OL | Organic Letters

Cite This: Org. Lett. XXXX, XXX, XXX−XXX

Letter

pubs.acs.org/OrgLett

Selective Synthesis of (+)-Dysoline

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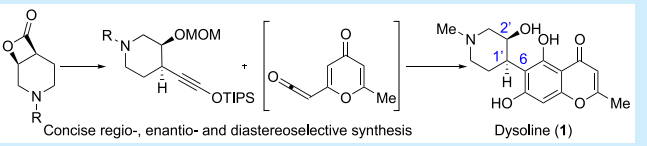
Supporting Information

ABSTRACT: Dysoline, a novel chromone alkaloid isolated from *Dysoxylum binectariferum*, was reported to have selective cytotoxicity for HT1080 fibrosarcoma cells (IC_{50} of 0.21 μM). Given the scarcity of natural material, a concise synthesis of (+)-dysoline was developed, allowing for further biological evaluation. An enantioselective nucleophile-catalyzed aldol lactonization formed the piperidine ring with control of relative and absolute stereochemistry. Construction of the C6-chromone core with complete regioselectivity was achieved using a Danheiser benzannulation.



Chromone and flavonoid alkaloids represent important scaffolds in medicinal chemistry. Secondary metabolites with these ring systems are found in a variety of plant species, many of which provide sources for traditional medicines.[1] Interest in chromone and flavonoid alkaloids has led to the identification and development of several members of this family that demonstrate an array of medicinal properties.[2] C8-Substituted chromone alkaloids in particular have been well-documented as potent cyclin-dependent kinase (Cdk) inhibitors.[3] Most notably rohitukine (**2**), first reported in 1983, provided the starting point for development of flavopiridol (**4**) and Riviciclib (**5**), both of which act through inhibition of the Cdk9/T1 complex (IC_{50} = 0.02 μM)[3,4,5d] and were given orphan drug status for treatment of myeloid leukemia.[5] In contrast, fewer reports have described biological activity associated with C6-substituted chromone and flavonoid alkaloids.[6]

Dysoline (**1**) is a C6-substituted chromone alkaloid that was isolated from *Dysoxylum binectariferum* in 2013.[7] Dysoline was reported to show cytotoxicity toward HT1080 fibrosarcoma cells (IC_{50} = 0.21 μM) while displaying little effect on a panel of six other human cancer cell lines (IC_{50} values > 10 μM). Additionally, dysoline was found to inhibit the production of TNF-α and IL-6 cytokines. In contrast, dysoline's C8-isomer rohitukine (**2**) arrests the growth of HCT116 (colon cancer) and HL60 (leukemia) cell lines.[5d,8] While possessing a similar structure, dysoline (**1**) demonstrated no activity against HCT116 and did not display substantial inhibition toward the Cdk family or several other kinases tested.[7] Given this reported biological activity and the scarcity of natural product, dysoline presents an attractive target for synthesis.

The regioselective synthesis of C6-substituted chromones remains challenging. Friedel−Crafts alkylation of the dihydroxy-chromone aryl ring results in a mixture of regioisomers with varying levels of selectivity.[9,10] Classical methods for the synthesis of C8-substituted chromone and flavonoid alkaloids involve construction of the chromone motif via a Claisen condensation or Vilsmeier−Haack or Baker-Venkataramanen rearrangement with regioselectivity dictated by the substitution of the starting trimethylphloroglucinol.[11]

A Danheiser-type benzannulation appeared capable of providing regioselective access to the C6-substituted chromone core of dysoline (Scheme 1B).[12] Additionally, the late-stage disconnection would provide a divergent synthesis, allowing for facile generation of analogues. This strategy identified ynol ether **7** as a key intermediate. Finally, a Wynberg−Romo nucleophile-catalyzed aldol lactonization (NCAL) was targeted for construction of the piperidine ring and establishment of the relative and absolute stereochemistry of the natural product.[13] The cis substitution pattern on the piperidine ring would translate into the cis relationship of the chromone ring and hydroxyl substituents. In this way, we hoped to avoid difficulties encountered in a prior synthesis of rohitukine (**2**), in which the alcohol stereocenter had to be corrected through a poorly selective oxidation/reduction sequence.[5a]

To access the requisite acid/aldehyde **9** for use in the NCAL, γ-aminobutyric acid (GABA) was alkylated and protected to give the allylic amine **11** (Scheme 2). Subsequent ozonolysis generated the acid/aldehyde **9** in good yield. While different enals could be employed in the reductive amination, superior results were observed with trans cinnamaldehyde. Subjection of **9a** to the original NCAL conditions reported by Romo (Mukiyama's reagent, triethylamine in acetonitrile) gratifyingly produced the desired bicyclic β-lactone **8a**, albeit in a moderate 28% yield (see Supporting Information). This yield could be improved by using the more soluble pyridinium salt, 2-bromo ethyl pyridinium tetrafluoroborate **12**.[14] A slight increase in yield was also observed using a catalytic amount of quinuclidine. Despite all attempts, different activating agents, bases, or the addition of Lewis acids,[15] the highest yield achievable in our hands was 51%. In addition, employing Cbz or Ts nitrogen protecting groups resulted in lower yield under these conditions (see Supporting Information).

Received: November 26, 2018

DOI: 10.1021/acs.orglett.8b03777
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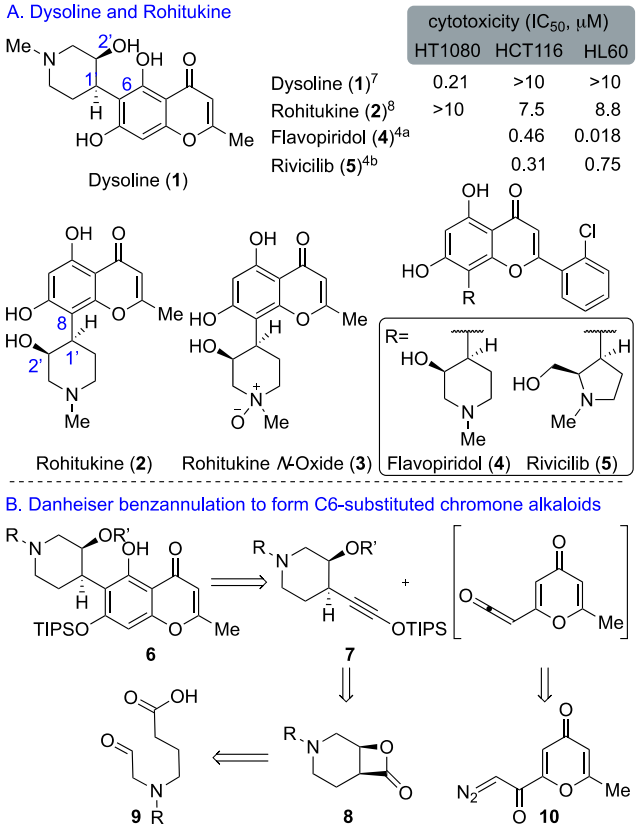
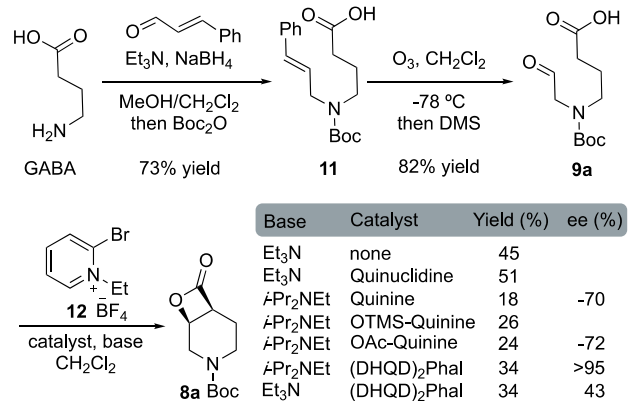
Scheme 1. (A) Chrome Alkaloids Isolated from *D. binectariferum* and (B) Retrosynthesis of Dysoline (1)



Scheme 2. NCAL Reaction for the Preparation of Bicyclic β-Lactone 8a



With optimized positive results for the racemic NCAL reaction, we turned to establishing the best conditions to obtain enantioenriched product. Different cinchona alkaloid catalysts were evaluated for this reaction, and the dimer $(DHQD)_2Phal$ provided the highest yield and enantioselectivity. Advantageously, both enantiomers of the β-lactone are accessible depending on which pseudoenantiomer of cinchona alkaloid was enlisted (Scheme 2).

Initial efforts for conversion of β-lactone **8a** to the desired ynol ether for use in the benzannulation focused on alkyne oxidation with LiOO*t*-Bu in accordance with the method of Julia et al. (Scheme 3).[16] Opening of the lactone with Weinreb's amine followed by MOM protection of the resulting alcohol gave Weinreb amide **17**. Subsequent reduction using Schwartz reagent provided aldehyde **18** in preparation for a Corey–Fuchs homologation.[17,18] Thus, aldehyde **18** was homologated to vinyl dibromide **19**, which was cleanly converted to terminal alkyne **20** with *n*-BuLi.[19] Unfortunately, attempts to oxidize this alkyne with LiOO*t*-Bu failed to yield the desired silyl ynol ether **7a**. Rather, unreacted alkyne was recovered intact. While oxidation of the des-hydroxy piperidine **14** proceeded smoothly from either the alkyne or directly from the vinyl dibromide, this reactivity could not be translated into our more elaborated substrate. Deprotonation of alkyne **20** with *n*-BuLi followed by quenching with CH_3OD resulted in complete deuteration of the alkyne, confirming formation and stability of the lithium acetylide. Use of an alternative oxidant (MeZnOO*t*-Bu) also failed to show desired reactivity. Potential interference from the MOM group led us to explore alternative protected alcohols to no avail.[20]

Unable to oxidize the terminal alkyne **20**, we explored an alternative preparation of the desired ynol ether leveraging chemistry developed by Kowalski.[21] Synthesis of the required substituted piperidine ethyl ester **22** was easily achieved through ethanolysis of the β-lactone followed by MOM protection. Subjection of ester **22** to the lithium anion of dibromomethane resulted in the desired dibromo ketone **23**. Enolate formation followed by lithium halogen exchange and alpha elimination initiated alkyl migration, leading to the desired ynolate, which could be trapped with TIPSOTf to furnish ynol ether **7a** in moderate yield. This sequence could also be done in one pot directly from ethyl ester **22**, although a slight decrease in overall yield was observed. By contrast, the monosubstituted piperidine **16** underwent homologation in substantially higher yield, indicating that steric hindrance by the OMOM could be retarding the reaction. Nonetheless, the Kowalski homologation provided the desired ynol ether **7a** without racemization, as indicated by complete retention of ee of dibromo ketone **23**.

We next turned our attention to accessing the requisite ketene. Accordingly, we targeted pyrone diazoketone **10** (Scheme 4) in anticipation of a Wolf rearrangement.[22] Preparation of the precursor pyrone carboxylic acid **26** was achieved in two steps utilizing a Claisen condensation followed by acid-promoted cyclization. Hydrolysis then furnished the desired carboxylic acid **26**. Conversion of pyrone carboxylic acid **26** to diazoketone **10** was achieved using slightly modified conditions established by Arndt and Eistert.[23] While only a moderate yield was observed for this transformation, the major byproduct was identified as pyrone ethyl ester **25**, which could be recycled into the sequence.

The propensity of pyrone diazo ketone **10** to undergo a Wolf rearrangement was explored by subjecting it to both thermal and photochemical conditions in the presence of ethanol. As expected, conversion of the diazo ketone to the homologated ethyl ester proceeded cleanly under both conditions. It is particularly noteworthy that the rate of conversion could be controlled through choice of light source. This element of control proved advantageous in the Danheiser benzannulation reaction as a means to minimize ketene dimerization.

With the necessary fragments in hand, we focused on the key annulation step to construct the aryl ring of dysoline (**1**). Irradiation of a solution of diazo ketone **10** and ynol ether **7a** with blue LED light (450 nm) formed the desired chromone core (Scheme 5). Further optimization revealed that increasing the temperature of this reaction improved the yield. The major

DOI: 10.1021/acs.orglett.8b03777
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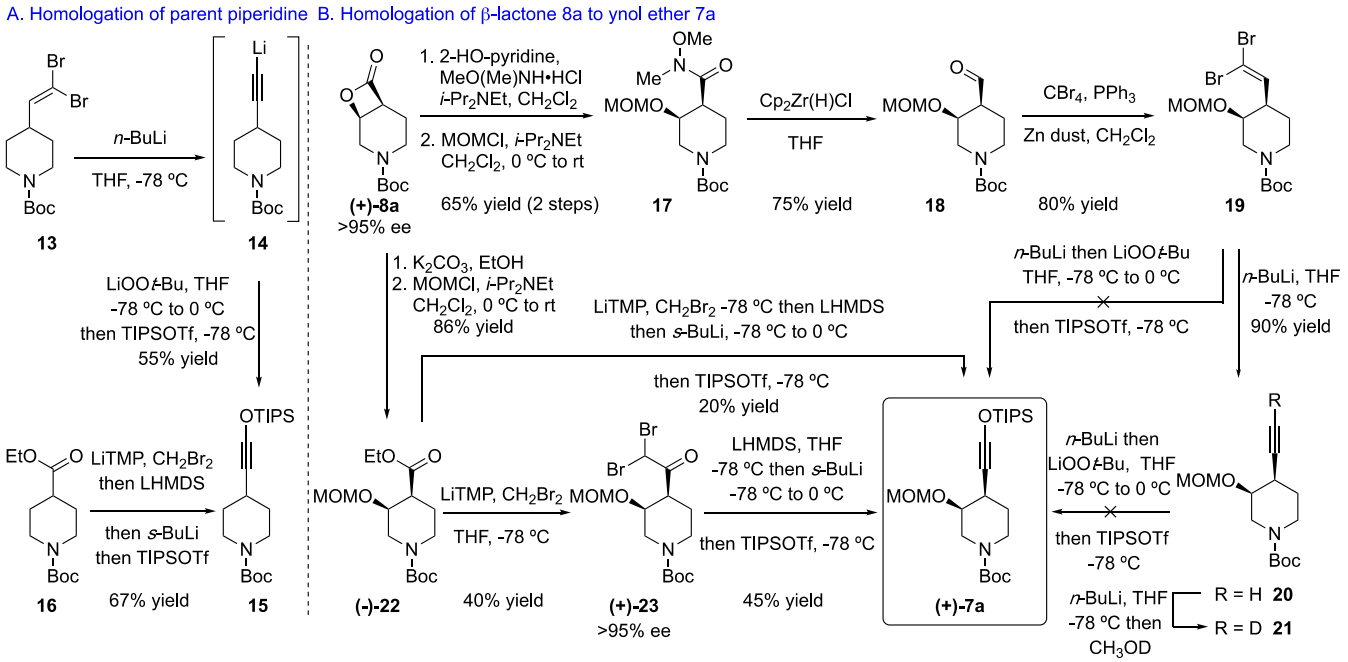
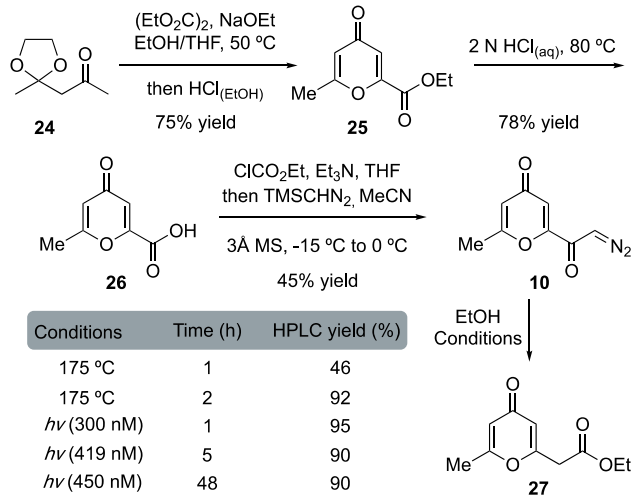
Scheme 3. Homologation and Preparation of Ynol Ether 7a: (A) Parent Piperdine As a Test Substrate and (B) Preparation of Desired Ynol Ether 7a



Scheme 4. Preparation of Diaketone and Homologation to Ethyl Ester



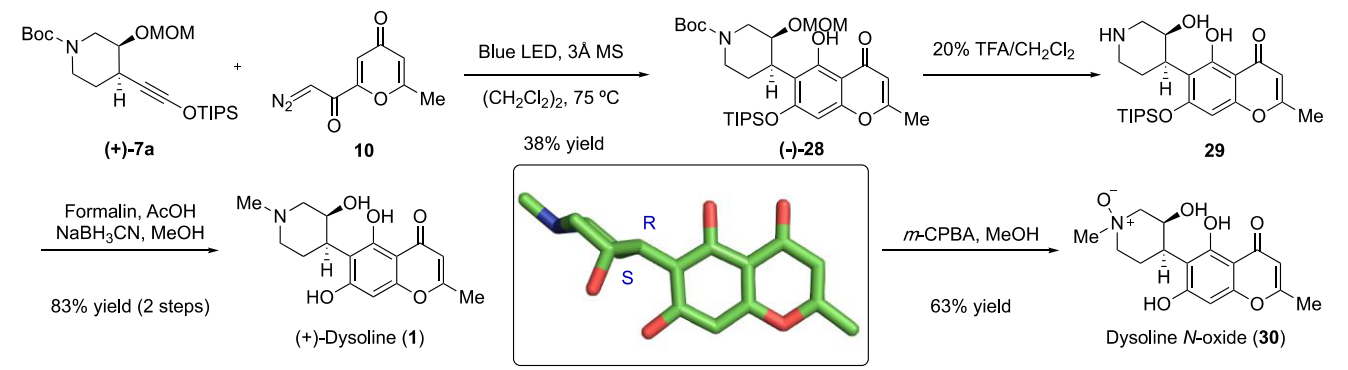
Conditions	Time (h)	HPLC yield (%)
175 °C	1	46
175 °C	2	92
hv (300 nM)	1	95
hv (419 nM)	5	90
hv (450 nM)	48	90

byproduct observed in this reaction was the dimethyl pyrone, presumably resulting from decarboxylation of the homologated carboxylic acid. Formation of this product was decreased, although not eradicated completely, with the use of molecular sieves.

Deprotection of the resulting chromone followed by methylation of the piperidine nitrogen furnished synthetic dysoline (**1**). Mass data and ^{13}C NMR of the synthetic product matched that of the isolation report, while small variation of the proton NMR was observed for the signals adjacent to the piperidine nitrogen.[24] The deviation is likely attributed to differences in protonation status of the synthetic sample compared to the natural sample. Further evidence of structure was obtained by X-ray crystallography of optically active, synthetic material with (−)-CSA. This crystal structure allowed for assignment of absolute (1′R, 2′S) as well as the relative stereochemistry of the natural product.

We next sought to confirm the reported biological activity of dysoline (**1**). Testing of racemic and nonracemic samples for cytotoxicity toward HT1080 and HCT116 cell lines unfortunately showed none of the reported activity. Addition-

Scheme 5. Benzannulation and Final Steps for the Synthesis of Dysoline (1)



DOI: 10.1021/acs.orglett.8b03777
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ally, synthetic dysoline did not inhibit IL-6 cytokine response, contrary to the reported 83% inhibition at 0.1 μM.[7]

With this result, we considered the possibility that the reported activity could arise from the oxidation of the piperidine nitrogen of dysoline (**1**). Although dysoline *N*-oxide (**30**) was not reported by the isolation group, rohitukine *N*-oxide (**3**) was isolated from the same source. We therefore considered N-oxidation as a potential explanation for the discrepancy in biological activity. Thus, treatment of the natural product with *m*-CPBA resulted in clean conversion to a single diastereomer of the *N*-oxide. Testing of this product failed to show any cytotoxicity toward HT1080 cells.

In conclusion, we developed a concise enantio-, diastereo-, and regioselective synthesis of dysoline (**1**). Despite the lack of biological activity, the chromone scaffold of dysoline (**1**) holds promise as a validated scaffold for drug discovery. In addition, the regioselective and divergent nature of this synthetic method should provide value as a means of accessing additional C6-chromone, flavone, or xanthone alkaloids.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03777.

Experimental methods, characterization data, and NMR spectra (PDF)

Accession Codes

CCDC 1876035 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Noelle Williams, Anthony Yuan, and Deepak Nijhawan (UT Southwestern) for assistance testing the biological activity of synthetic dysoline. X-ray crystallography was performed by Dr. V. Lynch (University of Texas at Austin). Financial support from CPRIT (RP180457) and the Welch Foundation (I-1612) is acknowledged.

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(24) Carbon signals matched with the exception of one peak at 56.7 ppm (observed). This difference is attributed to misannotation in the isolation paper as the spectra overlay without discrepancy.

DOI: 10.1021/acs.orglett.8b03777
Org. Lett. XXXX, XXX, XXX−XXX