"In silico and *In vitro* Studies of the Cytotoxicity and the Mode of Action of Dichapetalins A and M

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OUTLINE

- INTRODUCTION
- METHODS
- RESULTS
- DISCUSSION
- CONCLUSION

SOURCES OF DRUGS - approaches

- Traditional approach–Extracts
- Conventional approach- state of the art syst
- Receptor theory of drug action

a) chemicals in extracts are responsible for a drug's biological activity.

b) elucidate the chemical structures

c) rise of synthetic chemistry (1)

Evolving new approach

Application of computational techniques

E.g. *In silico* studies to understand the mode of action of *Cassia auriculata*(CA) (Fauzi *et al*, 2016) compounds

- Based on both *in silico* and *in vivo* studies, they concluded that CA mediates glucose/lipid metabolism via the PI3K* signalling pathway.

Present study - Aim

- *In vitro* cytotoxicity of two compounds from *D. madagascariense*
- Possible mode of action of two dichapetalins A and M with *in silico* studies and *in vitro* validation

Dichapetalum madagascariense

- Out of these 9 from *D. madagascariense*
- Bacterial infections
- Jaundice
- Viral hepatitis
- Rodenticides

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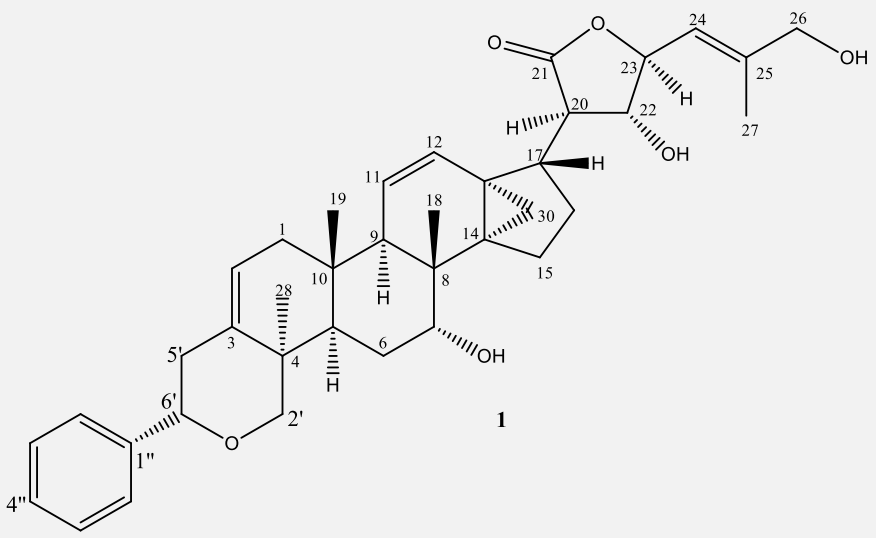
Confirmed Biological Activities

Cytotoxicity

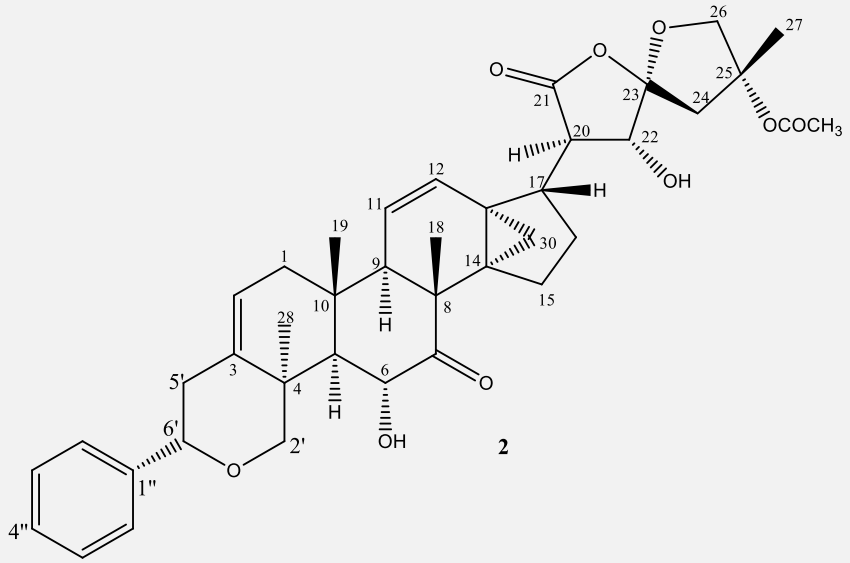
- Dichapetalin A exhibited anti-proliferative effects
- T-lymphocytic leukemia (Jurkat), acute promyelocytic leukemia (HL-60) and T-lymphoblast-like leukemia (CEM) cell lines (Osei-Safo et al., 2017).
- Cytotoxicity against various cancer cell lines (Achenbach et al., 1995; Addae-Mensah et al., 1996; Fang et al., 2006; Long et al., 2013).

Isolation and identification of dichapetalins

- Chromatographic separation of the root acetone extracts
- Comparative spectroscopic and physicochemical methods



A



M

IN SILICO METHODS

Target predictions using PIDGIN – a target prediction tool trained on CHEMBL

Molecular docking – GOLD software suite: understanding details of receptor active sites and receptor-ligand interactions

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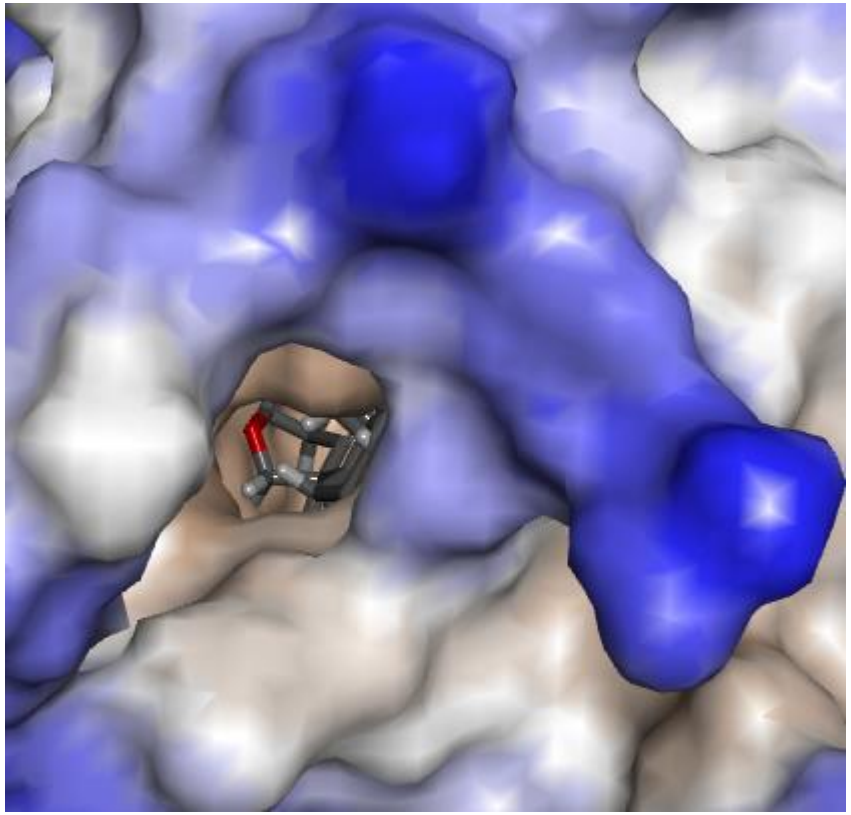
Table 1: Gene scores of predicted targets for dichapetalins A and M from *D. madagascariense*.

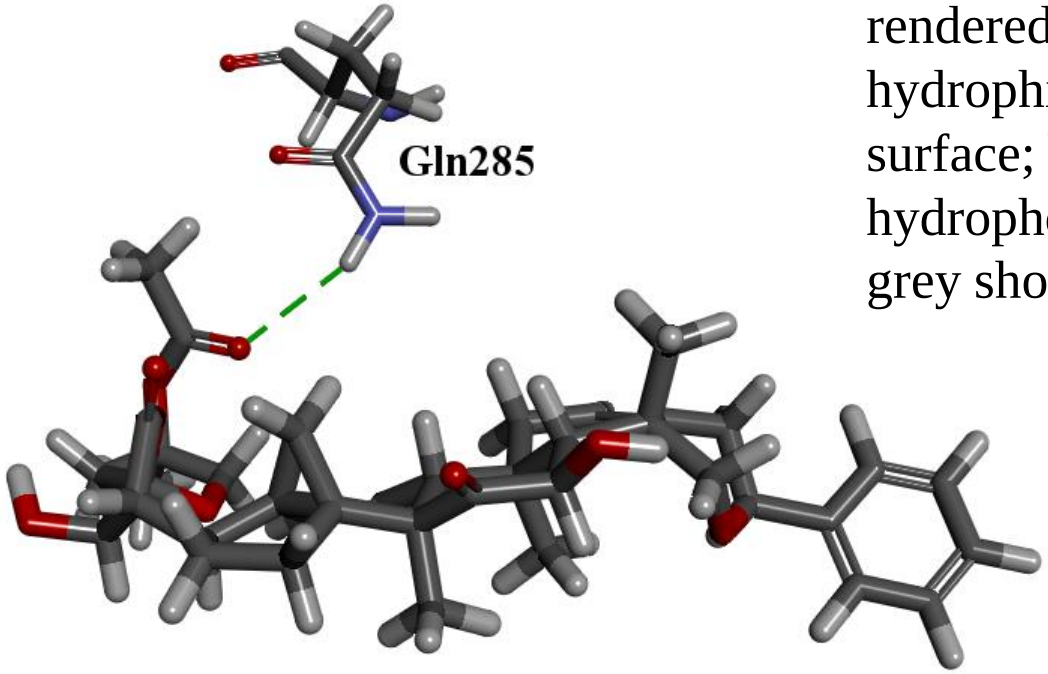
Gene ID	Gene name	Organism	Dichapetalin A	Dichapetalin M
pxr	**Pregnane X receptor**	**Rat**	**0.780**	**0.738**
SHBG	**Sex hormone-binding globulin**	**Human**	**0.764**	**0.754**
srd5a2	3-oxo-5-alpha-steroid 4-dehydrogenase 2	Rat	0.763	0.870
shbg	Sex hormone-binding globulin	Rat	0.733	0.514
ache	Acetylcholinesterase	Pacific electric ray	0.721	0.803
FXR	**Farnesoid X-activated receptor**	**Human**	**0.707**	**0.350**
ar	Androgen receptor	Mouse	0.678	0.451
fxr	Farnesoid X-activated receptor	Mouse	0.666	0.291
PPM1B	**Protein phosphatase 1B**	**Human**	**0.662**	**0.677**
PIP5KIC	**Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma**	**Human**	**0.641**	**0.512**
cpk1	Calcium-dependent protein kinase 1	P. falciparum (3D7)	0.6282	0.579

Docking pose of dichapetalins M

The docked configuration of **Dichapetalin M** in the binding site of NR112 as predicted by ChemPLP (**A**) The ligand occupies the binding pocket



(**A**)



(**B**)

The protein surface is rendered. Blue depicts a hydrophilic region on the surface; brown depicts hydrophobic region and grey shows neutral areas

. (**B**) Hydrogen bonds are shown as green line between **Dichapetalin M** and the amino acid Gln285..

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- . Table 2: Results of the scoring function for the ligands against NR112

Ligand	ASP	PLP	CS	GS
CO-crystallized ligand	27.2	71.9	29.2	59.3
Dichapetalin A	-19.7	2.6	18.7	-101.7
Dichapetalin M	-31.6	-37.4	10.2	-75.3

There was only one way dichapetalins M could bind

Nuclear hormone receptor family 1, group I member 2 (NR1I2, PXR)

- Controls inducible expression of xenobiotics handling genes including biotransformation enzymes and drug transporters
- It is activated by a range of compounds that induce CYP3A4, including rifampicin

Table 3: Cytotoxic effect of Dichapetalins A and M on MCF-7 cells via the MTT assay

Compounds	Treatment time (hrs)	IC_{50} (µM)	R^2	CI
Dichapetalin A	48	>100	-	-
	72	>100	-	-
Dichapetalin M	48	4.71	0.7509	2.813µM – 7.203µM
	72	3.95	0.8169	2.519µM - 6.007µM
Curcumin	48	17.49	0.8901	12.85µM - 23.07µM
	72	12.53	0.8878	8.915µM - 16.87µM

Dichapetalins M was more potent than the standard drug Curcumin

Target Validation

- To validate Dich M binding to NR1I2 (pxr) *in vitro*
- MCF-7 cell lines were treated with a PXR agonist control (rifampicin) to determine if dichapetalin M acts as an antagonist or an agonist to the receptor
- by evaluating its effect on CYP3A4, a downstream target of PXR
- Ketoconazole (Ket), an antagonist on both human HepG2 and MCF-7 cells

Fig. 1. Effect of dichapetalin M on the expression of CYP3A4 in MCF-7

- Real-time qPCR was performed after exposure of MCF-7 cell line to dichapetalin M (at 5%, 10% and 25% of its 72 hrs IC_{50} value) for 24 hrs and extracting RNA for expression studies.

Rifampicin treatment resulted in no significant change in the expression of CYP3A4 in MCF-7 but caused a significant increase in the expression of CYP3A4 (about 2.5-fold) after 24 hrs treatment of HepG2

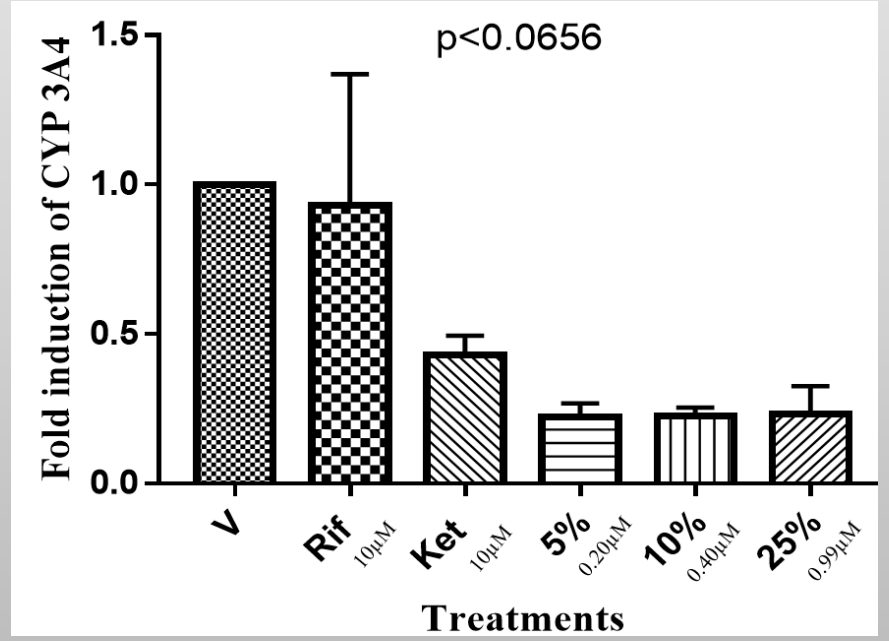

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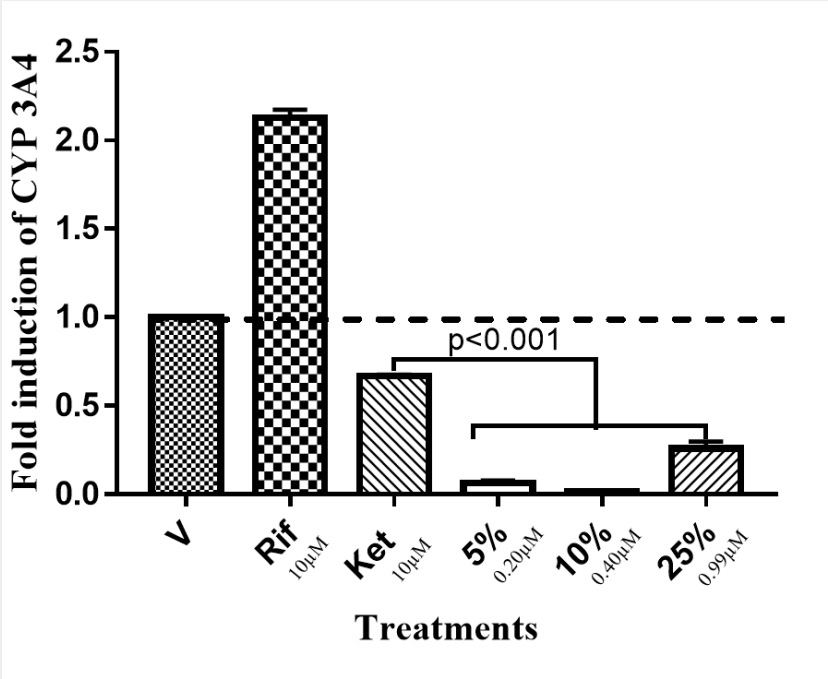
Fig. 2. Effect of dichapetalin M on the expression of CYP3A4 in HepG2 cells

- Real-time qPCR was performed after exposing of MCF-7 cell line to dichapetalin M (at 5%, 10% and 25% of its 72 hrs IC_{50} value) for 24 hrs and extracting RNA for expression studies.

Ketoconazole caused a significant decrease in the expression of CYP3A4 in both cell lines as expected.



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Discussion

- Dichapetalin M was the most potent cytotoxic dichapetalin against HCT116 (EC_{50} = 9.9x10-9 622 M) and WM 266-4 (EC_{50} = 7.8x10 623 -8 M) cell lines when tested together with 13 different dichapetalins (Long et al., 2013).

- Structurally, the stability of the spiroketal moiety, which is absent in dichapetalin A may contribute substantially to the significant activity of dichapetalin M against MCF-7 cell lines compared to dichapetalin A.

Discussion

- In terms of toxicity, previous study of dichapetalin M on brine shrimp indicated an LC_{50} of 0.011 µg/ml (Osei-safo et al., 2008), a value that is 20-fold more active than that of dichapetalin A (0.31 µg/ml

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Conclusion

- predict both human and non-human targets for dichapetalins A and M and validated the effects of dichapetalin M on the human PXR ortholog.

- Dichapetalin M acted as an antagonist to the PXR by significantly down regulating the expression of CYP3A4 in both cell lines, however, this observation did not occur 607 in a dose-dependent manner. 22 608

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