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REVERSAL OF MULTIDRUG RESISTANCE (MDR) BY ASPIDOFRACTININE-TYPE INDOLE ALKALOIDS

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Abstract: A series of indole alkaloids of the aspidofractinine-type was assessed for their potential in reversing MDR in vincristine-resistant KB cells. Of the compounds tested, kopsiflorine, kopsamine, pleiocarpine, 11-methoxykopsilongine, lahadinine A and *N*-methoxycarbonyl-11,12-methylenedioxy-$\Delta^{16,17}$-kopsinine were found to show appreciable activity.

One of the major problems associated with treatment of human neoplastic diseases is the phenomenon of multidrug resistance (MDR) to the many anti-cancer agents used in chemotherapy.[1,2] Among potential candidates which are being screened for their ability to reverse MDR are plant natural products, including indole alkaloids.[2-4] In the course of routine screening of compounds from our natural product work for various biological activity, we found that the indole alkaloid kopsiflorine **1** from *Kopsia dasyrachis* [5,6] showed promising activity in reversing MDR in vincristine-resistant KB cells. Since kopsiflorine is an indole alkaloid of the aspidofractinine group, and since we have in our ongoing studies of alkaloids from Malaysian plants uncovered numerous known, as well as new aspidofractinines,[5-22] we were prompted by this discovery to evaluate a whole series of aspidofractinine compounds for their potential in reversing MDR and would like to report our initial findings.

The results are presented in Table 1. The IC_{50} values of vincristine against sensitive (KB/S) and resistant (KB/VJ300) strains are 0.014 and 1.05 μg/mL respectively in the present experiments (75-fold resistance shown by the resistant strain).[23] All the compounds tested (except **7**) showed no appreciable cytotoxicity to both the sensitive (KB/S) as well as resistant (KB/VJ300) strains but compounds **1-6** significantly inhibited cell growth of the resistant strain (KB/VJ300) in a dose-dependent manner, when applied in the presence of vincristine (0.25 μg/mL). It can also be seen from the Table that some structure-activity correlations of these aspidofractinine compounds can be discerned. The aspidofractinine alkaloids **1-6** show appreciable activity in reversing MDR while the compounds **8-12** are practically inactive. It would appear from the results that the presence of carbamate and C(16)-ester functions on the basic aspidofractinine carbon skeleton are necessary; absence of the carbamate

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group results in substantial loss of activity (*c.f.* **4** *versus* **9**). An unsubstituted aromatic ring or presence of 11,12-methylenedioxy or 11,12-dimethoxy substituents do not adversely affect the activity (**1-6**), while the presence of only one aromatic methoxy substituent (**8**, **10**), or an aromatic hydroxy substituent (**12**) appear to adversely affect the activity. Replacement of the C(21)-H by a cyano function does not seem to have a negative effect (**3**), as does removal of C(16)-hydroxyl group (**4**), but the presence of a C(17)-hydroxyl function appears to be not desirable (**11**, **13**). The presence of unsaturation across C(16)-C(17) does not appear to exert an unfavorable effect provided the C(16)-ester function remains intact (**5**), but the presence of unsaturation in the piperidine ring (**11**) appears to be not favorable. In addition to the above compounds, other related aspidofractinine compounds including the semisynthetic dihydrokopsingine **13**,[12] 14,15-β-epoxykopsingine **14**,[13] kopsinganol **15**,[14] the heptacyclic carbonyl-bridged compound fruticosamine **16**,[15] methyl 11,12-methylenedioxychanofruticosinate **17**,[16] the heptacyclic oxo-bridged compound kopsidine B **18**,[17] the octacyclic caged compound, kopsinitarine D **19**,[18] the lactone bridged heptacyclic compound, paucidactine A **20**,[19] the pentacyclic indole pauciflorine A **21**,[20] the novel pentacyclic compound, danuphylline **22**[21] and the bisindole, nitaphylline[22] were also examined, but were all found to show only moderate or poor activity when tested at an initial concentration of 1.56 μg/mL. It would seem from this observation that too drastic a departure from the basic aspidofractinine skeleton results in loss of activity. Further studies focussing on the mechanism of reversal of MDR by kopsiflorine **1** have been initiated and the results indicate that **1** inhibits efflux of antitumour agents by its direct interaction with P-glycoprotein. Full details will be reported in a forthcoming disclosure.

Table 1. Cytotoxic activity of compounds **1-12**[a, b]

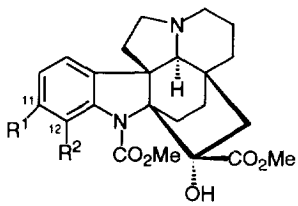
Compound	Reference	IC_{50}(μg/mL) (KB/VJ300)*
1	6	2.3
2	6, 7	1.6
3	8	2.5
4	6, 7	3.1
5	9	4.4
6	6, 7	5.6
7	9	*c*
8	6, 7	15.4
9	6, 7, 10	25
10	7	21
11	11	>25
12	7	>25

[a] KB/S and KB/VJ300 are vincristine-sensitive and -resistant human oral epidermoid carcinoma cell line respectively[23]

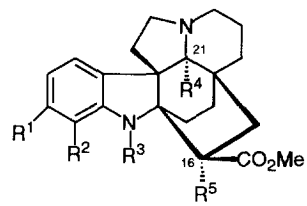
[b] All compounds tested (except **7**) showed no cytotoxicity towards KB/S and KB/VJ300 cell lines at the concentrations used

* with added vincristine 0.25 μg/mL, which did not affect the growth of the KB/VJ300 cells

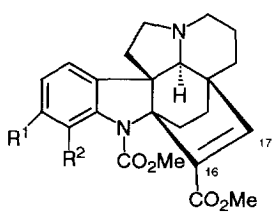
[c] cytotoxic (<50% cell growth shown at 7 μg/mL) to KB/S and KB/VJ300 cell lines



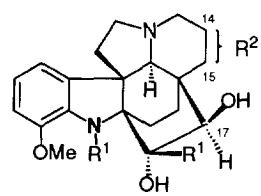
1 $R^1 = R^2 = H$ (kopsiflorine)
2 $R^1 = R^2 = OMe$ (11-methoxykopsilongine)
6 $R^1 = R^2 = OCH_2O$ (kopsamine)
8 $R^1 = H$, $R^2 = OMe$ (kopsilongine)
12 $R^1 = OH$, $R^2 = OMe$ (11-hydroxykopsilongine)



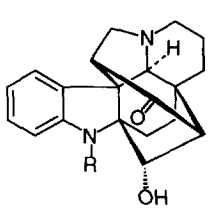
4 $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = CO_2Me$ (pleiocarpine)
9 $R^1 = R^2 = R^3 = R^4 = R^5 = H$ (kopsinine)
10 $R^1 = R^4 = R^5 = H$, $R^2 = OMe$, $R^3 = CO_2Me$ (12-methoxypleiocarpine)
3 $R^1 = R^2 = OCH_2O$, $R^3 = CO_2Me$, $R^4 = CN$, $R^5 = OH$ (lahadinine A)



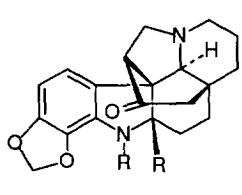
5 $R^1 = R^2 = OCH_2O$ (*N*-methoxycarbonyl-11,12-methylenedioxy-$\Delta^{16,17}$-kopsinine)
7 $R^1 = H$, $R^2 = OMe$ (*N*-methoxycarbonyl-12-methoxy-$\Delta^{16,17}$-kopsinine)



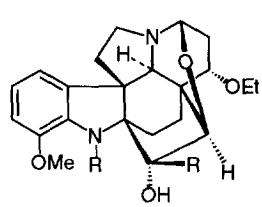
11 $R^1 = CO_2Me$, $R^2 = \Delta^{14,15}$ (kopsingine)
13 $R^1 = CO_2Me$, $R^2 = Nil$ (dihydrokopsingine)
14 $R^1 = CO_2Me$, $R^2 = \beta$–O (14,15-β-epoxykopsingine)
15 $R^1 = CO_2Me$, $R^2 = 15\alpha$-OH (kopsinganol)



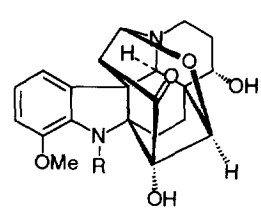
16 R = CO_2Me (fruticosamine)



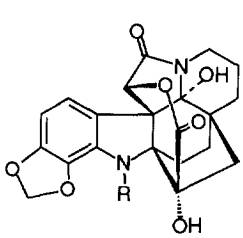
17 R = CO_2Me (methyl 11, 12-methylenedioxychanofruticosinate)



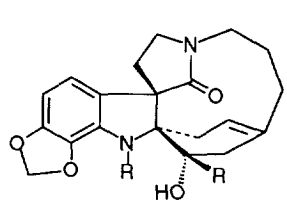
18 R = CO_2Me (kopsidine B)



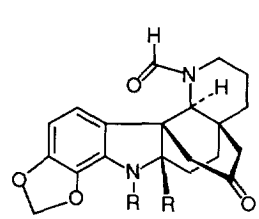
19 R = CO_2Me (kopsinitarine D)



20 R = CO_2Me (paucidactine A)



21 R = CO_2Me (pauciflorine A)



22 R = CO_2Me (danuphylline)

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