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Biomimetic Syntheses and Antiproliferative Activities of Racemic, Natural (−), and Unnatural (+) Glyceollin I

Rahul S. Khupse,[‡] Jeffrey G. Sarver,[†] Jill A. Trendel,[†] Nicole R. Bearss,[†] Michael D. Reese,[†] Thomas E. Wiese,[§] Stephen M. Boue,[∥] Matthew E. Burow,[⊥] Thomas E. Cleveland,[∥] Deepak Bhatnagar,[∥] and Paul W. Erhardt*[,†]

[†]Center for Drug Design and Development, Department of Medicinal and Biological Chemistry, College of Pharmacy, The University of Toledo, 2801 West Bancroft Street, Toledo, Ohio 43606, United States

[‡]University of Findlay, College of Pharmacy, Findlay, Ohio 45840, United States

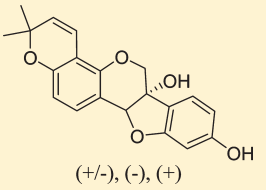
[§]Division of Basic Pharmaceutical Sciences, College of Pharmacy, Xavier University of Louisiana, New Orleans, Louisiana 70125, United States

[∥]Southern Regional Research Center, Agricultural Research Station, United States Department of Agriculture, New Orleans, Louisiana 70124, United States

[⊥]Department of Hematology and Medical Oncology, School of Medicine, and Center for Bioenvironmental Research, Tulane University, New Orleans, Louisiana 70112, United States

Supporting Information

ABSTRACT: A 14-step biomimetic synthetic route to glyceollin I (1.5% overall yield) was developed and deployed to produce the natural enantiomeric form in soy, its unnatural stereoisomer, and a racemic mixture. Enantiomeric excess was assessed by asymmetric NMR shift reagents and chiral HPLC. Antiproliferative effects were measured in human breast, ovarian, and prostate cancer cell lines, with all three chiral forms exhibiting growth inhibition (GI) in the low to mid μM range for all cells. The natural enantiomer, and in some cases the racemate, gave significantly greater GI than the unnatural stereoisomer for estrogen receptor positive (ER^+) versus ER^- breast/ovarian cell lines as well as for androgen receptor positive (AR^+) versus AR^- prostate cancer cells. Surprisingly, differences between ER^+ and ER^- cell lines were not altered by media estrogen conditions. These results suggest the antiproliferative mechanism of glyceollin I stereoisomers may be more complicated than strictly ER interactions.



(+/-), (-), (+)

INTRODUCTION

Interest in the potential for chemoprevention of cancer[1] has grown significantly over the past two decades.[2,3] Drawing from rapidly increasing knowledge about prognostic biomarkers, it may soon be possible to prevent or delay the onset of certain cancers in well-defined, high-risk populations as well as to reduce the recurrence of cancer in selected patients undergoing remission.[4] Within this context, the use of dietary supplements becomes particularly appealing as long-term, prophylactic treatments can be conveniently accommodated within a "normal" diet. To develop herbs and functional foods[5] as credible dietary supplements, definitive chemical fingerprinting[6] and systematic biological characterizations of both the natural matrices and their relevant constituents represent critical steps toward credentializing[7] a new treatment paradigm prior to clinical validation[7] and for assuring quality control (QC) of the marketed products. Because of the normal variation associated with the complex blend of natural materials,[6] and the sometimes tedious methods that may be required to purify their phytochemical constituents, these steps can be difficult to achieve. In addition, long-standing issues about the reliability of cancer prevention models to predict clinical outcomes[8] further complicates the development of naturally derived products for this type of indication. Given this combination of hurdles, we have adopted a strategy to examine promising dietary supplements as their individual nutraceutical[5] components while applying the same level of rigor during preclinical development that is typically afforded to the closely regulated process of drug discovery. Upon credentializing their potential utility, such components would then be ideally positioned for clinical validation within personalized treatment paradigms.[9] Ultimately, these well-defined studies can be coupled with subsequent preclinical evaluations directed toward evolving the optimal natural product mixtures and administration protocols that would best serve the more complex matrices' deployment as cancer preventative dietary supplements tailored for specific patient populations.

Our collaborating laboratories have been undertaking these investigations by systematically examining the possibility for stress-induced fortification[5] of beneficial anticancer principles within legumes that are staples of the U.S. agricultural industry.[10] The common soybean, *Glycine max*, can be considered to be a credible functional food that has well-established beneficial effects on the cardiovascular system. Its natural matrices and several of its purified constituents have also undergone studies

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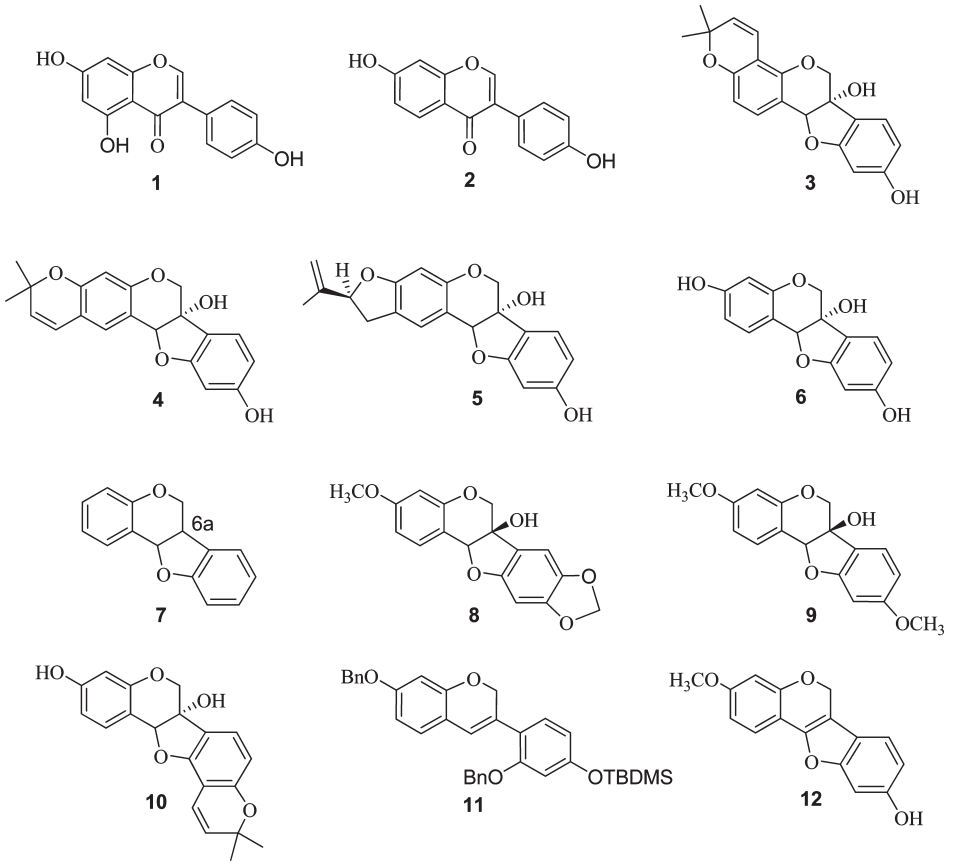

Figure 1. Selected structures. Genistein **1** and daidzein **2** are common isoflavones produced by soybean grown under normal conditions. The glyceollins, such as GLY I **3**, GLY II **4**, and GLY III **5**, are phytoalexins produced by soybean in response to specific stress conditions. All of the GLYs are derived from the phytochemical intermediate glycinol **6**. Structure **7** depicts the typical pterocarpan ring system and specifically denotes the "6a" position. Structures **8** and **9** are (+) pisatin and (+) variabilin, respectively; these compounds are 6a-hydroxypterocarpan natural products which have been previously synthesized by other investigators. Structure **10** was originally thought to be that of GLY I (see text for details). Structure **11** is an appropriately substituted isoflav-3-ene system that serves as a key intermediate for both our former total synthesis and the present biomimetic synthesis of the GLY I stereoisomers. Structure **12** is lespedezol A_1, which we previously prepared and use herein as a model system to explore alternative synthetic entries toward GLY I (see text for details).

for potential use in cancer treatment or prevention. None of these studies, however, have examined the potential anticancer benefits that may become enhanced when soy is subjected to specific stress conditions. Of particular note in soy are the glyceollins (GLYs), phytoalexins that belong to the pterocarpan class of natural products.[11] Phytoalexins are secondary metabolites thought to be produced as a defense mechanism when plants become stressed.[12] Because the phytoalexins are typically present in very low amounts for select periods of time, they represent an additional challenge toward potential development as either a nutraceutical or a dietary supplement. For example, the GLYs can be elicited in trace amounts and isolated as a complex mixture only when soybean plants are subjected to specific types of stress such as when soy cotyledons are infected with *Aspergillus*.[13] Unlike the abundant isoflavonoids genistein and daidzein (**1** and **2** in Figure 1) which are normally present in soy,[14] we have shown that the GLYs exhibit marked antiestrogenic activity in some tissues[15] as well as demonstrating unique anticancer properties[16] and promise for use as selective estrogen receptor modulators (SERMs).[17,18] To date, there have been three GLYs separately isolated from natural sources (**3**−**5** in Figure 1), for which GLY I, **3**, is the most prevalent and appears to be the most interesting family member in terms of exhibiting promising anticancer properties by virtue of its antiestrogenic activities.[19] Most recently, the GLYs' close phytochemical precursor, glycinol (**6** in Figure 1), also has been isolated and studied for these types of effects.[20] Likely owing to its display of two hydroxyl groups in a spatial arrangement that is similar to that for estradiol,[21] **6** exhibits potent estrogen receptor agonist activity that prompts its interest as a potential SERM.[20]

As part of our broad investigation to systematically examine the practical benefits of stressing soy for eventual use as a cancer-preventing dietary supplement, and to potentially develop one or more of the family members as distinct anticancer nutraceutical agents, we have undertaken medicinal chemistry studies directed toward synthesizing and rigorously evaluating each of the individual GLYs.[22−24] Herein, we report our biomimetic synthesis of GLY I and its characterization as an antiproliferative agent within a panel of human, hormone-related cancer cell lines. In addition, the racemate and GLY I's opposite enantiomer were prepared to probe the biological consequences resulting from the distinct stereochemistry that is exquisitely bestowed to this intriguing family of phytoalexin natural products by virtue of their unique 6a-position hydroxyl group.

CHEMISTRY

While the pterocarpans, as represented by **7** in Figure 1, are the second largest group of natural isoflavonoids,[25] their 6a-hydroxy-substituted family has only a few members. The first

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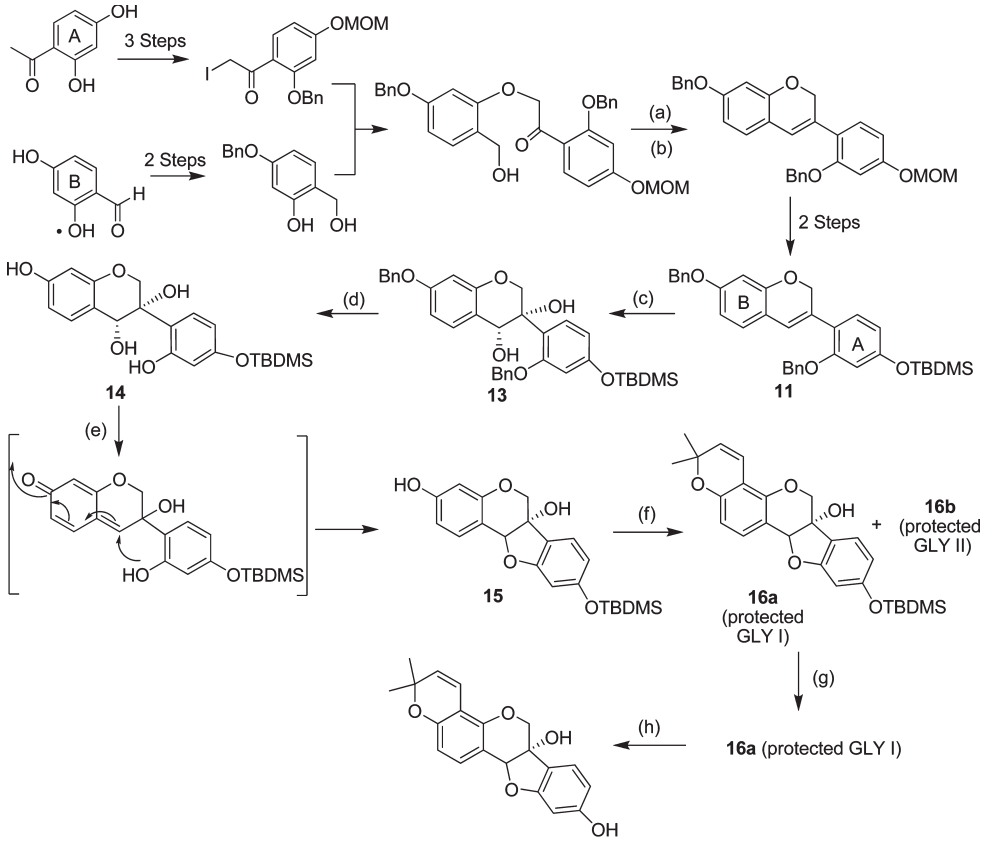
Scheme 1. Previous[23c] Total Synthesis of GLY I Utilizing a Nonbiomimetic Wittig Reaction (Steps (a) and (b)) to Obtain the Key Intermediate Isoflav-3-ene 11 (after Adjustment of Protecting Groups)[a]



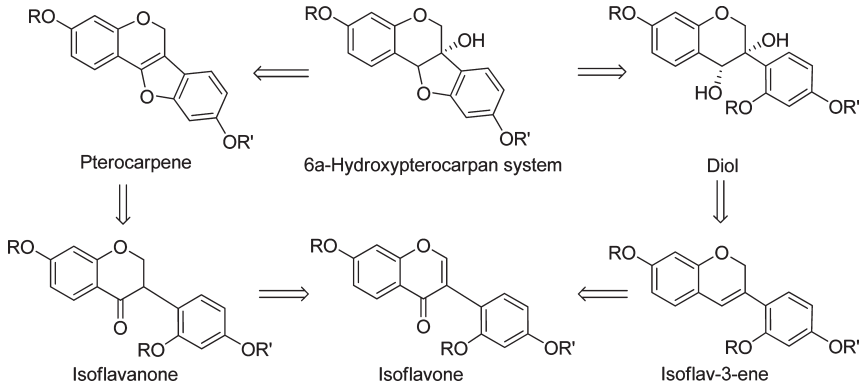
[a] Reagents and conditions: (a) $PPh_3 \cdot HBr$, CH_3CN, room temp; (b) *t*-BuONa, methanol, reflux, 78% (over two steps); (c) OsO_4, $(DHQD)_2PHAL$, CH_2Cl_2, −78 °C; (d) Pd−C(10%), H_2, acetone, 80%; (e) polymeric base, ethanol, molecular sieves 4 Å, 64%; (f) 1,1-diethoxy-3-methyl-2-butene, picoline, *p*-xylene, 130 °C, 61%; (g) silica gel column chromatography, (CH_2Cl_2:MeOH); (h) $N(Et)_3 \cdot 3HF$, CH_3CN, −20 to 4 °C, 77%.

6a-hydroxypterocarpan, (+)-pisatin, was isolated in 1960.[26] Owing in part to the lengthy, multistep routes needed to prepare these contiguous ring systems while maintaining stereocontrol, only four members of this family have been synthesized to date: pisatin,[27] variabilin,[28] and more recently in our laboratories GLY I[23c] and glycinol[24] (structures **8**, **9**, **3**, and **6** in Figure 1, respectively).

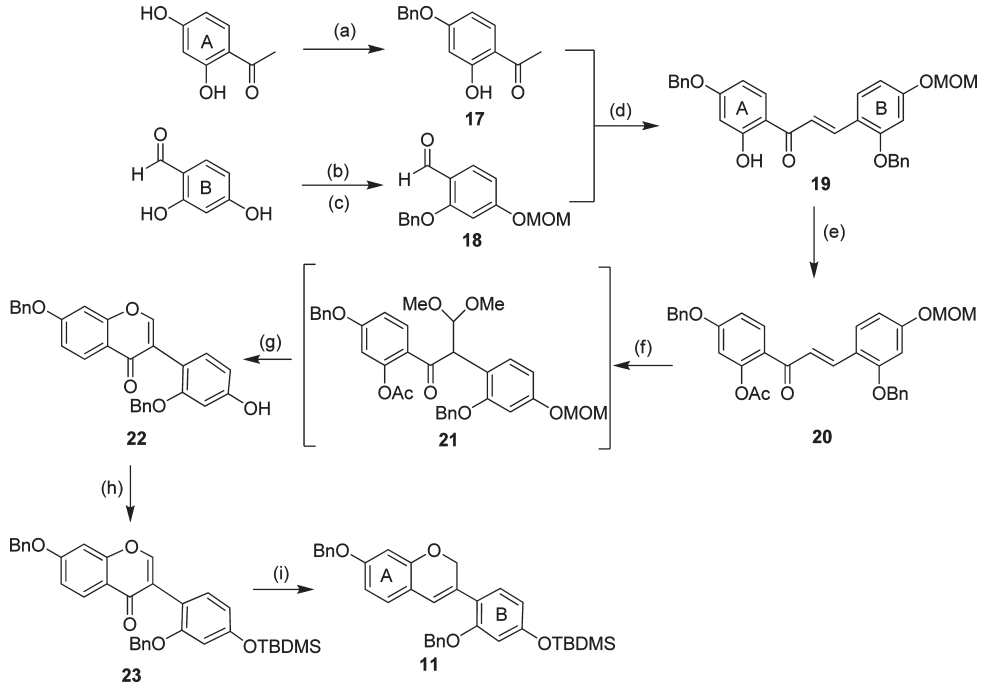
GLY I was first isolated in 1971 by Keen et al. from soybean hypocotyls inoculated with phytophthora which served as a stress factor to elicit the requisite phytochemical pathway.[29] However, they assigned the structure incorrectly and thus initially misnamed it as part of the phaseollin family. When Burden et al. later obtained 6a-hydroxyphaseollin, **10**, as a metabolite from phaseollin,[30] the spectroscopic properties did not match those given by Keen et al. To address the descrepancy, Burden et al. isolated the soybean phytoalexin and determined its correct structure by parallel degradation and mass spectroscopic studies of both compounds wherein each can be shown to have a distinct and characteristic retro-Diels−Alder fragmentation pattern.[31] In 1976, Lyne et al. isolated all of the GLYs from stressed soybean and provided definitive NMR data for the revised structure of GLY I along with the other two family members.[32] Subsequently, Keen et al. renamed the new phytoalexin framework as "glyceollin."[33] In our prior total synthesis of GLY I (Scheme 1), we took advantage of an internal Wittig reaction to form the key isoflav-3-ene intermediate **11**, which was then elaborated to **3** in six steps.[23c]

In addition to being amenable to the production of analogues for future SAR studies, we felt it was important to devise a biomimetic synthetic strategy so that we could also provide analytical standards that might be useful for tracking the chalcone-, isoflavone-, and pterocarpan-related biochemical intermediates that are traversed during stress-induced biosynthesis of the GLYs. The biomimetic approach described herein reserves construction of the isoprenyl-related ring system until the end, such that late-stage divergent chemistry can allow for both GLY I and GLY II analogues to be obtained from the same overall route. Introduction of the 6a-hydroxy-group, either within the context of the cis-fused ring system or as a prelude to the latter's construction, represents one of the most intricate steps leading to these natural products.[34] Previous strategies have typically utilized the isoflav-3-ene from which dihydroxylation and closure to the dihydrobenzofuran produces the natural and more stable cis-fused, multiring system.[35] Alternatively, as shown in retrosynthetic Scheme 2, we imagined that assembly of the GLYs' central skeleton might be accomplished by either of two routes. Both proceed from the same isoflavone and subsequently take advantage of what we perceived to be very accessible alkene intermediates for potential insertion of the 6a-hyroxy-group. The diol

Scheme 2. Retrosynthetic Approaches to the 6a-Hydroxypterocarpan System Required for GLY I



Scheme 3. Biomimetic Route to the Key Isoflav-3-ene Intermediate 11[a]



[a] Reagents and conditions: (a) BnBr, K_2CO_3, CH_3CN, reflux, 10 h, 88%; (b) MOMCl, K_2CO_3, acetone, room temp, 24 h, 70%; (c) BnBr, K_2CO_3, CH_3CN, reflux, 4 h, 80%; (d) piperidine, methanol, 60 °C, 6 h, 80%; (e) acetic anhydride, $N(Et)_3$, room temp, 92%; (f) $Tl(NO_3)_3$, $3H_2O$, methanol: TMOF (1:1), room temp, 8 h; (g) 10% HCl, THF, reflux, 6 h, 68% (over 2 steps); (h) TBDMSCl, $N(Et)_3$, CH_2Cl_2, 90%; (i) (1) $LiBH_4$, THF, 0 °C, 6 h, (2) 10% HCl, reflux, 2 h, 45% (over 2 steps).

route is similar to the literature,[35,36] while the other imagines adding a water molecule across the double-bond of a pterocarpene analogous to what has been reported for indene.[37] For this second route, however, we did not observe any indication of a successful hydration reaction after preparing lespedazol A_1, **12**,[23b] and using it as a model pterocarpene that we subjected to these types of conditions. Details for the reactions associated with our unsuccessful initial strategy can be found in the Supporting Information. The dihydroxylation approach was pursued next and proved to be successful. For this, we adopted the biomimetic route shown in Scheme 3 to produce the key isoflav-3-ene intermediate **11**. Interestingly, while this route begins with the same two starting materials that were used during our former total syntheses (Scheme 1), these initial building blocks are now destined to become reversed within the final product.

In Scheme 3, a Claisen−Schmidt condensation[38] produced chalcone **19** after regioselective protection of the ketone and aldehyde partners **17** and **18**, respectively. We found that the use of piperidine in anhydrous methanol[39] is advantageous to avoid concomitant cyclization of the chalcone to the flavone.[22,23,40] Conversions of chalcones to isoflavones are typically accomplished through an acetal intermediate **21** by using a thallium-mediated oxidative rearrangement.[41] We also attempted this conversion via a newer method that employs Kosher's reagent.[42] Yields for the latter were low (10−15%), however, when compared to the thallium-mediated approach (68%). The nature of the protecting groups influences this rearrangement where

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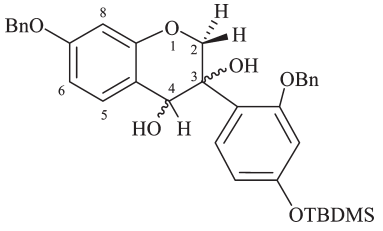
Table 1. NMR Shift (PPM) for Enantiomeric Diols and Their Diastereomeric Complexes with Chiral Shift Reagent (CSR) (the Molar Ratio of Diol:CSR was 5:1)



compd	Ar−H8	H4	H2 equatorial	H2 axial
(+) diol	6.59	5.51	4.74	4.03
(+) diol (+) CSR	6.61	5.75	4.88	4.25
(+) diol (−) CSR	6.62	5.94	5.0	4.45
(−) diol	6.59	5.52	4.74	4.03
(−) diol (−) CSR	6.61	5.78	4.90	4.28
(−) diol (+) CSR	6.62	5.91	4.85	4.41

ring "B" must migrate in preference to ring "A".[43] Because electron withdrawing groups decrease the migratory aptitude, we deployed Bn- and MOM-ether protecting groups for the aldehyde partner **18** which becomes ring "B" in **19**. Although the ketone partner's unprotected *ortho*-hydroxyl group, now present on phenyl ring "A", is needed for the subsequent ring closure, we found that formation of acetal **21** required that this hydroxyl group be transiently protected. Use of an acetyl group (to form **20**) became ideal because its electron withdrawing nature decreases the competing migratory aptitude of ring "A" while being labile enough to be lost under conditions envisioned for the subsequent ring closure.[41] Addition of trimethyl orthoformate led to a cleaner reaction and improved the yield of **21**, perhaps by removing water from the coordination sphere of thallium and promoting its formation of a chelate with **20**. When acidic conditions were deployed to form isoflavone **22**, the acetyl was lost and efficient ring closure was observed. These conditions also removed the MOM protecting group on ring "B", which we felt was additionally advantageous.[23c] Seeking a labile protecting group conducive to later steps in the overall synthesis, we chose TBDMS because it is stable to basic conditions and to hydrogenation while being subject to removal under neutral conditions.[23c] Reprotection of **22** with TBDMS to afford **23** was accomplished by standard methods.[44] The final reaction leading to the key isoflav-3-ene intermediate **11** involved lithium borohydride reduction of isoflavone **23** to an isoflavan-4-ol which undergoes 1,2-dehydration upon treatment with aqueous HCl to provide the desired product.[45] Because **11** is labile under these conditions, it is critical to remove all traces of acid during the workup. Even with such care, however, isolated yields for this final step can still vary from as much as 45% to as little as 20%.

The remaining steps in the overall synthesis involved: (i) construction of the dihydrobenzofuran ring while simultaneously establishing the 6a-hydroxy group in a stereospecific manner, (ii) elaboration of the isoprenyl-containing ring systems, and (iii) final deprotection. All of this chemistry was accomplished according to our previously published total syntheses for which these latter steps are explicitly shown in Scheme 1. Highlights include: (i) deployment of the Sharpless asymmetric dihydroxylation technology for stereospecific introductions of the 6a-hydroxy group,[46] (ii) generation of a quinone-methide to pull the dihydrobenzofuran ring together in its desired *cis*-orientation,[47] and (iii) use of a modified aldol condensation to form the final isoprenyl-ring system in a manner that predominately provides the regiochemistry desired for GLY I.[48] These steps were conducted in parallel for each enatiomer of **13** after refining the chemistry for the individual steps by first deploying the less precious racemic material as a model system. Absolute stereochemistry and ee were assessed at selected intermediate steps by using circular dichroism (CD) and NMR chiral shift reagent studies.[23c]

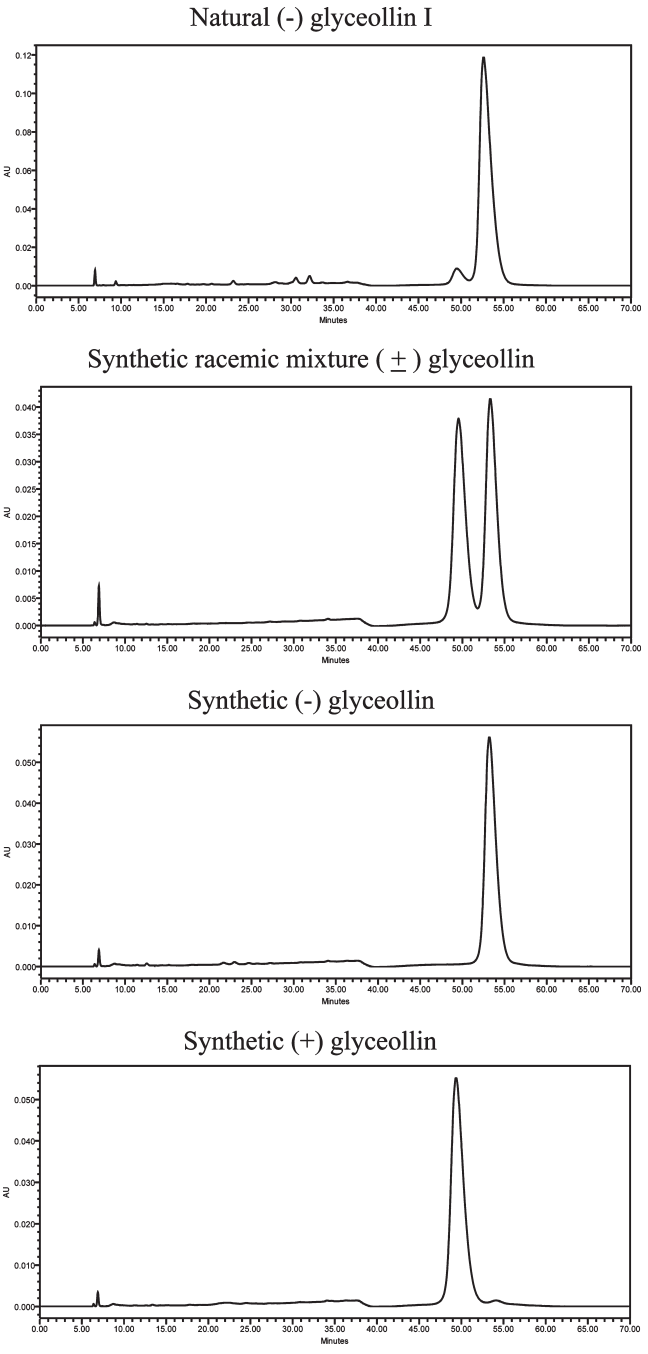


Figure 2. Glyceollin I chiral HPLC fingerprinting. Chiral column. Gradient solvent system utilizing water:methanol:acetonitrile (see Experimental Section for details).

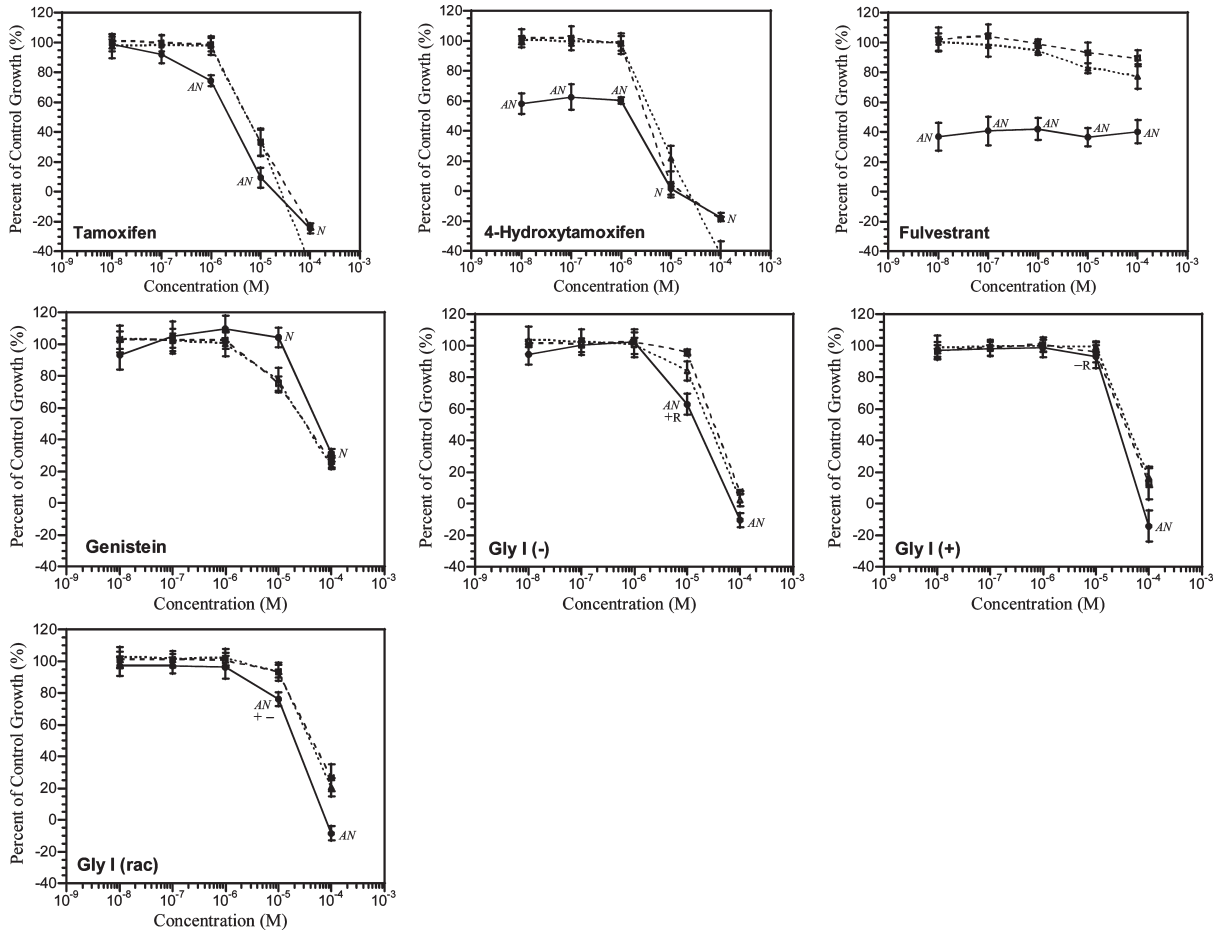


Figure 3. GI effects of standards and different enantiomeric forms of GLY I on the ER^+ MCF7 breast cancer cell line (solid circles, solid line), the ER^- MCF12A breast cell line (open squares, dashed line), and the ER^- NCI/ADR-RES ovarian cancer cell line (open triangles, dotted line), all cultured in low E2 media. Values are the average of eight sets of duplicate well measurements for each condition. Symbols indicate the value for MCF7 cells has a statistically significant difference ($p < 0.05$) versus MCF12A cells (*A*) or NCI-ADR-RES cells (*N*). For the GLY results, symbols further indicate the value for MCF7 cells has a statistically significant difference versus corresponding measurement for the −, +, or racemic (R) forms.

The overall yield to GLY I from the biomimetic route is ca. 1.4%, which is somewhat less than the 3.4% previously achieved via our nonbiomimetic route wherein a Wittig reaction was instead utilized to form the key isoflav-3-ene system **11**.

The proton NMR spectra of our synthesized GLYs resemble the data reported for the materials obtained from nature, all of which bear cis stereochemistry.[32] Most conspicuous are the protons at C-6, which appear as two separate doublets with the C-6 equatorial proton appearing downfield compared to the C-6 axial proton and wherein W coupling occurs with the C-11a and C-6 equatorial proton, an event not possible for the trans system. Both COSY and NOSEY spectra also confirm this relationship between the C-6 equatorial and C-11a protons. Absolute stereochemistry was confirmed by CD[49] and chiral HPLC[23c] studies, which deployed an authentic sample of (−)-(6a*S*,11a*S*)-GLY I obtained from nature as a standard. High-resolution mass spectroscopic data were in agreement with theoretical values for each enantiomer, as well as for racemic GLY I, and correctly supported a common molecular formula of $C_{20}H_{18}O_5$. Specific analytical details are conveyed in the Experimental Section and Supporting Information, the latter also providing NMR spectra, CD spectra, and chiral column chromatograms. Noteworthy for future QC purposes is the convenience of deploying chiral shift reagents during the routine NMR experiments performed upon completion of step c in Scheme 1 so as to be able to quickly assess the status of enantioselectivity for this key dihydroxylation reaction with about ±5% precision. Assessments for the latter are particularly relevant because these diol intermediates appear to have inherently low optical rotations. The NMR shift reagent data are summarized in Table 1. Similarly, a chiral column chromatography method proved to be very convenient for even more precisely determining the ee of the final product materials. Representative chromatograms from these types of assessments are shown in Figure 2 wherein it can be seen that a nearly baseline separation of the two enantiomers was achieved.

BIOLOGICAL RESULTS AND DISCUSSION

The cell growth inhibition (GI) activity of several drugs and selected natural product standards, along with the GLY I stereoisomers, were assessed in a panel of human cell lines that are relevant to hormone-related cancers, namely breast, ovarian, and prostate. Experiments in 96-well format were adapted from our previous methods using breast[50] and prostate[51] cancer cells so as to allow for a range of hormone conditions to be studied across a range of test agent concentrations.

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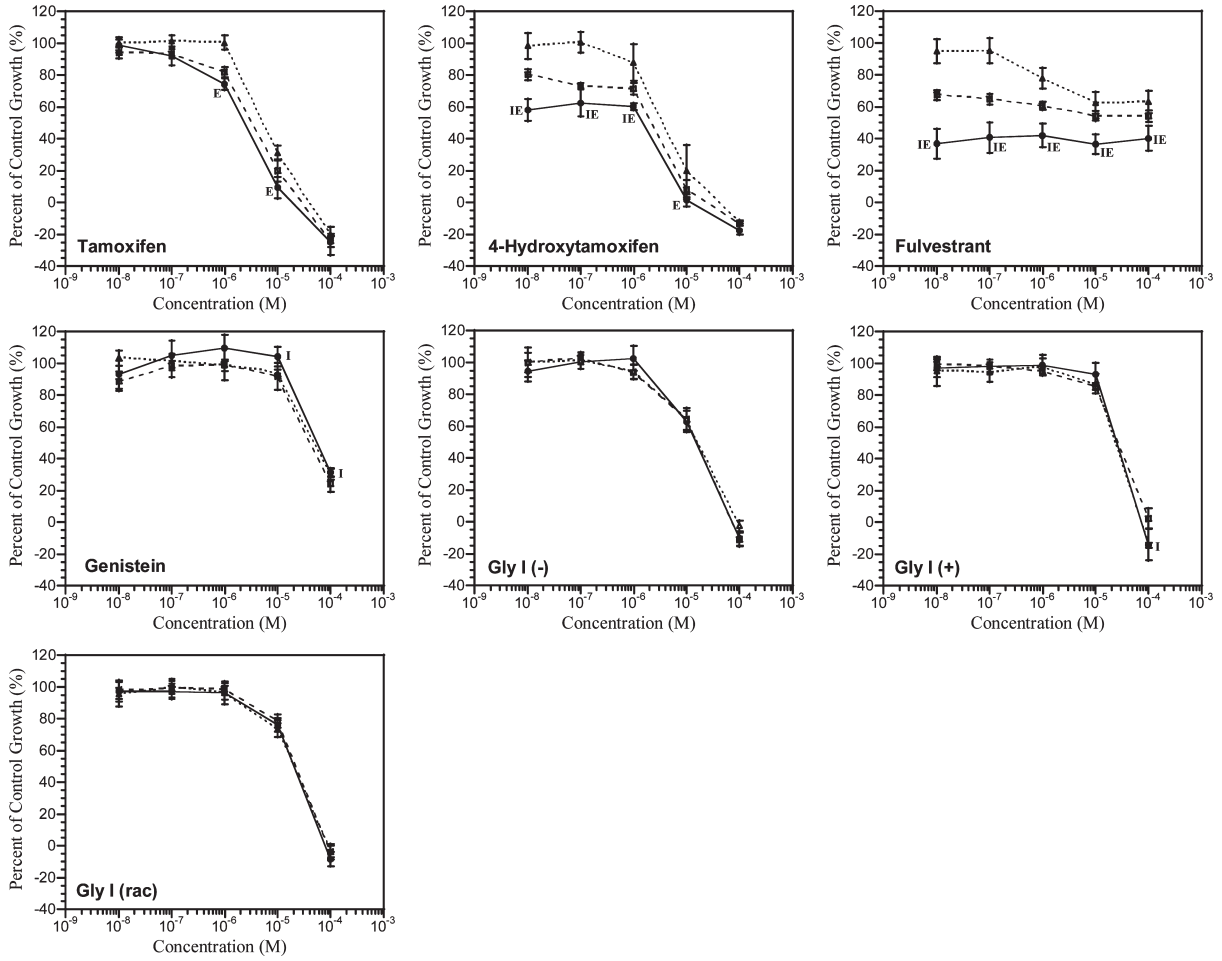

Figure 4. GI effects of standards and different enantiomeric forms of GLY I on the ER$^+$ MCF7 breast cancer cells cultured in low E2 (solid circles, solid line), intermediate E2 (open squares, dashed line), and high E2 (open triangles, dotted line). Values are the average of eight sets of duplicate well measurements for each condition. Symbols indicate the value for low E2 media has a statistically significant difference ($p < 0.05$) versus intermediate (**I**) or high E2 media (**E**).

Results From Human Breast and Ovarian Cell Lines. GI activities were measured on the ER$^+$ MCF7 breast cancer cell line, the ER$^-$ MCF12A immortalized normal breast epithelial cell line, and the ER$^-$ NCI/ADR-RES ovarian cancer cell line, wherein the ER$^+$ and the ER$^-$ designations reflect the presence or absence of functional estrogen receptors, respectively. Three different types of media conditions having specified levels of estradiol (E2) were used for each cell line: low E2 media (0.003 nM E2, 0.01 nM testosterone), intermediate E2 media (0.6 nM E2, 0.7 nM testosterone), and high E2 media (100 nM E2, 0.7 nM testosterone). A full graphical summary of the data from all three cell types in each media condition is provided in the Supporting Information. Selected subsets of these results that illustrate the overall findings are provided in Figures 3 and 4 and Table 2.

The results from all three cell lines at low E2 (Figure 3) clearly showed differences in the GI effects of the test agents on ER$^+$ versus ER$^-$ cell lines. Statistically significant differences ($p < 0.05$) between the ER$^+$ cell line and the two ER$^-$ cell lines are indicated in these graphs by italic letters *A* and *N*. In line with previous literature, the pure antiestrogenic drug fulvestrant significantly reduced cell growth of the ER$^+$ cells while having little or no impact on the growth of either of the ER$^-$ cell lines.[52] 4-Hydroxytamoxifen, the active metabolite of tamoxifen,[53] specifically inhibited only the ER$^+$ cell line at 10^{-8} M to 10^{-6} M, with both ER$^+$ and ER$^-$ cell lines being affected at higher concentrations. Tamoxifen itself showed a less ER-specific effect on growth but still yielded significant differences in response of ER$^+$ and ER$^-$ cells at intermediate concentrations. In agreement with previous findings that genistein can have differential estrogenic effects at different concentrations,[54] in our experiments this common soy natural product appeared to have some inhibitory effect on ER$^+$ cell growth at the lowest concentration while increasing estrogen-responsive cell growth at higher concentrations, although few of the differences between cell lines are statistically significant. The GLY I enantiomers and racemic mixture affected the growth of all three cell lines at higher concentrations. While the natural (−) enantiomer and the racemic mixture showed statistically significant differences between ER$^+$ and ER$^-$ cells at 10^{-5} M, the unnatural (+) enantiomer only caused differences at the highest concentration. Statistically significant differences between the effects of (−), (+), and racemic GLY I on MCF7 cells are also indicated by symbols on the graphs. All three GLY forms yielded significantly different levels of MCF7 GI at 10^{-5} M. Natural GLY I had the largest effect, the unnatural enantiomer had the least effect, and the racemic mixture was in-between with significantly different values versus each of the other forms.

Table 2. Measured $\log_{10}(GI_{50})$ Values ($n = 8$, GI_{50} in molar concentration) of Standards and Enantiomeric Forms of Gly I for the ER^+ MCF7 and the ER^- MCF12A Breast Cell Lines and for the NCI-ADR-RES Ovarian Cell Line[a]

	$\log_{10}[GI_{50}(M)] \pm 95\%$ CI								
	MCF12A			NCI-ADR-RES			MCF7		
agent	5% FBS	FBS/NS	FBS/NS +E2	5% FBS	FBS/NS	FBS/NS +E2	5% FBS	FBS/NS	FBS/NS +E2
tamoxifen	−5.24 ± 0.11	−5.16 ± 0.09	−5.24 ± 0.05	−5.24 ± 0.08 **IE**	−5.02 ± 0.08 **L**	−4.94 ± 0.03 **L**	−5.64 ± 0.06 *AN* **E**	−5.53 ± 0.05 *AN* **E**	−5.25 ± 0.05 *N* **LI**
4-hydroxy-tamoxifen	−5.48 ± 0.09 (≫−4.0)	−5.41 ± 0.03 (≫−4.0)	−5.49 ± 0.09 (≫−4.0)	−5.35 ± 0.09 **E** (≫−4.0)	−5.21 ± 0.05 (≫−4.0)	−5.11 ± 0.12 **L** (≫−4.0)	−5.85 ± 0.03 *AN* **E** (<−8.0) *AN* **IE**	−5.72 ± 0.04 *AN* **E** (−3.1) **L**	−5.46 ± 0.18 *N* **LI** (−3.1) **L**
fulvestrant									
genistein	−4.49 ± 0.09 **I**	−4.66 ± 0.11 **L**	−4.52 ± 0.13	−4.49 ± 0.09 **I**	−4.77 ± 0.12 **LE**	−4.57 ± 0.06 **I**	−4.21 ± 0.06 *AN*	−4.34 ± 0.10 *AN*	−4.28 ± 0.05 *AN*
Gly I (−)	−4.48 ± 0.06	−4.56 ± 0.06	−4.42 ± 0.06	−4.61 ± 0.07	−4.52 ± 0.06	−4.53 ± 0.10	−4.88 ± 0.05 *AN* +R	−4.86 ± 0.10 *AN* +R	−4.82 ± 0.08 *AN* +
Gly I (+)	−4.42 ± 0.12	−4.41 ± 0.13	−4.33 ± 0.09	−4.37 ± 0.05	−4.35 ± 0.10	−4.52 ± 0.09	−4.64 ± 0.06 *AN* −	−4.62 ± 0.09 *AN* −	−4.67 ± 0.04 *A* −
Gly I (rac)	−4.32 ± 0.10	−4.34 ± 0.04	−4.29 ± 0.07	−4.38 ± 0.07	−4.43 ± 0.07	−4.34 ± 0.05	−4.76 ± 0.05 *AN* −	−4.70 ± 0.08 *AN* −	−4.74 ± 0.05 *AN*

[a] Lower values indicate greater activity. Values in parentheses have been estimated by extrapolation beyond the range of measured concentrations. Italic symbols below an entry indicate the value for MCF7 cells has a statistically significant difference ($p < 0.05$) versus MCF12A cells (*A*) or NCI-ADR-RES cells (*N*) in the same media. Bold symbols indicate a statistically significant difference ($p < 0.05$) versus low (**L**), intermediate (**I**), or high (**E**) E_2 media for a given cell line. For GLY results, symbols below a value indicate a statistically significant difference versus corresponding measurement for the −, +, or racemic (R) forms in the same media for a given cell line.

GI results for the ER^+ MCF7 cell line at each of the three E2 media conditions are graphically summarized in Figure 4. In this series of graphs, statistically significant differences between the low E2 media versus the intermediate and high E2 media conditions are indicated by the bold letters **I** and **E**, respectively. Once again, fulvestrant provided the most consistent differences, with GI effects decreasing systematically with increasing E2 levels and significant differences between the low E2 media and the two elevated E2 levels at all concentrations tested. 4-Hydroxytamoxifen showed significant differences between low E2 and both elevated E2 levels at 10^{-8} M to 10^{-6} M, a difference between the low and highest E2 levels at 10^{-5} M, and no difference between E2 levels at 10^{-4} M. Tamoxifen only showed significant differences at intermediate agent levels, and the differences were only significant between the low and high E2 levels. The apparent low concentration GI effects and high concentration growth enhancement effects of genistein seem to be muted by increasing E2 levels, although differences were once again not generally statistically significant. Interestingly, none of the GLY I forms showed any differences in MCF7 growth inhibition with changing levels of E2, nor were there any significant differences between the effects of the different enantiomeric forms at any of the tested agent concentrations.

Another means of comparing GI effects is to consider specific GI_{50} values, the latter representing the estimated concentration of test agent that causes a 50% reduction in cell growth versus vehicle-only control wells. GI_{50} values for each cell line in each type of media with each test agent are summarized in Table 2. Results are recorded as $\log_{10}(GI_{50})$ values to make the table more legible and to account for our experience that GI_{50} data generally follows a log-normal distribution pattern.[50,51] Statistically significant differences between $\log_{10}(GI_{50})$ values have again been indicated by italic letters *A* or *N* for ER^+ MCF7 comparisons versus ER^- MCF12A and NCI-ADR-RES cell lines, respectively, and by bold letters **L**, **I**, or **E** for statistical significance versus low, intermediate, and high E2 levels, respectively. Fulvestrant's GI was less than 50% for all concentrations ($GI_{50} > 10^{-4}$ M) across all cell lines and conditions except ER^+ MCF7 cells in low E2 media for which inhibition is above 50% for all concentrations ($GI_{50} < 10^{-8}$ M). This resulted in fulvestrant's $\log_{10}(GI_{50})$ values being below −8.0 for this lone condition, or above −4.0 for all other conditions, with the MCF7 cells in low E2 media being statistically different from all of the other conditions and no further statistical analysis being possible. Tamoxifen and 4-hydroxytamoxifen $\log_{10}(GI_{50})$ values showed statistical differences between ER^+ MCF7 cells and both ER^- cells at low and intermediate E2 levels, and consistent differences between MCF7 results at high E2 levels versus both low and intermediate E2. Genistein gave consistent statistical differences between ER^+ and ER^- cell lines but no significant differences between ER^+ MCF7 cells at different E2 levels. Tamoxifen, 4-hydroxytamoxifen, and genistein all occasionally exhibited statistically significant differences between E2 levels for one or both ER^- cell lines, but none of these differences showed a consistent relationship between E2 level and effect (i.e., none have **E** > **I** > **L** or **L** > **I** > **E**). Conversely, results for the different forms of GLY I were highly consistent in showing no significant differences between E2 levels for any cell line. There were, however, significant differences for each GLY I form at all E2 levels between ER^+ MCF7 cells and both ER^- cell lines. Statistically significant differences between (−), (+), and racemic GLY I are indicated by −, +, and R symbols in Table 2, respectively. There were no significant differences

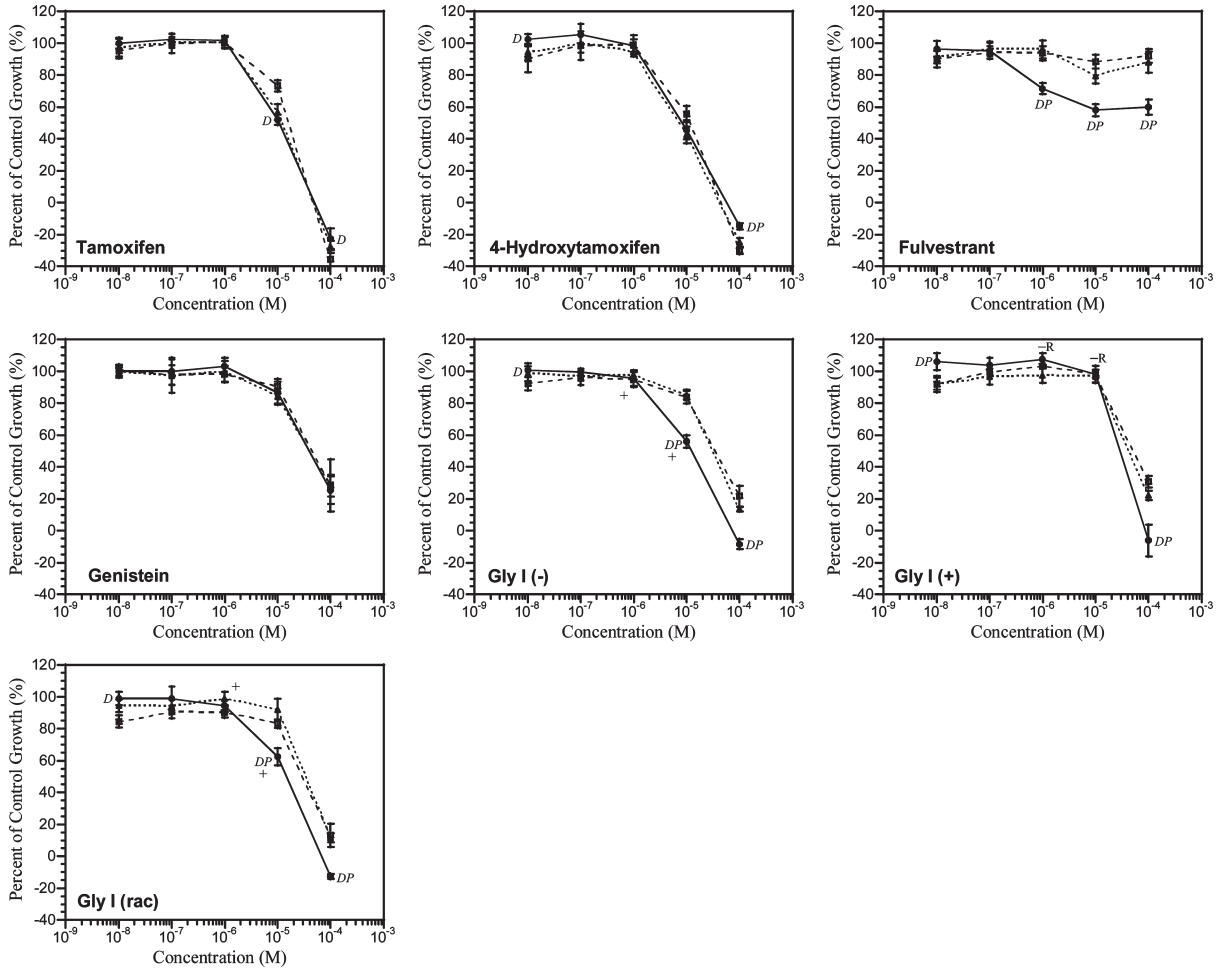

Figure 5. GI effects of standards and different enantiomeric forms of GLY I on the AR^+ LNCaP prostate cancer cell line (solid circles, solid line) and AR^- PC3 (open squares, dashed line) and DU145 (open triangles, dotted line) prostate cancer cell lines. Values are the average of eight sets of duplicate well measurements for each condition. Symbols indicate the value for LNCaP cells has a statistically significant difference ($p < 0.05$) versus DU145 cells (*D*) or PC3 cells (*P*). For the GLY results, symbols further indicate the value for LNCaP cells has a statistically significant difference versus corresponding measurement for the −, +, or racemic (R) forms.

between GLY I forms for either of the ER^- cell lines, but MCF7 cells had significant differences between the natural and both other forms at low and intermediate E2 levels, while the (−) and (+) forms remained significantly different for high E2 conditions.

Results From Human Prostate Cell Lines. The GI effects of the same standards and GLY I forms also were evaluated in media containing 0.6 nM E2 and 0.7 nM testosterone across a panel of human prostate cancer cell lines. The panel included AR^+ LNCaP cells and AR^- PC3 and DU145 cells wherein the AR^+ and AR^- designations reflect the presence or absence of functional androgen receptors, respectively. These GI results are summarized graphically in Figure 5, with statistically significant differences between LNCaP cells versus PC3 and DU145 cells indicated by italic letters *P* and *D*, respectively. As with the ER+ cell line, fulvestrant displayed a highly selective growth inhibitory effect on the AR^+ LNCaP cells, with significant differences versus both AR^- cell lines at the three highest concentrations. Tamoxifen and 4-hydroxytamoxifen exhibited only small and occasionally significant differences between cell lines, while genistein showed no significant differences between cell lines. Alternatively, each of the GLY I forms yielded significant differences in GI between the AR+ and AR− cell lines at the highest concentration, while the (−) and racemic forms also showed significant differences between cell lines at the 10^{-5} M level. The (−) and racemic forms also showed significant differences versus the (+) form at both 10^{-6} M and 10^{-5} M levels, as similarly indicated on these graphs by the −, +, and R symbols. Using the same symbols to indicate statistically significant differences, Table 3 shows that the $\log_{10}$ (GI_{50}) values for the prostate cancer cell lines exhibited little or no cell line differences for tamoxifen, 4-hydroxytamoxifen, or genistein but consistent significant differences between AR^+ and AR^- cell lines for each of the GLY I forms. LNCaP cells also showed significant differences in GI_{50} values for GLY I's natural and racemic forms versus the unnatural form. PC3 and DU145 cell lines exhibited smaller differences between the GLY I forms, albeit some of the differences remained statistically significant.

Discussion. Tamoxifen, 4-hydroxytamoxifen, and fulvestrant yielded GI effects that are dependent on ER expression and E2 level, as would be expected for agents acting primarily through antagonistic ER interactions. Genistein appeared to exhibit weak antagonistic effects at low concentrations and weak agonistic effects at high concentrations, in agreement with previously reported studies.[55]

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Table 3. Measured $\log_{10}$ (GI_{50}) Values (n = 8, GI_{50} in Molar Concentration) of Standards and Enantiomeric Forms of GLY I for the AR^+ LNCaP and the AR^- PC3 and DU145 Prostate Cell Lines[a]

	$\log_{10}$ [GI_{50} (M)] ± 95% CI		
agent	PC3	DU145	LNCaP
tamoxifen	−4.96 ± 0.05	−4.86 ± 0.04	−5.00 ± 0.04 *D*
4-hydroxytamoxifen	−5.12 ± 0.09	−4.97 ± 0.05	−5.08 ± 0.09
fulvestrant	(≫ −4.0)	(≫ −4.0)	(−3.4)
genistein	−4.37 ± 0.11	−4.30 ± 0.21	−4.41 ± 0.13
Gly I (−)	−4.51 ± 0.03	−4.44 ± 0.13	−4.94 ± 0.07
	+	+	*PD* +
Gly I (+)	−4.32 ± 0.05	−4.24 ± 0.10	−4.56 ± 0.11
	−	−R	*PD* −R
Gly I (rac)	−4.49 ± 0.09	−4.53 ± 0.10	−4.89 ± 0.05
		+	*PD* +

[a] Lower values indicate greater activity. Values in parentheses have been estimated by extrapolation beyond the measured range of concentrations. Italic symbols below an entry indicate the value for LNCaP cells has a statistically significant difference ($p < 0.05$) versus PC3 cells (*P*) or DU145 cells (*D*) in the same media. For the GLY results, symbols below the value further indicate a statistically significant difference versus corresponding measurement for the −, +, or racemic (R) forms in the same media for a given cell line.

The natural and racemic forms of GLY I, and to a lesser extent the unnatural form, displayed larger GI effects on ER^+ versus ER^- cells in low E2 media, as again expected based on the antiestrogenic activities reported for GLY mixtures and GLY I from some of our previous studies.[15d,19a] Surprisingly, however, all three GLY I forms exhibited substantial GI effects on ER^- cell lines, at concentrations that are not all that much greater than those inhibiting ER^+ cell growth. Further, the GI effects of the different forms of GLY I appeared to be completely unaffected by changes in E2 levels from 0.003 nM E2 (near the low end of the normal range for postmenopausal women[56]) to 100 nM E2 (nearly 2 orders of magnitude above the high end of the normal range for premenopausal women[56]). These results suggest that the primary mechanism for the GI effects observed for the GLY I forms in the present studies may not be through interactions with ERs.

The experiments with AR^+ and AR^- prostate cancer cell lines indicate that GLY I, particularly its natural and racemic forms, had a greater GI effect on AR^+ LNCaP cells than the AR^- PC3 and DU145 cell lines. Fulvestrant significantly and selectively inhibited the growth of AR^+ LNCaP cells, while tamoxifen, 4-hydroxytamoxifen, and genistein displayed little or no difference in effect on AR^+ versus AR^- cell lines. The GI effects of fulvestrant and GLY I could potentially be due to antiestrogenic effects against ERβ, which is reported to be the more dominant form of ER expressed in prostate cancer cells[57] (whereas ERα is generally the dominant form expressed in breast cancer cells[58]). Alternatively, these agents could be interacting with the AR expressed in LNCaP cells, which is known to have a mutation giving it relatively promiscuous steroid-binding characteristics.[59] Further testing will be necessary to elucidate whether the LNCaP GI activity is related to ERβ, a promiscuous AR, or possibly a previously unrecognized AR-related mechanism.

In addition to antagonizing estrogen- and androgen-mediated signaling pathways, other anticancer mechanisms for genistein detailed in the literature include antioxidant properties, cell cycle arrest, increased apoptosis, and inhibition of angiogenesis and metastasis.[14,16c,55c] Our preliminary mechanistic studies[16] with a mixture of GLY I, II, and III showed increased G0/G1 cell cycle arrest in LNCaP cells analogous to genistein induced changes, but in PC3 cells the GLY mix appeared to cause S phase arrest while genistein increased G2/M arrest. Also unlike genistein, the GLY mix did not appear to exhibit significant effects on apoptotic events or caspase activation in LNCaP cells.[16] The antioxidant properties of GLY I (+, −, and racemic) as well as GLY mix have been measured in our laboratory and have been found to be similar to genistein (data not shown). Further testing of GLY I effects on angiogenesis, metastasis, and signaling cascades will be needed to complete the comparison of genistein and GLY anticancer activities.

As stereochemical probes per se, the GLY I racemate and enantiomers appear to represent the first phytoestrogen-related anticancer or SERM natural products to be undergoing studies in this manner.[19b] The closest work in this regard would be that for equol, which is an asymmetric, gut-bacteria-derived human metabolite of the common soy natural product daidzein. Shown to be formed as its (*S*)-enantiomer,[60] stereochemical studies previously have demonstrated differential interactions between the two possible equol enantiomers for the ERs.[61] Alternatively, several studies have investigated the stereochemistry associated with common synthetic SERM compounds. For example, asymmetric derivatives of diethylstilbestrol,[62] dichlorodiphenyltrichloroethane,[63] raloxifene,[64] and diarylpropionitrile[65] have all shown differences in their biological activities that may be related to preferential binding of one of the enantiomers to ERs. Our prior studies involving specific characterization of interactions with the ERs suggest that while both of GLY I's enantiomers can bind with similar affinities to both receptor subtypes, natural GLY I exhibits only antagonistic actions, whereas (+) GLY I can serve as a weak agonist. Additionally, each GLY I enantiomer was found to *"induce unique gene expression profiles in a PCR array panel of genes commonly altered in breast cancer."*[19b] The data reported herein implies that the overall antiproliferative properties exhibited by the GLY I stereoisomers may be derived from a more complicated mechanistic picture than can be explained by relying upon just the classical types of interactions with ERs. This further prompts the need for additional investigations of this interesting compound that are directed toward delineating its principal mechanism(s) of action.

CONCLUSION

A biomimetic synthetic route to GLY I has been developed. In addition to being useful for the elaboration of analogues that can contribute to SAR, this method provides practical access to analytical standards that may be used for QC purposes when GLY I is obtained naturally from stressed soy bean. For the latter, asymmetric NMR shift reagents and chiral HPLC have been shown to be very practical methods for preliminary and final assessments of ee. Both enantiomers, as well as the GLY I racemate, were studied in antiproliferation studies using hormone-related human cancer cell lines. Within the breast and ovarian cell culture panel, all three GLY I forms exhibit substantial GI effects on ER^- cell lines, at concentrations that are not

all that much greater than those inhibiting ER^+ cell growth. Within the prostate cell culture panel, GLY I, particularly its natural and racemic forms, have a greater GI effect on AR^+ LNCaP cells than the AR^- PC3 and DU145 cell lines. Thus, although not pronounced, there do appear to be some differences between the GLY I enantiomers in their overall GI profiles. Perhaps more importantly, however, the composite of these studies suggest that the overall antiproliferative properties exhibited by the GLY I stereoisomers may be derived from a more complicated mechanistic picture than can be explained by relying upon just the classical types of interactions with ERs. This, in turn, further prompts the need for additional investigations of this interesting natural product that are directed toward delineating its principal mechanism(s) of action, as well as toward confirming its promise as a potential anticancer and cancer preventative agent.

EXPERIMENTAL SECTION

Chemistry. *General Methods.* Chemical reactions were conducted under nitrogen in anhydrous solvents unless stated otherwise. Reagents obtained from commercial suppliers were used without further purification. Acetone was dried over 4 Å molecular sieves. Tetrahydrofuran (THF) was distilled under nitrogen over sodium-benzophenone. Thin-layer chromatography (TLC) was done on 250 μ fluorescent plates and visualized by using UV light or iodine vapor. Normal phase flash column chromatography was performed using silica gel (200−425 mesh 60 Å pore size) and ACS grade solvents. Melting points (mps) are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on either a 600 or 400 MHz instrument. Peak locations were referenced using either tetramethyl silane (TMS) or residual nondeuterated solvent as an internal standard. Proton coupling constants (J values) are expressed in hertz using the following designations: s (singlet), d (doublet), br s (broad singlet), m (multiplet), and q (quartet). In some cases, overlapping signals occurred in the ^{13}C NMR spectra. Circular dichroism (CD) studies were performed on an AVIV CD spectrometer for which access was provided by Bowling Green State University, Ohio. High resolution mass spectroscopy (HR MS) was conducted on a Micromass LCT electrospray mass spectrometer via service provided by the Mass Spectroscopy and Proteomics Facility within The Ohio State University, Ohio. Chiral high performance liquid chromatography (HPLC) experiments are described below and gave acceptable results (peak area purity >98%). Combustion analyses were performed by Atlantic Microlab, Inc. (Norcross, GA) and gave satisfactory results (C, H, O within 0.4% of calculated values). In all cases, spectroscopic data are in agreement with known compounds and assigned structures. Structural integrity and sample purity for test compounds were established by the composite of TLC, NMR, CD, HR MS, chiral HPLC, and in some cases additionally included combustion analyses.

*4-Benzyloxy-2-hydroxy-acetophenone (**17**).* Oven-dried potassium carbonate (1.65 g, 12 mmol) was added to a solution of 2,4-dihydroxy-acetopheneone (1.52 g, 10 mmol) in 10 mL of acetonitrile, and the mixture was stirred for 1 h at room temp. Benzylbromide (1.3 mL, 11 mmol) was added, and the mixture was refluxed for 10 h. After disappearance of 2,4-dihydroxy-acetopheneone (TLC), the solvent was evaporated to one-third volume and poured into ice water with vigorous stirring. A pinkish−white solid precipitated. The solid was recrystallized from methanol (ca. 20 mL) to obtain 2.12 g (88%) of **17** as a white product: mp 109−110 °C [lit.[66] 108−109 °C]. TLC R_f 0.47 hexanes:EtOAc (5:1). ^1H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H, OH), 7.79 (d, 1H, J = 8.8 Hz, Ar−H6), 7.38−7.28 (m, 5H, C_6H_5), 6.54 (dd, 1H, ^2J = 8.8 Hz, ^3J = 2.4 Hz, Ar−H5), 6.5 (d, 1H, ^3J = 2.4 Hz, Ar−H3), 5.13 (s, 2H, O−CH_2), 2.5 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.9, 165.4, 164.6, 136.9, 134, 129.2, 128.8, 128.5, 114.6, 108.5, 102.4, 70.3, 27.3.

*2-Benzyloxy-4-methoxymethyloxy-benzaldehyde (**18**).* Oven-dried potassium carbonate (1.38 g, 10 mmol) was added to an ice-cooled solution of 2,4-dihydroxy-benzaldehyde (1.38 g, 10 mmol) in 10 mL of acetone. MOMCl (1.54 mL, 20 mmol) was added dropwise, and the mixture was stirred at 0 °C for 1 h, after which it was gradually allowed to come to room temp. The reaction was further stirred for 24 h at room temp and then poured into ice water with vigorous stirring. A buff-colored solid precipitated. The solid was filtered, dried, and recrystallized from MeOH:DCM (ca. 30:5 mL) to obtain 1.31 g (72%) of 2-hydroxy-4-methoxymethyloxy-benzaldehyde as a white solid: mp 52−53 °C [lit.[67] 50−51 °C]. TLC R_f 0.43 in hexanes:EtOAc (2:1). ^1H NMR (400 MHz, $CDCl_3$) δ 11.38 (s, 1H, OH), 9.74 (s, 1H, CHO), 7.45 (d, 1H, J = 8.4 Hz, Ar−H6), 6.65 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2 Hz, Ar−H5), 6.60 (d, 1H, ^3J = 2 Hz), 5.24 (s, 2H, O-CH_2-O), 3.48 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.8, 164.5, 164.4, 135.6, 116.2, 109.3, 103.6, 94.3, 56.7.

Oven-dried potassium carbonate (0.272 g, 2 mmol) was added to a solution of 2-hydroxy-4-methoxymethy-benzaldehyde (0.182 g, 1 mmol) in 10 mL of acetonitrile, and the mixture was stirred for 1 h at room temp. Benzylbromide (0.24 mL, 2 mmol) was added, and the mixture was refluxed for 10 h. After disappearance of 2-hydroxy-4-methoxymethy-benzaldehyde (TLC), the solvent was evaporated. The residue was chromatographed over silica using hexanes:EtOAc (5:1). The organic fractions were evaporated to obtain 0.218 g (80%) of **18** as a yellow oil. TLC R_f 0.27 hexanes:EtoAc (5:1). ^1H NMR (400 MHz, $CDCl_3$) δ 10.4 (s, 1H, CHO), 7.82 (d, 1H, J = 8.4 Hz, Ar−H6), 7.46−7.39 (m, 5H, C_6H_5), 6.7−6.69 (m, 2H, Ar−H3/Ar−H5), 5.21 (s, 2H, O−CH_2-O), 5.16 (s, 2H, $PhCH_2$), 3.48 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.6, 163.9, 162.9, 136.1, 130.5, 128.9, 128.5, 127.6, 120.2, 108.8, 101, 94.4, 70.7. Anal. ($C_{16}H_{16}O_4$) C, H, O.

*2-Benzyloxy-4-methoxymethyloxy-4′-benzyloxy-2′-hydroxy-chalcone (**19**).* A mixture of 2-benzyloxy-4-methoxymethyloxy-benzaldehyde (2.72 g, 10 mmol) and 4-benzyloxy-2-hydroxy-acetophenone (2.42 g, 10 mmol) in 50 mL of anhydrous methanol was refluxed in the presence of piperidine (1 mL, 10 mmol) for ca. 4 h, after which it was cooled to room temp. A yellow solid precipitated. The solid was filtered and washed with 100 mL of methanol. The filtrate was reduced to ca. 50 mL and refluxed for 2 h, after which it provided additional product upon cooling in the refrigerator. The combined yield of yellow solid **19** was 4.16 g (84%): mp 147−148 °C. TLC R_f 0.19 in hexanes:EtOAc (5:1). ^1H NMR (400 MHz, DMSO-d_6) δ 8.01 (d, 1H, J = 15.2, CH═), 7.84 (d, 1H, J = 15.2, CH═), 7.89 (d, 1H, J = 8.8 Hz, Ar−H6′), 7.73 (d, 1H, J = 9.2 Hz, Ar−H6), 7.56−7.36 (m, 10H, 2 × C_6H_6), 6.89 (d, 1H, J = 2.4 Hz, Ar−H3′), 6.73 (dd, 1H, ^2J = 8.8 Hz, ^3J = 2.4 Hz, Ar−H5′), 6.58 (d, 1H, ^3J = 2.8 Hz, Ar−H3), 6.48 (dd, 1H, ^2J = 9.2 Hz, ^3J = 2.4 Hz, Ar−H5), 5.29 (s, 2H, O−CH_2), 5.20 (s, 4H, Ph−CH_2), 3.40 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.6, 166.3, 166.4, 61.2, 160, 140.3, 137.1, 136.9, 132.8, 132.5, 129.4, 129.2, 129, 128.8, 128.6, 119.4, 117.6, 114.6, 109, 108.5, 102.6, 102.1, 94.5, 70.9, 70.4, 56.6. Anal. ($C_{31}H_{28}O_6$) C, H, O.

*2-Benzyloxy-4-methoxymethyloxy-4′-benzyloxy-2′-acetoxy-chalcone (**20**).* To a solution of 2-benzyloxy-4-methoxymethyloxy-4′-benzyloxy-2′-hydroxy-chalcone (0.496 g, 1 mmol) in 10 mL of acetic anhydride was added triethylamine (0.25 mL, 2 mmol) and the solution heated at 60 °C for ca. 6 h. After disappearance of starting material (TLC), the hot reaction mixture was poured into ice−water:methanol (1:1) ca. 50 mL. A solid precipitated upon vigorous stirring. The solid was filtered, dried, and recrystallized from methanol (ca. 15 mL) to obtain 0.495 g (92%) of **20** as a white solid; mp 94−96 °C. TLC R_f 0.21 in hexanes:EtOAc (3:1). ^1H NMR (400 MHz, DMSO-d_6) δ 7.78−7.75 (m, 2H), 7.70 (d, 1H, J = 8.8 Hz, Ar−H6′), 7.49−7.36 (m, 10 + 1H, 2 × C_6H_6 + Ar−H8), 6.98 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H5′), 6.90 (d, 1H, J = 2.4 Hz,

Ar−H3′), 6.85 (d, 1H, J = 2 Hz, Ar−H3), 6.70 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H5), 5.26 (s, 2H, O−CH_2), 5.20 (s, 4H, O−CH_2), 3.39 (s, 3H, OCH_3), 2.18 (s, 3H, CH_3 CO). ^{13}C NMR (100 MHz, DMSO-d_6) δ 189,169.5, 162.5, 160.9, 159.6, 151.4, 139.5, 137.2, 136.9, 132.3, 131.7, 129.3, 129.2, 128.8, 128.6, 128.6, 125.2, 123.3, 117.6, 113, 110.8, 109.1, 102.2, 94.5, 70.7, 70.5, 56.6, 21.5. Anal. ($C_{33}H_{30}O_7$) C, H, O.

*2′-7-Dibenzyloxy-4′-hydroxy-isoflavone (**22**).* 2-Benzyloxy-4-methoxymethyloxy-4′-benzyloxy-2′-acetoxy-chalcone (0.538 g, 1 mmol) was dissolved in 2 mL of methylene chloride and 25 mL of methanol: trimethyl orthoformate (1:1) was added, followed by addition of thallium nitrate-trihydrate (0.666 g, 1.5 mmol). After stirring the suspension for 2 h at room temp, it became a clear solution with white thallium(I) nitrate precipitating. After stirring for an additional 4 h, the TLC showed complete disappearance of chalcone **20**. Solvents were evaporated to reduce the volume to ca. one-third, and then 20 mL of DCM was added, followed by the addition of 0.2 g of sodium bisulfite. After stirring for 1 h, the entire solid was filtered, solvent evaporated, and the resulting residue passed through a short column of silica with hexanes:EtOAc (10:1) as eluant. The solvents were evaporated to obtain 0.48 g (80%) of the acetal **21** as a yellowish oil which was used directly in the next step without further purification. Crude acetal (ca. 0.48 g) was dissolved in 10 mL of methanol and refluxed with 2 mL of 1N HCl for about 12 h. After disappearance of the acetal (TLC), the reaction mixture was poured into ice water (100 mL). A yellowish−white solid precipitated. The precipitate was filtered, dried, and recrystallized from MeOH:DCM (ca. 15:2 mL) to obtain 0.306 g (68%) of 22 as a white powder having a yellow tinge; mp 116−117 °C. TLC R_f 0.25 hexanes:EtOAc (1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.59 (s, 1H, OH), 8.22 (s, 1H, OCH═), 8.02 (d, 1H, J = 8.8 Hz, Ar−H5), 7.50−7.23 (m, 10 + 1H, 2 × C_6H_5 + Ar−H8), 7.14 (dd, 1H, ^2J = 9.2 Hz, ^3J = 2.4 Hz, Ar−H6), 7.05 (d, 1H, J = 8 Hz, ArH-6′), 6.5 (d, 1H, J = 1.6 Hz, Ar−H3′), 6.41 (dd, 1H, ^2J = 8.4 Hz, Ar−H5′), 5.27 (s, 2H, Ph−CH_2), 5.02 (s, 2H, Ph−CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 75.3, 163.2, 159.5, 158.1, 158, 154.8, 138, 136.8, 132.7, 129.2, 128.9, 128.8, 128.7, 128.2, 127.7, 127.6, 123, 118.4, 115.8, 112.5, 108, 102.3, 101, 70.7, 70. Anal. ($C_{29}H_{22}O_5$) C, H, O.

*2′,7-Dibenzyloxy-4′-(t-butyldimethylsilyloxy)-isoflavone (**23**).* To a solution of 2′,7-dibenzyloxy-4′-hydroxy-isoflavone (0.450 g, 1 mmol) in 10 mL of DCM was added triethylamine (0.25 mL, 2 mmol) and TBDMSCl (0.225 g, 1.5 mmol). The reaction mixture was stirred at room temperature for ca. 6 h. After disappearance of starting material (TLC), the reaction was quenched with 1N HCl (ca. 2 mL). The mixture was extracted with DCM:water (2 × 10 mL:10 mL). The organic layer was dried over sodium sulfate and evaporated to give 0.507 g (90%) of **23** as a fluffy, white solid; mp 132−133 °C. TLC R_f 0.89 in hexanes:EtOAc (1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H, OCH═), 8.03 (d, 1H, J = 8.8 Hz, Ar−H5), 7.50−7.13 (m, 10 + 3H, C_6H_5, Ar−H6, H6′, H8), 6.53−6.48 (m, 2H, Ar−H3′/Ar−H5′), 5.27 (s, 2H, Ph−CH_2), 5.08 (s, 2H, $PhCH_2$), 0.93 {s, 9H, Si $(CH_3)_3$}, 0.16 {s, 6H, Si $(CH_3)_2$}. ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.1, 163.3, 158.1, 157.9, 157, 155, 137.9, 136.8, 132.7, 129.2, 128.9, 128.8, 128.6, 128.1, 127.7, 127.6, 122.7, 118.4, 115.9, 115.3, 112.1, 105.9, 102.4, 70.8, 70.1, 26.2, 18.6. Anal. ($C_{35}H_{36}O_5Si\cdot0.25H_2O$) C, H.

*2′,7-Dibenzyloxy-4′-(t-butyldimethylsilyloxy)-isoflav-3-ene (**11**).* To an ice-cooled solution of 2′,7-dibenzyloxy-4′-(*t*-butyldimethylsilyloxy)-isoflavone (0.564 g, 1 mmol) in 10 mL of THF was added 1.6 M lithium borohydride solution in THF (3 mmol) dropwise. The reaction mixture was allowed to come to room temp gradually and then stirred for ca. 2 h. After disappearance of starting material (TLC), the reaction was quenched with 1N HCl (pH 4). The reaction was further stirred at room temp followed by refluxing at 60 °C for ca. 2 h. The reaction mixture was extracted with DCM:water (2 × 15 mL:15 mL). The organic layers were combined and washed with 1N $NaHCO_3$ (2 × 15 mL), dried over sodium sulfate, and evaporated at 20 °C to obtain an oily residue. The residue was chromatographed over silica using DCM:hexanes (1:1). The organic fractions were evaporated to provide 0.247 g (45%) of **11** as a white solid; mp 105−107 °C. TLC R_f 0.75 in DCM:hexanes (2:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.46−7.32 (m, 10H, 2 × C_6H_5), 7.21 (d, 1H, J = 8.4 Hz, Ar−H5), 7.02 (d, 1H, J = 8.4 Hz), 6.63 (s, 1H, CH═), 6.58−6.55 (m, 2H, Ar−H3′/H5′), 6.49−6.46 (m, 2H, Ar−H6/8), 5.11 (s, 2H, Ph−CH_2), 5.07 (s, 2H, Ph−CH_2), 4.89 (s, 2H, OCH_2), 0.93 {s, 9H, Si $(CH_3)_3$}, 0.17 {s, 6H, Si $(CH_3)_2$}. ^{13}C NMR (100 MHz, DMSO-d_6) δ 199.6, 159.7, 157.5, 156.8, 154.7, 137.7, 137.5, 129.7, 129.2, 129.1, 128.6, 128.5, 128.4, 128.3, 128.1, 121.5, 121.4, 117.5, 112.8, 108.9, 105.9, 102.8, 70.5, 69.9, 68.3, 26.3, 18.7. Anal.($C_{35}H_{38}O_4Si\cdot0.25H_2O$) C, H.

*(±) 4′-t-Butyl Dimethylsilyloxy-2′,7-(dibenzyloxy)isoflavan-3,4-diol (±) (**13**).* 2′,7-Dibenzyloxy-4′-*t*-butyldimethylsilyloxy-isoflav-3-ene (0.537 g, 1 mmol) was dissolved in 10 mL of acetone, and 1 mL of water was added. Methane sulfonamide (0.095 g, 1 mmol) and 4-methylmorpholine-4-oxide (NMO) (0.140 g, 1.2 mmol) were then added, and the resulting mixture stirred at 0 °C for 30 min. Osmium tetroxide solution in water (1 mL = 0.004 mmol) was added slowly, upon which the reaction turned brownish−yellow. The reaction was gradually allowed to come to room temp and stirred for 24 h. After disappearance of the reactant (TLC), the reaction was quenched with 1 g of sodium bisulfate followed by evaporation of acetone. The residue was extracted with EtOAc:water (2 × 10 mL:10 mL). The organic layers were combined, dried over sodium sulfate, filtered, and evaporated. The residue was chromatographed over silica with hexanes:EtOAc (5:1). The organic fractions were evaporated to provide 0.525 g (90%) of **13** as a fluffy, white solid; mp 110−112 °C. TLC R_f 0.25 in hexanes:EtOAc (5:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.46 (d, 1H, J = 8.4 Hz, Ar−H5), 7.46−7.28 (m, 10 + 1H, 2 × C_6H_5 + Ar−H6′), 6.58−6.56 (m, 2H, Ar−H8/Ar−H5′), 6.48 (q, 1H, J = 8.4 Hz, J = 1.8 Hz, Ar−H6), 6.37 (d, 1H, J = 2.8 Hz, Ar−H3′), 5.51 (d, 1H, J = 7.2, H4), 5.19 (s, 2H, $PhCH_2$), 5.06 (s, 2H, $PhCH_2$), 4.72 (d, 1H, J = 11.4 Hz, H-2 equiv), 4.23 (d, 1H, J = 7.24, 4-OH), 4.19 (s, 1H, 3-OH), 4.02 (d, 1H, J = 11.4 Hz, H2 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.17 {s, 6H, Si $(CH_3)_2$}. ^{13}C NMR (100 MHz, acetone-d_6) δ 160.6, 158.2, 157.9, 156.4, 139.1, 138.6, 131.5, 130.8, 130.1, 129.9, 129.3, 129.1, 128.9, 128.8, 124.2, 119.1, 113.2, 109.4, 106.9, 102.8, 72.7, 71.6, 70.9, 68.3, 26.6, 19.3. Anal. ($C_{35}H_{40}O_6Si$) C, H.

*(+) 4′-t-Butyl Dimethylsilyloxy-2′,7-(dibenzyloxy)isoflavan-3,4-diol (+) (**13**).* Prepared according to previously published procedure.[23c] White solid: 0.385 g (66%). TLC R_f 0.25 in hexanes:EtOAc (5:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.46 (d, 1H, J = 8.4 Hz, Ar−H5), 7.46−7.28 (m, 10 + 1H, 2 × C_6H_5 + Ar−H6′), 6.58−6.56 (m, 2H, Ar−H8/Ar−H5′), 6.48 (q, 1H, J = 8.4 Hz, J = 1.8 Hz, Ar−H6), 6.37 (d, 1H, J = 2.8 Hz, Ar−H3′), 5.51 (d, 1H, J = 7.2, H4), 5.19 (s, 2H, $PhCH_2$), 5.06 (s, 2H, $PhCH_2$), 4.72 (d, 1H, J = 11.4 Hz, H-2 equiv), 4.23 (d, 1H, J = 7.24, 4-OH), 4.19 (s, 1H, 3-OH), 4.02 (d, 1H, J = 11.4 Hz, H2 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.17 {s, 6H, Si $(CH_3)_2$}.

*(−) 4′-t-Butyl Dimethylsilyloxy-2′,7-(dibenzyloxy)isoflavan-3,4-diol (−) (**13**).* Prepared according to previously published procedure.[23c] White solid: 0.410 g (70%). TLC R_f 0.25 in hexanes:EtOAc (5:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.46 (d, 1H, J = 8.4 Hz, Ar−H5), 7.46−7.28 (m, 10 + 1H, 2 5× C_6H_5 + Ar−H6′), 6.58−6.56 (m, 2H, Ar−H8/Ar−H5′), 6.48 (q, 1H, J = 8.4 Hz, J = 1.8 Hz, Ar−H6), 6.37 (d, 1H, J = 2.8 Hz, Ar−H3′), 5.51 (d, 1H, J = 7.2, H4), 5.19 (s, 2H, $PhCH_2$), 5.06 (s, 2H, $PhCH_2$), 4.72 (d, 1H, J = 11.4 Hz, H-2 equiv), 4.23 (d, 1H, J = 7.24, 4-OH), 4.19 (s, 1H, 3-OH), 4.02 (d, 1H, J = 11.4 Hz, H2 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.17 {s, 6H, Si $(CH_3)_2$}.

*(±) 4′-t-Butyldimethylsilyloxy-2′,7-dihydroxyisoflavan-3,4-diol (±) (**14**).* To a solution of 4′-*t*-butyldimethylsilyloxy-2′,7-dibenzyloxyisoflavan-3,4-diol (0.584 g, 1 mmol) in 6 mL of acetone was added 10% Pd−C (0.1 g) in 4 mL of cold acetone. This reaction mixture was hydrogenated at room temperature under 1 atm (15 psi) of H_2 pressure for ca. 4 h. After the disappearance of starting material (TLC), the catalyst was filtered through a pad of Celite. The Celite pad was washed with 10 × 2 mL of

methanol. The solution was filtered once more through filter paper and evaporated to obtain a white solid with red tinge: 0.360 g (89%); mp decomposes at 142 °C. TLC R_f 0.23 in hexanes:EtOAc (2:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.20 (d, 1H, J = 8.4 Hz, Ar−H5), 7.17 (d, 1H, J = 8.4 Hz, Ar−H6′), 6.42 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H6), 6.34 (d, 1H, J = 2.4 Hz, Ar−H8), 6.28 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H5′), 6.26 (d, 1H, J = 2.4 Hz), 5.09 (s, 1H, H4), 4.35 (d, 1H, J = 11.4 Hz, H2 equiv), 4.13 (d, 1H, J = 11.4 Hz, 4-OH), 4.19 (s, 1H, 3-OH), 4.02 (d, 1H, J = 11.4 Hz, H2 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.18 {s, 6H, Si $(CH_3)_2$}. ^{13}C NMR (100 MHz, acetone-d_6) δ 159.5, 150.8, 157.7, 155.9, 132.0, 129.0, 120.5, 116.7, 112.3, 110, 109.7, 103.4, 73.9, 70.1, 69.1, 26.5, 19.2. Anal. ($C_{21}H_{28}O_6Si$) C, H.

*(+) 4′-t-Butyldimethylsilyloxy-2′,7-dihydroxyisoflavan-3,4-diol (+) (**14**).* Prepared according to previously published procedure.[23c] White solid with red tinge: 0.364 g (90%); mp decomposes at 142 °C. TLC R_f 0.23 in hexanes:EtOAc (2:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.20 (d, 1H, J = 8.4 Hz, Ar−H5), 7.17 (d, 1H, J = 8.4 Hz, Ar−H6′), 6.42 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H6), 6.34 (d, 1H, J = 2.4 Hz, Ar−H8), 6.28 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H5′), 6.26 (d, 1H, J = 2.4 Hz), 5.09 (s, 1H, H4), 4.35 (d, 1H, J = 11.4 Hz, H2 equiv), 4.13 (d, 1H, J = 11.4 Hz, 4-OH), 4.19 (s, 1H, 3-OH), 4.02 (d, 1H, J = 11.4 Hz, H2 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.18 {s, 6H, Si $(CH_3)_2$}.

*(−) 4′-t-Butyldimethylsilyloxy-2′,7-dihydroxyisoflavan-3,4-diol (−) (**14**).* Prepared according to previously published procedure.[23c] White solid with red tinge: 0.34 g (84%); mp decomposes at 144 °C. TLC R_f 0.23 in hexanes: EtOAc (2:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.20 (d, 1H, J = 8.4 Hz, Ar−H5), 7.17 (d, 1H, J = 8.4 Hz, Ar−H6′), 6.42 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H6), 6.34 (d, 1H, J = 2.4 Hz, Ar−H8), 6.28 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H5′), 6.26 (d, 1H, J = 2.4 Hz), 5.09 (s, 1H, H4), 4.35 (d, 1H, J = 11.4 Hz, H2 equiv), 4.13 (d, 1H, J = 11.4 Hz, 4-OH), 4.19 (s, 1H, 3-OH), 4.02 (d, 1H, J = 11.4 Hz, H2 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.18 {s, 6H, Si $(CH_3)_2$}.

*(±) 9-(t-Butyl Dimethyl Silyloxy)-glycinol (±) (**15**).* To a solution of 4′-*t*-butyldimethylsilyloxy-2′,7-dihydroxyisoflavan-3,4-diol (0.041 g, 0.1 mmol) in 20 mL of anhydrous ethanol was added polymer-bound 1,3,4,6,7,8-hexahydro-2*H*-pyrimido(1,2-*a*)pyrimidine (4 mg, 0.01 mmol) and 4 Å molecular sieves (0.1 g). The reaction mixture was refluxed at 80 °C for 6 h with continuous distillation of the ethanol−water azeotrope. After disappearance of reactant (TLC), the molecular sieves and polymeric base were filtered. The filtrate was dried over sodium sulfate, filtered, and solvent evaporated at 20 °C. The residue was chromatographed over silica using hexanes:EtOAc (4:1). The organic fractions were evaporated at 20 °C to provide a pinkish−white solid: 0.023 g (60%); mp 197−199 °C. TLC R_f 0.38 in hexanes:EtOAc (2:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.55 (s.1H, −OH), 7.30 (d, 1H, J = 8.4 Hz, Ar−H1), 7.24 (d, 1H, J = 8.4, Ar−H7), 6.55 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H-2), 6.45 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H8), 6.3 (d, 1H, J = 2.4 Hz, ArH-4), 6.26 (d, 1H, J = 2.4 Hz, Ar H-10), 5.26 (s, 1H, 11a-H), 5.01 (s, 1H, 6a−OH), 4.14 (d, 1H, J = 12 Hz, H6 equiv), 4.02 (d, 1H, J = 12 Hz, H6 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.19 {s, 6H, Si $(CH_3)_2$}. ^{13}C NMR (100 MHz, acetone-d_6) δ 162.4, 160.3, 159.3, 157.7, 133.8, 125.6, 124.3, 114.1, 113.8, 111.4, 104.5, 103.9, 86.6, 77.4, 71.3, 26.6, 19.3. Anal. ($C_{21}H_{26}O_5Si$) C, H.

*(−) 9-(t-Butyl Dimethyl Silyloxy)-glycinol (−) (**15**).* Prepared according to previously published procedure.[23c] Pinkish−white solid: 0.025 g (64%). TLC R_f 0.38 in hexanes:EtOAc (2:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.55 (s.1H, −OH), 7.30 (d, 1H, J = 8.4 Hz, Ar−H1), 7.25 (d, 1H, J = 8.4, Ar−H7), 6.55 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H-2), 6.45 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H8), 6.3 (d, 1H, J = 2.4 Hz, ArH-4), 6.26 (d, 1H, J = 2.4 Hz, Ar H-10), 5.26 (s, 1H, 11a-H), 5.01 (s, 1H, 6a−OH), 4.14 (d, 1H, J = 12 Hz, H6 equiv), 4.02 (d, 1H, J = 12 Hz, H6 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.19 {s, 6H, Si $(CH_3)_2$}.

*(+) 9-(t-Butyl Dimethyl Silyloxy)-glycinol (+) (**15**).* Prepared according to previously published procedure.[23c] Pinkish−white solid: 0.023 g (60%). TLC R_f 0.38 in hexanes:EtOAc (2:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.55 (s.1H, −OH), 7.30 (d, 1H, J = 8.4 Hz, Ar−H1), 7.25 (d, 1H, J = 8.4, Ar−H7), 6.55 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H-2), 6.45 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H8), 6.3 (d, 1H, J = 2.4 Hz, ArH-4), 6.26 (d, 1H, J = 2.4 Hz, Ar H-10), 5.26 (s, 1H, 11a-H), 5.01 (s, 1H, 6a−OH), 4.14 (d, 1H, J = 12 Hz, H6 equiv), 4.02 (d, 1H, J = 12 Hz, H6 ax), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.19 {s, 6H, Si $(CH_3)_2$}.

*(±) 9-(t-Butyldimethylsilyloxy) Glyceollin I (±) (**16**).* To a solution of 9-(*t*-butyl-dimethylsilyloxy)-glycinol (0.040 g, 0.1 mmol) in 5 mL of xylene was added 1,1-diethoxy-3-methyl-2-butene (0.2 mmol in 0.2 mL of xylene) and 3-picoline (0.025 mmol in 0.3 mL xylene). The reaction mixture was refluxed at 130 °C for 18 h. After disappearance of reactant (TLC), the solvent was evaporated and the residue was chromatographed over silica using DCM as eluant. The organic fractions were evaporated to provide two yellowish oils: a major isomer I and a minor isomer II in ca. 5:1 ratio. Major yellow oil: 0.022 g (50%). TLC R_f 0.46 in toluene:methanol (10:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.27 (d, 1H, J = 8.4 Hz, Ar−H1), 7.24 (d, 1H, J = 8.4 Hz, Ar−H7), 6.57 (d, 1H, J = 9.6 Hz, H12), 6.48−6.45 (m, 2H, Ar−H7/Ar−H8), 6.3 (d, 1H, Ar-10H), 5.65 (d, 1H, J = 10.2 Hz, H13), 5.27 (s, 1H, 11a-H), 5.09 (s, 1H, 6a−OH), 4.20 (d, 1H, J = 11.4 Hz, H6 equiv), 4.08 (d, 1H, J = 11.4 Hz, H6 ax), 1.38,1.35 (2 s, 2 × 3H, H15/H16), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.19 {s, 6H, Si $(CH_3)_2$}. ^{13}C NMR (100 MHz, acetone-d_6) δ 162.3, 159.3, 155.4, 152.2, 132.7, 130.7, 125.6, 124, 117.6, 114.7, 114.1, 111.7, 111.2, 103.9, 86.5, 77.3, 77.2, 71.5, 28.6, 26.6, 19.3. HRMS calcd (M^+ + Na) 475.1917, found 475.1923.

*(−) 9-(t-Butyldimethylsilyloxy) Glyceollin I (−) (**16**).* Prepared according to previously published procedure.[23c] Major yellow oil: 0.027 g (61%). TLC R_f 0.46 in toluene:methanol (10:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.27 (d, 1H, J = 8.4 Hz, Ar−H1), 7.24 (d, 1H, J = 8.4 Hz, Ar−H7), 6.57 (d, 1H, J = 9.6 Hz, H12), 6.48−6.45 (m, 2H, Ar−H7/Ar−H8), 6.3 (d, 1H, Ar-10H), 5.65 (d, 1H, J = 10.2 Hz, H13), 5.27 (s, 1H, 11a-H), 5.09 (s, 1H, 6a−OH), 4.20 (d, 1H, J = 11.4 Hz, H6 equiv), 4.08 (d, 1H, J = 11.4 Hz, H6 ax), 1.38−1.35 (2 × s, 2 × 3H, H15/H16), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.19 {s, 6H, Si $(CH_3)_2$}.

The other material obtained during this reaction, (−) 9-(*t*-butyldimethylsilyloxy)glyceollin II (14b), eluted as an inseparable mixture with additional side products. No attempts were made to further isolate this isomer.

*(+) 9-(t-Butyldimethylsilyloxy) Glyceollin I (+) (**16**).* Prepared according to previously published procedure.[23c] Major yellow oil: 0.025 g (57%). TLC R_f 0.46 in toluene:methanol (10:1). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.27 (d, 1H, J = 8.4 Hz, Ar−H1), 7.24 (d, 1H, J = 8.4 Hz, Ar−H7), 6.57 (d, 1H, J = 9.6 Hz, H12), 6.48−6.45 (m, 2H, Ar−H7/Ar−H8), 6.3 (d, 1H, Ar-10H), 5.65 (d, 1H, J = 10.2 Hz, H13), 5.27 (s, 1H, 11a-H), 5.09 (s, 1H, 6a−OH), 4.20 (d, 1H, J = 11.4 Hz, H6 equiv), 4.08 (d, 1H, J = 11.4 Hz, H6 ax), 1.38−1.35 (2× s, 2 × 3H, H15/H16), 0.96 {s, 9H, Si $(CH_3)_3$}, 0.19 {s, 6H, Si $(CH_3)_2$}.

The other material obtained during this reaction, (+)9-(*t*-butyldimethylsilyloxy)glyceollin II (14b), eluted as an inseparable mixture with additional side products. No attempts were made to further isolate this isomer.

General Procedure for Deprotection of the TBDMS Group. 9-(*t*-Butyldimethyl-silyloxy)-glyceollin I (0.043 g, 0.1 mmol) was dissolved in 1 mL of acetonitrile, and the solution was cooled to −20 °C. $N(Et)_3 \cdot 3HF$ in acetonitrile (1.2 mL, 0.12 mmol) was added and the mixture stirred for 8 h at 4 °C. After disappearance of reactant (TLC), the pH was adjusted to 7−8 by addition of triethylamine and the mixture filtered through a silica column using DCM:MeOH (10:1). Evaporation of the solvent at 20 °C provided a brownish oily residue which was chromatographed over silica using hexanes:DCM:methanol (10:10:1). The organic fractions were evaporated at 20 °C to provide the solids specified below.

*(±) Glyceollin I (±) (**3**).* White solid with red tinge: 0.023 g (69%). TLC R_f 0.22 in hexanes:DCM:methanol (10:10:1). ^{1}H NMR (400 MHz,

methanol-d_4) δ 7.20 (d, 1H, J = 8.4 Hz, Ar−H1), 7.07 (d, 1H, J = 8.4 Hz, Ar−H7), 6.59 (d, 1H, J = 10.2 Hz, H12), 6.46 (d, 1H, J = 8.4 Hz, Ar−H2), 6.39 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H8), 6.22 (d, 1H, J = 2.4 Hz, Ar−H10), 5.61 (d, 1H, J = 10.2 Hz, H13), 5.16 (s, 1H, 11a-H), 4.90 (s, 1H, 6a−OH), 4.16 (d, 1H, J = 11.4 Hz, H6 equiv), 3.93 (d, 1H, J = 11.4 Hz, H6 ax), 1.38,1.37 (2 s, 2 × 3H, H15/H16). ^{13}C NMR (100 MHz, methanol d_4) δ 162.3, 161.3, 155.4, 151.9, 132.4, 130.5, 125.3, 121.3, 117.7, 114.3, 111.7, 111.4, 109.5, 99.1, 86.1, 77.3, 77.2, 71.3, 28.2. HRMS calcd (M^+ + Na) 361.1052, found 361.1052. Anal. ($C_{20}H_{18}O_5 \cdot 0.25H_2O$) C, H.

Glyceollin I (**3**). White solid with red tinge: 0.026 g (77%). TLC R_f 0.22 in hexanes:DCM:methanol (10:10:1). Chiral HPLC showed an essentially one peak chromatogram. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.20 (d, 1H, J = 8.4 Hz, Ar−H1), 7.07 (d, 1H, J = 8.4 Hz, Ar−H7), 6.50 (d, 1H, J = 10.2 Hz, H12), 6.37 (d.1H, J = 8.4 Hz, Ar−H7), 6.30 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H8), 6.13 (d, 1H, J = 2.4 Hz, Ar−H7), 5.52 (d, 1H, J = 10.2 Hz, H13), 5.06 (s, 1H, 11a-H), 4.81 (s, 1H, 6a−OH), 4.07 (d, 1H, J = 11.4 Hz, H6 equiv), 3.93 (d, 1H, J = 11.4 Hz, H 6ax), 1.38−1.35 (2 s, 2 × 3H, H15/H16). HRMS calcd [M^+ + Na] 361.1052; found 361.1059. Anal. ($C_{20}H_{18}O_5 \cdot 0.25H_2O$) C, H.

(+) Glyceollin I (**3**). White solid with yellow tinge: 0.025 g (75%). TLC R_f 0.22 in hexanes:DCM:methanol (10:10:1). Chiral HPLC showed an essentially one peak chromatogram. ^{1}H NMR (400 MHz, methanol-d_4) δ 7.21−7.19 (d, 1H, J = 8.4 Hz, Ar−H1), 7.07 (d, 1H, J = 8.4 Hz, Ar−H7), 6.51−6.49 (d, 1H, J = 10.2 Hz, H12), 6.38−6.36 (d.1H, J = 8.4 Hz, Ar−H7), 6.31−6.29 (dd, 1H, ^2J = 8.4 Hz, ^3J = 2.4 Hz, Ar−H8), 6.13 (d, 1H, J = 2.4 Hz, Ar−H7), 5.53−5.51 (d, 1H, J = 10.2 Hz, H13), 5.06 (s, 1H, 11a-H), 4.81 (s, 1H, 6a−OH), 4.08−4.06 (d, 1H, J = 11.4 Hz, H6 equiv), 3.94−3.92 (d, 1H, J = 11.4 Hz, H6 ax), 1.38−1.35 (2 s, 2 × 3H, H15/H16). HRMS calcd [M^+ + Na] 361.1052; found 361.1059.

Chiral Column Chromatography.[24,68] A chiral Cyclobond column from ASTEC Inc. was used in a Waters HPLC equipped with a model 2659 separation module, a quaternary pump, a degasser, an auto sampler/injector (syringe volume = 100 μL), a column oven, and a model 2996 photodiode array detector. The mobile phase used a gradient solvent system having water, acetonitrile, and methanol in the following percentages with time: 60, 0, 40 at the start, 45, 0, 55 at 30 min, and 60, 10, 30 at 48 min, after which the system was stepped back to start conditions and flushed for 12 min. Temperature was 35 °C, flow rate was 0.5 mL/min, and the detector was set at 254 nm. Retention times (min) were: 52.7 for authentic GLY I standard from stressed soybean, 53.2 for synthesized material, 49.4 for synthesized unnatural (+) material, and 49.5 + 53.3 for synthesized racemic material.

Chiral NMR Shift Reagent Studies.[69] Europium(III) tris-[3-(heptafluoro-propylhydroxy-methylene)-L-camphorate was deployed in 20% mole ratio by dissolving it in the same deuterated solvent as the routine NMR sample, adding the solution directly to the latter and then rerunning the NMR spectrum. Results for the key diol intermediate are summarized in Table 1.

Biology. *Cell Lines.* Androgen receptor negative (AR^-) human prostate cancer (PC) cell lines DU145 and PC3,[70] the estrogen receptor positive (ER^+) human breast cancer cell line MCF7,[71] and the estrogen receptor negative (ER^-) human ovarian cancer cell line NCI-ADR-RES[72] were generously provided by the National Cancer Institute Division of Cancer Treatment and Diagnosis Tumor Repository. The androgen receptor positive (AR^+) human PC cell line LNCaP[70] and ER^- immortalized "normal" human breast cell line MCF12A[73] were obtained from the American Type Culture Collection (ATCC).

Cell Culture. All cell lines except MCF12A were routinely maintained in RPMI-1640 media containing 2.0 mM L-glutamine supplemented with 20 mM HEPES, 0.2% sodium bicarbonate, 5% fetal bovine serum (FBS), 5% NuSerum (NS) IV (BD Biosciences, Bedford, MA), and 50 μg/mL gentamicin. MCF12A cells were maintained in a 1:1 mixture of DMEM and F-12 Ham media containing 2.5 mM L-glutamine and 15 mM HEPES, supplemented with 0.2% sodium bicarbonate, 10 mg/mL insulin, 500 μg/L hydrocortisone, 20 μg/L epidermal growth factor, 100 μg/L cholera toxin, 5% fetal bovine serum, and 50 μg/mL gentamicin.[73] All cells were cultured at 37 °C in a 5% CO_2/100% humidity environment.

Growth Inhibition Assays. Cell growth inhibition assays were performed by a sulforhodamine B staining method described previously.[50,51,74] Cell loading densities yielding exponential growth over the 48 h exposure period with final optical density (OD) values less than 2.0 were determined in preliminary tests. Three media conditions were employed during testing. For intermediate E2 level tests (0.6 nM E2, 0.7 nM testosterone), all cell lines were loaded in maintenance media and test agents were added in 5%FBS/5%NS RPMI maintenance media, using the following cell loading densities: DU145 2300 cells per well (cpw), PC3 1300 cpw, LNCaP 3600 cpw, MCF12A 1300 cpw, MCF7 2000 cpw, and NCI-ADR-RES 3500 cpw. For tests with MCF7, NCI-ADR-RES, and MCF12A cell lines in high E2 conditions, cell loading was carried out as described for the intermediate E2 conditions, but agents were loaded in 5%FBS/5%NS media with added E2 to give a final media concentration during the exposure period of 100 nM E2. For low E2 tests (0.003 nM E2, 0.01 nM testosterone) with MCF7, NCI-ADR-RES, and MCF12A cell lines, MCF7 and NCI-ADR-RES cells were precultured for at least 4 days in RPMI with 5% FBS (same as RPMI maintenance media but without added NS), after which these two cell lines were loaded in this 5% FBS media while MCF12A cells were loaded in their normal maintenance media at cell densities of MCF12A 1600 cpw, MCF7 3500 cpw, and NCI-ADR-RES 6000 cpw, and test agents were loaded in the 5% FBS RPMI media for all three cell lines. For each E2 condition tested, the percent of control cell growth was determined for each cell line at each agent concentration by comparing OD readings to those of matched vehicle-only control cells (0.25% DMSO). Agent concentrations causing 50% growth inhibition (GI_{50}) versus vehicle-only control cells were estimated by a Hill equation fit of the data. A log-normal distribution provided the best approximation for the GI_{50} measurements, so statistical analyses were performed using $\log_{10}$ (GI_{50}) values. All results have been listed as mean ±95% confidence interval (CI). Statistically significant differences were determined by analysis of variance (ANOVA) using a Bonferroni posthoc analysis for pairwise comparisons, with $p < 0.05$ used as the criteria for statistical significance performed using SYSTAT 12 (SYSTAT Software Inc., San Jose, CA).

■ ASSOCIATED CONTENT

Supporting Information. Historical structural assignment; phytochemical pathway; attempted addition of water across the pterocarpene "6a−11a" double-bond; NMR and CD studies with selected spectra; chiral HPLC studies with selected chromatograms; HR MS and combustion analyses data; biological data; NMR spectra for new intermediates and final test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 419-530-2167. Fax: 419-530-1994. E-mail: paul.erhardt@utoledo.edu.

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■ ABBREVIATIONS USED

GI, growth inhibitory; ER, estrogen receptor; AR, androgen receptor; QC, quality control; GLYs, glyceollins; SERMs-selective estrogen receptor modulatorsCD, circular dichroism; E2, estradiol; THF, tetrahydrofuran; TLC, thin-layer chromatography; mps, melting points; TMS, tetramethyl silane; HR MS, high resolution mass spectroscopy

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(5) The following definitions, as meant for use herein, have been adapted from a Health Canada Policy Paper which appeared on www.ana-jana.org (a) *functional foods* are items consumed as part of the usual diet that have been demonstrated to have physiological benefits or to reduce the risk of disease beyond basic nutritional factors, (b) *nutraceuticals* are items isolated or purified from foods generally taken in medicinal forms not associated with the usual diet, that have been demonstrated to have physiological benefits or to reduce the risk of disease, and (c) *fortification* is a process that alters the content of a dietary supplement's constituents such that there is an enhancement of the beneficial physiological effects or further reduction in the risk of disease. For a brief review of these terms and topic, see:: Monge, A.; Cardozo, T.; Barreiro, E. J.; Huenchunir, P.; Pinzon, R.; Mora, G.; Nunez, A.; Chiriboga, X.; Caceres, A.; Rivera, G.; Bocanegra-Garcia, V.; Gupta, M.; Ferro, E. A.; Peralta, I.; Lock, O.; Flores, D.; Salazar, L.; Guzman, O. D.; Cerecetto, H.; Gonzalez, M. Functional foods, reflections on an expanding market. *Chem. Int.* **2008**, Sept−Oct, 9−13.

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(7) The following definitions, as meant for use herein, have been adapted from analogies to the development of small molecule pharmaceutical agents wherein the attrition rates observed during drug development candidates derived from new mechanistic pathways thought to represent novel therapeutic targets, and likewise for their accompanying "drug wannabe" compounds, is so high that success in preclinical models is now regarded by many as being only a *credentialization* of such pathways and their associated compounds, rather than as a *validation*. Actual *validation* is then reserved for success observed in clinical studies. For discussions of this topic, see:(a) Erhardt, P. W.; Proudfoot, J. R. Drug Discovery: Historical Perspective, Current Status, and Outlook. In *Comprehensive Medicinal Chemistry II*; Taylor, J. B., Triggle, D. J., Kennewell, P. D., Eds.; Elsevier, Ltd.: Oxford, U.K, 2007; pp 29−96. (b) Erhardt, P. W. Drug Discovery. In *Pharmacology—Principles and Practice*; Hacker, M., Bachmann, K., Messer, W., Eds.; Elsevier, Ltd.: Oxford, 2009; pp 475−560.

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