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Synthesis and anticancer activity of sclerophytin-inspired hydroisobenzofurans

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ABSTRACT

Three structurally related sets of hydroisobenzofuran analogs of sclerophytin A were prepared in three or four steps from (*S*)-(+)-carvone via an aldol-cycloaldol sequence. The most potent members of each set of analogs exhibited IC_{50}'s of 1–3 μM in growth inhibitory assays against KB3 cells. The NCI 60-cell line 5-dose assay for analog **6h** revealed a GI_{50} = 0.148 μM and LC_{50} = 9.36 μM for the RPMI-8226 leukemia cell line, and a GI_{50} = 0.552 μM and LC_{50} = 26.8 μM for the HOP-92 non-small cell lung cancer cell line.

The 2,11-cyclized cembranoids are a class of diterpenoids isolated from a variety of marine sources that exhibit a wide range of biological activities.[1,2] Sclerophytin A, for example, was reported to exhibit growth inhibitory activity against the murine L1210 leukemia cell line with an IC_{50} = 1.0 ng/mL (Fig. 1).[3,4]

A considerable amount of synthetic effort has been directed toward the synthesis of sclerophytin A and related cembranoids.[5,6] Total syntheses of these complex targets typically require in excess of 20 steps from commercially available starting materials. The majority of these diterpenoids possess a *cis*-fused hydroisobenzofuran core structure.[1,2] We hypothesized that seco analogs containing the hydroisobenzofuran core might exhibit some of the same anticancer activities as the parent compounds. We designated sclerophytin A as the nominal target of the analoging study and named the resulting compounds 'sclerologs'.[7] As part of the design principle, we sought to construct novel scaffolds that would readily lend themselves to diversification. To this end, we chose aryl groups as C2 substituents and an ester as the C9 substituent (Scheme 1, cf. Fig. 1). In order to keep the sclerolog syntheses as short as possible, we elected to retain the isopropenyl alkene. It seems unlikely that the steric and/or electronic differences between the isopropenyl and isopropyl groups would have a significant effect on anticancer activity.

In our own studies toward the total synthesis of selected 2,11-cyclized cembranoids, we developed a three-step protocol for assembling the hydroisobenzofuran core structure from (*S*)-(+)-carvone.[8] Following the same procedure, intermolecular aldol reaction of carvone and aryl aldehydes **2a**–**f** gave *anti*-aldol adducts **3a**–**f** with varying levels of diastereoselectivity (1:1–10:1 *anti*:*syn*) (Scheme 1). Etherification of alcohols **3a**–**f** generally proceeded in good yields to give glycolate esters **4a**–**f**. Cycloaldolization under the influence of KHMDS afforded hydroisobenzofurans **5a**–**f** as single diastereomers based on ^{1}H NMR analysis. The stereochemical assignments were made by analogy to earlier examples whose structure was determined by X-ray crystallographic analysis.[8a]

A series of acyl derivatives of ester **5a** were prepared to evaluate the effect of the carboxyl substituent on activity (Scheme 2). Esters **5a.i**–**iii** were prepared by transesterification of ethyl ester **5a** in the presence of excess alcohol and Bu_3SnOAc catalyst, while carboxylic acid **5a.iv** was prepared by saponification.

In the course of optimizing the cycloaldol reaction of glycolate **4a** to ester **5a**, we found that diene **6a** was formed in significant amounts if the reaction mixture was not rapidly quenched with HOAc after addition of KHMDS (Scheme 3). For methyl glycolates **4g** and **4h**, the diene was the only cyclized product isolated from the reaction mixture.

The dienes were presumably formed by in situ lactonization of the aldol intermediates to form β-lactones **7**, which underwent unusually facile loss of CO_2 to give the dienes (Scheme 3).[9] Since the dienes also constituted novel scaffolds, we included several in the subsequent assays (vide infra).

Sclerophytin A and all other hydroisobenzofuran-containing 2,11-cyclized cembranoids possess a C10 stereocenter in the alkane, rather than alcohol, oxidation state (Scheme 3, cf. Fig. 1).[1,2]

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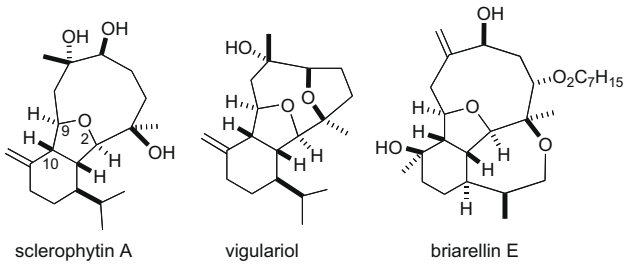
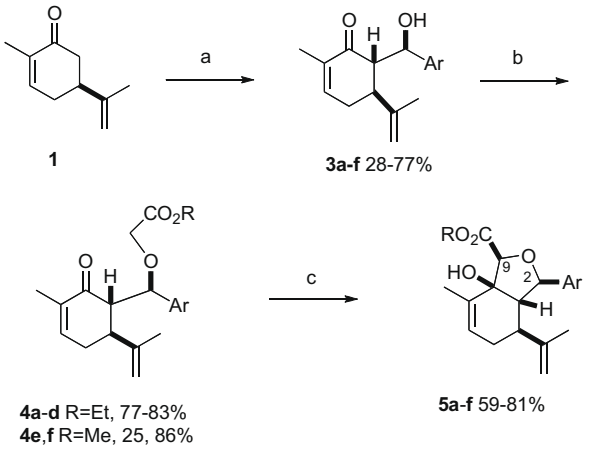


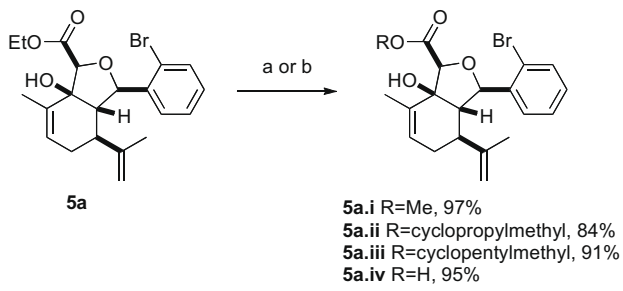
Figure 1. Representative hydroisobenzofuran-containing 2,11-cyclized cembranoids.



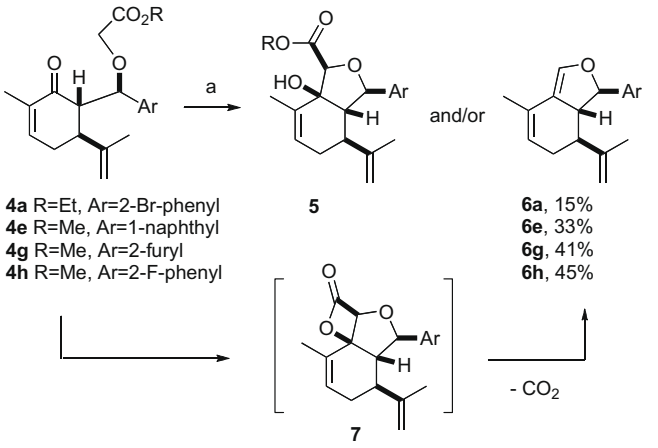
Scheme 1. Sclerolog synthesis via an aldol-cycloaldol sequence. Reagents and conditions: (a) (1) LDA, THF, −78 °C; (2) **2a**–**f**; (3) HOAc; (b) Ag_2O, $BrCH_2CO_2R$, DMF, 2,6-lutidine, rt; (c) (1) KHMDS, THF, −78 °C; (2) HOAc.[a] **2a**–**d**: 2-bromo-, 3-bromo-, 2,3-dichloro-, 2,4-dichlorobenzaldehyde, respectively; **2e**: 1-naphthaldehyde; **2f**: pyridine-2-carboxaldehyde.

We therefore prepared analog **10a** to more closely mimic the natural structures. We have previously reported the reduction of alcohols similar to **5a**–**d** in a three-step oxidative/reductive rearrangement process (Scheme 4).[8a,10] Enones **8a**–**d** were prepared by oxidative rearrangement of the corresponding 3° alcohols.[11] Conversion of enone **6a** to tosyl hydrazone **9a** was followed by reductive transposition to afford *cis*-fused hydroisobenzofuran **10a**. As the enones also constituted a novel structural class, we included them in the biological assays.

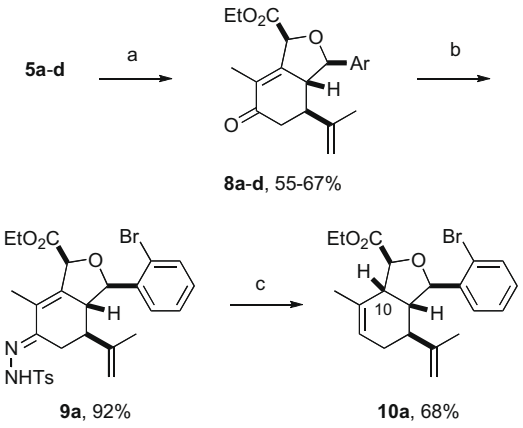
The human KB-3 carcinoma cell line was used to perform the MTT colorimetric assays.[12–14] Cells were treated with increasing concentrations of compounds to assess their growth inhibitory properties. IC_{50} values (concentration of the drug required to reduce cell viability by 50%) ranged from 1 to >100 μM (Table 1).



Scheme 2. Variation of the C9 ester moiety. Reagents: (a) Bu_3SnOAc, ROH; (b) LiOH, $THF/MeOH/H_2O$.



Scheme 3. Proposed sequence for formation of dienes **6**. Reagents and conditions: (a) (1) KHMDS, THF, −78 °C; (2) HOAc.



Scheme 4. Oxidative–reductive rearrangement sequence. Reagents and conditions: (a) PCC, silica gel, CH_2Cl_2; (b) $TsNHNH_2$, EtOH, HOAc; (c) (1) catecholborane, $CHCl_3$, 0 °C; (2) $NaOAc–3H_2O$, reflux.

Several notable features become apparent upon examination of the assay data. Firstly, for the 2-Br esters **5a** and **5a.i**–**iii** (entries 1–4), the smaller the alcohol moiety of the ester, the lower the IC_{50}, that is, Me < Et < cyclopropylmethyl < cyclopentylmethyl. Secondly, two or more members of each class (alcohols **5**, dienes **6** and enones **8**) possess single digit micromolar activity. Reduction of C10 from the alcohol to the alkane oxidation state significantly diminished activity (**5a** vs **10a**)

The essentially equal potency of the alcohols and dienes is intriguing and initially tempted us to speculate that the esters might undergo lactonization and decarboxylation to the dienes under the assay conditions (cf. Scheme 3). This notion is supported by the substantial difference in activity between C10–OH ester **5a**, which can undergo lactonization, and the corresponding C10 reduction product **10a**, which cannot. However, the 1-napthyl methyl ester **5e** is at least 10-fold more active than the corresponding diene **6e**. Furthermore, enones **8a**–**d** exhibit essentially equal potency to the C10–OH methyl and ethyl esters, and also cannot undergo β-lactone formation. Further studies will be needed to determine whether the alcohols, dienes and enones are inhibiting growth by the same or different mechanisms of action.

The most active representatives of the compound classes (alcohol **5d**, diene **6h** and enone **8d**) were submitted to NCI's Developmental Therapeutics program for 60-cell line screening (see Supplementary data for complete results). Single dose assays

Table 1
Inhibition of KB-3 cell survival assessed by MTT viability assay[a]

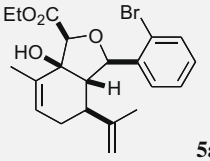
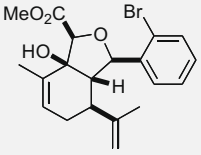
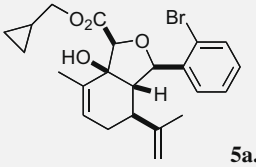
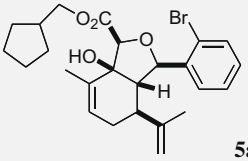
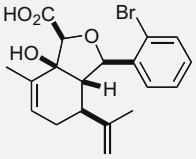
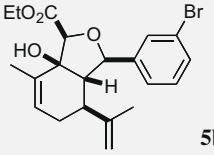
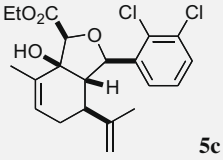
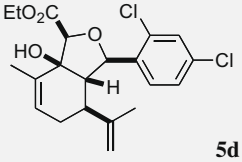
Entry	Compound	IC_{50} (μM)
1	 **5a**	20
2	 **5a.i**	5
3	 **5a.ii**	50
4	 **5a.iii**	>100
5	 **5a.iv**	>100
6	 **5b**	3
7	 **5c**	5
8	 **5d**	3

Table 1 (*continued*)

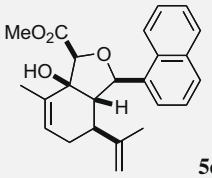
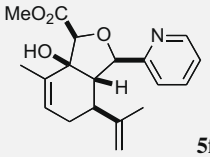
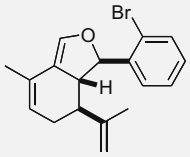
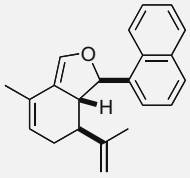
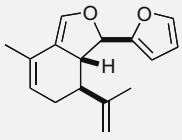
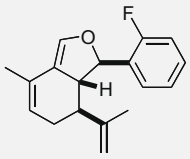
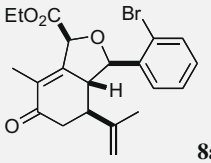
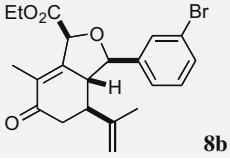
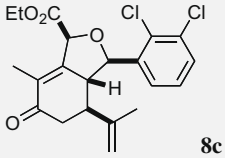
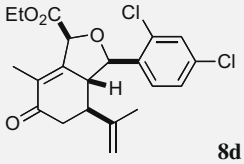
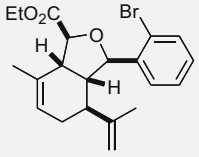
Entry	Compound	IC_{50} (μM)
9	 **5e**	10
10	 **5f**	100
11	 **6a**	4
12	 **6e**	>100
13	 **6g**	2
14	 **6h**	1
15	 **8a**	70
16	 **8b**	30

Table 1 (*continued*)

Entry	Compound	IC_{50} (μM)
17	 **8c**	5
18	 **8d**	3
19	 **10a**	>100

[a] Cells were treated with increasing concentration of compounds and MTT viability assays were performed after 96 h. Values are the means of triplicate assays and are expressed as mean relative to untreated controls (see Supplementary data for details and representative concentration curves).

revealed no significant activity for alcohol **5d**. Enone **8d** exhibited significant differential activity against the RPMI-8226 leukemia and the PC-3 prostate cancer cell lines. Diene **6h** was the most active, possessing significant differential activity against the entire leukemia panel and the NCI-H522 non-small cell lung cancer cell line. Subsequent 5-dose testing of **6h** revealed a GI_{50} = 0.148 μM and LC_{50} = 9.36 μM for the RPMI-8226 leukemia cell line, and a GI_{50} = 0.552 μM and LC_{50} = 26.8 μM for the HOP-92 non-small cell lung cancer line.

The results described herein suggest that the three novel hydroisobenzofuran-containing scaffolds **5**, **6** and **8** exhibit promising anticancer activity. Further SAR studies and investigations into their mechanism of action are pending.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.079.

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