-R<sub>7</sub> have the meaning defined in claim 1;

5 A is H, halogen, a trifluoromethylsulfonyl group, or a trialkylsilyl group;

B is a halogen, a trifluoromethylsulfonyl group, or a trialkylsilyl group;

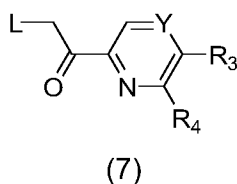
with the proviso that one of A and B is a trifluoromethylsulfonyl group or a trialkylsilyl group.

12. The method according to claim 11, which further comprises a prior step of preparing the  
 10 compound of formula (4) by means of a method comprising the condensation reaction between a compound of formula (6):



- 15 wherein R<sub>1</sub> and R<sub>2</sub> are selected independently from H; a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; a leaving functional group; and a C(O)OMe group;

and a compound of formula (7):



20

or a hydrobromide salt thereof;

wherein,

- 25 Y is N; CR<sub>12</sub>, wherein R<sub>12</sub> is H; a C<sub>1</sub>-C<sub>6</sub> alkyl group optionally substituted at the end of the chain by a halogen or hydroxyl; a leaving functional group; or C(O)OMe;

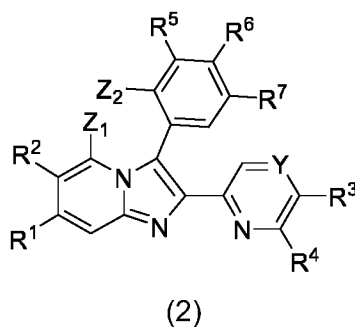
R<sub>3</sub> and R<sub>4</sub> are selected independently from H; a C<sub>1</sub>-C<sub>6</sub> alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; a leaving functional group; and a C(O)OMe group;

L represents a halogen, a methylsulfonyl group, or a p-toluenesulfonyl group.

13. The method according to claim 11 or 12, which further comprises, when any of  $R_1$ ,  $R_2$ ,  $R_3$ , or  $R_4$  is a  $C(O)OMe$  ester group, the substitution of said ester by a methyl group substituted at the end of the chain by a halogen or a hydroxyl; or by a leaving functional group.

5

14. A compound of formula (2):



10

or a salt, solvate, or stereoisomer thereof;

wherein,

$Z_1$  is hydrogen or can form a single covalent bond together with  $Z_2$ ;

$Z_2$  is hydrogen, halogen, or can form a single covalent bond together with  $Z_1$ , or can be attached to  $R_5$  forming an aryl group optionally substituted by at least one  $C_1$ - $C_6$  alkyl group, halogen, or OH;

15

$Y$  is N or  $CR_{12}$ , wherein  $R_{12}$  is H; a  $C_1$ - $C_6$  alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; or a leaving functional group;

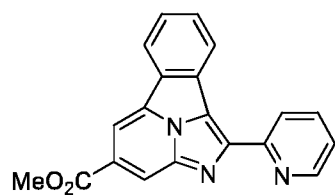
$R_1$ - $R_4$  are selected independently from H; a  $C_1$ - $C_6$  alkyl group, optionally substituted at the end of the chain by a halogen or a hydroxyl; a  $C(O)OMe$  group; and a leaving functional group;

20

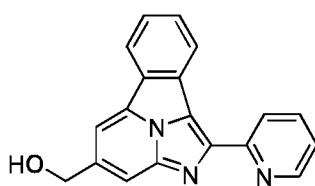
$R_5$ - $R_7$  are all H, or grouped in  $R_5$ - $R_6$  or  $R_6$ - $R_7$  pairs, can form a  $C_6$  aryl group optionally substituted by at least one  $C_1$ - $C_6$  alkyl group, halogen, or hydroxyl, with the proviso that the compound for which  $Z_1=Z_2=R_1=R_2=R_3=R_4=R_5=R_6=R_7=H$  and  $Y=CR^{12}=CH$ , is not included.

25

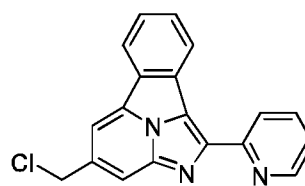
15. The compound of formula (2) according to claim 14, selected from the following:



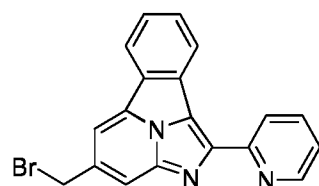
(2a)



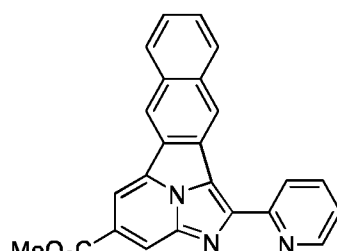
(2b)



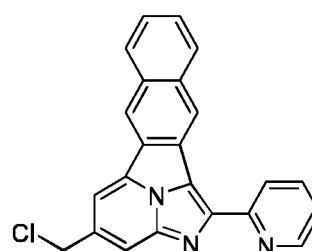
(2c)



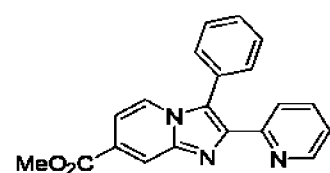
(2d)



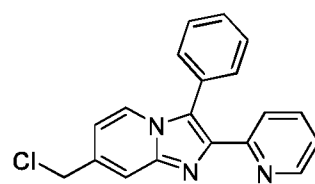
(2e)



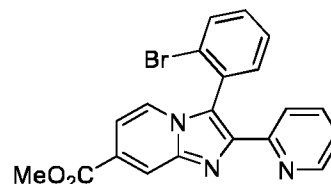
(2f)



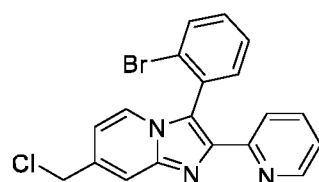
(2g)



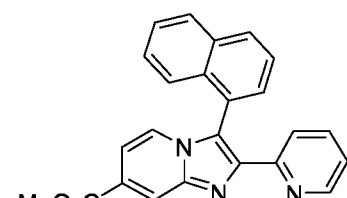
(2h)



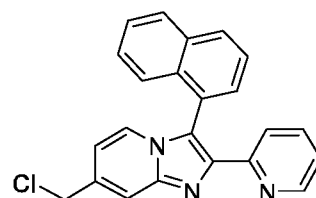
(2i)



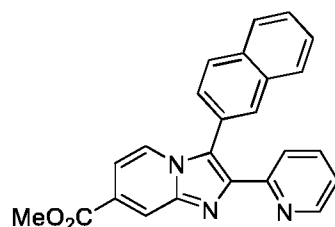
(2j)



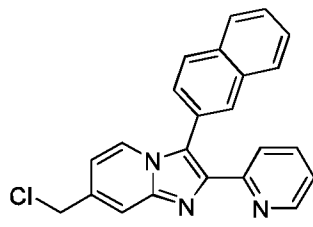
(2k)



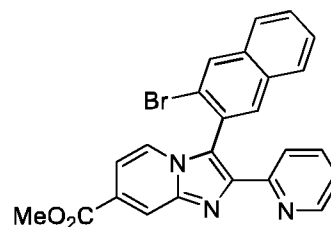
(2l)



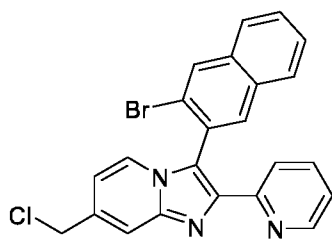
(2m)



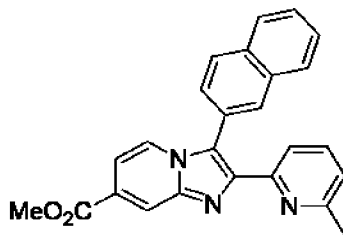
(2n)



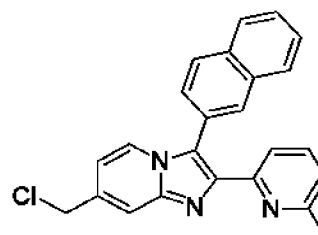
(2o)



(2p)



(2q)



(2r)

or a salt, solvate, or stereoisomer thereof.

16. A pharmaceutical composition comprising a compound of formula (1) as defined in any of claims 1 to 9, or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof, and a pharmaceutically acceptable excipient or vehicle thereof.

17. The compound of general formula (1) as defined in any of claims 1 to 9, or a pharmaceutically acceptable salt, or stereoisomer, or solvate, or prodrug thereof, or a pharmaceutical composition as defined in claim 16, for use as a medicinal product.

18. The compound of formula (1) as defined in any of claims 1 to 9, or a pharmaceutically acceptable salt, or stereoisomer, or solvate, or prodrug thereof, or a pharmaceutical composition as defined in claim 16, for use in the treatment of proliferative diseases, autoimmune diseases, and viral infections.

19. The compound or composition for use according to claim 18, wherein the proliferative disease is a cancer selected from intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, ovarian cancer, and breast cancer.

20. The compound or composition for use according to claim 19, wherein the cancer is produced in the A2780Cis ovarian cancer cell line, or in the MDA-453 and MDA-231 breast cancer cell lines, or in the EGI-1 and HUCCT1 extra- and intrahepatic cholangiocarcinoma cell lines.

21. The compound or composition for use according to any one of claims 19 to 20, wherein the cancer has developed resistance to other anticancer agents as a result of previous chemotherapy cycles.

Figure 1.

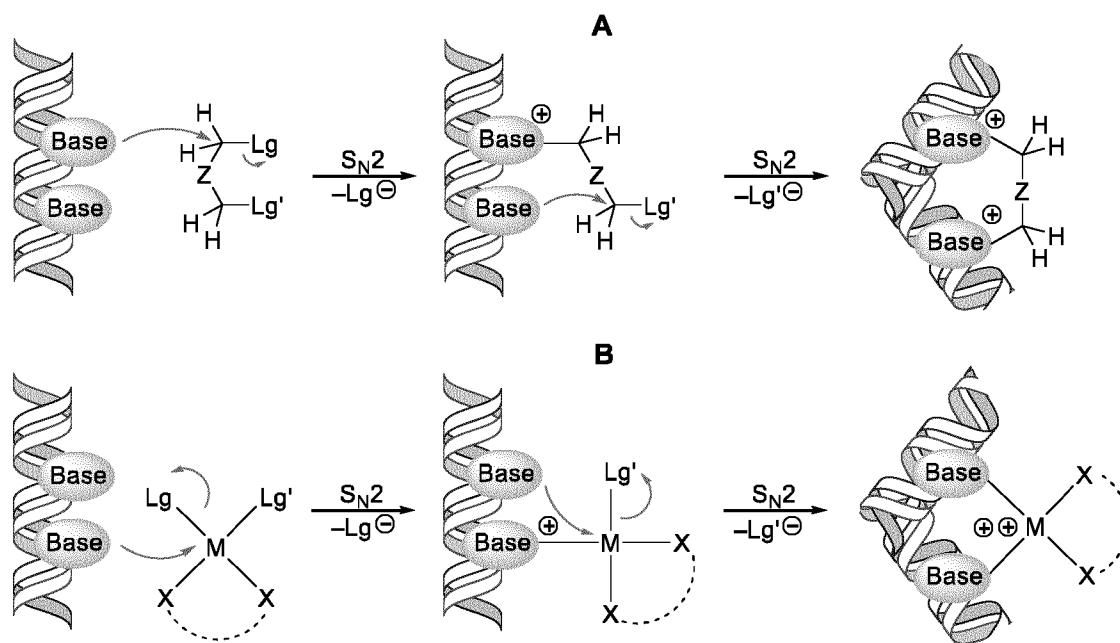
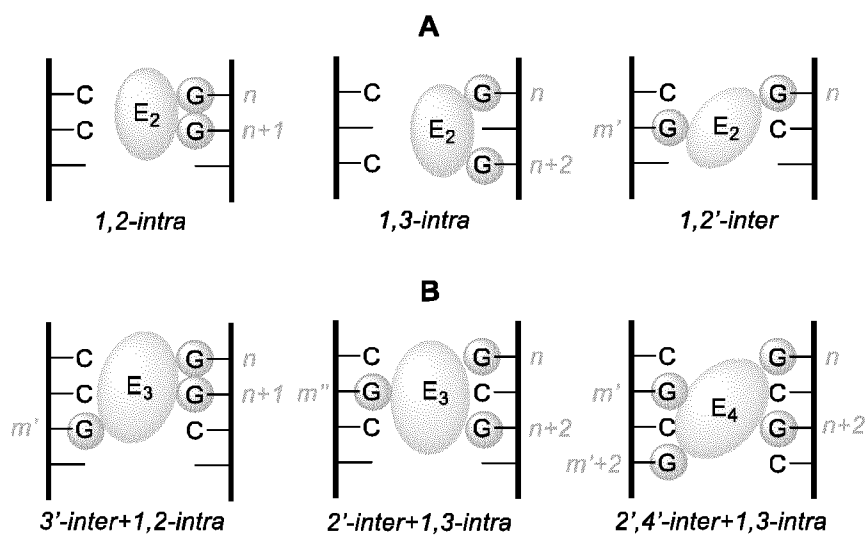


Figure 2.



2/15

Figure 3

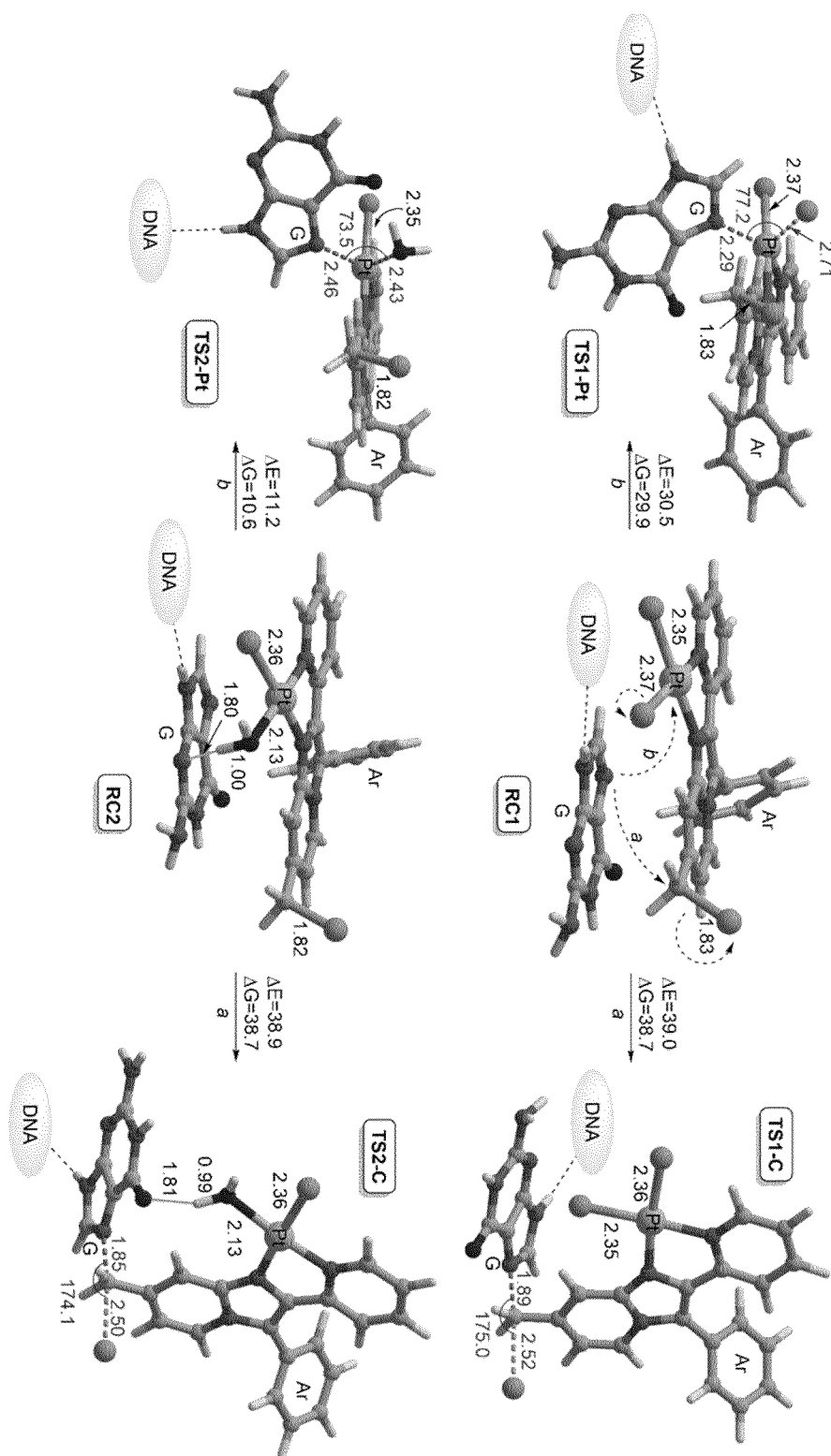


Figure 4.

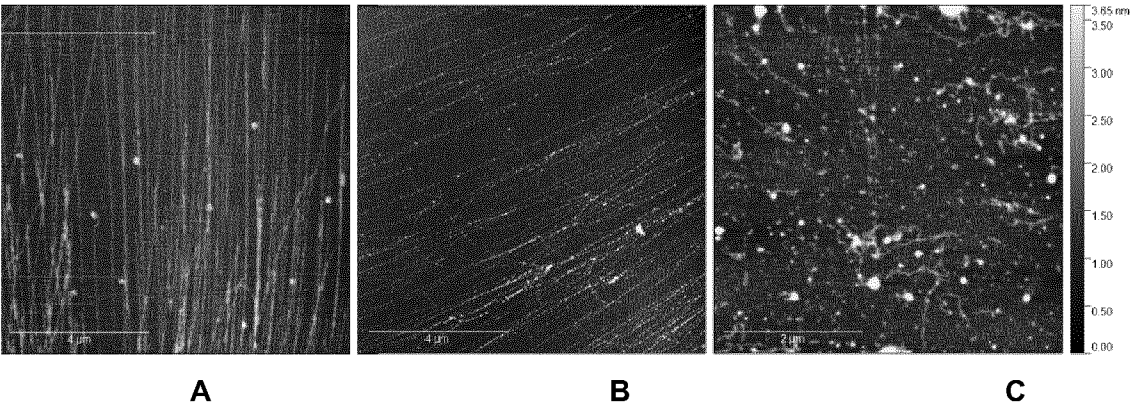


Figure 5.

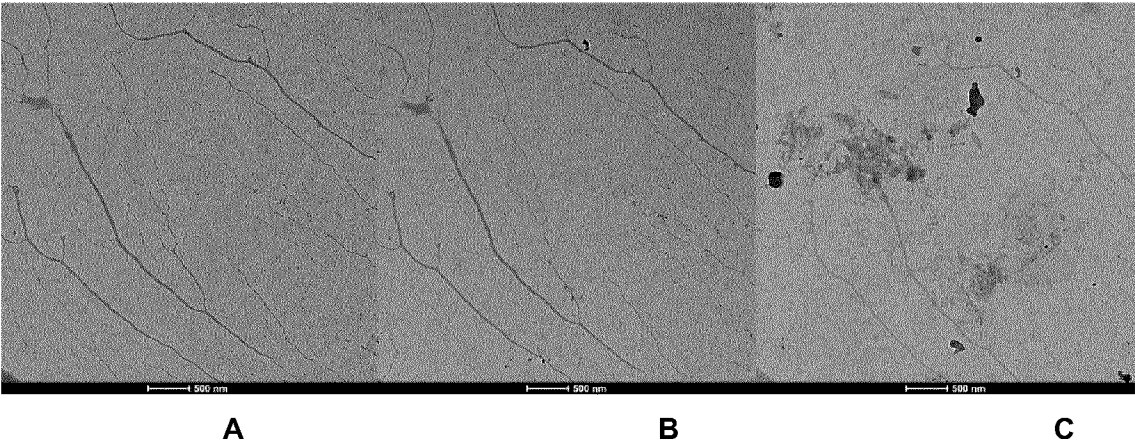


Figure 6.

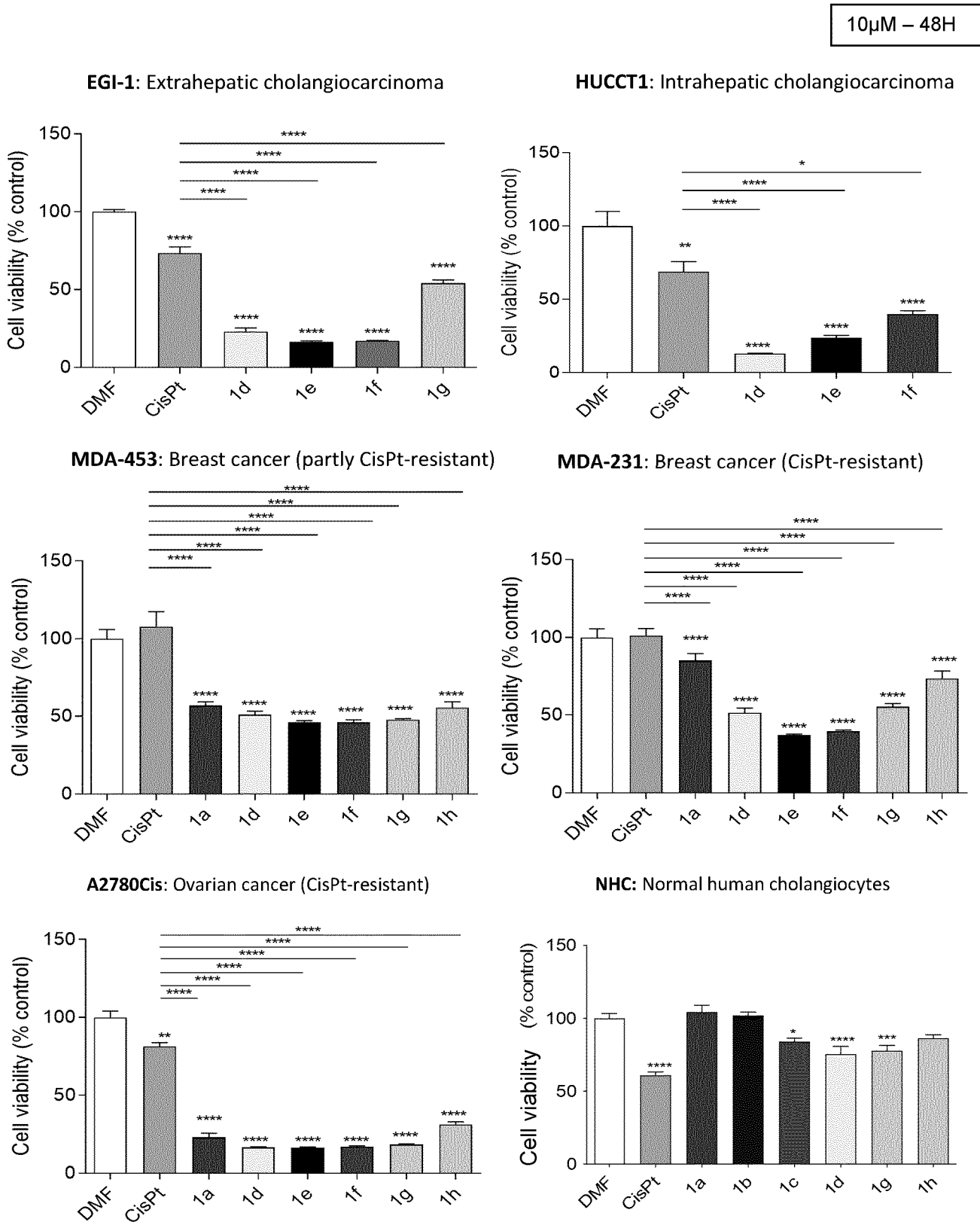


Figure 7.

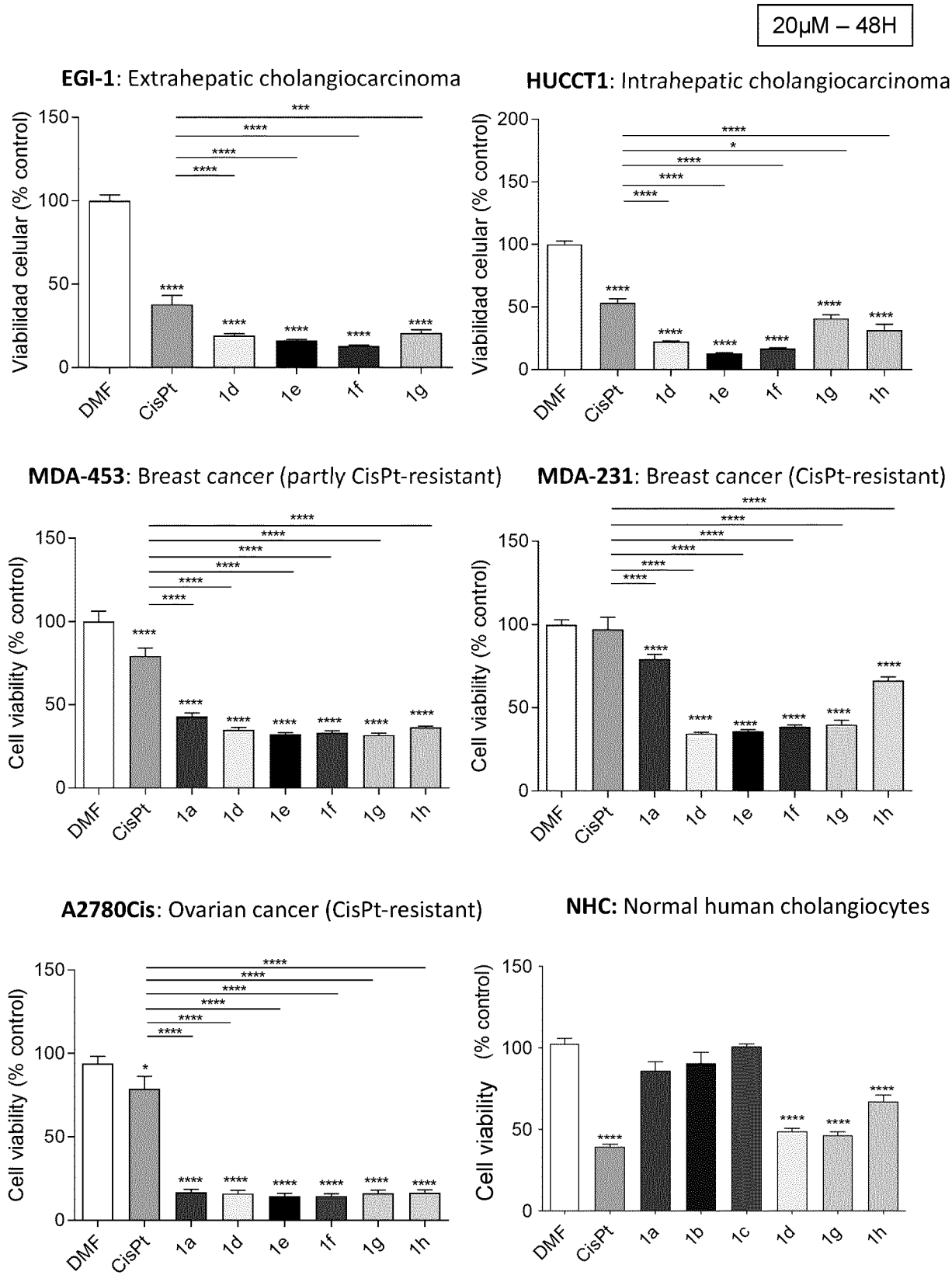


Figure 8.

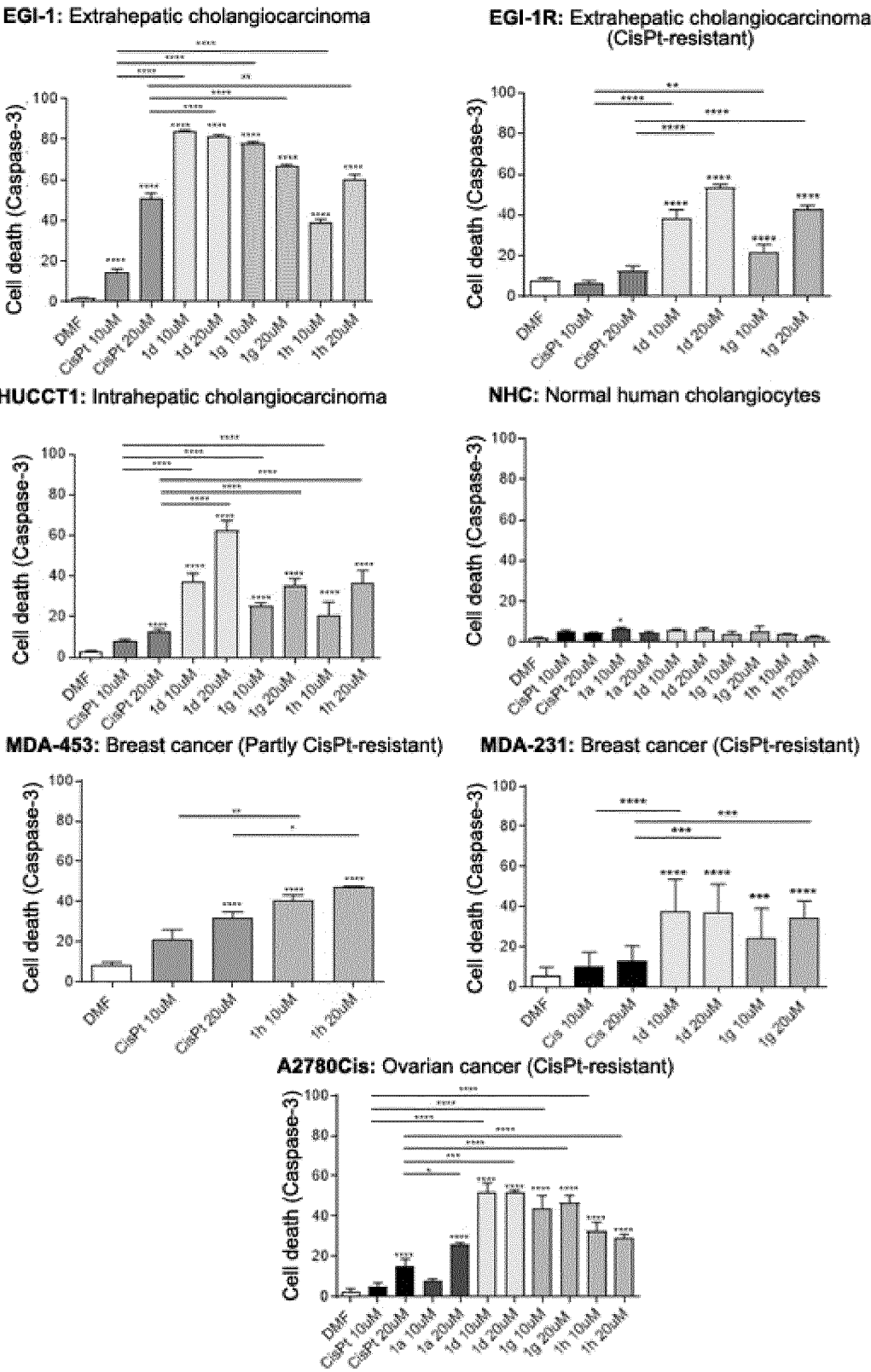


Figure 9.

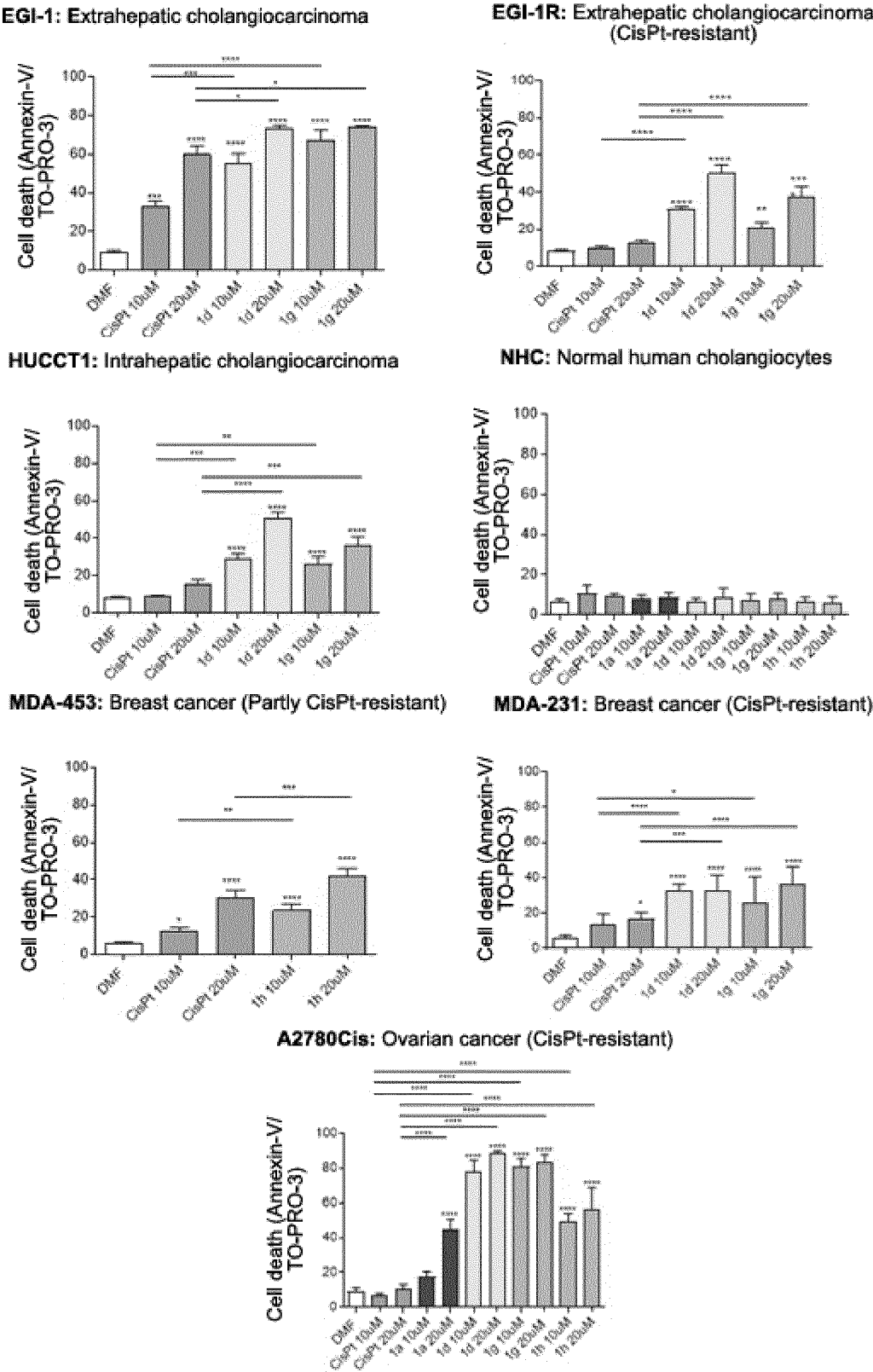
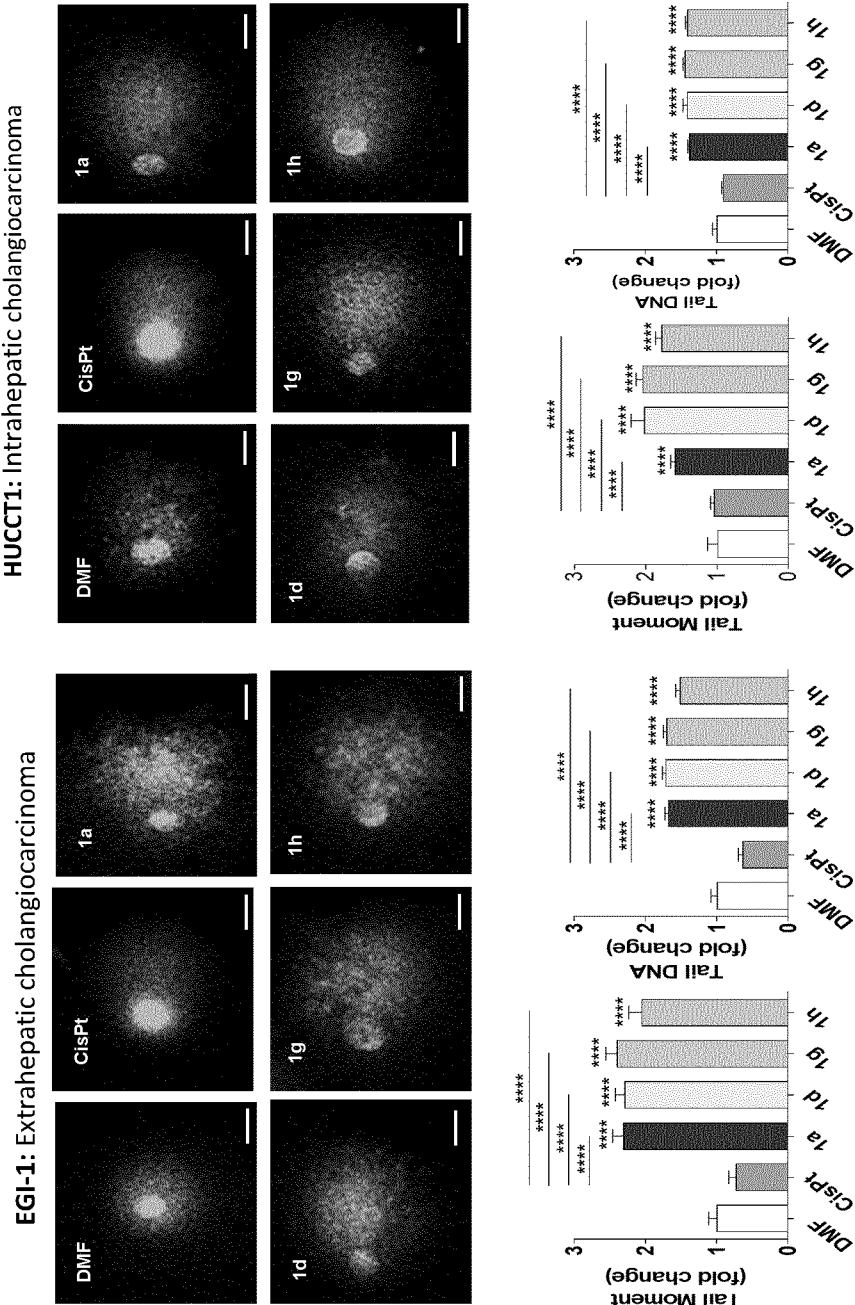


Figure 10 (a)



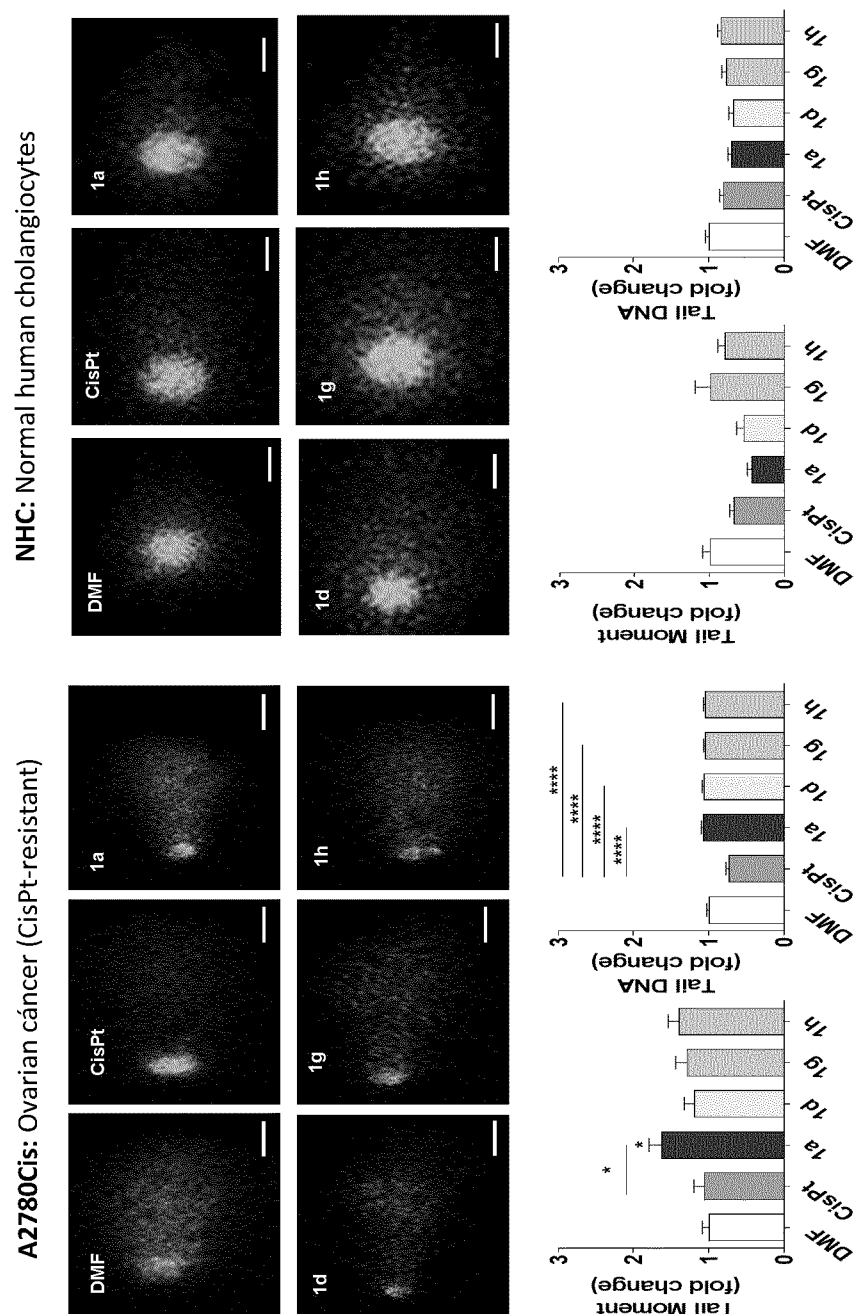


Figure 11.

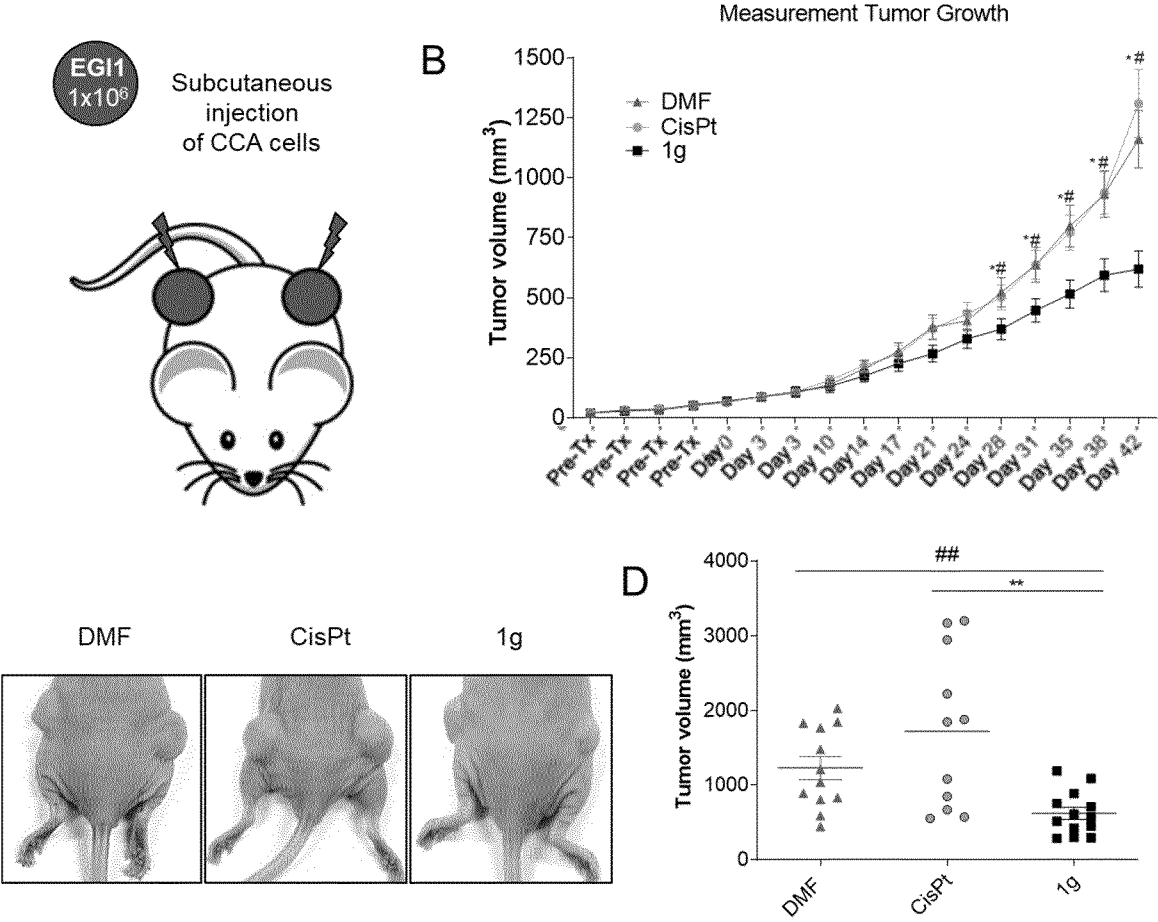


Figure 12.

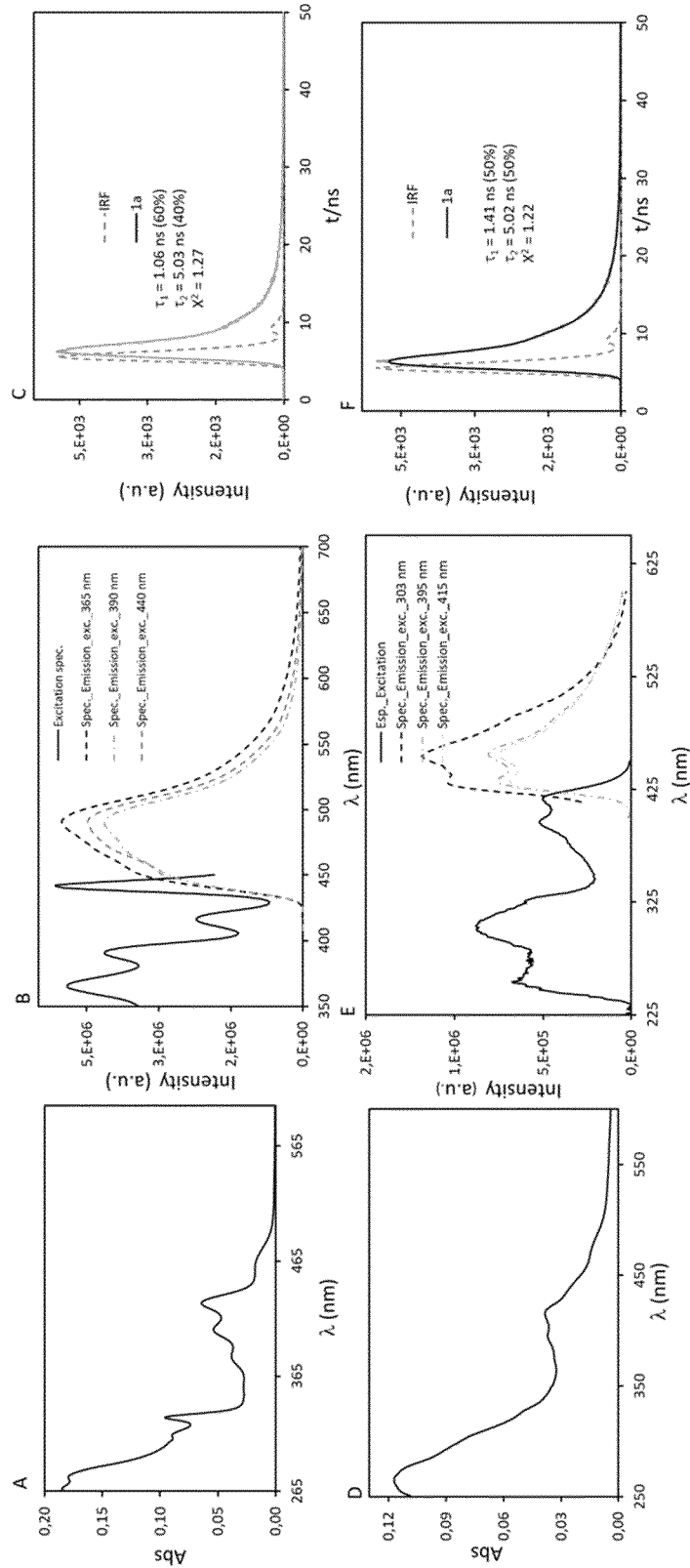


Figure 13.

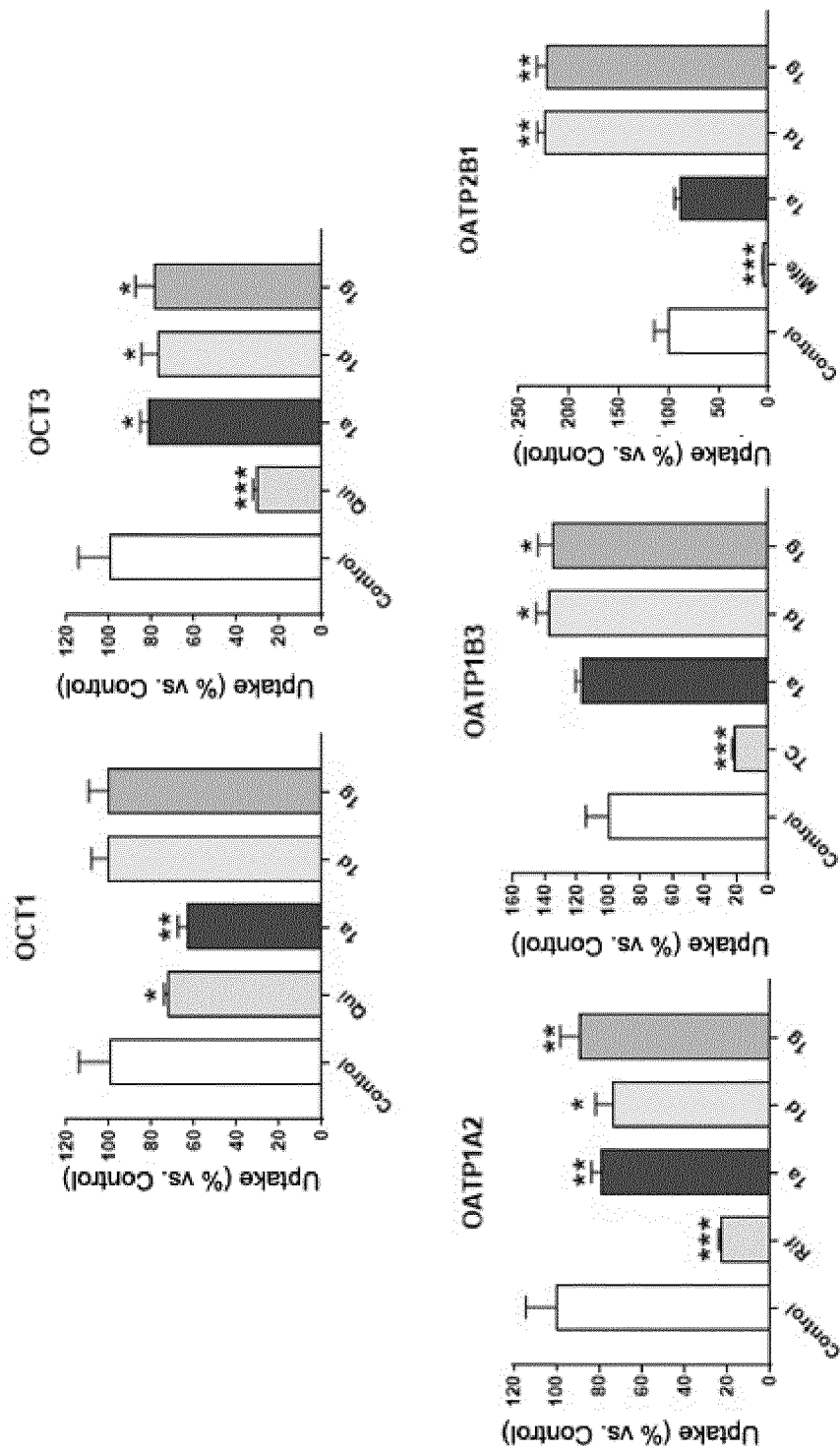


Figure 14.

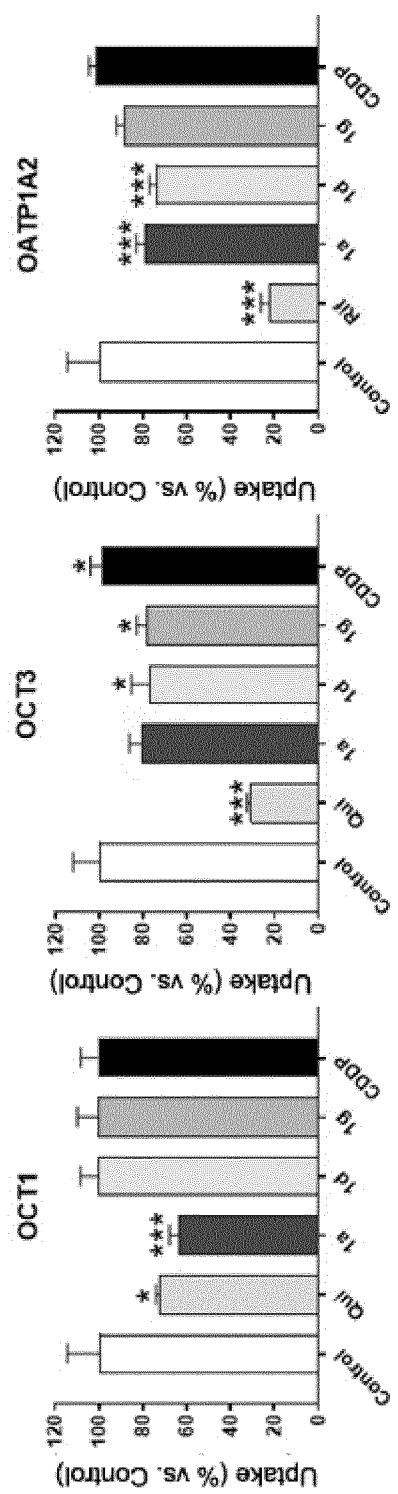


Figure 15.

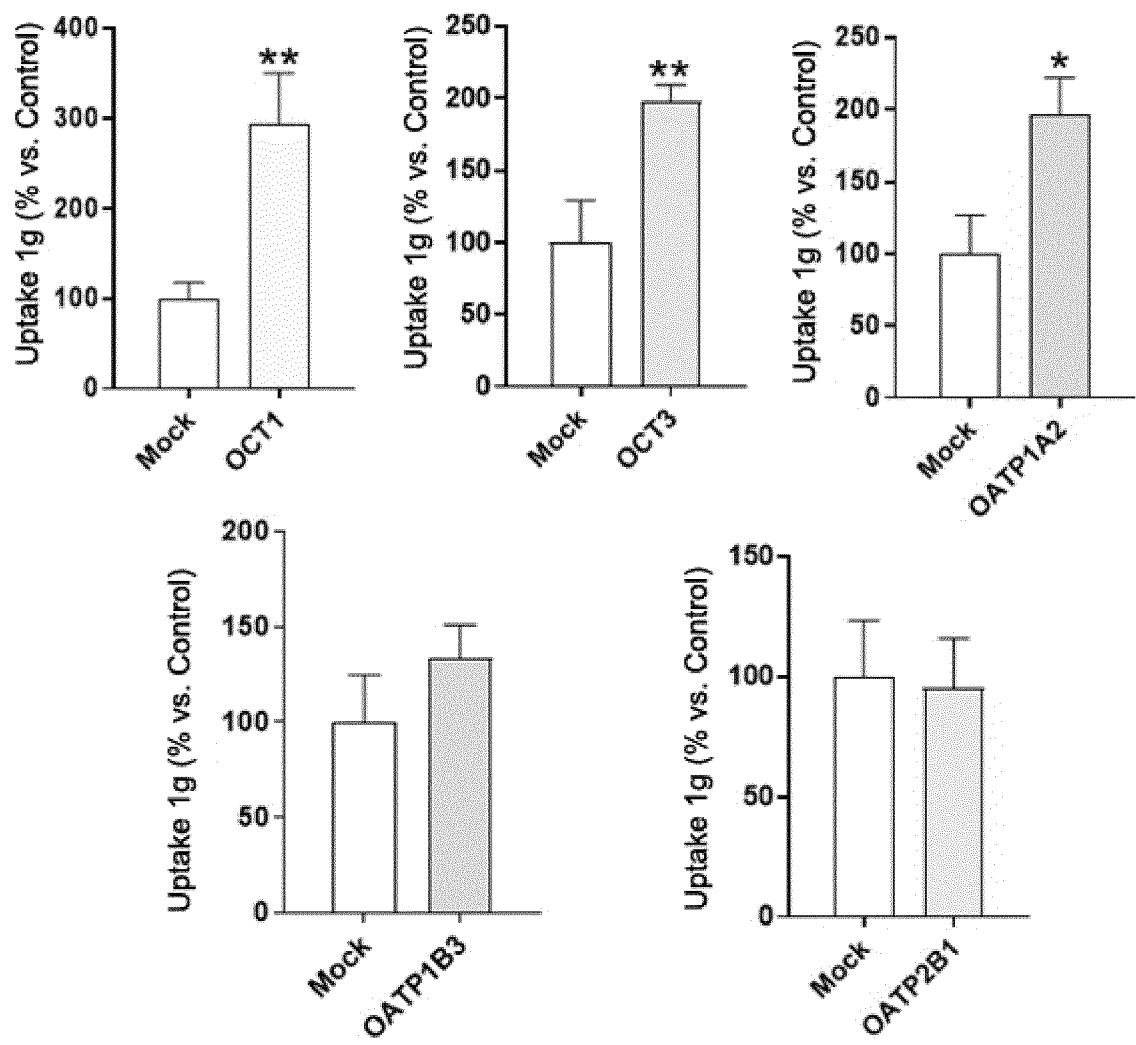
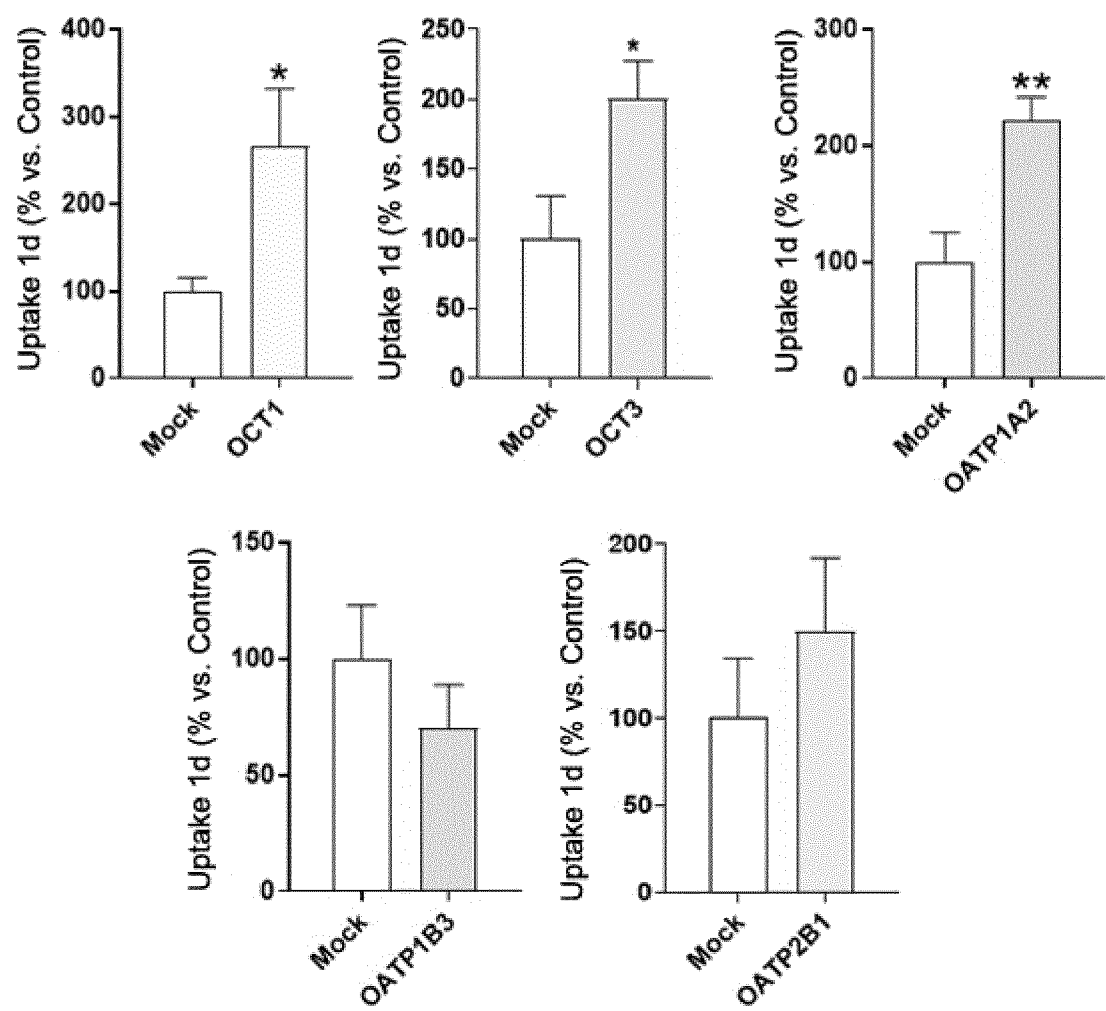


Figure 16.



## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2023/083500

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| INV. C07F1/00 C07F15/00 A61K33/243 A61P35/00 C07D471/04<br>C07D471/16                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| ADD.<br>According to International Patent Classification (IPC) or to both national classification and IPC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| <b>B. FIELDS SEARCHED</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| Minimum documentation searched (classification system followed by classification symbols)<br>C07F A61K A61P C07D                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br>EPO-Internal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| Category*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                           | Relevant to claim No.                                                |
| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Q. PEÑA, ET AL.: "Metallodrugs in cancer nanomedicine",<br>CHEMICAL SOCIETY REVIEWS,<br>vol. 51, no. 7, 4 April 2022 (2022-04-04),<br>pages 2544-2582, XP093112046,<br>Royal Society of Chemistry, Cambridge, GB<br>ISSN: 0306-0012, DOI: 10.1039/d1cs00468a<br>cited in the application<br>page 2548 - page 2549; figure 3<br>-----<br>-/-- | 1-21                                                                 |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> |                                                                                                                                                                                                                                                                                                                                              |                                                                      |
| Date of the actual completion of the international search<br><br>28 March 2024                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                              | Date of mailing of the international search report<br><br>08/04/2024 |
| Name and mailing address of the ISA/<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                              | Authorized officer<br><br>English, Russell                           |

## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2023/083500

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                       |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                    | Relevant to claim No. |
| A                                                    | <p>S. KARMAKAR, ET AL.: "Anticancer activity of a cis-dichloridoplatinum(II) complex of a chelating nitrogen mustard: insight into unusual guanine binding mode and low deactivation by glutathione", DALTON TRANSACTIONS, vol. 45, no. 8, 12 January 2016 (2016-01-12), pages 3599-3615, XP093142988, Royal Society of Chemistry, Cambridge, GB ISSN: 1477-9226, DOI: 10.1039/c5dt04459f cited in the application scheme 3, pages; 3599, 3601, 3610</p> <p>-----</p> | 1-21                  |