Synthesis and *in vitro* characterization of anticancer platinum(II) coordinates: NCI Compare and FTIR spectroscopy for drug candidate profiling

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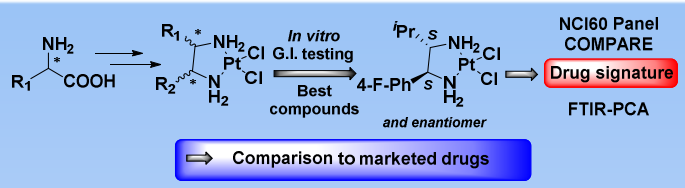
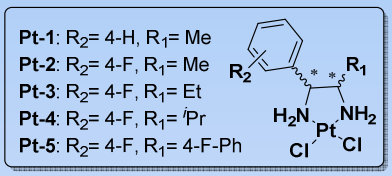
1. Introduction

Current efforts on platinum compounds are driven by the need to broaden the classes of tumor that respond to the treatment and moreover, to overcome resistance arising on platinum chemotherapy. This may come through the discovery of new compounds exhibiting an atypical activity profile. Fingerprinting the biochemical impression on cells using a quick and powerful technique to compare new compounds, may help to rapidly identify good candidates for further work and *in vivo* experiments.



2. Preliminary screening

Best candidates for this study were selected on the basis of in crystal violet cytotoxicity assays from previous work by our group [1]. These first screening phase revealed a strong dependence of the anti-proliferative effect to the stereochemistry. If little enantiomeric effect was observed, diastereomeric selectivity is important and *trans* compounds are much more active than *cis* isomers. Also, adding a second aromatic substituent on the five-membered ring decreases the activity. **Table 1** summarized T/C values obtained from these studies.



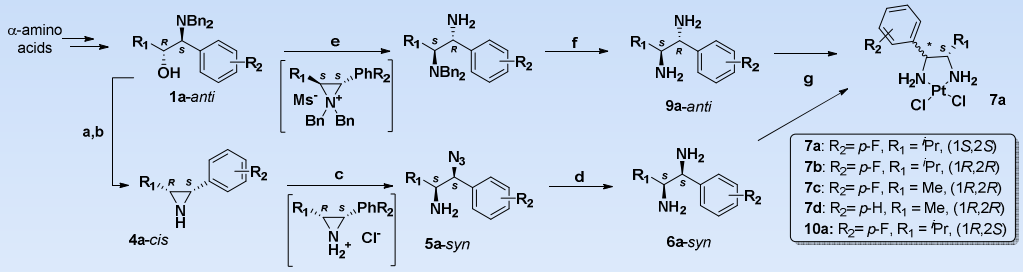
Stereochemistry	Pt-1	Pt-2	Pt-3	Pt-4	Pt-5
(1*R*,2*S*)	33	6	7	26	0 (*meso*)
(1*S*,2*R*)	29	6	28	5	0 (*meso*)
(1*S*,2*S*)	**10**	**-52**	**-32**	**-27**	**-5**
(1*R*,2*R*)	**-2**	**-41**	**-23**	**-51**	**0**

Table 1. *T/C (%) values obtained for the whole compounds series.*

- *Cytostatic effect:* $\frac{T}{C} = \frac{100.(T-C_O)}{C-C_O}$
- *Cytocidal effect:* $\frac{T}{C} = \frac{100.(T-C_O)}{C_O}$

3. Synthesis

Two distinct pathways were used to produce *syn* and *anti* configured diamines from their common amino alcohol precursors **1**. Both proceeds through aziridinium intermediates regio- and stereoselectively opened by a nitrogen containing nucleophile. *Anti*-configured diamine **10a** was prepared through a dibenzylaziridinium intermediate, regiospecifically opened on the benzylic position by NH_3. For the production of the *syn* isomers, aziridine **4a-d** were easily activated by the addition of HCl to produce the protonated aziridinium species, prior to opening with sodium azide.



Scheme 1. *Reagents and conditions: (a) $SOCl_2$, toluene, 40 °C; (b) NaOH, MeOH, 50 °C; (c) NaN_3, HCl, MeCN, 80 °C; (d) LAH, Et_2O, rt; (e) MsCl, NH_4OH, toluene, 0 °C/rt; (f) H_2 (P_{atm}), $Pd(OH)_2$/C, HCl, MeOH, rt. (Example from **1a**)*

4. *In vitro* growth inhibitory concentration - IC_{50}

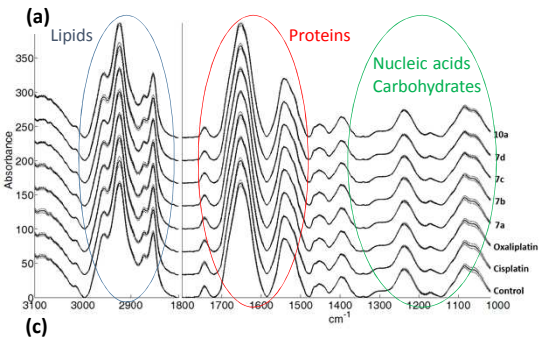
IC_{50} values from MTT test on six cell lines for compounds **7a-d**, **10a**, cisplatin and oxaliplatin are given in Table 2.

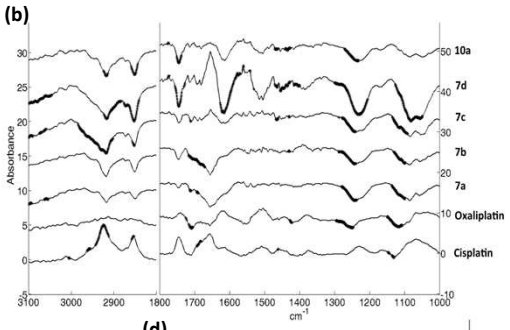
	A549	*U373*	*SKMEL*	*OE21*	*Hs683*	*B16F10*	Mean
Cisplatin	7,0	1,7	11	3,3	3,3	3,0	**5 ± 1**
Oxaliplatin	2,3	0,9	3,7	0,83	3	0,17	**1.8 ± 0.5**
7a	0,36	0,17	3	0,47	0,47	0,10	**0.8 ± 0.4**
7b	1,3	0,37	3	1,6	1,5	0,10	**1.3 ± 0.4**
7c	2,7	0,7	6	2,7	3,7	0,27	**2.7 ± 0.9**
7d	4,0	2,0	4	7	4,3	0,70	**3.7 ± 0.8**
10a	49	19	58	60	57	6,7	**42 ± 11**

Table 2. *In vitro growth inhibitory potency determined by MTT assays for 6 cancerous cell lines.*

5. FTIR spectroscopy

FTIR spectroscopy was used to produce a biochemical signature of the compounds [2]. FTIR spectroscopy provides a sensitive tool for fingerprinting metabolic changes arising inside cells upon drug treatment, allowing investigation for the action mode and the classification of compounds. Hence, when having chosen potent compounds as potential lead from a series of compounds, atypical IR fingerprints may give the compound the ability to overcome the limitations of current drugs, and would be used as a selection criterion within a chemical series.





(c)

MANOVA	Control	CisPt	OxaliPt	7a	7b	7c	7d
Control	1						
Cisplatin	2.55 10^{-2}	1					
Oxaliplatin	9.28 10^{-5}	6.53 10^{-4}	1				
7a	2.17 10^{-5}	8.25 10^{-4}	3.09 10^{-1}	1			
7b	1.78 10^{-5}	1.07 10^{-5}	6.04 10^{-3}	3.40 10^{-1}	1		
7c	5.57 10^{-5}	7.74 10^{-4}	1.67 10^{-2}	2.76 10^{-3}	4.82 10^{-2}	1	
7d	4.10 10^{-6}	3.97 10^{-5}	2.30 10^{-2}	9.23 10^{-2}	6.07 10^{-3}	1.05 10^{-2}	1
10a	3.26 10^{-3}	2.34 10^{-3}	4.91 10^{-3}	1.63 10^{-2}	1.64 10^{-2}	3.82 10^{-2}	1.05 10^{-1}

(d)

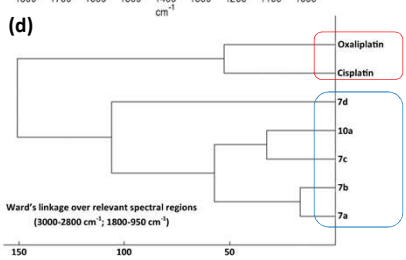
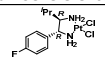
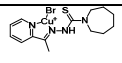
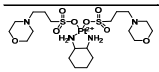
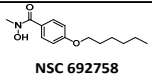


Figure 1*. Mean spectra (solid lines) ± standard deviation (dotted lines) of A549 cells exposed to the drug indicated in the right margin during 6 hrs at their IC_{50} concentration (**Fig. 1a**). Difference between mean spectra after 6 hours treatment. Student t-test was computed at every wavenumber with a significance level of p = 0.005 (**Fig. 1b**). MANOVA P-values on the first 6 principal components, computed between 3000-2800 cm^{-1}, 1800-1700 cm^{-1} and 1600-1000 cm^{-1} (**Fig. 1c**). Hierarchical clustering using the Ward's algorithm (**Fig. 1d**).*

4. NCI COMPARE

The two most interesting compounds were sent to the NCI to confirm their *in vitro* activity and to assess their resemblance to current drugs. The NCI COMPARE program consists in an online database and comparison tool which analyzes cytotoxicity data from the 60 cell line panel for similar activity profiles with all the compounds screened previously by the DTP [3].

Chemical structure and NSC reference	Pearson's correlation coefficient
 **7b**	***0.91*** *(n = 56)*
 **NSC 635450**	***0.726*** *(n = 43)*
 **NSC 614887**	***0.722*** *(n = 47)*
 **NSC 625299**	***0.713*** *(n = 42)*
 **NSC 692758**	***0.71*** *(n = 55)*
 **NSC 691081**	***0.703*** *(n = 53)*

6. Conclusion

The herein proposed workflow has been able to efficiently select promising compounds from of a chemical series. The selected compounds exhibit an interesting profile from both FTIR spectroscopy and from the COMPARE algorithm. The COMPARE data supports the findings from FTIR spectroscopy. Indeed, if no high correlation has been found between 7a/7b and cisplatin or oxaliplatin (< 0.7), oxaliplatin correlates to 7a with a rather high coefficient (0.69). Interestingly, looking at the MANOVA analysis of the IR experiments, 7a and oxaliplatin do not show significantly different mean spectra ($p = 0.3$).

[1] (a) F. Dufrasne, M. Gelbcke, B. Schnurr, R. Gust, Arch. Pharm. 335 (2002) 229-239. (b) A. Dullin, F. Dufrasne, M. Gelbcke, R. Gust, ChemMedChem. 1 (2006) 644-653.
[2] (a) R. Gasper, J. Dewelle, R. Kiss, T. Mijatovic, E. Goormaghtigh, Biochim. Biophys. Acta 1788 (2009) 1263-1270. (b) G. Berger, R. Gasper, D. Lamoral-Theys, A. Wellner, M. Gelbcke, R. Gust, et al., Int. J. Oncol. 37 (2010) 679-686.
[3] (a) Developmental Therapeutic Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, MD, U.S. (http://dtp.cancer.gov). (b) K. D. Paull, R. H. Shoemaker, L. Hodes, A. Monks, D. A. Scudiero, L. Rubinstein, J. Plowman, M. R. Boyd, J. Natl. Cancer Inst. 81 (1989) 1088-1092.