Discovery of a Series of Nonpeptide Small Molecules That Inhibit the Binding of Insulin-like Growth Factor (IGF) to IGF-Binding Proteins

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Insulin-like growth factors (IGF-I and II) play an important role in metabolic and mitogenic activities through stimulation of the IGF-I receptor on the cell surface. Although the concentration of IGF in blood and cerebrospinal fluid is quite high (>100 nM), this large pool of IGF is biologically inactive because of its association with six distinct binding proteins, which form high-affinity complexes with IGF. Thus, inhibitors of IGF-binding proteins (IGFBPs), especially IGFBP-3, could potentially alter the distribution between the "free" and "bound" forms of IGF and thereby elevate biologically active IGF-I to exert a beneficial effect on those patients with diseases that respond to the application of exogenous IGF-I. Whereas IGF-I peptide variants, which bind to IGFBPs but not the IGF-I receptor, have been shown to be potent IGF/IGFBP inhibitors, small molecule nonpeptide IGF/IGFBP inhibitors have the potential advantages of oral bioavailability and flexible dosing regimen. Here we report the discovery of several isoquinoline analogues, exemplified by **1** and **2**, which bind IGFBP-3 as well as other IGFBPs at low nanomolar concentrations. More importantly, both compounds were shown to be able to release biologically active IGF-I from the IGF-I/IGFBP-3 complex. These results point to the feasibility of developing orally active therapeutics to treat IGF-responsive diseases by optimization of the lead molecules **1** and **2**.

Introduction

The insulin-like growth factors (IGF-I and II) are small polypeptide hormones regulating cell proliferation, cell differentiation, cell death, and cell metabolic activities.[1] The mitogenic and metabolic actions of the IGFs are mediated by their binding and activation of the cell surface IGF-I receptor, a $\alpha_2\beta_2$ heterotetramer closely related to the insulin receptor.[2] The affinity of the IGF-I receptor for IGF-II is much lower than it is for IGF-I.[1] Upon binding the IGF ligand, the IGF-I receptor changes its conformation, resulting in autophosphorylation of the intracellular β-subunits and activation of the receptor's intrinsic tyrosine kinase activity to propagate the signal to the nucleus.[3] In blood and interstitial fluids, including the cerebrospinal fluid, the concentration of freely circulating IGF is exceedingly low because most of the IGFs are bound to one or more of six high-affinity IGF-binding proteins (IGFBPs), which inhibit their interaction with the IGF-I receptor.[4] The major binding protein in circulation is IGFBP-3, which together with an acid labile subunit forms a trimolecular complex with IGF to sequester most of the IGFs in blood.[5] In addition, IGFBP-5 can also form a similar trimolecular complex,[6] whereas the remaining four binding proteins form dimolecular complexes with IGF.

The multiple activities of IGF-I, from regulation of glucose homeostasis to whole body growth, have prompted its use in clinical trials to treat insulin resistant diabetes and growth failure, as well as other IGF-responsive diseases.[7] Because of the intrinsic nature of a peptide therapeutic, patients participating in these trials had to be injected with various doses of recombinant IGF-I, once or twice daily, throughout the trial period. Thus, orally active compounds that can stimulate the IGF-I receptor would have a distinct advantage over IGF-I. Despite the publication of a preliminary report on the identification of a small molecule insulin-receptor activator from screening of a natural product library,[8] discovery of small molecule agonists for the IGF-I receptor could be difficult, as the agonist has to bind both β subunits of the receptor simultaneously to induce the activation. A further complication in the design of an orally active IGF-I receptor agonist is that IGF-I, upon binding to its receptor, undergoes a conformational change prior to activation of the receptor[9], and, thus, a small molecule agonist would presumably have to mimic this change. An alternative approach to the development of an orally active compound that can potentiate the action of endogenous IGF-I could be the use of a small molecule ligand that displaces the bound IGF-I from the large pool of IGF/IGFBP complexes in the body fluids.

IGF-I could be liberated from its trimolecular or dimolecular complexes with the IGFBPs, either in the blood or in tissue-specific interstitial fluids in the body, and the "free" biologically active IGF-I could then exert its effect on the responding cells. Indeed, Loddick et al. have recently demonstrated an IGF-I-like neuroprotective effect in rat brain by using a peptide IGF/IGFBP inhibitor derived from IGF-I that binds to the IGFBPs but is inactive on the IGF-I receptor.[10] Lowman et al., using a similar peptide IGF/IGFBP inhibitor, have also shown that the inhibitor mimicked the IGF-I action on animal models of diabetes and growth failure.[11] Moreover, the same group also discovered a small peptide (18 amino acid), which binds to human IGFBP-1 and

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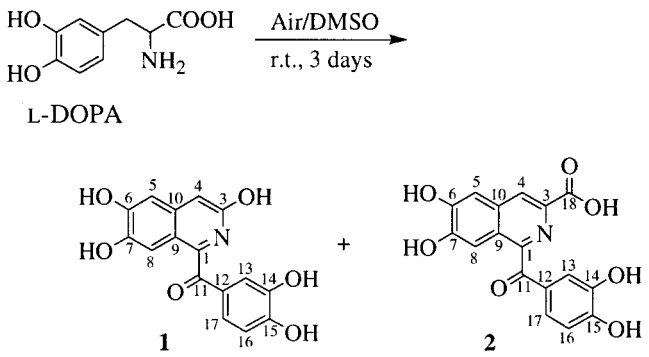
Scheme 1



displaces IGF-I from the IGF-I/IGFBP-1 complex.[11] This peptide, identified from a phage-displayed peptide library, has a well-defined turn-helix structure in aqueous solution, suggesting the possibility of designing small molecule mimics. However, it remains a formidable challenge to develop a small molecule IGF/IGFBP inhibitor based on this peptide. As a result, we took the direct approach to identify small molecule IGF/IGFBP inhibitors from screening of our in-house chemical library collection by using the [^{125}I]-IGF-I/IGFBP-3 binding assay. Here we report the discovery of a series of nonpeptide IGFBP inhibitors, the structures of which are amenable to optimization for development of orally active compounds that could displace bound IGF-I from its binding protein complexes.

Results and Discussion

High throughput screening of the in-house chemical libraries resulted in the identification of an initial hit compound, which was designated as L-(3,4-dihydroxyphenyl)-alanine (L-DOPA) in the library collection. However, this result could not be confirmed by using a freshly prepared sample of L-(3,4-dihydroxyphenyl)-alanine powder dissolved in DMSO, but the same sample solution did show activity after being stirred at room temperature for 3 days in DMSO with the container opened to air. HPLC analysis of the stirred sample revealed several small peaks distinct from L-(3,4-dihydroxyphenyl)alanine, which exhibited IGF/IGFBP-3 binding inhibitory activity. Preparative HPLC separation of these peaks (see Experimental Section for details) resulted in the isolation of several fractions, two of which showed low nanomolar inhibitory activity on the IGFBP-3 binding assay. These two components were then fully characterized by chemical analysis, and the structures of these two compounds (**1** and **2** in Scheme 1) were elucidated on the basis of NMR (proton, carbon, HMQC, and HMBC), IR, MS, and elemental analysis methods as illustrated below.

Structural Elucidation of the Active Components. Compound **1** was obtained as a yellow solid. Ion spray mass spectrometric analysis of the compound showed a parent peak at *m/e* 314 $(M+H)^+$. In negative charge ionization mode, a parent peak at *m/e* 312 $(M-H)^-$ was detected. These results indicated that the molecular weight of this compound was 313 and that the molecule might have both acidic and basic functional groups. Moreover, the odd number molecular weight implied it contained an odd number of nitrogen atoms (which could be the basic center for protonation in mass spectrometry). High-resolution mass spectrometric analysis and elemental analysis confirmed the molecular formula of **1** as $C_{16}H_{11}NO_6$. An intense absorption band at 3300 cm^{-1} in the FT-IR spectrum indicated the presence of hydroxyl groups in **1**. Also, absorption at 1640 cm^{-1} in conjunction with a ^{13}C NMR signal at δ 192.5 were in agreement with the presence of a conjugated ketone carbonyl group.

Table 1. $^{2-4}J_{C-H}$ Correlations from HMBC Experiments for Compounds **1** and **2** (in CD_3OD)

	long-range correlations to carbon number	
proton number	compound **1**	compound **2**
H-4	C-5, C-9	C-5, C-9
H-5	C-4, C-6, C-7, C-9	C-4, C-7, C-9
H-8	C-1, C-6, C-7, C-10	C-1, C-6, C-7, C-9
H-13	C-11, C-14, C-15, C-17	C-11, C-15, C-17
H-16	C-12, C-14, C-15	C-12, C-14, C-15
H-17	C-11, C-13, C-15	C-11, C-13, C-15

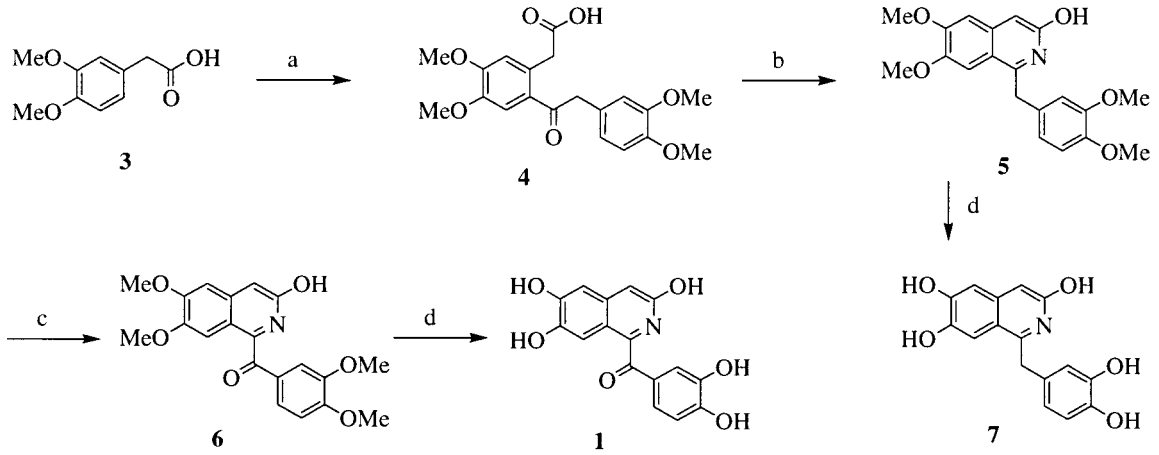
Table 2. ^{1}H NMR and ^{13}C NMR Data for Compounds **1** and **2**

	compound **1**		compound **2**	
no.	proton	carbon	proton	carbon
1	- - -	149.9	- - -	154.5
3	- - -	157.5	- - -	138
4	6.95 (s)	103.6	8.52 (s)	122.4
5	7.02 (s)	107.5	7.41 (s)	110.1
6	- - -	155.7	- - -	151.9
7	- - -	148.7	- - -	150.4
8	6.96 (s)	107.6	7.21 (s)	107.1
9	- - -	118.5	- - -	122.6
10	- - -	141.9	- - -	132.3
11	- - -	192.5	- - -	193.2
12	- - -	129.5	- - -	127.7
13	7.37 (d, J = 2.0 Hz)	117.6	7.37 (d, J = 2.0 Hz)	116.5
14	- - -	147.1	- - -	145.3
15	- - -	154.2	- - -	151.8
16	6.84 (d, J = 8.3 Hz)	116.4	6.83 (d, J = 8.3 Hz)	115.4
17	7.18 (dd, J = 2.0, 8.3 Hz)	126.4	7.18 (dd, J = 1.9, 8.3 Hz)	124.1
18	- - -		- - -	166.5

The ^{1}H NMR spectrum of **1** in CD_3OD showed only six aromatic protons that were not exchangeable with deuteriomethanol, and the ^{13}C NMR spectrum of **1** (Table 2) confirmed the presence of 16 carbons. These NMR results indicated a high degree of unsaturation, as all carbon resonances were downfield of 100 ppm. A benzene ring bearing 1,3,4-tri-substituents was identified by ^{1}H NMR analysis: H-13 (δ 7.37, doublet, J_{meta} = 2.0 Hz), H-16 (δ 6.84, doublet, J_{ortho} = 8.3 Hz), and H-17 (δ 7.18, doublet of doublet, J_{ortho} = 8.3 Hz, J_{meta} = 2.0 Hz). All carbons attached to a proton were assigned on the basis of a ^{1}H-detected heteronuclear one bond ^{1}H-^{13}C correlation experiment (HMQC). Thus, C-13 (δ 117.6), C-16 (δ 116.4), and C-17 (δ 126.4) for the 1,3,4-tri-substitued phenyl group were identified from the ^{13}C NMR spectrum. The remaining protons in ^{1}H NMR spectrum, three singlets at δ 6.95 (H-4), 7.02 (H-5), and 6.96 (H-8) were correlated to carbons at δ 103.6 (C-4), 107.5 (C-5), and 107.6 (C-8) on the basis of the HMQC experiment.

Because most carbons in compound **1** were not bonded to a proton, further information regarding the skeletal framework was sought from multiple bond proton−carbon couplings, which were identified by a ^{1}H-detected heteronuclear multiple bond ^{1}H−^{13}C correlation experiment (HMBC) (Table 1). The presence of a 1,3,4-

Scheme 2[a]



[a] Reagents and conditions. (a) PPA/110 °C. (b) NH_4OAc/AcOH/reflux. (c) SeO_2/dioxane/reflux, 1 h. (d) 48% HBr/reflux, o/n.

trisubstituted phenyl ring (C-12, C-13, C-14, C-15, C-16, and C-17) was further confirmed by cross-peaks of H-13 to C-14, C-15, and C-17; H-16 to C-12, C-14, and C-15; and H-17 to C-13 and C-15. The $^3J_{C-H}$ coupling of H-13 and H-17 to the carbonyl carbon (δ 192.5) indicated that this carbonyl group was attached to the phenyl ring with the two protons located at its ortho positions. The relative downfield chemical shifts of C-14 and C-15 (δ 147.1 and 154.2) implied that they were bonded to a hydroxy group. Thus, a 3,4-dihydroxybenzoyl group was identified.

The presence of a second benzene ring (C-5, C-6, C-7, C-8, C-9, and C-10) was identified from $^3J_{C-H}$ couplings of H-5 to C-7 and C-9, and H-8 to C-6 and C-10, and also $^2J_{C-H}$ couplings of H-5 to C-6 and H-8 to C-7. The downfield chemical shifts of C-6 and C-7 (δ 155.7 and 148.7) indicated a hydroxy substituent was attached to each of these two carbons. The cross-peaks of H-5 to C-4 and H-8 to C-1 revealed that C-1 and C-4 were connected to this benzene ring. This finding indicated the presence of a 1,2,4,5-tetra-substituted benzene ring and explained why H-5 and H-8 appeared as singlets (no para coupling observed) in the proton NMR spectrum. This substitution pattern was confirmed further by the $^3J_{C-H}$ coupling of H-4 to C-5 and C-9. The downfield chemical shifts of C-1 (δ 149.9) and C-3 (δ 157.5) suggested that they were bonded to an aromatic nitrogen. Thus, a benzene ring fused to a pyridine ring (C-1, N-2, C-3, C-4, C-10, and C-9) moiety was established on the basis of intensive $^3J_{C-H}$ coupling of H-4 to C-9. By comparing the NMR spectrum of isoquinoline with that of 3-hydroxyisoquinoline, the chemical shift of C-3 (δ 157.5) in **1** implied that this carbon was bonded to a hydroxy group like 3-hydroxyisoquinoline. As a result, the 3,4-dihydroxybenzoyl moiety was logically placed at C-1 and not at C-3 because no long-range $^3J_{C-H}$ coupling in the HMBC experiment was observed between H-4 and the C-11 carbonyl carbon. Therefore, the chemical structure of **1** was elucidated as 1-(3,4-dihydroxybenzoyl)-3,6,7-trihydroxy-isoquinoline (Scheme 1).

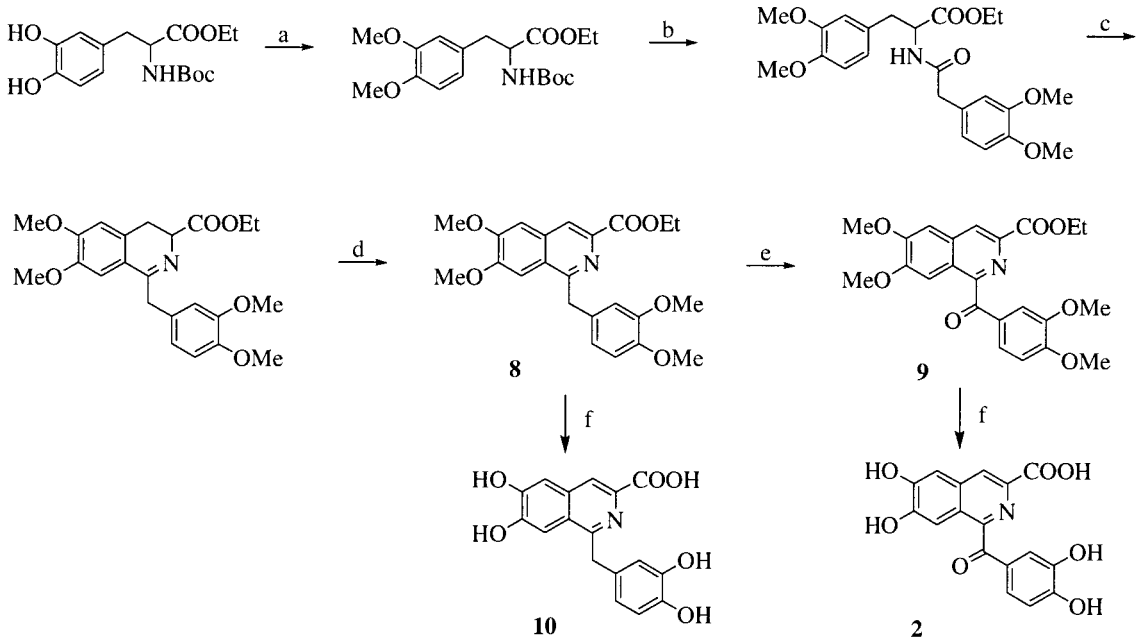
Compound **2** was also obtained as a yellow solid. Its molecular formula, $C_{17}H_{11}NO_7$, was established by ion spray mass spectrometry (*m/e* 342 in positive charge ionization mode, *m/e* 340 in negative charge ionization mode) and confirmed by high-resolution EIMS and microanalysis. A very strong IR absorption band at 3400 suggested the presence of a carboxylic acid group along with hydroxyl moieties. An IR band at 1640 cm^{-1} and two ^{13}C NMR signals at δ 194.6 and 167.9 indicated the presence of a conjugated ketone carbonyl group and a carboxylic acid carbonyl group in **2**. The ^1H NMR spectrum of **2** (Table 2) possessed many similarities to that of **1**, including three protons on a 1,3,4-trisubstituted benzene ring and two singlets on another aromatic moiety. The significant difference in chemical shift was H-4, which appeared at δ 8.52 (compared with δ 6.95 in **1**). This finding implied the presence of an electron-withdrawing carboxylic acid group on the pyridine ring. The ^{13}C NMR spectrum of **2** (Table 2) revealed 17 carbon signals, which is one more carbon than that of **1**. The extra carbonyl carbon signal at δ 167.9 matched with a carboxylic acid group. HMQC and HMBC NMR experiments of **2** showed results very similar to those of compound **1**. Therefore, the chemical structure of **2** was elucidated as 1-(3,4-dihydroxybenzoyl)-3-hydroxycarbonyl-6,7-dihydroxy-isoquinoline (Scheme 1).

Chemical Synthesis of the Active Components and Their Analogues. The structures of compounds **1** and **2** were further confirmed by chemical synthesis. The preparation of 1-(3,4-dihydroxybenzoyl)-3,6,7-trihydroxyisoquinoline **1** is outlined in Scheme 2.

2-[(3,4,-Dimethoxyphenyl)acetyl]-4,5-dimethoxyphenylacetic acid **4**, synthesized from self-condensation of 3,4-dimethoxyphenylacetic acid **3** in polyphosphoric acid (PPA), was subjected to a cyclization with ammonium acetate in refluxing acetic acid to give 1-(3,4-dimethoxybenzyl)-3-hydroxy-6,7-dimethoxyisoquinoline **5** according to a reported protocol.[12] Oxidation of compound **5** with selenium dioxide in dioxane or acetic acid gave the corresponding ketone **6**, but in low yield (16%). We also tried a direct oxidation of papaverine with vanadium pentoxide as reported[13] but no desired product was isolated. Demethylation of **6** was accomplished in 48% HBr after reflux overnight to give the designed compound **1**. Chemical characterization of this compound verified that it matched the active component **1** isolated from the L-(3,4-dihydroxyphenyl)-alanine/DMSO solution. Similarly, demethylation of intermediate **5** afforded 1-(3,4-dihydroxybenzyl)-3,6,7-trihydroxyisoquinoline **7** in good yield.

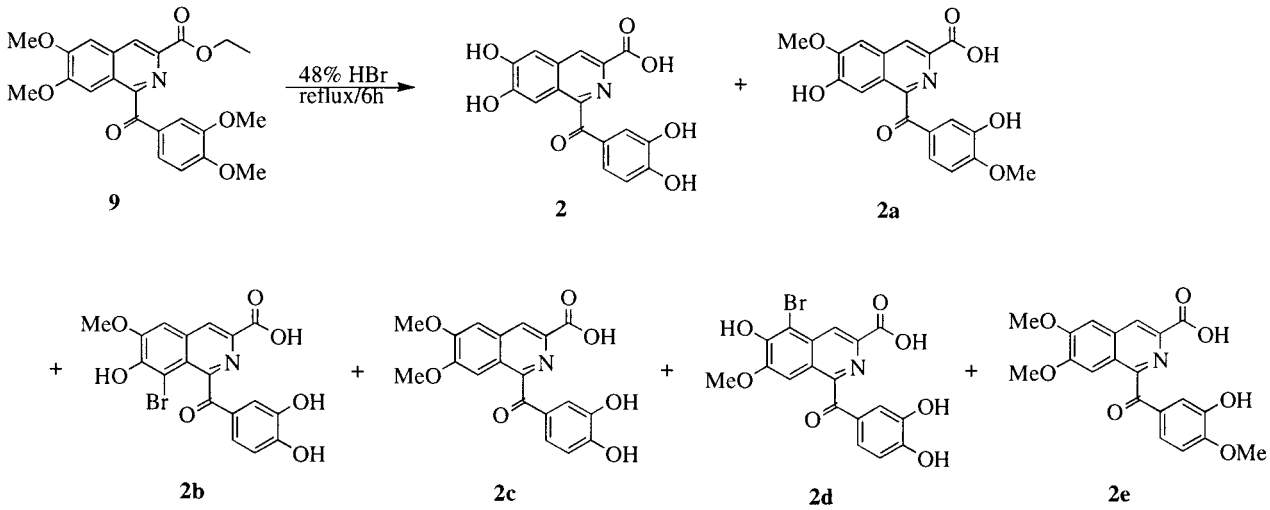
Scheme 3 depicts the synthesis of 1-(3,4-dihydroxybenzoyl)-3-hydroxycarbonyl-6,7-dihydroxyisoquinoline **2**.

Scheme 3[a]



[a] Reagents and conditions. (a) MeI/NaOH/EtOH. (b) TFA/r.t., then 3,4-dimethoxyphenylacetyl chloride/$NaHCO_3$/H_2O/EtOAc. (c) $POCl_3$/MeCN/reflux. (d) S/160 °C. (e) SeO_2/dioxane/reflux, 1 h. (f) 48% HBr/reflux, o/n.

Scheme 4



1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-3-ethoxycarbonylisoquinoline **8** was synthesized from D,L-(3,4-dihydroxyphenyl)-alanine according to a literature procedure.[14] Compound **8** was then oxidized with selenium dioxide in refluxing dioxane to give the corresponding ketone **9** in excellent yield. Deprotection of compound **9** in 48% hydrobromic acid under reflux overnight afforded the designed compound **2,** which matched the HPLC retention time, as well as the NMR spectra of, the active component **2** isolated from the L-(3,4-dihydroxyphenyl)alanine/DMSO solution. Deprotection of compound **8** in refluxing hydrobromic acid gave a related compound **10** in good yield.

To explore the importance of the hydroxyl groups in the two catechol moieties for binding activity, compound **9** was partially hydrolyzed in aqueous HBr (reflux, 6 h) to afford several partially demethylated compounds (**2a**−**e**), which were isolated by HPLC on a reversed phase C-18 column (Scheme 4). Their structures were confirmed by HMQC and HMBC NMR experiments.

Oxidation of papaverine **11** with selenium dioxide in dioxane gave the corresponding 1-(3,4-dimethoxybenzoyl)-6,7-dimethoxyisoquinoline **12**, which was demethylated in refluxing hydrobromic acid to afford the corresponding 1-(3,4-dihydroxybenzoyl)-6,7-dihydroxyisoquinoline **13** (Scheme 5).

Isoquinoline analogues **14a**−**d** were also synthesized from D,L-(3,4-dihydroxyphenyl)alanine and 3,4-methylenedioxyphenyl-, 2,4-dichlorophenyl-, 1-naphthyl-, or 2-naphthylacetic acid, respectively, according to a procedure similar to that described in Scheme 3.

In vitro Binding Activity of Compounds 1 and 2 and Their Analogues. The synthesized compounds **1, 2, 2a**−**e, 6, 7, 9, 10, 13,** and **14a**−**d** were evaluated for their ability to inhibit the binding of radiolabled IGF-I to IGFBP-3 in a binding assay as described in the Experimental Section. The data obtained were used to calculate the K_i value for each compound using Prism Software, and are summarized in Table 3. Compound **1** showed very good potency (K_i = 28 nM), but its

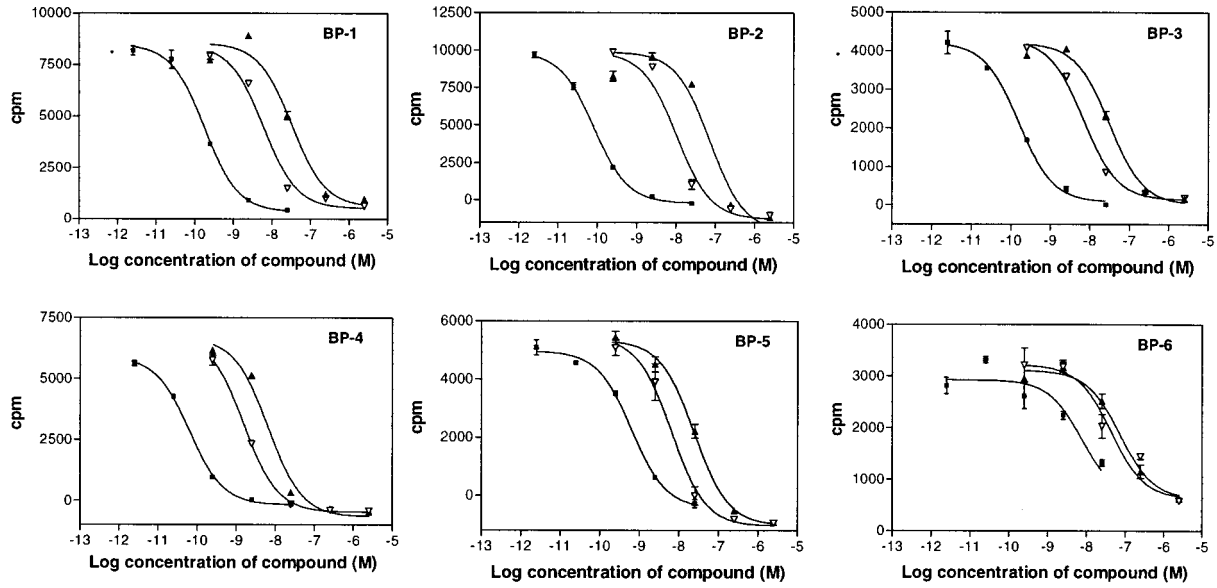
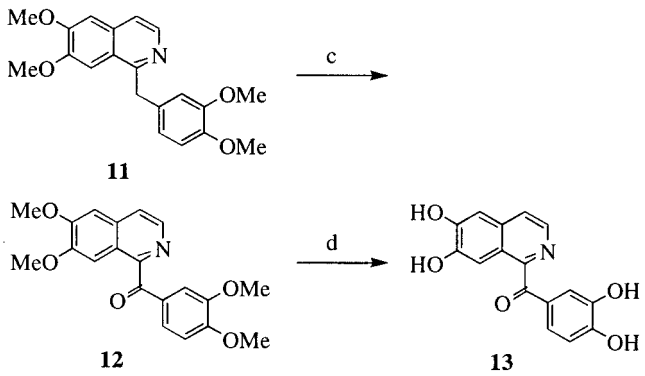


Figure 1. Dose-dependent inhibition of [^{125}I]hIGF-I binding to IGFBP-1, BP-2, BP-3, BP-4, BP-5, and BP-6 by hIGF-I (■), compound **1** (▲), and compound **2** (▽). The radioligand-binding assay was carried out in duplicate in glass test tubes with 0.5 pmol binding protein, labeled hIGF-I (30,000 cpm), plus increasing concentrations of hIGF-I, **1**, or **2**. After incubation at room temperature for 2 h, the IGF/IGFBP complex was precipitated by the addition of 20% BSA and 20% PEG-800 solutions, and the radioactivity in the pellet was counted on a γ-counter. The data obtained were analyzed by the Prism software to generate the dose−response curves. The standard errors are within the boundaries of the symbols for those points on the curves that do not have error bars.

Scheme 5[a]



[a] Reagents and conditions. (a) SeO_2/dioxane/reflux, 1 h. (b) 48% HBr/reflux, o/n.

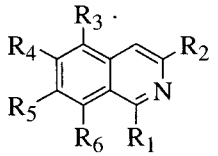
precursor **6** was completely inactive, indicating that one or more of the catechol hydroxy groups are essential for activity. Replacement of the 3,4-dihydroxybenzoyl moiety of **1** at position-1 with a 3,4-dihydroxybenzyl group resulted in a compound with the same binding affinity (compound **7**, K_i = 29 nM). Although removal of the hydroxyl group on C-3 of the isoquinoline ring (compound **13**, K_i = 58 nM) caused a 2-fold decrease in activity compared to **1**, replacement of the same hydroxyl group with a more acidic carboxylic moiety yielded a molecule with 5-fold increase in affinity (compound **2**, K_i = 5.6 nM). The corresponding benzylic analogue of **2** (compound **10**, K_i = 7.5 nM) showed only slightly decreased activity, whereas the precursor of **2**, compound **9**, displayed no activity. These data suggested that the ketone functional group in compounds **1** and **2** had minimum effect on IGFBP-3 binding affinity, position-3 of the isoquinoline ring required an acidic group for high potency, and one or both of the two catechol moieties were crucial for IGFBP-3 activity.

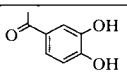
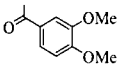
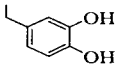
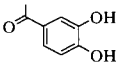
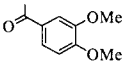
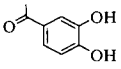
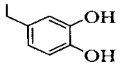
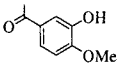
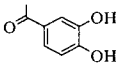
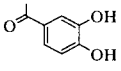
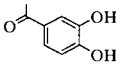
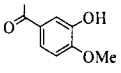
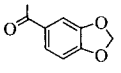
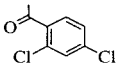
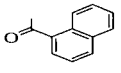
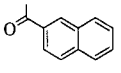
Next, we examined several partially methylated compounds to determine the importance of the catechol hydroxyl groups on compound **2**. Whereas methylation of both 6- and 7-hydroxyl groups of compound **2** abolished its binding affinity (compound **2c** and **2e**, K_i >10,000 nM), demethylation at position-7 of compound **2e** resulted in an active analogue **2a** (K_i = 320 nM). Introduction of a bromine atom at position-8 and demethylation at position-15 of **2a** increased the activity of the resulting compound (compound **2b**, K_i = 140 nM) about 2-fold. A closely related analogue of **2b** with a 5-bromo-6-hydroxy-7-methoxy-isoquinoline moiety (compound **2d**, K_i = 70 nM) also showed relatively high activity.

The catechol functional group was crucial for imparting high affinity to the isoquinoline nucleus. By contrast, the catechol moiety in the 1-benzoyl group of compounds **1** and **2** was not critical for activity. This conclusion was based on the SAR data of compounds **14a**−**d**, which only lost 3- to 12-fold activity in comparison with the parent compound **2**. Compound **14d** was only 3-fold less active than compound **2**, and more importantly it was much more lipophilic than compound **2**. These compounds could serve as leads for the design of orally bioavailable small molecules that disrupt the binding of IGFs to their binding proteins. The isoquinoline derivatives **1** and **2** also showed high binding activity on other IGFBPs in addition to IGFBP-3 as shown in Figure 1 and their respective affinity constants for the six IGFBPs are presented in Table 4.

In vitro Bioactivity of Compounds 1 and 2. To demonstrate the ability of compounds **1** and **2** to release bioactive IGF-I from its bound form with IGFBP-3, we employed a cell proliferation assay using 3T3 fibroblasts,[15] which expressed IGF-I receptors and responded to IGF-I stimulation through cell proliferation. The cell proliferation effect was monitored by quantitation of [^{3}H]-thymidine incorporation into the cells. As seen in Figure 2, human IGF-I (2 nM) induced a robust proliferation of 3T3 fibroblasts as reflected by a >2-fold

Table 3. IGFBP-3 Inhibitory Activity of Compounds **1** and **2** and Their Analogues



Entry	Compound	R_1	R_2	R_3	R_4	R_5	R_6	K_i (nM)
1	**1**		OH	H	OH	OH	H	28 ± 8
3	**6**		OH	H	OMe	OMe	H	>10,000
4	**7**		OH	H	OH	OH	H	29 ± 9
5	**13**		H	H	OH	OH	H	58 ± 25
6	**9**		COOEt	H	OMe	OMe	H	>10,000
7	**2**		COOH	H	OH	OH	H	5.6 ± 0.5
8	**10**		COOH	H	OH	OH	H	7.5 ± 8.1
9	**2a**		COOH	H	OMe	OH	H	320*
10	**2b**		COOH	H	OMe	OH	Br	140 ± 70
11	**2c**		COOH	H	OMe	OMe	H	>10,000
12	**2d**		COOH	Br	OH	OMe	H	70 ± 40
13	**2e**		COOH	H	OMe	OMe	H	>10,000
14	**14a**		COOH	H	OH	OH	H	70 ± 24
15	**14b**		COOH	H	OH	OH	H	44 ± 41
16	**14c**		COOH	H	OH	OH	H	27 ± 7
17	**14d**		COOH	H	OH	OH	H	13 ± 6

* Single test.

increase of [^{3}H]-thymidine incorporation above basal level into the cells and the hIGF-I-induced proliferation was substantially blocked by addition of hIGFBP-3 (4 nM). Addition of compounds **1** (Figure 2A) and **2** (Figure 2B) dose-dependently reversed the neutralization effect of hIGFBP-3 on the activity of hIGF-I, demonstrating the ability of **1** and **2** to displace bioactive IGF-I bound to IGFBP-3. Moreover, maximal reversal of the IGFBP-3 inhibitory was reached at 1 μM concentration for compound **2** but at 10 μM concentration for compound **1**. These results are in agreement with the binding assay data (Table 3), showing that **2** is 5-fold more potent than **1**. Compounds **1** or **2** (10 μM) alone had no effect on [^{3}H]-thymidine incorporation in these cells. These in vitro data clearly demonstrate that isoquinolines **1** and **2** are capable of interacting with the binding protein in a specific manner to displace and release "free" bioactive IGF-I.

Conclusion

A series of highly active, nonpeptide small molecule inhibitors of IGF/IGFBP-3 binding was identified from high throughput screening, followed by structural elucidation of the active components isolated from the

Table 4. Binding Affinities of Compounds **1** and **2** and IGF-I on IGFBP-1 to IGFBP-6

	K_i (nM)		
IGFBP	hIGF-I	1	2
BP-1	0.17 ± 0.03	34.23 ± 11.41	5.07 ± 0.74
BP-2	0.07 ± 0.02	17.13 ± 5.65	2.07 ± 0.03
BP-3	0.21 ± 0.05	28.23 ± 8.06	5.63 ± 0.46
BP-4	0.06 ± 0.02	6.23 ± 0.62	1.04 ± 0.19
BP-5	0.20 ± 0.04	20.95 ± 0.05	5.20 ± 0.30
BP-6	3.10 ± 0.20	84.65 ± 12.35	23.65 ± 3.35

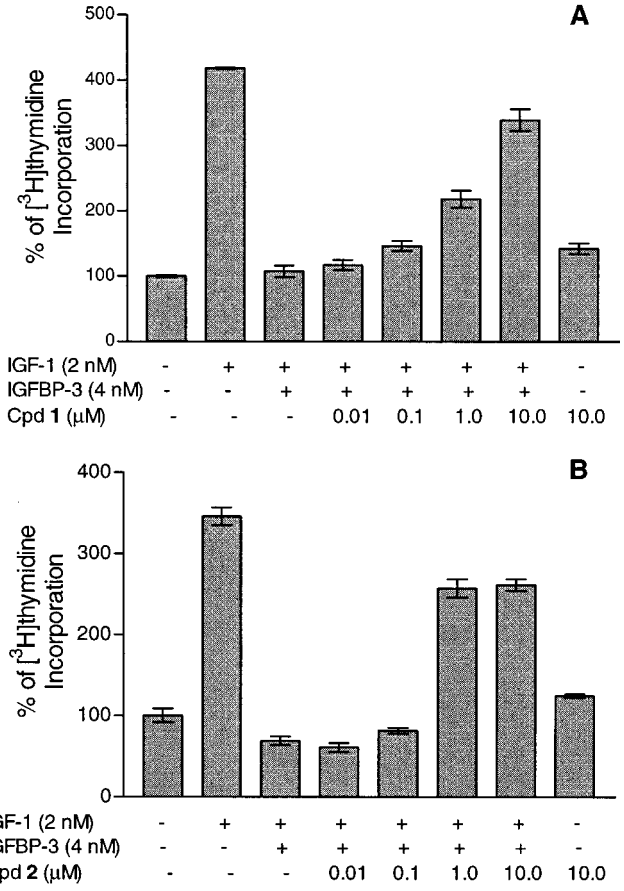


Figure 2. Reversal of IGFBP-3 inhibition of IGF-I-stimulated Balb/c 3T3 fibroblast proliferation by compounds **1** (panel A) and **2** (panel B). Human IGF-I (2 nM) induced a >2-fold increase in proliferation of the fibroblast compared to that of the control as measured by the incorporation of [^{3}H]thymidine. Human IGFBP-3 (4 nM) completely inhibited the proliferative effect of 2 nM IGF-I. Addition of compound **1** (panel A) or compound **2** (panel B) dose-dependently reversed this inhibition. Both **1** and **2** alone have no effect on fibroblast proliferation. Data represent the mean ± SEM.

screening hit solution. These compounds inhibited IGF-I binding to IGFBP-3 and thus were able to release "free" IGF-I from its binding protein complex at low nanomolar concentrations. Furthermore, the IGF-I released by compounds **1** and **2** was shown to be biologically active in an in vitro bioassay. These results demonstrated that nonpeptide small molecule IGF/IGFBP binding inhibitors could be developed and possibly used as therapeutics to treat IGF-responsive diseases.

Experimental Section

^{1}H NMR and ^{13}C NMR were recorded on a Varian Mercury FT-300 NMR spectrometer, HMQC and HMBC experiments were performed on a Varian FT-500 NMR spectrometer. Electrospray ionization mass spectra (EI-MS) were recorded on a PE Sciex API LC/MS System. FT-IR spectra were recorded on a Perkin-Elmer 1605 IR Spectrophotometer (KBr). HPLC analyses were carried out on a Beckman 322 gradient HPLC system, whereas preparative HPLC separations were performed on a Biotage Kiloprep 100G HPLC system. All reagents and solvents were of ACS reagent grade or higher and were used as provided.

Separation of the Active Components from L-3,4-(Dihydroxyphenyl)-alanine. One gram of l-3,4-dihyroxyphenyl-alanine (Sigma) was stirred in 200 mL of dimethyl sulfoxide at room temperature in an open flask for 3 days. The resulting brown solution was purified on a Kiloprep 100G preparative HPLC system equipped with a C18 cartridge and in an acetonitrile gradient in aqueous 0.1% trifluoroacetic acid. The collected fractions were monitored by the IGFBP-3 radioligand-binding assay after removal of the solvent. The major active fractions from four repetitive runs were pooled, and the solvent was evaporated; the residue was characterized by MS, IR, and NMR (including NOE, HMQC, and HMBC) analyses.

1-(3,4-Dimethoxybenzyl)-3-hydroxy-6,7-dimethoxyisoquinoline (5).[12] 3,4-Dimethoxyphenyl-acetic acid (5 g) was mixed with PPA (100 g), and the mixture was heated on a water bath for 0.5 h and then stirred at room-temperature overnight. The dark brown syrup was poured into ice−water (800 mL) and allowed to stand for 3 h. The yellow solid was collected by filtration. The collected solid was recrystallized from ethanol to afford a yellowish solid (2 g), which was mixed with ammonium acetate (4 g) in acetic acid (5 mL) and heated at reflux for 1 h and then allowed to cool. The cold solution was diluted with methylene chloride and washed with water. The organic phase was dried over sodium carbonate and concentrated in vacuo to give the desired compound as a yellow solid (1.8 g). ^{1}H NMR ($CDCl_3$/TMS): 3.79 (s, 3H), 3.80 (s, 3H), 3.87 (s, 3H), 3.95 (s, 3H), 4.37 (s, 2H), 6.65 (s, 1H), 6.68 (s, 1H), 6.75 (d, J = 8.4 Hz, 1H), 6.80 (dd, J = 1.0, 8.4 Hz, 1H), 6.95 (d, J = 1.0 Hz, 1H), 6.96 (s, 1H). ^{13}C NMR ($CDCl_3$/TMS): 98.0, 98.4, 100.9, 106.9, 107.5, 109.3, 116.0, 124.9, 137.9, 143.3, 143.5, 143.7, 144.7, 150.7, 156.4. EI-MS *m/e* 355 $(M+H)^+$.

1-(3,4-Dimethoxybenzoyl)-3-hydroxy-6,7-dimethoxyisoquinoline (6). 1-(3,4-Dimethoxybenzyl)-3-hydroxy-6,7-dimethoxyisoquinoline (355 mg) was mixed with selenium dioxide (1.1 equiv) in dioxane (5 mL) and heated at reflux for 2 h. The resultant mixture was purified on silica gel with 5% MeOH in methylene chloride as the eluent to give the product as a yellow oil (60 mg). ^{1}H NMR: ($CDCl_3$/TMS): 3.93 (s, 3H), 3.95 (s, 3H), 4.00 (s, 3H), 4.09 (s, 3H), 6.82 (s, 1H), 6.89 (d, J = 1.8 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 7.02 (dd, J = 1.8, 8.4 Hz, 1H), 7.30 (s, 1H), 7.62 (s, 1H), 8.88 (brs, 1H). EI-MS *m/e* 370 $(M+H)^+$. HR-MS Anal. for $C_{20}H_{20}NO_6$: calcd. *m/e*, 370.1291; found *m/e*, 370.1280.

1-(3,4-Dihydroxybenzoyl)-3-hydroxy-6,7-dihydroxyisoquinoline (1). 1-(3,4-Dimethoxybenzoyl)-3-hydroxy-6,7-dimethoxyisoquinoline (15 mg) was suspended in 48% HBr (3 mL) and heated at reflux for 4 h. The crude product was purified by reversed-phase HPLC on a C-18 column using a gradient of acetonitrile in 0.1% TFA/H_2O to give the product as a yellowish powder (2 mg). ^{1}H NMR (CD_3OD/TMS): 6.83 (d, J = 8.3 Hz, 1H), 6.94 (s, 1H), 6.95 (s, 1H), 7.01 (s, 1H), 7.17 (dd, J = 2.0, 8.4 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H). ^{13}C NMR: 103.9, 107.6, 116.4, 117.6, 118.6, 126.6, 129.5, 141.9, 147.2, 148.8, 150.0, 154.2, 155.9, 192.3. IR (KBr): 3300 (s), 1629 (s), 1590 (m), 1465 (m). EI-MS *m/e* 314 $(M+H)^+$; (negative charge mode): *m/e* 312 $(M-H)^-$. HR-MS Anal. for $C_{16}H_{12}NO_6$: calcd. *m/e*, 314.0665; found *m/e*, 314.0660. Anal. ($C_{16}H_{11}NO_6 \cdot 0.5\ H_2O$) C, H, N.

1-(3,4-Dihydroxybenzyl)-3-hydroxy-6,7-dihydroxyisoquinoline (7). 1-(3,4-Dimethoxybenzyl)-3-hydroxy-6,7-dimethoxyisoquinoline (15 mg) was suspended in 48% HBr (3 mL) and heated at reflux for 4 h. The crude product was purified with HPLC on a reversed-phase column to give the product as a yellowish powder (2 mg). ^{1}H NMR ($CDCl_3$/TMS): 4.43 (s, 2H), 6.53 (dd, J = 2.1, 8.4 Hz, 1H), 6.60 (d, J = 2.1 Hz, 1H),

6.70 (d, J = 8.4 Hz, 1H), 7.00 (s, 1H), 7.08 (s, 1H), 7.43 (s, 1H). MS (EI) *m/e* 300 $(M+H)^+$.

1-(3,4-Dimethoxybenzyl)-3-ethoxycarbonyl-6,7-dimethoxyisoquinoline (8).[14] N-Boc-D,L-3,4-dimethoxyphenylalanine ethyl ester (82.0 g, 0.232 mol) was stirred in trifluoroacetic acid (70 mL, 0.58 mol) until bubbling ceased (approximately 30 min). The trifluoroacetic acid was removed in vacuo, and the residue was coevaporated with toluene twice to give D,L-3,4-dimethoxyphenylalanine ethyl ester trifluoroacetic acid salt as an oil. ^{1}H NMR ($CDCl_3$/TMS): 1.31 (t, J = 7.0 Hz, 3H), 3.13 (dd, J = 7.8, 14.4 Hz, 1H), 3.30 (dd, J = 4.8, 14.4 Hz, 1H), 3.83 (s, 3H), 3.84 (s, 3H), 4.27 (m, 3H), 6.60 (brs, 3H), 6.73 (d, J = 2.1 Hz, 1H), 6.74 (dd, J = 2.1, 9.0 Hz, 1H), 6.82 (d, J = 9.0 Hz, 1H).

The deprotected amino acid ethyl ester was dissolved in 600 mL of H_2O containing sodium bicarbonate (60 g, 0.510 mol). To this mixture was added slowly a solution of 3,4-dimethoxyphenylacetyl chloride, synthesized freshly from 3,4-dimethoxyphenylacetic acid (47.8 g, 0.244 mol) and oxalyl chloride (40.5 mL, 0.464 mol) in 400 mL of ethyl acetate. The mixture was stirred for 16 h at room temperature. The ethyl acetate and H_2O layers were separated, and the organic layer was washed with 2 × 300 mL of saturated sodium bicarbonate, dried over $MgSO_4$, filtered, and concentrated in vacuo to give N−(3,4-dimethoxyphenylacetyl)-D,L-3,4-dimethoxyphenylalanine ethyl ester as a tan solid (81 g). ^{1}H NMR ($CDCl_3$/TMS): 1.24 (t, J = 7.2 Hz, 3H), 2.98 (m, 2H), 3.50 (s, 2H), 3.78 (s, 3H), 3.82 (s, 3H), 3.86 (s, 3H), 3.89 (s, 3H), 4.15 (q, J = 7.2 Hz, 2H), 4.78 (m, 1H), 5.90 (d, J = 7.5 Hz, 1H), 6.40 (dd, J = 2.1, 8.1 Hz, 1H), 6.51 (d, J = 2.1 Hz, 1H), 6.65 (d, J = 8.1 Hz, 1H), 6.70 (d, J = 1.8 Hz, 1H), 6.71 (dd, J = 7.8, 1.8 Hz, 1H), 6.80 (d, J = 7.8 Hz, 1H).

N−(3,4-dimethoxyphenylacetyl)-D,L-3,4-dimethoxyphenylalanine ethyl ester (81 g, 0.188 mol) was dissolved in 300 mL of acetonitrile. Phosphorus oxychloride (88 mL, 0.94 mol) was added and the reaction mixture was heated to reflux for 2 h. After the mixture was cooled to room temperature, the solvent and excess phosphorus oxychloride were removed under vacuum using a KOH trap. The residual dark oil was then diluted with 100 mL of methylene chloride and 400 mL of ice−water, and neutralized with Na_2CO_3 while stirring vigorously on an ice−water bath. The mixture was then diluted with 200 mL of ethyl acetate, the organic and aqueous layers were separated, and the aqueous layer was extracted with 2 × 300 mL of ethyl acetate. The combined organic layers were washed with 2 × 300 mL of saturated sodium bicarbonate, dried over $MgSO_4$, and concentrated to give 1-(3,4-dimethoxybenzyl)-3-ethoxycarbonyl-3,4-dihydro-6,7-dimethoxyisoquinoline as an orange solid, which was carried forward without purification. ^{1}H NMR ($CDCl_3$/TMS): 1.31 (t, J = 7.2 Hz, 3H), 2.95 (d, J = 9.0 Hz, 2H), 3.72 (s, 3H), 3.83 (s, 6H), 3.88 (s, 3H), 4.03 (d, J = 14.8 Hz, 1H), 4.10 (d, J = 14.8 Hz, 1H), 4.27 (q, J = 7.2 Hz, 2H), 4.39 (t, J = 9.0 Hz, 1H), 6.66 (s, 1H), 6.78 (d, J = 8.1 Hz, 1H), 6.88 (dd, J = 8.1, 1.8 Hz, 1H), 6.94 (d, J = 1.8 Hz, 1H), 7.00 (s, 1H), 7.27 (s, 1H). EI-MS *m/e* 414 $(M+H)^+$.

1-(3,4-Dimethoxybenzyl)-3-ethoxycarbonyl-3,4-dihydro-6,7-dimethoxyisoquinoline (77.7 g, 0.188 mol) was dissolved in 30 mL of methylene chloride. Sulfur (9.04 g, 0.282 mol) was added, and the mixture was heated to 160 °C until bubbling ceased in approximately 30 min. The mixture was cooled to approximately 50 °C, diluted with ethyl acetate, and stirred while being cooled to room temperature, followed by filtration through a short silica gel column with ethyl acetate. The product precipitated from the filtrate was collected to give a yellow solid (48 g). ^{1}H NMR ($CDCl_3$/TMS): 1.48 (t, J = 7.0 Hz, 3H), 3.76 (s, 3H), 3.82 (s, 3H), 3.87 (s, 3H), 4.02 (s, 3H), 4.53 (q, J = 7.0 Hz, 2H), 4.65 (s, 2H), 6.75 (d, J = 8.1 Hz, 1H), 6.80 (dd, J = 8.1, 1.8 Hz, 1H), 6.83 (d, J = 1.8 Hz, 1H), 7.17 (s, 1H), 7.36 (s, 1H). EI-MS *m/e* 412 $(M+H)^+$.

1-(3,4-Dimethoxybenzoyl)-3-ethoxycarbonyl-6,7-dimethoxyisoquinoline (9). 1-(3,4-Dimethoxybenzyl)-3-ethoxycarbonyl-6,7-dimethoxyisoquinoline (**8**, 20.55 g, 0.05 mol) was stirred in 500 mL of dioxane and heated to reflux to give a clear solution. Selenium dioxide (5.55 g, 0.05 mol) was added and the refluxing continued for 0.5 h. After the mixtured cooled to room temperature, methylene chloride was added, and the solution was filtered through a short silica column to remove a black solid. The filtrate was concentrated to give a tan solid, which was washed with ether. Filtration isolated the desired product as a white solid (19.5 g). ^{1}H NMR ($CDCl_3$/TMS): 1.44 (t, J = 7.5 Hz, 3H), 3.96 (s, 3H), 3.97 (s, 3H), 4.01 (s, 3H), 4.08 (s, 3H), 4.49 (q, 2H), 6.87 (d, J = 8.4 Hz, 1H), 7.28 (s, 1H), 7.52 (dd, J = 1.8, 8.4 Hz, 1H), 7.57 (s, 1H), 7.81 (d, J = 1.8 Hz, 1H), 8.55 (s, 1H). EI-MS *m/e* 426 $(M+H)^+$. Anal. ($C_{23}H_{23}NO_7$) C, H, N.

1-(3,4-Dihydroxybenzoyl)-3-hydroxycarbonyl-6,7-dihydroxyisoquinoline (2). 1-(3,4-dimethoxybenzoyl)-3-ethoxycarbonyl-6,7-dimethoxyisoquinoline (**9**, 2 g) was refluxed in 48% HBr (15 mL) for 40 h. A yellow solid that precipitated upon cooling was collected by filtration, washed with ethyl acetate, and dried under vacuum with P_2O_5 to give a yellow solid (1.4 g). A 1-g portion of the compound was dissolved in refluxing MeOH (20 mL), and boiling water (about 40 mL) was added until the solution was cloudy. This mixture was allowed to sit overnight, and the precipitated light yellow solid was collected by filtration to give 0.78 g of the pure product. ^{1}H NMR (DMSO-D_6/TMS): 6.82 (d, J = 8.1 Hz, 1H), 7.09 (dd, J = 1.5, 8.1 Hz, 1H), 7.11 (s, 1H), 7.24 (d, J = 1.5 Hz, 1H), 7.43 (s, 1H), 8.43 (s, 1H), 9.20 (brs, 1H), 10.06 (brs, 1H), 10.50 (brs, 2H). ^{13}C NMR: 109.1, 111.3, 116.3, 117.7, 124.6, 124.8, 126.5, 129.8, 135.2, 138.1, 147.0, 152.3, 153.9, 154.3, 156.0, 167.9, 194.6; IR (KBr): 3408, 1650, 1588, 1516, 1409, 1286, 1197. EI-MS *m/e* 342 $(M+H)^+$, (negative charge mode): *m/e* 340 $(M-H)^-$. HR-MS for $C_{17}H_{12}NO_7$ $(M+H)^+$: calcd. *m/e*, 342.0614; found *m/e*, 342.0618. Anal. ($C_{17}H_{11}NO_7 \cdot 2.7H_2O$) C, H, N.

1-(3,4-Methylenedioxybenzoyl)-3-hydroxycarbonyl-6,7-dihydroxyisoquinoline (14a). This compound was prepared in a manner similar to the procedure described for **2** from methylenedioxyphenylacetic acid. White powder. ^{1}H NMR (DMSO-D_6/TMS): 6.30 (s, 2H), 7.14 (d, J = 8. Hz, 1H), 7.31 (s, 1H), 7.40 (d, J = 8.1 Hz, 1H), 7.55 (s, 1H), 7.57 (s, 1H), 8.58 (s, 1H), 10.70 (brs, 2H). EI-MS *m/e* 354 $(M+H)^+$. Anal. ($C_{18}H_{11}NO_7 \cdot 0.67\ H_2O \cdot 0.33$ HBr) C, H, N.

1-(2,4-Dichlorobenzoyl)-3-hydroxycarbonyl-6,7-dihydroxyisoquinoline (14b). This compound was prepared in a manner similar to the procedure described for **2** from 2,4-dichlorophenylacetic acid. Yellowish powder. ^{1}H NMR (DMSO-D_6/TMS): 7.40 (s, 1H), 7.51 (dd, J = 1.8, 8.1 Hz, 1H), 7.60 (d, J = 8.1 Hz, 1H), 7.67 (d, J = 2.1 Hz, 1H), 8.01 (s, 1H), 8.45 (s, 1H), 10.60 (brs, 1H), 10.79 (brs, 1H). EI-MS *m/e* 378 $(M+H)^+$. Anal. ($C_{17}H_9NCl_2O_5 \cdot 0.5\ H_2O \cdot 0.5$ MeOH) C, H, N.

1-(1-Naphthyl)-3-hydroxycarbonyl-6,7-dihydroxyisoquinoline (14c). This compound was prepared in a manner similar to the procedure described for **2** from 1-naphthylacetic acid. Yellowish powde. ^{1}H NMR (CD_3OD/TMS): 7.29 (s, 1H), 7.32 (d, J = 7.2 Hz, 1H), 7.36 (d, J = 1.8 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.48 (dt, J = 1.5, 6.6 Hz, 1H), 7.55 (dt, J = 1.2, 6.9 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 8.05 (dd, J = 2.1, 7.2 Hz, 1H), 8.31 (s, 1H), 8.62 (d, J = 8.4 Hz, 1H), 10.45 (brs, 1H). EI-MS *m/e* 360 $(M+H)^+$. Anal. ($C_{21}H_{13}NO_5 \cdot 2\ H_2O$) C, H, N.

1-(2-Naphthyl)-3-hydroxycarbonyl-6,7-dihydroxyisoquinoline (14d). This compound was prepared in a manner similar to the procedure described for **2** from 2-naphthylacetic acid. Yellowish powder. ^{1}H NMR (CD_3OD/TMS): 7.33 (s, 1H), 7.41 (s, 1H), 7.53 (t, J = 7.2 Hz, 1H), 7.64 (d, J = 7.2 Hz, 1H), 7.89 (d, J = 7.5 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 9.0 Hz, 1H), 8.09 (dd, J = 1.8, 9.0 Hz, 1H), 8.26 (s, 1H), 8.55 (s, 1H). EI-MS *m/e* 360 $(M+H)^+$. Anal. ($C_{21}H_{13}NO_5 \cdot 1.3\ H_2O$) C, H, N.

Partial Hydrolysis of 1-(3,4-Dimethoxybenzoyl)-3-ethoxycarbonyl-6,7-dimethoxyisoquinoline (9). 1-(3,4-dimethoxybenzoyl)-3-ethoxycarbonyl-6,7-dimethoxyisoquinoline (200 mg) was refluxed in 48% HBr (5 mL) for 8 h. The following compounds, in addition of compound **2**, were separated by reversed-phase HPLC on a C-18 column. The structures of these compounds were assigned on the basis of NMR studies including HMBC NMR experiments.

1-(3-Hydroxy-4-methoxybenzoyl)-3-hydroxycarbonyl-6-methoxy-7-hydroxyisoquinoline (2a). White powder. HPLC: >96% purity, $t_R = 12.4$ min. ^{1}H NMR (DMSO-D_6/TMS, 500 MHz): 3.84 (s, 3H), 3.97 (s, 3H), 7.03 (d, $J = 8.5$ Hz, 1H), 7.16 (s, 1H), 7.18 (dd, $J = 1.6$, 8.5 Hz, 1H), 7.30 (d, $J = 1.6$ Hz, 1H), 7.70 (s, 1H), 8.56 (s, 1H), 9.55 (brs, 2H). ^{13}C NMR (DMSO-D_6/TMS, 500 MHz): 55.8, 56.0, 106.7, 107.3, 111.5, 115.7, 123.1, 123.3, 124.1, 128.9, 132.2, 138.3, 146.6, 150.8, 153.1, 153.2, 154.0, 166.4, 193.3. EI-MS *m/e* 370 $(M+H)^+$.

1-(3,4-Dihydroxybenzoyl)-3-hydroxycarbonyl-8-bromo-7-hydroxy-6-methoxyisoquinoline (2b). White powder. HPLC: 92% purity, $t_R = 11.95$ min. ^{1}H NMR (DMSO-D_6/TMS, 500 MHz): 4.07 (s, 3H), 6.81 (d, $J = 8.5$ Hz, 1H), 7.00 (d, $J = 8.1$ Hz, 1H), 7.20 (s, 1H), 7.85 (s, 1H), 8.60 (s, 1H), 9.40 (brs, 1H), 9.98 (brs, 1H), 10.92 (brs, 1H). ^{13}C NMR (DMSO-D_6/TMS, 500 MHz): 56.8, 102.3, 107.3, 115.3, 116.3, 122.5, 123.2, 123.6, 129.3, 133.6, 138.8, 145.2, 148.4, 151.1, 152.0, 155.5, 166.1, 192.8. EI-MS *m/e* 436 $(M+H)^+$.

1-(3,4-Dihydroxybenzoyl)-3-hydroxycarbonyl-6,7-dimethoxyisoquinoline (2c). White powder. HPLC: >99%, $t_R = 13.3$ min. ^{1}H NMR (CD_3OD/TMS): 3.93 (s, 3H), 4.08 (s, 3H), 6.87 (d, $J = 8.1$ Hz, 1H), 7.28 (s, 1H), 7.34 (dd, $J = 8.1$ Hz, 1H), 7.50 (d, $J = 2.4$ Hz, 1H), 7.52 (s, 1H), 8.56 (s, 1H). EI-MS *m/e* 370 $(M+H)^+$.

1-(3,4-Dihydroxybenzoyl)-3-hydroxycarbonyl-5-bromo-6-hydroxy-7-methoxyisoquinoline (2d). White powder. HPLC: 95% purity, $t_R = 13.9$ min. ^{1}H NMR (CD_3OD/TMS, 500 MHz): 3.93 (s, 3H), 6.78 (d, $J = 8.5$ Hz, 1H), 7.04 (d, $J = 2.1$, 8.5 Hz, 1H), 7.09 (d, $J = 2.1$ Hz, 1H), 7.13 (s, 1H), 8.28 (s, 1H). ^{13}C NMR (CD_3OD/TMS, 500 MHz): 57.0, 107.0, 113.2, 116.6, 118.0, 124.3, 124.6, 126.4, 129.5, 137.9, 139.4, 146.7, 152.7, 153.7, 156.8, 157.1, 167.6, 195.2. EI-MS *m/e* 436 $(M+H)^+$.

1-(3-Hydroxy-4-methoxybenzoyl)-3-hydroxycarbonyl-6,7-dimethoxyisoquinoline (2e). White powder. HPLC: >99% purity, $t_R = 17.65$ min. ^{1}H NMR (CD_3OD/TMS, 500 MHz): 3.97 (s, 3H), 3.98 (s, 3H), 4.09 (s, 3H), 6.90 (d, $J = 8.1$ Hz, 1H), 7.32 (s, 1H), 7.42 (dd, $J = 2.4$, 8.1 Hz, 1H), 7.48 (s, 1H), 7.57 (d, $J = 2.4$ Hz, 1H), 8.60 (s, 1H). EI-MS *m/e* 384 $(M+H)^+$.

1-(3,4-Dihydroxybenzyl)-3-hydroxycarbonyl-6,7-dihydroxyisoquinoline (10). 1-(3,4-Dimethoxybenzyl)-3-ethoxycarbonyl-6,7-dimethoxyisoquinoline (100 mg) was refluxed in 48% HBr (3 mL) for 20 h. After cooling, the solid was collected to give the product as a white powder. ^{1}H NMR (CD_3OD/TMS): 5.80 (s, 2H), 6.57 (d, $J = 8.0$ Hz, 1H), 6.65 (s, 1H), 6.72 (d, 1H), 7.56 (s, 1H), 7.78 (s, 1H), 8.62 (s, 1H). EI-MS *m/e* 328 $(M+H)^+$. Anal. ($C_{17}H_{13}NO_6 \cdot 0.67\ H_2O$) C, H, N.

1-(3,4-Dihydroxybenzoyl)-6,7-dihydroxyisoquinoline (13). Papaverine (1 g) was mixed with selenium dioxide (1.1 equiv) in dioxane (20 mL) and heated at reflux for 2 h. The resultant mixture was purified on silica gel with 5% MeOH in methylene chloride to give 1-(3,4-dimethoxybenzoyl)-6,7-dimethoxyisoquinoline as a white solid. EI-MS 354 $(M+H)^+$. This compound (200 mg) was suspended in 48% HBr (3 mL) and heated at reflux for 4 h. The mixture was cooled to room temperature and a gray solid precipitated. The solid was collected by vacuum filtration to give the product as a brownish solid (130 mg). ^{1}H NMR (CD_3OD/TMS, 500 MHz): 6.89 (d, $J = 8.1$ Hz, 1H), 7.12 (dd, $J = 2.1$, 8.1 Hz, 1H), 7.27 (s, 1H), 7.41 (d, $J = 2.1$ Hz, 1H), 7.52 (s, 1H), 8.20 (d, $J = 6.6$ Hz, 1H), 8.28 (d, $J = 6.6$ Hz, 1H). EI-MS *m/e* 298 $(M+H)^+$. Anal. ($C_{16}H_{11}NO_5$) C, H, N.

IGFBP Radioligand-Binding Assay. Human IGFBP-1, BP-2, BP-4, BP-5, and BP-6 cDNA's were used to express the five binding proteins in the BaculoGold Expression System (PharMingen, San Diego, CA) in Sf9 insect cells according to the manufacturer's instruction, and the proteins were purified by affinity chromatography on a hIGF-II-coupled Affi-Gel 15 column, followed by reversed-phase HPLC as described.[4b] IGFBP-3 was isolated from out-dated human plasma as reported previously.[4b] The radioligand-binding assay was performed at room temperature in duplicate in 0.02% NP-40/PBS buffer, pH 7.2. Briefly, for the human IGFBP-3 binding assay, the reaction was started by the addition of 100 μL of buffer, 100 μL of hIGF-I (Sigma, St. Louis, MO) in buffer solution, followed by 100 μL of [^{125}I]hIGF-I (30,000 cpm, specific activity ~2,000 Ci/mmol; New England Nuclear, Boston, MA) to a 12 × 75-mm glass test tube. Then 200 μL of a 2.5 nM hIGFBP-3 in buffer solution (0.5 pmol) was added, and the mixture was incubated for 2 h. After incubation, 100 μL of ice-cold 20% BSA and 500 μL of ice-cold 20% PEG-8000 in PBS buffer were added and the mixture was vortexed and then centrifuged for 30 min at 3000 rpm. The supernatant was carefully removed by suction, and the pellet was counted on a γ-counter. One-half pmol of hIGFBP-3 bound ~24% of [^{125}I]-hIGF-I, and the nonspecific binding was ~2%. Binding assays for the other five human IGFBPs were set up in a similar manner. The data obtained were used to calculate the K_i values by the Prism Software (GraphPad Software, Inc., San Diego, CA).

High-Throughput Screening. To identify molecules that could displace IGF from its binding protein, an in-house library of ~85,000 compounds was screened by an adaptation of the hIGFBP-3 radioligand-binding assay on a Biomek 2000 Laboratory Automation Workstation (Beckman-Coulter, Fullerton, CA). Compounds predissolved in dimethyl sulfoxide were screened at a final concentration of 10 μM in a 96-well format, and the criterion for a positive hit was set at >50% inhibition of [^{125}I]hIGF-I binding to hIGFBP-3 at this concentration.

Fibroblast Proliferation Assay. Balb/c 3T3 fibroblast cells (ATCC, Rockville, MD) were cultured in DMEM medium (Fisher Scientific, Springfield, NJ). The cells grown up in the culture flask were trypsinized and diluted to 50,000 cells/mL in 10% FBS (HyClone, Logan, UT) in DMEM. The cells were aliquoted to 96-well tissue culture plates (Corning, Corning, NY) at 180 μL/well. After 48 h of incubation at 37 °C and 5% CO_2, the plates were washed twice with serum-free DMEM and incubated with 180 μL of serum-free DMEM for an additional 2 h. To each well, 20 μL of sample was then added, and the cells were incubated for 4 h. (Compounds **1** and **2** were predissolved in dimethyl sulfoxide at 10 mM concentration and diluted 1:10 in serum-free DMEM before being added to the cells.) Then 1 μCi [^{3}H]thymidine (New England Nuclear) was added to each well and the plates were incubated for another 20 h. Following the incubation, the cells in each well were transferred to a 96-well GF/B filter plate (Packard, Meriden, CT) and dried at 65 °C for 1 h. Scintillation fluid (50 μL/well) was added, and the plates were counted on a microplate scintillation counter (Packard Instruments, Meriden, CT).

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