Novel β-carboline indole alkaloids from the leaves of *Tabernaemontana elegans*

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ABSTRACT

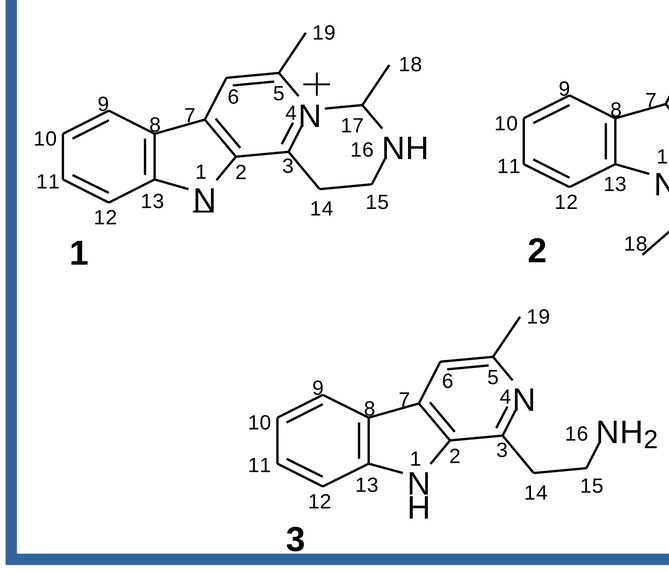
We have isolated three β-carboline indole alkaloids (**1**-**3**) from the MeOH extract of the leaves of *Tabernaemontana elegans*. The chemical structures of these novel entities were established by means of spectroscopic techniques including 2D NMR spectroscopic experiments. The new skeletal features of compounds **1** and **2** were the presence of a two-carbon unit, attached to a structurally related β-carboline skeleton, resulting in the formation of additional six and seven-membered new rings in **1** and **2**, respectively. To the best of our knowledge, it appears to be the first report on the isolation of β-carboline indole alkaloids from the genus Tabernaemontana. Compounds **1**-**3** were evaluated for their potential P-glycoprotein modulating properties using the rhodamine-123 assay, in both MDR1-gene transfected and parental mouse lymphoma cell lines. Compounds **1** and **3** exhibited a weak activity.

Tabernaemontana elegans

RESULTS AND DISCUSSION

The genus *Tabernaemontana* (Apocynaceae) has a wide distribution and plants belonging to this genus are used in traditional medicine to treat cancer [1]. These plants are characterized to produce indole alkaloids of unusual structures as well as novel bioactivity. The new feature shared by β-carboline indole alkaloids **1–3** is the presence of a methyl group at C-5. Furthermore, compounds **1** and **2** contained an additional two-carbon unit (C-17 and C-18) at N-16, which is connected to N-4 in compund **1** and N-1 in compound **2**, to form an additional six and seven-membered rings, respectively. Therefore, the β-carbolines **1** and **2** can be considered as compounds with new skeletal features.

Chemical structures of indole alkaloids **1-3**



Compounds **1**–**3** were evaluated for their P-gp modulating properties on human MDR1 gene-transfected and parental L5178 mouse lymphoma cell lines, by flow cytometry, using the rhodamine-123 exclusion test. The results are summarized in Table. Their antiproliferative effects on these cell lines are also presented below. Compounds **1** and **3** displayed weak MDR reversal activity when tested at the highest concentration (FAR = 1.73 and 1.43, at 20 µM, respectively). Small molecules are not Pgp modulators and the range of appropriate molecular weighs varies between 250 and 2000.[22] Therefore, the low molecular weight of the compounds **1**–**3** (228 for **3** and 251 for compounds **1** and **2**) may contribute for their lack of activity.

Multidrug resistance reversal effects of **1-3**

Compounds	Conc. (µM)	FSC[a]	SSC[a]	FL-1[a]	FAR[a]
PAR[b]	-	443	185	972	-
PAR	-	443	175	891	-
MDR[c]	-	452	251	10.7	-
Verapamil	22.2	439	251	98.9	9.25
1	20	454	239	18.5	1.73
	2	450	244	7.7	0.72
2	20	458	243	10.7	0.99
	2	454	243	8.9	0.78
3	20	446	242	15.3	1.43
	2	460	232	8.4	0.79
DMSO	-	466	242	10.3	0.97

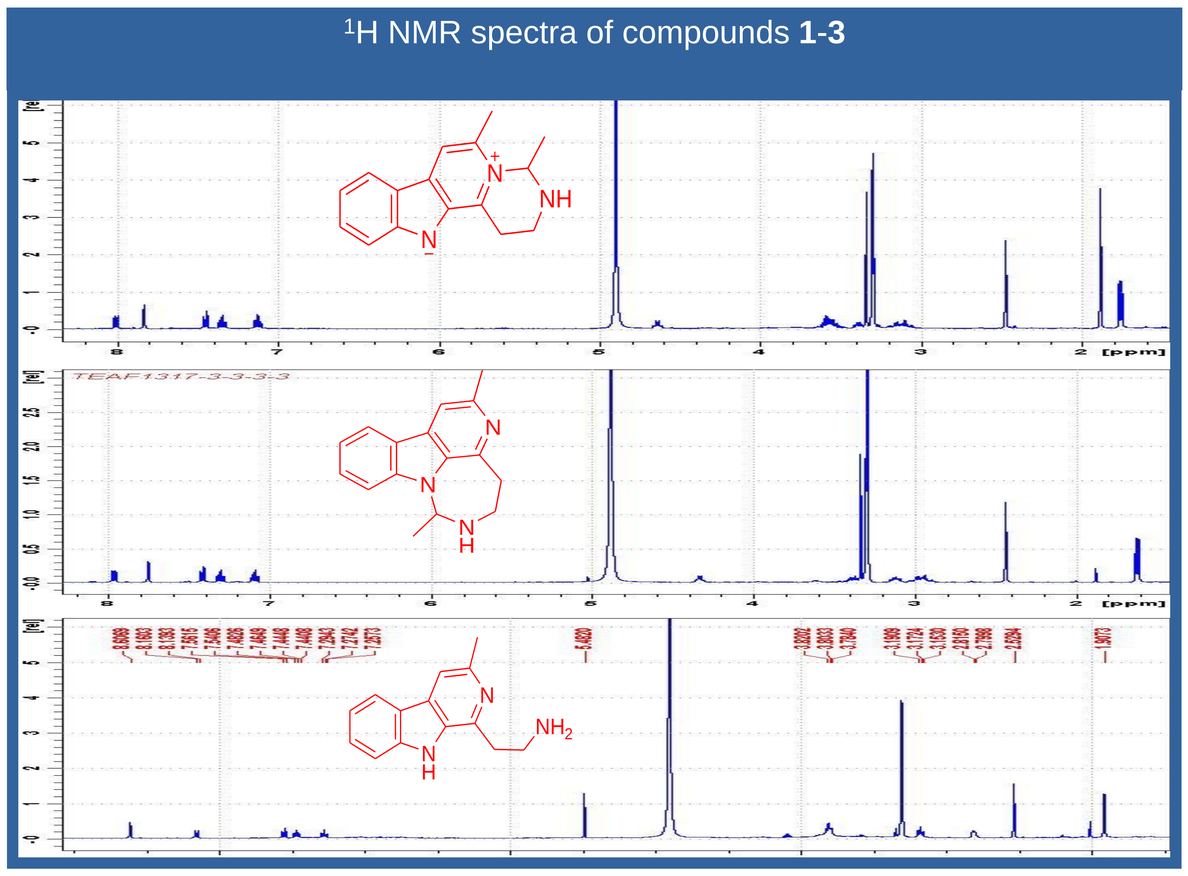
[a] FSC: Forward scatter count of cells in the samples; SSC: Side scatter count of cells in the samples; FL-1: Mean fluorescence intensity of the cells. FAR: fluorescence activity ratio: values were calculated by using the equation given in the experimental section. [b]PAR control: a parental cell without MDR gene. [c]MDR: a parental cell line transfected with human MDR1 gene.

Antiproliferative effects of **1–3**

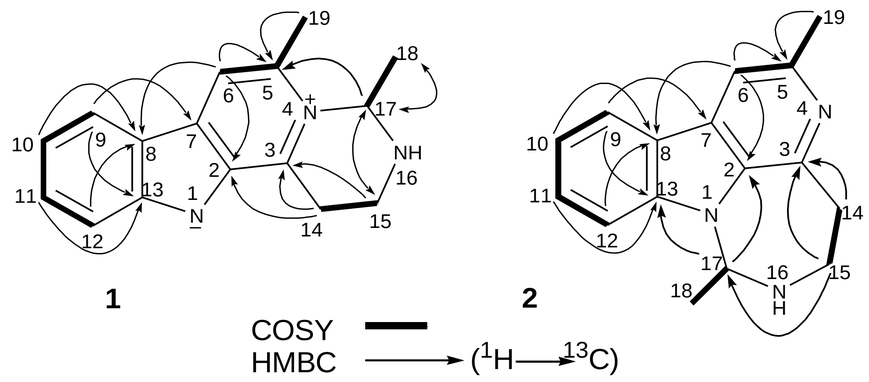
Compounds	PAR-L5178* ID_{50} (µM)	MDR-L5178* ID_{50} (µM)
1	45.9 ±6.4	37.5 ±2.1
2	46.6 ±9.2	39.7 ±0.7
3	70.6 ±2.1	51.5 ±0.7

*Parental (PAR) and Multidrug Resistance (MDR) Mouse Lymphoma Cells (L5178)

^{1}H NMR spectra of compounds **1-3**

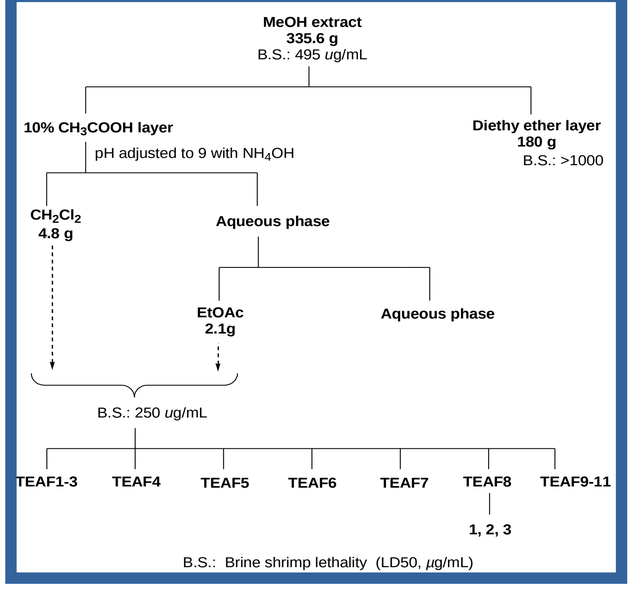


Key HMBC and COSY correlations of **1** and **2**



The MeOH extract of *Tabernaemontana elegans* was extracted with dichloromethane and ethyl acetate solvents. The CH_2Cl_2 and EtOAc soluble fractions were combined and subjected to further chromatographic procedures to isolate compounds **1**–**3**.

Isolation Scheme



CONCLUSION

Three novel β-carboline indole alkaloids (**1**–**3**) have been isolated from a MeOH extract of the leaves of *Tabernaemontana elegans* (Apocynaceae). To the best of our knowledge, this is the first report of β-carboline indole alkaloids from the genus *Tabernaemontana.* Compounds **1** and **3** exhibited a weak MDR activity in mouse lymphoma cell lines.

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References: [1] Graham, J. et al. (2000) J. Ethnopharm. 73:347 – 377.