Supplementary Material

Our aim was to find some correlation between the functional activity of the four compounds and properties that are commonly considered in drug design and medicinal chemistry [S1, S2]. To this end, we used several cheminformatic platforms to estimate molecular structure, lipophilicity, water solubility, ability to interact with P-glycoprotein or P450 cytochromes, and to induce mutations on DNA (see Supplemental Methods for details).

Methods

Structures for the four compounds were drawn in ACD/ChemSketch freeware 2012 (https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/). They were exported to 2D SDF files. These were loaded into the Jmol application (https://jmol.org) and optimized to 3D with the Universal Force Field coercing them to square planar coordination of the platinum atom with cis configuration of the two chlorine or methyl substituents, and sodium removed. The resulting 3D SDF file or the corresponding SMILES notation were used to feed the property prediction servers.

The following servers were used to estimate the predictions of several properties for the platinum compounds used in this study:

1. SwissADME. Molecular Modelling Group of the University of Lausanne and SIB Swiss Institute of Bioinformatics. http://www.swissadme.ch/ (reference S3)

Provides estimates of topological polar surface area, logP, logS, CYP450 inhibition, glycoprotein P activity, among others.

2. Virtual Computational Chemistry Laboratory, ALOGPS 2.1 programme. https://vcclab.org/lab/alogps/ (reference S4)

Provides estimates of logP (1-octanol/water partition coefficient) and logS (aqueous solubility).

3. Virtual logP on-line: Bernard Testa's virtual logP calculator. https://www.ddl.unimi.it/vegaol/vlogp.htm (reference S5)

Provides estimates of logP (1-octanol/water partition coefficient).

4. OCHEM Predictor - Online chemical database. https://ochem.eu/predictor/show.do (reference S6).

It provides several estimates, among them logP, logS and the Ames assay. A preliminary model was used, which is specifically designed for predicting logS in platinum compounds, with molecules required to be in neutral form (sodium sulfonate) (https://ochem.eu/model/26433871, version of 4 June 2024).

Molecular geometry

The properties shown below were calculated using the Jmol application on the 3D-optimized structure (SDF file). There are no large differences in geometric properties among the four compounds. The molecular mass ranges from 611 to 694, spanning

a 13% difference overall. Based on solvent-excluded molecular surface, the volume varies between 422 and 477 cubic Angstroms (12%), and the surface area ranges between 390 and 423 square Angstroms (8% difference). The topological polar surface area (computed at SwissADME) is the same for the four compounds, 108 square Angstroms. This suggests the feasibility of the compounds permeating cell membranes.

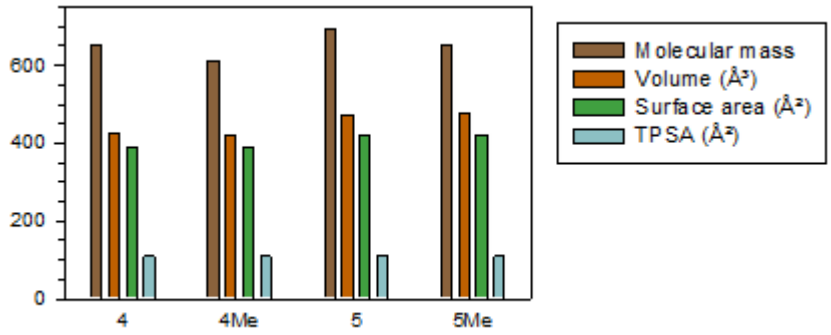


Figure S1: Geometric properties of the molecules. Molecular mass was calculated in both ChemSketch and Jmol. Volume and surface area were measured on the solvent-excluded molecular surface computed in Jmol. Topological polar surface area (TPSA) was calculated in Swiss-ADME.

Lipophilicity

This is measured as the partition coefficient between octanol and water (logP or logPow), an estimation of lipophilicity. The SwissADME server predicts logP values between 2.18 for compound 4Me and 3.18 for compound **5** (consensus values averaging four calculation methods). According to this, **5** would be the most lipophilic and **4Me** the least one. Using the ALOGPS and Testa's servers gives values that follow the same trend.

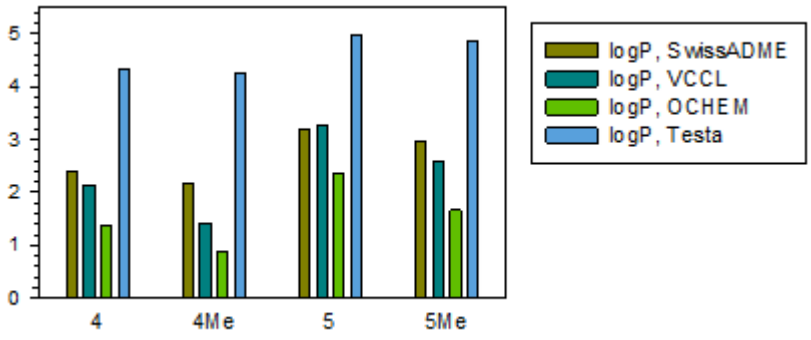


Figure S2: Estimation of lipophilicity. Four algorithms (XLOGP3, WLOGP, MLOGP, Silicos-IT) were averaged in the Consensus logP provided by SwissADME. ALOGPS 2.1 method in Virtual Computational Chemistry Laboratory provided another estimate. Additionally, ALOGPS 2.1 was provided by OCHEM and Bernard Testa's Virtual logP.

Water solubility

Several methods and servers for estimating solubility provide different values, but comparison among the four compounds offers the same trend in all of them: compound **5** is predicted as the least soluble (within the poorly soluble category in SwissADME), while compound **4Me** is the most soluble of the four (the only one tagged as moderately soluble). The overall variation is between 1.1 and 1.8 in log units, depending on the method.

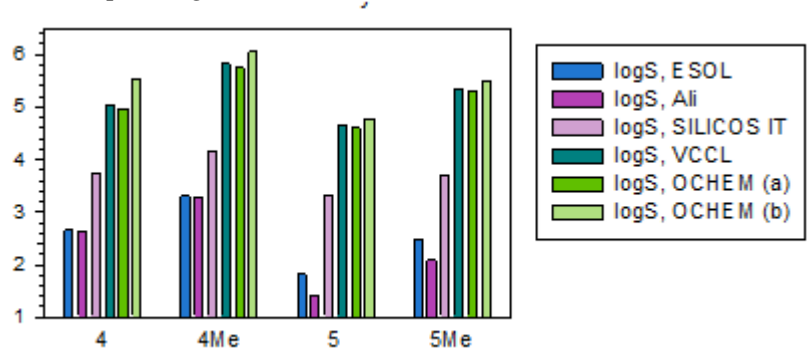


Figure S3: Estimation of solubility in water. Logarithm of solubility in nmol per litre was estimated by several methods in prediction servers: ESOL, Ali and SILICOS-IT in Swiss-ADME; ALOGPS 2.1 method in Virtual Computational Chemistry Laboratory; ALOGPS 2.1 method in OCHEM (a) and solubility based on logP and melting point in OCHEM (b).

Mutagenicity

The Ames test, a strain-dependent bacterial reverse mutation assay, is a frequent method for in vitro evaluation of substances eliciting mutagenicity via short-chain-length DNA damage. The algorithm at OCHEM, using the Ames prediction, classifies all four compounds as active with an estimated accuracy of 73% and no difference among them.

Pharmacokinetics

The SwissADME server provides estimations of the potential ability to interact as a substrate for glycoprotein P; the result is positive just for compounds **4Me**, **5** and **5Me**. On the other hand, the prediction of inhibitory action on the cytochrome P450 family reports that none of the four compounds would inhibit CYP1A2 or CYP2C9, all four would inhibit CYP2C19 and CYP3A4, and only 4Me would inhibit CYP2D6.

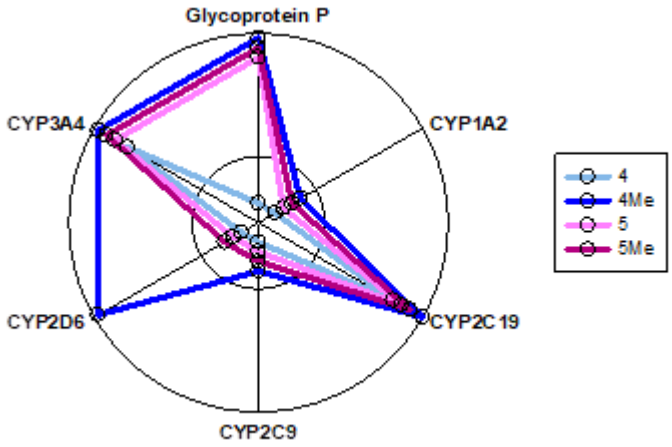


Figure S4: Estimation of potential as substrate for glycoprotein P and inhibitor of the five major members of the cytochrome P450 family that are responsible for more than 90% of the metabolism of clinical drugs. All estimates were obtained at SwissADME. Data in the outer circle mean positive action, while those in the inner circle mean poor estimated activity.

The molecular size of the four compounds is not widely different, staying within a range of 13% in mass, 12% in volume and 8% in surface area (Appendix A, figure S1). The topological polar surface area [S7, S8] has been deemed a relevant indicator: drugs with higher TPSA values will usually be less lipid-soluble and will, in general, experience less and slower absorption and distribution. In the four platinum complexes tested in this current study, the TPSA value was found to be identical (figure S1), which is probable due to them having the same polar atoms and given that TPSA calculation does not depend on the 3D conformation. The value found, 108 $Å^2$, is low enough to suggest the feasibility of the compounds permeating cell membranes [S2].

Lipophilicity is a key indicator in assessing the ability of chemicals to permeate cell membranes and to be absorbed from the gastrointestinal tract into the portal circulation. Both the experimentally determined and computationally predicted lipophilicity are characterized through *logP*, the logarithm of partition coefficient between *n*-octanol and water. This was calculated through several algorithms. The compound with the highest lipophilicity is **5** (figure S2), followed by **5 Me**. Several estimations of water solubility (logS) assessed compound **4 Me** as moderately soluble, while the other three complexes showed poorer solubility. The lipophilicity of complexes **5** and **5 Me** is higher than that of complexes 4 and 4 Me (figure S3).

From these predictions, it might be considered that the slightly higher lipophilicity of complexes **5** and **5 Me** could play some role in the effectivity of these two complexes, mainly manifested in the

observed increase in apoptosis in NB4 leukemia cells by favoured passage through the cell membrane.

Additionally, we studied predicted pharmacokinetics through the possible interaction with members of the cytochrome P450 family (involved in several steps of xenobiotic metabolism) and with P-glycoprotein (a transporter across cell membranes that may pump drugs out of cells). The result obtained indicates that all four complexes may presumably interact with CYP3A4 and CYP2C9 and with P-glycoprotein. Only complex **4Me** might interact with cytochrome CYP2D6, which might explain its higher induction of apoptosis (figure S4).

The predicted interaction with P-glycoprotein could explain, at least in part, the influence of the concentration of the complexes on the results. In acute myeloid leukemia, the modulation of P-glycoprotein is relevant with respect to the action of antitumor compounds such as doxorubicin [S9]. In prostate cancer, the interaction with P-glycoprotein may be critical considering the therapeutic efficacy of the antitumor compound [S10].

References

1. van de Waterbeemd, H. (2008) Physicochemical properties in drug profiling. In: Molecular Drug Properties. Measurement and Prediction. R. Mannhold (ed.) pp. 25-52. Wiley, Weinheim.
2. Silva, A.M., Martins-Gomes, C., Ferreira, S.S., Souto, E.B., Andreani, T. (2022) Molecular physicochemical properties of selected pesticides as predictive factors for oxidative stress and apoptosis-dependent cell death in Caco-2 and HepG2 cells. Int. J. Mol. Sci. 23:8107. doi: 10.3390/ijms23158107
3. A. Daina, O. Michielin, V. Zoete (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7:42717. https://doi.org/10.1038/srep42717
4. I. V. Tetko, P. Bruneau (2004) Application of ALOGPS to predict 1-octanol/water distribution coefficients, logP, and logD, of AstraZeneca in-house database. J. Pharm. Sci. 93:3103-3110. https://doi.org/10.1002/jps.20217
5. 5. P. Gaillard, P.A. Carrupt, B. Testa, A. Boudon (1994) Molecular lipophilicity potential, a tool in 3D QSAR: method and applications, J. Comput. Aided Mol. Design 8:83-96. https:/doi.org/ 10.1007/BF00119860
6. 6. Sushko, S. Novotarskyi, R. Körner, A.K. Pandey, M. Rupp, W. Teetz, S. Brandmaier, A. Abdelaziz, V.V. Prokopenko, V.Y. Tanchuk, R. Todeschini, A. Varnek, G. Marcou, P. Ertl, V. Potemkin, M. Grishina, J. Gasteiger, C. Schwab, I.I. Baskin, V.A. Palyulin, E.V. Radchenko, W.J. Welsh, V. Kholodovych, D. Chekmarev, A. Cherkasov, J. Aires-de-Sousa, Q.Y. Zhang, A. Bender, F. Nigsch, L. Patiny, A. Williams, V. Tkachenko, I.V. Tetko (2011). Online chemical modeling environment (OCHEM): web platform for data storage, model development and publishing of chemical information. J. Comput. Aided Mol. Des. 25:533-554. https//doi.org/10.1007/s10822-011-9440-2.
7. Ertl, P. (2008) Polar surface area. In: Molecular Drug Properties. Measurement and Prediction.R. Mannhold (ed.) pp. 111-126. Wiley, Weinheim.
8. Prasanna,S., Doerksen, R.J. (2009) Topological polar surface area: A useful descriptor in 2D-QSAR. Curr. Med. Chem. 16:21-41. doi: 10.2174/092986709787002817

9. Pom, P., Labbozzetta, M., Ramarosandratana, A.V., Rosselli, S., Tutone, M., Sajeva, M., Notarbartolo, M. (2021) In vitro modulation of P-glycoprotein activity by Euphorbia intisy essential oil on acute myeloid leukemia cell line HL-60R. Pharmaceuticals (Basel). 14:111. doi: 10.3390/ph14020111
10. Levrier, C., Rockstroh, A., Gabrielli, B., Kavallaris, M., Lehman, M., Davis,R.A., Sadowski, M.C., Nelson, C.C. (2018) Discovery of thalicthuberine as a novel antimitotic agent from nature that disrupts microtubule dynamics and induces apoptosis in prostate cancer cells. Cell Cycle 17:652-668. doi: 10.1080/15384101.2017.1356512