Parallel Synthesis of Indolylquinones and Their Cell-Based Insulin Mimicry

Michael C. Pirrung,*[,†,‡] Zhitao Li,[‡] Erika Hensley,[‡] Yufa Liu,[‡] Aparna Tanksale,[†,‡] Bo Lin,[§] Ashok Pai,[§] and Nicholas J. G. Webster[§]

Department of Chemistry, University of California, Riverside, California 92521-0403, Department of Chemistry, Duke University, Durham, North Carolina 27708-0317, and Veterans Medical Research Foundation and the VASDHS, San Diego, California 92161

Received April 23, 2007

A synthetic route to bis-indolyldihydroxybenzoquinones was adapted for parallel organic synthesis. The route involves selective conjugate addition of an indole to dichlorobenzoquinone promoted by Brønsted acid, followed by a Lewis acid-promoted conjugate addition of a second indole and a final hydrolysis. Methods for high-throughput purification of the products of this synthesis were also developed. Using these methods, we prepared a library whose structures are based on asterriquinone natural products, which have a wide range of biological activities. In this report, the activities of the library members in activation of the insulin receptor on mammalian cells were examined. Novel compounds were discovered that fall outside earlier developed structure−activity relationships for insulin mimics, supporting the value of systematic investigation (inspired by Nature) for the discovery of novel biologically active molecules.

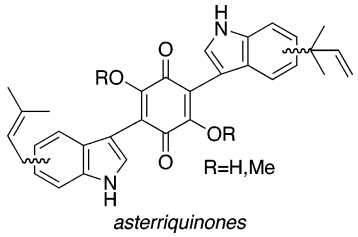
Introduction

A class of tryptophan-derived[1] fungal natural products, the asterriquinones, has been known for over two decades. Their structures consist of a central quinone ring bearing hydroxy or methoxy groups and two flanking indoles bearing prenyl or reverse prenyl groups (Chart 1). Reduced analogs of this bis-indolylquinone structure are also known. The biological activities that have been ascribed to members of this family are broad, including antitumor/cytotoxic activity in cell-based assays.[2] A possible mechanism for this action is inhibition of the interaction between phosphorylated tyrosine kinases, such as the epidermal growth factor receptor, and the SH2 domains of their adapter proteins, such as Grb2.[3] Activities of asterriquinones against HIV protease[4] and HIV reverse transcriptase[5] have been disclosed, as has activity against serine proteases in the clotting cascade.[6]

Our interest in the asterriquinones arose from the activation of the human insulin receptor by demethylasterriquinone B1 (DAQ B1; Chart 2) and its consequent oral insulin mimetic activity in mice.[7] DAQ B1 was also subsequently shown to activate the TrkA nerve growth factor receptor.[8] An analog of DAQ B1 that is slightly more selective for the insulin receptor has been reported, and its study has included a significant amount of pharmacology in animals.[9] Once daily oral dosing of *db/db* mice (5 mg/kg) causes a dose-dependent decrease in glucose, and pharmacokinetics in rats, dogs, and primates show high systemic availability, long serum half-life, and no overt toxicity. Through systematic synthesis and evaluation of methylated DAQ B1 derivatives, we have identified an essential pharmacophore of DAQ B1 as represented in the indolylquinone analog ZL-196,[10] which has oral activity in transiently lowering glucose in lean, nondiabetic mice and persistently lowering glucose in *ob/ob* diabetic mice.[11] We have also shown that synthetic indolylquinone relatives of the asterriquinones and ZL-196 inhibit the Cdc25B phosphatase.[12]

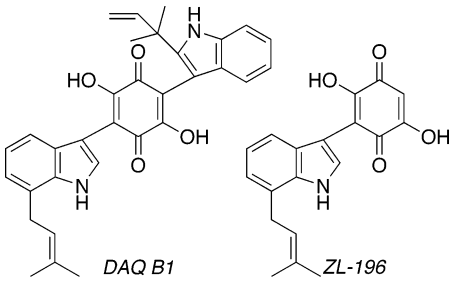
The ability of diverse members of the asterriquinone family to interact with a wide range of growth factor receptors and phosphatases, as well as with other enzymes, suggests that they might be considered privileged structures. They seem particularly relevant to tyrosine kinase/protein phosphatase relationships. Access to a wider variety of asterriquinones

Chart 1



asterriquinones

Chart 2

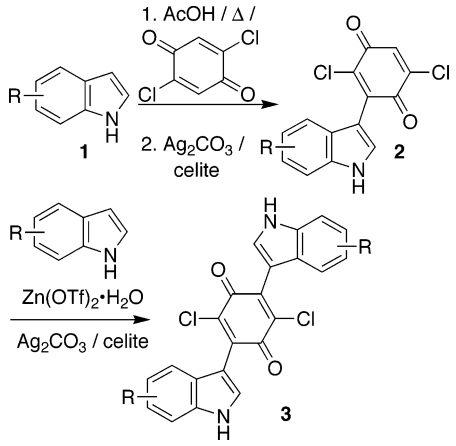


DAQ B1 *ZL-196*

* To whom correspondence should be addressed. E-mail: michael.pirrung@ucr.edu.
† University of California.
‡ Duke University.
§ Veterans Medical Research Foundation and the VASDHS.

10.1021/cc070062m CCC: $37.00
Published on Web 06/27/2007

Scheme 1



for biological evaluation against diverse targets is therefore desirable. Specifically, examination of a large collection of indolylquinones whose structures are unbiased by known active structures would be the best way to discover novel structural types, broadening the structural definition of the "privilege", and to define structural limits on biological activity within the insulin mimics. One of the most attractive features of the asterriquinones as potential privileged structures is their inherent modularity, which both calls for and facilitates combinatorial approaches to the synthesis of large molecular libraries based on their indolylquinone structures. Synthetic methods developed in our laboratory over the past several years have facilitated the modular synthesis of a wide variety of asterriquinone derivatives.[10,13] Two processes we developed can be used to assemble an asterriquinone library. The Brønsted acid-catalyzed condensation of indoles **1** with dichlorobenzoquinone gives, after oxidation, the indolylquinones **2** with only very minor amounts of addition of a second indole to **2**. This second process requires Lewis acid catalysis and provides the targets **3** (Scheme 1). Noteworthy in both of these reactions is the ability to form carbon−carbon bonds without using air- or water-sensitive reagents or catalysts, facilitating the preparation of molecular libraries. Both reactions also require only underivatized indoles as reactants, unlike some of our total syntheses of DAQ B1[13b] that would have been much more difficult to apply in combinatorial synthesis.

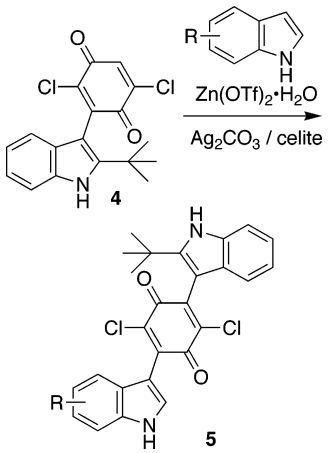
Results

Rehearsal. While we were very familiar with the foregoing reactions necessary to assemble asterriquinone libraries, we needed experience applying them in parallel. On an Argonaut Quest 210 synthesizer that accommodates 20 reaction vessels, 20 indoles that had been examined[12a,c] earlier were subjected to simultaneous acetic acid-catalyzed condensation with dichlorobenzoquinone. The results are summarized in Table 1. The yields are 69−98%; the products are obtained in high purity, and good quantities of material are obtained (63−100 mg). For the Lewis acid-promoted addition of the same twenty indoles to an indolylquinone, a reactant (**4**, ≡**2**{*6*}) that was expected to perform well because of its bulky indole 2-substituent was chosen (Scheme 2). This indolylquinone, each indole, zinc triflate hydrate, and silver carbonate on celite were heated at reflux in THF for 24 h. The results are summarized in Table 2. The yields are 24−86%; the products are obtained in high purity, and reasonable quantities of material are obtained (24−91 mg). A noteworthy aspect of the second set of reactions was that these indoles were not prequalified for success in the Lewis acid-promoted addition.

Table 1. Results of the Simultaneous Acetic Acid-Catalyzed Condensation With Dichlorobenzoquinone of 20 Indoles

	indole	yield (%)	HPLC purity (%)
2{*1*}	indole	86	95
2{*2*}	*N*-methylindole	90	95
2{*3*}	2-methylindole	93	98
2{*4*}	2-cyclopropylindole	78	95
2{*5*}	2-isopropylindole	59	96
2{*6*}	2-*tert*-butylindole	91	95
2{*7*}	2-phenylindole	98	97
2{*8*}	4-methoxyindole	71	91
2{*9*}	4-benzyloxyindole	79	94
2{*10*}	5-fluoroindole	81	92
2{*11*}	5-methoxyindole	72	95
2{*12*}	5-benzyloxyindole	69	94
2{*13*}	5-methylindole	69	94
2{*14*}	6-fluoroindole	77	98
2{*15*}	6-methylindole	76	94
2{*16*}	7-methylindole	90	93
2{*17*}	7-tert-butylindole	89	94
2{*18*}	2,5-dimethylindole	86	98
2{*19*}	2-methyl-5-methoxyindole	82	97
2{*20*}	2-methyl-5-chloroindole	84	97

Scheme 2



For expediency, these reaction mixtures were purified by silica gel chromatography, which would not be practical in the synthesis of a large library. In solution-phase combinatorial synthesis, workup is often the most difficult step. Since the main impurities in these reaction mixtures are the starting materials (indoles and indolylquinones), which are much less polar than the product, it is possible to purify the product by filtration through a plug of silica gel. A 10 mL Quest reaction vessel packed with silica gel to half of its capacity is used as the column. A small amount of silica gel loaded with the crude product (by evaporation) is added to the top of this column, and it is washed successively with 20% ethyl acetate in hexanes, followed by 40% ethyl acetate in hexanes. The less polar eluent gives starting materials (indolylquinones) in the first band, and the more polar eluent gives the

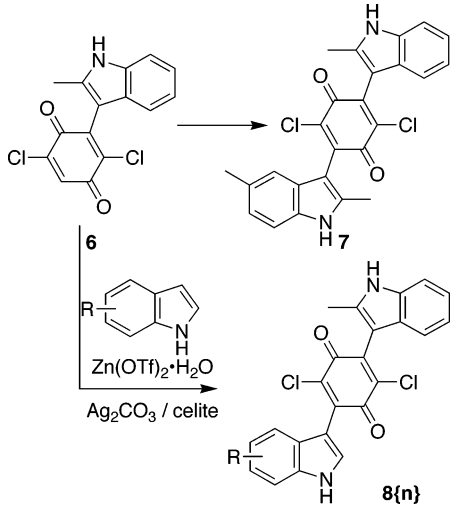
Table 2. Results of Indolylquinone, Each Indole, Zinc Triflate Hydrate, and Silver Carbonate on Celite Heated at Reflux in THF

	indole	yield (%)	HPLC purity (%)
5{*1*}	indole	60	99
5{*2*}	*N*-methylindole	86	99
5{*3*}	2-methylindole	74	99
5{*4*}	2-cyclopropylindole	58	99
5{*5*}	2-isopropylindole	52	99
5{*6*}	2-*tert*-butylindole	79	99
5{*7*}	2-phenylindole	84	93
5{*8*}	4-methoxyindole	24	99
5{*9*}	4-benzyloxyindole	34	99
5{*10*}	5-fluoroindole	53	99
5{*11*}	5-methoxyindole	58	98
5{*12*}	5-benzyloxyindole	60	99
5{*13*}	5-methylindole	67	99
5{*14*}	6-fluoroindole	45	99
5{*15*}	6-methylindole	25	99
5{*16*}	7-methylindole	43	98
5{*17*}	7-tert-butylindole	80	98
5{*18*}	2,5-dimethylindole	84	95
5{*19*}	2-methyl-5-methoxyindole	68	98
5{*20*}	2-methyl-5-chloroindole	76	97

Table 3. Results of a Full Run of 20 Indoles Reacting with Indolylquinone **6**

	indole	yield (%)	HPLC purity (% quinone/ %hydroquinone)
8{*1*}	indole	89	50/29
8{*2*}	*N*-methylindole	88	73/12
8{*3*}	2-methylindole	89	85
8{*4*}	2-phenylindole	86	91
8{*5*}	4-methoxyindole	0	
8{*6*}	4-benzyloxyindole	76	63
8{*7*}	5-fluoroindole	74	84
8{*8*}	5-methoxyindole	81	86
8{*9*}	2-methyl-5-chloroindole	100	81
8{*10*}	2-methyl-5-methoxyindole	67	91/9
8{*11*}	5-benzyloxyindole	76	77/10
8{*12*}	5-methylindole	82	78/17
8{*13*}	7-chloroindole	19	40/45
8{*14*}	5-iodolindole	49	88
8{*15*}	5-hydroxyindole	0	
8{*16*}	5-chloroindole	51	90
8{*17*}	6-chloroindole	63	84
8{*18*}	5,6-methylenedioxyindole	44	73
8{*19*}	6-fluoroindole	61	68
8{*20*}	2-isopropylindole	86	83

Scheme 3



product in the second band. Because of the different colors of the starting material and product bands, they are easily recognized on the column. Evaporation gives the product, often in solid form.

A model reaction of indolylquinone **6** (≡**2**{*3*}) with 2,5-dimethylindole to give bisindolylquinone **7** was used to test this purification procedure (Scheme 3). Additionally, this reaction examined the scope of the Lewis acid-promoted coupling reaction between indoles and indolylquinones. We expected nucleophilic addition of indoles to dichloroindolylquinones to be more difficult than that to dichlorobenzoquinone because of the electron-donating properties of the indole. For **4**, the bulky group at the 2-position of the indole tends to prevent the indole and the quinone from becoming coplanar, minimizing this electronic effect and presumably enhancing the reactivity of the quinone. Success of reactions with indolylquinone **6**, with only a relatively small methyl group at the 2-position of the indole, would encourage use of a wider range of 2-substituted and even 2-unsubstituted indoles. The reaction in Scheme 3 was performed overnight on the Quest synthesizer in a 5 mL reaction vessel equipped with a frit. TLC showed that the indolylquinone was completely consumed, and a new spot appeared with a lower R_f value. The reaction mixture was filtered through the frit and purified by the high-throughput method described above to give the desired product **7** in 92% yield.

These small silica gel columns could be installed on the Quest synthesizer, and elution could be driven by its high-pressure nitrogen gas, much like a flash chromatography column. In this way twenty reactions could be worked up simultaneously, a purification procedure much more efficient than traditional methods.

A full run of twenty indoles reacting with indolylquinone **6** was examined under the conditions planned for parallel synthesis. The results given in Table 3 indicate that indoles with electron-donating groups (compounds *2*, *3*, *4*, *8*, *9*, *10*, *11*, *12*, and *20*) give the best yields (>70%) in the coupling reaction, while indoles bearing only halogens (compounds *7*, *13*, *14*, *16*, *17*, and *19*) give lower yields (19−74%). Two 4-substituted indoles, 4-benzyloxy indole (compound *6*) and 4-methoxyindole (compound *5*), gave quite different results, yields of 76% and no product, respectively. 5-Hydroxyindole (compound *15*) also gave no product. The products were subjected to LC-MS to determine the purity and establish identity. The purity of most products is good (above 80%), while that of some is poor. Six products (compounds *1*, *2*, *10*, *11*, *12*, and *13*) showed the corresponding hydroquinone as a major impurity, as identified by mass spectrometry. Because the polarities of the hydroquinones are very similar to the desired benzoquinone products, it was impossible to separate them from intended products on such short columns. Hydroquinones, as intermediates in the coupling reaction, were expected to be oxidized by silver carbonate on celite. A solution to this problem is provided in a later section.

To test the reactivity of different indolylquinones in the coupling reaction, twenty different indolylquinones **9** were chosen to react with indole on the Quest synthesizer (Scheme

Scheme 4

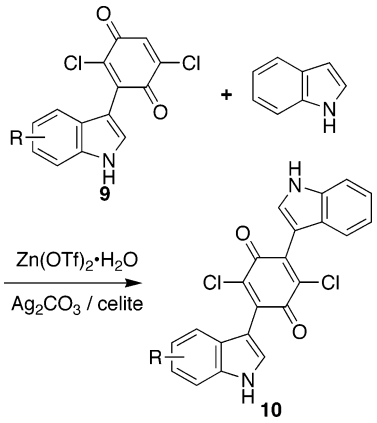


Table 4. Reactivity of Different Indolylquinones in the Coupling Reaction

	substituted indolylquinone from	yield (%)
10{*1*}	indole	45
10{*2*}	*N*-methylindole	91
10{*3*}	6-fluoroindole	36
10{*4*}	2-phenylindole	82
10{*5*}	4-methoxyindole	64
10{*6*}	5-fluoroindole	70
10{*7*}	2-cyclopropylindole	80
10{*8*}	5-methoxyindole	75
10{*9*}	2-methyl-5-chloroindole	100
10{*10*}	2-methyl-5-methoxyindole	80
10{*11*}	2-ethylindole	70
10{*12*}	5-methylindole	80
10{*13*}	7-methylindole	60
10{*14*}	6-methylindole	73
10{*15*}	2-(1-methyl)cyclopropylindole	32
10{*16*}	4-fluoroindole	100
10{*17*}	6-chloroindole	49
10{*18*}	2,5-dimethylindole	48
10{*19*}	7-*tert*-butylindole	70
10{*20*}	2-isopropylindole	86

4). The reactions were worked up using the high-throughput procedure. The results are shown in Table 4. Most reactions gave an acceptable yield (36−100%). Even though different indolylquinones gave different yields, no obvious relationship between the structure of indolylquinone and the reaction yield could be established at this stage.

As described in Scheme 1, the combinatorial synthesis of bis-indolylquinones can be conducted through variation of the indoles. One indole will react with dichlorobenzoquinone via a Brønsted acid-catalyzed reaction in the first step to give an indolylquinone, which will be used as one building block in the combinatorial synthesis. The other indole building block will react with the indolylquinone via a Lewis acid-promoted reaction in the second step. There are therefore two possible routes to prepare each bis-indolylquinone target, one in which each indole enters in the first step and one in which it enters in the second step. On the basis of our experience with the reactions in question and our preliminary studies of the reactivity of indoles with indolylquinones, the following guidelines were developed to decide which indole should be introduced first. (1) Indoles with electron-donating groups, which are expected to give good yields in the Lewis acid-promoted coupling reactions, should be introduced in the second step. (2) Indoles with poor reactivity in this reaction, such as those with electron-withdrawing groups or 4-substituents, should be introduced in the first step via the Brønsted acid-catalyzed reaction with dichlorobenzoquinone, because it is more tolerant of substitution.

Scheme 5

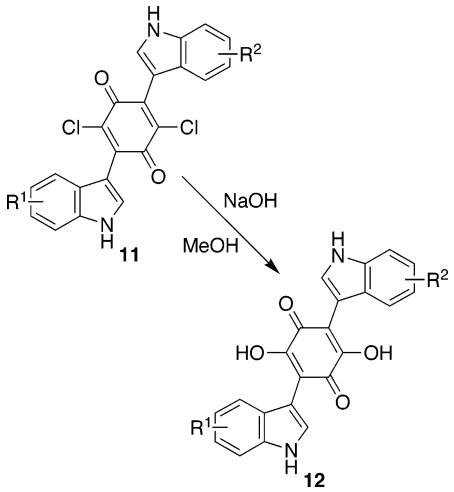


Table 5. Results of Typical Hydrolysis Reactions

	indole	yield (%)	HPLC purity (%)	$(M + H)^+$ calcd/obsd
12{*2,1*}	5-benzyloxylindole	95	82	553/553
12{*2,2*}	5-methylindole	50	65	461/461
12{*2,3*}	*N*-methylindole	63	73	461/461
12{*2,4*}	6-chloroindole	95	78	480/480
12{*2,5*}	4-methoxyindole	95	92	476/476
12{*2,6*}	4-benzyloxyindole	80	88	553/553
12{*2,7*}	2-isopropylindole	95	96	489/489
12{*2,8*}	5-methoxyindole	0		
12{*2,9*}	2-methyl-5-chloroindole	86	93	495/495
12{*2,10*}	2-methyl-5-methoxyindole	95	84	491/491

For this library, we aimed only to hydrolyze the dichlorobenzoquinones **11** to the dihydroxybenzoquinones **12** (Scheme 5). However, additional diversity could certainly be incorporated into the library through variation in the nucleophile used in reaction with the dichloride. For conventional synthesis of dihydroxybenzoquinones, hydrolysis is conducted under basic conditions, and the product is purified by chromatography on oxalic acid precoated silica gel. A parallel hydrolysis protocol was tested with ten dichlorobenzoquinones **11**{*2, n*}, which all include 2-phenylindole. NaOH solution was added to refluxing solutions of dichlorobenzoquinones **11** in methanol in Quest reaction vessels; the mixtures were refluxed for 30 min, and the reactions were cooled to room temperature. To avoid chromatography, we first tested purification by crystallization on the synthesizer to give dihydroxybenzoquinones **12**{*2, n*}. After acidification with sulfuric acid, a precipitate formed that was retained in the reaction vessel after filtration. The crude product was then dissolved in hot toluene. Hexane was added and the mixture was cooled to room temperature. After filtration, the purified product was retained in the reaction vessel. The final product was collected by dissolution in ethyl acetate. Table 5 shows the results of typical hydrolysis reactions. Most gave good yields and high purities of products. LC/MS mostly gave the expected molecular ions.

Table 6. Thirty-five Indoles **13**{*n*} Used in the Lewis Acid-Promoted Coupling Step

	indole
13{*1*}	5-benzyloxylindole
13{*2*}	5-methylindole
13{*3*}	*N*-methylindole
13{*4*}	6-chloroindole
13{*5*}	4-methoxyindole
13{*6*}	4-benzyloxyindole
13{*7*}	2-isopropylindole
13{*8*}	5-methoxyindole
13{*9*}	2-methyl-5-chloroindole
13{*10*}	2-methyl-5-methoxyindole
13{*11*}	5-chloroindole
13{*12*}	2-cyclopropylindole
13{*13*}	5-iodoindole
13{*14*}	2-ethylindole
13{*15*}	2-propylindole
13{*16*}	2-phenylindole
13{*17*}	2-methylindole
13{*18*}	indole
13{*19*}	5-fluoroindole
13{*20*}	7-chloroindole
13{*21*}	5,6-methylenedioxyindole
13{*22*}	6-fluoroindole
13{*23*}	2-*tert*-butylindole
13{*24*}	2-(3′,5′-dimethoxyphenyl)indole
13{*25*}	7-methylindole
13{*26*}	7-*tert*-butylindole
13{*27*}	2,5-dimethylindole
13{*29*}	5-bromoindole
13{*29*}	2-pentylindole
13{*30*}	7-ethylindole
13{*31*}	7-methoxyindole
13{*32*}	2-isobutylindole
13{*33*}	2-methyl-7-bromoindole
13{*34*}	2-phenyl-1-ethylindole
13{*35*}	2-(*p*-chlorophenyl)indole

Solid-phase extraction (SPE) using ion-exchange resins has been used for the purification of benzoic acids.[14] Since dihydroxyquinones have a pK_a (4−5) similar to that of benzoic acid, this method was also tested. Dowex 1 × 8−400 resin was successively treated with NaOH and HCO_2H to convert it to the formate form. The crude product (following sulfuric acid treatment) was dissolved in methanol and treated with triethylamine; then ion-exchange resin was added, followed by agitation for 30 min. The resin, which was loaded with product, was then washed with DMF, THF, methanol, and dichloromethane before being treated with formic acid/dichloromethane solution to release the product. The product obtained from this method showed purity similar to that of the crystallization method.

Building Block Selection and Preparation. Our aim was to prepare an unbiased library of bis-indolylquinones that would be generally useful for discovering biological activity against a wide range of targets and discovering new structural types with activity against the insulin receptor. Therefore, the basis of selection of building blocks for this library was primarily the commercial availability of indoles consistent with the structural requirements of the coupling reactions. A total of 35 indoles **13**{*n*} (Table 6) and 21 indolylquinones **9**{*n*} (Table 7) were used in the Lewis acid-promoted coupling step. These building blocks can create a library of 535 possible bis-indolyldichloroquinones. This numerical diversity is not simply the product of the number of building blocks in each set because multiple combinations of building blocks can give the same bis-indolyldichloroquinone. Of the indoles, 26 were commercially available, and nine were prepared in our lab. Of the indolylquinones, 17 were known compounds from our earlier work on the Brønsted acid-promoted coupling reaction,[13a,c] while the other four were new compounds prepared using that reaction.

Table 7. Twenty-one Indolylquinones **9**{*n*} Used in the Lewis Acid-Promoted Coupling Step

	dichloroindolylquinone derived from this indole
9{*1*}	2-methylindole
9{*2*}	2-phenylindole
9{*3*}	2-isopropylindole
9{*4*}	2-*tert*-butylindole
9{*5*}	5-bromoindole
9{*6*}	1-methylindole
9{*7*}	2-*n*-propylindole
9{*8*}	5-iodoindole
9{*9*}	7-methylindole
9{*10*}	5-chloroindole
9{*11*}	7-chloroindole
9{*12*}	6-chloroindole
9{*13*}	4-methoxyindole
9{*14*}	4-benzyloxyindole
9{*15*}	7-ethylindole
9{*16*}	5-methylindole
9{*17*}	5-methoxyindole
9{*18*}	6-fluoroindole
9{*19*}	7-methoxyindole
9{*20*}	4-methyl-5-methoxyindole
9{*21*}	5-fluoroindole

The preparation of the two building block sets was performed conventionally, and all compounds were purified by chromatography. The indolylquinones were made from the corresponding indoles and dichlorobenzoquinone using the acid-promoted coupling reaction. The acetic acid conditions (Method A) or the sulfuric acid/THF conditions (Method S) were used, depending on the nature of the indole. The yields are given in Table 8. The indoles not commercially available were prepared by several different traditional routes summarized in the Supporting Information.

Library Production. In the performance of twenty reactions simultaneously, there are at least two ways to simplify reaction setup: use one indolylquinone to react with twenty different indoles or use one indole to react with twenty different indolylquinones. While they result in the same overall library, they are quite different in their execution, and each has advantages and disadvantages. To use one indolylquinone in reactions with diverse indoles has several advantages. First, the reaction is easier to monitor. The products, the bis-indolylquinones, and one of the starting materials, the indolylquinones, of the coupling reaction have different colors and R_f values, which makes it quite convenient to monitor the reaction via TLC. The other starting materials, the indoles, are much less polar than the indolylquinones and bisindolylquinones and do not interfere with them on TLC. Figure 1 shows a typical TLC plate after one run of ten reactions. For most of the reactions, there are two spots on TLC, the more mobile ones being the indolylquinone, while the less mobile spots are the products. Reactions 3 and 6 show only one spot on TLC. Because the

Table 8. Yields of Indolylquinones Made from the Corresponding Indoles and Dichlorobenzoquinone Using the Acid-Promoted Coupling Reaction

	indole	yield (%)	method
9{*1*}	2-methylindole	84	S
9{*2*}	2-phenylindole	92	S
9{*3*}	2-isopropylindole	88	S
9{*4*}	2-*tert*-butylindole	77	A
9{*5*}	5-bromoindole	64	S
9{*6*}	*N*-methylindole	92	S
9{*7*}	2-propylindole	44	S
9{*8*}	5-iodoindole	59	A
9{*9*}	7-methylindole	84	S
9{*10*}	5-chloroindole	69	A
9{*11*}	7-chloroindole	58	A
9{*12*}	5-methylindole	72	S
9{*13*}	6-chloroindole	66	A
9{*14*}	4-methoxyindole	63	A
9{*15*}	4-benzyloxyindole	79	S
9{*16*}	5-methylindole	84	S
9{*17*}	5-methoxyindole	82	S
9{*18*}	6-fluoroindole	61	A
9{*19*}	7-methoxyindole	72	S
9{*20*}	4-methyl-5-methoxyindole	85	A
9{*21*}	5-fluoroindole	71	A

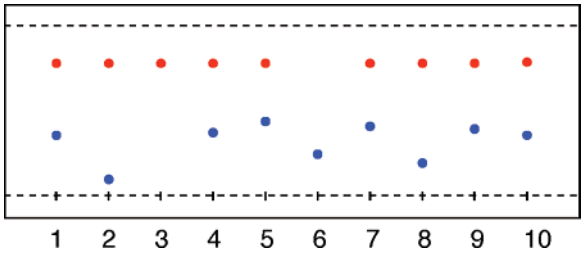


Figure 1. Example thin-layer chromatogram for the reaction of one indolylquinone with ten different indoles.

same indolylquinone was used in all reactions, it is easy to see by comparison with other reactions that the only spot in reaction 3 is starting material and no product is present, while the only spot in reaction 6 is the product and no starting material remains.

Second, for reasons identical to the considerations in TLC analysis, the reaction is easier to work up when using one indolylquinone, particularly in collection and recovery. After silica gel plug elution, all indolylquinone fractions can be combined and repurified, enabling it to be recycled. This is especially important when the indolylquinone is precious. The disadvantage of this method is a very practical one. Since these reactions are run on a fairly small scale (0.05−0.1 mmol), only about 30 mg of indolylquinone and less than 20 mg of indole are needed for each reaction. While all of the indolylquinones used here are crystalline and easy to handle, many indoles are liquids or oils or even sticky solids. Given the number of reactions to be run, it is inconvenient to measure and transfer such small amounts of oils. This might be the main disadvantage of this method, though it is significant.

The other method is to allow one indole to react with twenty different indolylquinones, as was done in testing the reactivity of different indolylquinones (Table 4), which overcomes the disadvantage of the previous method. Since all the indolylquinones are crystalline, 0.1 mmol of each can

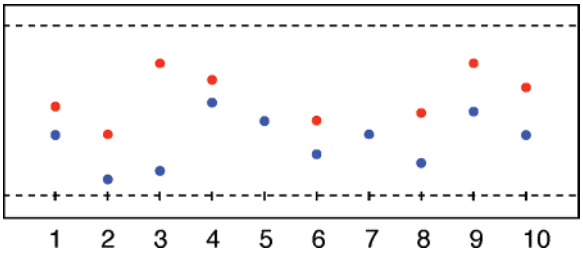


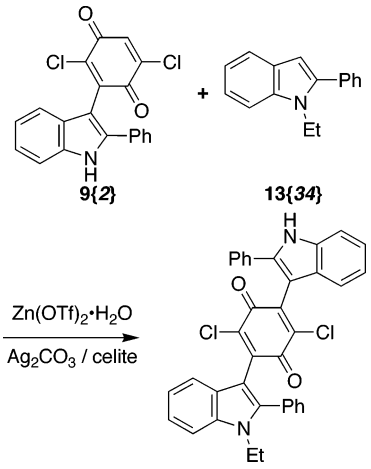
Figure 2. Example thin-layer chromatogram for the reaction of one indole with ten different indolylquinones.

be weighed easily. The total amount of indole needed for twenty reactions (2 mmol) can also be weighed conveniently, and the stock can be used immediately. Another advantage is the possibility of recovering the excess indoles used in the reaction when they are valuable. The disadvantage of this method is the inconvenience in monitoring and workup. As shown in Figure 2, indolylquinone starting materials often have different R_f values. When there are two spots, the more mobile spot is generally the starting material, but if there is only one spot, as in reactions 5 and 7, it is difficult to tell if the spot is unreacted starting material or product (unless compared with authentic samples). Purification of the products by silica gel plug elution will also be more difficult than when one indolylquinone is used with twenty indoles, where the single starting material can be eluted from all columns by the same solvent, while the products will remain on the column to be eluted with more polar solvents. As is evident from Figure 2, when one indole is used with twenty indolylquinones, each reaction may require a different step gradient, and each elution may require individual monitoring. Another disadvantage is the difficulty in recovering the indolylquinones compared to the first method. Generally, because the indolylquinones must all be prepared, while some of the indoles are commercial, it is more important to recover the former.

After evaluation of both methods in model experiments, the first was chosen considering its great convenience in the workup process. To overcome its major shortcoming, inconvenience in weighing the indoles, all indoles were made up as 0.1 M stock solutions in THF. One indolylquinone was used to react with ten different indoles, with each indole added as its THF solution. This method worked well initially, but we soon found that some indoles are not stable in stock solutions. They decompose upon storage, introducing impurities into the coupling reactions and reducing the yields. We were therefore forced to develop a compromise between the two methods involving the use of four different indolylquinones to react with five different indoles (each used four times). The amount of each indole required is 0.4 mmol, which can be weighed easily and accurately into a stock that is used immediately.

Several different reaction protocols were used for the Lewis-acid promoted coupling of indolylquinones with indoles. The first, protocol A, was the following: all reactants, including indolylquinone, indole, zinc triflate, silver carbonate on celite, and solvent (THF), were mixed in 10 mL Quest reaction vessels and stirred at 60 °C for 20 h. The mixture was filtered through the frit of the reaction vessel and collected into vials. A small amount of silica gel was

Scheme 6



added to the solution, and the solvent was evaporated. The initial use of a rotary evaporator proved impractical and was replaced by a centrifugal concentrator. The capacity of the concentrator used is 50 vials (20 mL, 28 mm O.D. × 60 mm height) or 2.5 times the capacity of the Quest synthesizer. Evaporation of 50 vials with the centrifugal concentrator was conducted at a temperature and pressure to require about 20 h. This method is appealing because it is automatic. After the solvent was evaporated, the dried silica gel loaded with crude product was loaded onto a silica gel plug for the earlier-described elution. However, another problem was later discovered in this protocol. As described earlier, side products sometimes include hydroquinones from incomplete oxidation of the conjugate addition product. These were normally only around 10% of the reaction mixture. When the centrifugal concentrator was used, however, the amount of hydroquinone sometimes rose.

The initial observation of this phenomenon was made with the reactants in Scheme 6, and this reaction was used in trouble-shooting this problem. With protocol A, the reaction is very slow as monitored by TLC; after 24 h, significant amounts of indolylquinone **9**{*2*} remain, together with some desired product, but no hydroquinone was observed. After workup and purification by silica gel plug elution, no starting material was recovered, while 80% of the product was the hydroquinone. One possible explanation for this observation is that the coupling reaction was completed during the evaporation process, and since there was no oxidant in the solution (because it had been removed by filtration), the hydroquinone formed from the coupling reaction could not be oxidized. To test this hypothesis, 0.1 mmol of indolylquinone **9**{*2*}, 0.2 mmol of indole **13**{*34*}, 0.2 mmol of zinc triflate hydrate, and 0.2 mmol of silver carbonate on celite, together with a small amount of silica gel, were mixed in a vial in 2 mL of THF. The vial was then put into the centrifugal concentrator to be evaporated. After 20 h, the reaction mixture was checked by TLC, which showed complete consumption of the indolylquinone and formation of the desired product. The dried silica gel was subjected to the plug elution protocol to give the desired product in almost quantitative yield. LC/MS indicated high purity of the desired bis-indolylquinone product, and no hydroquinone was observed.

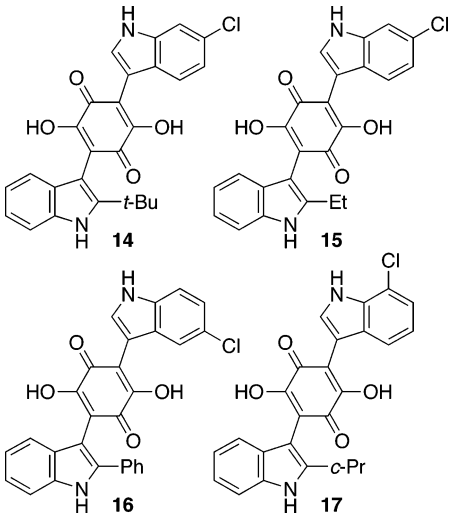
This result suggested a revised set of coupling reaction conditions, protocol B, in which silica gel may play a role as a cocatalyst with zinc triflate. Another positive influence on the reaction might be the much higher concentration that is created during the evaporation. This method also has other advantages: it is more economical and convenient; the vials used for these reactions are much cheaper than the Teflon reaction vessels originally used; the centrifugal concentrator has a larger capacity (50 vials) than the Quest synthesizer (20 reaction vessels) and is easier to maintain; and the procedure is simpler, because the crude product is ready for purification immediately following the reaction.

Many other reactions were conducted using protocol B, and the results are tabulated in Table 1 in the Supporting Information. For many coupling reactions, significant amounts of hydroquinone were still present in the product, even though excess silver carbonate on celite had been used. These results suggested that silver carbonate on celite might not be the best oxidant for this heterogeneous coupling protocol. Because the oxidant need not be removed from the reaction mixture, oxidants other than solid-phase reagents can be used. When silver acetate was used instead of silver carbonate on celite, better product purity was often obtained. The substitution of silver acetate for silver carbonate on celite as oxidant constituted protocol C. This protocol generally gives higher product purity than other protocols. In coupling reactions involving 2-arylindoles or haloindoles, Protocol C also gave better yields than other protocols. However, the yields with Protocol C were not always the best, especially in coupling reactions involving **9**{*13*}, where yields using protocol A were much superior. As summarized in the Supporting Information, all compounds in this library were characterized for purity and molecular weight by LCMS. Without direct evidence for the regiochemistry of the addition of the indole to the quinone, one might be concerned that some indoles could have added at the 2-position (or other sites). We have characterized many such reaction products by NMR, including many compounds described in rehearsal reactions in this paper that were also present in the main library. We have never observed products of addition at sites other than the indole 3-position.

The alkaline hydrolysis of the bis-indolyldichloroquinones **11** was conducted using the method described earlier. The crude products **12** were purified by the precipitation method, with the results listed in Table 2 in the Supporting Information. These hydrolysis reactions depend strongly on the nature of the indoles. Empirical observations have been made that are helpful for the prediction of results, though no absolute rules have been established. (1) A bis-indolyldichloroquinone including at least one 2-alkylindole is more likely to give good results. (2) A bis-indolyldichloroquinone including one 5-alkylindole or 5-alkoxyindole is more likely to give poor results. (3) A bis-indolyldichloroquinone including 5-fluoroindole or 6-fluoroindole is more likely to give poor results. (4) A bis-indolyldichloroquinone including two 2-unsubstituted indoles is more likely to give poor results.

Of the potential diversity available from 21 indolylquinones and 35 indoles, 424 bis-indolyldichloroquinones were prepared by solution-phase combinatorial synthesis. The

Chart 3



yields ranged from 6 to 100%, with an average yield of greater than 60%. The purities of the products ranged from 50 to 100%, with an average purity of greater than 80%. Over 70% were prepared in quantities of more than 10 mg and purities of >80%. These 305 compounds were selected for hydrolysis, and 269 (88%) yielded the desired product. The hydrolysis yields ranged from 14 to 100%, with an average yield of greater than 50%. The purities ranged from 29 to 96%, with an average purity of greater than 70%.

Library Screening. Stock solutions (10 mM in DMSO) were prepared of each of the 269 compounds that passed quality control as described above. They were evaluated for their ability to activate the human insulin receptor tyrosine kinase domain in an engineered CHO cell line. Receptor autophosphorylation was determined by SDS-PAGE and immunoblotting (direct immunofluorescence with an Odyssey imager or enzyme-mediated chemiluminescence and autoradiography) using methods described earlier.[10,11] Initial screening was performed at 10−30 μM, and 37 compounds showing some IR activation were rescreened at 1, 3, and 10 μM to estimate potency. The most potent of these compounds proved to be **14**, and three other actives are **15**−**17** (Chart 3). The potency of both DAQ B1 and ZL-196 in such cell-based assays are in the low micromolar regime, comparable to these new compounds.

The general cytotoxicity toward CHO cells of all members of this screening library was examined using a mitochondrial dye-based assay. Compounds most impacting cell viability were bis-indolyldihydroxybenzoquinones derived from indole **13**{*26*}. Cell viability data for compound treatments at 100 μM were subjected to QSAR analysis using the Tsar software package (Accelrys). Either neural net or multiple regression models accounted for ~50% of the variation in toxicity. Both models show the least impact on cell viability with an aryl group at the indole 2-position. To provide a data set for insulin receptor activation that could be readily compared to the cytotoxicity data set, a novel well-plate assay using CHO.IR cells was developed. Compound treatments were conducted at 30 μM and cell lysates were added to wells coated with an anti-phosphotyrosine antibody, capturing activated insulin receptors. An ELISA assay for immobilized insulin receptor then provided activation data, which were subjected to QSAR analysis. A neural net model (using 25 parameters/substituent position on the asterriquinone) accounted for ~50% of the variation in activity and gave a good fit to the data ($r = 0.69$). This model shows enhancement of activity with a bulky substituent at the indole 2-position, in concert with structures **14**−**17**. Importantly, comparison of the activity data for cytotoxicity and for insulin receptor activation demonstrates that there is *no* correlation between the two (Figure 3).

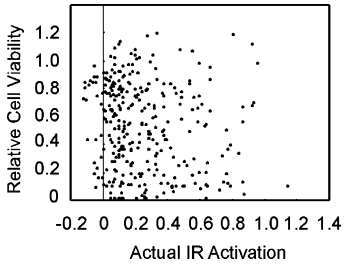


Figure 3. Scatter plot of the relationship between compound effects on cell viability (at 100 μM) and insulin receptor activation (at 30 μM). Data are normalized to no effect. Activity is relative to 100 ng/mL of insulin, 1.0 being 100% of the effect of insulin. The cell viability is relative to time 0, 1.0 being an equivalent absorption to the cells at time 0.

Discussion

The preparation of molecular libraries based on the bis-indolylquinone structure can be efficiently performed as demonstrated in this work. The overall strategy of using a Brønsted acid to introduce a first indole and a Lewis acid to introduce a second indole provides the intrinsic selectivity necessary to prepare unsymmetrical asterriquinone analogues. Their lack of symmetry increases the possible diversity of a library and, as evidenced in demethylasterriquinone B1 itself, may be important to biological activity. The only shortcoming of this strategy is that it cannot be executed to directly provide targets using dihydroxybenzoquinone itself. This quinone is not electrophilic enough to react with indoles in a conjugate addition reaction, requiring dichlorobenzoquinone to be used in its place. This in turn requires a subsequent hydrolysis reaction of the bis-indolyldichloroquinone to the bis-indolyldihydroxyquinone. While this transformation appears trivial, formally the hydrolysis of a vinylogous acid chloride to a vinylogous acid, in fact it turned out to be a major challenge of the synthesis. The effects of structure on the efficiency of this reaction suggest that bis-indolyldichloroquinones that prefer nonplanar biaryl conformations are better hydrolysis substrates. A speculation is that electron donation from the indoles impedes nucleophilic addition of hydroxide ion to the quinone. This consideration might also explain the deleterious effect of electron-releasing substituents at the indole 5-position.

The design of this library was not necessarily based on known structure−activity relationships for insulin mimicry. Rather, it was intended as a general purpose resource that could be used to examine a wide variety of targets in the tyrosine kinase/phosphatase families to identify key leads that could be further elaborated. It was also envisioned as a means to move outside known structural types among insulin

mimics. Hence, readily available indoles were used rather than the 7-alkyl substituted indoles that are most often present in effective insulin mimics.[11]

The activities of library members in mimicking insulin discovered in this study are surprising in light of the previously developed pharmacophore model embodied in ZL-196. Whereas past work suggested that 7-alkyl indole substituents are essential (and sufficient) for activity, here a chlorinated indole in tandem with another indole with a bulky 2-substituent allows insulin mimicry. The position of indole chlorination does not appear crucial, yet there is subtle interplay between the substituents on each indole. That is, when the 2-substituent is phenyl, the indole must be chlorinated at the 5-position, but when the 2-substituent is cyclopropyl, the indole must be chlorinated at the 7-position. This is known because the other combinations of indole chlorination and 2-substituents were present in this library. Without knowledge beforehand of where activity would be found, only these specific combinations emerged as actives.

An important outgrowth of this study is the demonstration that the cytotoxicity of asterriquinone analogues is not activity-dependent. Predictors of toxicity derived from the QSAR study are quite different from the predictors of insulin receptor activation, implying that it should be possible to develop compounds that separate the two properties.

The demonstrated activities of asterriquinones in modulating tyrosine kinase/phosphatase signaling pathways suggests that they might constitute a novel privileged structure. While the criteria and standards for privileged structures are somewhat vague and subjective, data on the interactions of a large number of compounds with a large number of biological targets are essential to the case. Library-based synthesis techniques such as those described in this work provide the former. The latter will typically arise not from the efforts of a single laboratory but from many laboratories. To identify and validate new privileged structures, conventions for expression of the biological activity of compounds in a wide range biological assays will be essential, as will incentives for investigators to contribute such activity data to databases like ChemBank.[15]

Experimental Section

All compounds **2**{*n*} in Table 1 are known,[13c] and the reaction products were analyzed in this work using the following HPLC conditions: 25 °C, flow rate = 1 mL/min, 10 μL injection, UV detection (273 nM), gradient elution A/water, B/acetonitrile, T = 0 min 100% A/0% B, T = 6 min 0% A/100% B, T = 8 min 0% A/100% B, T = 10 min 100% A/0% B.

Parallel Synthesis of Compounds 5{*n*}. Quest 210 reaction vessels (5 mL) were used. To each was added zinc triflate monohydrate (305 mg, 0.80 mmol), silver carbonate (on celite, 50 wt %, 221 mg, 0.40 mmol), compound **4** (70 mg, 0.20 mmol), an indole (0.40 mmol), and THF (3 mL). The reaction vessels were flushed with nitrogen and sealed. The reaction mixture was stirred at 64 °C for 2 days, cooled to room temperature, filtered, washed with ethyl acetate (8 mL), and purified by flash chromatography (ethyl acetate/hexane 1:5). The results are listed in Table 2, and the compounds are characterized below. HPLC conditions were as follows: 25 °C, flow rate = 1 mL/min, 10 μL injection, UV detection (268 nM), gradient elution A/water, B/acetonitrile, T = 0 min 60% A/40% B, T = 5 min 20% A/80% B, T = 16 min 20% A/80% B, T = 20 min 60% A/40% B.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(1*H*-indol-3-yl)-[1,4]benzoquinone (5{*1*}). ^{1}H NMR (acetone-d_6): δ 11.03 (1H, s), 10.42 (1H, s), 7.81−6.95 (9H, m), 1.43 (9H, s). MS: 462.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(1-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*2*}). ^{1}H NMR (acetone-d_6): δ 10.42 (1H, s), 7.70 (1H, s), 7.52−6.95 (8H, m), 3.96 (3H, s), 1.43 (9H, s). MS: 476.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(2-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*3*}). ^{1}H NMR (acetone-d_6): δ 10.64 (1H, s), 10.43 (1H, s), 7.41−6.95 (8H, m), 2.44 and 2.40 (3H, s), 1.45 and 1.43 (9H, s). MS: 476.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(2-cyclopropyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*4*}). ^{1}H NMR (acetone-d_6): δ 10.44 (1H, s), 10.39 and 10.31 (1H, s), 7.38−6.95 (8H, m), 3.89−3.50 (1H, m), 1.45−1.43 (9H, m), 1.29−0.84 (4H, m). MS: 502.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(2-*iso*propyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*5*}). ^{1}H NMR (acetone-d_6): δ 10.68 and 10.66 (1H, s), 10.44 (1H, s), 7.41−6.86 (8H, m), 3.20−2.97 (1H, m), 1.45−1.34 (15H, m). MS: 504

2,5-Di[(2-*tert*-butyl-1*H*-indol-3-yl)]-3,6-dichloro-[1,4]-benzoquinone (5{*6*}). ^{1}H NMR (acetone-d_6): δ 10.49 and 10.46 (1H, s), 10.25 (1H, s), 7.40−6.91 (8H, m), 1.48−1.42 (9H, s). MS: 518.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(2-phenyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*7*}). ^{1}H NMR (acetone-d_6): δ 11.14 and 10.82 (1H, s), 10.44 and 10.27 (1H, s), 7.78−6.96 (13H, m), 1.47−1.44 (9H, m). MS: 538.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(4-methoxy-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*8*}). ^{1}H NMR (acetone-d_6): δ 10.86 (1H, s), 10.44 (1H, s), 7.65−6.58 (8H, m), 3.87 and 3.79 (3H, s), 1.46−1.43 (9H, s). MS: 492.

2-(4-Benzyloxy-1*H*-indol-3-yl)-3,6-dichloro-5-(2-*tert*-butyl-1*H*-indol-3-yl-[1,4]benzoquinone (5{*9*}). ^{1}H NMR (acetone-d_6): δ 10.87 (1H, s), 10.40 (1H, s), 7.61−6.61 (13H, m), 5.20 and 5.17 (2H, s), 1.40 and 1.28 (9H, s). MS: 568.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(5-fluoro-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*10*}). ^{1}H NMR (acetone-d_6): δ 11.11 (1H, s), 10.42 (1H, s), 7.88−6.95 (8H, m), 1.43 (9H, s). MS: 480.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(5-methoxy-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*11*}). ^{1}H NMR (acetone-d_6): δ 10.93 (1H, s), 10.42 (1H, s), 7.76−6.86 (8H, m), 3.79 (3H, s), 1.43 (9H, s). MS: 492.

2-(5-Benzyloxy-1*H*-indol-3-yl)-3,6-dichloro-5-(2-*tert*-butyl-1*H*-indol-3-yl-[1,4]benzoquinone (5{*12*}). ^{1}H NMR (acetone-d_6): δ 10.94 (1H, s), 10.42 (1H, s), 7.77−6.95 (8H, m), 5.12 (2H, s), 1.43 (9H, s). MS: 568.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(5-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*13*}). ^{1}H NMR (acetone-d_6): δ 11.00 (1H, s), 10.42 (1H, s), 7.77−6.95 (8H, m), 2.43 (3H, s), 1.43 (9H, s). MS: 476.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(6-fluoro-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*14*}). ^{1}H NMR (acetone-d_6): δ 11.06 (1H, s), 10.43 (1H, s), 7.81−6.94 (8H, m), 1.43 (9H, s). MS: 480.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(6-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*15*}). ^{1}H NMR (acetone-d_6): δ 10.89 (1H, s), 10.42 (1H, s), 7.74−6.95 (8H, m), 1.43 (9H, s). MS: 476.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(7-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*16*}). ^{1}H NMR (acetone-d_6): δ 11.00 (1H, s), 10.42 (1H, s), 7.77−6.95 (8H, m), 2.58 (3H, s), 1.43 (9H, s). MS: 476.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(7-*tert*-butyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*17*}). ^{1}H NMR (acetone-d_6): δ 10.80 (1H, s), 10.43 (1H, s), 7.73−6.95 (8H, m), 1.56 (9H, s), 1.43 (9H, s). MS: 518.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(2,5-dimethyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*18*}). ^{1}H NMR (acetone-d_6): δ 10.52 (1H, s), 10.43 (1H, s), 7.39−6.93 (7H, m), 2.41 and 2.38 (3H, s), 2.37 (3H, s), 1.45 and 1.43 (9H, s). MS: 490.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(5-methoxy-2-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*19*}). ^{1}H NMR (acetone-d_6): δ 10.52 (1H, s), 10.43 (1H, s), 7.39−6.75 (8H, m), 3.74 (3H, s), 2.41 and 2.36 (3H, s), 1.43 (9H, s). MS: 506.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(5-chloro-2-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (5{*20*}). ^{1}H NMR (acetone-d_6): δ 10.81 (1H, s), 10.43 (1H, s), 7.52−6.94 (7H, m), 2.44 and 2.41 (3H, s), 1.45 and 1.43 (9H, s). MS: 510.

Compounds **8**{*n*} in Table 3 were characterized as part of the larger library (see Table 1 in the Supporting Information). For this and subsequent tables, the bisindolyl-dichlorobenzoquinones were analyzed by LC/MS (flow rate = 1 mL/min, UV detection (280 nM), gradient elution A (95% water, 5% acetonitrile, 0.03% formic acid), B (5% water, 95% acetonitrile, 0.03% formic acid); T = 0 min 75% A, 25% B; T = 15 min: 15% A, 85% B. MS data were obtained in positive-ion mode in an ion-trap mass spectrometer.

Compounds **10**{*n*} in Table 4 were characterized as part of the larger library (see Table 1 in the Supporting Information).

Compounds **12**{*2, n*} in Table 5 were characterized as part of the larger library (see Table 2 in the Supporting Information).

Most compounds **13**{*n*} in Table 6 are commercially available, with the following exceptions that were prepared following Smith's procedure for indole synthesis.[16]

2-Cyclopropyl-1H-indole (13{*12*}).[17] ^{1}H NMR ($CDCl_3$): δ 7.95 (br s, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.29−7.26 (m, 1H), 7.13−7.02 (m, 2H), 6.15 (s, 1H), 2.01−1.92 (m, 1H), 0.99−0.93 (m, 2H), 0.80−0.75 (m, 2H).

2-Propyl-1H-indole (13{*15*}).[18] ^{1}H NMR ($CDCl_3$): δ 7.88 (1H, br s), 7.52 (d, J = 7.8 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.14−7.03 (m, 2H), 6.24 (s, 1H), 2.74 (t, J = 7.5 Hz, 2H), 1.75 (m, 2H), 1.01 (t, J = 7.2 Hz, 3H).

2-(3,5-Dimethoxy-phenyl)-1H-indole (13{*24*}).[19] mp: 124−125 °C (lit. 125 °C). ^{1}H NMR ($CDCl_3$): δ 8.32 (br s, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.23−7.09 (m, 3H), 6.82−6.81 (m, 2H), 6.45 (s,1H), 3.87 (s, 6H).

2-Pentyl-1H-indole (13{*29*}).[20] ^{1}H NMR ($CDCl_3$): δ 7.87 (br s, 1H), 7.52 (d, J = 6.3 Hz, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.13−7.03 (m, 2H), 6.24 (s, 1H), 2.75 (t, J = 7.2 Hz, 2H), 1.78−1.68 (m, 2H), 1.40−1.22 (m, 4H), 0.89 (t, J = 7.2 Hz, 3H).

2-Isobutyl-1H-indole (13{*32*}).[21] ^{1}H NMR ($CDCl_3$): δ 7.84 (br s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.14−7.03 (m, 2H), 6.23 (m, 1H), 2.62 (d, J = 7.2 Hz, 2H), 1.99 (m, 1H), 0.98 (d, J = 6.6 Hz, 6H).

Compounds **9**{*n*} in Table 7 are known,[13c] with the following exceptions.

2,5-Dichloro-3-(2-propyl-1H-indol-3-yl)-[1,4]benzoquinone (9{*7*}). IR (KBr): ν 3386, 3064, 2962, 2928, 1676, 1660, 1569, 1459, 1442, 1269, 1250, 1028, 877, 745 cm^{-1}. ^{1}H NMR (acetone-d_6): δ 10.68 (br s, 1H), 7.40 (s, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.09 (td, J = 7.2, 1.5 Hz, 1H), 7.01 (td, J = 7.8, 1.2 Hz, 1H), 2.67 (t, J = 7.5 Hz, 2H), 1.74 (m, 2H), 0.88 (t, J = 7.5 Hz, 3H). ^{13}C NMR (acetone-d_6): δ 178.2, 177.2, 144.1, 141.3, 140.6, 140.3, 136.3, 133.6, 127.2, 121.4, 119.8, 111.2, 104.5, 29.5, 22.6, 13.4. HRMS-FAB (*m/z*): [M$^+$] calcd for $C_{17}H_{13}Cl_2NO_2$, 333.0323; found, 333.0117. mp: 73−74 °C.

2,5-Dichloro-3-(5-iodo-1H-indol-3-yl)-[1,4]benzoquinone (9{*8*}). IR (KBr): ν 3376, 3052, 1730, 1671, 1650, 1572, 1453, 1249, 1112, 1007, 886, 791 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 11.98 (s, 1H), 7.74 (d, J = 1.2 Hz, 1H), 7.63 (d, J = 2.7 Hz, 1H), 7.52 (s, 1H), 7.42−7.39 (m, 1H), 7.33−7.30 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 178.6, 177.6, 143.8, 139.0, 137.2, 135.6, 133.7, 131.8, 130.4, 130.2, 128.7, 115.2, 105.8, 84.6. HRMS-FAB (*m/z*): [M + 2H]$^+$ calcd for $C_{14}H_8Cl_2INO_2$, 418.8977; found, 418.8987. mp: 236−237 °C.

2,5-Dichloro-3-(7-ethyl-1H-indol-3-yl)-[1,4]benzoquinone (9{*15*}). IR (KBr): ν 3397, 2965, 2929, 1677, 1656, 1564, 1434, 1269, 1236, 1108, 1017, 750 cm^{-1}. ^{1}H NMR (acetone-d_6): δ 11.00 (br s, 1H), 7.68 (d, J = 3.0 Hz, 1H), 7.36 (s, 1H), 7.25 (dd, J = 7.2, 2.4 Hz, 1H), 7.10−7.04 (m, 2H), 2.97 (q, J = 7.5 Hz, 2H), 1.34 (t, J = 7.5 Hz, 3H). ^{13}C NMR (acetone-d_6): δ 178.0, 177.7, 143.8, 139.3, 136.7, 135.2, 133.5, 130.0, 127.9, 125.9, 121.1, 125.9, 121.1, 120.7, 119.5, 107.2, 24.0, 14.1. HRMS-FAB (*m/z*): [M$^+$] calcd for $C_{16}H_{11}Cl_2NO_2$, 319.0167; found, 319.0157. mp: 182−183 °C.

2,5-Dichloro-3-(5-methoxy-4-methyl-1H-indol-3-yl)-[1,4]-benzoquinone (9{*20*}). IR (KBr): ν 3395, 3062, 2937, 2835, 1676, 1654, 1561, 1491, 1267, 1124, 1010, 883 cm^{-1}. ^{1}H NMR (acetone-d_6): δ 10.64 (br s, 1H), 7.47 (d, J = 2.7 Hz, 1H), 7.41 (s, 1H), 7.32 (d, J = 8.7 Hz, 1H), 6.97 (d, J = 8.7 Hz, 1H). ^{13}C NMR (acetone-d_6): δ 178.2, 177.9, 152.5, 143.6, 142.0, 139.6, 133.8, 132.4, 128.8, 127.4, 116.9, 109.8, 109.4, 107.0, 56.8, 12.2. HRMS-FAB (*m/z*): [M$^+$] calcd for $C_{16}H_{11}Cl_2NO_3$, 335.0116; found, 335.0108. mp: 162−163 °C.

Acknowledgment. Financial support from the NIH (DK-60532, AG023191) and the American Diabetes Association is gratefully acknowledged. The assistance of J. Wessels and V. Smola in administrative support of this work is greatly appreciated.

Supporting Information Available. Experimental description of library synthesis and characterization and Screening for both insulin receptor activation and cytotoxicity (113 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Arai, K.; Yamamoto, Y. *Chem. Pharm. Bull*. **1990**, *38*, 2929. (b) Bok, J. W.; Hoffmeister, D.; Maggio-Hall, L. A.; Murillo, R.; Glasner, J. D.; Keller, N. P. *Chem. Biol*. **2006**, *13*, 31. (c) Misiek, M.; Hoffmeister, D. *Planta Med.* **2007**, *73*, 103.

(2) (a) Shimizu, S.; Yamamoto, Y.; Koshimura, S. *Chem. Pharm. Bull*. **1982**, *30*, 1896. (b) Kaji, A.; Saito, R.; Nomura, M.; Miyamoto, K.-i.; Kiriyama, N. *Anticancer Res.* **1997**, *17*, 3675. (c) Kaji, A.; Kimura, K.; Teranishi, M.; Kiriyama, N.; Nomura, M.; Miyamoto, K.-i. *Chem. Pharm. Bull*. **1998**, *46*, 1325. (d) Kaji, A.; Iwata, T.; Kiriyama, N.; Nomura, M.; Miyamoto, K.-i. *J. Antibiot*. **1998**, *51*, 235. (e) Kaji, A.; Saito, R.; Nomura, M.; Miyamoto, K.-i.; Kiriyama, N. *Biol. Pharm. Bull*. **1998**, *21*, 945.

(3) (a) Harris, G. D.; Nguyen, A.; Strawn, L.; Fong, A.; App, H.; Le, T.; Sutton, B.; Tang, P. C. Presented at the 215th ACS National Meeting, Dallas, TX, March 29 to April 2, 1998; MEDI 163. (b) Harris, G. D.; Nguyen, A.; App, H.; Hirth, P.; McMahon, G.; Tang, C. *Org. Lett.* **1999**, *1*, 431. (c) Alvi, K. A.; Pu, H. Luche, M.; Rice, A.; App, H.; McMahon, G.; Dare, H.; Margolis, B. *J. Antibiot*. **1999**, *52*, 215.

(4) (a) Fredenhagen, A.; Petersen, F.; Tintelnot-Blomley, M.; Rosel, J.; Mett, H.; Hug, P. *J. Antibiot*. **1997**, *50*, 395. (b) Singh, S. B.; Ondeyka, J. G.; Tsipouras, N.; Ruby, C.; Sardana, V.; Schulman, M.; Sanchez, M.; Pelaez, F.; Stahlhut, M. W.; Munshi, S.; Olsen, D. B.; Lingham, R. B. *Biochem. Biophys. Res. Commun*. **2004**, *324*, 108.

(5) Ono, K.; Nakane, H.; Shimizu, S.; Koshimura, S. *Biochem. Biophys. Res. Commun*. **1991**, *174*, 56.

(6) Mocek, U.; Schultz, L.; Buchan, T.; Baek, C.; Fretto, L.; Nzerem, J.; Sehl, L.; Sinha, U. *J. Antibiot*. **1996**, *49*, 854.

(7) Zhang, B.; Salituro, G.; Szalkowski, D.; Li, Z.; Zhang, Y.; Royo, I.; Vilella, D.; Diez, M. T.; Pelaez, F.; Ruby, C.; Kendall, R. L.; Mao, X.; Griffin, P.; Calaycay, J.; Zierath, J. R.; Heck, J. V.; Smith, R. G.; Moller, D. E. *Science* **1999**, *284*, 974.

(8) Wilkie, N.; Wingrove, P. B.; Bilsland, J. G.; Young, L.; Harper, S. J.; Hefti, F.; Ellis, S.; Pollack, S. J. *J. Neurochem.* **2001**, *78*, 1135.

(9) Liu, K.; Xu, L.; Szalkowski, D.; Li, Z.; Ding, V.; Kwei, G.; Huskey, S.; Moller, D. E.; Heck, J. V.; Zhang, B. B.; Jones, A. B. *J. Med. Chem*. **2000**, *43*, 3487.

(10) Pirrung, M. C.; Liu, Y.; Deng, L.; Halstead, D. K.; Li, Z.; May, J. F.; Wedel, M.; Austin, D. A.; Webster, N. J. *J. Am. Chem. Soc*. **2005**, *127*, 4609.

(11) Lin, B.; Li, Z.; Park, K.; Deng, L.; Pai, A.; Zhong, L.; Pirrung, M. C.; Webster, N. J. G. *J. Pharmacol. Exp. Ther*. Submitted for publication.

(12) Sohn, J.; Kiburz, B.; Li, Z.; Deng, L.; Safi, A.; Pirrung, M. C.; Rudolph, J. *J. Med. Chem*. **2003**, *46*, 2580−8.

(13) (a) Pirrung, M. C.; Park, K.; Li, Z. *Org. Lett*. **2001**, *3*, 365. (b) Pirrung, M. C.; Li, Z.; Park, K.; Zhu, J. *J. Org. Chem*. **2002**, *67*, 7919. (c) Pirrung, M. C.; Deng, L.; Park, K.; Li, Z. *J. Org. Chem*. **2002**, *67*, 8374. (d) Pirrung, M. C.; Fujita, K.; Park, K. *J. Org. Chem*. **2005**, *70*, 2537.

(14) Bookser, B. C.; Zhu, S. *J. Comb. Chem*. **2001**, *3*, 205.

(15) Tolliday, N.; Clemons, P. A.; Ferraiolo, P.; Koehler, A. N.; Lewis, T. A.; Li, X.; Schreiber, S. L.; Gerhard, D. S.; Eliasof, S. *Cancer Res.* **2006**, *66*, 8935.

(16) (a) Smith, A. B., III; Visnick, M. *Tetrahedron Lett.* **1985**, *26*, 3757. (b) Smith, A. B., III; Visnick, M.; Haseltine, J. N.; Sprengeler, P. A. T*etrahedron* **1986**, *42*, 2957.

(17) Augustine, R. L.; Gustavsen, A. J.; Wanat, S. F.; Pattison, I. C.; Houghton, K. S.; Koletar, G. *J. Org. Chem.* **1973**, *38*, 3004.

(18) Castro, C. E.; Gaughan, E. J.; Owsley, D. C. *J. Org. Chem*. **1966**, *31*, 4071.

(19) Pigerol, C.; Chandavoine, M. M.; De Cointet de Fillain, P.; Nanthavong, S. Ger. Offen. DE 2524659, Oct 26, 1977.

(20) Piozzi, F.; Langella, M. R. *Gazz. Chim. Ital.* **1963**, *93*, 1382.

(21) Ito, Y.; Kobayashi, K.; Saegusa, T. *J. Org. Chem.* **1979**, *44*, 2030.

CC070062M