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Synthesis and biological evaluation of caulibugulones A–E†

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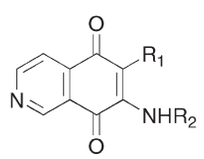
The marine bryozoan metabolites caulibugulone A–E were prepared from a readily available isoquinoline dione. These natural products were found to be potent and selective inhibitors of the dual specificity phosphatase Cdc25B.

Recently, the Gustafson group at the National Cancer Institute (NCI) reported the isolation of caulibugulones A–F, a series of novel cytotoxic isoquinoline quinones and iminoquinones, from an extract of the marine bryozoan *Caulibugula intermis* (Fig. 1).[1] They also disclosed that caulibugulones A–F exhibited IC_{50}'s of 0.03–1.67 μg mL^{-1} against murine tumor cells based on an *in vitro* cytotoxicity assay. These compounds attracted our attention because of our prior extensive work on the synthesis and SAR of novel (iso)quinoline diones.[2] Herein, we now report the synthesis and biological evaluation of caulibugulones A–E.

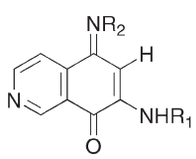


Caulibugulone A
1, R_1 = H, R_2 = Me

Caulibugulone B
2, R_1 = Br, R_2 = Me

Caulibugulone C
3, R_1 = Cl, R_2 = Me

Caulibugulone D
4, R_1 = H, R_2 = CH_2CH_2OH



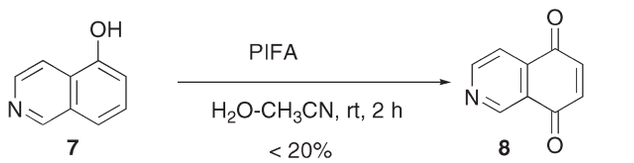
Caulibugulone E
5, R_1 = Me, R_2 = H

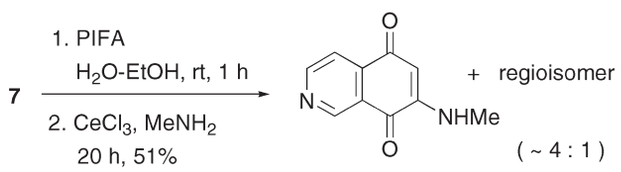
Caulibugulone F
6, R_1 = Me, R_2 = CH_2CH_2OH

Fig. 1 Structures of caulibugulones A–F.

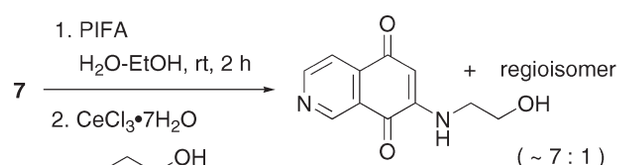
The synthesis of caulibugulones began with the preparation of isoquinoline dione **8** (Scheme 1). According to a literature procedure,[3] we prepared dione **8** from isoquinolin-5-ol, but to our disappointment, this compound was too unstable to be isolated in high yield and purity. Therefore, we proceeded without isolation of intermediate **8** directly toward caulibugulone A. The oxidation of 5-hydroxyisoquinoline by iodobenzene bis(trifluoroacetate) PIFA in a H_2O–EtOH solution and the subsequent *in situ* addition of $CeCl_3$[4] and methyl amine were performed in one pot and provided a ~4 : 1 mixture[5] of **1** and its regioisomer in 51% yield after aqueous work-up and chromatography. Further separation by repeated chromatography on SiO_2 provided pure caulibugulone A as a red solid.[6] Caulibugulone D (**4**) was subsequently synthesized from 5-hydroxyisoquinoline in moderate yield *via* a related two step–one pot sequence using 2-aminoethanol. The low yield can be attributed to poor solubility of this product in organic solvents.

Caulibugulones B and C were synthesized by halogenation of caulibugulone A according to literature procedures (Scheme 2).[1]



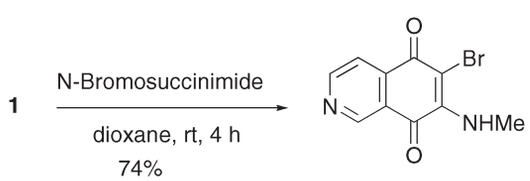


Caulibugulone A (**1**)

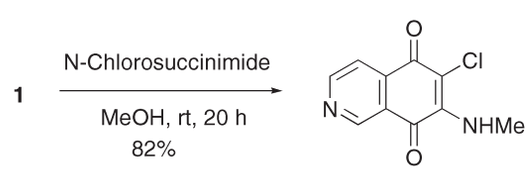


Caulibugulone D (**4**)

Scheme 1 Synthesis of caulibugulones A and D.



Caulibugulone B (**2**)



Caulibugulone C (**3**)

Scheme 2 Synthesis of caulibugulones B and C.

Thus, treatment of **1** with NBS and NCS provided caulibugulone B (**2**) in 74% yield and caulibugulone C (**3**) in 82% yield. ^1H and ^{13}C NMR spectra of these synthetic samples matched the reported data.

Finally, caulibugulone E was prepared by the treatment of caulibugulone A with ammonia in the presence of $Ti(O–iPr)_4$ (Scheme 3).[7] Although the reaction was sluggish, we obtained caulibugulone E in 74% yield, along with recovered calibugulone A in 15% yield after 7 days at room temperature.

Biological evaluation of caulibugulones A–E confirmed our expectation that these compounds were potent inhibitors of the dual specificity phosphatase (DSPase) Cdc25.

† Electronic supplementary information (ESI) available: experimental procedures and ^1H and ^{13}C NMR spectra for all new compounds. See http://www.rsc.org/suppdata/ob/b4/b408184f/

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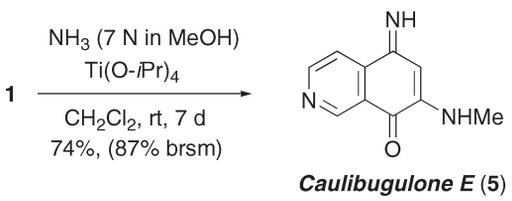
Table 1 IC_{50} concentrations of caulibugulones for inhibition of recombinant human protein phosphatases. All values are μM and are the mean ± SEM of 3 independent determinations

Caulibugulone	Cdc25B	VHR	PTP1B
A (**1**)	6.7 ± 1.3	>500	>1000
B (**2**)	2.7 ± 0.5	130 ± 23	183 ± 24
C (**3**)	5.4 ± 0.7	175 ± 2	322 ± 32
D (**4**)	19.1 ± 0.3	>1000	>1000
E (**5**)	32.5 ± 3.6	>1000	>1000



Scheme 3 Synthesis of caulibugulone E.

As illustrated in Table 1, all five caulibugulones inhibited full-length human Cdc25B *in vitro* with IC_{50} values ranging from 2.7 to 32.5 μM, with caulibugulones B and E being the most and least potent, respectively.[8] Moreover, all caulibugulones exhibited a minimum 30-fold preference as inhibitors against the dual specificity phosphatase Cdc25B compared to the dual specificity phosphatase VHR or the protein tyrosine phosphatase PTP1B. In addition, we have observed cell growth inhibitory activity with human tumor cells consistent with Cdc25 inhibition.[9]

In conclusion, the first total synthesis of the naturally occuring cytotoxic caulibugulones proceeded efficiently in high overall yields from readily available isoquinolin-5-ol *via* hypervalent iodine oxidation,[10] regioselective halogenations, and amination reactions. Biological assays established this class of natural products as new phosphatase inhibitors with considerable selectivity against the Cdc25 family of DSPases. Since this is a potentially clinically relevant biological mechanism of action, studies continue to determine the antitumor activity of caulibugulones.

Acknowledgements

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Notes and references

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5 Regioisomeric ratio was determined by ^{1}H NMR.

6 Each fraction was checked by ^{1}H NMR because TLC analysis could not differentiate between the two isomers. ^{1}H NMR and ^{13}C NMR spectra matched well with the reported spectra.

7 The regiochemistry of caulibugulone E was confirmed by analysis of the HMBC spectrum.

8 Epitope-tagged (His_6) full length human $Cdc25B_2$ was expressed in *E. coli* and purified as previously described.[2] Human recombinant VHR and PTP1B were purchased from BIOMOL (Plymouth Meeting, PA). Activities of all phosphatases were measured using the substrate *O*-methyl fluorescein phosphate (Sigma, St. Louis, MO) at concentrations varying with the K_m of each enzyme in a 96-well microtiter plate assay (25 μL final volume) based on previously described methods.[2] Fluorescence emission from the product was measured after a 20 or 60 min incubation period at ambient temperature with a multiwell plate reader (PerSeptive Biosystems Cytofluor II; Framingham, MA; excitation filter, 485 nm/bandwidth 20 nm; emission filter, 530 nm/bandwidth 30 nm).

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