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Phenanthroindolizidines and Phenanthroquinolizidines: Promising Alkaloids for Anti-Cancer Therapy

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Abstract

The phenanthroindolizidine and phenanthroquinolizidine alkaloids, typified by tylophorine and cryptopleurine, are a family of plant-derived small molecules with significant therapeutic potential. The plant extracts have been used in herbal medicine and the isolated compounds have displayed a range of promising therapeutic activity such as anti-ameobicidal, anti-viral, anti-inflammatory and anti-cancer activity. Despite their therapeutic protential, no compounds in this class have fully passed clinical trials. Drawbacks include low *in vivo* anti-cancer activity, central nervous system toxicity and low natural availability. A number of biological effects of these compounds, such as protein and nucleic acid synthesis suppression, have been identified, but the specific biomolecular targets have not yet been identified. Significant effort has been expended in the synthesis and structure-activity-relationship (SAR) studies of these compounds with the hope that a new drug will emerge. This review will highlight important contributions to the isolation, synthesis, SAR and mechanism of action of the phenanthroindolizidine and pheanthroquinolizidine alkaloids.

INTRODUCTION

Isolation and Structure

The phenanthroindolizidine and phenanthroquinolizine alkaloids are a family of plant-derived alkaloids composed of over sixty compounds Fig. (1) [1]. They have been isolated from the Asclepiadaceae and Moraceae plant families primarily from India and Japan [2]. One of these plants, *Tylophora indica*, was included as an official drug in the Bengal pharmacoepia of 1884 [3]. The leaves of this plant have been used for the treatment of asthma as well as bronchitis, rheumatism and dysentery in India.

The medicinal properties of these plants spurred the isolation and characterization of the individual alkaloid constituents. The isolation of the major alkaloid from *Tylophora indica*, tylophorine, was reported in 1935 [4] and its structure and stereochemistry were reported in 1960 [5]. Tylophorine was initially reported as having the (S)-absolute stereochemistry, but a total synthesis and optical rotation measurement led to the revision to (R) [6].

The most recent comprehensive review on the isolation, synthesis and structure-activity-relationship studies of the phenanthroindolizidine and phenanthroquinolizidine natural products was published by Huang and co-workers in 2001 [1,2,7,8]. This review will highlight the synthetic and medicinal studies that have appeared subsequently.

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Therapeutic Potential

The biological properties of these alkaloids range from cancer cell growth inhibition (*in vitro* and *in vivo*) [1,9–16] and anti-inflammatory [3,17] activity to anti-ameobicidal [18] and anti-viral [19] activity. A summary of their GI_{50} concentrations in various cancer cell lines is given in Table 1.

Although some phenanthroindolizidines are too toxic, several, e.g. both enantiomers of tylophorine, have demonstrated low toxicity in rodents. (R)-Tylophorine was non-toxic in rats up to a dose of 500 mg/kg [3] and non-toxic in hamsters up to 60 mg/kg [18]. Some CNS toxicity, ptosis, sedation, decreased motor activity and staggering gait, has been observed in rats at high doses of (R)-tylophorine [3].

In the early 1960's tylocrebrine, Fig. (1), was advanced to clinical trials but failed due to CNS toxicity, manifested as disorientation and ataxia [20]. Suffness proposed that more polar analogs, which cannot clear the blood brain barrier, could have less side effects [20].

Medicinal interest in the phenanthroindolizidines was revived in the 1990's when it was shown that a number of these compounds are highly potent (10^{-8}–10^{-12} M) growth inhibitors in the NCI's 60 cell line assay and that their mode of action appears to be different than other known anti-cancer compounds based on their COMPARE [21] analysis [13]. Both drug-sensitive and multidrug-resistant cancer cells demonstrate growth inhibition in the low nanomolar range with this class of compounds (vide infra) [11–13].

MECHANISM OF ACTION

The specific cellular target of the phenanthroindolizidines and quinolizidines is unknown, but a significant amount of cellular activity of these compounds is known. It was determined in the 1970's that tylocrebrine, tylophorine and cryptopleurine inhibit protein synthesis, and to a lesser extent RNA and DNA synthesis [9,22]. Inhibition of protein synthesis in Ehrlich ascites-tumor cells by 50% was achieved using 10^{-6} M tylophorine, 10^{-7} M tylocrebrine and 10^{-8} M cryptopleurine [9]. A much higher amount was required for nucleic acid synthesis inhibition. Tylocrebrine inhibits protein and DNA synthesis irreversibly in cells, as determined by lack of incorporation of carbon-14 labeled lysine and thymidine, respectively. Tylocrebrine reversibly inhibited RNA synthesis as measured by the amount of ^{14}C-labeled uridine incorporation [22]. It was observed that tylocrebrine and cryptopleurine prevent the breakdown of polyribosomes and release of nascent peptides and it was inferred that they inhibit peptide chain elongation [22,23]. It was further determined that tylocrebrine was not an inhibitor of peptide bond formation [22]. The 40S ribosomal subunit has been postulated to house the binding site of these drugs [23,24].

Other Targets

Besides the ribosome, other targets have been shown to interact with these compounds. Thymidylate synthase, an enzyme important in DNA synthesis, was inhibited by micromolar amounts of tylophorinidine [25]. Dihydrofolate reductase, another enzyme important in nucleic acid biosynthesis, was also inhibited by tylophorinidine [26]. Antofine has been shown to bind bulged DNA [27] and tabacco mosaic virus [19]. Nuclear Factor-kB, a transcription factor, has been suggested to be a mechanism of drug resistance because of its anti-apoptotic role [28, 29]. NF-kB mediated transcription was inhibited by (*S*)-tylophorine and analogs [13,15]. Thus, inhibition of NF-kB may account for the cytostatic activity of these drugs in multi-drug-resistant cancer cell lines.

To date, the specific cellular target protein or nucleic acid has not been identified. That the molecules can interact with a number of protein and nucleic acid synthesis cellular machinery

is clear. While it is likely that the compounds operate *via* a number of inhibition mechanisms, which might be to their advantage, to date no protein isolation *via* pull-down (affinity chromatography) experiments has been reported and no specific nucleic acid targets have been identified. It would be advantageous to drug development to understand how these drugs interact with specific cellular targets.

Many targets may be involved as the compounds inhibit both protein and nucleic acid synthesis. Since protein synthesis is more potently inhibited than nucleic acid synthesis, this is probably the drug's primary mode of growth inhibition. The nucleic acid synthesis inhibition, including transcription *via* NF-kB may serve as important supplementary activity that increases potency, especially in MDR cells.

Total Synthesis of Phenanthroindolizidines and Phenanthroquinolizidines

The potent medicinal properties and interesting structures of the phenanthroindolizidines and phenanthroquinolizidines have attracted synthetic organic chemists and medicinal chemists for several decades [1]. The total synthesis of these compounds provides a sustainable source as well as ready access to derivatives for structure-activity-relationship studies. Tylophorine, antofine, cryptopleurine and septicine have been the most popular total synthesis targets to date. Since the target molecules contain a chiral center, their stereoselective synthesis is important to SAR studies and subsequent drug development, where the development of an enantiomerically pure compound would be ideal in order to avoid possible deleterious activity of the other enantiomer as well as use of unnecessarily high doses. Therefore, although a number of racemic routes for the syntheses of these natural products have been reported [1,2, 16,30–40] (several of them are quite concise) this review will summarize only the enantioselective syntheses in further detail.

Both the (*R*)- and (*S*)-tylophorine enantiomers have been synthesized selectively using the chiron approach, starting with either proline [41,42], glutamic acid [6] or pyroglutamate [43] as well as the chiral auxiliary approach manifested in diastereoselective Grignard additions [44] and double Michael reactions [45], respectively. Recently, we reported the first catalytic asymmetric route to (S)-tylophorine [46].

(*R*) and (*S*)-Antofine have been synthesized enantioselectively starting from proline [42], pyroglutamate [47], and from an allylic alcohol obtained enantioselectively *via* an enzymatic resolution [48]. Antofine has also been synthesized enantioselectively *via* enantioselective catalytic phase transfer alkylation [49].

(*R*)- and (*S*)-Cryptopleurine has been synthesized enantioselectively starting from (*S*)-(+)-α-aminoadipate [6] and an allylic alcohol obtained *via* an enzymatic resolution [48], and by using a proline-derived chiral auxiliary to direct the facial selectivity of an alkylation reaction [50].

The naturally occurring seco analogs of tylophorine and cryptopleurine, septicine and julandine, respectively, have been synthesized enantioselectively as well. (*R*)-Septicine has been synthesized en route to (*R*)-tylophorine *via* an enantioselective alkylation controlled by a chiral ester [44]. (*R*)-Julandine has been synthesized using the same enantioselective alkylation route as employed in the synthesis of (*R*)-cryptopleurine, where a proline-derived auxilliary directed the facial selectivity [50].

Asymmetric Synthesis *via* the Chiral Pool

The first enantioselective synthesis of (*S*)-(+)-tylophorine, the unnatural enantiomer, was reported by Rapoport and co-workers in 1983 [6]. This synthesis established the absolute configuration of the natural compound, which was opposite the one synthesized by these researchers. The source of chirality was (*S*)-glutamic acid. The synthesis began with

condensation of veratraldehyde (**1**) with (3,4-dimethoxyphenyl)acetonitrile (**2**), Scheme 1. The resulting stilbene **3** was oxidized to the phenanthrene with VOF_3. Subsequent reduction of the nitrile with diisobutyl-aluminum hydride (DIBAL-H) provided phenanthryl aldehyde **4** in 89% overall yield. Condensation of aldehyde **4** with (*S*)-diisopropylglutamate provided an initial Schiff base that was rapidly converted to the corresponding aminal upon reaction with an additional equivalent of (*S*)-diisopropylglutamate (to prevent epimerization). The crude aminal was reduced with $NaCNBH_3$, yielding the desired N-(phenanthrylmethyl)-glutamate **5**. Amide formation in warm MeOH/HOAc and hydrolysis of the remaining ester provided acid **6** in 88% yield (four steps). Conversion of the acid to the acid chloride (oxalyl chloride, DMF) and subsequent intramolecular Friedel-Crafts alkylation promoted by $SnCl_4$ provided ketone **7**. Depending upon the reducing agent used, ketone **7** provided either the C(14)-R or C(14)-S alcohols, **8** or **9**, respectively (tylophorine numbering). Thus, hydrogenation (H_2, Pd/C) of **7** provided **8** selectively. Alcohol **8** could be taken on to the pyrrolidine **10**. This compound has demonstrated good anti-cancer activity *in vitro* (vide infra). The C14-(*S*)-alcohol **9** was obtained by reduction of ketone **7** with L-Selectride. Subsequent reduction of **9** with $LiAlH_4$ provided pyrrolidine **11**, which demonstrated very good *in vitro* and *in vivo* anti-cancer activity (vide infra). (*S*)-Alcohol **9** was deoxygenated by conversion to the corresponding chloride and subsequent reduction. The resulting amide was reduced with $LiAlH_4$ to provide (*S*)-tylophorine. The observed optical rotation of +15° for this compound is somewhat lower than that obtained by other research groups (vide infra). It has been noted that tylophorine decomposes at room temperature, with concomitant decrease in optical rotation, so this may be the source of the relatively lower optical rotation of Rapoport's synthetic compound [45]. Interestingly, Rapoport and co-workers were unable to perform the de-oxygenation *via* chlorination/reduction on alcohol **8**. Using an approach similar to their tylophorine synthesis, Rapoport and coworkers synthesized (*S*)-(+)-cryptopleurine starting from (*S*)-α-aminoadipic acid [6].

Nordlander and Njoroge subsequently reported a very short, enantioselective synthesis of (*S*)-tylophorine in 1987 starting from (*S*)-*N*-(trifluoroacetyl)prolyl chloride (Scheme 2). 2,3,6,7-Tetramethoxyphenanthrene (**14**), which can be synthesized in as few as three steps [51,52] (vide infra) was subjected to Friedel-Crafts acylation with (*S*)-*N*-(trifluoroacetyl)prolyl chloride (**13**) in the presence of $AlCl_3$ in refluxing CH_2Cl_2. Crystalline ketone **15** was obtained in 51% yield in >98% enantiomeric purity. Deketonization of **15** was accomplished in 64% yield by treatment with Et_3SiH in neat $BF_3{\bullet}OEt_2$ at room temperature. Subsequent deacylation in methanolic ammonia provided the amine **17** in 71% yield. Pictet-Spengler annulation afforded (*S*)-tylophorine in 53% yield in high optical purity.

Huang and co-workers reported a concise, enantioselective synthesis of (+)-tylophorine using *tert*-butyl L-pyroglutamate as the chiral pyrrolidine source in 2004 (Scheme 3) [43]. Condensation of veratraldehyde (**1**) and homoveratric acid (**18**) in the presence of acetic anhydride and triethylamine afforded an inconsequential mixture of olefin isomers **19** (Scheme 3). Methylation of the carboxylic acid and oxidative cyclization with vanadium oxytrichloride in dichloromethane gave the phenanthryl ester in 99% yield. Reduction with $LiAlH_4$ gave the alcohol that was converted to the phenanthryl chloride **20** in 95% yield. Alkylation of *tert*-butyl L-pyroglutamate (**21**) with phenanthryl chloride **20** (NaH, DMSO, rt) provided the phenanthryl amide **22** in 96% yield. Carboxylic acid deprotection, conversion to the acid chloride and subsequent Lewis-acid catalyzed intramolecular Friedel-Crafts cyclization provided the amido ketone **7** in 92% yield. Reduction with $LiAlH_4$ provided a diastereomeric mixture (55:45) of alcohols. Deoxygenation with triethylsilane in trifluoroacetic acid provided (+)-tylophorine in 92% combined yield with high optical purity ($[\alpha]_D^{25} = +74.9°$).

Furstner and co-workers reported an enantioselective route to (−)-antofine and (−)-tylophorine using commercially available D-(−)-prolinol as the source of chirality in 2006 (Scheme 4)

[42]. Selective N-protection of prolinol (**23**) [53] was followed by oxidation of the primary alcohol and Wittig olefination of the resulting aldehyde to give **24**. Hydroboration then provided primary alcohol **25** that was oxidized and coupled with the Bestmann-Ohira reagent **26** to provide terminal alkyne **27**.

Alkyne **27** underwent Sonogashira-type coupling to aryl iodide **28** (synthesized three steps from meta-iodoanisole, see lower part of Scheme 4), providing the biphenylalkyne **29** in 58% yield. Platinum(II)-catalyzed cycloisomerization provided phenanthrene **30** in 72% yield. Removal of the Boc-protecting group and Pictet-Spengler cyclization in one pot afforded (−)-antofine in 91% yield and >98% ee. (−)-Tylophorine was synthesized in the same manner but using a tetramethoxyiodobiphenyl coupling partner in the Sonogoshira-type fragment coupling.

Auxilliary-Directed Asymmetric Synthesis

Fukumoto and co-workers reported the first asymmetric total synthesis of the natural (*R*)-enantiomer of tylophorine in 1990 (Scheme 5). In this synthesis, a stereoselective intramolecular Michael addition of an amide nucleophile onto a chiral α,β-unsaturated ester provided the stereocenter found in the natural product. The Michael addition was performed in tandem with another Michael addition, thereby synthesizing two ring of the natural product in one pot. (−)-Phenylmenthol was the chiral auxiliary used to obtain the stereocenter in the natural product.

The (E)-acid **35** [54] was converted into amide **37** by condensing its acid chloride with 4-aminobutyraldehyde diethyl acetal (**36**). Conversion of the acetal to its aldehyde and Horner-Wadsworth-Emmons olefination with (−)-phenylmenthyl(trip[henylphosphoranylidene) acetate (**38**) [55] in refluxing acetonitrile afforded **39**. Stereoselective intramolecular double Michael reaction of **39** in the presence of TBSOTf and triethylamine afforded an inconsequential mixture of diastereomers **40**. Oxidation with thallium(III) trifluoroacetate afforded phenanthrene **41**. Supponification of the ester and decarboxylation followed by reduction of the amide provided the natural product in high enantiopurity.

Kibayashi and co-workers reported the enantioselective syntheses of (*R*)-(−)-cryptopleurine and (*R*)-(−)-julandine using a proline derived-auxilliary to direct an amido alkylation in 1995 (Scheme 6). (*S*)-*N*-nitrosoprolinol (**42**) [56] was converted in 5 steps to hemi-aminal **43**. The imminium ion formed by treatment of **43** with BF_3•OEt_2 reacted with enolsilanes **44** and **45** to provide β-aminoketones **46** and **47** in 76% and 70% yield, respectively, with greater than 99% selectivity. The ketone and amide functional groups of **46** were reduced with BH_3•THF, along with concomitant removal of the auxiliary. Amide formation with (4-methoxyphenyl) acetyl chloride (**50**) in the presence of 5% NaOH in CH_2Cl_2 followed by hydrolysis of the intermediate N,O-bis(phenylacetyl) derivative afforded hydroxy amide **51** in 85% yield. Oxidation of the hydroxyl group with pyridinium dichromate and subsequent aldol condensation in refluxing 5% ethanolic KOH formed quinolizidinone **52** in 53% combined yield. Reduction of the amide with 3:1 $LiAlH_4/AlCl_3$ and recrystallization provided (*R*)-julandine in 58% yield.

(*R*)-Cryptopleurine was synthesized from the aryl bromide **47** in like manner (Scheme 7). Treatment of the seco-arylbromide intermediate **55** with light or Bu_3SnH, AIBN provided the phenyanthryl amide **56**. Reduction of **56** with $LiAlH_4$ to provide the natural product.

(−)-Tylophorine and (−)-Septicine, Chiral Auxiliary Approach

Commins and co-workers reported the development of chiral auxiliaries that could substitute for (−)-8-phenylmenthanol, which is available in only one naturally occurring enantiomer. In

particular, trans-2-(α-cumyl)cyclohexanol proved readily available in both antipodes in high enantiomeric purity [57]. Commins' syntheses of (−)-tylophorine and (−)-septicine used (−)-trans-2-(α-cumyl) cyclohexyl ester as a chiral auxiliary to guide the facial selectivity of a Grignard reagent to a functionalized pyridinium salt (Scheme 8) [44]. Thus, pyridinium salt **57** underwent diasteroselective addition of 3-butenyl magnesium bromide, providing **58** in high diasteremeric purity after recrystallization. Oxidative cleavage of the terminal alkene followed by reduction of the resulting aldehyde provided alcohol **59**. Conversion to the chloride **60** and treatment with basic methanol provided the mono-unsaturated indolizidine **61**. Electrophilic substitution of bromine for silicon and 1,4-reduction followed by enolate oxygen trapping as the triflate afforded the difunctionalized indolizidine **64**. Negishi cross-coupling with 3,4-dimethoxyphenyl zinc bromide (**65**) provided (−)-septicine in 67% yield. (−)-Tylophorine was obtained in 68% yield by oxidation of (−)-septicine with VOF_3 in trifluoroacetic acid and dichloromethane.

Asymmetric Synthesis *via* Chiral Catalysis

In 2003, Kim and co-workers reported an asymmetric total synthesis of (−)-antofine using an enantioselective catalytic phase transfer alkylation (Scheme 9) [49]. The key alkylation of glycine imine **67** with phenanthrylalkyl bromide **66**, mediated by the phase transfer catalyst **68**, occurred in 97% yield and with 96% enantioselectivity. Subsequent conversion to the unsaturated pyrrolidine **71** was possible *via* a ring-closing-metathesis route. Reduction of **71** and Pictet-Spengler annulation with formaldehyde generated the natural product.

Phenanthryl bromide **66** was synthesized (see lower part of Scheme 9) from alcohol **73** which in turn was synthesized in a four-step sequence starting from condensation of homovaleric acid (**18**) with p-anisaldehyde (**72**) as described by Weinreb *et al.* [58].

Another asymmetric route to antofine was reported by Kim and co-workers in 2004 (Schemes 10–12) [48]. In this route, which was also amenable to the enantioselective synthesis of (−)-cryptopleurine, a common chiral synthon, (*R*)-(*E*)-4-(tributylstannanyl)but-3-en-2-ol (**74**) was the source of chirality. The chiral vinylstannane **74** underwent Pd-catalyzed Stille coupling with phenanthrylbromide **66**, providing allylalcohol **75** in 95% yield (Scheme 10). Trichloroacetimidate formation and Overman rearrangement provided the chiral amide **77** in 92% combined yield. Protecting group exchange, *N*-allylation and ring closing metathesis provided unsaturated pyrrolidine **78** in 85% combined yield. Hydrogenation and Pictet-Spengler annulation provided (−)-antofine in 50% combined yield.

(*R*)-(*E*)-4-(Tributylstannanyl)but-3-en-2-ol (**74**) was obtained in three steps as shown in Scheme 11 [59]. Catalytic hydrostannylation of 3-butyne-2-ol (**79**) with Bu_3SnH in THF at −78°C in the presence of $Pd(PPh_3)_2Cl_2$ (1 mol %) afforded vinylstannanes **80** in 81% yield. Lipozyme-mediated kinetic resolution with vinyl acetate in diisopropylether at 35°C provided 49% of (*R*)-**81** (>99% ee) and 48% of (*S*)-**74** (>99% ee). Alkaline methanolysis of the acetate (*R*)-**81** provided the necessary (*R*)-**74** in 98% yield.

Intermediate **82** (derived from **77**, Scheme 10) was also used en route to a total synthesis of (−)-cryptopleurine by Kim and co-workers (Scheme 12) [48]. Cross-metathesis with homoallyl acetate dimer **83** (5 mol % Grubbs II, refluxing CH_2Cl_2, 1 d) gave an inseparable E/Z mixture of alkene **84** (82% yield, 8:1 E/Z) along with recovered starting material (14%). Hydrogenation of the alkene and concomitant Cbz deprotection followed by supponification of the acetate provided alcohol **85**. Mitsunobu cyclization provided the piperidine in 68% yield. Pictet-Spengler annulation then provided (−)-cryptopleurine in 67% yield.

We have recently accomplished the enantioselective total synthesis of (*S*)-(+)-tylophorine (Scheme 13) [46]. The synthesis relies on the introduction of the indolizidine ring of

tylophorine through a copper(II)-catalyzed enantioselective carboamination reaction developed recently in our lab [60]. Entry into the chiral indolizidine core of these compounds has been referred to as the "limiting synthetic factor" in the synthesis of this class of compounds [61].

Conversion of commercially available 3,4-dimethoxy benzylalcohol (**86**) to the bromide **87** followed by radical-induced dimerization [62] provided **88** (73% yield for two steps). Oxidation [51,52] with phenyliodine(III)bis(trifluoroacetate) (PIFA) provided phenanthrene **14** (66% yield) which was sulfonylated with chlorosulfonic acid to provide the phenanthrene fragment **89**. Convergent fragment coupling of aryl sulfonyl chloride **89** and pentenylamine (**90**) provided the aryl sulfonamide intermediate **91**. Enantioselective copper(II)-catalyzed carboamination with the bis(oxazoline) complex **92**, a process recently developed by our research group [60], provided sultam **93** in 64% yield. Reductive removal of the sulfur dioxide group provided the required pyrrolidine **17** in moderate yield (44%). The yield in the conversion of **93** to **17** is moderate because the phenanthrene methoxy groups are labile under the Li/NH_3 reaction conditions. Pictet-Spengler cyclization with formaldehyde afforded (*S*)-tylophorine in 89% yield. The enantiomeric excess of synthetic (*S*)-tylophorine was 81% as measured by chiral HPLC. Both enantiomers of the chiral bis(oxazoline) ligand, Ph-Box (**92**), are commercially available and easily synthesized, thus the sulfonamide **91** can in principle provide for the synthesis of either enantiomer of tylophorine. This route should allow for the coupling of diverse arylsulfonyl chlorides with a range of amines for structure-activity-relationship studies. The synthesis of pyrrolidine-substituted analogs of tylophorine is currently underway in our labs.

Structure-Activity-Relationship Studies

The therapeutic properties of the phenanthroindolizidines and phenanthroquinolizidines have attracted the interest of a number of medicinal chemists. An extensive but incomplete understanding of the structure-activity-relationship of these compounds has been achieved. A more full understanding has thus far been limited by a lack of robust and versatile organic synthesis methods (vide infra). As seen above, however, a number of recently reported synthetic routes should allow access to a diversity of analogs for future studies.

The phenanthroindolizidines are composed of a substituted phenanthrene fused to an indolizine Fig. (2). Thus far, researchers have studied the effect of the phenanthrolene substitution pattern, presence or lack of indolizidine ring fusion, presence or lack of C14-hydroxyl groups on the indolizine ring and the oxidation state of the amine (vide infra). Very little information is available on the affect of substituents on the pyrrolidine subunit.

Phenanthrene Substitution

It was found early on that hydroxy and alkoxy substituents on the phenanthrene unit are mandatory for biological activity. The unsubstituted phenanthro[9,10-b]quinolizidine was inactive in *in vitro* protein synthesis studies and *in vivo* anti-tumor evaluation, while the unsubstituted phenanthroindolizidine was inactive in *in vitro* antiamoebic activity assays [9, 18].

The phenanthrene substitution pattern greatly affects the *in vitro* anti-cancer activity of the phenanthroindolizidines. In an SAR study of antofine (Fig. (3) and Table 2), Kim and co-workers found that changing the C2 substituent from MeO to HO, *i*-PrO, or OCH_2O (shared with C3) all had significantly deleterious effects on the cytotoxicity of the compounds. Thus, the C2 substituent must be a small, H-bond acceptor but not H-bond donor. C3 and C6 could tolerate such changes, and when C6 was changed to HO, the activity increased relative to the parent antofine (Fig. 3 and Table 2).

Phenanthrene Ring Fusion

Seco analogs of antofine, secoantofine, 6-*O*-desmethylsecoantofine and secoantofine *N*-oxide are all substantially less active (IC_{50} 400–3000 nM, Fig. 1 and Table 1) *in vitro* [11,12]. Tyloindicine I, however, which contains more unsaturation in the indolizidine ring, is highly potent (GI_{50} 10^{-9} M) in the NCI's 60 cell line assay. Tyloindicine I is one of the most potent compounds in this family of structures. The greater rotational flexibility of the aryl rings and the decreased basicity of the enamine nitrogen might be beneficial to the activity of this compound.

Nitrogen

The oxidation level of the indolizidine amine affects the *in vitro* cell growth inhibitory activity of these compounds. Some *N*-oxides of tylophorine analogs are naturally occurring [e.g. tylophoridicin C and tylophoridicine F and antofine *N*-oxide, Fig. (1)]. These *N*-oxides are 5–10 times less active *in vitro* than their free amine counterparts in KB, HepG2 and PANC-1 cell lines (60–160 nM) [11,14]. It is interesting to note that while *N*-oxidation diminishes potentcy, the *N*-oxides of antofine and tylophoridicine are still active compounds (Table 1).

C9-Keto analogs, amide structures of various phenanthroindolizidines and phenanthroquinolizidines, have also been found to be much less potent in cancer cell growth assays [31,34,63].

Interestingly, one study found that the pH of the cell growth medium has a strong effect on the activity of the phenanthroindolizidines and phenanthroquinolizidines. Tylophorine and cryptopleurine were substantially less cytostatic to yeast at pH 5.8 than they were at pH 7.0 [23]. This may indicate that an unprotonated amine (pKa of indolizidines are ca. 10–11) is necessary for transport into the cell or cell membranes. It could also indicate that the unprotonated form of the molecule binds to its receptor.

C(13a) Stereochemistry

Some of the phenanthroindolizidines have C13a(R) stereochemistry while others have C**13a** (*S*)-stereochemistry. In human colorectal (HCT 116) and lung (A549) carcinoma cells, the natural (*R*)-enantiomer of antofine is more cytostatic (IC_{50} = 9.9 and 10.4 nM, respectively) than the unnatural (*S*)-enantiomer (IC_{50} of racemate = 29.4 and 25.1 nM, respectively) [16]. In contrast, as measured in human nasopharyngeal carcinoma (KB) cells, the natural (*R*)-enantiomer of tylophorine is less cytostatic (IC_{50} = 173 nM) [12] than the unnatural (*S*)-enantiomer (IC_{50} = 12 nM) [13]. The C(**13a**) stereochemistry of tylocrebrine is (*S*). It is interesting to note that tylocrebrine and isotylocrebrine are enantiomeric at C(**13a**) but similar in biological activity and identical in phenanthrene alignment if one allows the pyrrolidine nitrogen to shift, Fig. (4). In the R-series, C-2 methoxylation is very important for activity while in the S-series, C-7 methoxylation is very important for activity.

C(14) Hydroxylation

In vitro antiproliferative assays (cancer cells) show the same or a slight loss of activity for C (14) hydroxylated analogs of tylophorine, Fig. (5). Gao and co-workers [13,14] tested the C (14) hydroxylated analogs **10** and **11** of (*S*)-tylophorine originally synthesized by Rapoport and coworkers [6] (vide supra). The C(14) S, C(13a) S tylophorine analog **11** has an GI_{50} of 24–40 nM in KB and HepG2 cell lines while the C(14) R, C(13a) S tylophorine analog **10** has GI_{50} around 100 nM in these cell lines [13]. For comparison, (S)-tylophorine has a GI_{50} of 9–15 nM in these two cell lines. Naturally occurring tylophorinidine, however, which has C(14) S, C(13a) S stereochemistry and a slightly different phenanthrene substitution pattern, is as

active *in vitro* (GI_{50} in HepG2 = 11 nM) as (S)-tylophorine [14]. Thus, the C(14) S, C(13a) S stereochemistry may be optimal for these compounds [14].

Lack of Indolizidine, Quinolizidine

Phenanthrene-based tylophorine derivative (PBT's) that lack the phenanthroindolizidine ring fusion (fused middle ring) have been studied. The synthesis of the fused indolizidine ring is considered to be the "limiting synthetic factor" of these molecules, thus removal of one of the C-C bonds that make up the indolizidine ring greatly decreases the synthesis burden. A large number of PBTs have been synthesized [61].

In general, the cytotoxicity is in the micromolar to sub-micromolar range and inhibition of tumor growth *in vivo* is modest [64]. A comparison on the mechanism of action has been made between the PBTs and (*S*)-tylophorine, and Gao concluded that the PBTs do not operate by the same biological mechanism as (*S*)-tylophorine [14]. Similar analogs synthesized by other groups confirm that tylophorine and cryptopleurine analogs lacking the indolizidine and quinolizidine rings are much less active in cancer cell growth assays [65]. Interestingly, though, because their structures are similar to the poly-substituted cis-stilbene combretastin A4 [66], a potent anti-mitotic and anti-angiogenic agent, they were tested in anti-angiogenesis assays and found to be quite promising lead compounds [65].

Pyrrolidine Substitution

There is no SAR information on pyrrolidine substitution in the indolizidine class of compounds, likely due to difficulty of their synthesis (vide supra). Most enantioselective syntheses of phenanthroindolizidines start from proline or other naturally occurring compounds that are not easily derivatized. Recently reported syntheses that use chiral auxiliary and chiral catalyst methodology to install the pyrrolidine ring should allow for greater access to pyrrolidine-substituted analogs (vide supra).

In Vivo Activity and Pharmakokinetics

In 1968, Donaldson and co-workers reported that mice infected by Ehrlich ascites-tumor cells (manifested as breast cancer) showed 50% tumor growth inhibition upon administration of 3 micromoles/kg twice daily for ten days with cryptopleurine [9]. Later studies by the NCI found this compound generally toxic. While tylocrebrine showed some promise *in vivo*, Phase I studies were terminated due to CNS toxicity, manifested as disorientation and ataxia [20]. Decreasing diffusion through the blood brain barrier should minimize the CNS toxicity of the compounds.

Recently, interest has been renewed in the phenanthroindolizidines as anti-cancer compounds. Gao and coworkers recently found that C14 hydroxylated analogs, Fig. (5), are more efficient at reducing tumor growth *in vivo*. While (*S*)-tylophorine is equipotent to tylophorinidine *in vitro* (HepG2, human hepatocarcinoma, cells), it is far less efficacious *in vivo* (mice) [13,14]. Likewise, (*S*)-tylophorine is more active than the synthetic **11** *in vitro*,Fig. (5), but far less potent *in vivo* [13]. Both tylophorinidine and **11** share the same C(**14**) hydroxylation of the indolizidine, which may give them favorable pharmacokinetics *in vivo* [14]. Antofine, though potent *in vitro*, is metabolically unstable, resulting in a poor *in vivo* pharmacokinetic profile (hypothesized to be due to a methoxy moiety on the phenanthrene) [16]. Antofine also lacks a C(14) hydroxyl group. While a couple promising (ca. 60% tumor growth suppression relative to control) [14] compounds have been identified in *in vivo* studies, a balance between complete inhibition of tumor growth *in vivo* and mimimization of general toxicity has not been achieved. The factors that affect the pharmacokinetics of these compounds must be more fully understood in order for an optimal compound to be developed into an anti-cancer drug. A better

understanding of the structure-activity-relationship of these compounds should facilitate development of a potent anti-cancer compound without undesirable side effects.

Anti-Inflammatory Effects

It should be noted that tylophorine (natural source) is also a promising lead in prevention and treatment of inflammation. The leaves of Tylophora indica have been used for decades in India to treat inflammatory-related conditions such as asthma, bronchitis and rheumatism [3,17]. *In vitro* experiments demonstrated that tylophorine prevents NO production in murine macrophage cells (RAW264.7) with an IC_{50} of 1.8 μM [67]. Tylophorine also inhibits expression of several proinflammatory factors such as iNOS and COX-II. It enhanced Akt activation and decreased c-Jun expression. Akt and c-Jun activity are involved in both apoptosis and stress-induced inflammation [67]. *In vivo* studies in rats indicated that within hours of ip administered tylophorine (natural source), 25 mg/kg produced significant anti-inflammatory effects. [3] CNS depression was observed at 100 mg/kg or greater. Importantly, tylophorine is very effective *in vivo* when its effects can be observed shortly after it is administered, when drug metabolism is still at a minimum. 7-Methoxycryptopleurine also exerted potent anti-inflammatory activity *in vitro* as well as *in vivo* (rats) [68].

Acknowledgments

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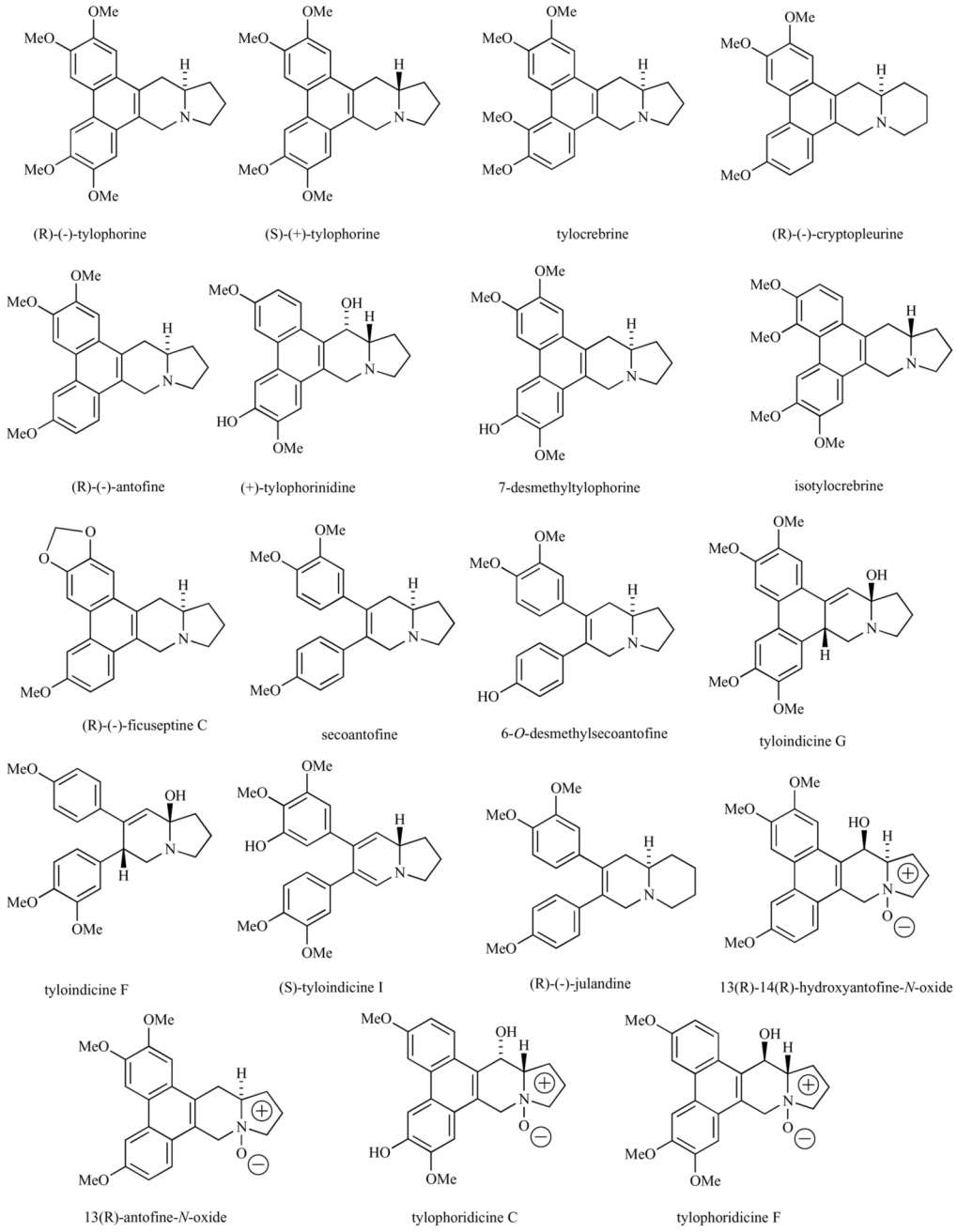


Fig. 1.
Structures of several phenanthroindolizidines and phenanthroquinolizidines.

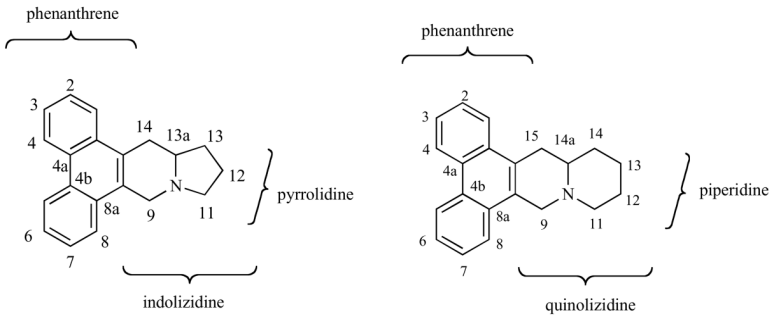


Fig. 2.
Phenanthroindolizidine and phenanthroquinolizidine.

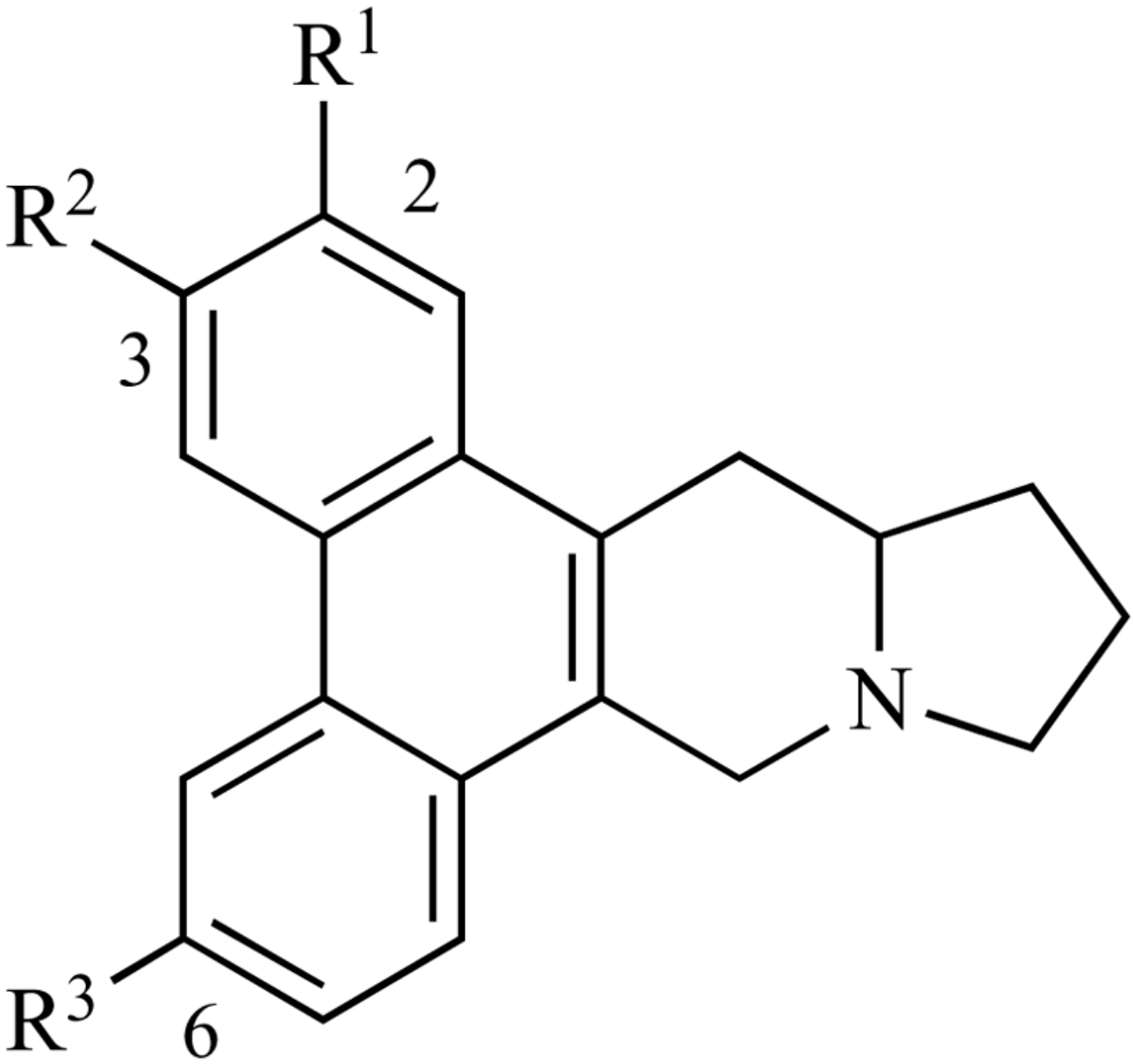


Fig. 3.
Antofine analogs.

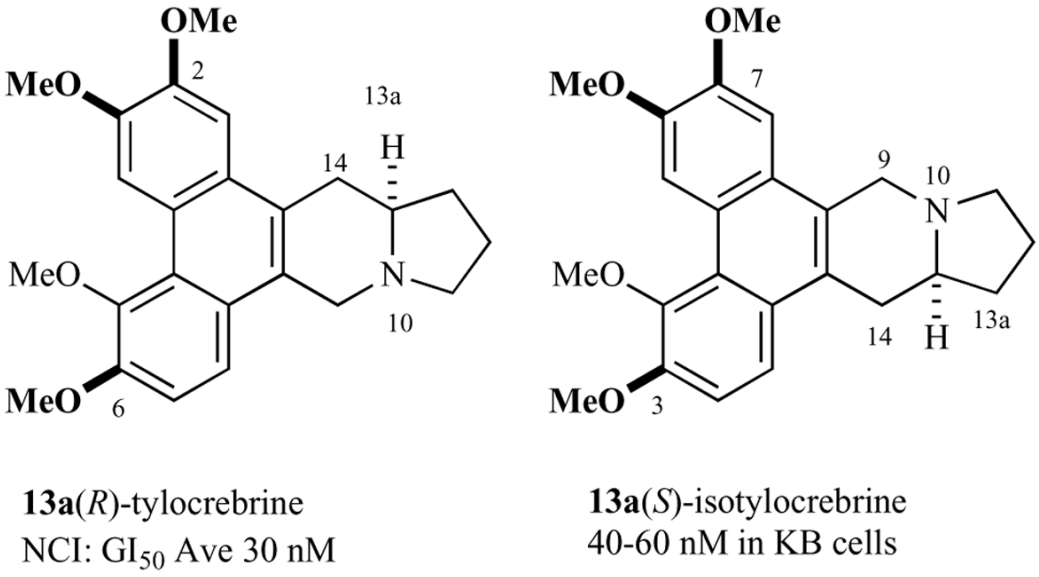
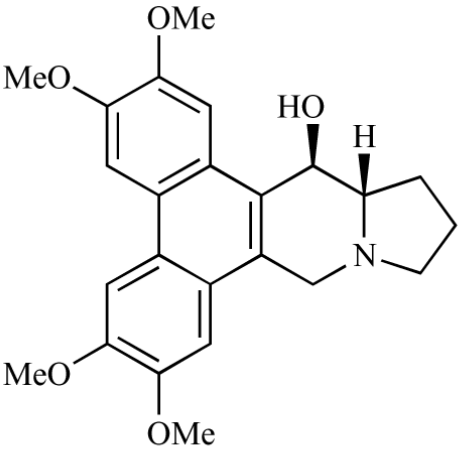
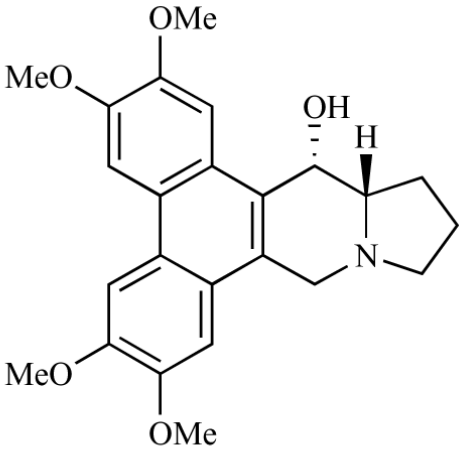


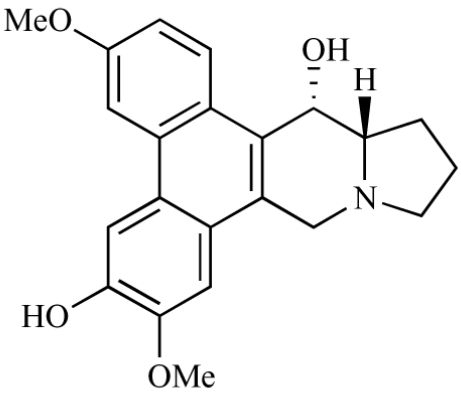
Fig. 4.
Structural similarities of enantiomers.



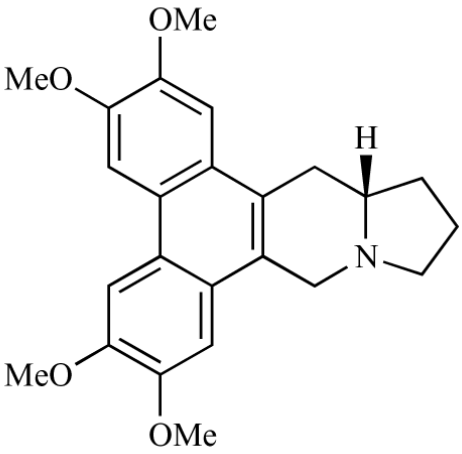
C13(*S*),C14-(*R*)-**10** (NSC-716802)
GI_{50} KB = 106 ± 84 nM
HepG2 = 110 ± 45 nM



C13-(*S*),C14-(*S*)-**11** (NSC-716802)
GI_{50} KB = 28 ± 4 nM
HepG2 = 35 ± 5 nM

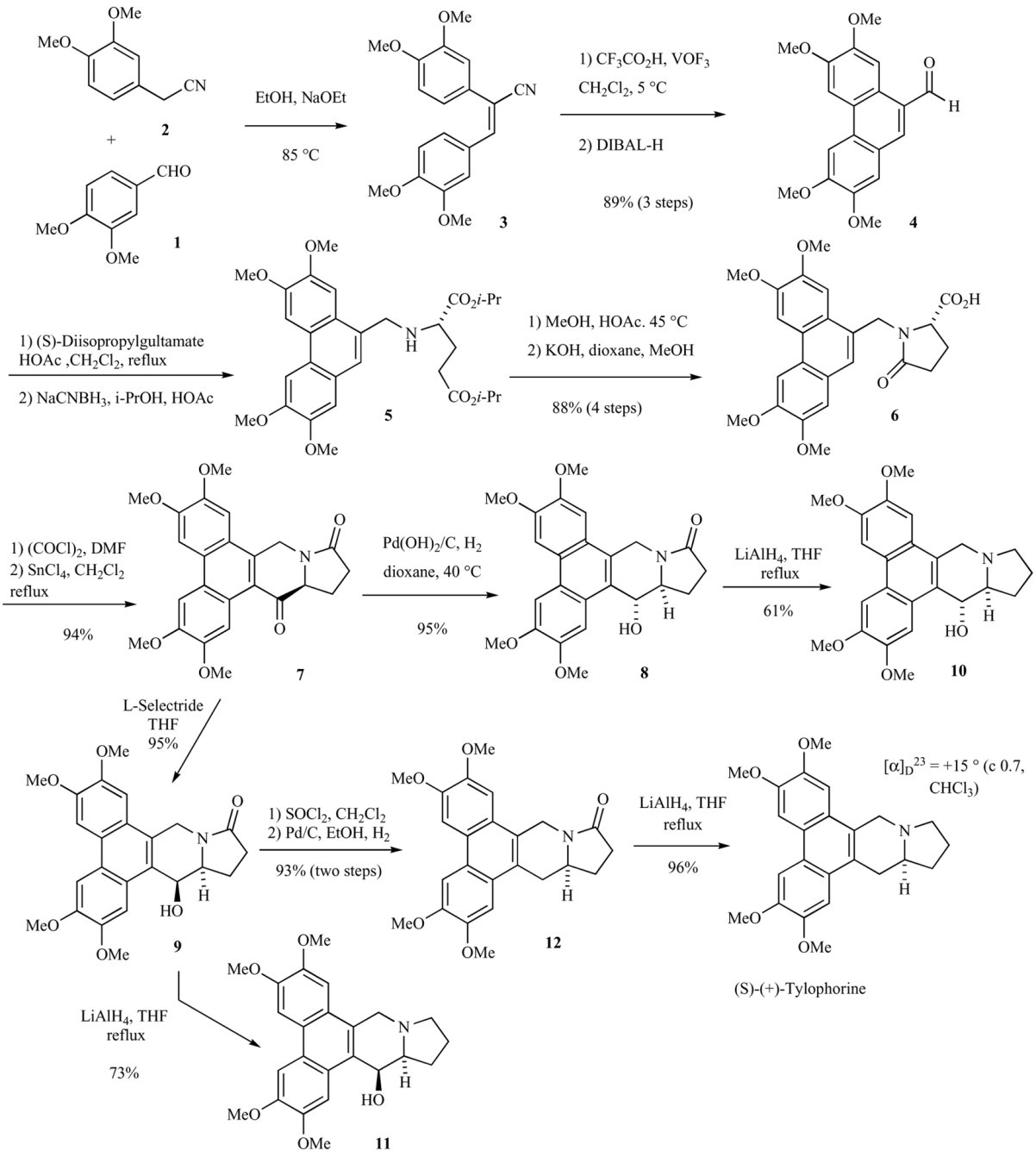


(+)-tylophorinidine
GI_{50} HepG2 = 11 ± 5 nM

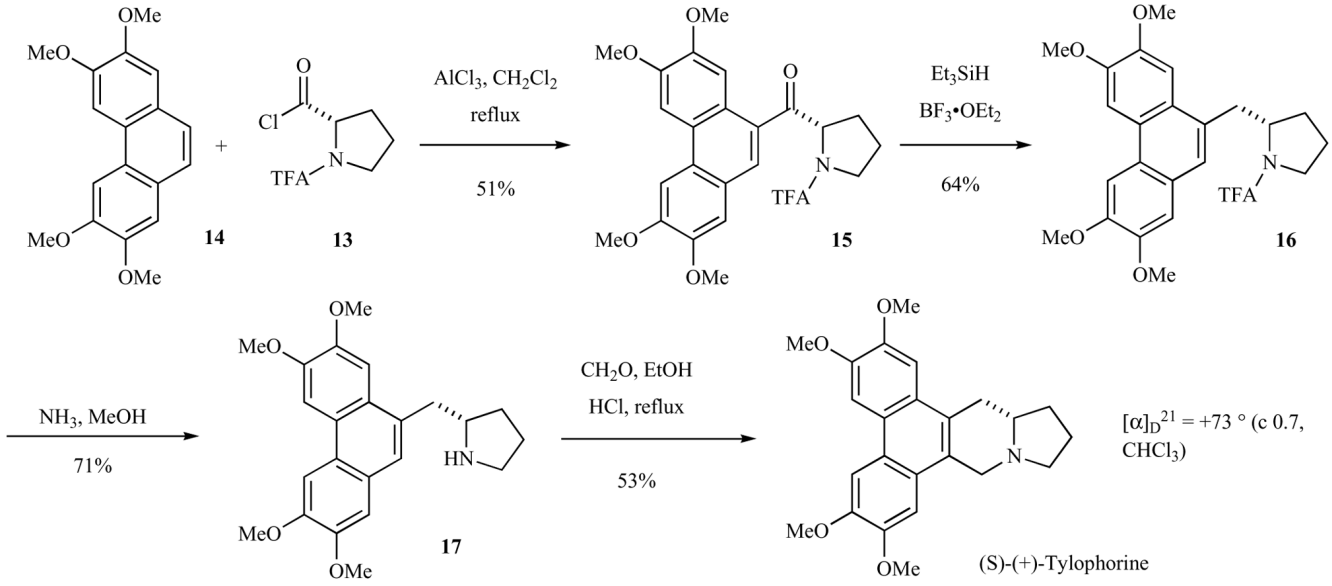


(*S*)-(+)-tylophorine
GI_{50} KB = 12 ± 3 nM
HepG2 = 11 ± 4 nM

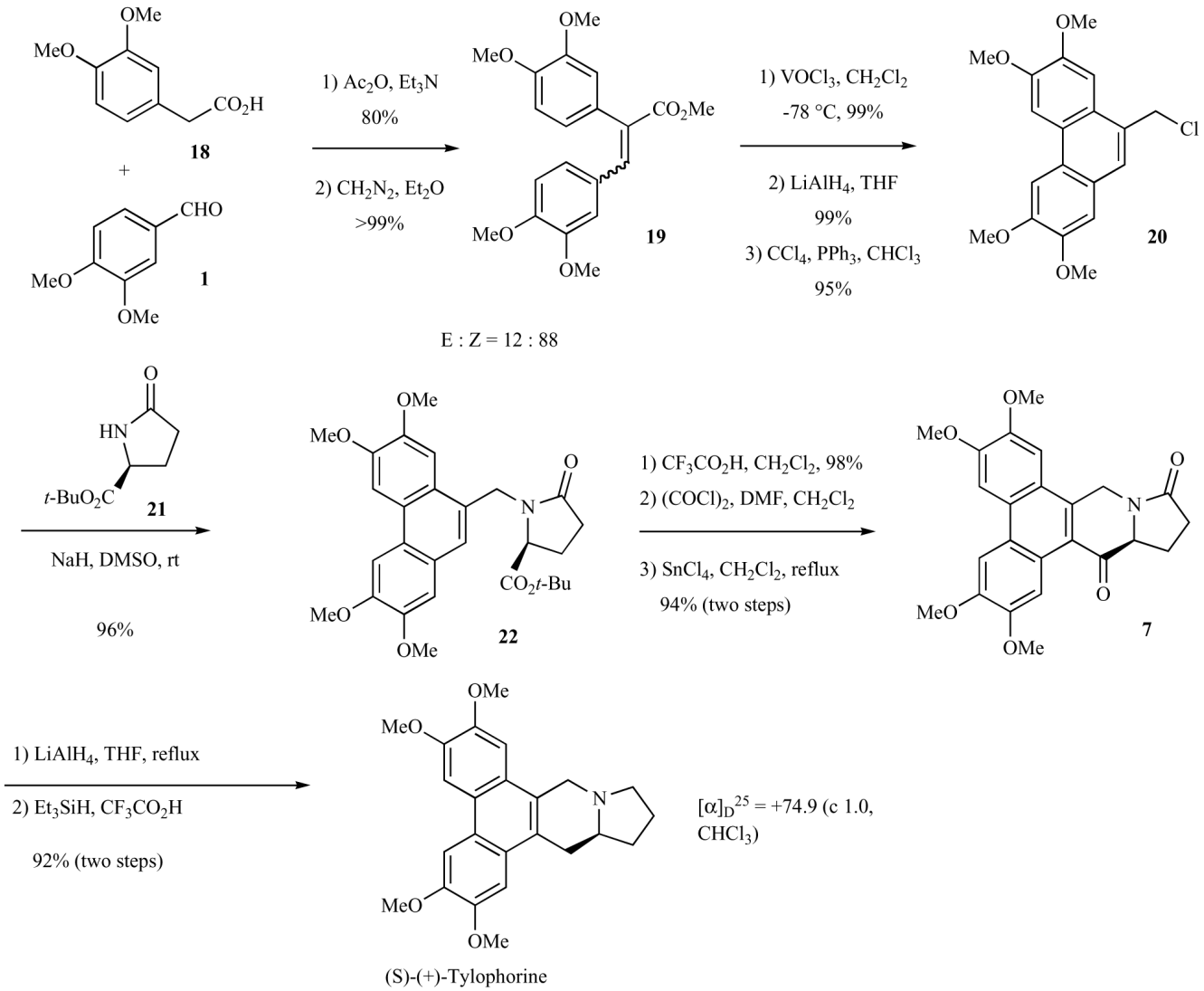
Fig. 5.
C14-Hydroxylated analogs compared to tylophorine.



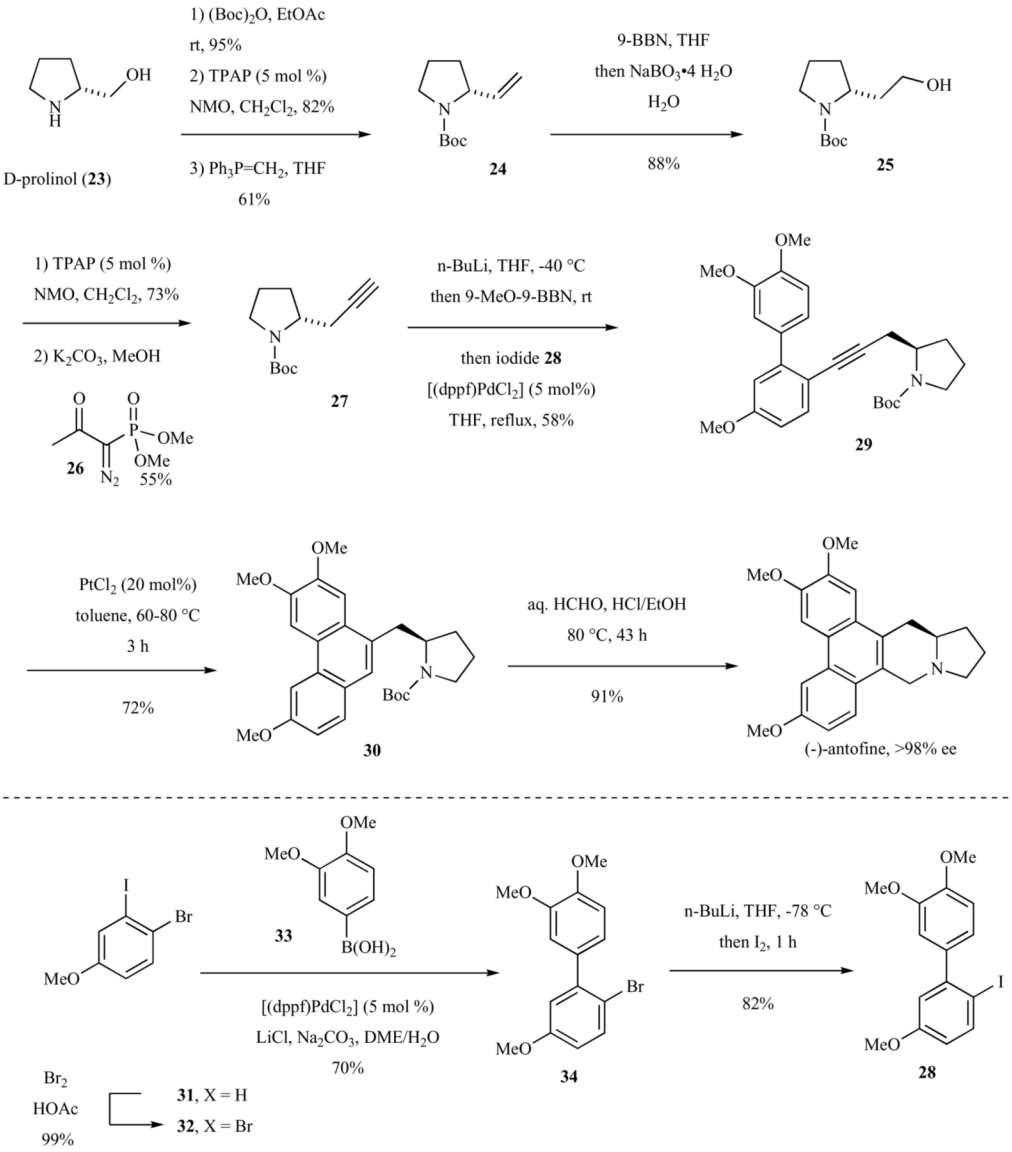
Scheme 1.
Glutamic acid approach to the synthesis of (+)-tylophorine



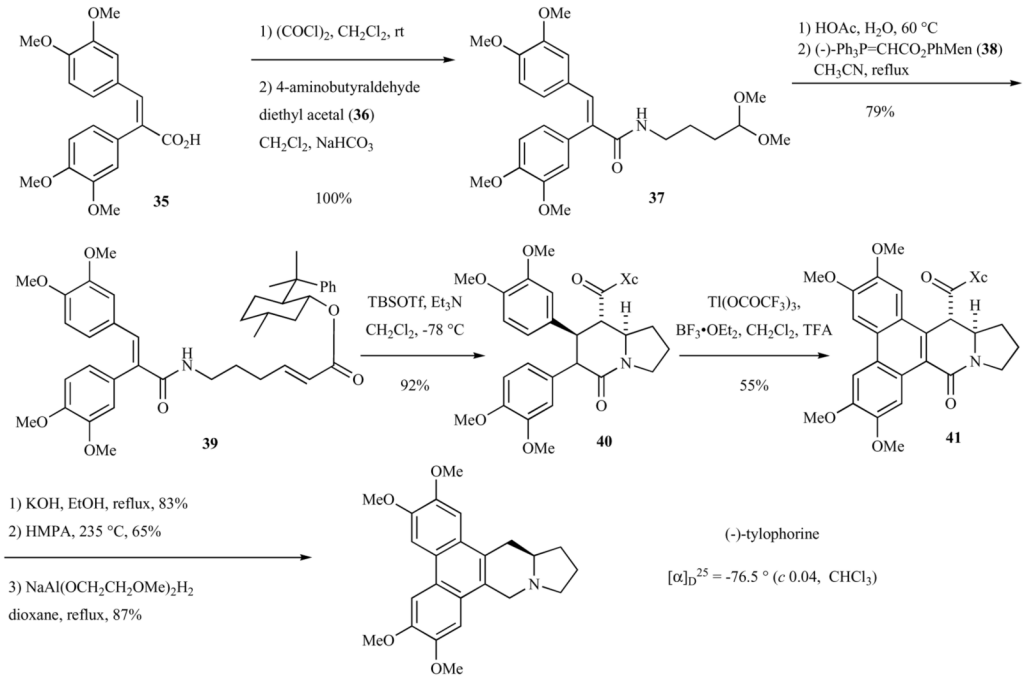
Scheme 2.
Proline approach to the synthesis of (+)-tylophorine.



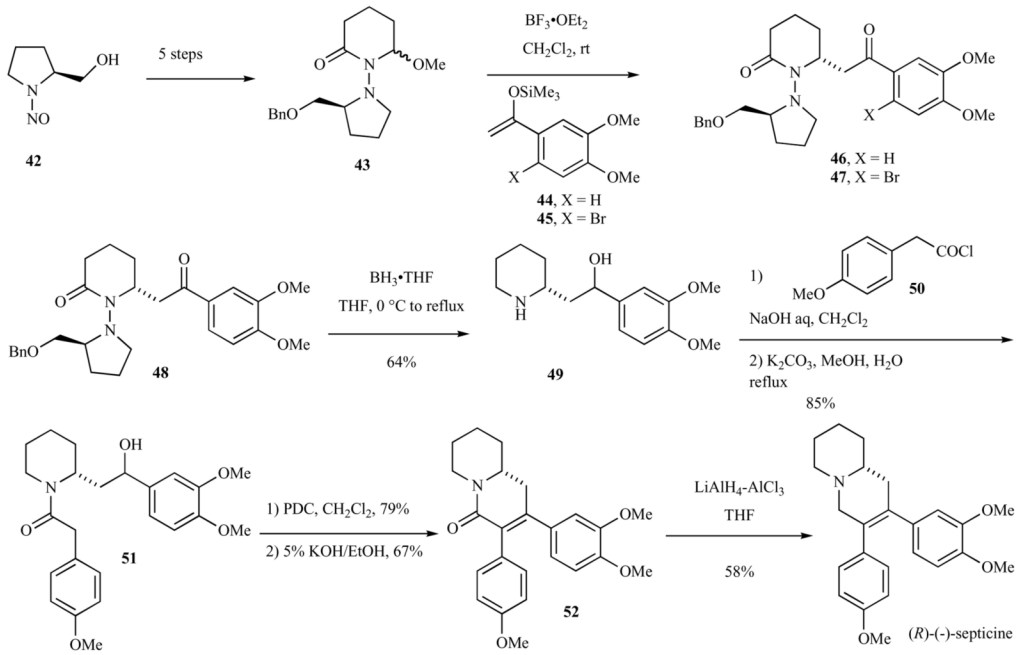
Scheme 3.
Pyroglutamate approach to the synthesis of (+)-tylophorine.



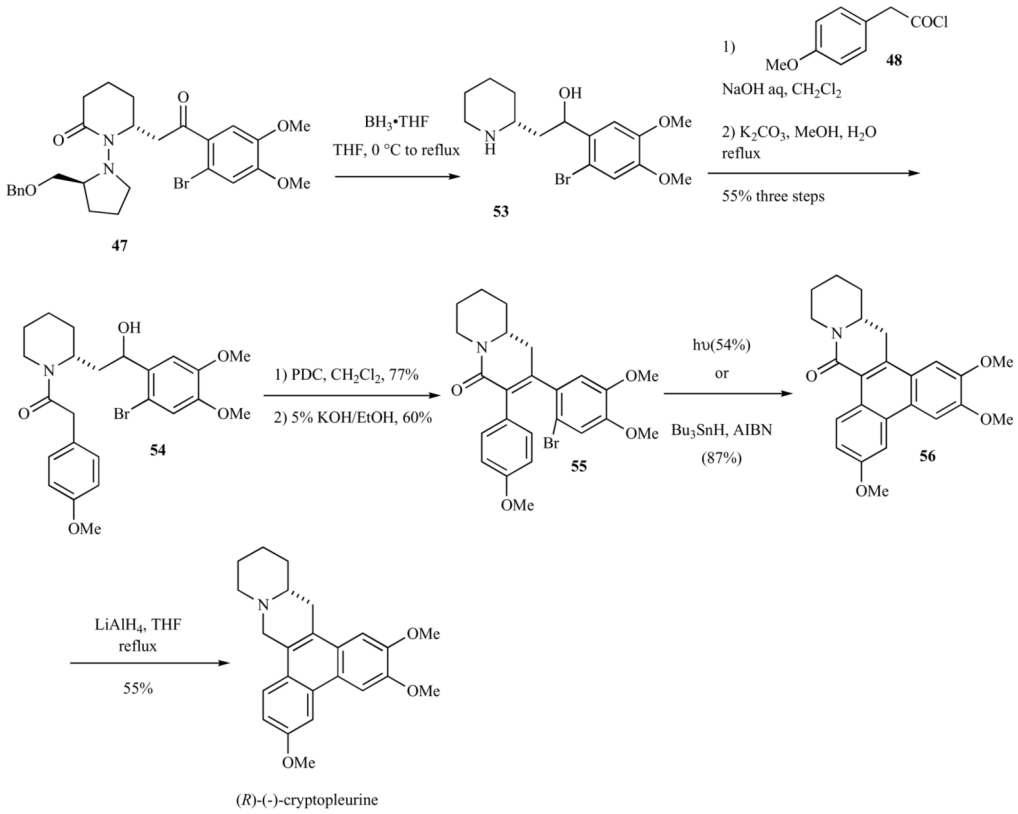
Scheme 4.
Cycloisomerization route to (−)-antofine.



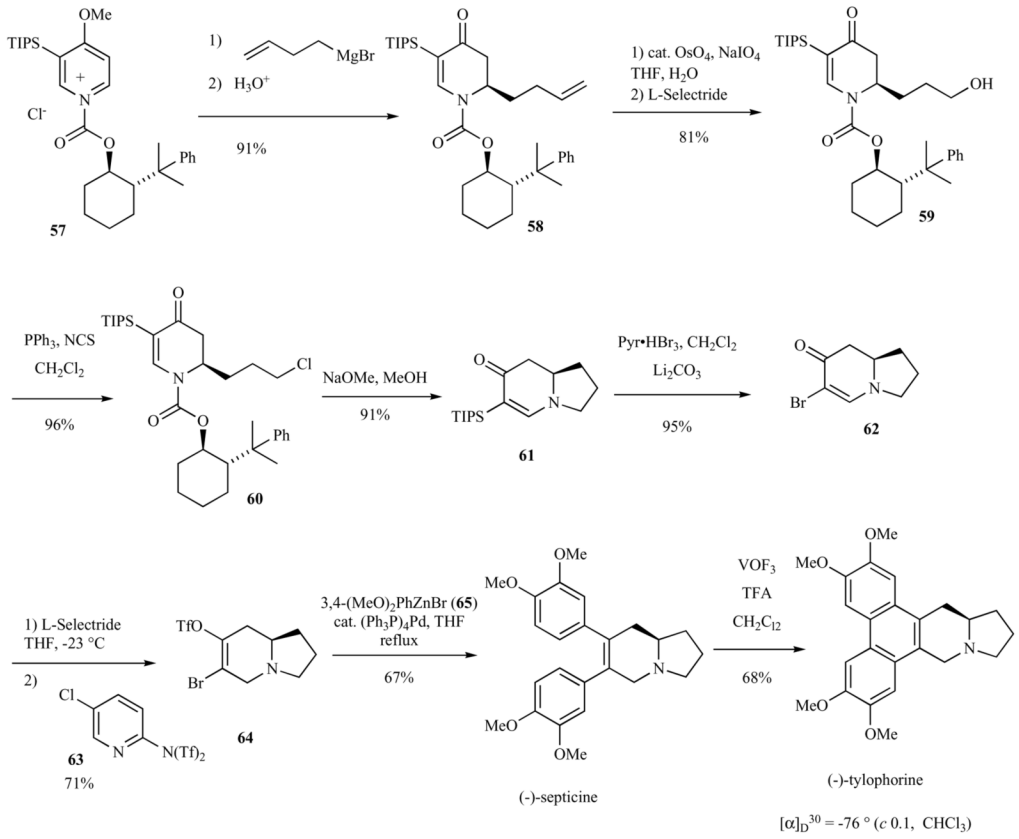
Scheme 5.
Synthesis of (−)-tylophorine *via* intramolecular double Michael reaction.



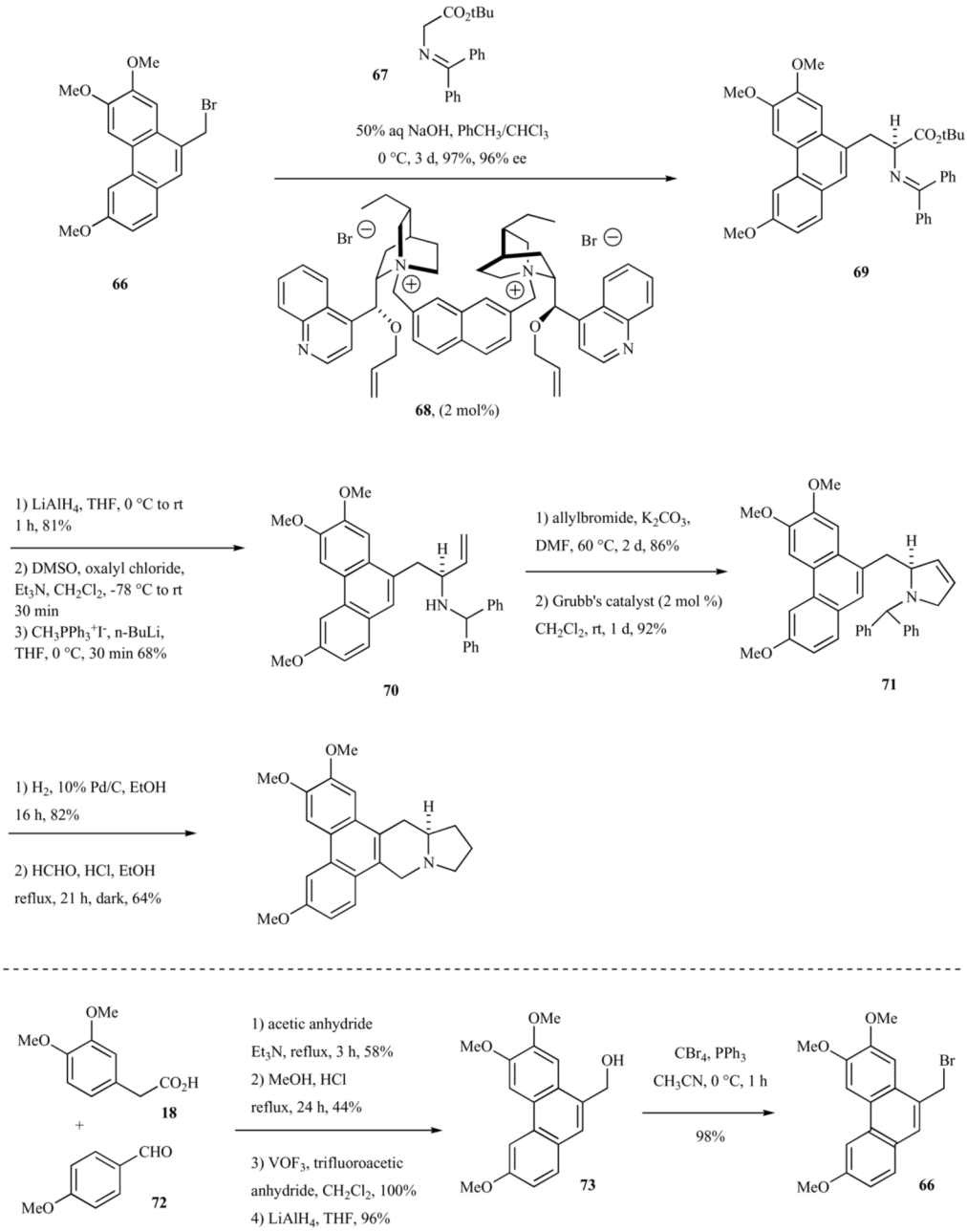
Scheme 6.
Synthesis of (R)-(−)-julandine.



Scheme 7.
Synthesis of (*R*)-(−)-cryptopleurine.

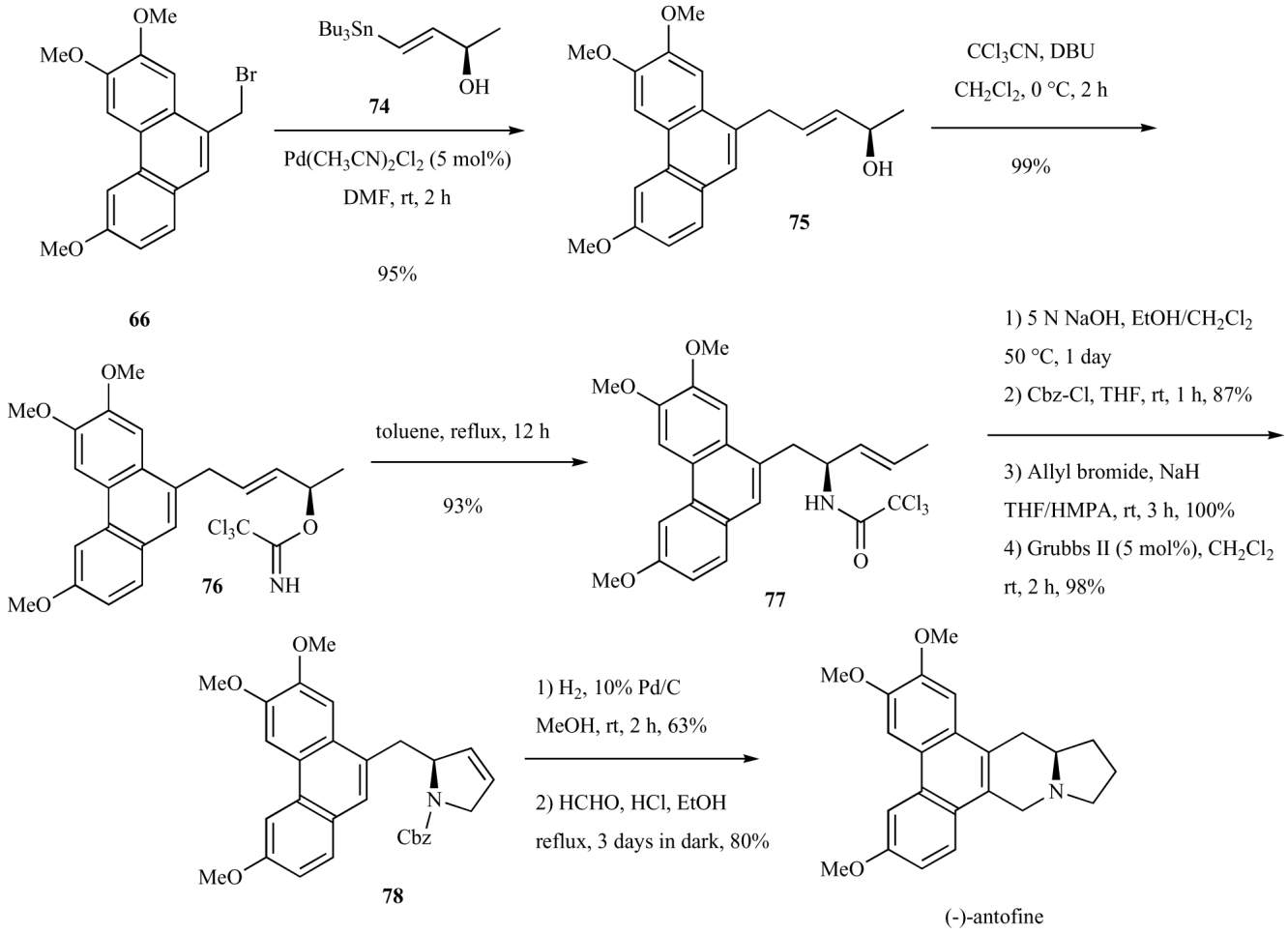


Scheme 8.
Synthesis of (−)-tylophorine asymmetric 1,4-addition and cross-coupling.

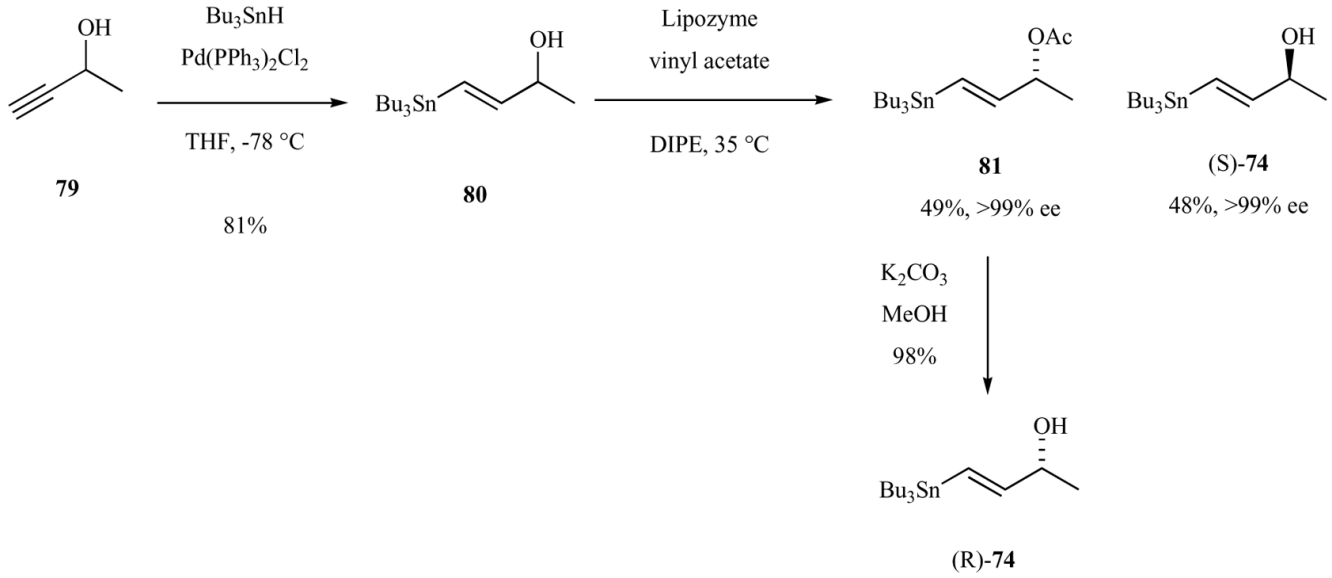


Scheme 9.

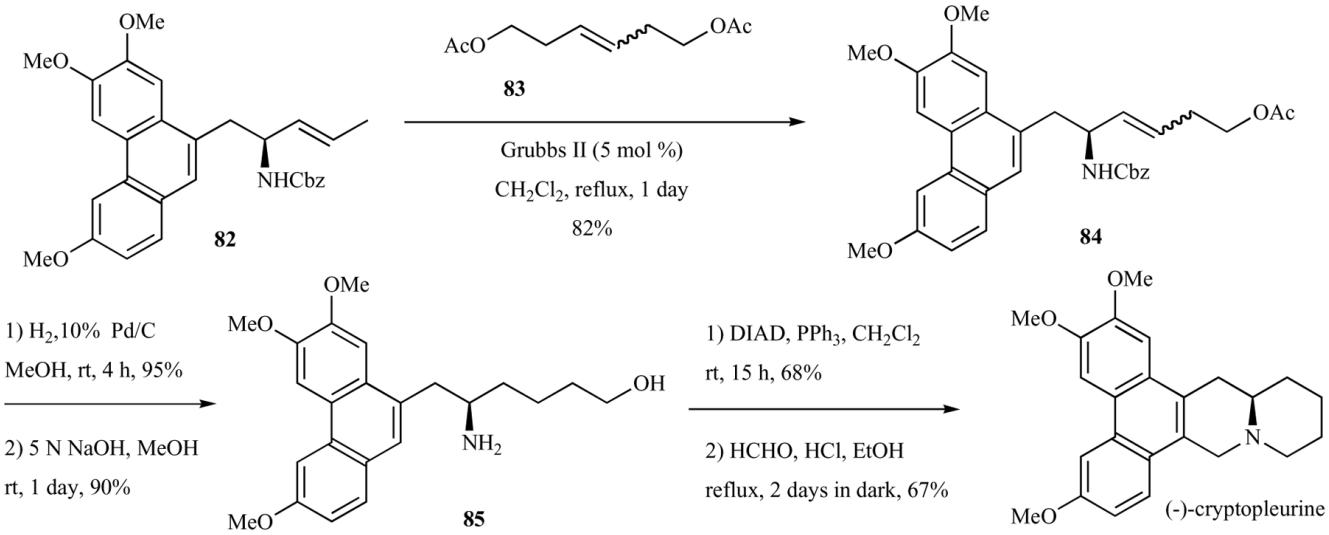
Synthesis of (−)-antofine *via* enantioselective phase transfer alkylation.



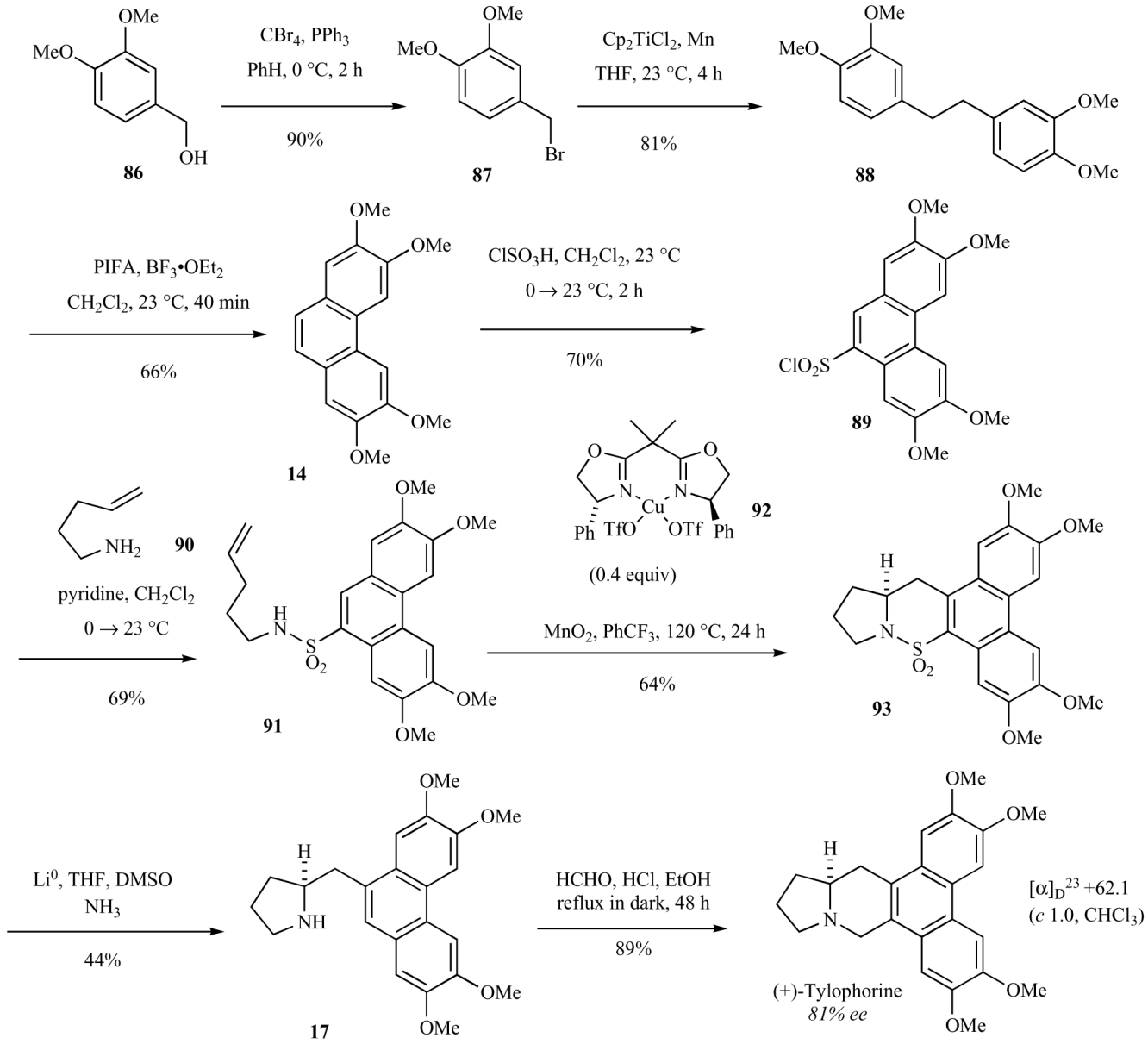
Scheme 10.
Total Synthesis of antofine *via* Overman rearrangement and ring-closing metathesis.



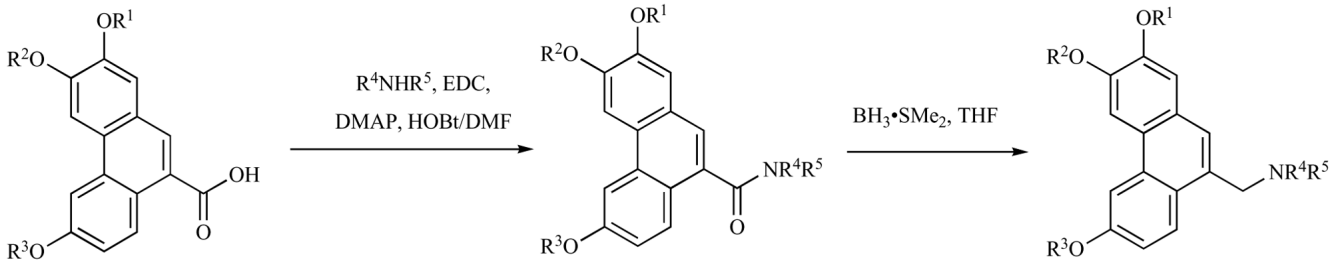
Scheme 11.
Synthesis of chiral vinylalcohol *via* kinetic resolution.



Scheme 12.
Total synthesis of (−)-cryptopleurine.



Scheme 13.
Enantioselective total synthesis of (*S*)-(+)-tylophorine.



Scheme 14.
Synthesis of phenanthrene-based tylophorine derivatives (PBTs).

Table 1

GI_{50} Values of Some Phenanthroindolizidines and Phenanthroquinolizidines in Various Cancer Cell Lines

Entry	Compound	Cell Type[a]	GI_{50} (M)	Reference
1	(*R*)-tylophorine	KB	17×10^{-8}	[12]
2	(*S*)-tylophorine	NCI panel[b]	1.9×10^{-8}	*c*
3	tylocrebrine	NCI panel	2.95×10^{-8}	*c*
4	(*R*)-cryptopleurine	NCI panel	1×10^{-12}	*c*
5	(*R*)-antofine	KB	1.5×10^{-8}	[12]
6	(+)-tylophorinidine	HepG2 and PANC-1	1×10^{-8}	[14]
7	7-desmethyltylophorine	KB	1.5×10^{-8}	[12]
8	(*S*)-isotylocrebrine	KB	4.5×10^{-8}	[12]
9	secoantofine	KB	2.5×10^{-6}	[12]
10	6-*O*-desmethylsecoantofine	KB	4.0×10^{-7}	[12]
11	tyloindicine G	NCI panel	1×10^{-10}	*c*
12	tyloindicine F	NCI panel	1×10^{-10}	*c*
13	(*S*)-tyloindicine I	NCI panel	4.4×10^{-9}	*c*
14	13(*R*)-14(*R*)-hydroxyantofine-*N*-oxide	KB	1.4×10^{-7}	[11]
15	13(*R*)-antofine-*N*-oxide	KB	1.4×10^{-7}	[11]
16	tylophoridicine C	HepG2 and PANC-1	8×10^{-8}	[14]
17	tylophoridicine F	HepG2 and PANC-1	7×10^{-8}	[14]

[a] Cell Type: KB = human nasopharyngeal carcinoma (average of one or more cell lines), HepG2 = human hepatocyte carcinoma, PANC-1 = human pancreatic carcinoma.

[b] Average GI_{50} for NCI 60 cell line panel.

[c] The Ave GI_{50} values for the NCI cell line panels can be obtained at http://dtp.nci.nih.gov/dtpstandard/dwindex/index.jsp

Table 2

Phenanthrene Substituted Antofine Analogs: Activity in HCT 116[a] [16]

Entry	R^1	R^2	R^3	IC_{50} (nM)
1, (*R*)-antofine	OMe	OMe	OMe	9.9
2 (±)-antofine	OMe	OMe	OMe	29.4
3	O-i-Pr	OMe	OMe	783.2
4	OMe	O-i-Pr	OMe	24.7
5	OMe	OMe	O-i-Pr	19.2
6	OH	OMe	OMe	68.3
7	OMe	OH	OMe	21.4
8	OMe	OMe	OH	1.1
9	OMe	OMe	H	49.3
10	$-OCH_2O-$		OMe	364.1

[a]Human colorectal carcinoma.