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C8c–C15 monoseco-analogues of the phenanthroquinolizidine alkaloids julandine and cryptopleurine exhibiting potent anti-angiogenic properties

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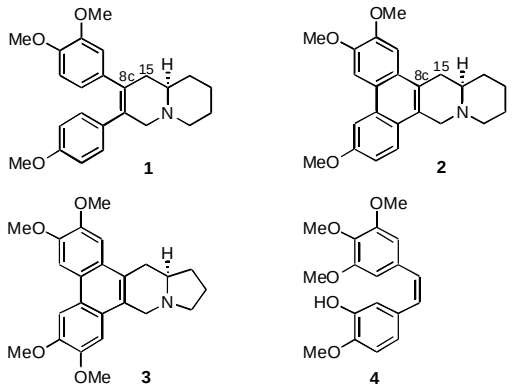
Abstract—Four enantiomerically pure monoseco-analogues, **5**, **7**, **9**, and **11**, of the phenanthroquinolizidine alkaloid julandine (**1**) and four of congener cryptopleurine (**2**), viz. compounds **6**, **8**, **10**, and **12**, have been prepared and subjected to preliminary biological evaluation. These analogues show dramatically reduced cytotoxicity compared with the parent system **2** but they are, nevertheless, potent anti-angiogenic agents.

Plant-derived phenanthroquinolizidine alkaloids such as julandine (**1**)[1,2] and cryptopleurine (**2**)[3] exert interesting anti-viral, anti-fungal, and cancerostatic effects as well as inhibiting proteosynthesis in eukaryotic cells.[4] Closely related phenanthroindolizidine alkaloids such as (−)-tylophorine (**3**) have recently been shown to inhibit the growth of various human cancer cell lines, especially multi-drug resistant ones such as KB-V1, with IC_{50} values in the low nanomolar range and being comparable, therefore, to certain clinically used drugs.[5] In addition, compound **3** and some readily accessible derivatives have proven to be active, both in vitro and in vivo, against HEPG2 tumor cells and the unique mode of action involved has led to suggestions that this natural product and its analogues may have potential clinical utility in the treatment of certain refractory cancers.[6] As a consequence of their interesting biological profiles, the phenanthroquinolizidine and phenanthroindolizidine alkaloids have been the subject of a number of synthetic studies[4] most of which have been summarized in a recent review by Huang and co-workers.[4a] In addition, a number of analogues of these natural products have been prepared and subjected to various forms of biological screening.[4a,7] These generally rather dated studies indicate that certain analogues can display useful properties including anti-fungal and cytotoxic activities.[4a] However, almost nothing appears to be known about the anti-angiogenic profile of the title alkaloids and their derivatives and this is despite their rather close structural resemblance to the potent anti-mitotic and anti-angiogenic agent combretastatin A4 (**4**) which is now in clinical trials (in a pro-drug form) for the treatment of patients with advanced solid tumors.[8] Herein, therefore, we report on the identification of new and readily acces-



Keywords: Anti-angiogenic; *cis*-Stilbene; Combretastatin A4; Phenanthroquinolizidine.
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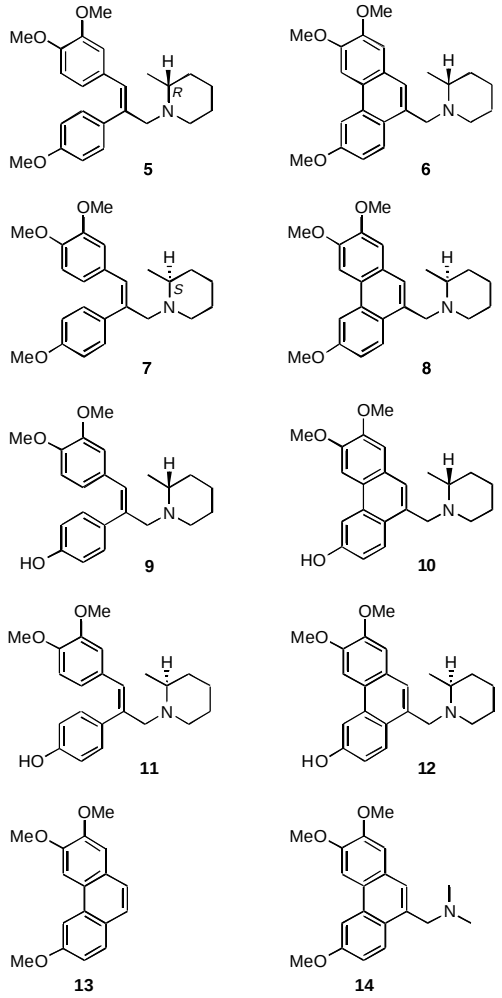
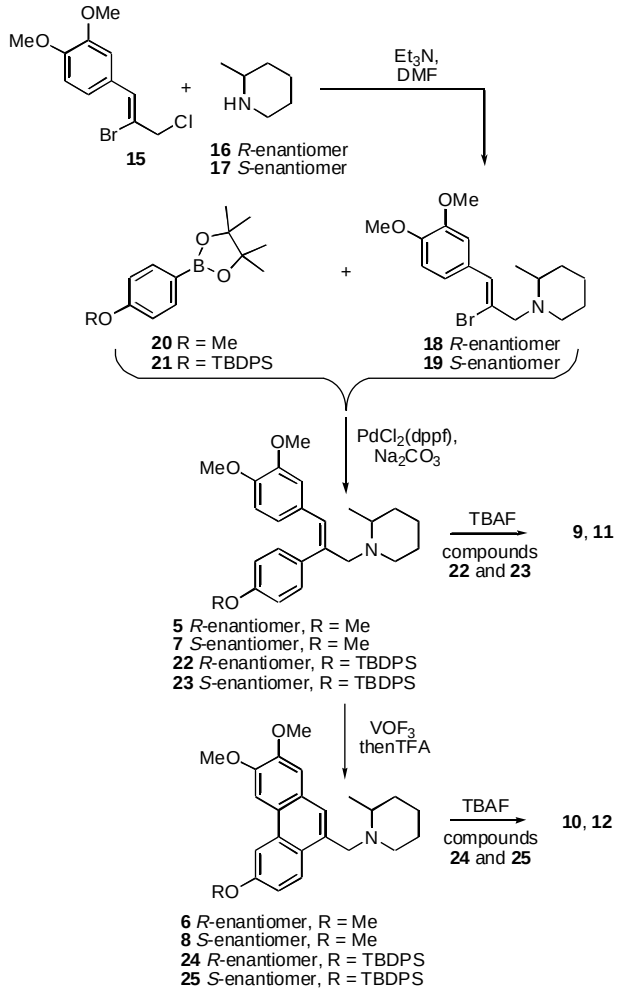
sible C8c–C15 monoseco-analogues, **5**–**12**, of the title alkaloids that do indeed exhibit significant anti-angiogenic activity whilst being orders of magnitude less cytotoxic than the parent natural product **2**.

We have recently detailed[9] the preparation of certain C15-functionalized C8c–C15 monoseco-analogues of compounds **1** and **2** by using *gem*-dihalocyclopropanes as (readily available) building blocks for this purpose.[10] Unfortunately, and contrary to expectations engendered by an earlier report,[11] these analogues failed to cyclize,[9] in the presence of base, to give the title alkaloids. Nevertheless, as detailed immediately below, this chemistry has allowed for the rapid assembly of the hitherto unreported and biologically interesting C8c–C15 seco-analogues **5**–**12** of the (+)- and (−)-forms of the title natural products. In order to develop some understanding of the SAR profile of the title class of analogues the simpler phenanthrenes **13** and **14** were also prepared.



The route used in the synthesis of analogues **5**–**12** is shown in Scheme 1 and starts with alkylation of the previously described[9] allylic chloride **15** using the relevant enantiomeric form, **16** or **17**, of 2-methylpiperidine, each

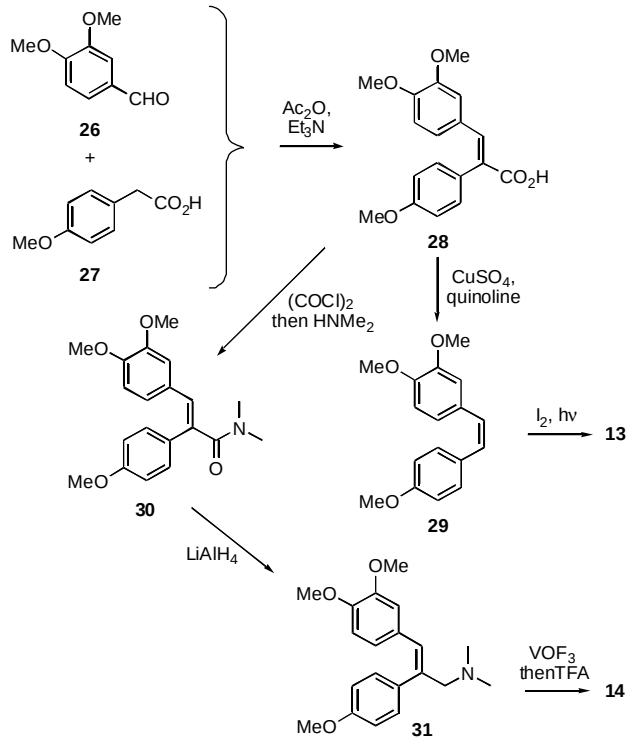


Scheme 1.

of which is readily obtained from the commercially available racemate using the resolving agent (+)-mandelic acid to give the *R*-enantiomer and (−)-mandelic acid to give the *S*-enantiomer.[12] The resulting conjugates, **18** and **19** (obtained in ca. 98% yield in each case), were subjected to Suzuki–Miyaura cross-coupling with the previously reported aryl boronates **20**[9] or **21**[13] to give *cis*-stilbenes **5** (74%), **7** (73%), **22** (73%) or **23** (69%), the first two of which represent the enantiomeric forms of the C8c–C15 seco-analogues of julandine (**1**). The assignment of the illustrated *Z*-configuration associated with the double bond in each of these products follows from the observation of significant NOE interactions between the relevant olefinic hydrogen and the methylene protons attached to the carbon linking the *cis*-stilbene moiety to the nitrogen of the piperidine ring. Reaction of compounds **22** and **23** with tetra-*n*-butylammonium fluoride (TBAF) afforded the C6-hydroxy analogues **9** (85%) and **11** (87%), respectively. Access to the equivalent cryptopleurine-type analogues involved oxidative cyclization of the *cis*-stilbenes **5**, **7**, **22**, and **23** with Liepa's reagent (VOF_3)[14] followed by treatment with trifluoroacetic acid (TFA). In this manner, the corresponding phenanthrenes **6** (63%), **8** (48%), **24** (68%), and **25** (58%), respectively, were obtained. Removal of the TBDPS-groups associated with the last two of these

products was achieved using TBAF and thereby affording the remaining target analogues, namely phenols **10** (93%) and **12** (82%), respectively. The spectral data obtained on each of compounds **5**–**12** were consistent with the assigned structures and each member of the relevant enantiomeric pair displayed optical rotations of essentially the same magnitude but opposite sign. The *R*-configured materials were laevorotatory in each case.

The reaction sequence (Scheme 2) leading to the trimethoxyphenanthrene **13** started with the condensation of the commercially available aldehyde **26** and arylacetic acid **27** under conditions defined by Oishi and Kurosawa.[15] The resulting α-arylcinnamic acid **28** (45%) was decarboxylated by heating with copper(II) sulfate in refluxing quinoline[16] and the major product of the reaction was the *cis*-stilbene **29** (70%), although this was accompanied by small amounts (5%) of the corresponding *trans*-isomer. Attempts to convert the former product into the target phenanthrene **13** by treating it with Liepa's reagent then TFA only resulted in the formation of the *trans*-stilbene observed in the previous step. However, when an ether/dichloromethane solution of compound **29** containing catalytic amounts of iodine was irradiated with light from a medium-pressure mercury vapor lamp[17] the desired phenanthrene **13**[18] could be obtained in 51% yield. The preparation of the *N*,*N*-dimethylaminomethyl derivative, **14**, of phenanthrene **13** involved initial conversion of the acid **28** into the corresponding *N*,*N*-dimethylamide **30** (68%) under standard conditions. $LiAlH_4$-promoted reduction of the latter compound to the corresponding amine **31** (74%) followed by oxidative cyclization of this material using VOF_3 and TFA then gave target **14**[19] in 56% yield.



Scheme 2.

Each of compounds **5**–**12** was screened, at eight different concentrations, against a panel of nineteen human and other cancer cell lines as listed in Table 1.[20] An authentic sample of natural product **2** was also tested against the same panel. As a consequence it became clear that the monoseco-analogues (**6**, **8**, **10**, and **12**) of cryptopleurine (**2**) are ca. three orders of magnitude less cytotoxic than the parent compound while the related *cis*-stilbenes show essentially no toxicity whatsoever. Furthermore, the configuration (*R* vs *S*) at the single stereogenic center within these analogues has essentially no impact on activity. Clearly, then, the scission of the C8c–C15 bond within the title natural products leads to derivatives with dramatically reduced cytotoxicity profiles. The phenanthrenes **13** and **14** also proved to be only weakly cytotoxic.

The anti-angiogenic properties of compounds **5**–**14** were determined in an in vitro assay using rat aorta blood vessel fragments.[21] Unfortunately, solubility problems prevented analogous testing of cryptopleurine itself. Nevertheless, the results shown in Table 2 indicate that most of the analogues **5**–**12** completely inhibited blood vessel growth at 100 μg/mL. Even more significantly, compounds **6** and **8** also completely inhibited blood vessel growth at the 10 μg/mL level, while several others were still able to inhibit growth by more than 50% at the 1 μg/mL level. It is worth noting that every single one of these analogues of alkaloids **1** and **2** is more active, at least at the 100 μg/mL level, than PI-88, a polysulfated oligosaccharide which exhibits anti-angiogenic properties in vivo and which is now in clinical development as an agent for the treatment of certain cancers.[22] A further important facet of these results is that the phenanthrenes seem to be more active than the corresponding *cis*-stilbenes, while chirality has little or no impact on the anti-angiogenic properties of the title analogues. In addition, those phenanthrenes incorporating a free hydroxy group are slightly less active than their methoxy counterparts, perhaps because of a reduction in their lipophilic properties. Interestingly, the phenanthrene and aminomethyl subunits associated with compounds **5**–**12** both seem to be making important contributions to their anti-angiogenic properties as judged by the test results observed for the simpler analogues **13** and **14**.

The origins of the significant anti-angiogenic properties of compounds **5**–**14** have not been established thus far. However, the capacity of certain combretastatin A4 derivatives/analogues to act as vascular targeting agents, by binding to tubulin in newly formed endothelial cells lining the tumor vasculature,[23] suggests this mode of action may be involved in the present case. This situation, coupled with the considerable interest in the possibility of separating any cytotoxic activity of combretastatin-type compounds from their ability to effect vascular shutdown,[8,23b] serves to highlight the therapeutic potential of C8c–C15

Table 1. IC_{50} values (μM) determined for compounds **2** and **5**–**14** in cytotoxicity testing against a range of cancer cell lines[a]

Cell line[b]	Compound										
	(−)-**2**[c]	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
TFI	—	>20	7.65	>20	7.20	>20	8.40	>20	9.80	>20	6.75
CTLL2	—	>20	8.10	>20	7.61	>20	8.10	>20	9.09	17.50	3.39
BT20	0.003	>20	10.89	>20	10.24	>20	11.57	>20	13.73	>20	7.38
MatLyLu	0.003	>20	~15	>20	~15	>20	>20	>20	>20	>20	17.34
KHOS-NP	0.010	>20	13.99	>20	12.28	>20	13.42	>20	15.43	>20	17.14
A431	0.003	>20	8.09	>20	8.12	>20	20.00	>20	13.77	>20	5.37
A375	0.003	>20	10.05	>20	8.96	>20	9.38	>20	11.25	>20	7.1
A549	0.003	>20	12.02	>20	11.25	>20	15.18	>20	16.55	>20	17.03
HCT-15	0.003	>20	8.33	>20	7.82	>20	7.97	>20	8.41	>20	8.27
HT1376	0.004	>20	9.51	>20	9.68	>20	6.04	>20	8.31	>20	7.88
PA-1	0.002	>20	7.27	>20	7.38	>20	—	>20	~8	>20	4.16
HEPG2	—	>20	7.27	>20	8.12	>20	—	>20	8.33	—	—
HEK293	—	>20	17.08	>20	15.50	>20	—	>20	14.83	—	—
BUD-8	0.006	>20	>20	>20	>20	>20	—	>20	18.24	>20	>20
RAMOS	0.003	>20	15.50	>20	>20	>20	—	>20	18.16	>20	16.65
DAUDI	0.002	>20	12.04	>20	9.08	>20	—	>20	13.50	>20	15.71
MES-SA	0.003	>20	9.04	>20	10.07	>20	—	>20	14.12	>20	9.1
MES-SA-Dx5	0.003	>20	8.46	>20	11.73	>20	—	>20	11.79	>20	16.78
MCF-7	0.003	—	—	—	—	—	—	—	—	>20	18.59

— = not tested.

[a] DMSO was used as solvent in these assays unless otherwise stated.

[b] TFI = human erythroleukemia, CTLL2 = murine cytotoxic T cells, BT-20 = human breast carcinoma, MatLyLu = rat prostate carcinoma, KHOS-P = human osteosarcoma, A431 = human epidermoid carcinoma, A375 = human melanoma, A549 = human lung carcinoma, HCT-15 = human colon carcinoma, HT1376 = human bladder carcinoma, PA-1 = human ovarian teratocarcinoma, HEPG2 = human hepatoma, HEK293 = human embryonic kidney cells, BUD-8 = human fibroblast, RAMOS = Burkitt lymphoma, DAUDI = Burkitt lymphoma, MES-SA = human uterine sarcoma, MES-SA-Dx5 = human uterine sarcoma derived from MES-SA, and MCF-7 = human breast carcinoma.

[c] Methanol used as solvent in the assaying of this compound because of difficulties dissolving it in DMSO.

Table 2. Anti-angiogenic properties of compounds **5**–**14** as determined in a rat aorta assay[a]

Compound	% inhibition of blood vessel growth			
	at 100 μg/mL	at 10 μg/mL	at 1 μg/mL	at 0.1 μg/mL
5	100	62	59	36
6	—[b]	100	48	29
7	100	43	68	22
8	100	100	42	17
9	100	65	43	—
10	100	85	47	18
11	100	42	53	31
12	100	74	58	35
13	83	42	—	—
14	—	100	22	7
PI-88	74	—	—	—

[a] Assays conducted according to the method of Parish et al.[21] using DMSO as solvent.

[b] Not tested at this concentration due to lack of solubility. — = not tested.

monoseco-analogues, such as compounds **5**–**12**, of the title alkaloids. Clearly further studies in this area are warranted and are being planned.

Acknowledgments

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