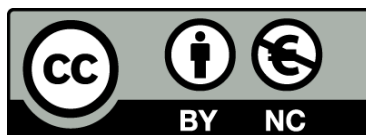


# Iminociclitoles como inhibidores de glicosidasas y su posible aplicación como coadyuvantes en el tratamiento de trastornos metabólicos e infecciones

Livia Gómez Cortés



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INSTITUTO DE QUÍMICA AVANZADA DE CATALUÑA (IQAC)  
Departamento de química biológica y modelización molecular  
Grupo de biotransformaciones y moléculas bioactivas

BIOGLANE S.L.N.E.

Amb el suport del Comissionat per a Universitats i Recerca del Departament  
d'Innovació, Universitats i Empresa de la Generalitat de Catalunya

UNIVERSIDAD DE BARCELONA  
FACULTAD DE FARMACIA  
Departamento de Bioquímica y Biología Molecular  
Programa de Doctorado de Biotecnología

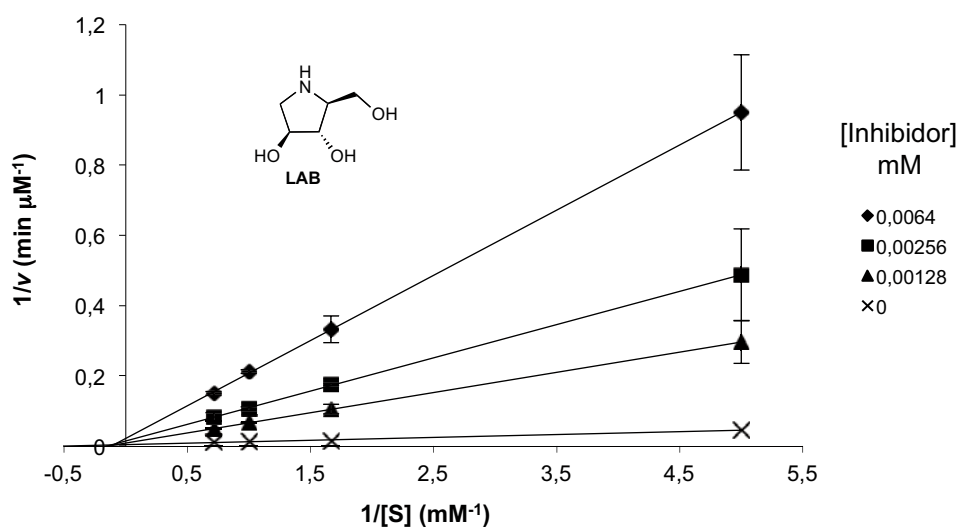
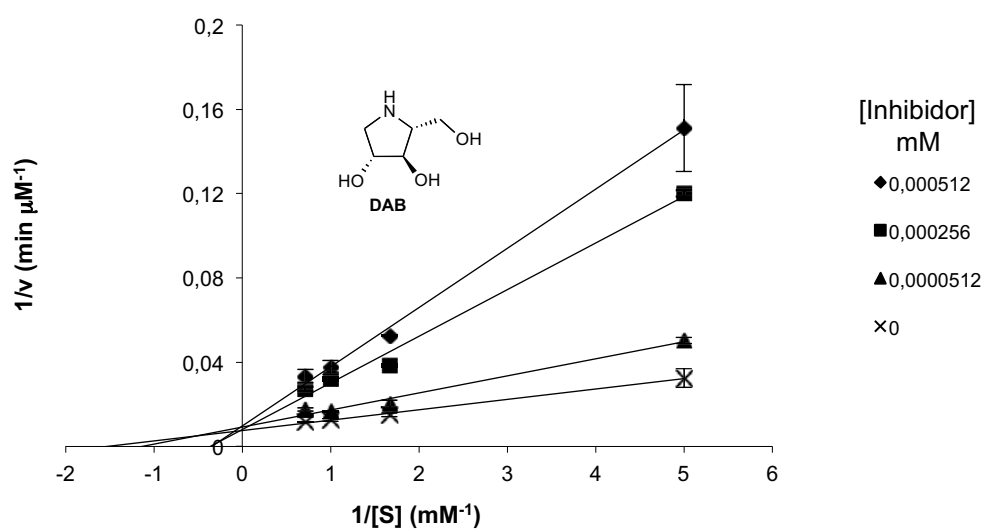
**Iminociclitoles como inhibidores de glicosidasas y su posible aplicación  
como coadyuvantes en el tratamiento de trastornos metabólicos e  
infecciones**

**Livia Gómez Cortés**

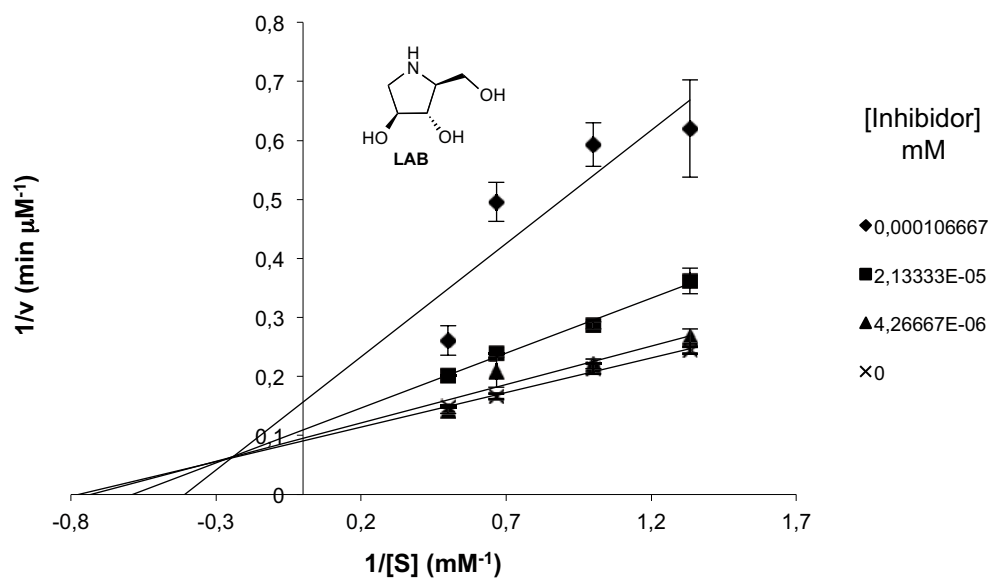
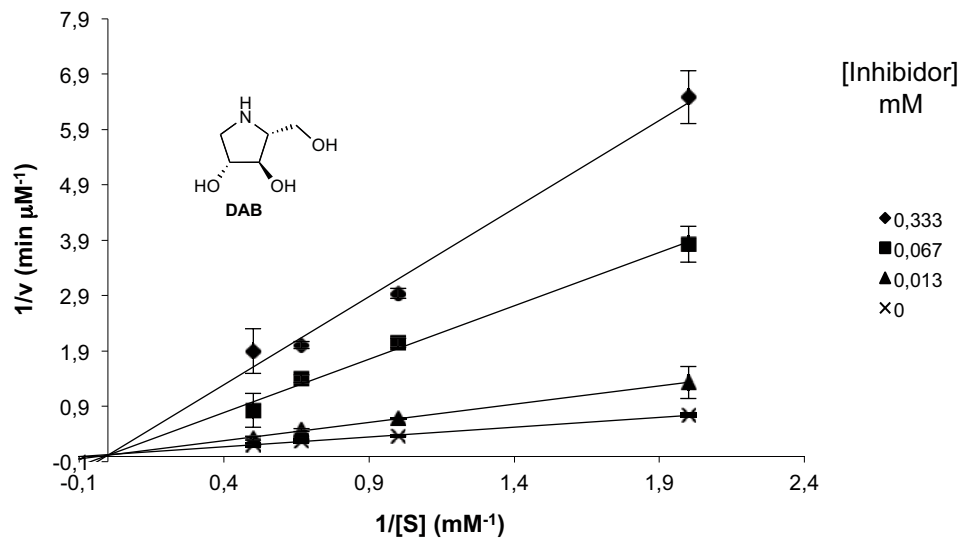
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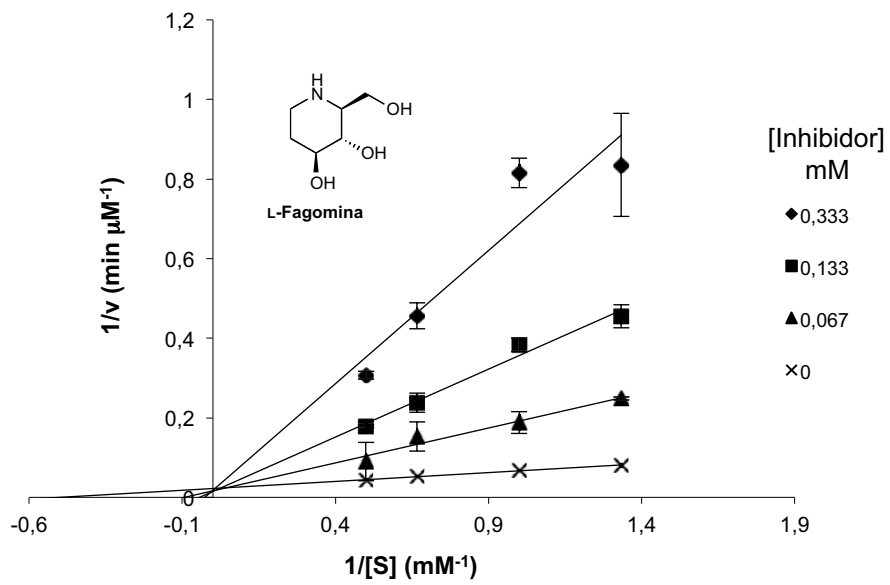
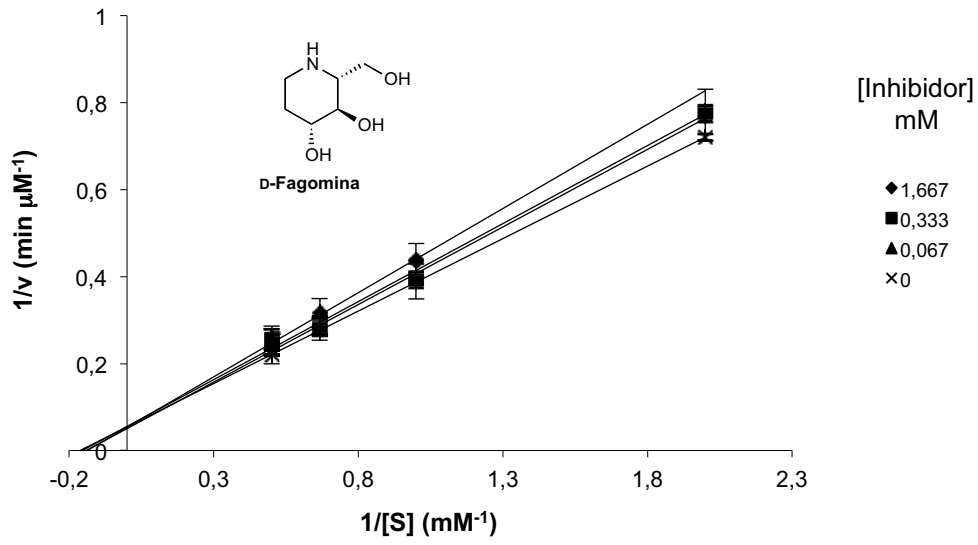
3.1.1 Diagramas dobles recíprocos de Lineweaver-Burk de los compuestos de referencia, DAB, LAB, D-fagomina y L-fagomina, frente a las diferentes glicosidasas comerciales estudiadas: a)  $\alpha$ -D-glucosidasa de levadura, b)  $\alpha$ -D-glucosidasa de arroz, c)  $\beta$ -D-glucosidasa de almendra dulce, d)  $\beta$ -D-galactosidasa de hígado bovino, e)  $\alpha$ -L-rhamnosidasa de *Penicillium decumbens*, f)  $\alpha$ -D-mannosidasa de judía y g)  $\alpha$ -L-fucosidasa de riñón bovino

a)  $\alpha$ -D-Glucosidasa de levadura

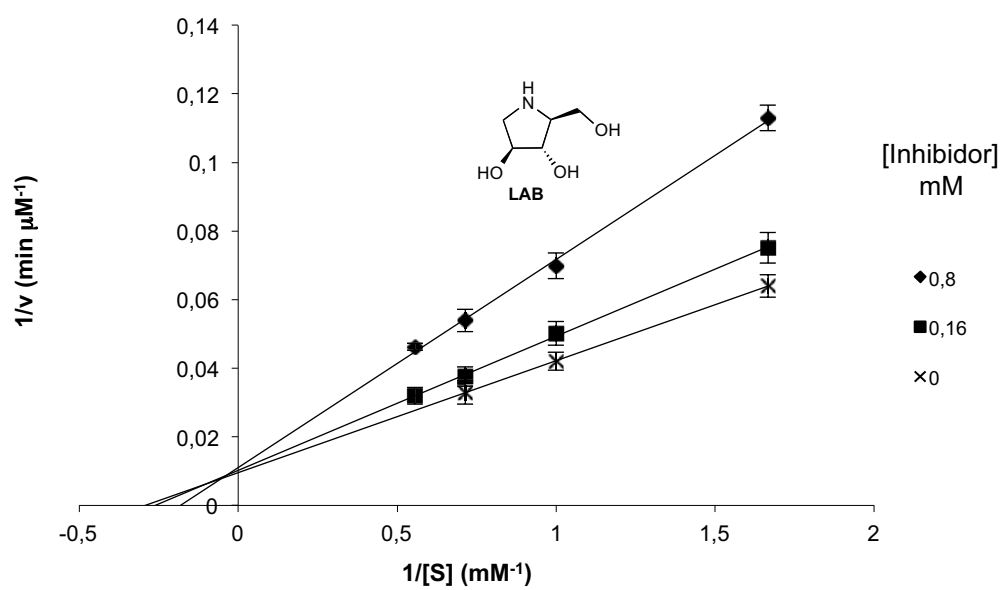
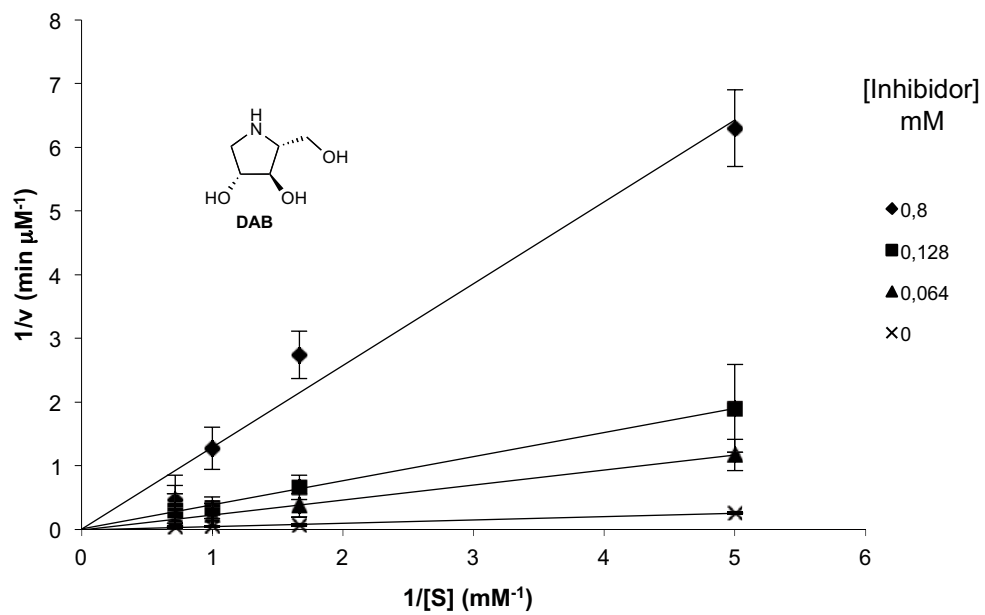


b)  $\alpha$ -D-glucosidasa de arroz

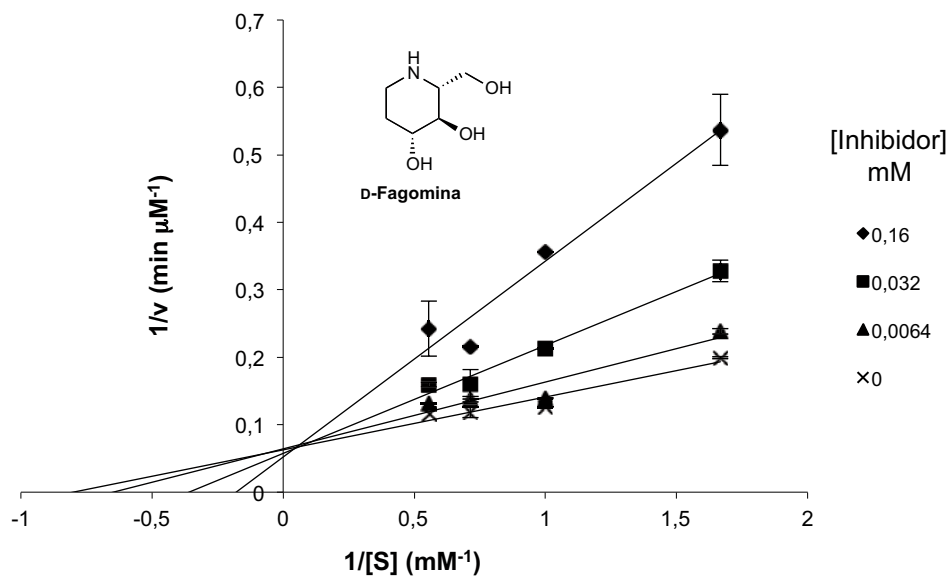




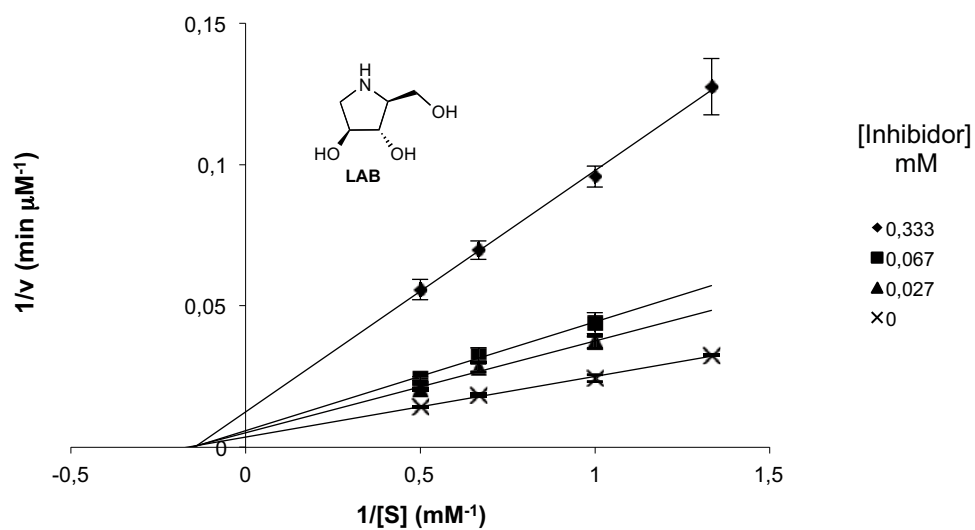
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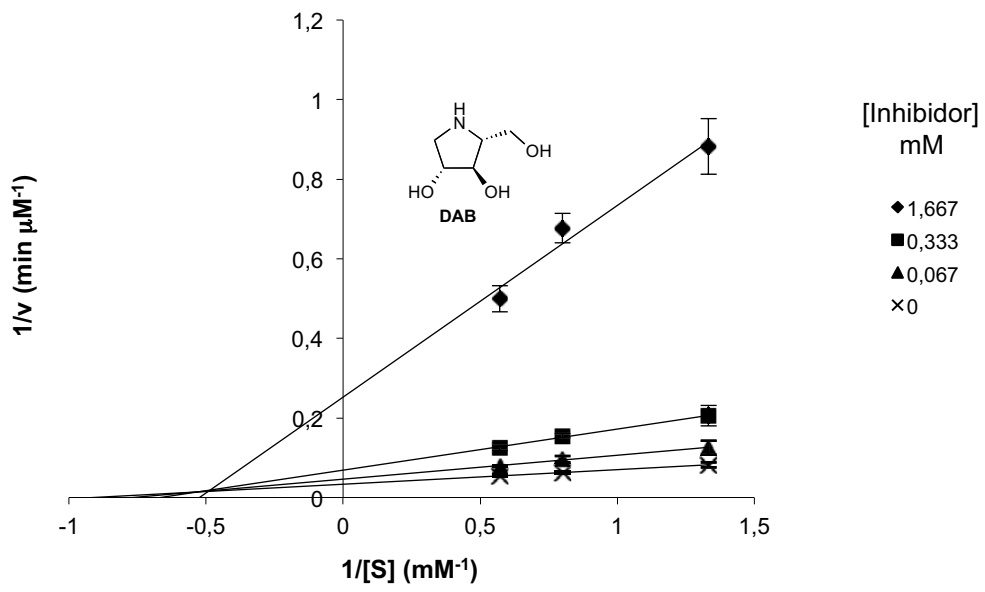
d) β-D-galactosidasa de hígado bovino



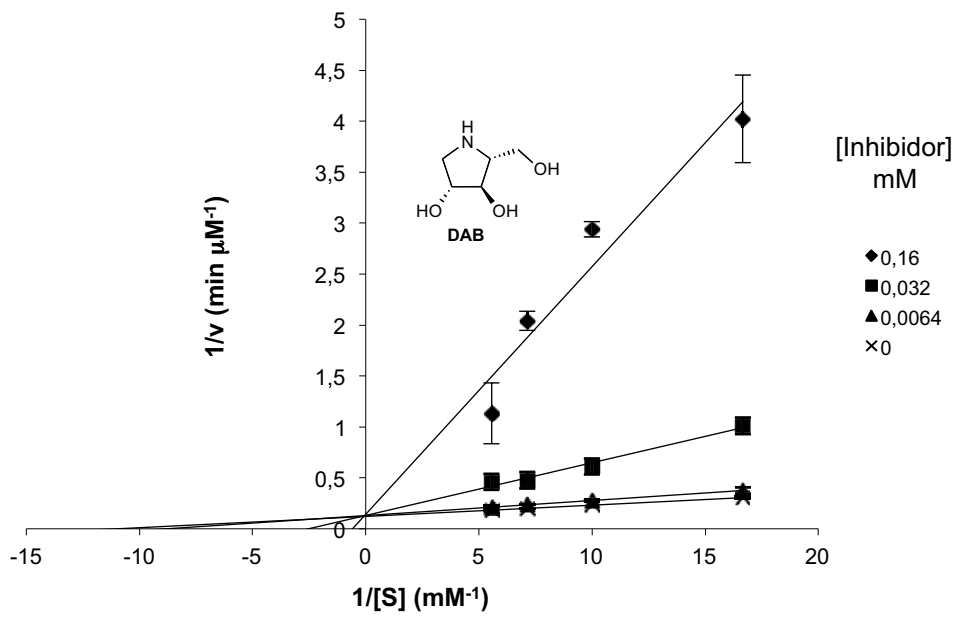
e)  $\alpha$ -L-ramnosidasa de *Penicillium decumbens*



f)  $\alpha$ -D-mannosidasa de judía



g)  $\alpha$ -L-fucosidasa de riñón bovino



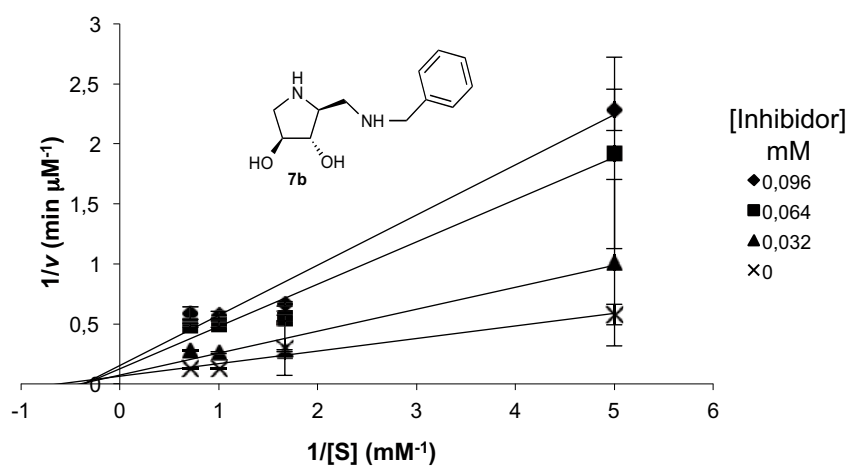
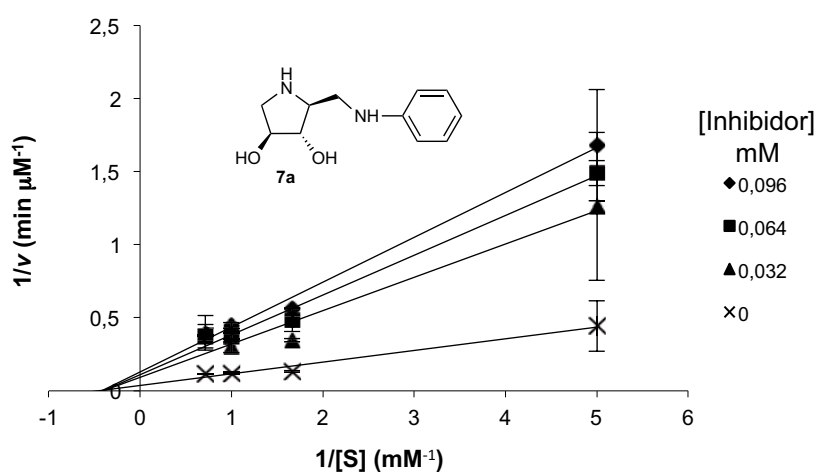


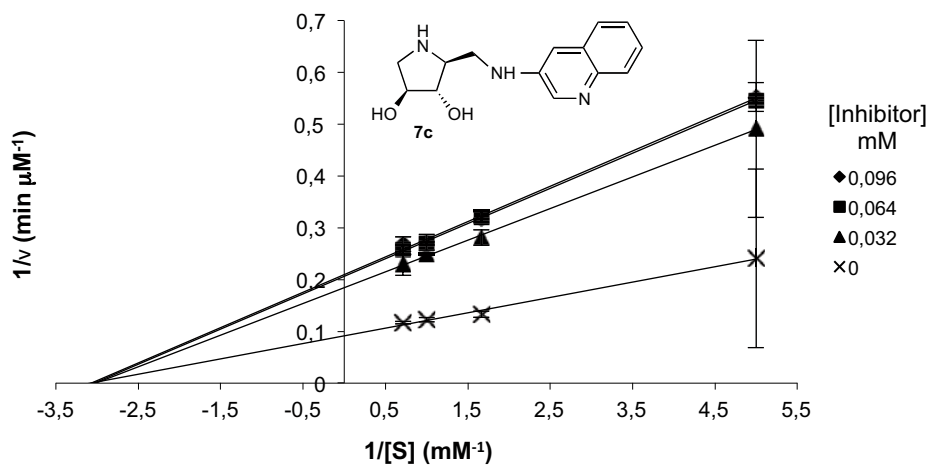
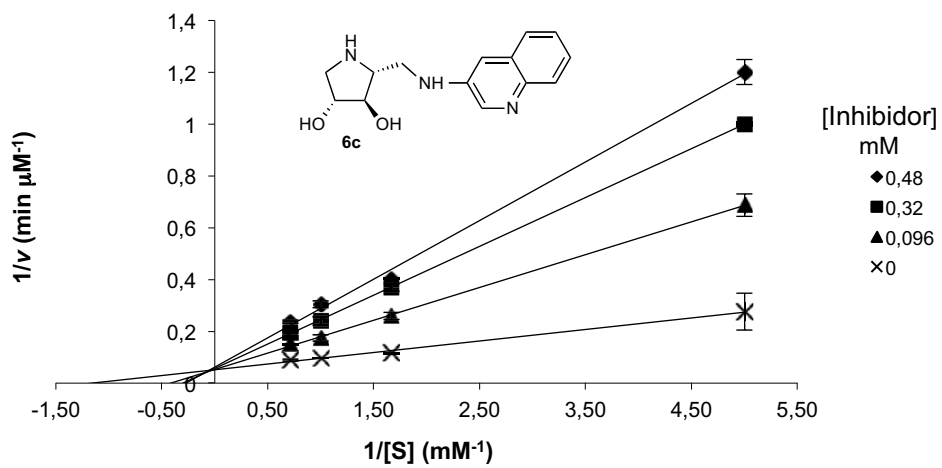




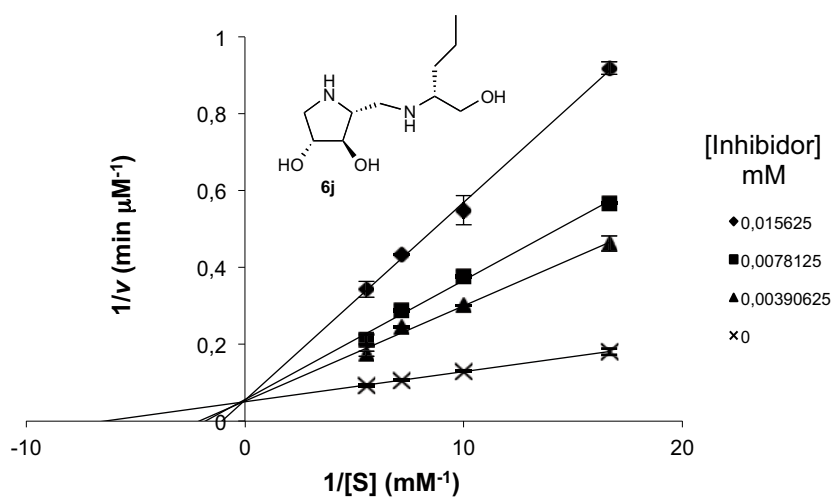
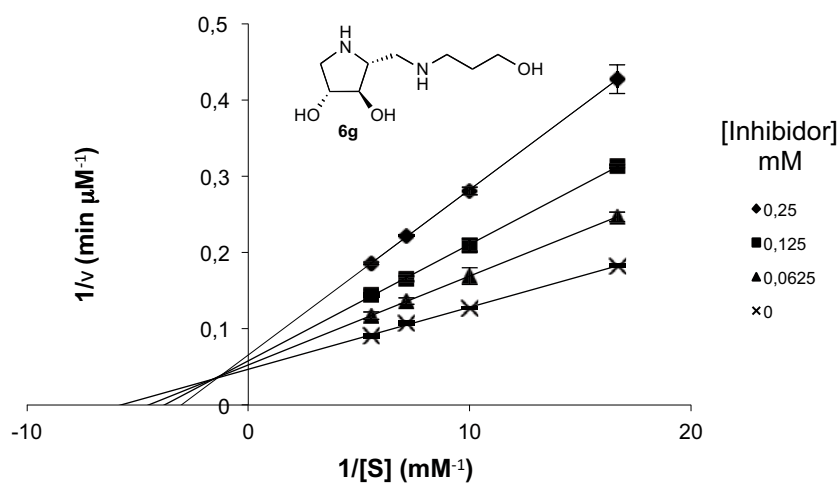
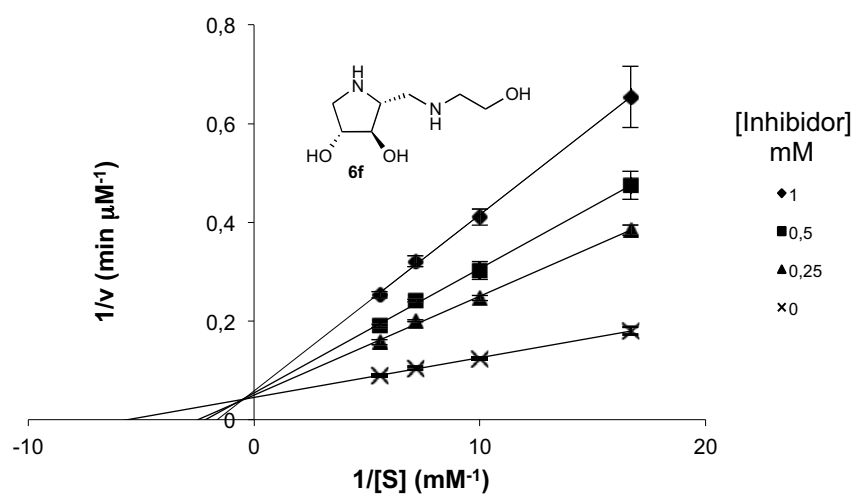
3.1.2 Diagramas dobles recíprocos de Lineweaver-Burk de los compuestos derivados de DAB y LAB frente a las diferentes glucosidasas comerciales estudiadas: a)  $\alpha$ -D-glucosidasa de levadura, b)  $\alpha$ -D-glucosidasa de arroz, c)  $\beta$ -D-glucosidasa de almendra dulce, d)  $\beta$ -D-galactosidasa de hígado bovino y e)  $\alpha$ -L-rhamnosidasa de *Penicillium decumbens*.

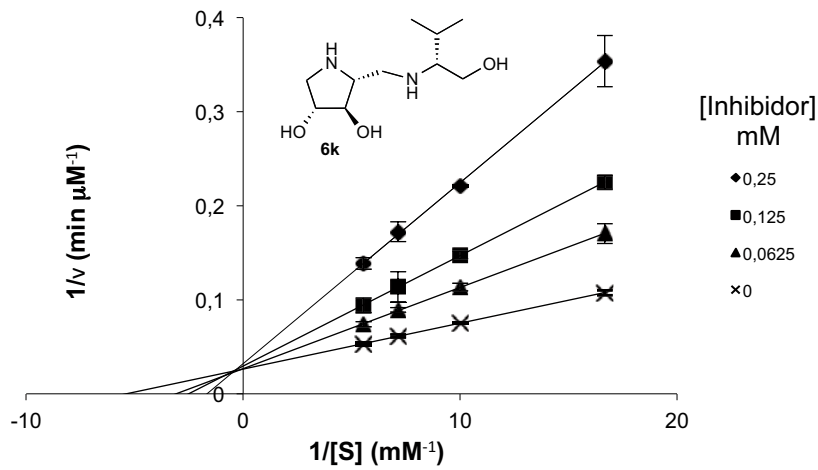
a)  $\alpha$ -D-Glucosidasa de levadura (*Saccharomyces cerevisiae*)  
conjugados con aminas aromáticas



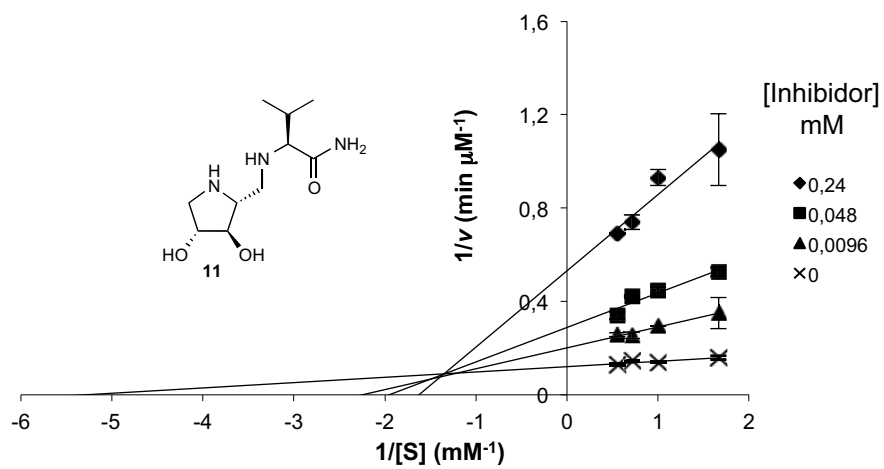
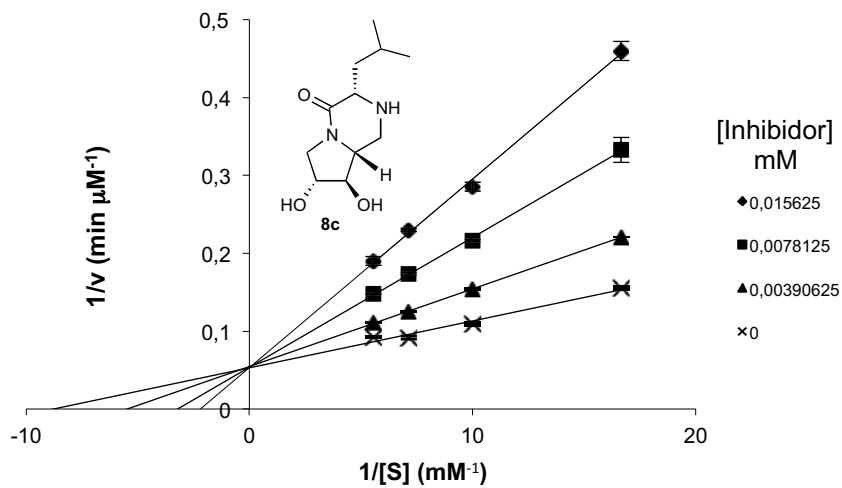


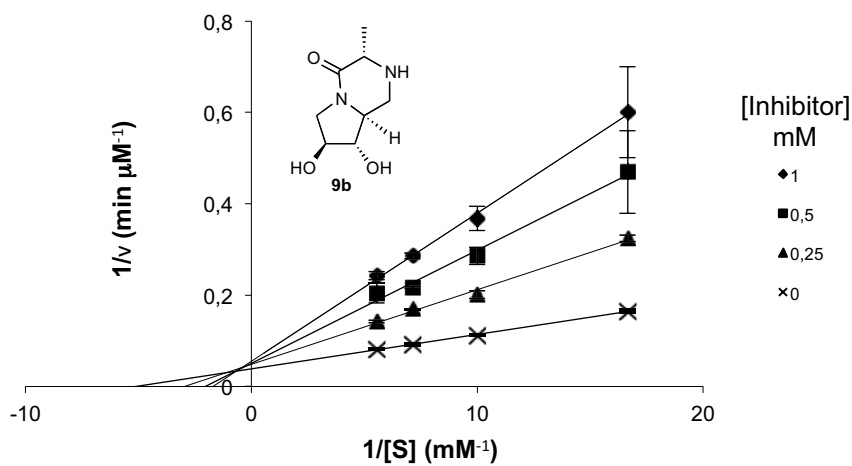
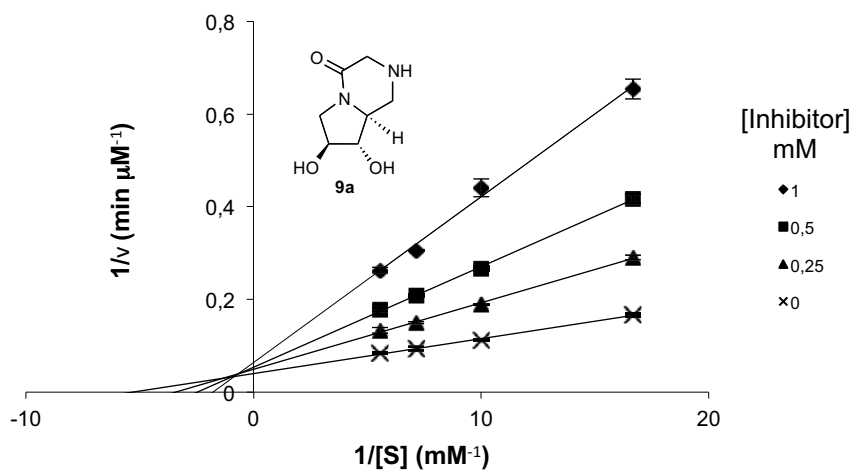
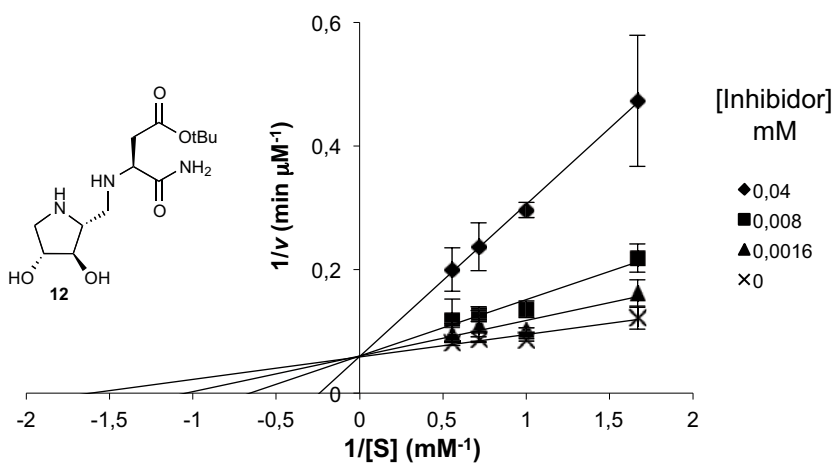
## Conjugados con aminoalcoholes

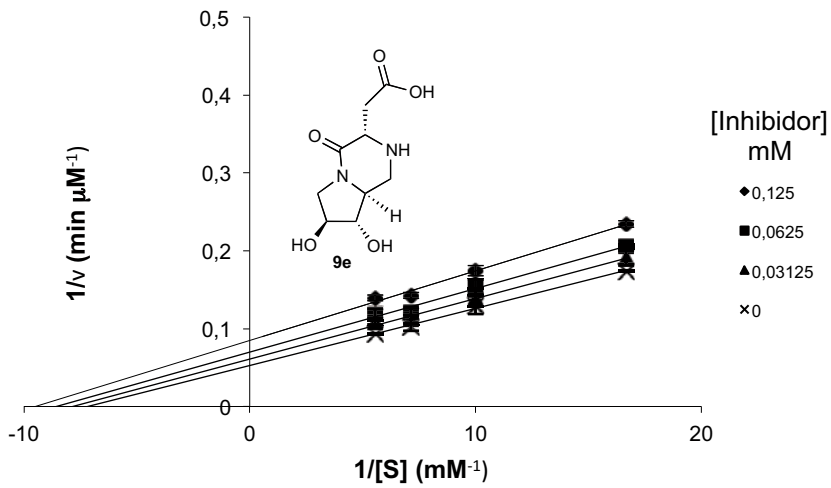
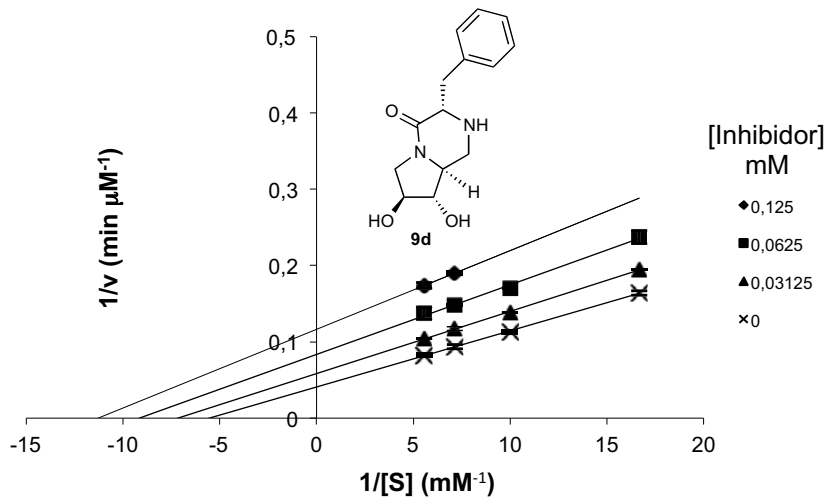
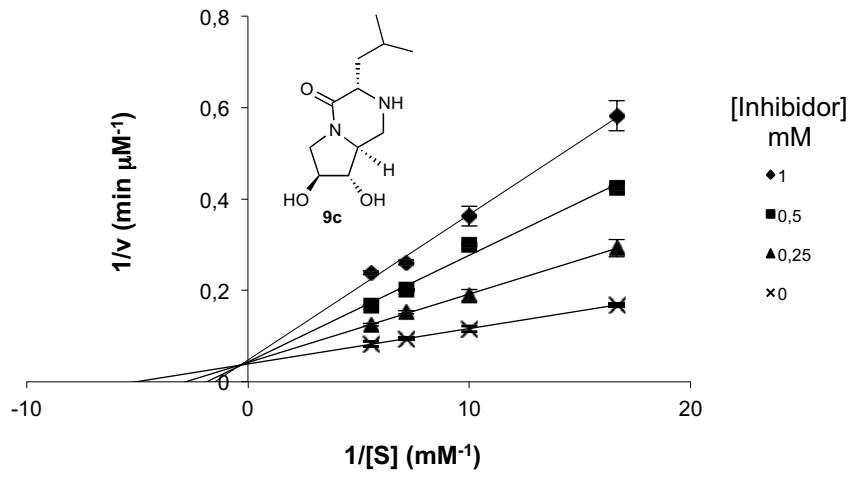




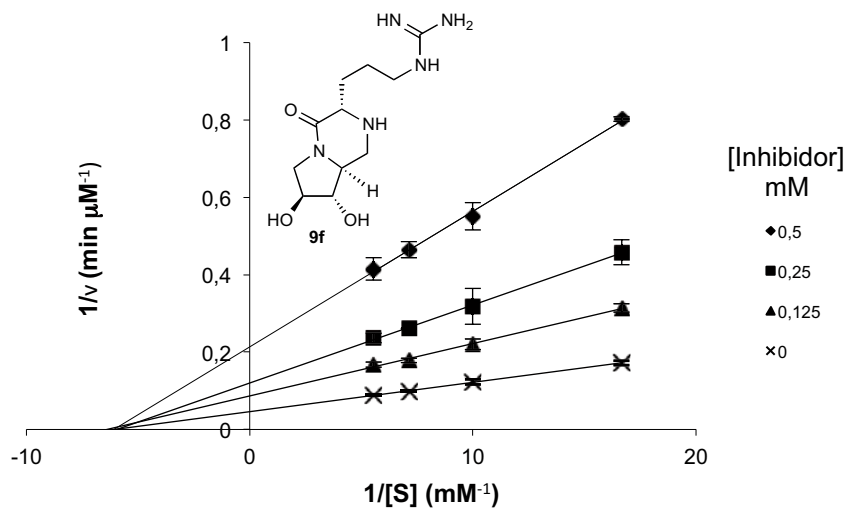
conjugados con aminoácidos y derivados del tipo 2-oxopiperacina





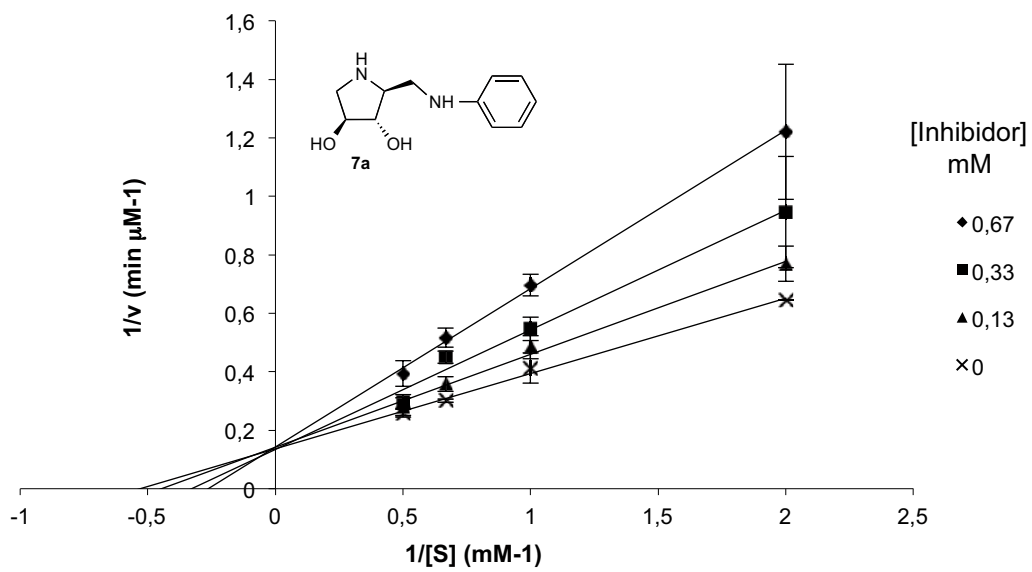




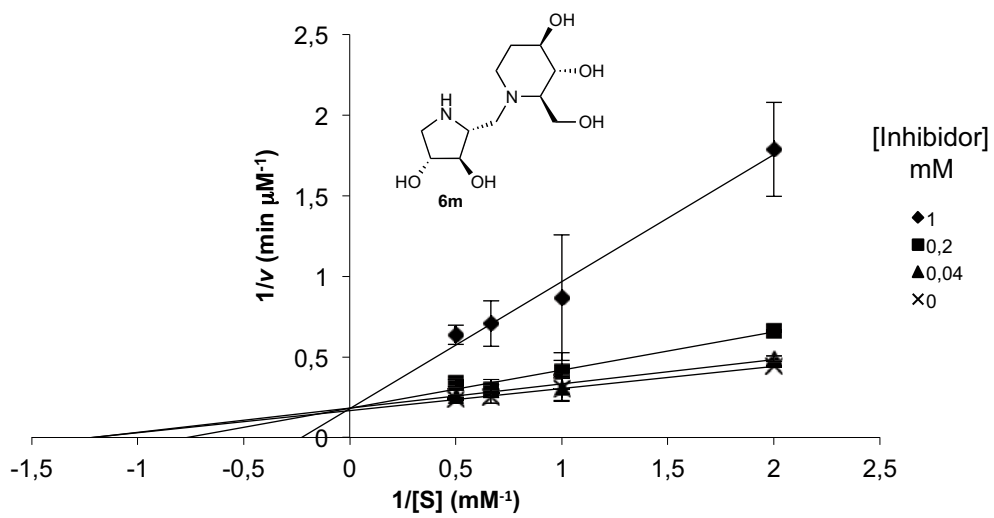
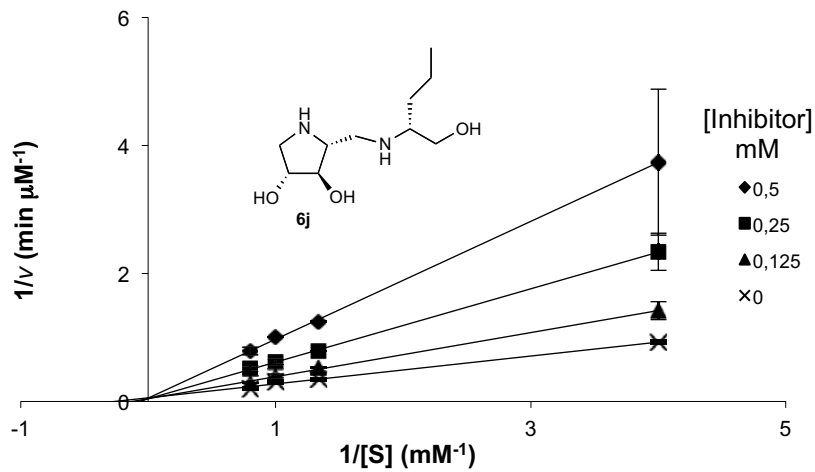


b) α-D-glucosidasa de arroz

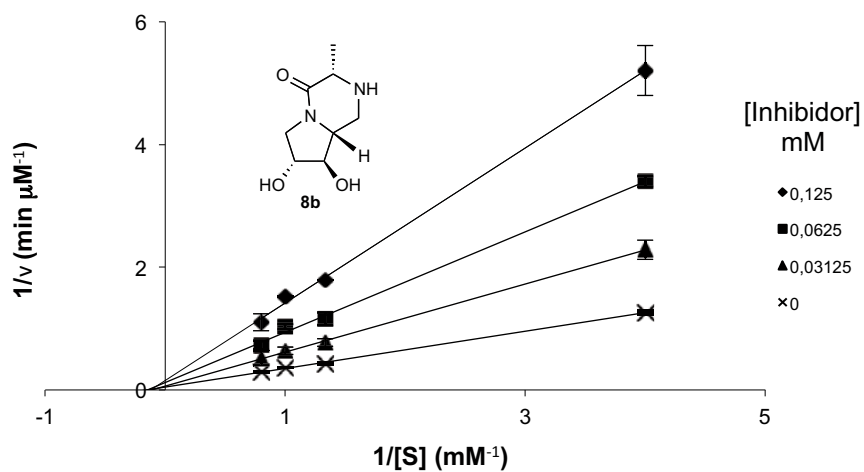
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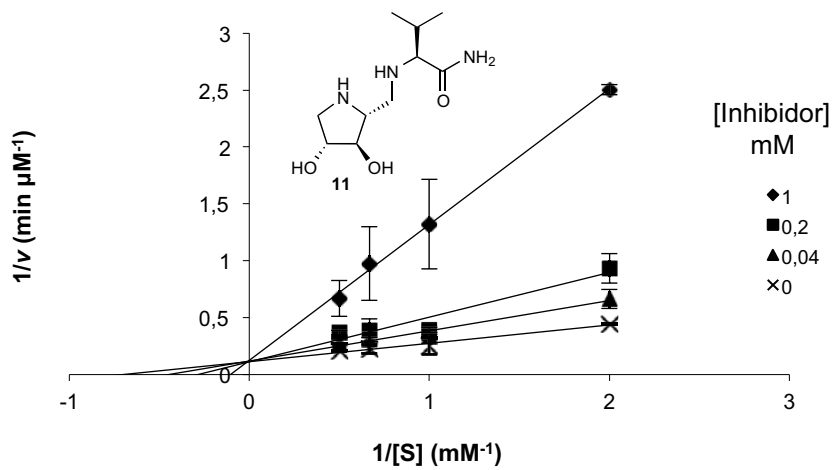
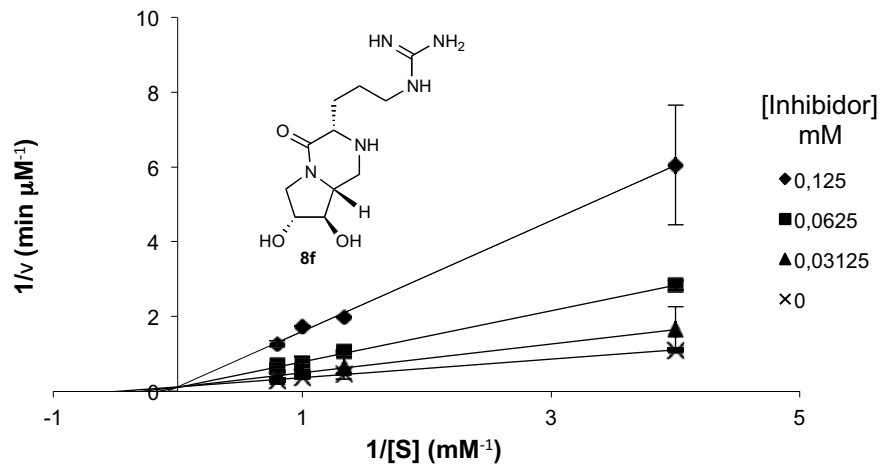
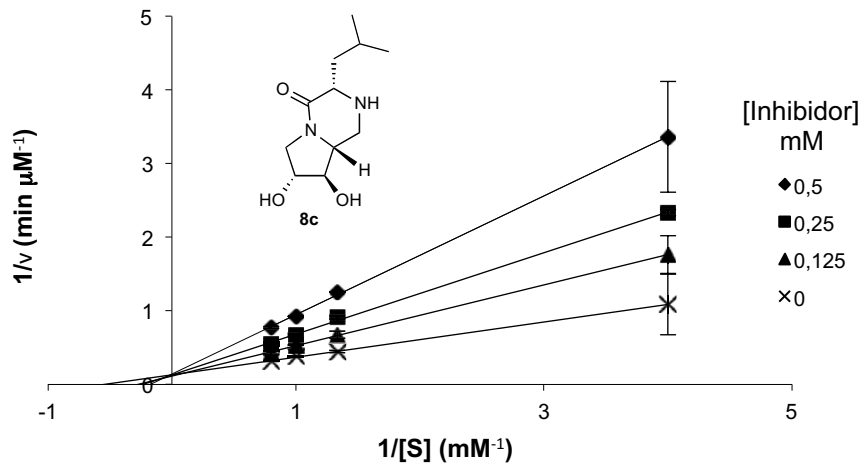


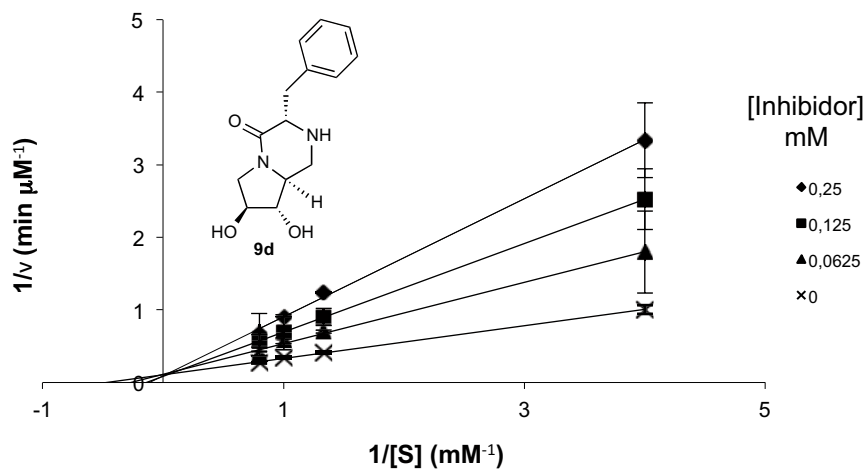
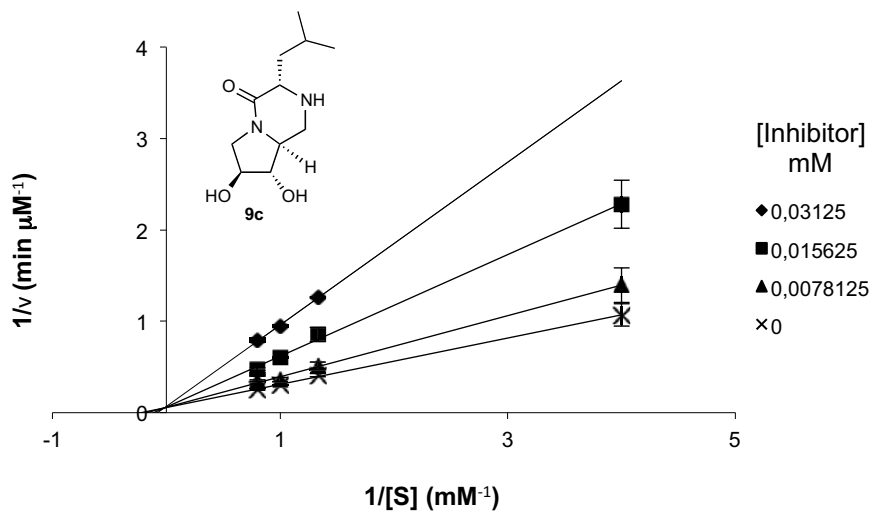
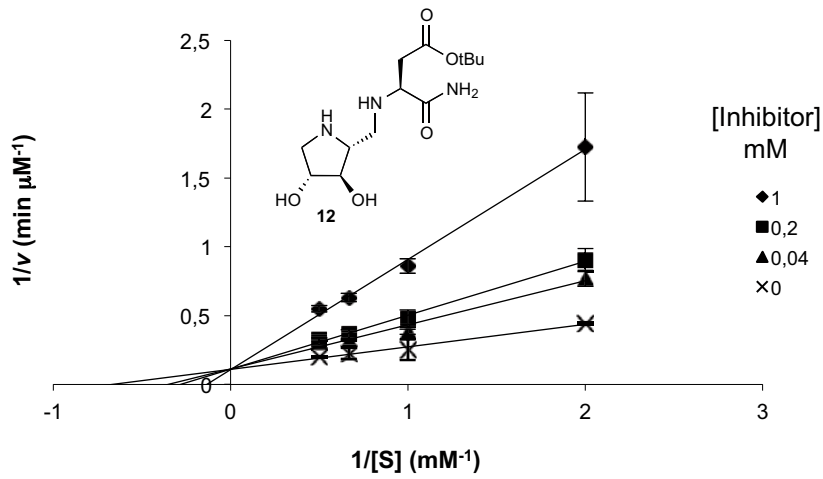
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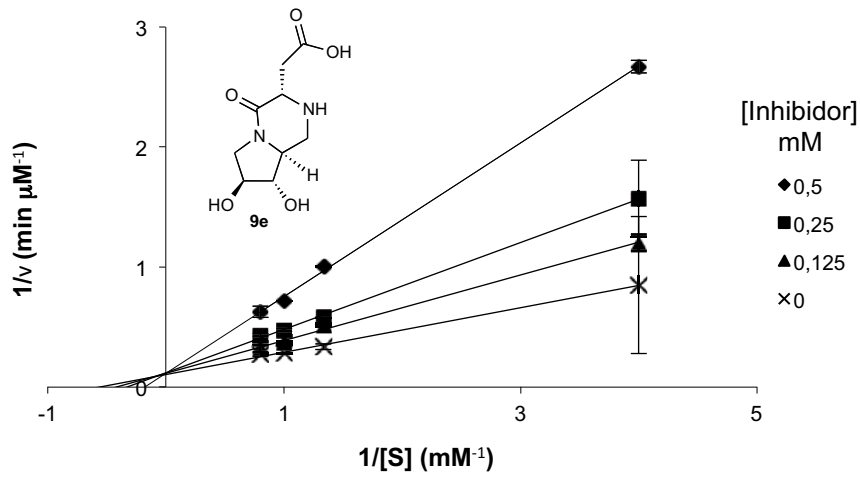


conjugados con aminoácidos y derivados 2-oxopiperacina



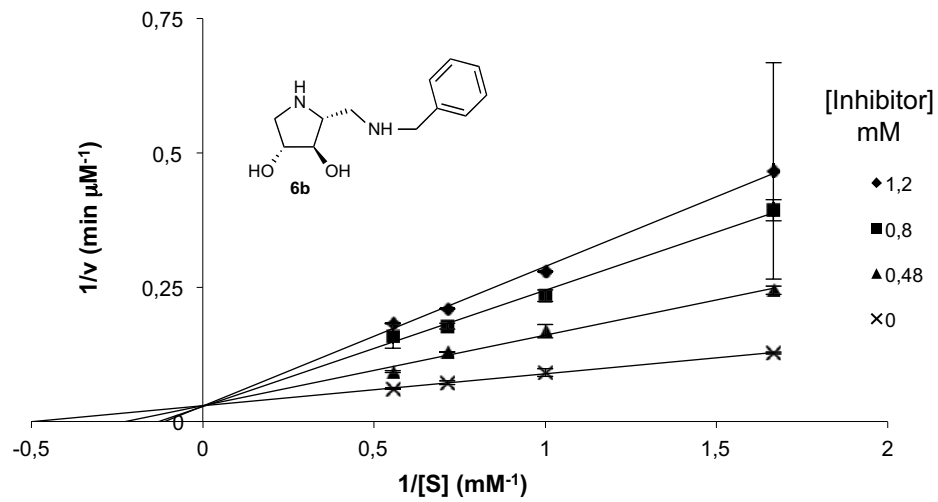
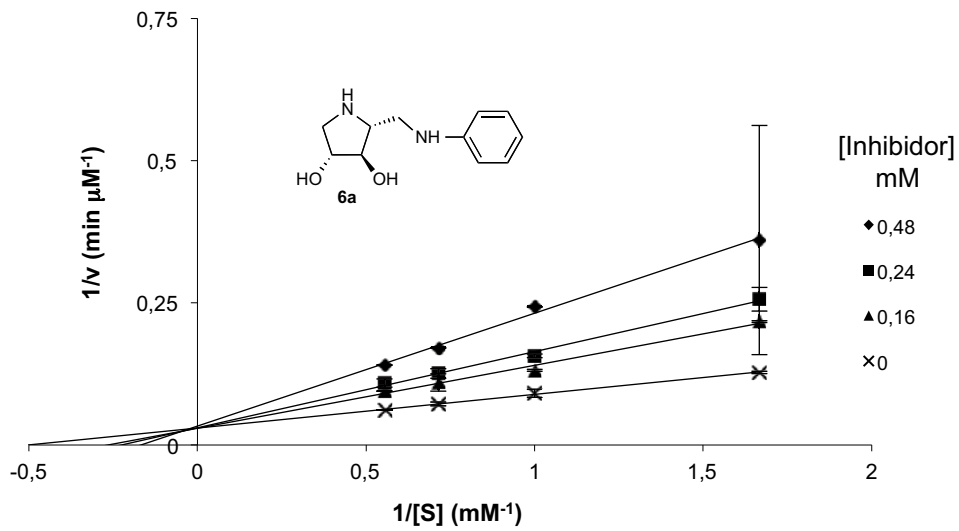


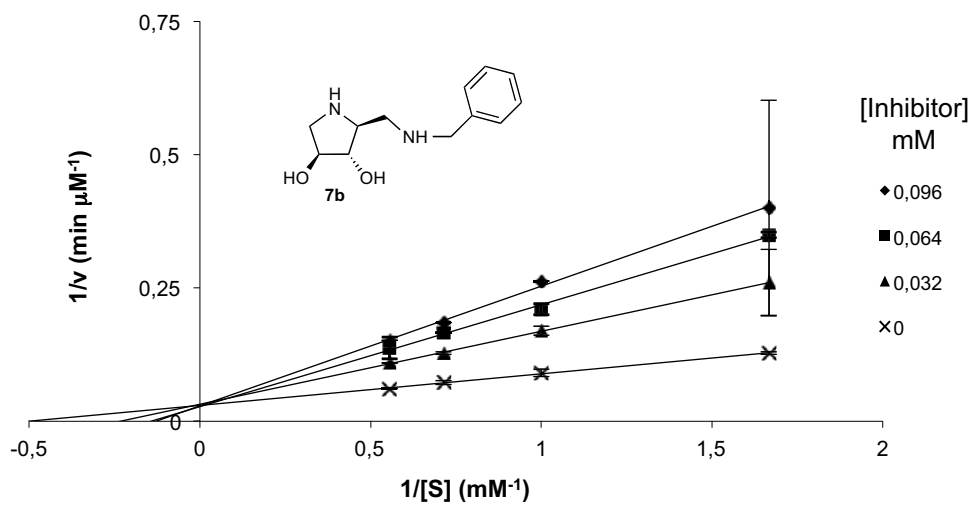




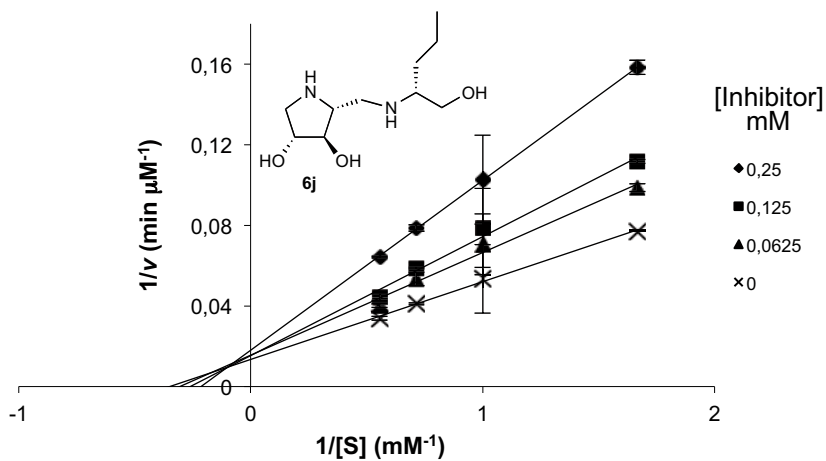
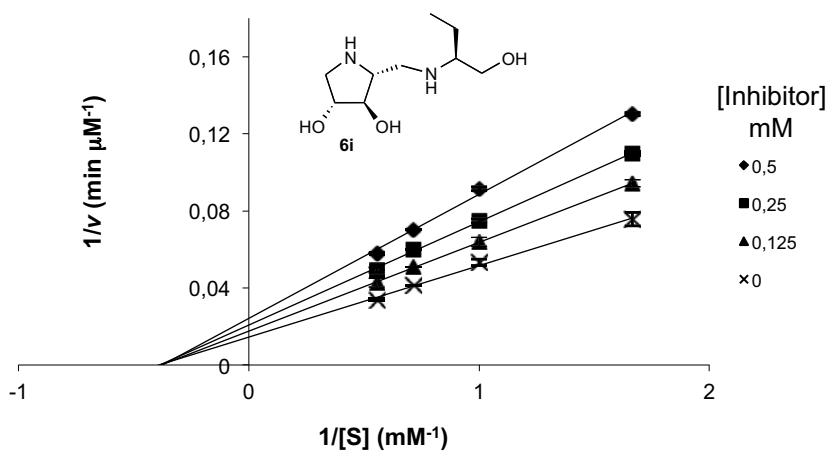
c)  $\beta$ -D-glucosidasa de almendra dulce

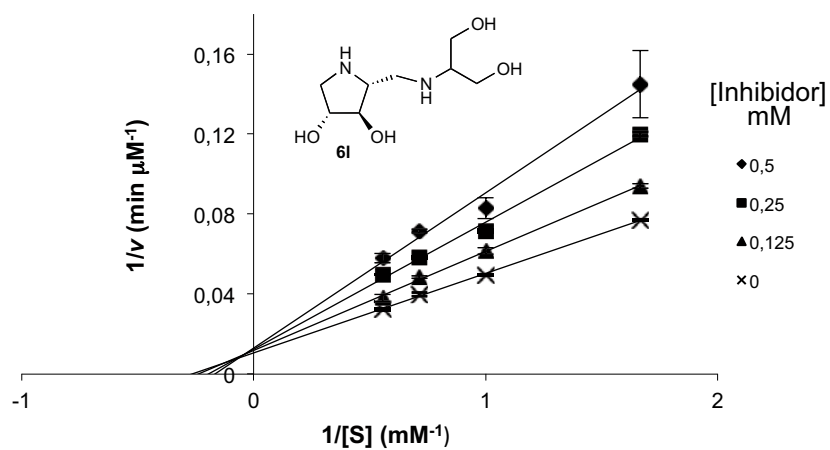
derivados conjugados con aminas aromáticas



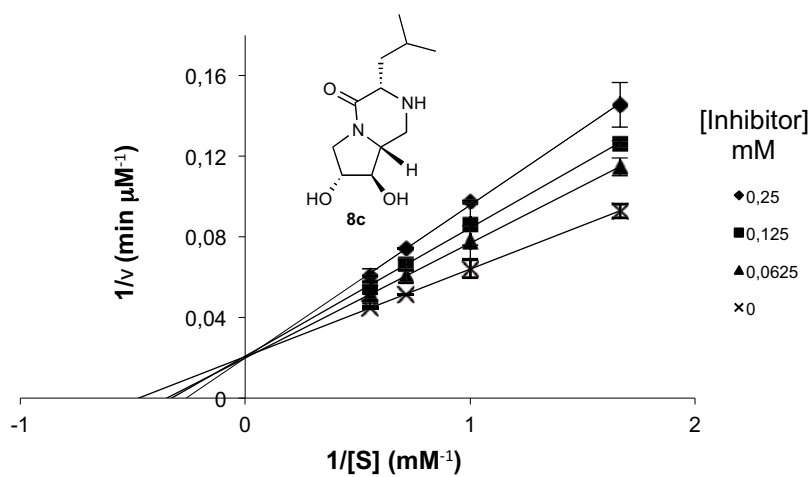


derivados conjugados con aminoalcoholes



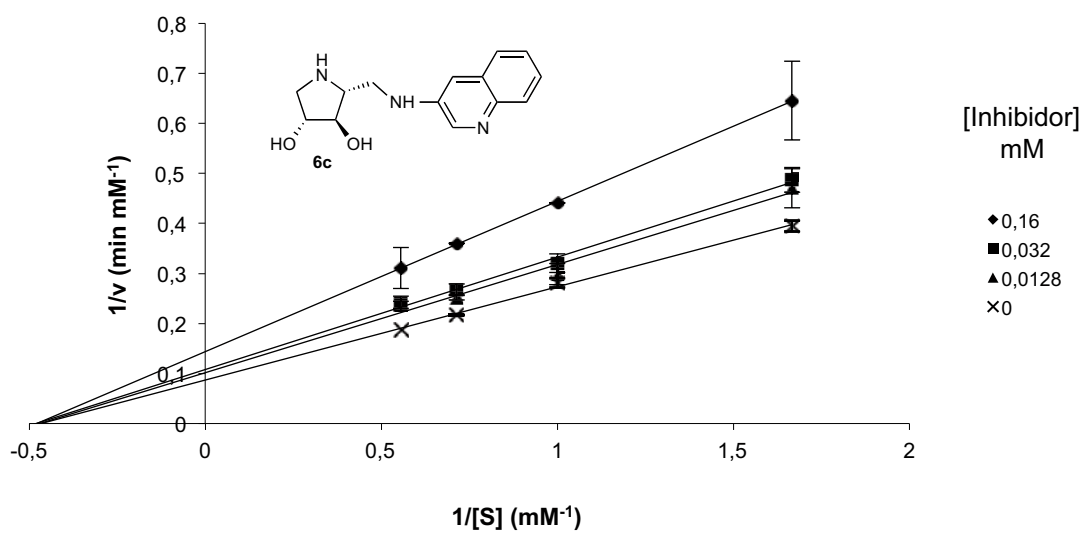
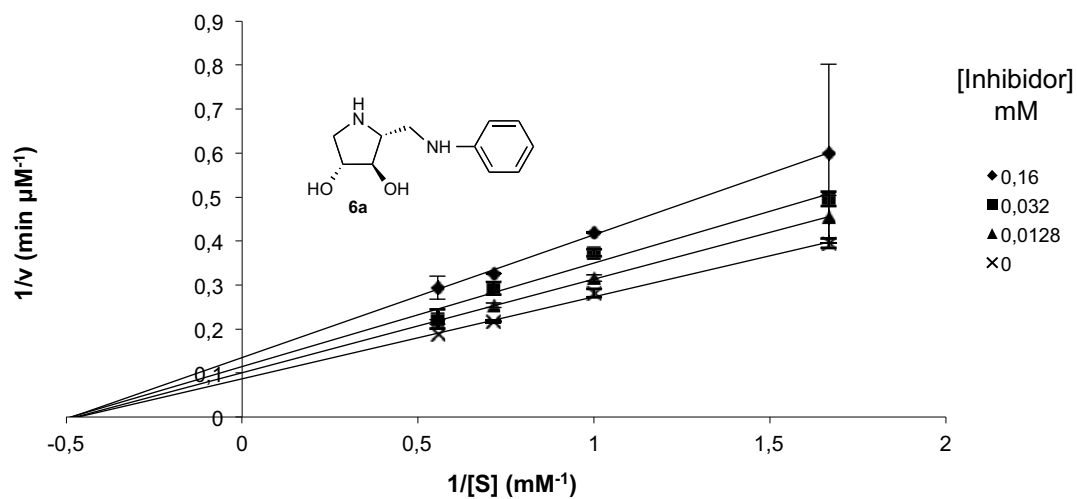


conjugados con aminoácidos y derivados 2-oxopiperacina



d) β-D-galactosidasa de hígado bovino

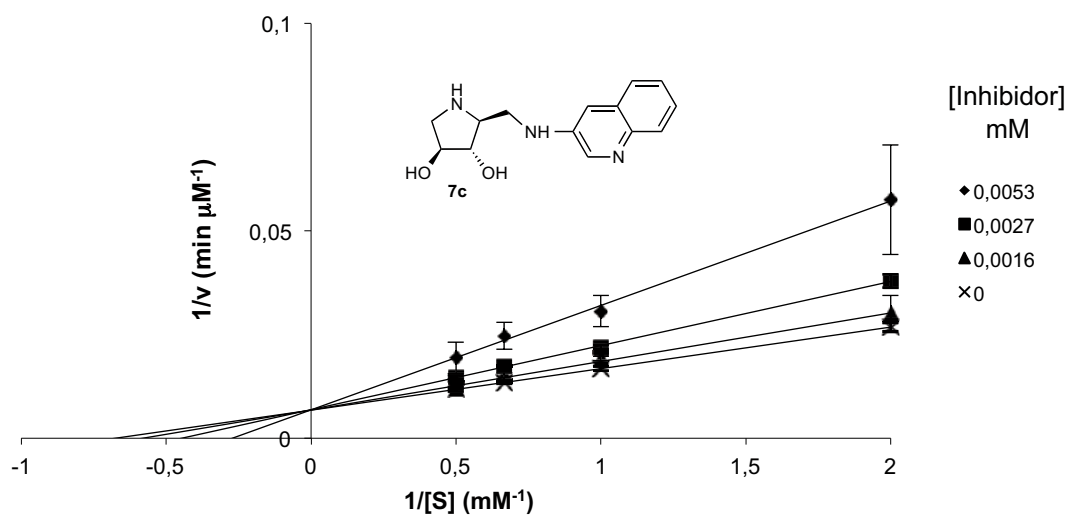
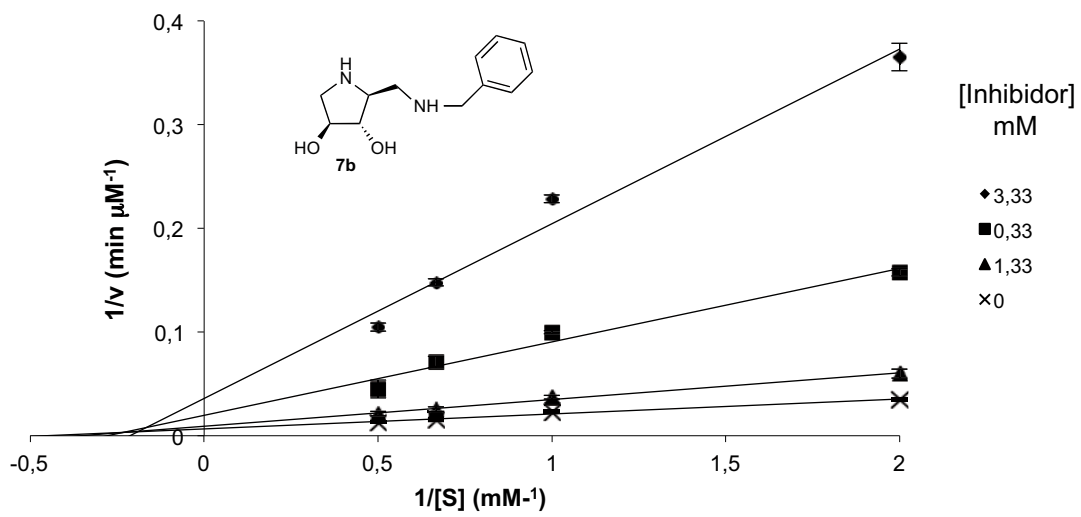
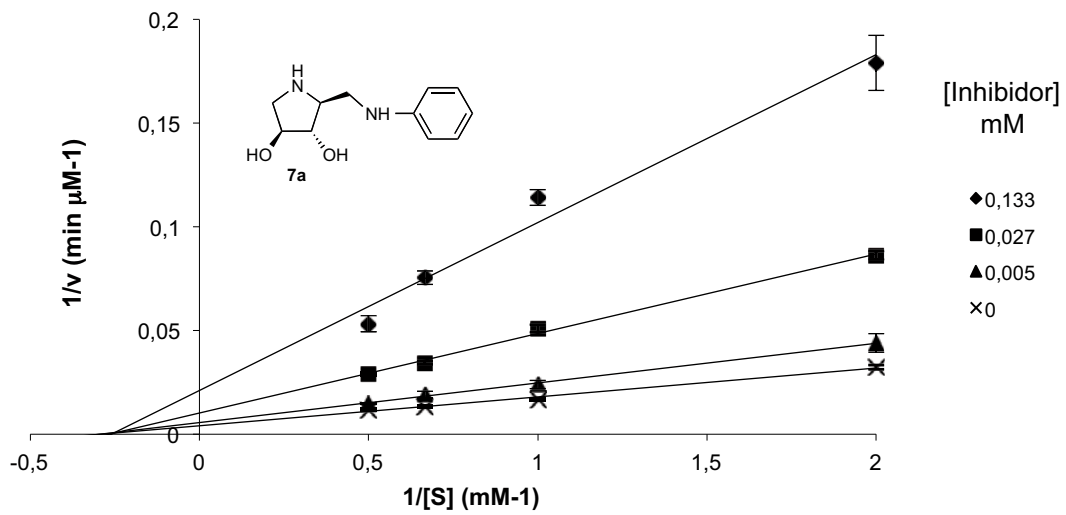
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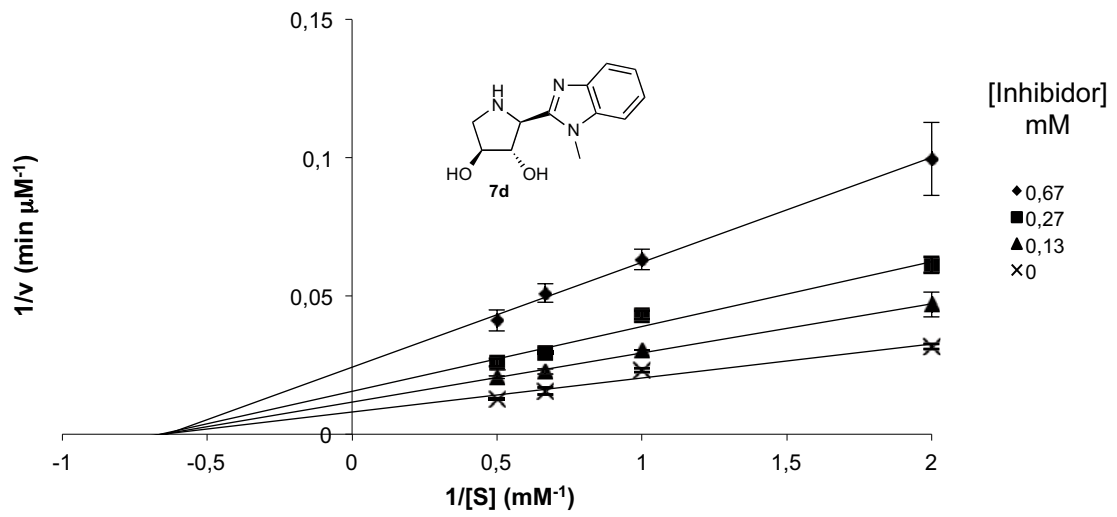


e)  $\alpha$ -L-rhamnosidasa de *Penicillium decumbens*

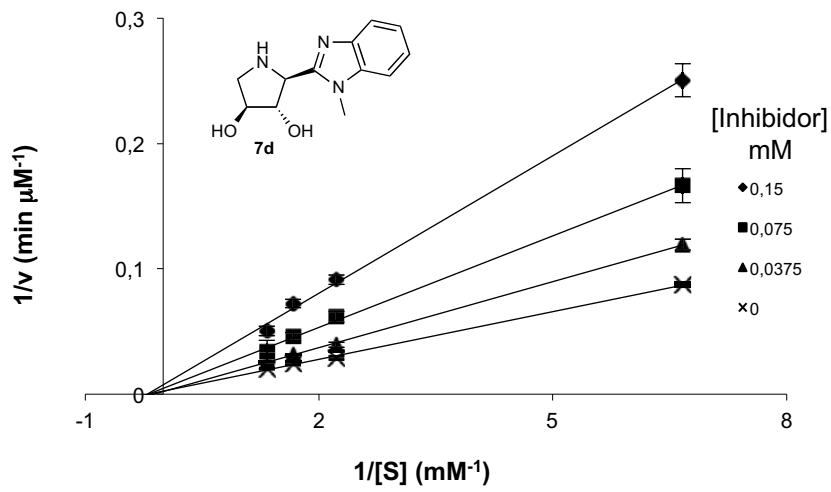
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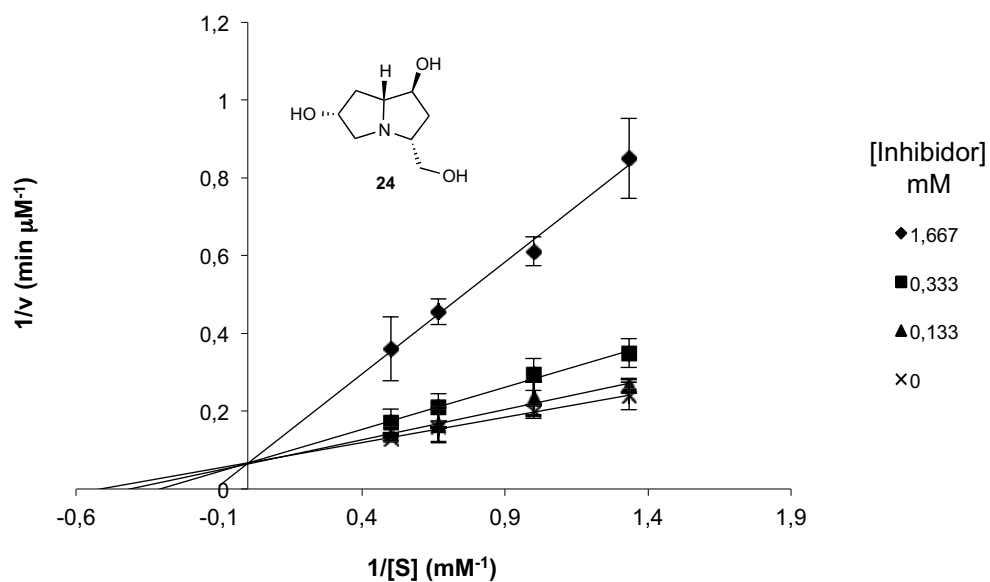
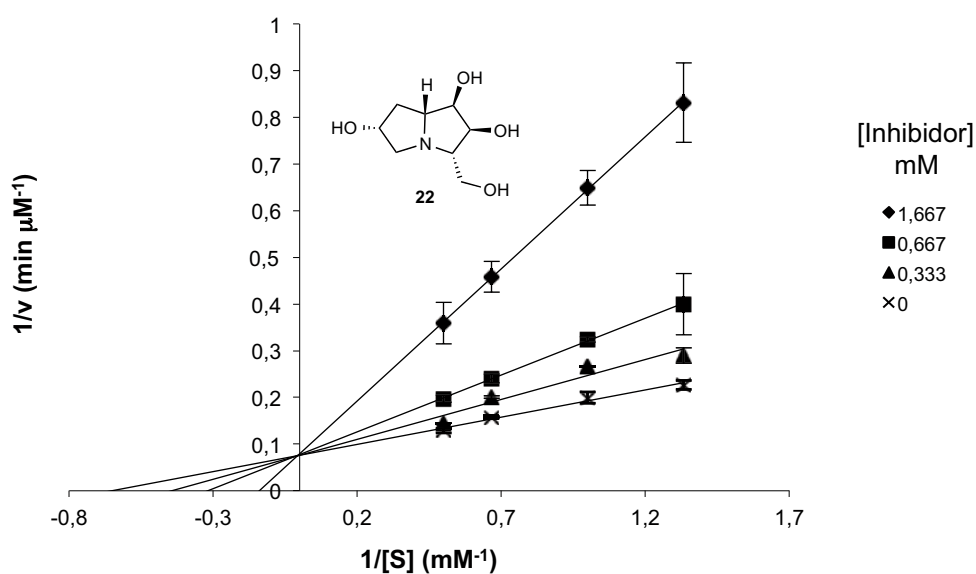


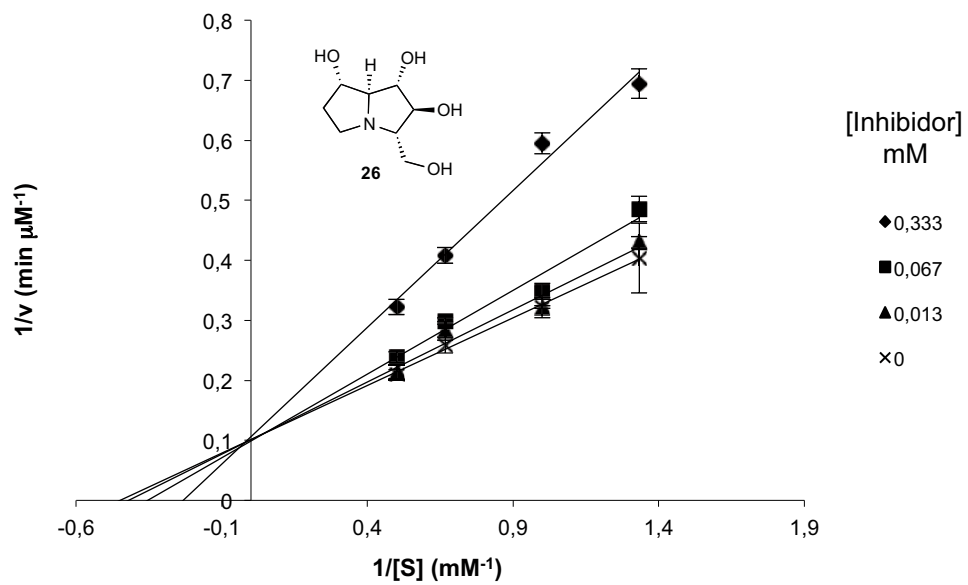
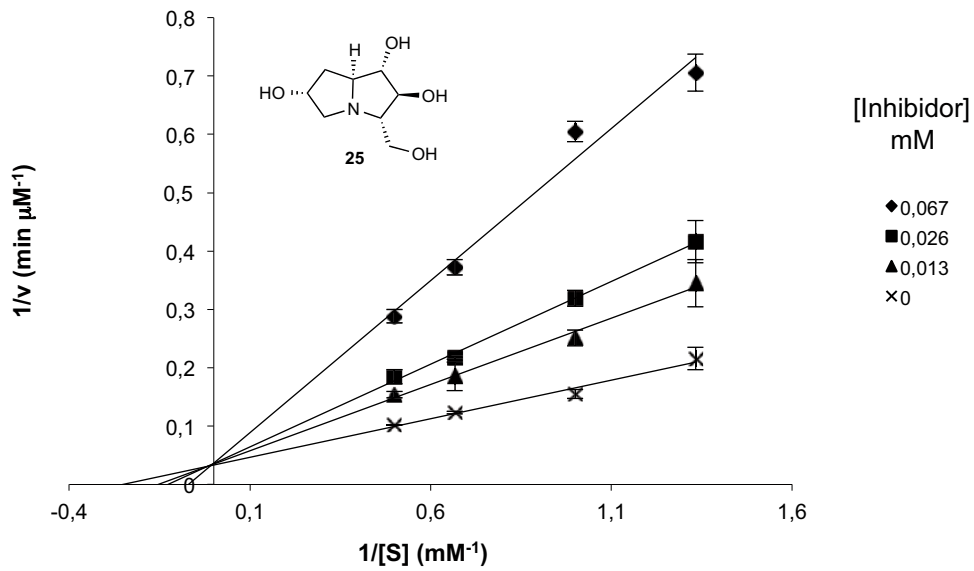
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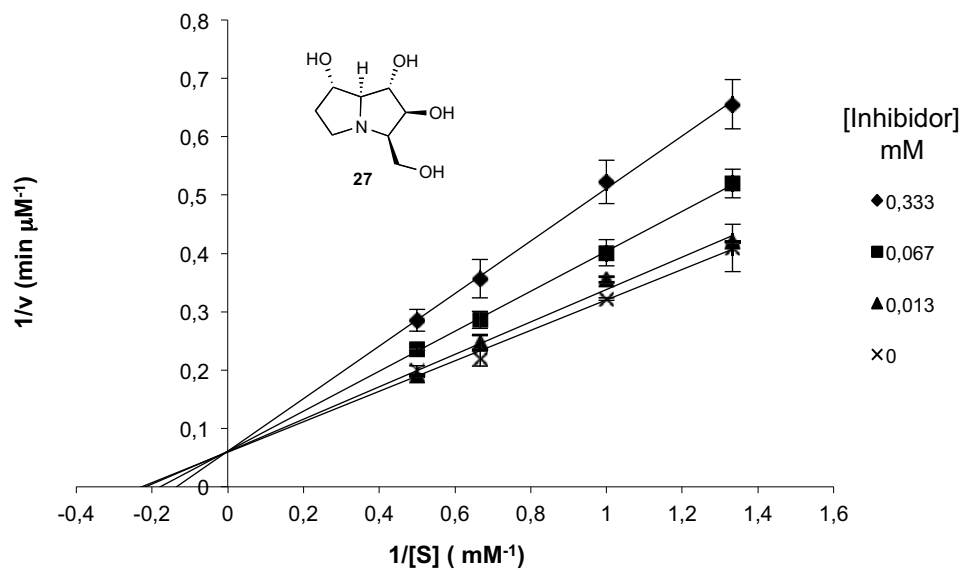


3.1.3 Diagramas dobles recíprocos de Lineweaver-Burk de las pirrolizidinas frente a las glicosidasas comerciales estudiadas: a)  $\alpha$ -D-glucosidasa de arroz, b)  $\beta$ -D-glucosidasa de almendra dulce, c)  $\beta$ -D-galactosidasa de hígado bovino, d)  $\alpha$ -L-rhamnosidasa de *Penicillium decumbens*, e)  $\alpha$ -D-manosidasa de judía y f)  $\alpha$ -L-fucosidasa de riñón bovino

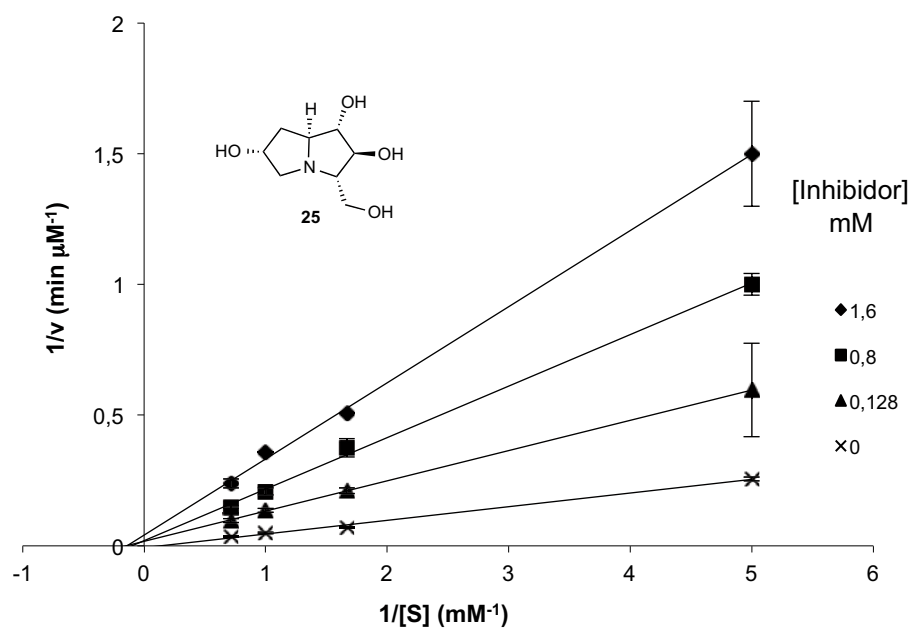
a)  $\alpha$ -D-Glucosidasa de arroz



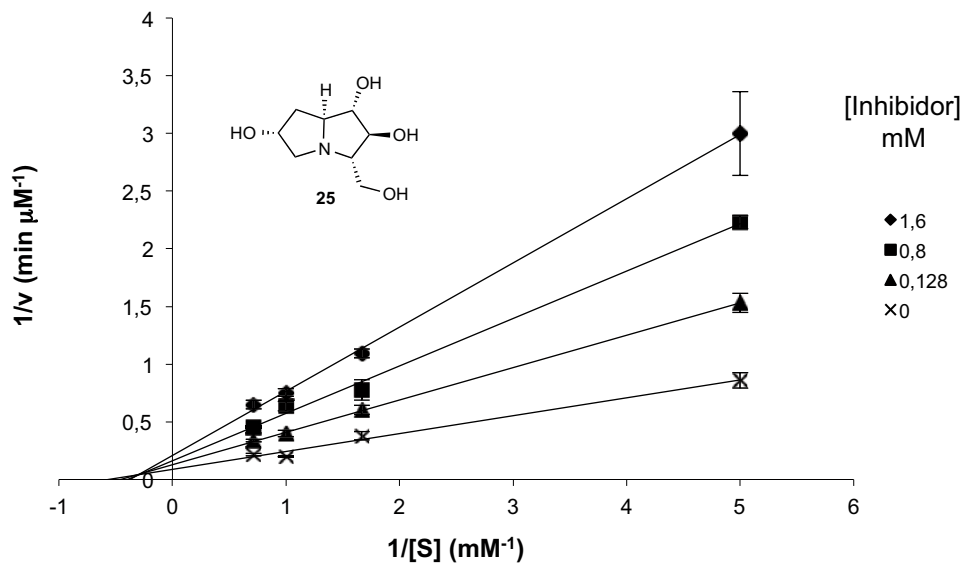




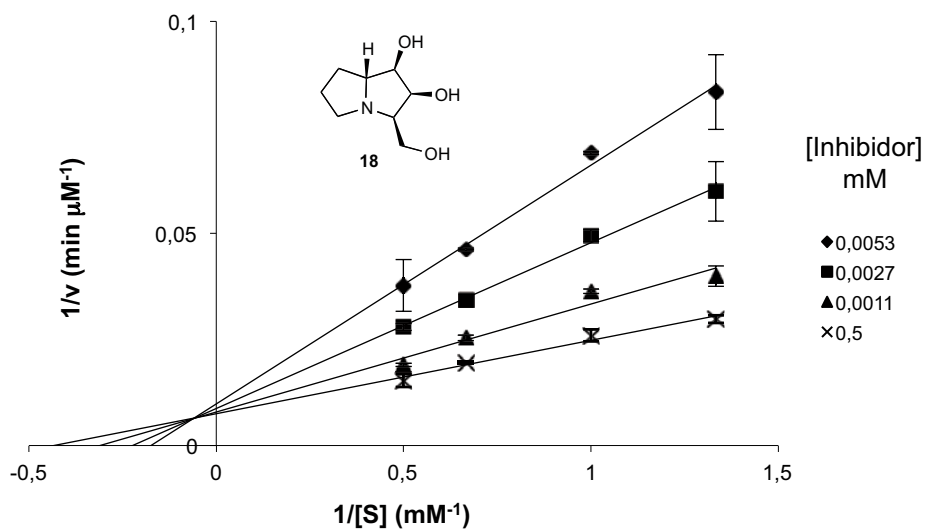
b)  $\beta$ -D-glucosidasa de almendra dulce

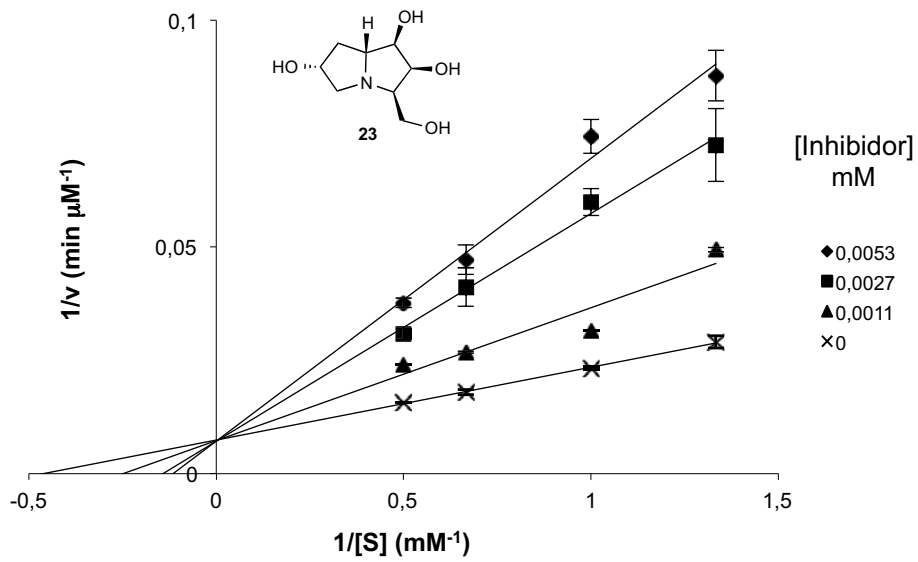
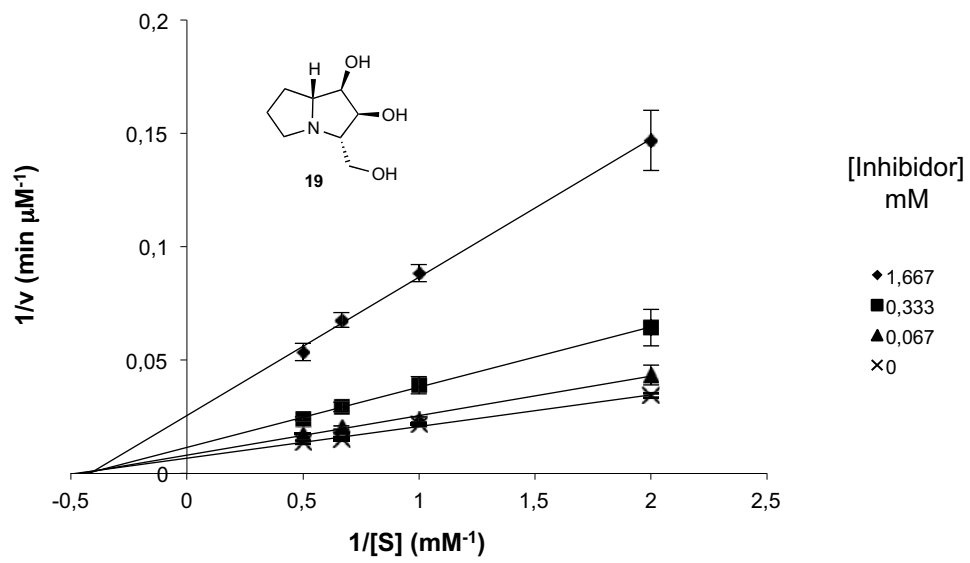


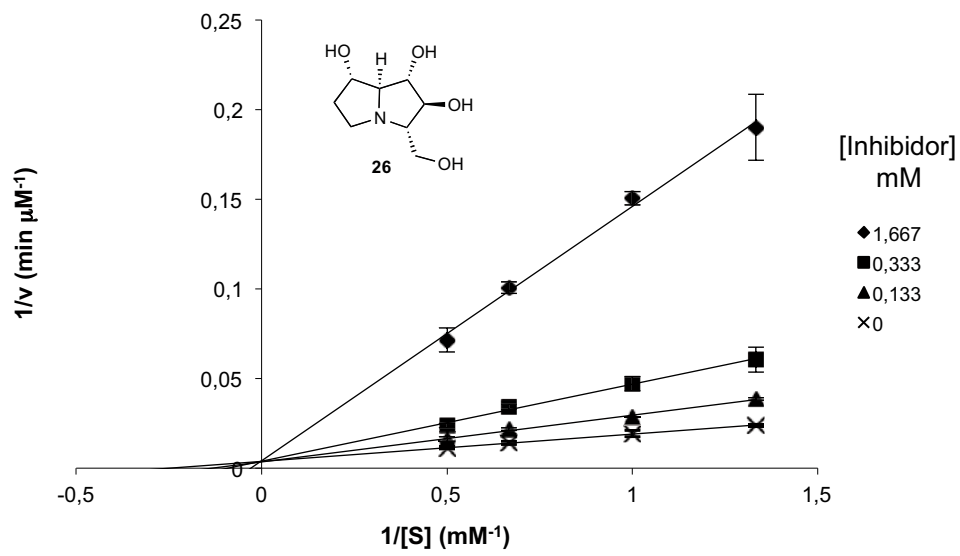
c)  $\beta$ -D-galactosidasa de hígado bovino



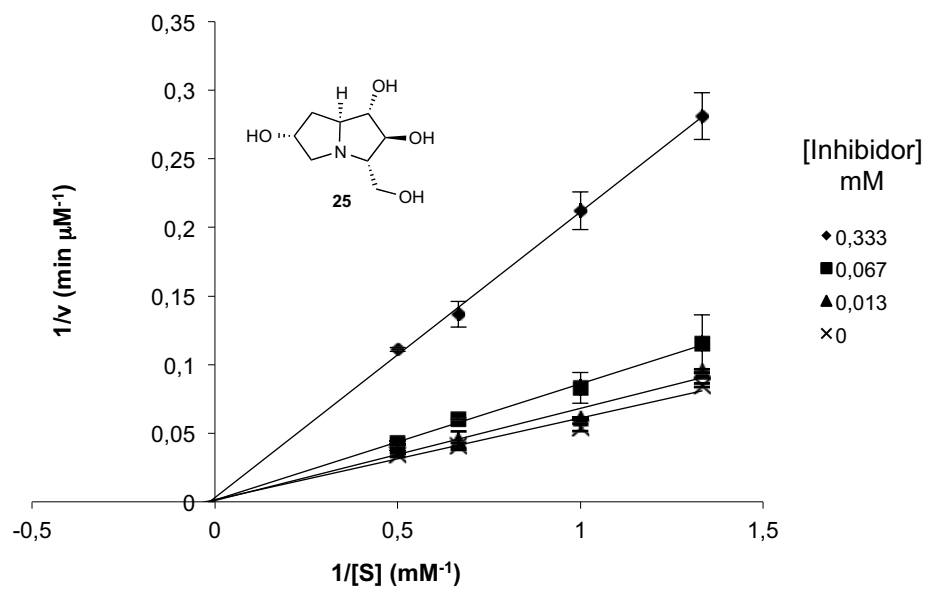
d)  $\alpha$ -L-rhamnosidasa de *Penicillium decumbens*



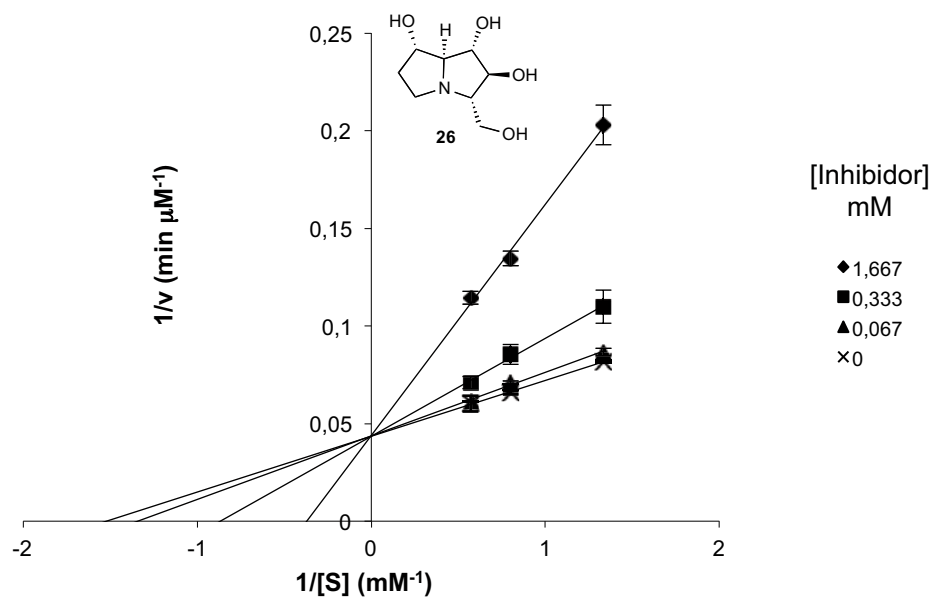




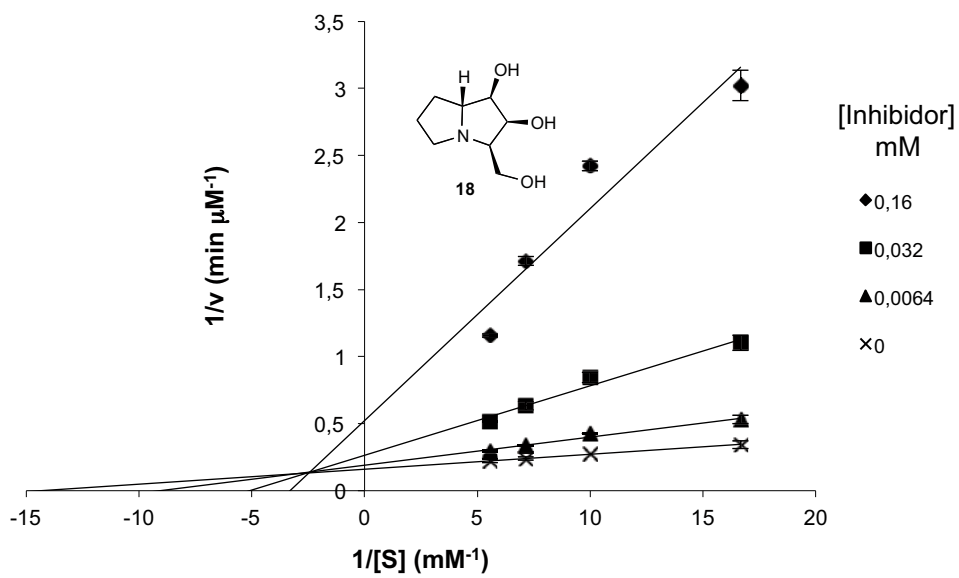
e)  $\alpha$ -D-manosidasa de judía

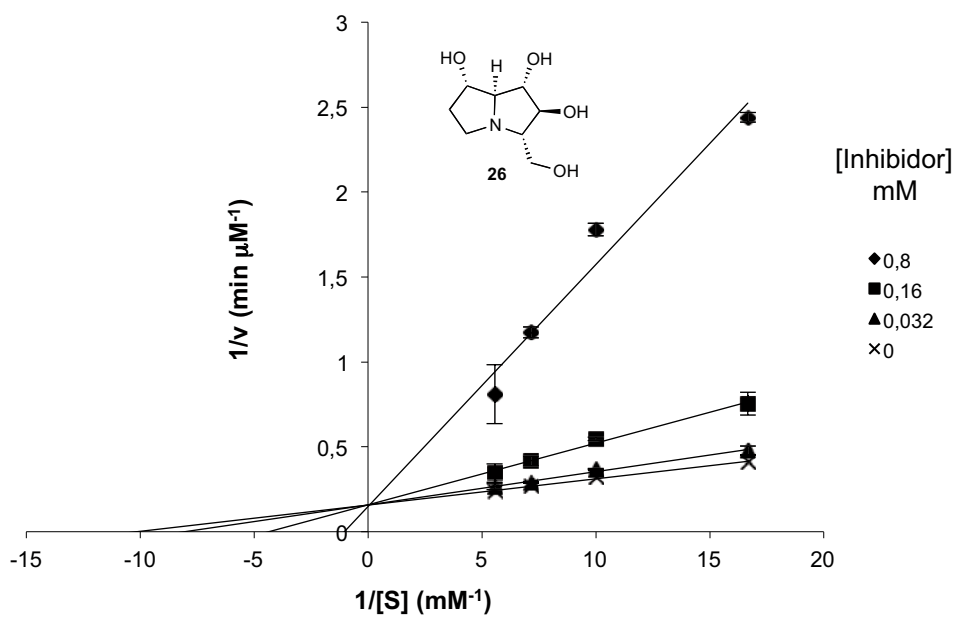
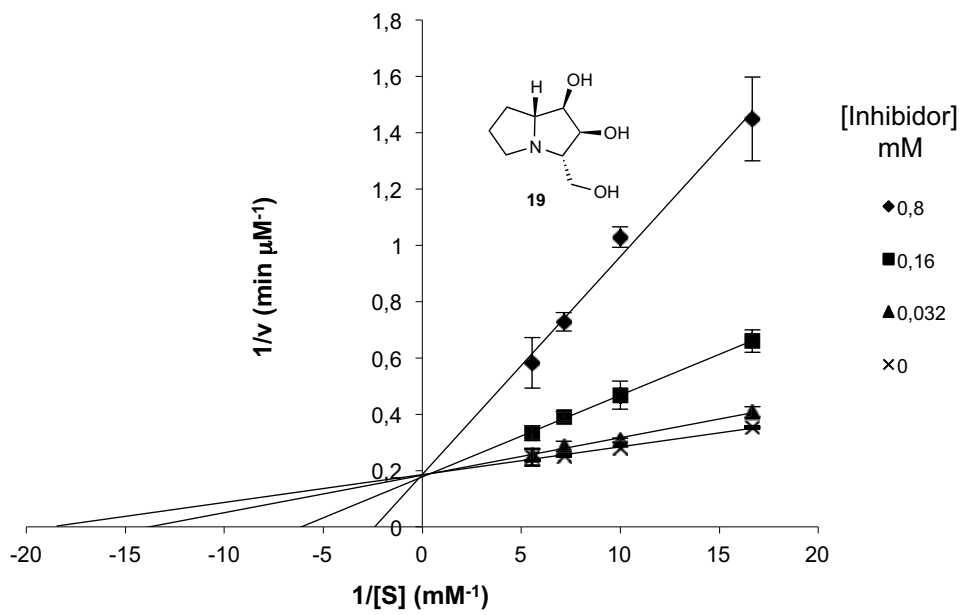






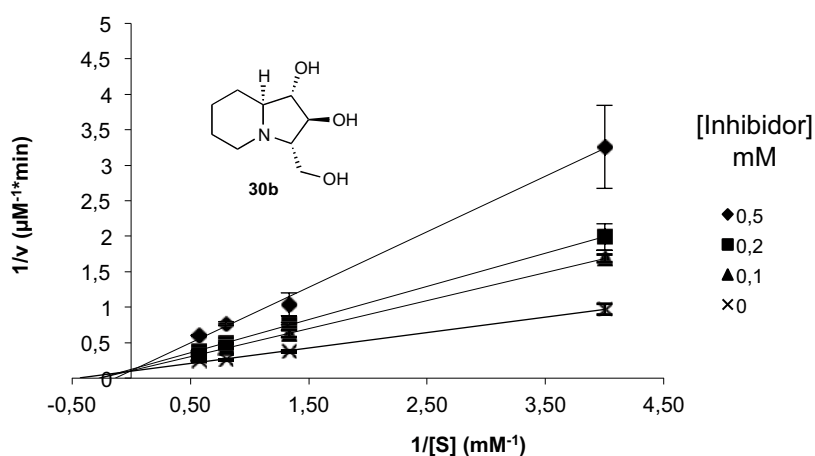
f)  $\alpha$ -L-fucosidasa de riñón bovino



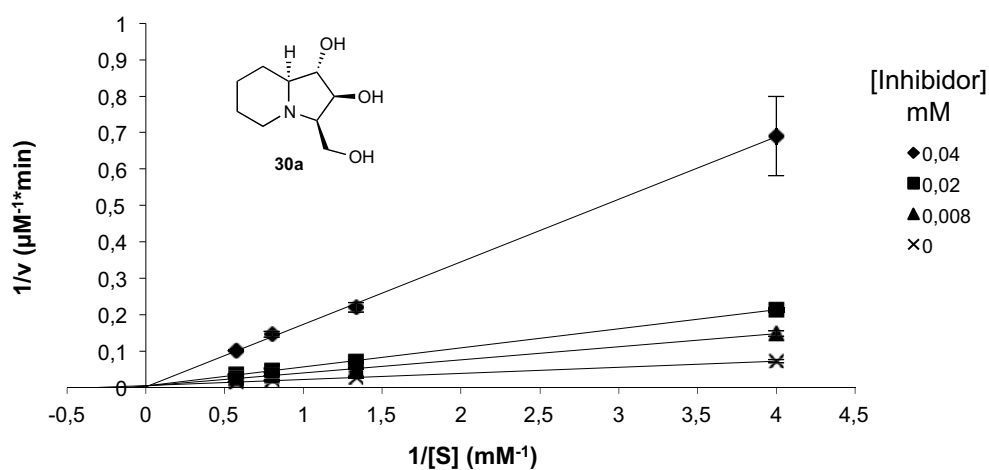


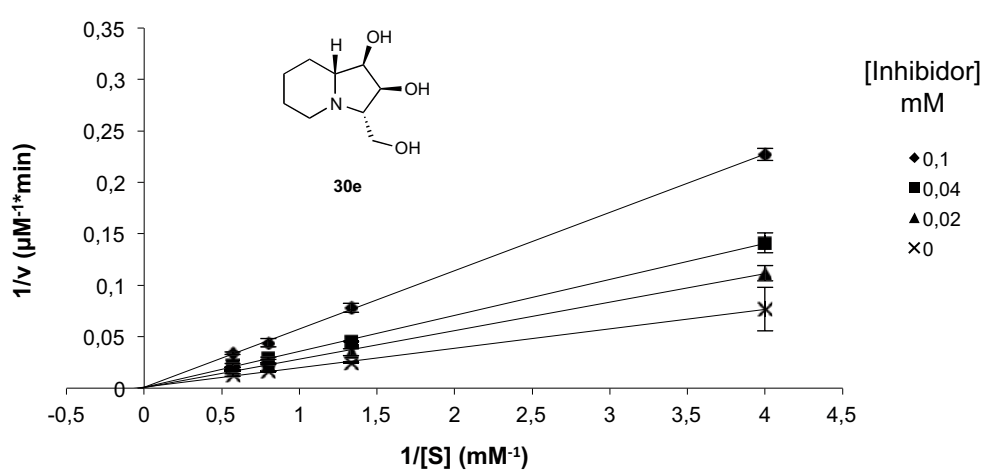
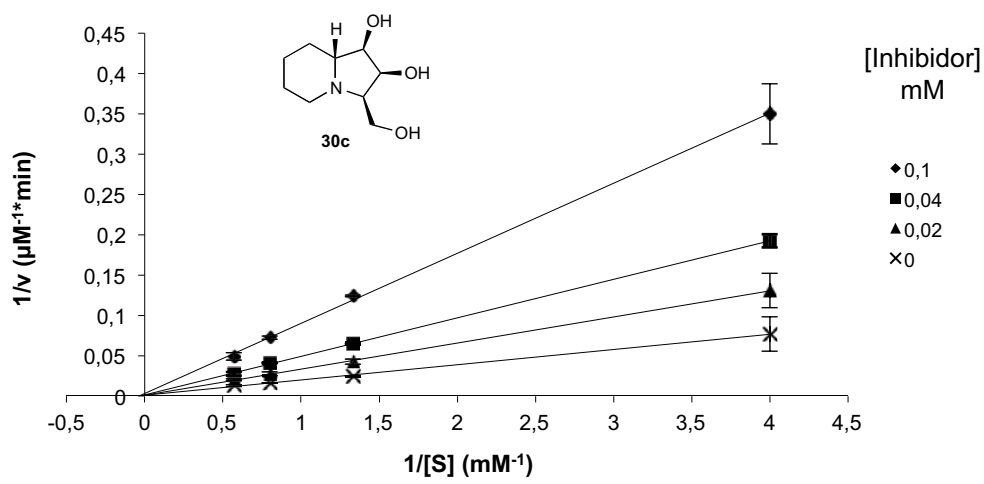
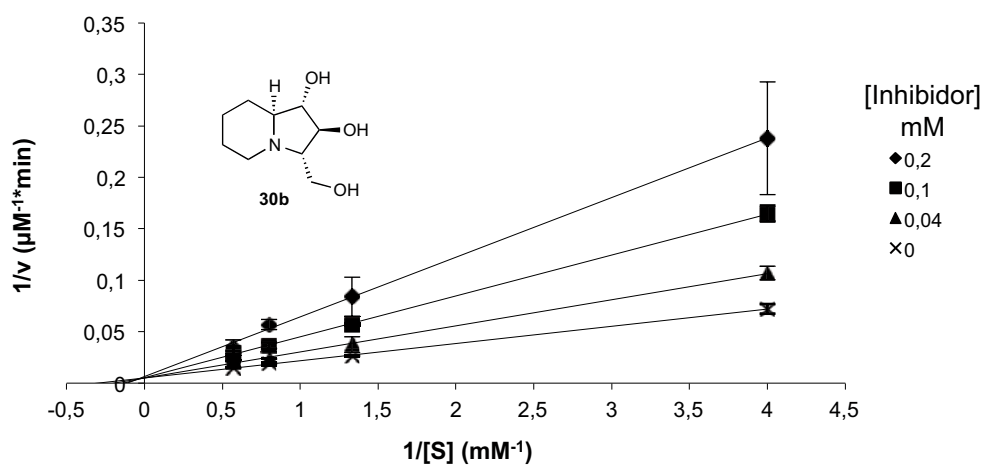
3.1.4 Diagramas dobles recíprocos de Lineweaver-Burk de las indolizidinas y quinolizidians frente a las glicosidasas comerciales estudiadas: a)  $\alpha$ -D-glucosidasa de arroz, b)  $\alpha$ -L-rhamnosidasa de *Penicillium decumbens*, c)  $\alpha$ -D-manosidasa de judía, y d)  $\alpha$ -L-fucosidasa de riñón bovino

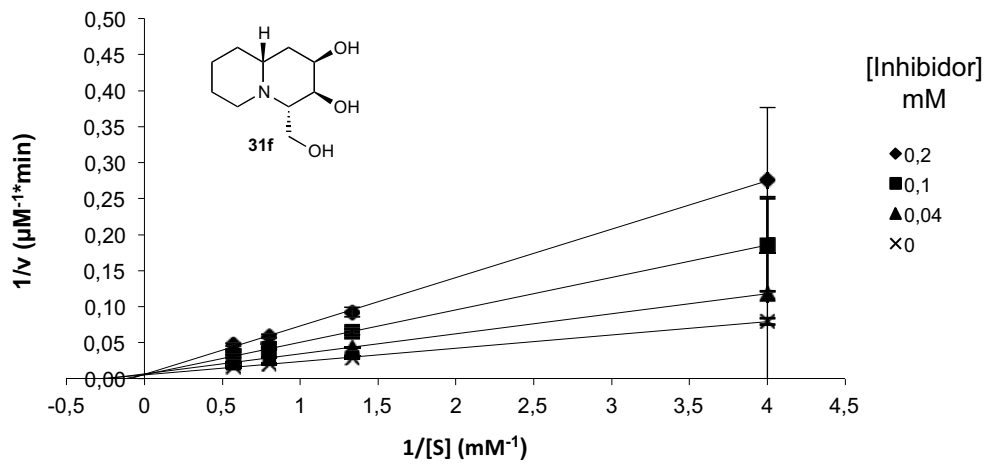
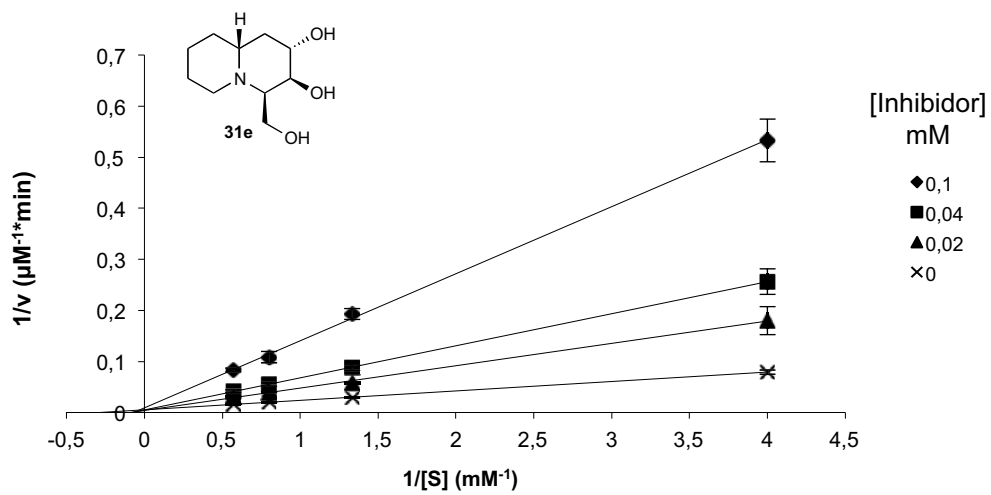
a)  $\alpha$ -D-glucosidasa de arroz



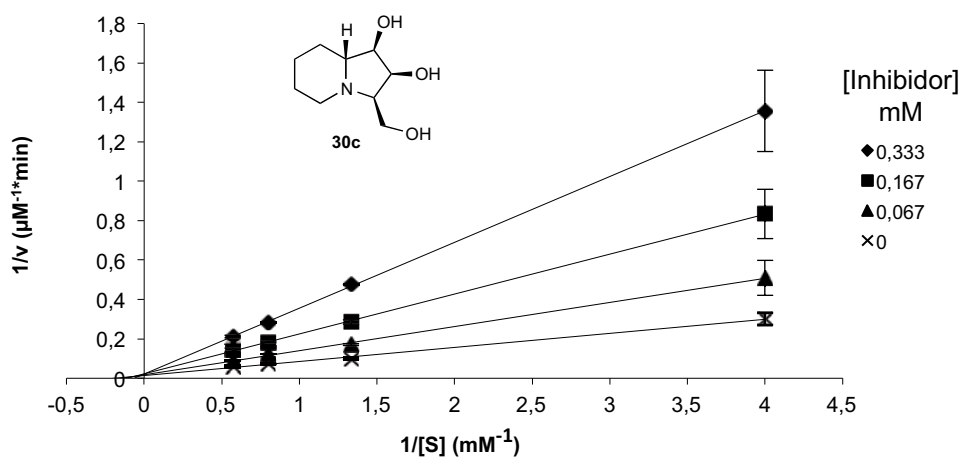
b)  $\alpha$ -L-rhamnosidasa de *Penicillium decumbens*



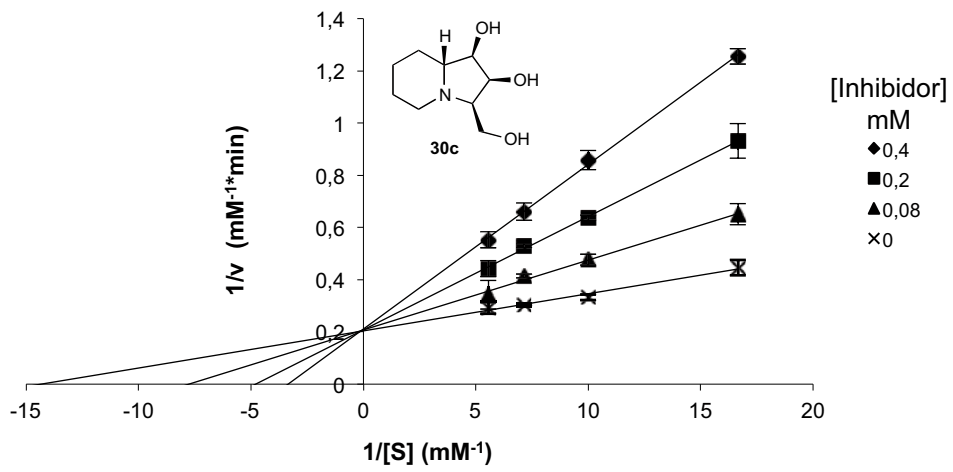




c)  $\alpha$ -D-manosidasa de judía

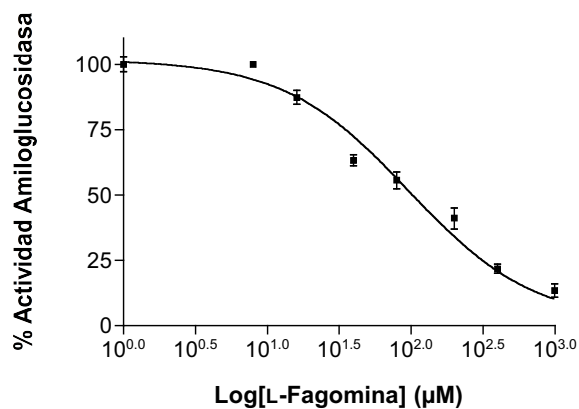
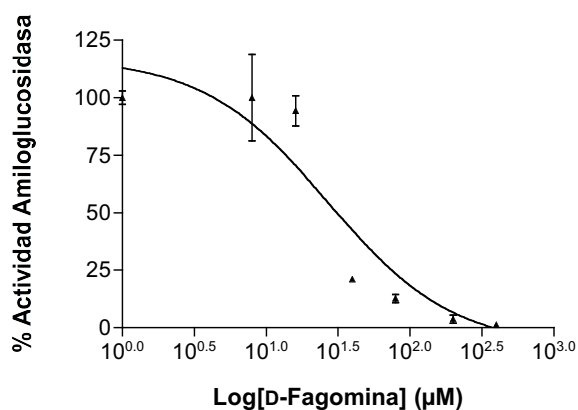
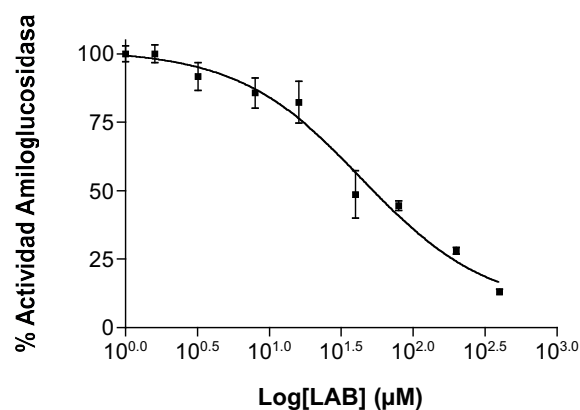
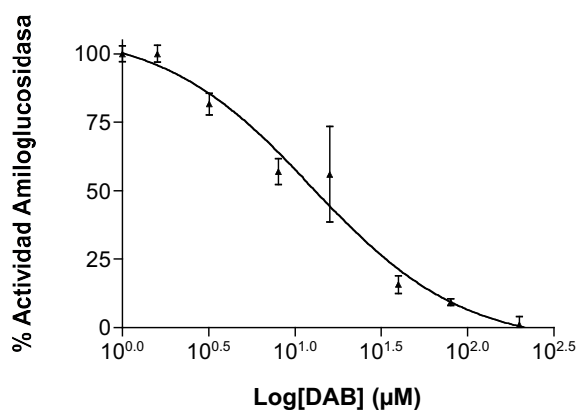


$\alpha$ -L-fucosidasa de riñón bovino

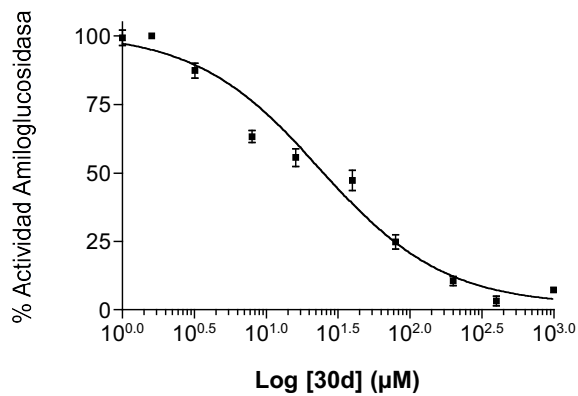
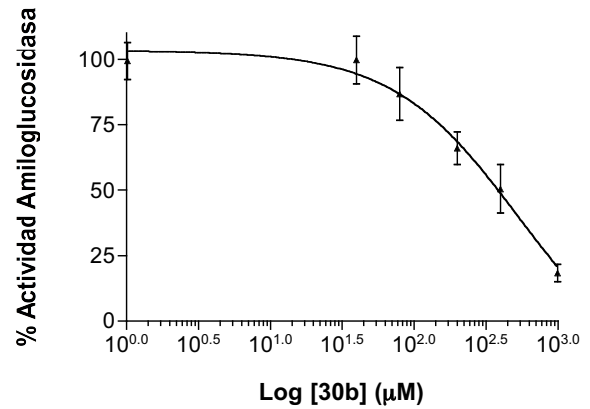
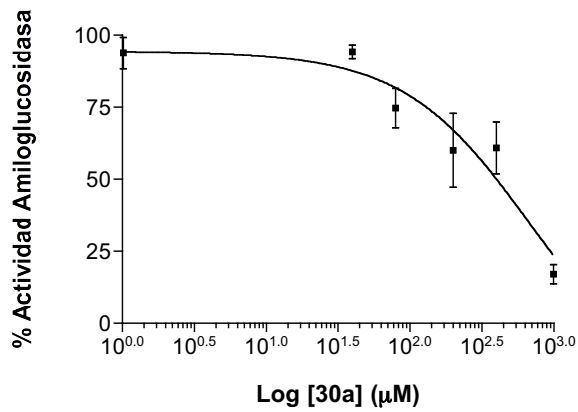


### 3.2.1 Determinación de valores de IC<sub>50</sub> de los compuestos de referencia DAB, LAB, D-fagomina y L-fagomina frente a la enzima amiloglucosidasas de *Aspergillus niger*.

Curvas dosis-respuesta. Representaciones gráficas del % de actividad de hidrólisis amiloglucosidasa en función del logaritmo de la concentración de inhibidor. Para la determinación de los valores de IC<sub>50</sub>, se aplicó un modelo de de aproximación no lineal utilizando el programa Graph Pad Prism 4.

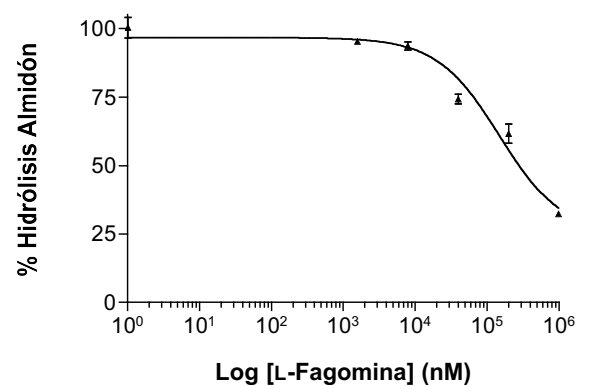
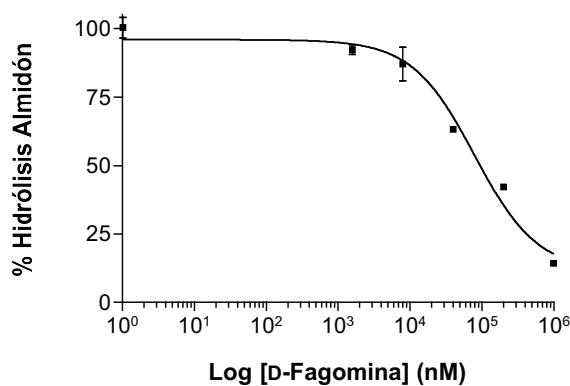
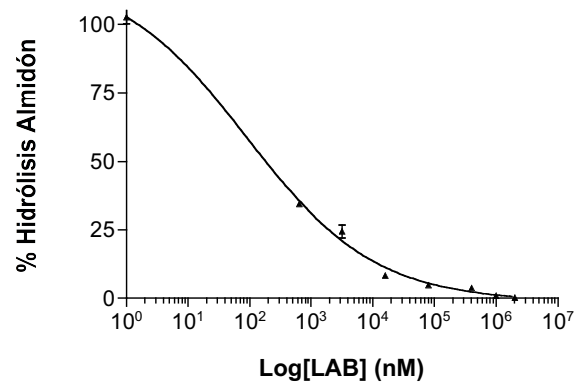
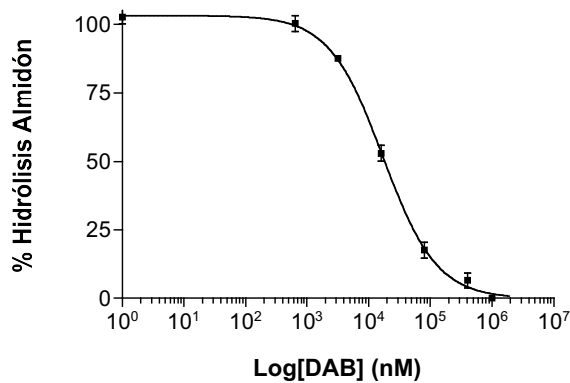


3.2.4 Determinación de valores de  $IC_{50}$  de las indolizidians frente a la enzima amiloglucosidasa de *Aspergillus niger*. Curvas dosis-respuesta. Representaciones gráficas del % de actividad amiloglucosidasa en función del logaritmo de la concentración de inhibidor. Para la determinación de los valores de  $IC_{50}$ , se aplicó un modelo de de aproximación no lineal utilizando el programa Graph Pad Prism 4.

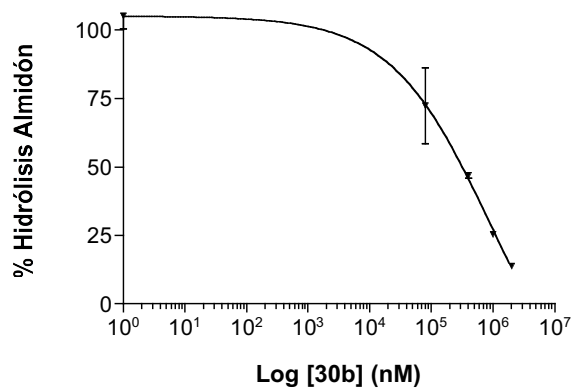




3.2.1 Determinación de valores de  $IC_{50}$  de los compuestos de referencia DAB, LAB, D-fagomina y L-fagomina frente a la capacidad de hidrólisis de almidón de homogeneizados de mucosa intestinal de rata. Curvas dosis-respuesta. Representaciones gráficas del % de actividad de hidrólisis de almidón en la mucosa intestinal en función del logaritmo de la concentración de inhibidor. Para la determinación de los valores de  $IC_{50}$ , se aplicó un modelo de de aproximación no lineal utilizando el programa Graph Pad Prism 4.

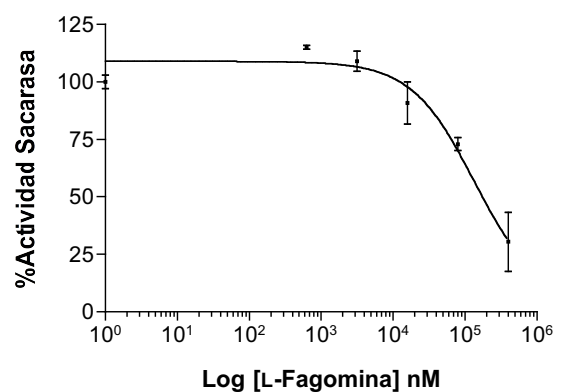
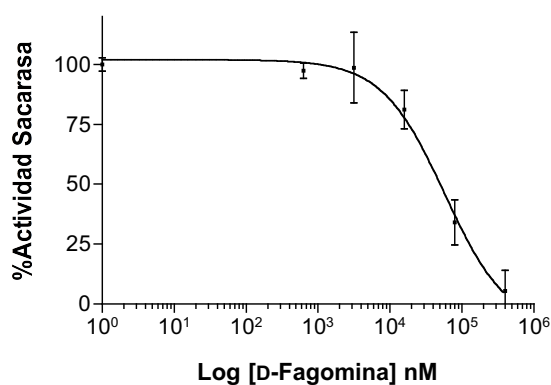
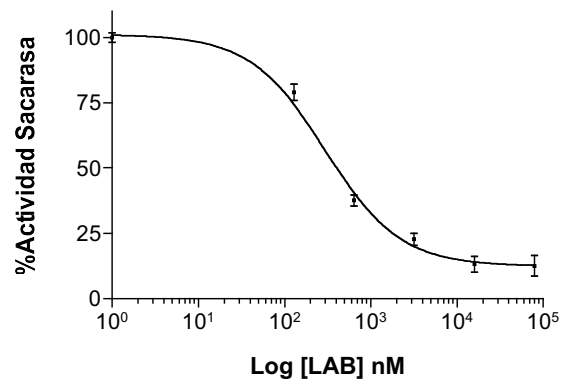
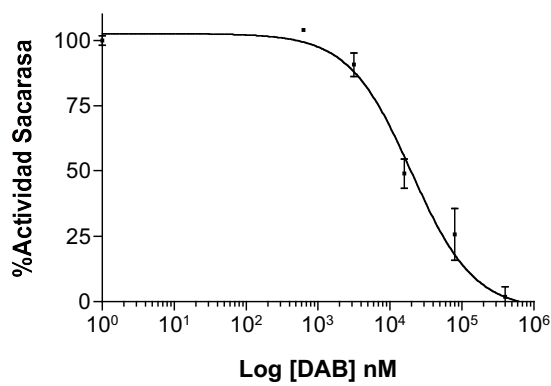


3.2.4 Determinación de valores de  $IC_{50}$  de las indolizidinas frente a la capacidad de hidrólisis de almidón de homogeneizados de mucosa intestinal de rata. Curvas dosis-respuesta. Representaciones gráficas del % de actividad de hidrólisis de almidón en la mucosa intestinal en función del logaritmo de la concentración de inhibidor. Para la determinación de los valores de  $IC_{50}$ , se aplicó un modelo de de aproximación no lineal utilizando el programa Graph Pad Prism 4.

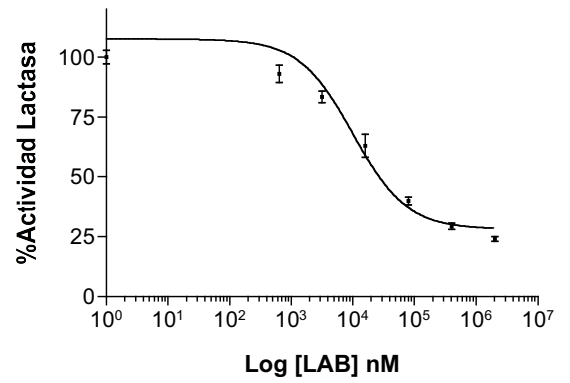
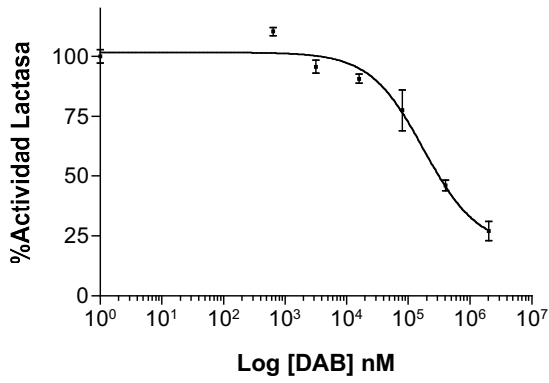


3.2.1 Curvas dosis-respuesta empleadas para la determinación de valores de  $IC_{50}$  de DAB, LAB, D-fagomina y L-fagomina frente a disacaridasas de la mucosa intestinal. Los estudios se realizaron sobre homogeneizados de mucosa intestinal de rata. A continuación se resumen las representaciones gráficas del % de actividad de las disacaridasas intestinales : a) sacarasa, b) lactasa, c) trehalasa, y d) maltasa, en función del logaritmo de la concentración de inhibidor. Para la determinación de los valores de  $IC_{50}$ , se aplicó un modelo de de aproximación no lineal utilizando el programa Graph Pad Prism 4.

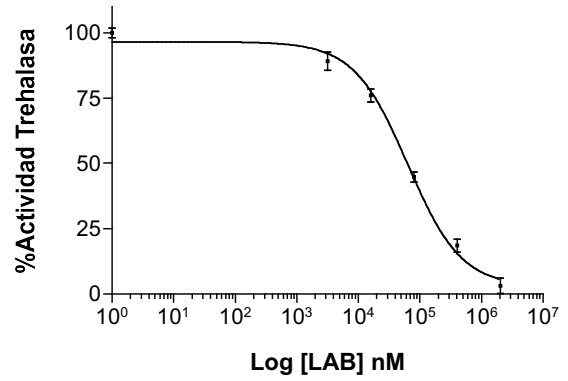
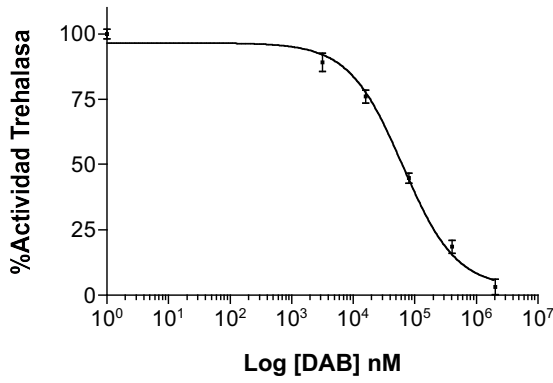
a)



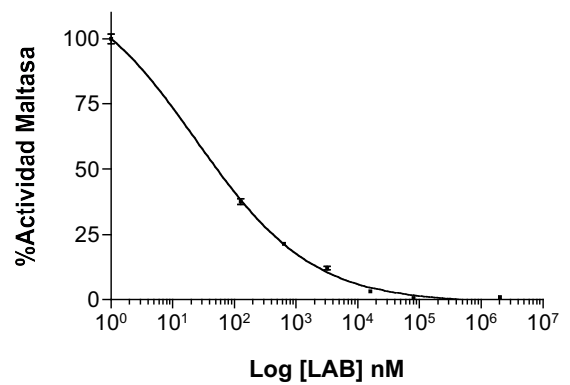
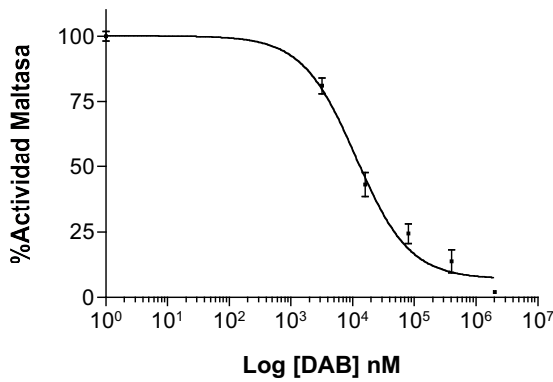
b)

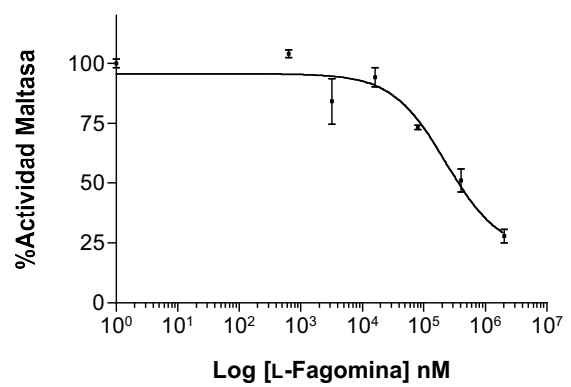


c)



d)

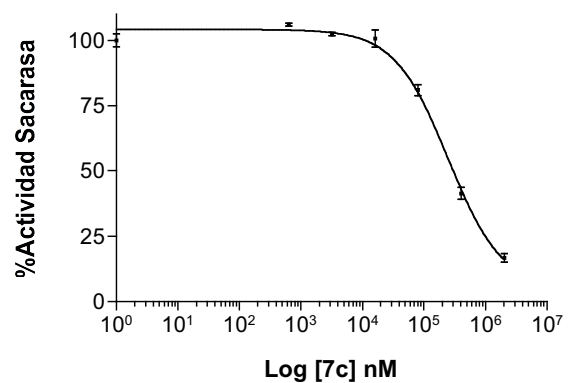
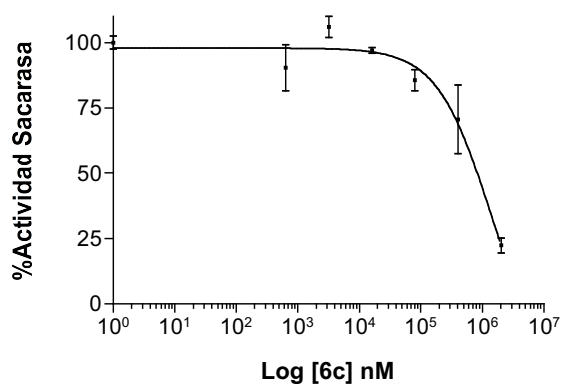
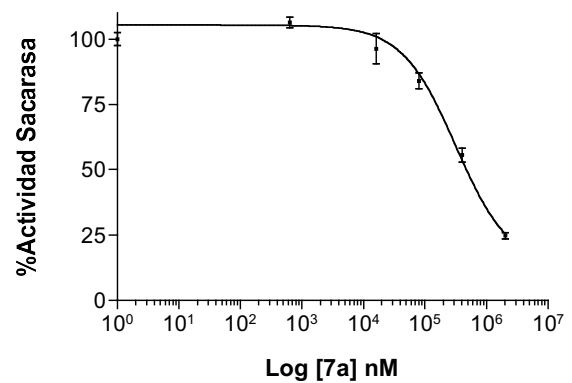
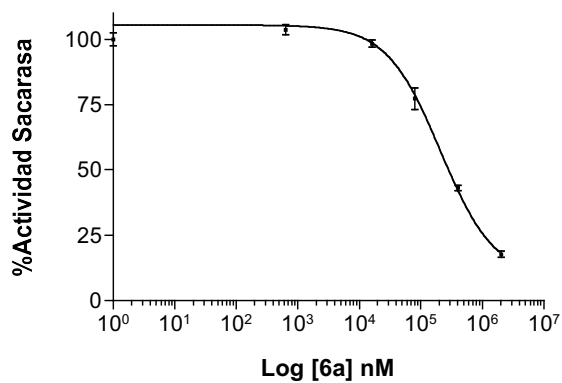


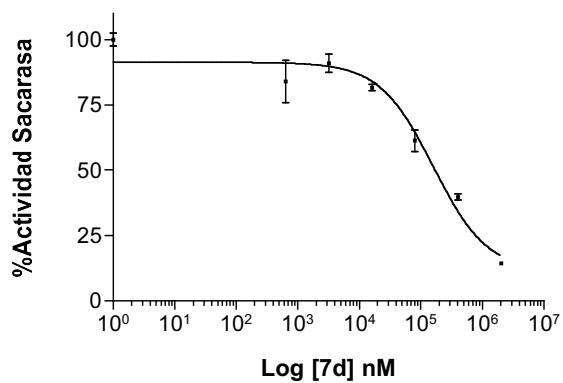


3.2.2 Curvas dosis-respuesta empleadas para la determinación de valores de  $IC_{50}$  de los derivados de DAB y LAB frente a disacaridasas de la mucosa intestinal. Los estudios se realizaron sobre homogeneizados de mucosa intestinal de rata. A continuación se resumen las representaciones gráficas del % de actividad de las disacaridasas intestinales : a) sacarasa, b) maltasa, en función del logaritmo de la concentración de inhibidor. Para la determinación de los valores de  $IC_{50}$ , se aplicó un modelo de de aproximación no lineal utilizando el programa Graph Pad Prism 4.

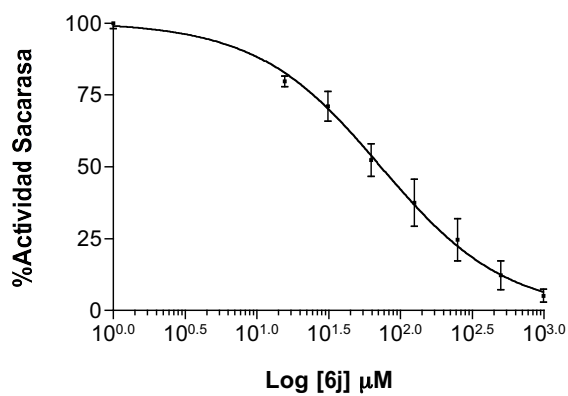
a) Sacarasa

aminas aromáticas

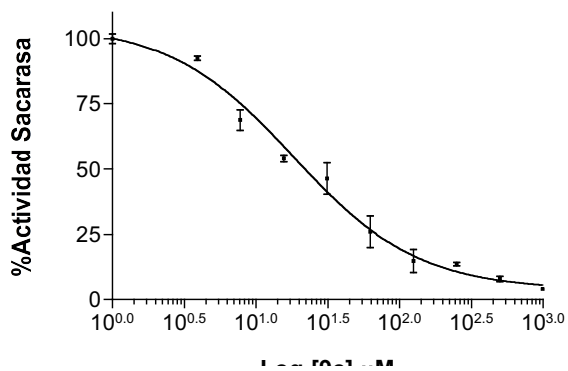
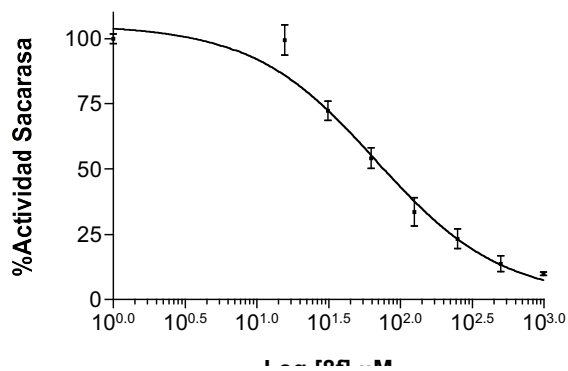
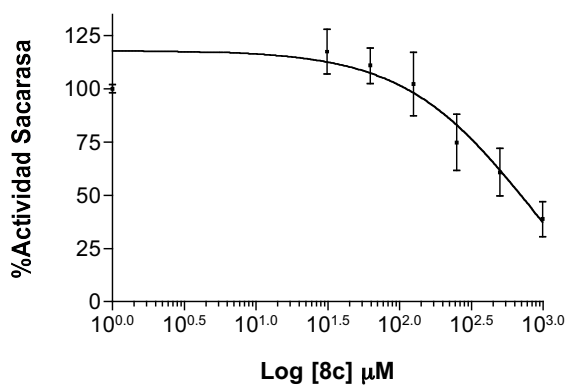
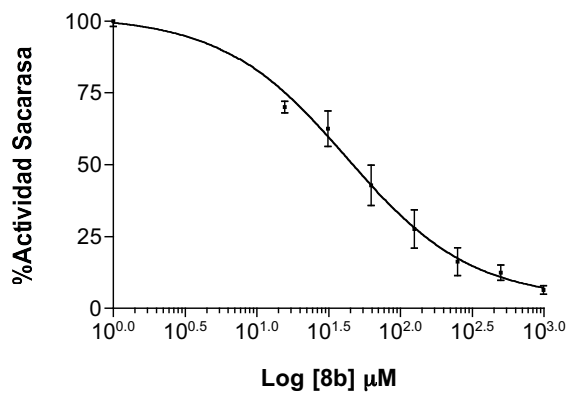


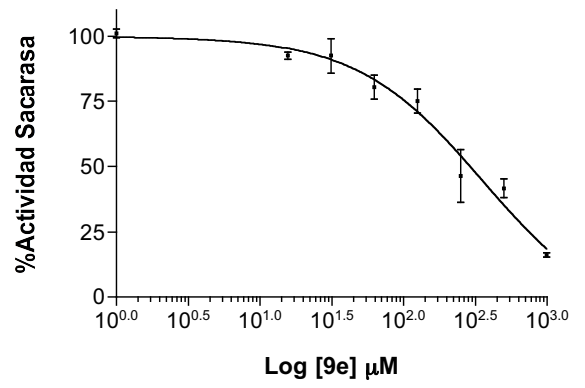
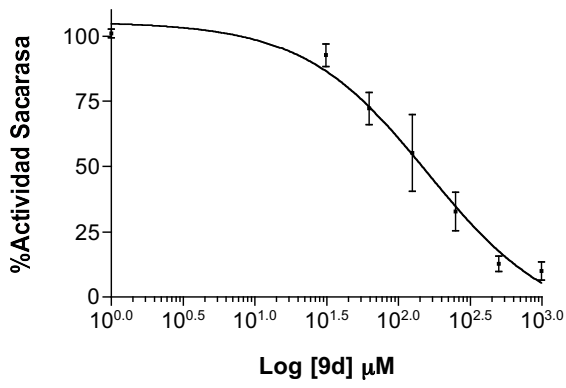


aminoalcoholes

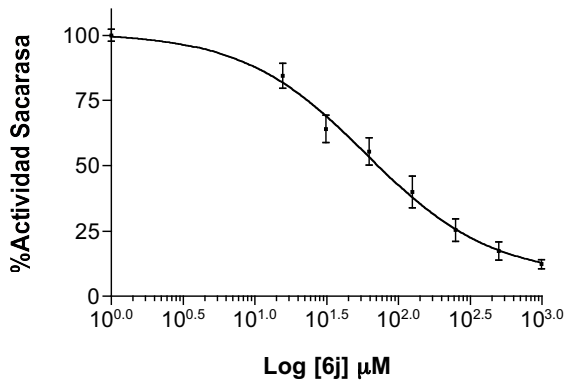


c) 2-oxopiperazina

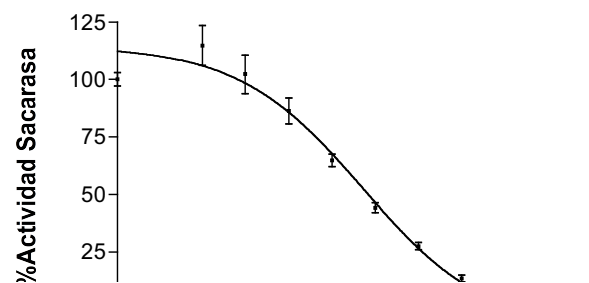
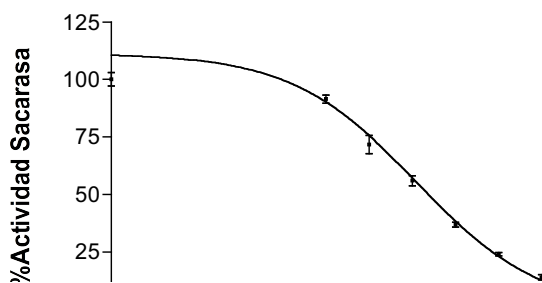
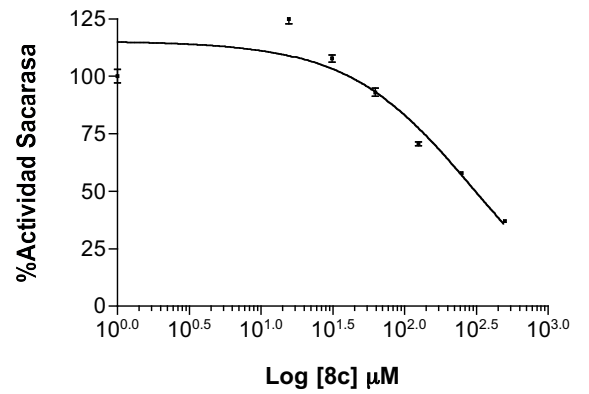
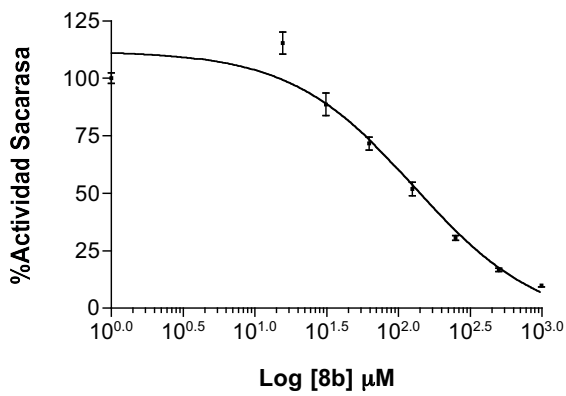




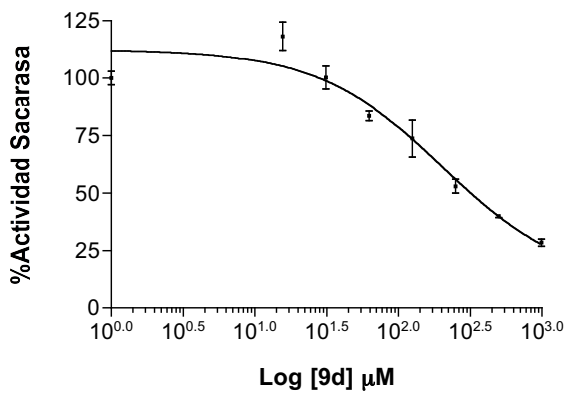
b) Maltasa  
aminoalcoholes



2-oxopiperazina

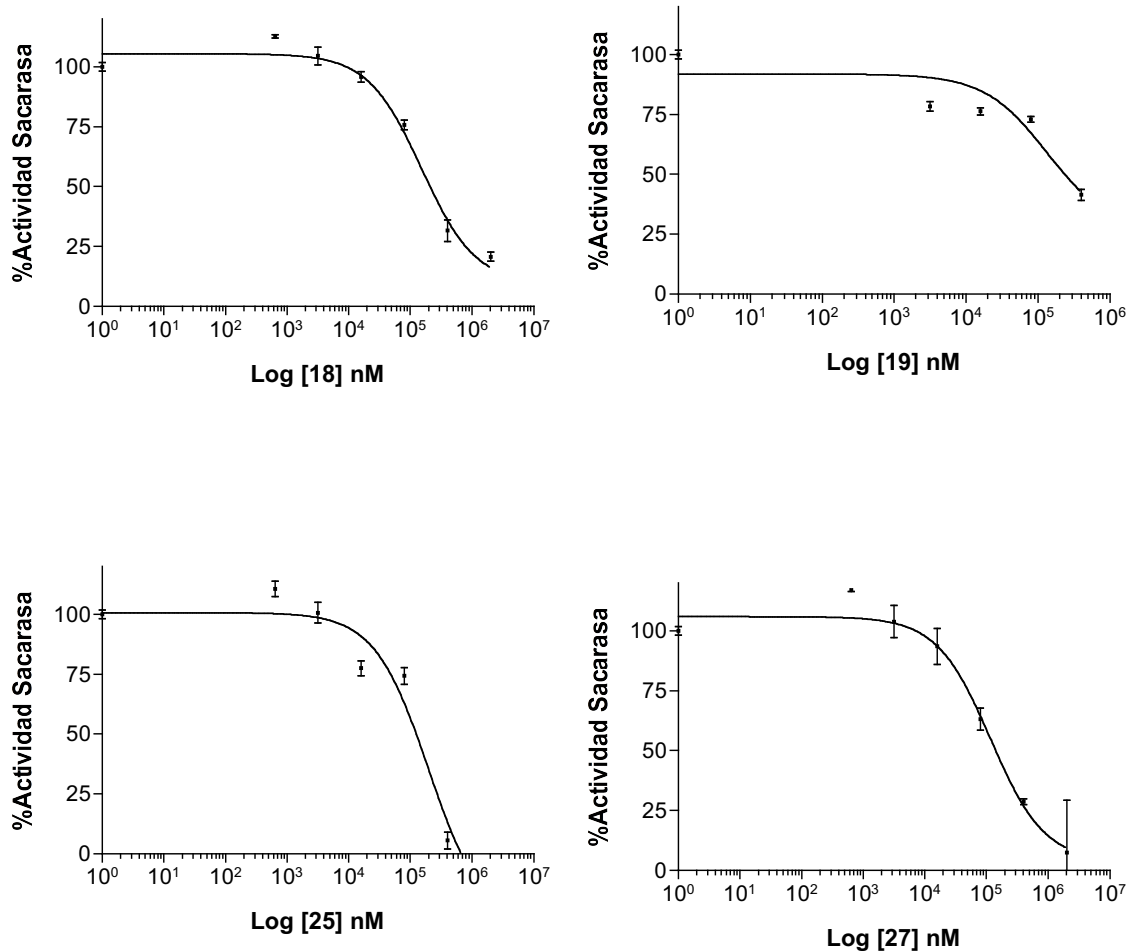




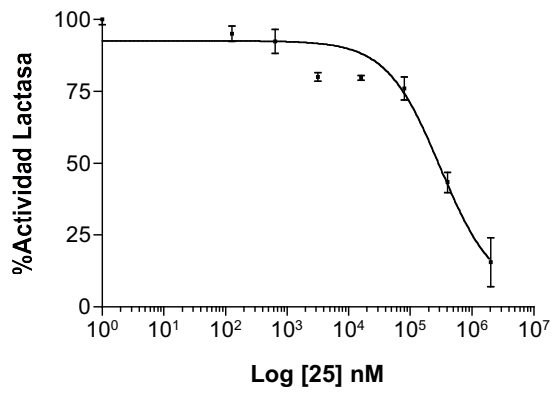


3.2.3 Curvas dosis-respuesta empleadas para la determinación de valores de  $IC_{50}$  de las pirrolizidinas frente a disacaridasas de la mucosa intestinal. Los estudios se realizaron sobre homogeneizados de mucosa intestinal de rata. A continuación se resumen las representaciones gráficas del % de actividad de las disacaridasas intestinales : a) sacarasa, b) lactasa, c) trehalasa, y d) maltasa, en función del logaritmo de la concentración de inhibidor. Para la determinación de los valores de  $IC_{50}$ , se aplicó un modelo de de aproximación no lineal utilizando el programa Graph Pad Prism 4.

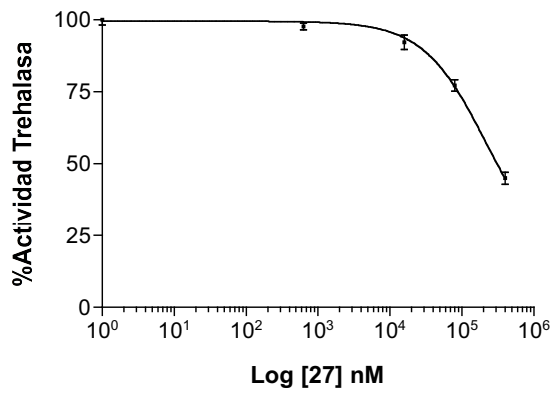
a) Sacarasa



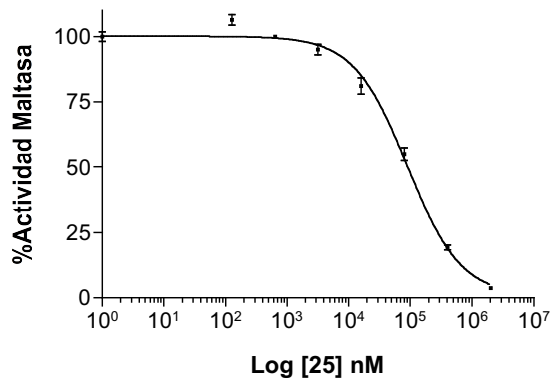
b) Lactasa



c) Trehalasa

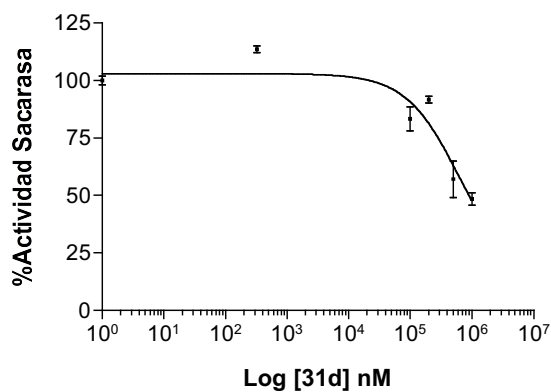
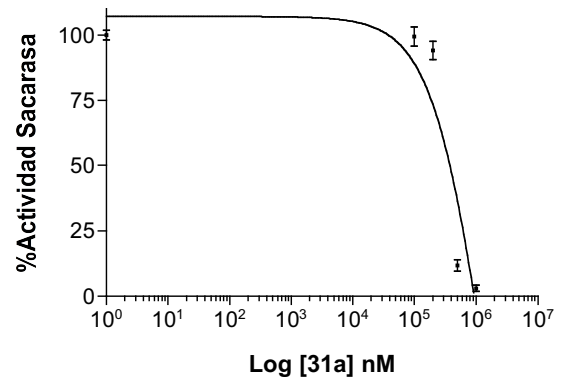
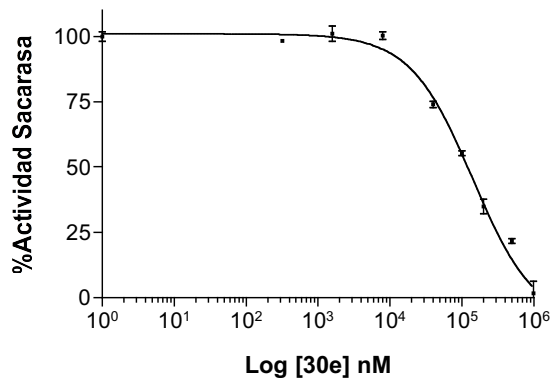
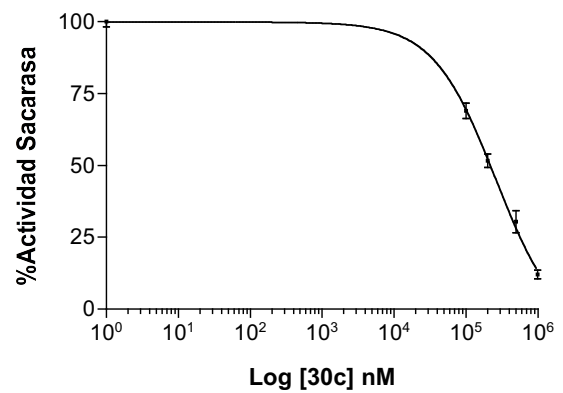
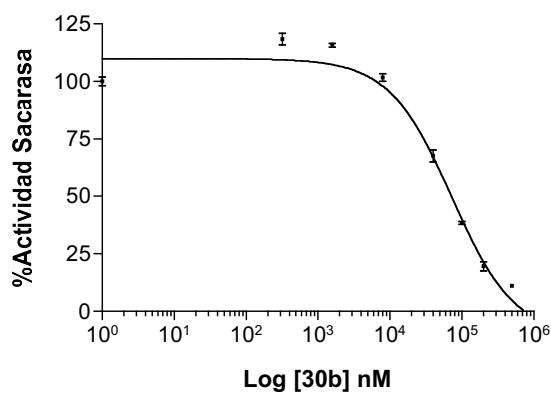


d) Maltasa

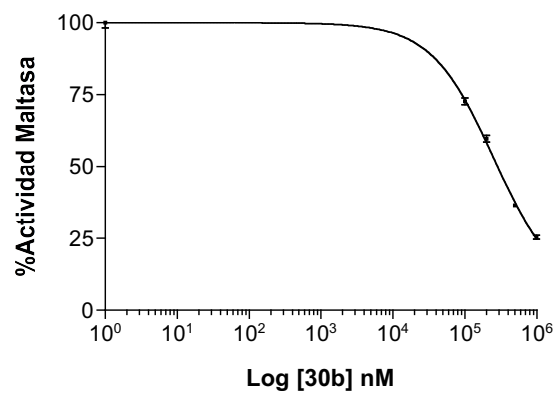


3.2.4 Curvas dosis-respuesta empleadas para la determinación de valores de  $IC_{50}$  de las indolizidinas y quinolizidinas frente a disacaridasas de la mucosa intestinal. Los estudios se realizaron sobre homogeneizados de mucosa intestinal de rata. A continuación se resumen las representaciones gráficas del % de actividad de las disacaridasas intestinales: a) sacarasa, b) maltasa, en función del logaritmo de la concentración de inhibidor. Para la determinación de los valores de  $IC_{50}$ , se aplicó un modelo de de aproximación no lineal utilizando el programa Graph Pad Prism 4.

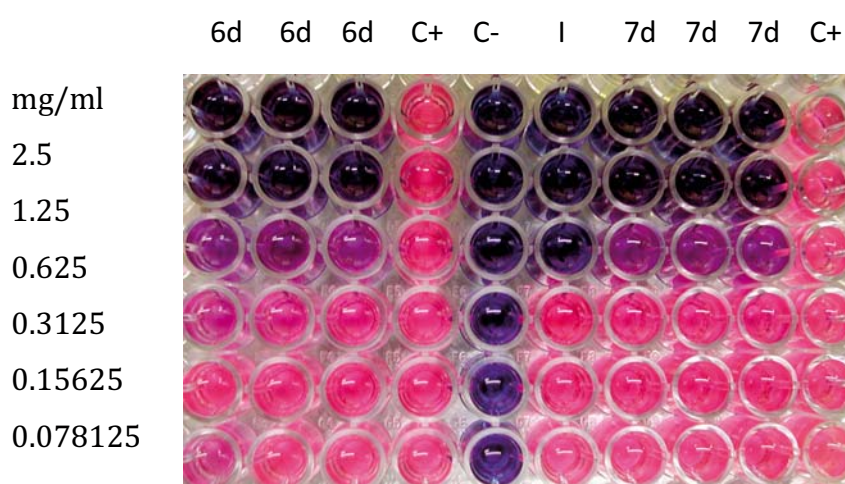
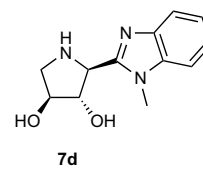
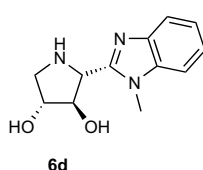
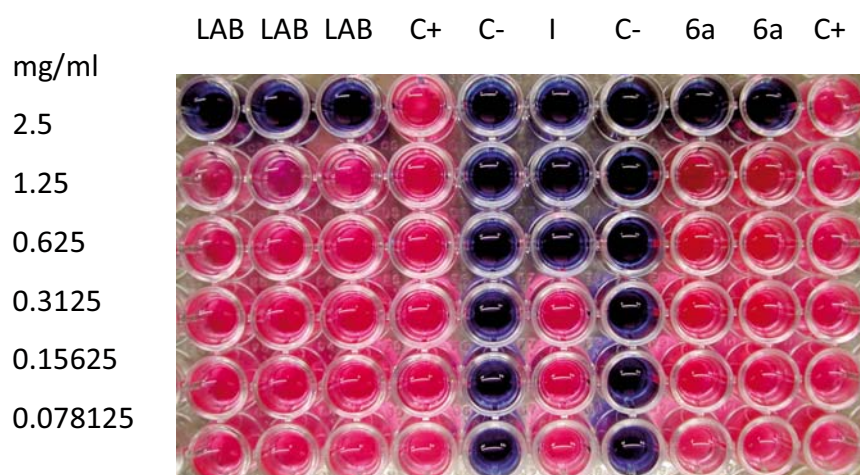
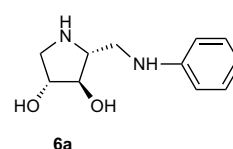
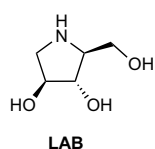
a) sacarasa

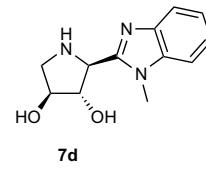
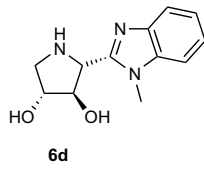


b) maltasa



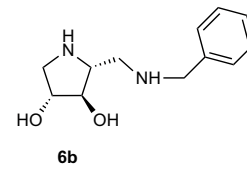
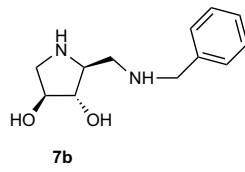
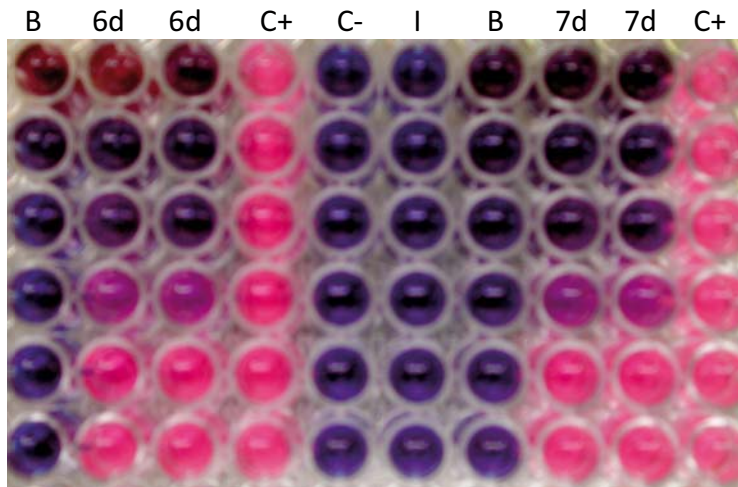
3.4.2 Fotografías de las placas de los experimentos de determinación de MIC (Concentración Mínima Inhibitoria) de los productos que resultaron inhibidores de  $\alpha$ -L-rhamnosidasa de *Penicillium decumbens*: derivados de **LAB** conjugados con aminas aromáticas (**7a-d**), y sus enantiómeros: **DAB** y sus derivados aromáticos (**6a-d**) sobre el crecimiento de *Mycobacterium tuberculosis*. El valor de MIC corresponde a la concentración mínima a la que no se ha producido el cambio de color (de azul a rosa). C+, control positivo de crecimiento, sin inhibidor; C-, control negativo de crecimiento, sin bacilo; I isoniazida (MIC 0.25  $\mu$ g/ml); y B, blanco de producto.





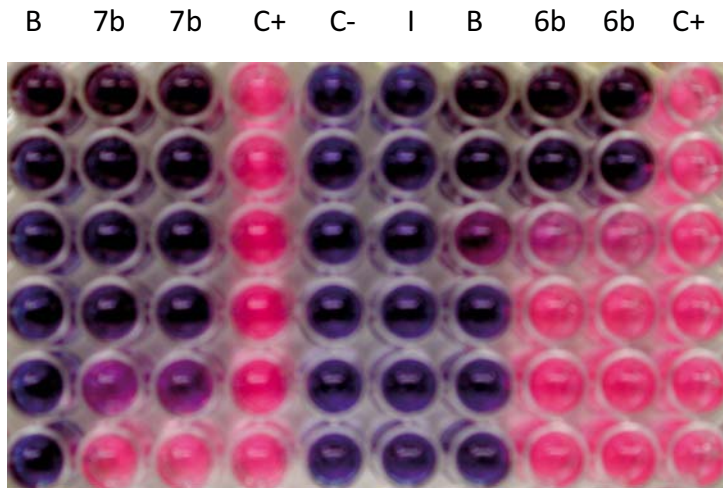
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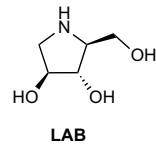
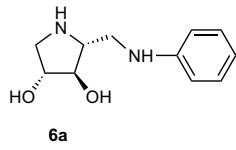
5  
2.5  
1.25  
0.625  
0.3125  
0.15625



mg/ml

5  
2.5  
1.25  
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0.3125  
0.15625





mg/ml

5

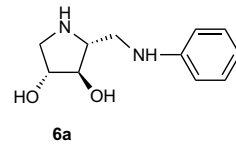
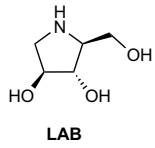
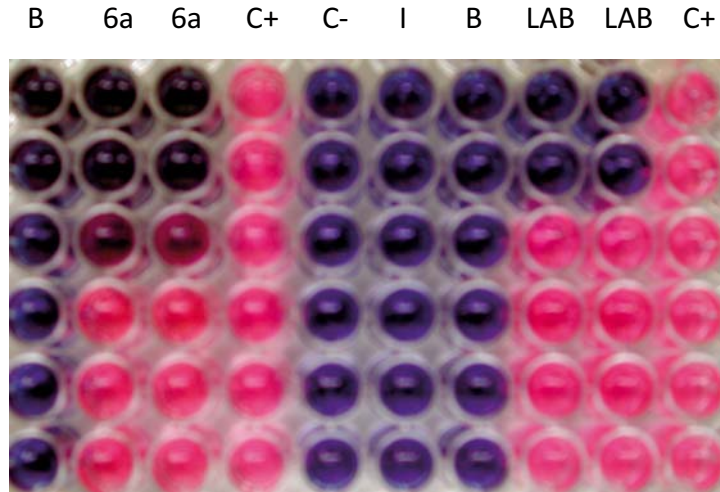
2.5

1.25

0.625

0.3125

0.15625



mg/ml

2.5

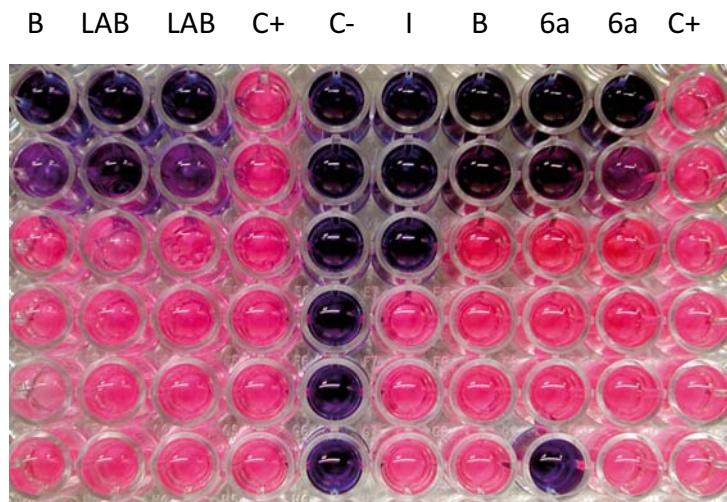
1.25

0.625

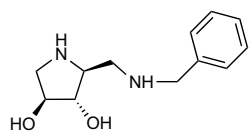
0.3125

0.15625

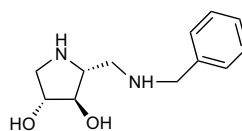
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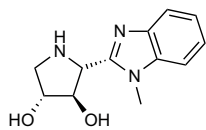
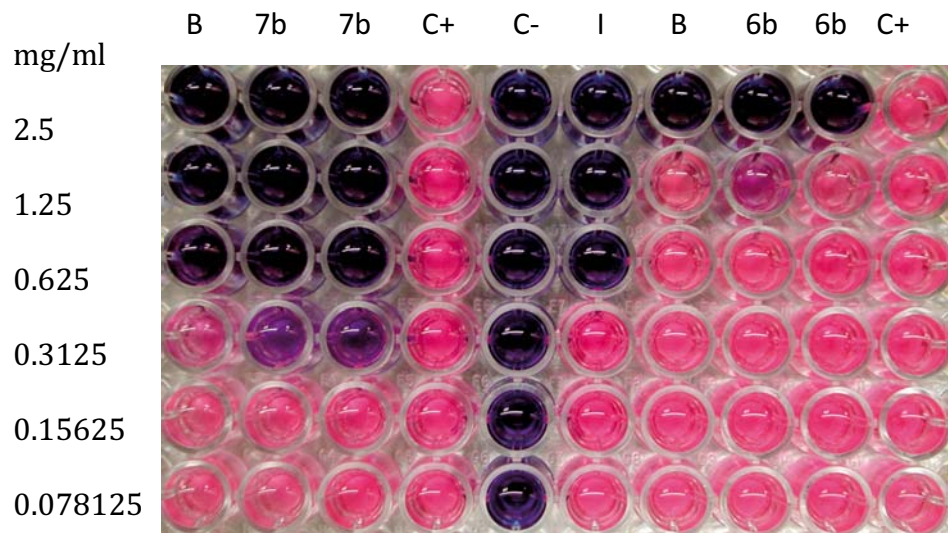




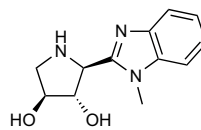
7b



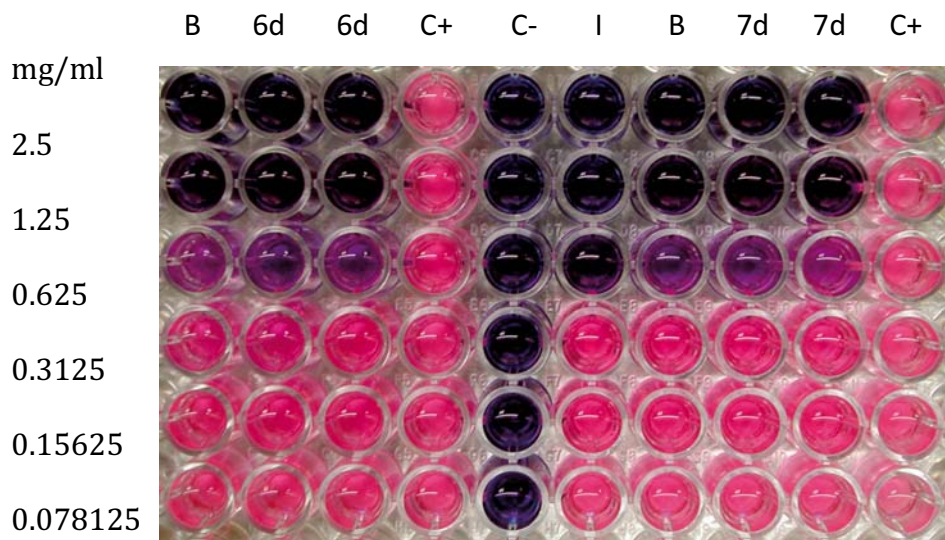
6b

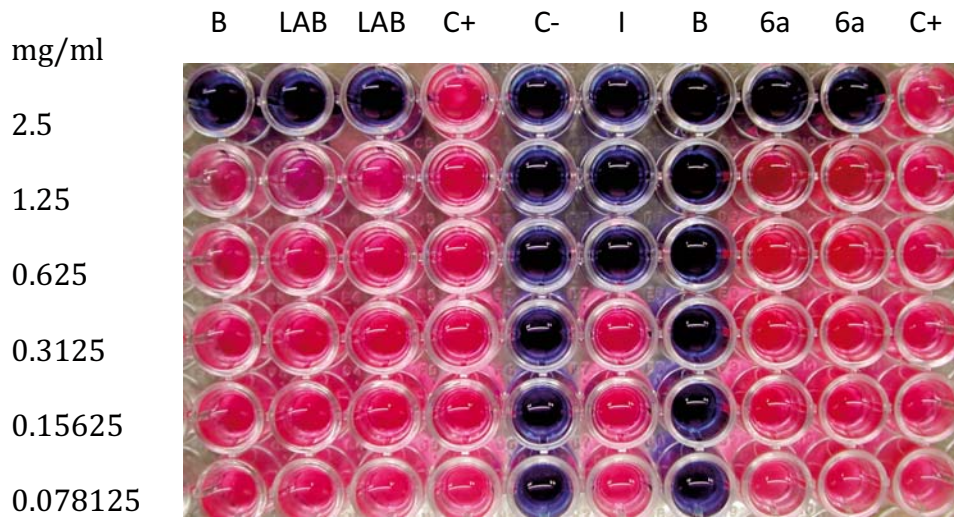
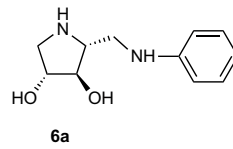
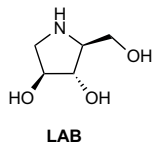
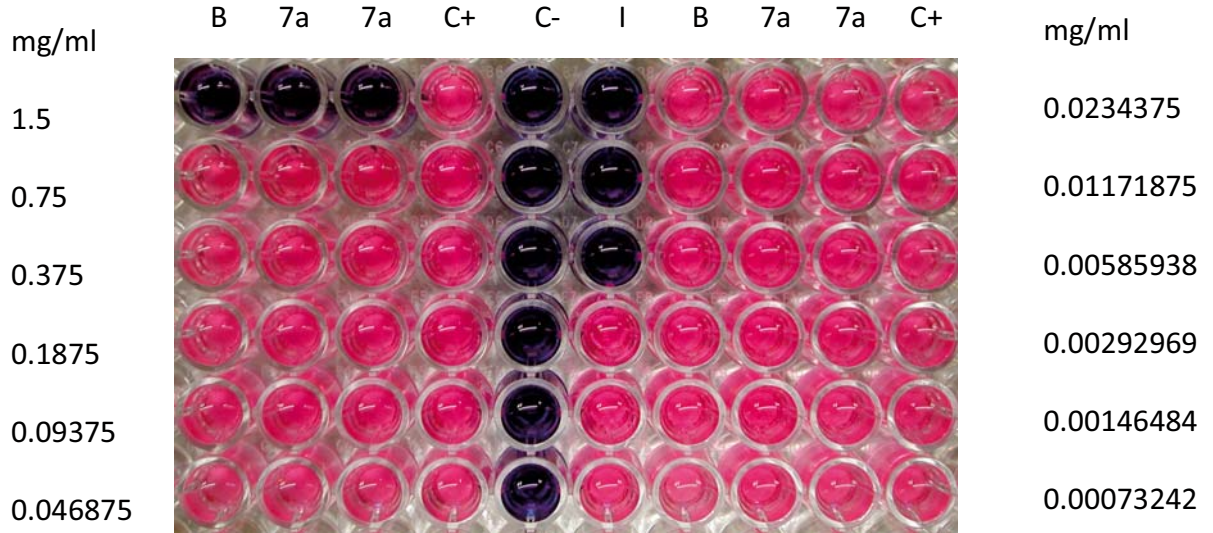
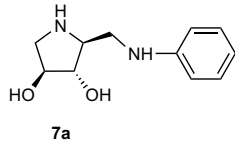


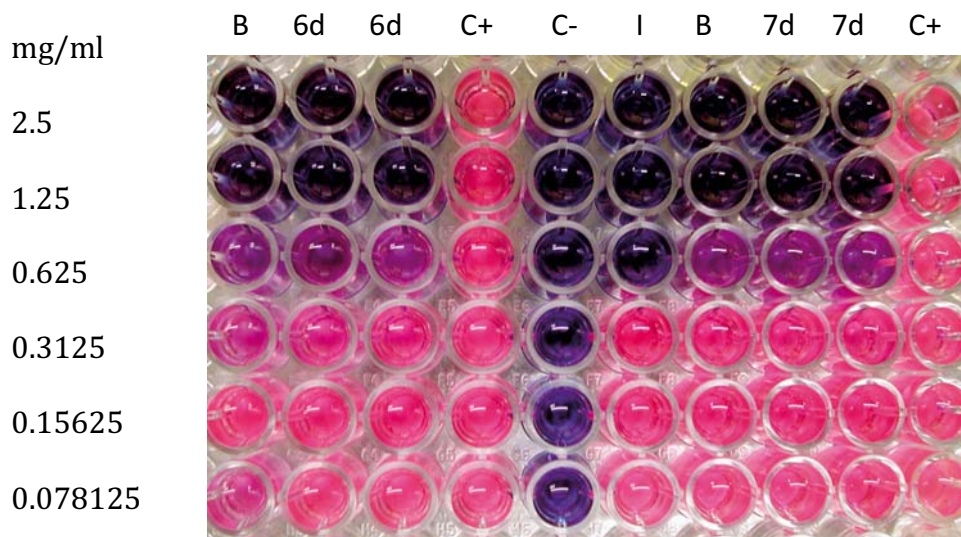
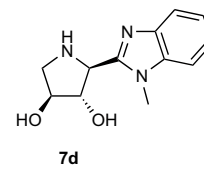
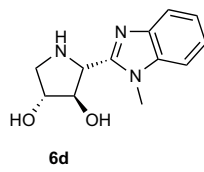
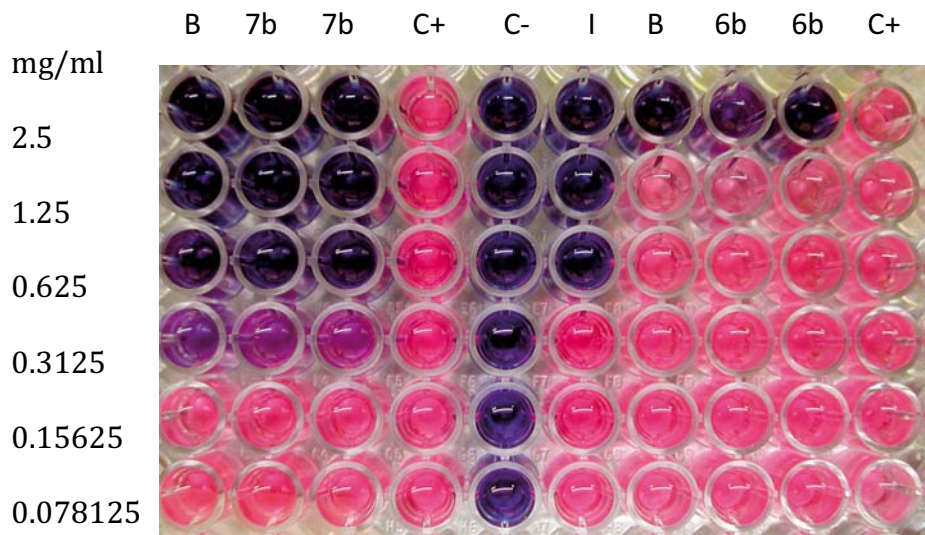
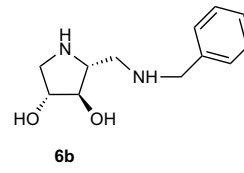
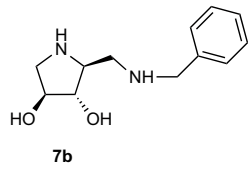
6d

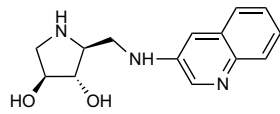


7d

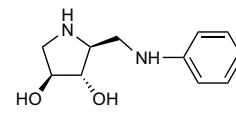








7c



7a

mg/ml

0.5

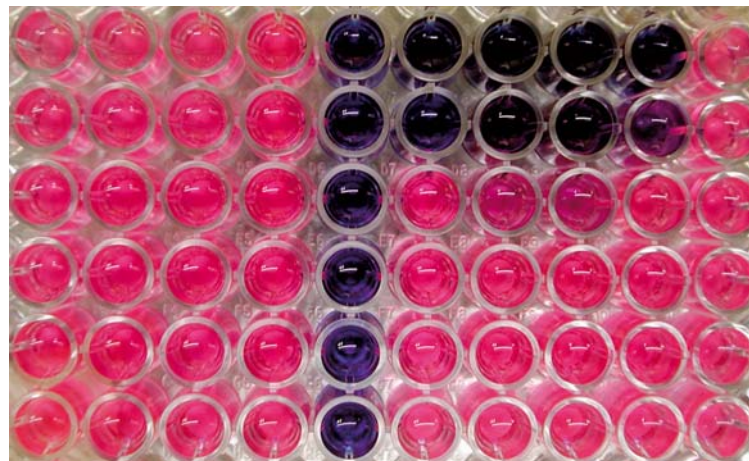
0.25

0.125

0.0625

0.03125

0.015625



mg/ml

1.5

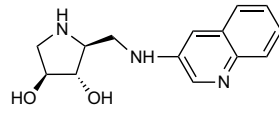
0.75

0.375

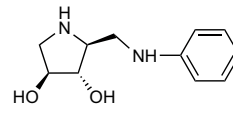
0.1875

0.09375

0.046875



7c



7a

mg/ml

5

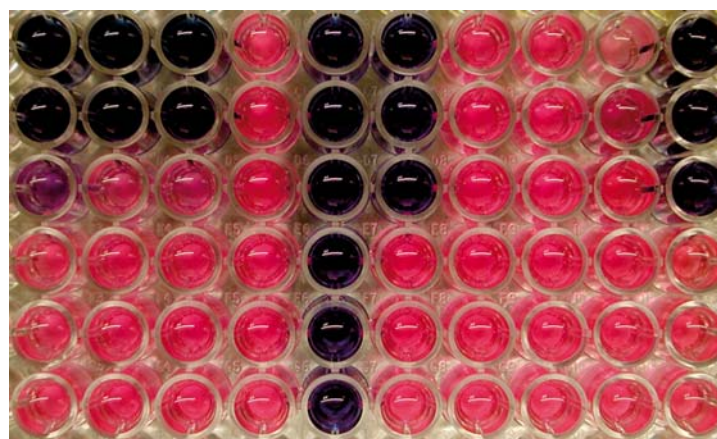
2.5

1.25

0.625

0.3125

0.15625



mg/ml

8

4

2

1

0.5

0.25