The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
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P-gp modulation and biosynthetic relationship of isolated compounds from *Plectranthus mutabilis* Codd

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pharmaceuticals MDPI

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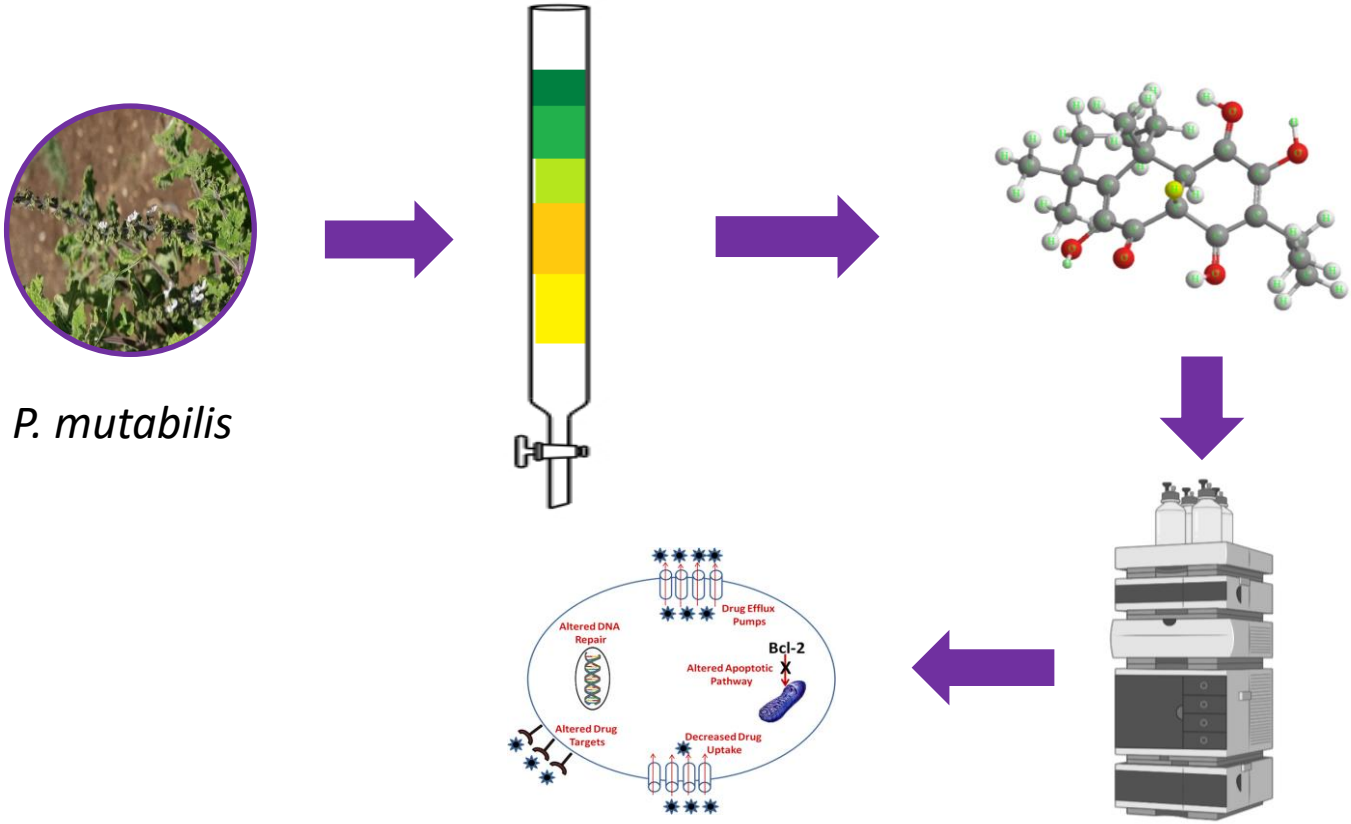
P-gp modulation and biosynthetic relationship of isolated compounds from *Plectranthus mutabilis* Codd

Graphical Abstract



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Abstract: The development of multidrug resistance (MDR) is one of the major challenges in the successful treatment of cancer. MDR is often associated with the P-glycoprotein efflux pump. Natural products are a source of promising lead compounds to overcome MDR and, among them, diterpenoids from Plectranthus spp. are known as P-gp modulators. Bioguided fractionation of P. mutabilis acetone extract led to the isolation of one new nor-abietane diterpene, mutabilol (1), and three coleon compounds (coleon-U-quinone (2), 8α,9α-epoxycoleon-U-quinone (3), and coleon U (4)). Moreover, an additional acetoxy derivative of an abietane diterpenoid was tentatively identified using HPLC-MS/MS. The compounds were quantified using HPLC-DAD and coleon U was found to be the major compound in the extract. Using computational studies, a biosynthetic relationship between compounds 2 - 4 revealed that both compounds 2 and 3 were formed directly from compound 4. Compounds 2 - 4 were found to be selective towards the cancer cell lines and their anticancer effect was not compromised by the P-gp activity in resistant NCI-H460/R cells. Importantly 2, 3, and 4 were able to inhibit P-gp activity in NCI-H460/R cells at longer exposure (72 h) and revert doxorubicin (DOX) resistance in combined treatment. None of the compounds influenced the P-gp expression in NCI-H460/R cells, while the extract significantly increased it. Our study identified abietane diterpenoids from P. mutabilis that can evade MDR in cancer cells and inhibit the P-gp activity in prolonged treatment.

Keywords: *Plecthranthus*; *P. mutabilis*; isolation; P-glycoprotein

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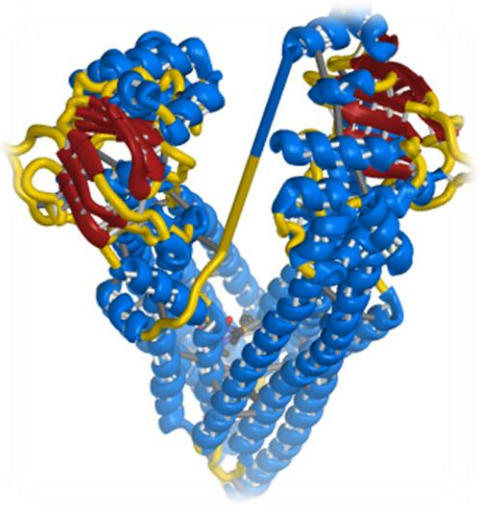
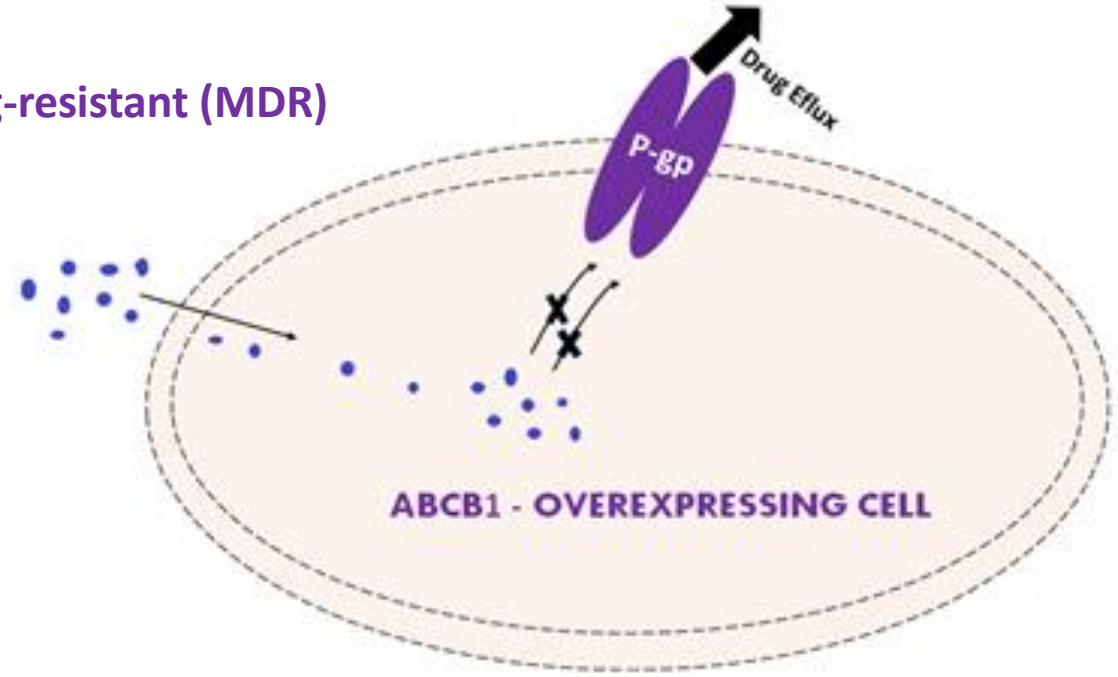
Introduction

Multidrug-resistant (MDR)



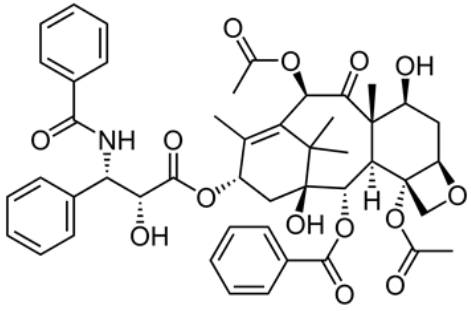


- Greatly hinders the efficacy of chemotherapy
- Major challenge to cancer therapy
- **P-gp** is one of the major contributors to MDR
- Need to develop new reversal MDR agents

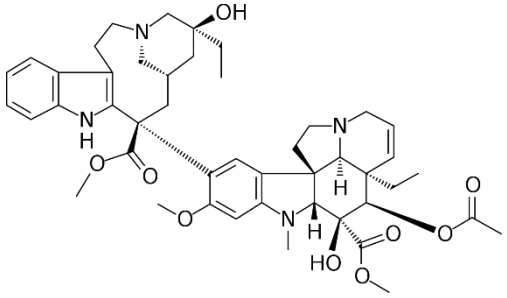
Introduction

Natural products

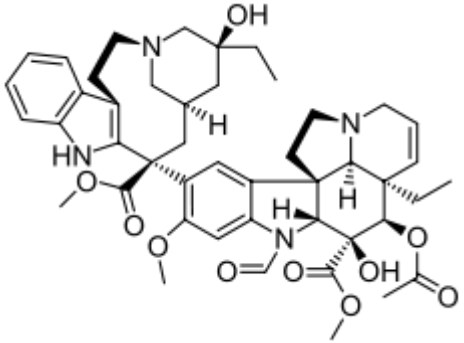
- Source of bioactive compounds
- Source of anti-cancer drugs
- 60% of available anticancer drugs in clinic are derived from natural sources



Paclitaxel (Taxol®)



Vinblastine



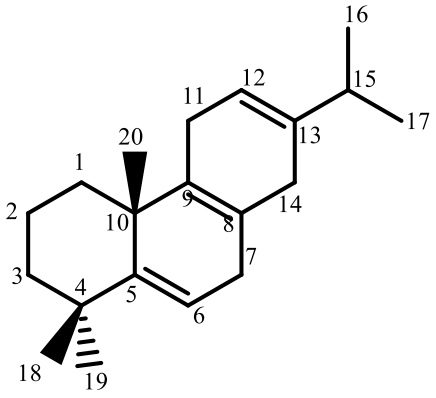
Vincristine

Nat Rev Drug Discov 20, 200–216 (2021). https://doi.org/10.1038/s41573-020-00114-z

Introduction

Plectranthus genus (Lamiaceae)

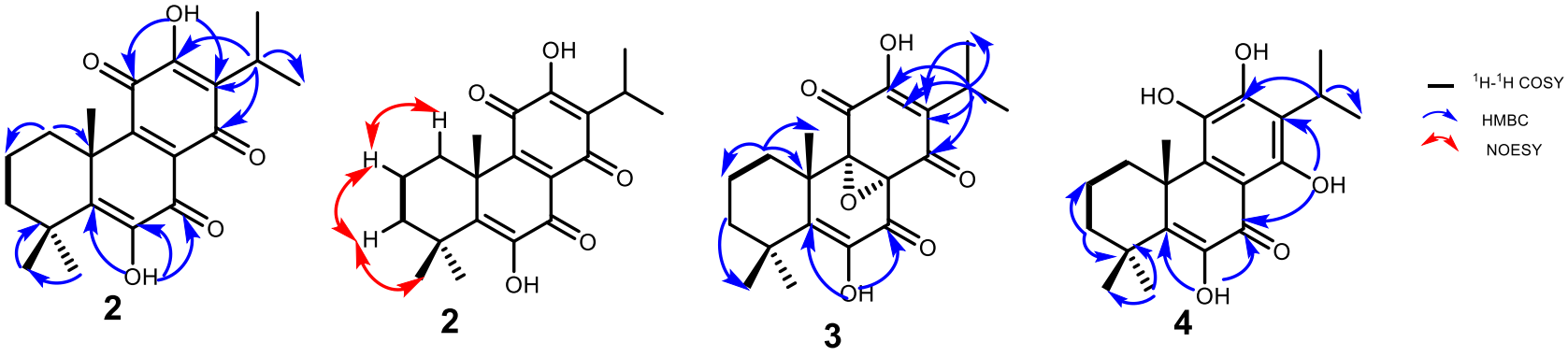
- Traditional medicinal practice
- Treatment of ailments
- Treatment of different types of cancer

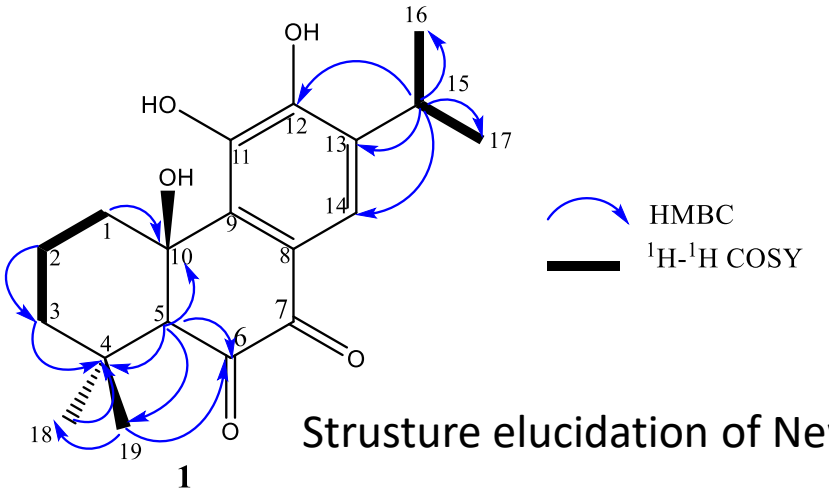


- **Source of bioactive compounds:**
- abietane-type diterpenoids
- antibacterial, antifungal, antiplasmodial, and **antitumoral**

Results and discussion

Plectranthus mutabilis Codd. phytochemical study





1. Mutabilol, (+)-(5S,10R)-10,11,12-trihydroxy 6,7-dioxo-20-nor-abieta-8,11,13-triene
2. Coleon U quinone
3. 8α,9α-Epoxycoleon U quinone
4. Coleon U

Strusture elucidation of New C_{20}-nor-abietane

Epole N. Ntungwe; *et al*. *ACS Medicinal Chemistry Letters,* 2022, https://doi.org/10.1021/acsmedchemlett.1c00711

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Results and discussion

Plectranthus mutabilis Codd. phytochemical study

Abietane Diterpenoids compositions of *P. mutabilis* extract by HPLC–DAD

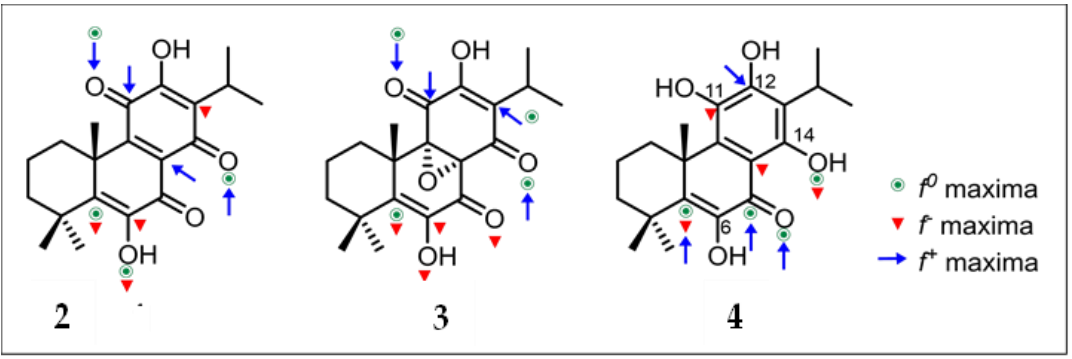
Compounds	Concentration µg/mg	LOD µg/mg	LOQ µg/mg
Mutabilol **(1)**	51±0.008	1.120	3.39
Coleon U quinone **(2)**	35±0.005	0.102	0.310
8α,9α-Epoxycoleon U quinone **(3)**	36±0.018	0.828	2.510
Coleon U **(4)**	96±0.048	0.78	2.35

Results are expressed as average ± standard deviation (SD) of three determinations. Compounds 2 and 3 were quantified at 270 nm and compounds 1 and 4 at 254 nm.

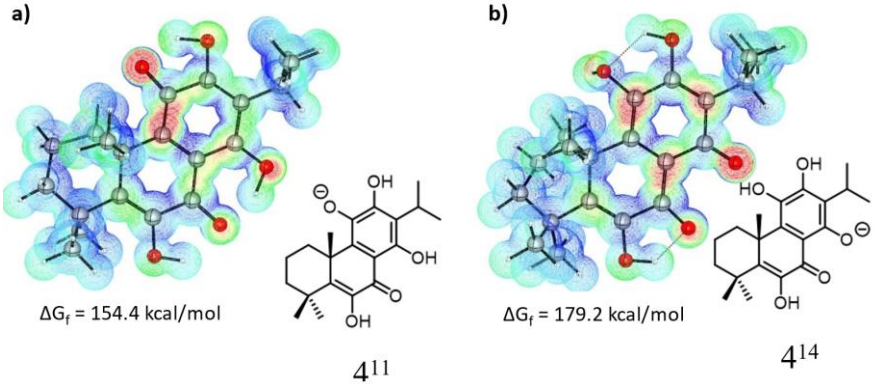
Epole N. Ntungwe; *et al*. ACS *Medicinal Chemistry Letters,* 2022, https://doi.org/10.1021/acsmedchemlett.1c00711

Results and discussion

Positions with higher Fukui indexes



Electrophilic (f_k^-), nucleophilic (f_k^+) and radical (f_k^0) Fukui functions of **2-4**. The higher condensed Fukui indexes are indicated as green circles, red triangles, and blue arrows, respectively representing the sites in the molecules that are most susceptible for a radical attack, most nucleophilic, and most electrophilic.



Biosynthetic relation between 2-4

Hydrogen Bond Dissociation Energies (BDEs; kcal/mol) of O-H bonds in 4.

Position of OH in 4	O-H Bond Dissociation Energy (kcal/mol)
C6	103.8
C11	84.1
C12	103.0
C14	116.5

- BDE O-H at the C11 is lowest -the hydroquinone to quinone
- 25 kcal/mol difference in the **Gibbs free energy**
- That the hydroxyl deprotonation at position 11 is preferable
- both the quinone **2** and the epoxyquinone **3** are formed directly from Coleon U

Epole N. Ntungwe; *et al*. *ACS Medicinal Chemistry Letters,* 2022, https://doi.org/10.1021/acsmedchemlett.1c00711

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Results and discussion

Selectivity towards cancer cells

Inhibition of cell viability assayed by MTT

Human non-small cell lung carcinoma cells (NCI-H460), its MDR variant with P-gp ex-pression (NCI-H460/R), and human embryonic pulmonary fibroblasts (MRC-5)

Compounds	NCI-H460	NCI-H460/R	MRC-5	Selectivity index [a]
1	> 50	> 50	> 50	n.a.
2	22.96±0.56[b]	20.37±0.43	44.13±1.19	2.0
3	20.23±0.59	17.26±0.26	40.22±0.44	2.0
4	14.11±0.19	14.50±0.18	35.47±0.56	2.5
extract	15.30±0.37[c]	15.66±0.47	16.68±0.69	1.0
Paclitaxel	0.0006 ± 0.0001	0.117 ± 0.013	0.523 ± 0.001	872

[a]Selectivity index was calculated as a relation between IC_{50} for MRC-5 cells and IC_{50} for NCI-H460
[b]IC_{50} values in μM for compounds,
[c]IC_{50} values in μg/mL for extract

- Reduction of cancer cell viability
- All compounds and the extract are not substrates for P-gp

Results and discussion

Interaction with P-gp

Rho 123 accumulation assay in MDR NCI-H460/R cells

Compounds/(Cell Lines)		MFI[a]	FAR±S.E.[b]	SI±S.E.[c]
NCI-H460[d]		2479.3	3.06±0.61	
NCI-H460/R		811.1		32.71±1.65
TQ	50 nM	3004.0	3.70±0.54[e]	121.16±0.88[e]
Extract	5 µg/mL	339.5	0.42±0.20[f]	13.69±3.24[f]
	10 µg/mL	307.7	0.38±0.20	12.41±3.15
1	5 µM	265.9	0.33±0.21	10.72±3.55
	10 µM	254.5	0.31±0.22	10.26±3.63
2	5 µM	277.3	0.34±0.20	11.18±3.29
	10 µM	242.0	0.30±0.20	9.76±3.44
3	5 µM	289.7	0.36±0.20	11.68±3.29
	10 µM	276.6	0.34±0.21	11.16±3.41
4	5 µM	173.5	0.21±0.24	7.00±3.99
	10 µM	109.7	0.14±0.29	4.43±4.71

Tariquidar (TQ) – Positive control

- Mean fluorescence intensity (MFI)
- Fluorescence activity ratio (FAR)
- Sensitivity index (SI)

❖ TQ increases the accumulation of Rho123 and thus inhibits P-gp activity

❖ Compounds and extract stimulate P-gp activity in a direct interaction assessed after 30 min

Epole N. Ntungwe; et al. ACS Medicinal Chemistry Letters, 2022, https://doi.org/10.1021/acsmedchemlett.1c00711

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Results and discussion

Rho123 accumulation after 72 h treatment with compounds and extract

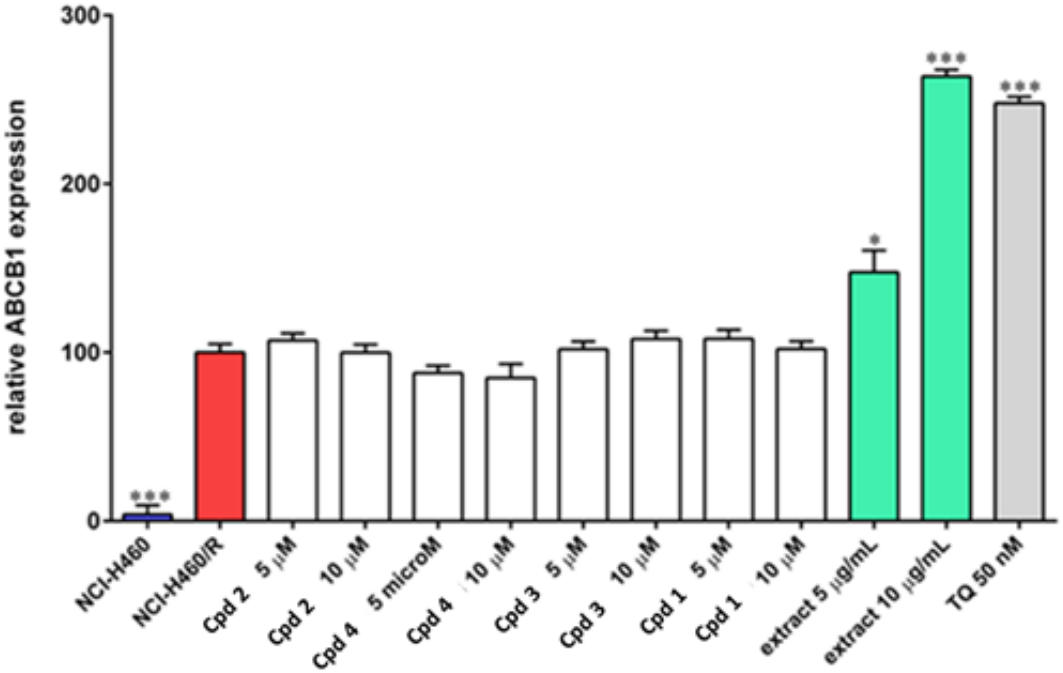
Compounds/(Cell Lines)		MFI[a]	FAR±S.E.[b]	SI±S.E.[c]
NCI-H460[d]		2151.0	4.19±0.45	
NCI-H460/R		513.7		23.88±2.22
TQ	50 nM	3004.0	3.70±0.54[e]	121.16±0.88[e]
Extract	5 μg/mL	654.0	1.27±0.96[e]	30.40±2.14
	10 μg/mL	473.4	0.92±0.96[f]	22.01±2.14
1	5 μM	595.3	1.16±0.98	27.68±2.18
	10 μM	573.0	1.12±1.03	26.64±2.29
2	5 μM	669.6	1.30±1.00	31.13±2.22
	10 μM	991.3	1.93±0.86	46.09±1.90
3	5 μM	711.6	1.39±0.98	33.08±2.19
	10 μM	666.1	1.30±1.02	30.97±2.26
4	5 μM	1258.8	2.45±0.78	58.52±1.73
	10 μM	253.0	0.49±1.25	11.76±2.78

- Lower concentration -increased the Rho123 accumulation- inhibiting P-gp
- At higher conc. the extract and **4** decreased Rho123 accumulation – stimulating P-gp

Epole N. Ntungwe; *et al*. ACS *Medicinal Chemistry Letters,* 2022, https://doi.org/10.1021/acsmedchemlett.1c00711

Results and discussion

Effects on P-gp (ABCB1) expression after 72 h treatment of NCI-H460/R cells



- None of the compounds had influence on ABCB1 expression
- The extract significantly increased ABCB1 expression in a concentration-dependent manner

The extract can increase resistance by stimulating P-gp expression. Therefore, it would not be wise to use the extract in combination with chemotherapeutics which are P-gp substrates.

Epole N. Ntungwe; *et al*. ACS *Medicinal Chemistry Letters,* 2022, https://doi.org/10.1021/acsmedchemlett.1c00711

Results and discussion

Reversal of doxorubicin (DOX) resistance in NCI-H460/R cells

Compounds	Concentration	IC_{50} for DOX	Relative reversal index
DOX		1.620±0.084	
	1 µM	0.565±0.012	2.867***
2	2 µM	0.482±0.010	3.361***
	5 µM	0.217±0.004	7.465***
3	1 µM	0.820±0.016	1.976***
	2 µM	0.345±0.007	4.696***
	5 µM	0.343±0.006	4.723***
	1 µM	0.625±0.013	2.592***
4	2 µM	0.540±0.012	3.000***
	5 µM	0.268±0.006	6.045***

DOX (0.1, 0.2, 0.5, 1 and 2 µM) was administrated after 72 h pre-treatment with 2, 3, and 4 applied in three concentrations below their IC_{50} (1, 2, and 5 µM).

- All combinations of all compounds with DOX showed significant reversal potential
- The most potent sensitization of NCI-H460/R cells to DOX was achieved with 5 µM of **2** and **4**

Epole N. Ntungwe; *et al*. ACS *Medicinal Chemistry Letters,* 2022, https://doi.org/10.1021/acsmedchemlett.1c00711

Conclusions

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C_{20}-*nor*-Abietane and Three Abietane Diterpenoids from *Plectranthus mutabilis* Leaves as P-Glycoprotein Modulators

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Epole N. Ntungwe,◆ Sofija Jovanović Stojanov,◆ Noélia M. Duarte, Nuno R. Candeias, Ana M. Díaz-Lanza, Máté Vágvölgyi, Attila Hunyadi, Milica Pešić,* and Patrícia Rijo*

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