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The inhibitory capacity of organometallic compounds with anticancer features in GST P1-1 enzyme activity: an automatic approach

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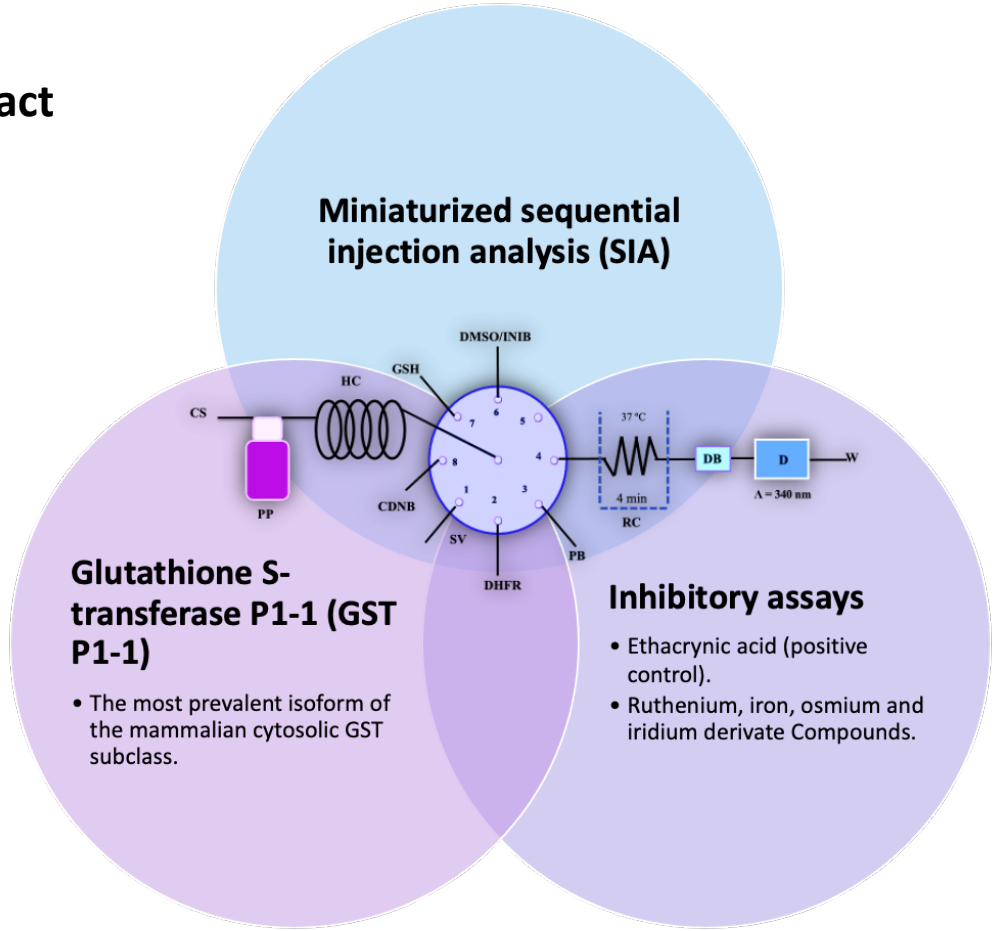
The inhibitory capacity of organometallic compounds with anticancer features in GST P1-1 enzyme activity: an automatic approach

Graphical Abstract



Abstract

Glutathione *S*-transferases (GSTs) are an important group of isoenzymes that play an essential role in the detoxification of carcinogens. In this way, GST P1-1 is considered a suitable and excellent targetable biomarker to differentiate cancer from normal cells.
Ruthenium-, iron-, osmium- and iridium-based compounds are considered promising candidates for the next generation of metal anticancer drugs in order to overcome the side effects produced by the anticancer platinum drugs used in clinic.
In this work, it was developed a miniaturized approach based on sequential injection analysis (SIA) for the determination of the half maximal inhibitory concentration (IC_{50}) of GSTs. Beyond the advantages presented by SIA systems such as versatility, simplicity, robustness, reliability and efficiency, this specific one present two more important advantages allowing the use of three times less reagent solutions than in batch method and reducing the analysis time from 8 minutes to 5 minutes.
The newly developed method was applied to 22 ruthenium, iron, osmium and iridium derivates compounds ethacrynic acid (EA) and flurbiprofen, a cyclooxygenase inhibitor, were shown to be potent GST P1-1 inhibitors. The iridium compound tested is the strongest inhibitor of GST P1-1 (IC_{50} = 6.7 ± 0.7 µM) in the present work, more effective than EA itself and the ruthenium analogue.

Keywords: cancer; organometallic compounds; glutathione S-transferase P1; sequential injection analysis

Introduction

Sequential injection analysis (SIA)

Automatic approach

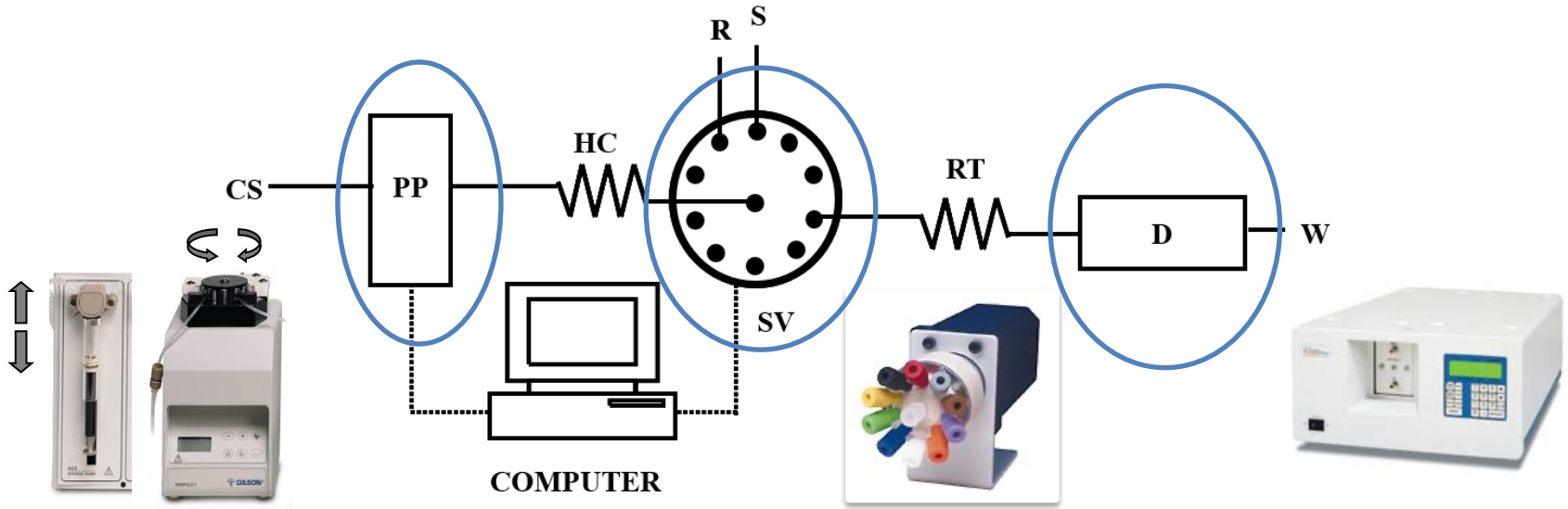
Apply to enzyme based assays

Smaller volumes

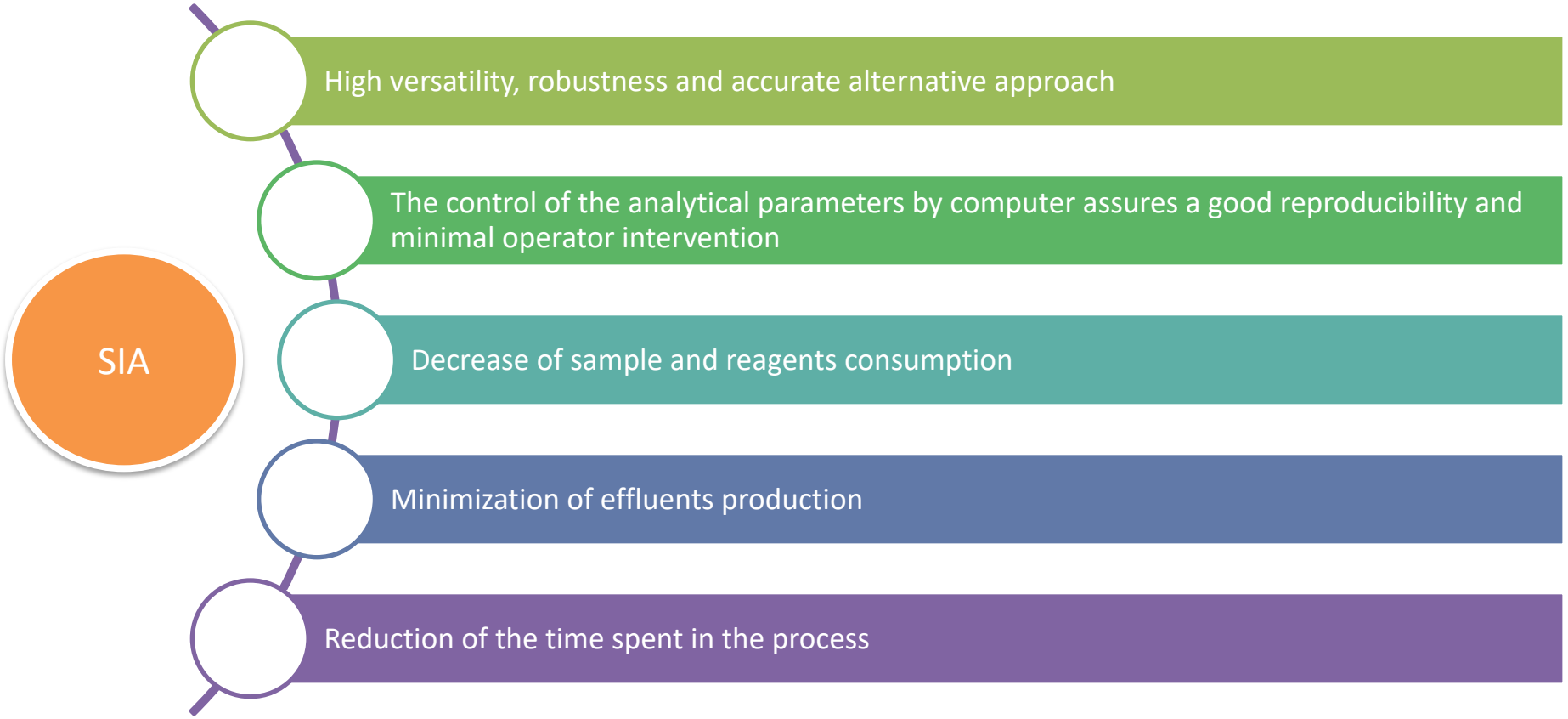
Multiple reagentes handling

More suitable for high-cost enzymes/reagents

Complicated multistep reactions



Introduction



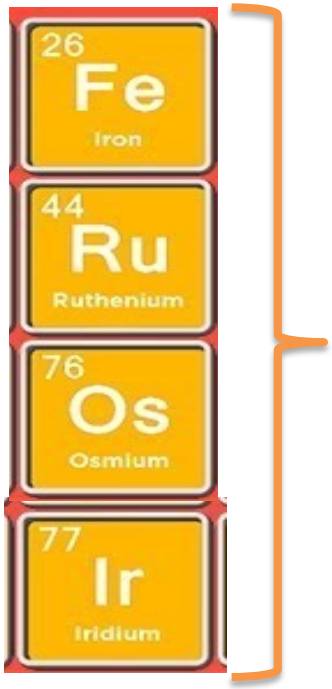
Introduction

Superfamily widely distributed in phase II metabolism enzymes

The most prevalent isoform of the mammalian cytosolic GST subclass.
Usually overexpressed in human tumors including ovarian, kidney and breast carcinoma

Introduction

Metal-based compounds



Promising candidates for the next generation of metal anticancer drugs

Overcome the side effects produced by anticancer platinum drugs

Potential inhibition effect on GST P1-1 enzyme

Aim of the study

Optimization of the new SIA system

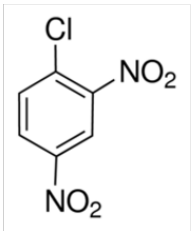
- reaction time
- temperature
- volumes of the aliquots solutions
- order of aspiration

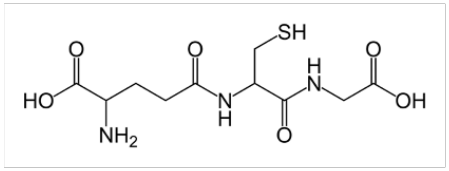
Metal-based compounds test

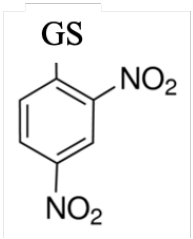
- Ruthenium (Ru)
- Iron (Fe)
- Osmium (Os)
- Iridium (Ir)

Determination of the half maximal inhibitory concentration (IC_{50})

1-chloro 2,4-dinitrobenzene + L- glutathione (GSH) **GST P1-1** GS-DNB Conjugate + HCl







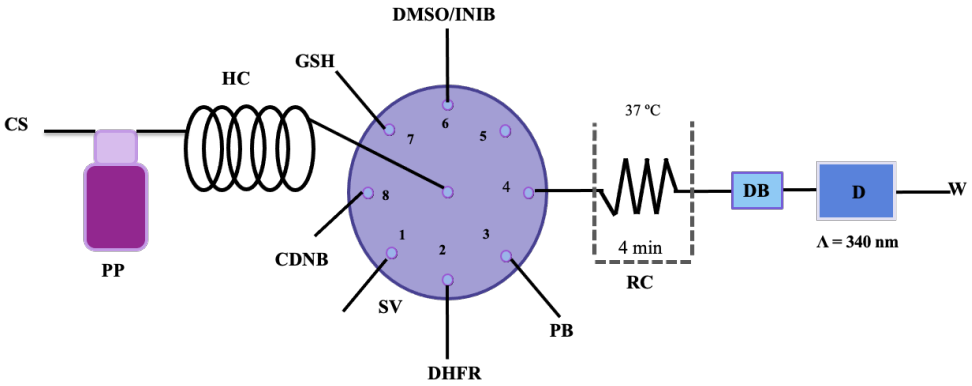
Results and discussion

Optimization of SIA system

Condition	Range	Selected value
Stop period (min)	0 - 5	4
Aspiration order	GSH - DMSO/inhibitor - GST P1-1 - CDNB GSH - GST P1-1 - DMSO/inhibitor - CDNB CDNB - DMSO/inhibitor - GST P1-1 - GSH CDNB - GST P1-1- DMSO/inhibitor - GSH	CDNB - DMSO/inhibitor - GST P1-1 - GSH
Temperature (ºC)	25 - 37	37
GST volume (µL)	10 - 25	20
GSH volume (µL)	15 - 25	20

Results and discussion

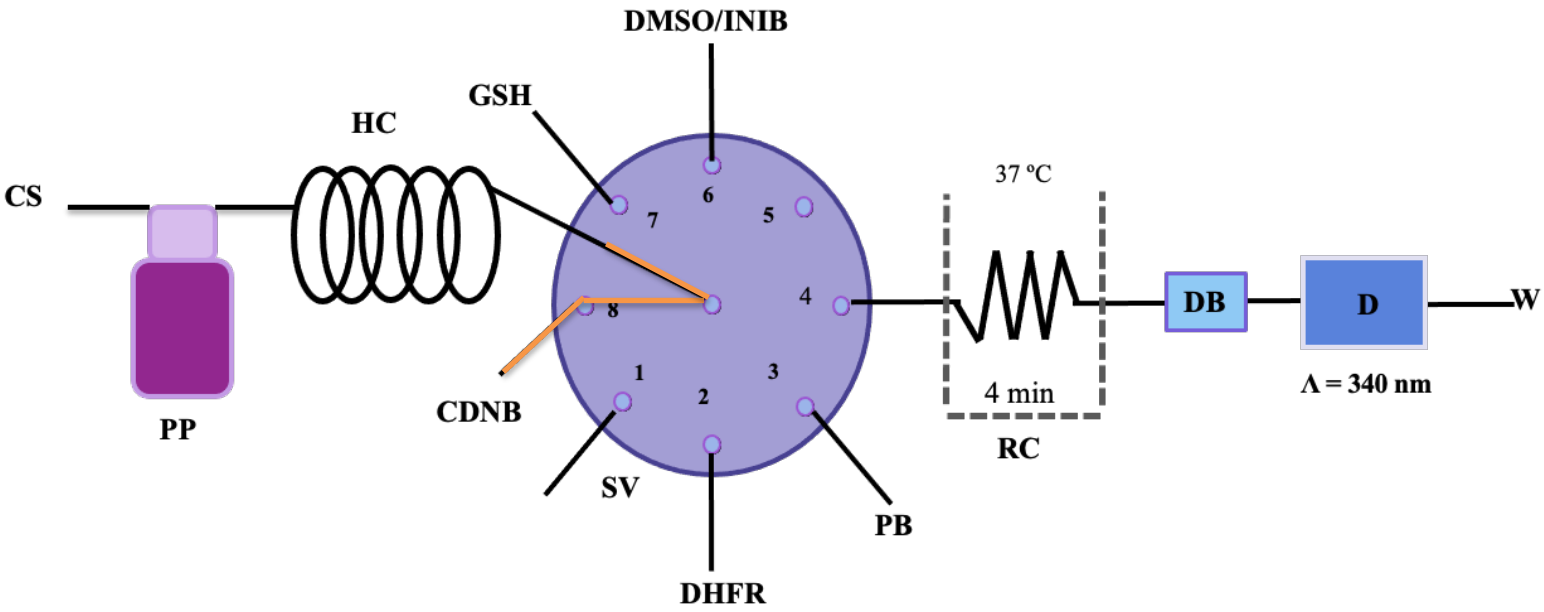
Schematic representation of the SIA system optimized Analytical cycle



Step	Position valve	Reagent	Volume (µL)	Time (s)	Flow rate (mL min $^{-1}$)	Action
1	8	CDNB	10	1.2	0.5	**Aspiration of CDNB**
2	6	DMSO/ inhibitor	10	1.2	0.5	**Aspiration of DMSO/inhibitor**
3	2	GST P1-1	20	2.4	0.5	**Aspiration of GST P1-1**
4	7	GSH	20	2.4	0.5	**Aspiration of GSH**
5	4	Mixture	333	20	1	**Propulsion to the reactor coil**
6	4		----	240	0	**Stop flow period in reactor coil**
7	4	Mixture	2000	60	2	**Propulsion to the detector**

Results and discussion

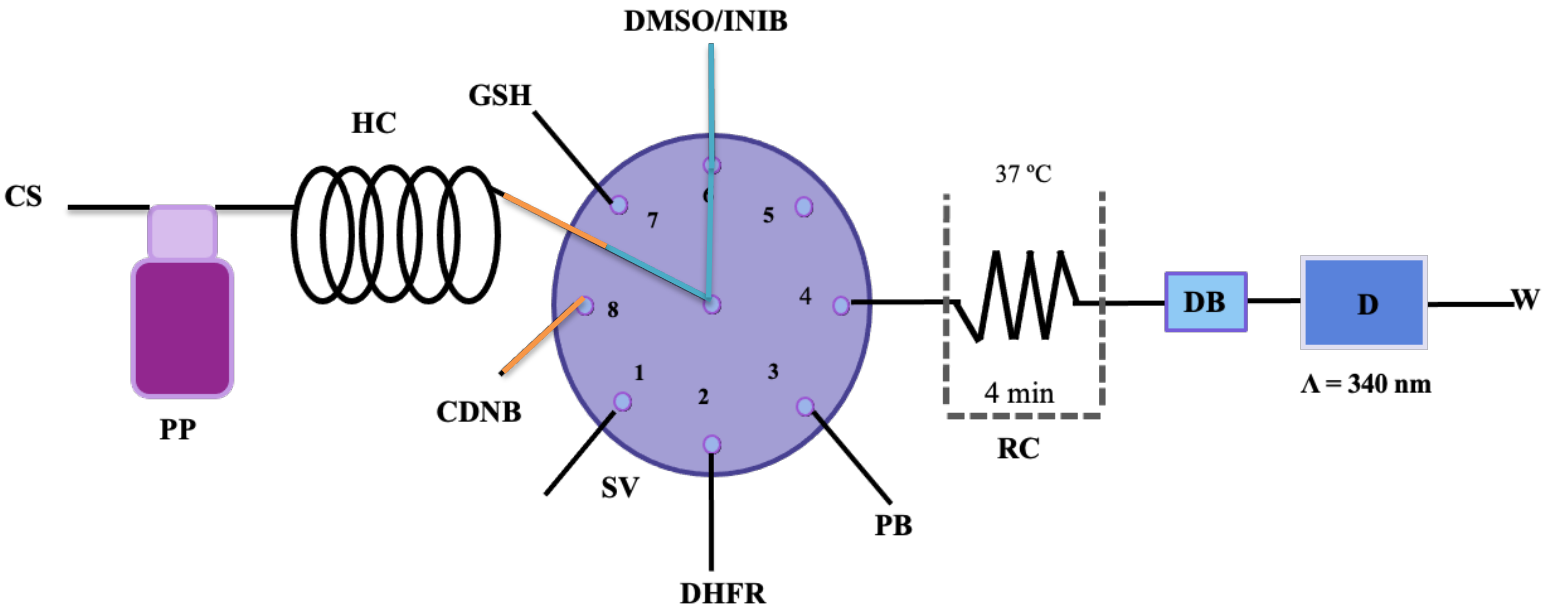
Analytical cycle



Aspiration of 10 µL of CDNB

Results and discussion

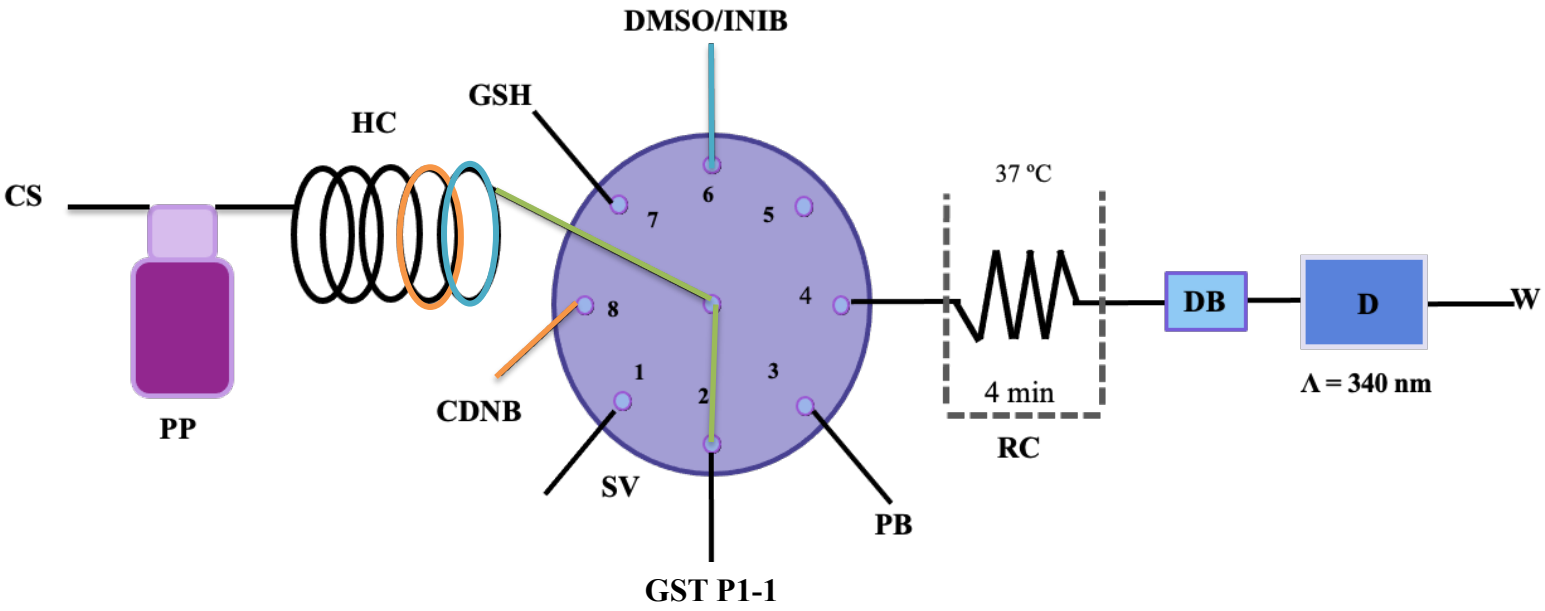
Analytical cycle



Aspiration of 10 µL of DMSO/INIB

Results and discussion

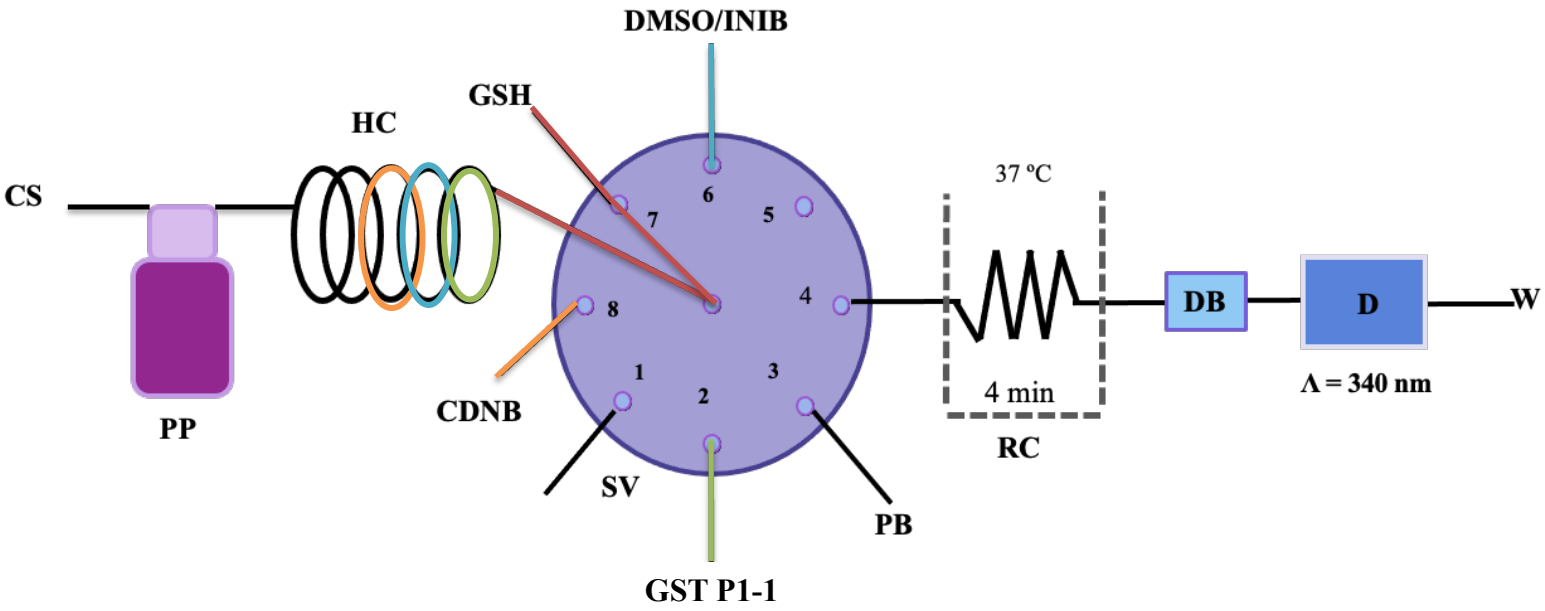
Analytical cycle



Aspiration of 20 µL of GST P1-1

Results and discussion

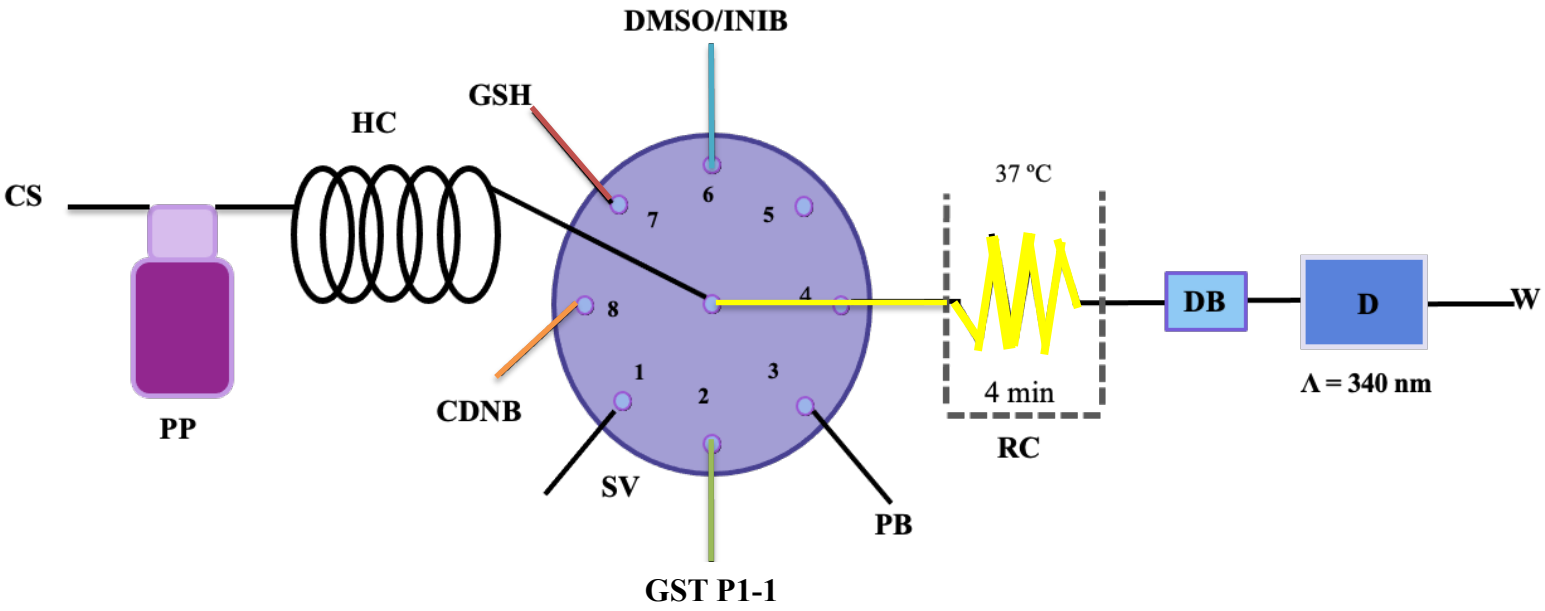
Analytical cycle



Aspiration of 20 µL of GSH

Results and discussion

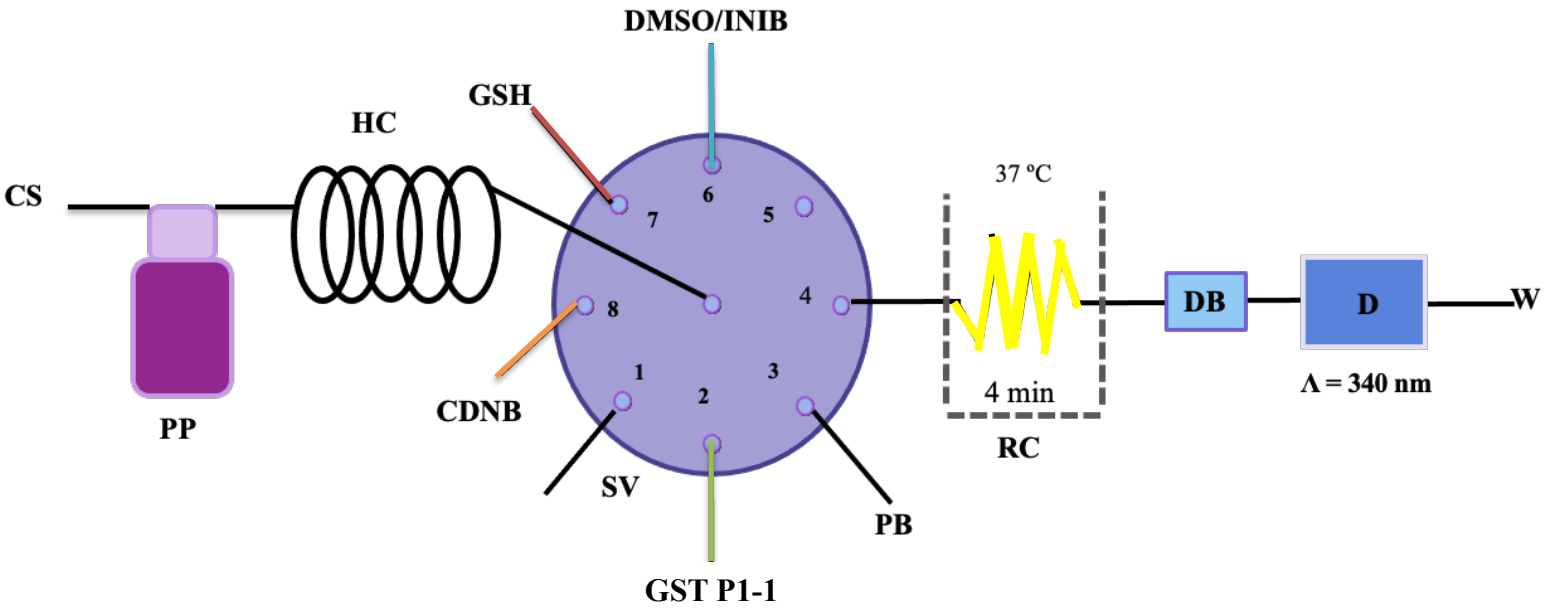
Analytical cycle



Propulsion to the reactor coil

Results and discussion

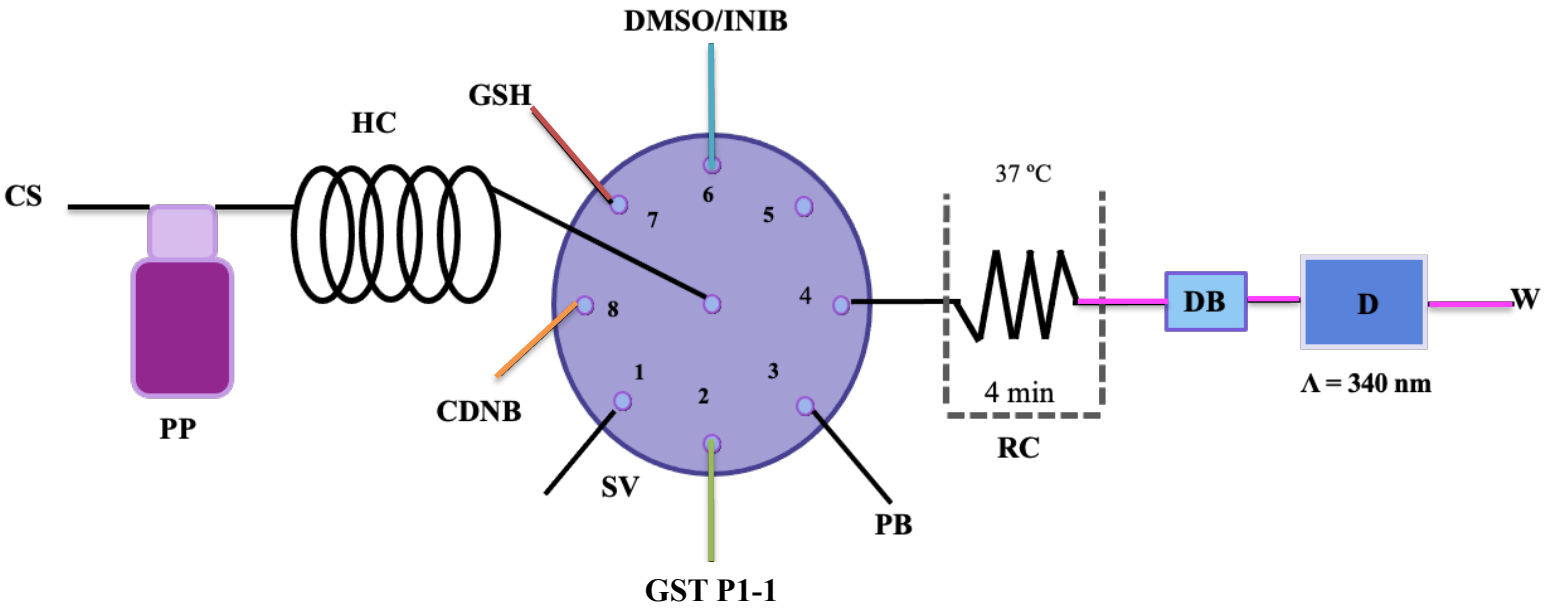
Analytical cycle



Stop flow period in reactor coil

Results and discussion

Analytical cycle

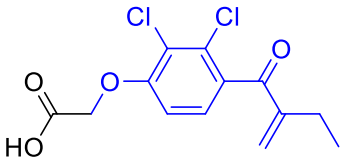


Propulsion to the detector

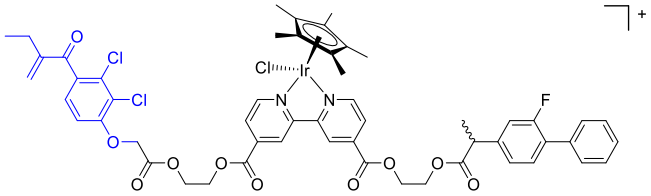
Results and discussion

Evaluation of the half maximal inhibitory concentration (IC_{50}) of organometallic compounds

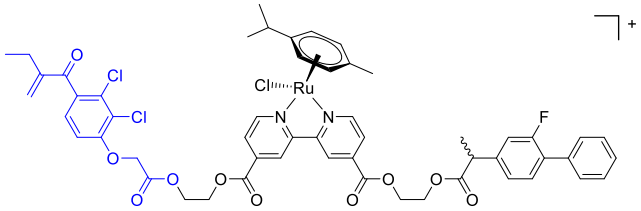
Ethacrynic acid



IC_{50} = 11.3 ± 0.8



IC_{50} = 6.7 ± 0.7

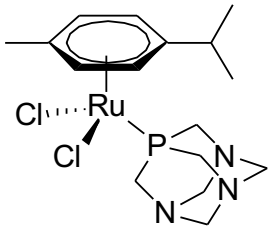


IC_{50} = 12.1 ± 1.8

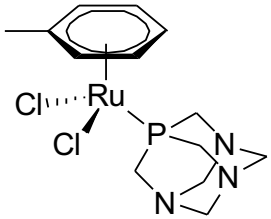
Results and discussion

Evaluation of the half maximal inhibitory concentration (IC_{50}) of organometallic compounds

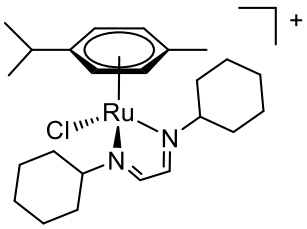
RAPTA



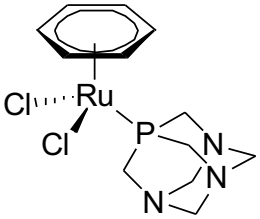
IC_{50} = 56.9 ± 4.3



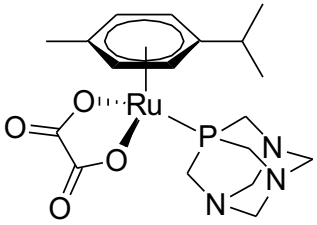
IC_{50} = 75.8 ± 5.6



IC_{50} = 235.0 ± 1.6



IC_{50} = 24.7 ± 1.4

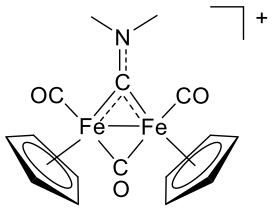


IC_{50} = 60.7 ± 5.3

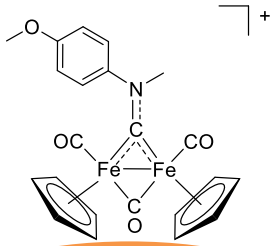
Results and discussion

Evaluation of the half maximal inhibitory concentration (IC_{50}) of organometallic compounds

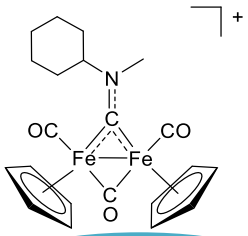
Aminocarbyne derivatives



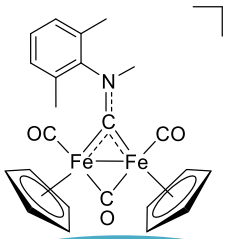
IC_{50} = 275.1 ± 9.4



IC_{50} = 113.0 ± 5.3

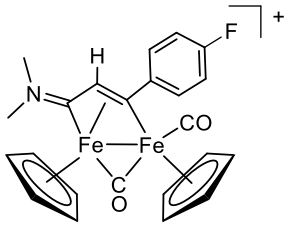


IC_{50} = 219.1 ± 8.4

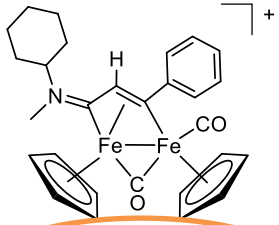


IC_{50} = 72.4 ± 4.9

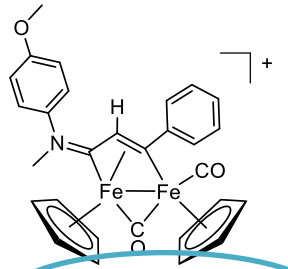
Vinyliminium derivatives



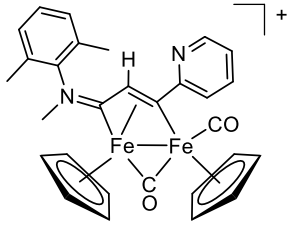
IC_{50} = 82.9 ± 8.7



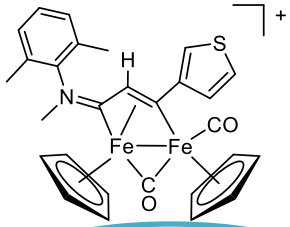
IC_{50} = 90.7 ± 8.6



IC_{50} = 46.8 ± 2.8



IC_{50} = 34.0 ± 4.3

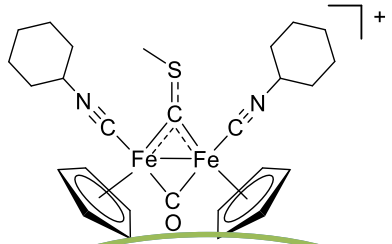


IC_{50} = 30.3 ± 8.3

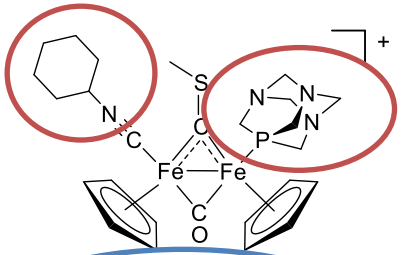
Results and discussion

Evaluation of the half maximal inhibitory concentration (IC_{50}) of organometallic compounds

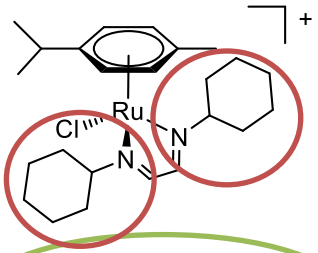
Thiocarbyne complex



IC_{50} = 17.4 ± 2.8



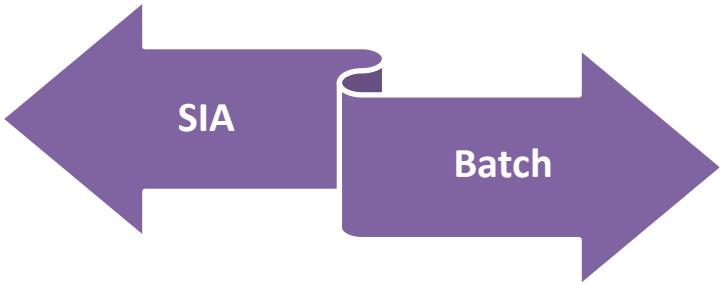
IC_{50} = 165.0 ± 3.8



IC_{50} = 235.0 ± 1.6

Results and discussion

Validation method



Compounds	IC_{50} batch ± SD (µM)	IC_{50} SIA ± SD (µM)
1	13.58 ± 0.02 [1]	11.3 ± 0.8
4a	226.4 ± 3.2	275.1 ± 9.4
4c	60.7 ± 2.9	113 ± 5.3
4d	32.8 ± 5.5	72.4 ± 4.9
6a	11.5 ± 0.7	17.4 ± 2.8
7e	11.6 ± 0.5	12.1 ± 1.8

IC_{50} SIA = (1.2 ± 0.3) IC_{50} BATCH - (12.4 ± 30.9)

Pearson coefficient

T-student

Good correlation between the two sets of results.

[1] L. Biancalana, L. K. Batchelor, S. A. P. Pereira, P.-J. Tseng, S. Zacchini, G. Pampaloni, L. M. F. S. Saraiva, P. J. Dyson, F. Marchetti, *Chem. Eur. J.* **2020**, *26*, 17525.

Results and discussion

Advantages of SIA system comparing with batch procedure

	Batch procedure	SIA system
Reaction time	8 min	5 min
Volume of GSH spent	5 times less than batch procedure	
Volume of CDNB spent	1.25 times less than batch procedure	
Volume of GST P1 spent	2.3 times less than batch procedure	

Conclusions

A SIA system was optimized to evaluate the inhibition capacity of different organometallic complexes with anticancer activity against GST P1-1 enzyme.

Most of the organometallic compounds tested exhibited good inhibition profiles where the IC_{50} values are in the low μM range.

The SIA system allows the decrease of the reagents volumes spent (three times less) and brief waiting times to obtain the analytical signal (5 minutes instead of 8 minutes).

SIA methodology showed to be a valuable automatic alternative for the analysis of the inhibitory effect on the GST P1-1 of organometallic compounds with potential anticancer activity.

Acknowledgments

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Professor Paul Dyson (EPFL) (co-supervisor)
Prof. Fabio Marchetti and Lorenzo (Università de Pisa)

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Thank you for your attention!