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Synthesis and structure–activity studies of antofine analogues as potential anticancer agents

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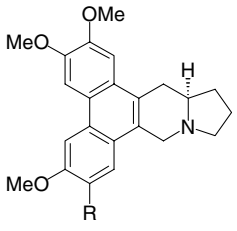
Abstract—Due to the profound cytotoxicities and interesting biochemical aspects, phenanthroindolizidine alkaloids have received an attention as potential therapeutic leads. To define the features of the molecule that are essential for cytotoxicity, we have synthesized and evaluated a series of phenanthroindolizidine alkaloid, antofine, analogues with different substituents on the phenanthrene ring. The systematic structure activity relationship studies elucidate the essential functional group requirement of phenanthrene ring, providing the basis for further development of phenanthroindolizidine alkaloids.

Phenanthroindolizidine alkaloids are a small group of alkaloids isolated mainly from *Cynanchum*, *Pergularia*, *Tylophora*, and some genera of the Asclepiadaceae family.[1,2] These pentacyclic natural products exhibit a variety of biological effects including antitumor, antiamoebic, antibacterial, and antifungal activities.[1–3] Among these interesting biological activities, the most intriguing property is the profound cytotoxic activity against various cancer cell lines. For example, (−)-antofine ((−)-**1**, Fig. 1) has IC_{50} values in the low nanomolar range against drug-sensitive KB-3-1 and multidrug-resistant KB-V1 cancer cell lines, comparable to that of clinically employed cytotoxic drugs.[4,5] While (−)-tylophorine ((−)-**2**), the representative phenanthroindolizidine alkaloid, showed a decreased cytotoxicity toward the KB cancer cell lines, 7-desmethyl analogue of tylophorine **3** exhibited similar cytotoxicity as antofine.[4] In addition, (+)-tylophorine ((+)-**2**), the optical antipode of natural product, inhibited the growth of KB variants and HepG2 cells to noteworthy extent with EC_{50} values in the low nanomolar range.[6] Beyond the potent cytotoxicity, an additional interesting biological property of phenanthroindolizidine alkaloids in antitumor activity is that the mode of action is unique and distinctly different from other known antitumor drugs.[5,6]



R = H (-)-antofine ((-)-**1**)
R = OMe (-)-tylophorine ((-)-**2**)
R = OH (-)-7-desmethyltylophorine (-)-**3**

Figure 1. Chemical structures of compounds **1**–**3**.

The structure–activity relationship (SAR), obtained by comparing the cytotoxic activities of the various natural phenanthroindolizidine alkaloids, indicates that (i) a rigid phenanthrene structure is a prerequisite for a high cytotoxic activity, (ii) the unshared electron pair on nitrogen is important for a high activity, and

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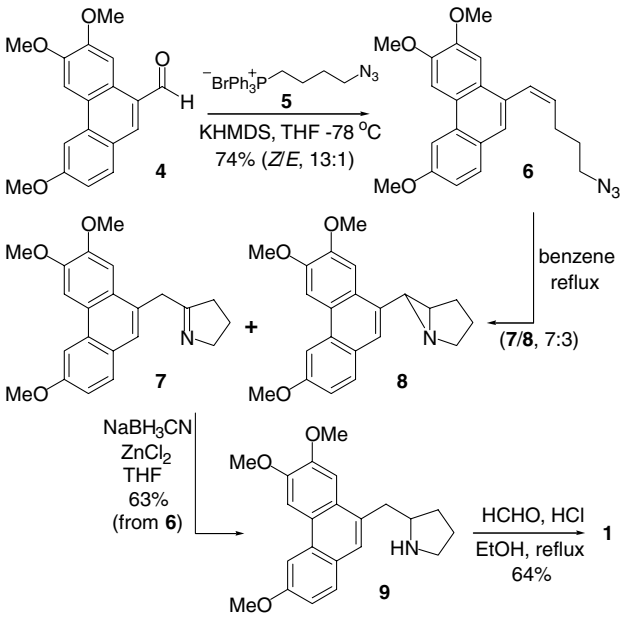
(iii) the cytotoxic potency is highly sensitive to the substitution types and patterns on the phenanthrene ring.[3,4,7] Although the preliminary SAR of phenanthroindolizidine alkaloids has been partially elucidated, the molecular target site and mechanism of action are still not clear. Recent mechanism studies by one of us indicated that antofine exhibits an inhibitory activity on cell proliferation by arresting the G2/M phase of the cell cycle.[5] Cheng and co-workers reported that tylophorine analogues may alter the activity of several transcription factors, subsequently modulating the expression of target genes involved in the control of cell growth, differentiation, and survival.[6] Very recently, Xi reported that antofine has favorable molecular interaction with the bulged regions of DNA and RNA.[8]

Due to the profound cytotoxic activity and interesting biochemical aspects, phenanthroindolizidine alkaloids have received a significant attention as potential therapeutic leads. However, this class of natural products has not yet been developed for clinical use. The major drawbacks to the potential therapeutic use of this compound class are the serious central nervous side effects[9] and low water solubility. Furthermore, our unpublished mouse studies revealed that (−)-antofine (**1**) is metabolically unstable, resulting in a poor pharmacokinetic profile. The methoxy moiety on the phenanthrene ring proved to be the major cause of the in vivo metabolic instability. This finding prompted us to perform the systematic structure activity relationship studies of phenanthrene ring of antofine to identify the essential structural elements required for the cytotoxic activity and to optimize the therapeutic index of these compounds. We present here the synthesis and cytotoxicity evaluation of a series of antofine analogues with functional group modifications on the phenanthrene ring.

Due to their exceptional bioactivity and unusual architecture coupled with low natural abundance, many synthetic methodologies have been developed by medicinal and synthetic chemist for the synthesis of natural phenanthroindolizidine alkaloids and their derivatives.[2,3,10] As part of our ongoing program, we have previously developed two different asymmetric synthetic approaches to (−)-antofine.[11] Although our previous approaches are efficient enough to apply for the synthesis of antofine analogues, a different synthetic route was explored for the rapid preparation of antofine analogues in racemic form.

Racemic antofine (**1**) was synthesized by employing the intramolecular 1,3-dipolar cycloaddition[12] as a key step (Scheme 1). The starting material for our synthesis was the known phenanthryl aldehyde **4**, which was obtained from the commercially available homoveratric acid and *p*-anisaldehyde via the conventional five-step sequence according to the previously reported procedure.[13] Wittig reaction of aldehyde **4** with the known phosphonium salt **5**[14] in the presence of KHMDS gave mainly (*Z*)-ω-azidoalkene **6** as an inseparable *Z*/*E* mixture (74%, 13:1 *Z*/*E*). Upon heating the azidoalkene **6** in refluxing benzene, a mixture of imine **7** and aziridine **8** was obtained in a 7:3 ratio. Since the isolated imine **7** and



Scheme 1. Synthesis of antofine (**1**).

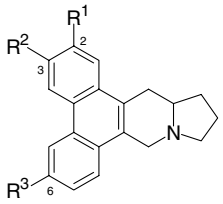
aziridine **8** were turned out to be unstable, the crude reaction mixture of above reaction was directly treated with $ZnCl_2$ and $NaBH_3CN$[15] to give the previously known pyrrolidine **9**[11,16] in 63% two-step yield. Finally, the Pictet–Spengler cyclomethylenation of amine **9**, using the previously reported reaction conditions[11,16,17] (formaldehyde, HCl, EtOH, reflux), afforded the racemic antofine (**1**)[18] in 64% yield.

With the new facile synthetic route, we could properly prepare the antofine analogues **10**–**17** (Table 1) from the appropriate starting materials in similar overall yield. Further, in order to introduce phenolic hydroxyl groups in compounds **13**–**15**, the benzyl protecting group was employed from the beginning and removed by hydrogenation at the final step of the synthesis.

The cytotoxic activity of antofine and its analogues in various cancer cells was determined by the sulforhodamine B (SRB) assay as described previously.[5,19] As shown in Table 1, the naturally occurring antofine ((−)-**1**) was approximately 2-fold more potent than the racemic form **1**. This result led us to the reasonable assumption that cytotoxicity is largely due to only one enantiomer in the racemic mixture without the significant interference of the other enantiomer.

By comparing the cytotoxic activities of the racemic analogues **10**–**17** it shows that the cytotoxic potency was highly dependent, as expected, on the substitution types and patterns on the phenanthrene ring. Replacement of the methoxy group at the 3- or 6-position with a bulkier isopropoxy group (compounds **11** and **12**) did not significantly alter the cytotoxicities against the cancer cells tested. These results indicated that bulky alkoxy groups are well tolerated at 3- or 6-position of the phenanthrene ring and modest alterations of these positions are seemingly allowed. However, replacing the 2-meth-

Table 1. Structures and cytotoxic activities for antofine ((−)-**1** and **1**) and its analogues (**10**–**17**)



Compound	R^1	R^2	R^3	IC_{50}[a] (nM)		
				HCT 116[b]	HT-1080[c]	A549[d]
(−)-**1**	OMe	OMe	OMe	9.9	9.6	10.4
1	OMe	OMe	OMe	29.4	27.5	25.1
10	O^{i}Pr	OMe	OMe	783.2	>1000	>1000
11	OMe	O^{i}Pr	OMe	24.7	37.4	53.9
12	OMe	OMe	O^{i}Pr	19.2	23.6	40.5
13	OH	OMe	OMe	68.3	72.6	>100
14	OMe	OH	OMe	21.4	19.8	49.8
15	OMe	OMe	OH	1.1	1.1	1.4
16	OMe	OMe	H	49.3	59.1	>100
17	–OCH_2O–		OMe	364.1	453.0	747.2

[a] All values are means of a minimum of three experiments.
[b] Human colorectal carcinoma.
[c] Human colorectal adenocarcinoma.
[d] Human lung carcinoma.

oxy group of antofine with an isopropoxy group resulted in a complete loss of activity (compound **10**), which is probably caused by unfavorable steric interactions of the bulky hydrophobic alkyl group at 2-position with the binding site.

In another variation, we replaced the electron-donating methoxy group of antofine with a polar phenolic hydroxyl group. Replacing the methoxy group at 2-position with a hydroxyl group (compound **13**) caused more than 2-fold decrease in potency, whereas modification at 3-position (compound **14**) did not significantly affect the activity. Replacement of the 6-methoxy group in antofine by a hydroxyl group, which led to the naturally occurring 6-*O*-desmethylantofine **15**, caused a significant increasing activity (IC_{50}, ca. 1 nM) as compared to racemic antofine. The pronounced cytotoxicity of natural (−)-**15** toward the KB cancer cell lines has been reported previously by Stærk.[4] Our result is in agreement with previous studies and indicates the importance of a hydroxyl group at 6-position for high cytotoxicity.

To further examine the influence of substitutions at the 6-position, compound **16**[20] was prepared and tested. Compound **16**, possessing a hydrogen atom at 6-position instead of the methoxy group of antofine, was only ~2-fold less potent than racemic antofine but >50-fold less potent than the hydroxyl group containing **15**. These results together with the reported bioactivities of **2** and **3** suggested that the binding site of antofine might contain a hydrogen-bond acceptor close to the 6-methoxy group of antofine.

Next, replacement of the 2,3-dimethoxy substituents by 2,3-dioxymethylene group was investigated. The antofine analogue **17** has been previously isolated from *Ficus septica* and named as ficuseptine C, of which cytotoxicity has not been reported yet.[21] Compound **17** was >100 times less potent than racemic antofine. This result together with the results obtained with compounds **10** and **11** indicates that one or both methyl groups are important for activity and steric bulkiness is not tolerated at position 2. Alternatively, If we consider Xi's proposed binding model of antofine with bulged DNA,[8a] the decreased potency of **17** could be explained by the repulsive steric interactions between the ribose ring of DNA and the conformationally restricted dioxymethylene group.

In conclusion, we have synthesized and evaluated a series of antofine analogues with different substituents on the phenanthrene ring. The present survey revealed that steric bulkiness is not tolerated at 2-position of antofine, while modest alteration at position 3 is probably allowed. The substituent at the 6-position seems to be involved in hydrogen bonding as a donor. These results are of interest for establishing the SAR of phenanthrene ring of phenanthroindolizidine alkaloids and providing the basis for further development. Further studies toward more potent and pharmacologically suitable phenanthroindolizidine alkaloids, based on these findings, are currently in progress in our laboratory.

Acknowledgments

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